Book of Abstracts

21st ANNUAL WORKSHOP ON EMERGING HRMS AND LC-MS/MS APPLICATIONS IN ENVIRONMENTAL ANALYSIS AND FOOD SAFETY

Organized and hosted by:

Environment and
Climate Change Canada

Environnement et
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07 – 08 October 2025, Ottawa, Canada

Sandman Signature Hotel, 250 West Hunt Club Rd, Ottawa

https://lcmsms2025.ca/

Dear Colleagues,

On behalf of the Organizing Committee, it is my great pleasure to welcome you to the 21st Annual Workshop on Emerging HRMS and LC-MS Applications in Environmental Analysis and Food Safety.

This workshop provides a forum for scientists from government, academia, and industry to exchange knowledge, discuss challenges, and advance the application of HRMS and LC-MS/MS in environmental and food safety research. Our goal is to highlight innovative and practical applications of these techniques for the screening and quantification of organic contaminants in environmental and food matrices.

Together with the Scientific Committee, we have prepared an exciting and diverse scientific program. Highlights include plenary and invited lectures by Diana Aga, Damià Barceló Cullerès, Stéphane Bayen, Robert Letcher, Paul Mayer, Hui Peng, and Jeff Smith. Poster sessions, held mid-day, further enrich the program and provide opportunities for interaction and discussion.

It is important to highlight that this workshop would not have been possible without the involvement of many people who have worked on planning and organizing this annual event. In particular, I would like to thank the Scientific Committee members for overseeing all scientific aspects of this meeting, providing advice, assisting in planning the plenary lectures and the overall technical program, session chairpersons, and selecting the best presenters for awards. The Scientific Committee members include:

Prof. Diana Aga (University at Buffalo, Buffalo, USA)
Prof. Stéphane Bayen (McGill University, Montréal, Canada)
Prof. Damià Barceló Cullerès (IDAEA-CSIC, ICRA, Barcelona, Spain)
Prof. Robert Letcher (ECCC, Carleton University, Ottawa, Canada)
Prof. Amadeo Rodríguez Fernández-Alba (University of Almeria, Spain)
Dr. Andrzej Wnorowski (Chair), (Environment and Climate Change Canada, Ottawa)

I am also deeply grateful to the Organizing Committee, who have worked tirelessly since last year to ensure that every detail of the Workshop is carefully planned. Their support with logistics, audio-visual preparations, website design, and registration have been invaluable. The members are:

Dr. Alana Greaves (ECCC, Canada)
Gillian Chapman (ECCC, Canada)
Devon Gouveia (ECCC, Canada)
David Harnish (ECCC, Canada)
Philippe Lalonde (ECCC, Canada)
Anita Pall (ECCC, Canada)
Dr. Tristan Smythe (Carleton University, ECCC, Canada)
Dr. Andrzej Wnorowski (ECCC, Canada)

We would also like to acknowledge our Sponsors, whose generous contributions allowed this event to remain free of charge for all participants, and whose support helps us recognize outstanding young scientists for their research achievements. The contributing partners are:

Prince Sultan Bin Abdulaziz International Prize for Water
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SCIEX
Canadian Society for Mass Spectrometry

Finally, I extend my sincere thanks to all presenters for sharing their findings, for their dedication in the laboratory, and for preparing their presentations so that we can all benefit from new knowledge and the latest applications of HRMS and LC-MS/MS in environmental and food safety analysis. I am equally grateful to all attendees for your participation, questions, and discussions. Your engagement is what makes this Workshop successful.

I hope your participation in this event sparks new ways of thinking, inspires fresh ideas and approaches, and fosters partnerships and collaborations. This spirit of exchange and connection is the true purpose of the Workshop.

Kind regards,

Andrzej Wnorowski, Ph.D.
21st HRMS and LC-MS/MS Workshop Chair

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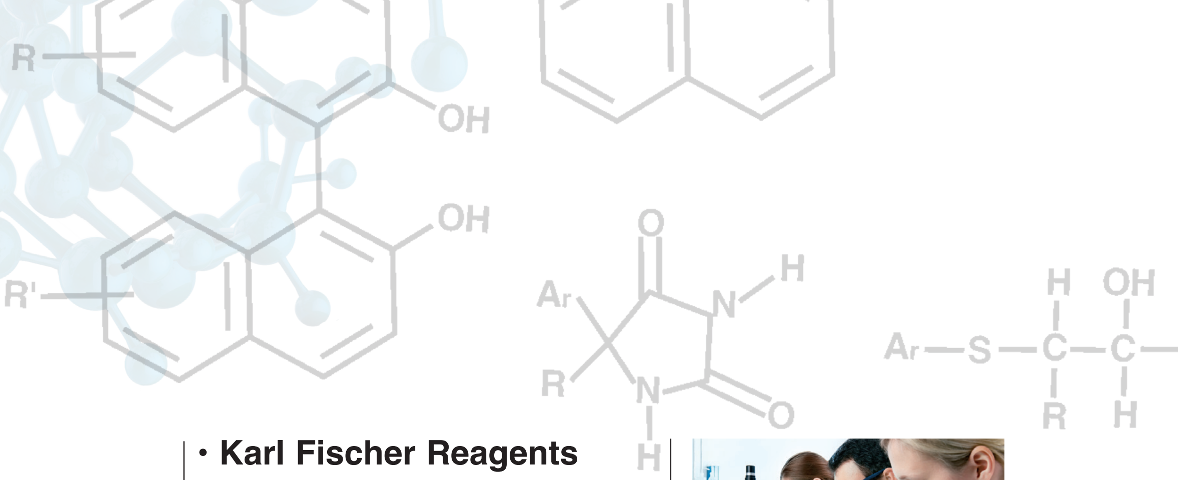

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Oral Presentations

OP-01

Immunoproteomics and Emerging Contaminants Determination followed by Maldi-TOF and LC-orbitrap-MS in Wastewater Based Epidemiology

Jaxaira Maggi[1], E. Sánchez-Jiimenez[1], Joaquin Abian[1], Antoni Ginebreda[2], Montserrat Carrascal[1], Damià Barceló[3]

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Abstract

Wastewater-based epidemiology (WBE) offers a unique window into the health and habits of communities through the analysis of pollutants and biomarkers in sewage. Traditionally focused on small molecules, such as pharmaceuticals and illegal drugs, recent advances in environmental proteomics have expanded WBE to include large biomolecules such as proteins. Notably, novel sampling methods using polymeric probes and high-resolution mass spectrometry have facilitated the detection of human and animal proteins, both soluble and in particulate material, linking them to specific populations and industrial activities. An immunological dimension to this approach is fundamental to include the recognition of host immunoglobulins, immune-response proteins, and pathogen antigens in wastewater, potentially serving as indicators of community immune status, infection prevalence, and vaccination coverage.

In this work, a summary of the methods developed in our facilities[1,2] for the separation of the soluble and particulate fractions of wastewater and their concentration, and conventional shotgun proteomics will be presented. We developed *Aquasearch*, a novel software application designed to identify protein biomarkers from MALDI-TOF raw data. We have validated the effectiveness and accuracy of this tool in identifying protein biomarkers through the analysis of a set of wastewater samples and by comparing the data obtained with LC-Orbitrap MS. Aquasearch and its source code are available at ref [2]. The number of identifications ranged between 244 and 386 for the soluble part and between 488 and 935 in the particulate . The proteins and the origin of these proteins in each fraction is very different . While in the particulate fraction Bacteria proteins dominate, in the soluble fraction the number of Eukaryote proteins increases up to 42% of total protein. In the case of the soluble fractions, the 3 most abundant proteins are amylase enzymes from humans with the double of Peptide-Spectrum Matches (PSMs) than the next most abundant protein. These human amylases are followed by amylase enzymes from murids, human albumin and by albumins from livestock (cow, pig, sheep or rabbit). In contrast, in the particulate fraction, though the most

abundant protein is a human elastase (with 4 times less PSMs than the most abundant soluble proteins), it is followed for a mix of proteins from human and different kinds of bacteria. The protein composition in the influent and effluent waters of 3 wastewater treatment plants (WWTPs) in Catalonia which serve communities of different population and industrial profile was comprehensively studied. The results indicate that excreta (urine and faeces) from humans, and blood and other residues from livestock were the two main protein sources [1] .Furthermore, our findings enable the proposal of specific bioindicators for wastewater-based environmental monitoring. A list of candidates with different application in environmental and health-related fields is selected and will be quantified. This includes human and animal population monitoring, particularly for rodent pest control (amylases) and livestock processing industry monitoring (albumins). Protein-related WBE enables to detect rodent pests in cities and can be a tool with potential for their population control. In short our studies offers a complete set of complementary information that enhances the understanding of the processes occurring at water treatment plants at a protein level. Proteins are retained in the WWTP and only some recalcitrant molecules remains. This data will be invaluable for implementing new control methodologies in the WWTPs and investigating the impact of wastewater discharge in environmental buffers.

Looking forward, the next frontier is expanding multi-omics integration in WBE [3]. This includes not only proteomics and immunology, but also metagenomics, metabolomics, and beyond. This comprehensive framework could enhance understanding of disease transmission, immunity, and population health. In turn, such insights may inform more targeted public health strategies and more efficient resource allocation. Ultimately, by linking environmental surveillance with clinical medicine, WBE stands poised to become a proactive, cost-effective, and community-centred pillar of global disease monitoring.

Acknowledgements and References

This work is funded by the Spanish Ministry of Science and Innovation, ref. PID2024-158804OB-100 under the project entitled: “Proteomics applied to Wastewater-Based Epidemiology(WBE) New strategies for the surveillance of human health and the improvement of environmental diagnostics”.

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OP-02
Identification of new markers of AFFF contamination in surface water and groundwater near a hotspot in Atlantic Canada

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Abstract

The widespread use of Aqueous Film Forming Foams (AFFF) at airports, military bases, and firefighter training facilities has left thousands of contaminated “hotspots” across North America. Per-/polyfluoroalkyl substances (PFAS) are the key ingredients of legacy AFFFs that pose the greatest risk of contaminating neighboring drinking water systems[1]. In Canada, regulations have been introduced to limit exposure to a small number of PFAS in drinking water (30 ng/L of 25 common PFAS)2.

In the present study, we use liquid chromatography-ion mobility mass spectrometry (LC-IMS-MS) to identify both regulated and unregulated legacy PFAS in surface water samples collected near a former firefighter training facility, as well as drinking water wells downgradient.

We identified over 57 different PFAS in surface water, including 5 new PFAS that have not been previously reported in environmental waters: nH-ketone-5:2-, ketone-8:2-, and unsaturated 8:2 fluorotelomer sulfonates, tridecafluoro-N-methylsulfonyl-hexane-1-sulfonamide, and heptadecafluoro-N-methylsulfonyl-octane-1-sulfonamide. The total concentration of PFAS was diluted from 1500 ng/L to 320 ng/L as the water entered the North Atlantic approximately 5 km away. In two drinking water wells characterized by exceedances >100 ng/L and located 1.5 km and 3.5 km from the hotspot, 27 PFAS were identified, including 13 that are unregulated and 6 that likely result from degradation of AFFF formulations. While the presence of unregulated PFAS did not significantly change the total PFAS concentrations, the identification of new markers of AFFF contamination may inform forensic studies aimed at establishing links between a source of pollution and its receptors.

The present study identifies 5 new PFAS in AFFF-impacted surface water, as well as 6 potential markers of legacy contamination in drinking water systems.

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OP-03

Targeted and non-targeted analysis of endocrine disrupting chemicals and their metabolites: implications for reproductive effects

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Abstract

Exposure to endocrine disrupting chemicals (EDCs) has been associated with adverse human health effects, including those relating to the reproductive system; some EDCs have been completely banned (e.g., bisphenol A in baby bottles) in Canada. Consequently, replacement/alternative chemicals have been introduced into the market. Since information on exposure levels of these replacements is limited, chemical exposure characterization via targeted analysis poses a significant challenge. Non-targeted analysis (NTA) has emerged as a complementary technique to identify new EDCs and their metabolites, and aid in the identification of compounds for prioritization by risk assessment. The goal of this study was to develop an NTA method to screen for EDCs in human urine and identify markers of exposure related to adverse reproductive health effects.

Urine samples analysed were collected as part of a male fertility study; in total, 41 samples were collected from males that had known fertility issues and 105 samples were collected from males that had no fertility issues (control group). NTA workflow/procedure included the development of a sample preparation method using solid phase extraction (validated with 55 EDCs deemed “high priority” by Health Canada) and sample analysis via the Thermo Fisher LC-MS Orbitrap. Samples were screened via targeted analysis (TraceFinder) and NTA (Compound Discoverer) to identify target EDCs and unknown metabolites. Differential and statistical analysis was performed to identify any differences between the two sample groups.

EDCs included in the method validation were part of Health Canada’s bisphenol A functional alternatives list. Our method is the first to include many of these chemicals, including 2,2-methylenedi-p-cresol and methyl 2,2-bis-4-hydroxyphenyl acetate as part of an NTA workflow for analysis of human urine samples. Five sample preparation methods were compared with respect to the chemical coverage and extraction efficiencies of 55 target EDCs. Target EDCs were selected in a way that would ensure inclusion of various chemical groups, including monophthalate metabolites, bisphenols, benzophenones, and plastic additives. The optimized sample preparation method was based on solid phase extraction with HLB cartridges and all chemical groups demonstrated extraction efficiencies above 40%.

Targeted analysis was able to identify 11 phthalate metabolites, including monopentyl, monohexyl, monoheptyl, and monopropyl phthalate. Bisphenol alternatives such as BPS-MAE, 2,4 bisphenol S, TGSH, and D8, were also identified. We were also able to identify (for the first time) butyl bis (4-hydroxyphenyl) acetate in urine samples. This compound has been found to be associated with thermal resistant materials, such as thermal papers. Using the NTA workflow, we identified (for the first time) other novel metabolites in urine samples, which include sulfonated 2,2-methylenedi-p-cresol, glucuronated butyl bis (4-hydroxyphenyl) acetate, glucuronated diphenolic acid, and glucuronated methyl 2,2-bis-4-hydroxyphenyl acetate.

Statistical analysis indicated that only monoisononyl and monoheptyl phthalate demonstrated significant differences between the two urine sample groups. It was noted that the concentrations of monoisononyl and monoheptyl phthalate were higher in the control group samples compared to those from males with known fertility issues. This finding suggests that reproductive effects may be due to co-exposure between different chemicals, as well as other lifestyle factors. Pearson correlations were performed to identify co-exposure; correlations were found between benzylparaben and monoheptyl pth, as well as between monoisononyl and benzylparaben in the control group. This finding may indicate that there is a co-exposure of phthalates and parabens for the participants in the control group.

Novelty: Identification of butyl bis (4-hydroxyphenyl acetate) and metabolite in urine. Co-exposure between monoheptyl and benzylparaben in urine samples.

OP-04
Bridging non-targeted studies across LC-MS platforms using machine learning: honey fingerprinting as a case study

Stéphane Bayen[1*], Shawninder Chahal[1], Lei Tian[1], Ferenc Balogh[2], Ralph Hindle[3], Kathy Hunt[3], Anca Baesu[4], Yong-Lai Feng[4], Salwa Karboune[1], Lorna De Leoz[5], Tarun Anumol[5], Daniel Cuthbertson[5]

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Abstract

Honey one of the most frequently frauded foods globally, with cases of adulteration with foreign sugars, mislabelling of botanical origin, or contamination with prohibited veterinary drugs. Recent advances in analytical sciences, notably non-targeted analysis (NTA) via liquid chromatography-mass spectrometry (LC-MS), have opened the door to the comprehensive chemical fingerprinting of food, and signals for thousands of molecules can be recorded simultaneously for a single sample. However, a key challenge in the implementation of NTA for food authentication is that it is unlikely that the exact same fingerprint will be observed across multiple LC-MS instruments due to small shifts in sensitivity, observed mass, and retention time.

In the present study, we use the botanical origin of honey as a case study for the application of LC-MS based fingerprinting for food authenticity purposes. Several hundreds of honey samples self-reported as monofloral blueberry, buckwheat, clover, goldenrod, linden, or other were collected in Canada. The LC-MS spectra of these samples were recorded for four LC-QTOF-MS instruments with different models. We then developed various automated machine learning pipelines capable of classifying honeys with different floral types and identifying markers of authenticity. We also developed a strategy for bridging the molecular feature space across the four LC-QTOF-MS instruments.

Using our data analysis pipelines, the LC-HRMS data set was reduced from several hundreds of gigabits to only key a few dozens of features (markers) sufficient to classify honeys based on their floral origin and to detect mislabelled honey samples. A logistic regression model was then trained on a set of samples from one instrument and then tested on a different set of samples on another instrument. Three of the instruments showed excellent

classification performance when training and testing on different instruments, with few statistically significant differences in classification performance when compared to training and testing on the same instrument. The fourth instrument still showed acceptable cross-instrument performance, but often showed reductions in classification performance compared to the same-instrument case. It was also noted that this instrument had an LC elution profile that was quite different from the other three instruments and was likely the cause of its reduced performance. Overall, when training and testing on the same instrument, the accuracy ranged from 83 – 88%, and when training and testing on different instruments, the accuracy ranged from 77 – 85% showing that even when differences were statistically significant, they were relatively low in magnitude.

This novel bridging approach opens the use of NTA across platforms and enhances cross-laboratory collaboration in various fields including food authentication, metabolomics and environmental monitoring.

Acknowledgments

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OP-05

Forced degradation, LC-UV and LC- HRMS studies on Mometasone Furoate and Glycopyrrolate: Characterization of degradation products

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Drug purity is crucial for efficacy and safety, as impurities significantly impact stability and therapeutic performance. Mometasone Furoate (MF), a synthetic corticosteroid that suppresses pro-inflammatory cytokines, and Glycopyrrolate (Gly), a long-acting muscarinic antagonist, are used in fixed dose combination (FDC) products for the treatment of Chronic Obstructive Pulmonary Disease (COPD). Both drugs contain functional groups such as hydroxyl, ester, ketone, halogenated sites, and quaternary ammonium groups, rendering them susceptible to degradation. Regulatory guidelines mandate impurity profiling; therefore, force degradation studies are essential for assessing drug stability under hydrolytic, thermal, photolytic, and oxidative conditions. However, no comprehensive study currently reports the degradation and interaction products of MF and Gly in combination therapy.

Forced degradation studies of MF and Gly were performed according to ICH Q1A(R2) guidelines. Stress conditions included hydrolytic (acidic, alkaline), thermal, humidity, and UV exposures. Chromatographic separation was achieved using a Sunfire™ RP C18 column (5 µm; 4.6 × 250 mm). The mobile phase consisted of acetonitrile and ammonium acetate buffer (0.02 M, pH 4.5). HPLC was employed for separation of parent drugs and degradation products. LC-MS-Q-TOF was utilized for characterization of degradation products, and structural elucidation was conducted by analyzing fragmentation pathways. The method ensured resolution of critical degradation products, enabling a systematic investigation of stability-indicating parameters.

The forced degradation study demonstrated that both MF and Gly are highly susceptible to degradation in acidic and alkaline conditions, including in the presence of trace sodium carbonate. Chromatographic analysis allowed efficient separation of degradation products, designated as DP1–DP5, H1–H3, and S1–S3. LC-MS-Q-TOF analysis revealed detailed mass fragmentation pathways, providing structural insights into the formation of degradation products. MF showed degradation primarily through hydrolysis of ester linkages and modification of halogenated moieties, while Gly exhibited instability due to quaternary ammonium group cleavage and hydrolytic pathways. Photolytic and thermal stresses resulted in minor degradation compared to hydrolytic stresses. The developed HPLC method successfully resolved co-eluting peaks and was stability-indicating. Importantly, this study provides the first comprehensive assessment of the degradation and interaction behaviour of MF and Gly in fixed-dose combination therapy. The findings enhance understanding of their degradation liabilities, which is critical for selecting optimal formulation conditions, defining packaging requirements, and establishing storage parameters. These insights will ultimately help to extend the shelf life and improve the therapeutic safety of MF-Gly FDC products.

Novelty: First comprehensive impurity profiling and forced degradation study of MF and Gly combination, with structural elucidation of degradation products using LC-MS-Q-TOF.

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OP-06
Enhancing our understanding of viroceuticals using an optimized mass spectrometry-based lipidomics approach

Jocelyn A. Menard[1], Tilia Zinnecker[2], Joshua A. Roberts[1], Elena Godbout[3], Rozanne Arulanandam[3], Andrew Chen[3], Anne Landry[3], Christopher N. Boddy[4], Udo Reichl[2,5], Jean-Simon Diallo[3,6], Yvonne Genzel[2], Jeffrey C. Smith[1,7,8]

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Abstract

Viroceuticals are effective emerging tools for vaccination, genetic therapies, and cancer treatment, yet have large costs associated with their manufacturing and delivery. We have conducted novel studies on viroceutical products and production via MS-based lipidomics. Lentivirus (LV) technology descends from human immunodeficiency virus (HIV), whose lipid envelope has been previously shown to have a direct impact on its transduction efficiency. Madin-Darby Canine Kidney (MDCK) cells are a widely used host cell line for influenza A virus (IAV) production as an alternative to egg-based production and exhibit a pronounced heterogeneity that can significantly affect viral yields. We have developed a rapid, robust, and sensitive untargeted lipidomics pipeline to elucidate novel lipidomic signatures that affect both LV and IAV biotherapeutic production pipelines.

HEK 293T cells were cultured to confluence and divided into three populations: untreated, treated with a VSV G plasmid, or treated with all four plasmids coding the production of LV. Each population was left for 24, 48, and 72 hours before harvesting. Both the cellular and viral fractions were harvested and the lipids were extracted via a Bligh and Dyer solvent extraction. A portion of all samples were pooled and analyzed in DDA mode using iterative injections employing exclusion lists using an Agilent 1260 HPLC coupled to an Agilent 6546 QToF mass spectrometer to identify all lipid species. Samples were then analyzed in triplicate in MS-only mode for quantitative analysis. A similar process was employed for MDCK cells producing influenza.

The impact of 48 hours of LV production on the lipidome of HEK 293T cells was measured and 151 lipids were identified, 84 of which had fold changes with FDR-corrected $P<0.05$ compared to HEK 293T treated with media. LV contained 102 lipids, half of which were determined to be unique LV virion lipids after subtracting the media lipidome. Following this, an untargeted lipidomics experiment was conducted on HEK 293T packaging cells producing LV at three time points: 24, 48 and 72 hr. Lipids were identified and significant changes to the HEK 293T lipidome from LV production were recorded at each time point. Triacylglycerol (TG) and phosphatidylcholine (PC) species were the most affected lipid classes throughout the time course. Large increases in TG species abundance were observed with significant depletions for many PC species, suggesting lipid droplet formation occurs during LV production. We also investigated two MDCK clones (C59 and C113) that differ in biochemical properties and viral production attributes and examined their lipid dynamics upon influenza A virus (IAV) infection at 24, 48, and 72 hours. C113 had elevated levels of lipid species across all major lipid classes with the exception of ether lipids compared to C59. IAV infection in C59 led to lipid droplet (LD) accumulation, elevated levels of ceramides and diacylglycerols as well as lysophospholipid and phospholipid depletion. IAV

infection in C113 led to a decrease in LDs and lysophospholipids. Lipidomic analysis of the purified progeny virions from C59 and C113 yielded only subtle differences with an overall strong positive correlation in lipid profile (R2 = 0.77), suggesting similar lipid raft domain composition between clones. This approach offers the potential to elucidate critical lipidomic dynamics, enabling the potential refinement of current production systems and achieving more efficient influenza production, contributing to the advancement of vaccine manufacturing capabilities.

Novelty: For the first time, we report the temporal dynamics of lipids during viroceutical production in both producer cells and in the viral product in both lentiviral and influenza models.

OP-07
High throughput tandem mass spectrometry analysis of para-phenylene diamine quinones for spatiotemporal analysis of tire wear toxicants in urban waters

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Abstract

Para-phenylene diamine quinones (PPDQs) are an emerging class of environmental contaminants derived from the transformation of antiozonant para-phenylene diamines (PPDs) which are added to rubber tires to prevent cracking and extent their service life. N-(1,4-dimethylbutyl)-N'-phenyl-p-phenylene diamine (6PPDQ) has been shown to leach from tire wear particles and be acutely toxic to salmonids at ng/L concentrations.1 6PPDQ is transported to receiving waters during rain events and associated with 'urban run-off mortality syndrome'. Characterizing the fate and distribution of PPDQs over complex urban landscapes requires widescale intensive sampling enabled by high throughput analytical workflows.

We have developed a direct mass spectrometry approach using an on-line sample clean up via a capillary hollow fibre membrane immersion probe.2 Hydrophobic analytes in aqueous samples permeate through the membrane and are entrained into a flowing (10 uL/min) solvent acceptor phase (85% methanol/15% heptane/0.1% formic acid) which is continuously injected into an electrospray ionization source. PPDQs are quantified by multiple reaction monitoring of [M+H]+. Combined with a purpose-built smartphone sample collection app, low-cost auto-sampler, and data processing tools we describe end-to-end automation that supports rapid reporting and adaptive sampling.

We report on the method optimization and performance metrics providing a 'fit-for-purpose' method for direct analysis 6PPDQ in complex storm and surface waters. Key performance metrics include detection limits of ~ 2 ng/L with a complete analytical duty cycle of 3 mins/sample. This workflow allows for the analysis of up to 200 samples/day enabling rapid reporting and adaptive sampling.

We will present results from a large citizen science sampling campaign including nearly 5000 samples on Vancouver Island (BC, Canada) representing the single largest PPDQ dataset globally. Working with 38 community and First Nation partners, we characterize spatiotemporal trends and environmental exposures in 78 urban streams. During rain events, 40% of stream samples were observed in exceedance of the BC Water Quality Guideline for 6PPDQ and 15% exceeded the lethal concentration for juvenile coho. We have employed this data to develop predictive models that indicate that the number of antecedent dry days and turbidity are important drivers. Combined with the convenience of geo-located sample collection, automated data processing and geospatial mapping, this program has been employed to inform future sampling sites and maintain active community engagements across multiple stakeholders for over two years. The results indicate that 6PPDQ concentrations vary widely over time and space raising important questions about obtaining a representative sample.3 This work has resulted in the identification of important point sources and emphasizes the importance

of working with local government agencies to assess stormwater inputs to inform risk assessments and mitigation efforts. We have recently adapted this technique to report the first on-site real-time measurements of 6PPDQ, monitoring in-stream concentrations during a rain event.

Novelty: End-to-end analysis automation of ultra trace level emerging tire wear toxicants supports widescale surveillance and citizen science campaigns to characterize the fate and distribution of 6PPDQ in urban waters.

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OP-08

ASSESSING CHEMICAL LEACHABLES FROM MICROPLASTICS AND BULK PLASTIC SINGLE-USE FOOD CONTACT MATERIALS

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Single-use plastic packages (SUPs) are designed for one-time or short-term use before disposal. During storage and their usage, microplastics (MPs) and chemical migrants can leach from these containers into food. This study aimed to develop a protocol for generating standard MPs from bulk food contact materials (FCM)and assess the rate of migration of chemicals from the different microplastic sizes and bulk plastic materials. Take-out containers were cleaned thoroughly with Milli-Q water and then cut into pieces measuring 2-4 mm. The samples were then precooled in liquid nitrogen (-196 °C) for about 20 minutes before milling. The resulting samples were separated into various sizes using stainless steel sieves. High-purity sodium sulphate blanks were prepared to monitor contamination. ATR-FTIR was employed to determine the polymer type and assess changes in the chemical composition of the polymer after milling. For the migration test, procedural blanks were prepared by extracting 0.05 g of sodium sulphate in 5 mL of 3% acetic acid for 10 days. Leachates of MPs were obtained by extracting 0.1 g of MPs in 10 mL over the same period. Leachates from bulk plastics were obtained by extracting scratched and non-scratched samples in 20 mL of 3% acetic acid for 10 days. Leachables from those MP fractions and bulk material were tested using LC-QToF-MS/MS (Agilent Technologies). Results showed higher recoveries of MP and blank fractions for cycle 6. Spectral matching based on ATR-FTIR analysis identified polypropylene as the polymer type and sodium sulphate as the blank material, suggesting little to no changes in their chemical composition during milling. LC-MS non-targeted analysis extracted 1,734 features from the MP fractions and 2,337 from the bulk material leachate, respectively. Principal component analysis revealed differences among the LC-MS profiles of the blanks, the MPs, and the bulk samples. MS/MS identified previously unreported suspects leaching into food from the plastic containers. This work lays the foundation for developing standard protocols to produce MPs through cryogenic milling and investigating the leachables they release into food.

OP-09

Impact of daily usage and washing on the contaminant and microplastics loads released from reusable drinking water bottles and food storage bags

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Plastic packaging pollution is a major threat to ecosystems. To mitigate this issue, international organizations have proposed plastic reusability as a potential solution. Plastic containers, for example, can be reused multiple times to store food. Reuse, however, may influence the degradation of the bulk material and induce the release of microplastics into food. This contamination adds to the load of microplastics which may already be present in the raw plant animal ingredients, which may have accumulated those from their environment. In this case, microplastics can contribute to the contamination of food with harmful chemicals through vectorization of environmental contaminants (e.g. POPs or trace elements), or through the leaching of additives used in plastic formulation. The exact role and contribution of microplastics to food contamination remain however poorly understood.

This study aimed to assess the impact of daily usage on the release of chemical contaminants from reusable plastic food contact materials, and understand the role of microplastics in this context. More specifically, reusable plastic bags and sports bottles were subjected to various weathering conditions, including squeezing, scratching, and washing. Food simulants were used to evaluate potential chemical release into actual food systems. The simulants were analyzed using liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-QTOF), and non-targeted chemical profiles were compared across bulk materials and microplastic fragments to identify unknown contaminants of concern and assess the effects of weathering.

Consumer-driven weathering has a significant impact on the release of contaminants from reusable plastic items. To refine the analytical interpretation, several LC-HRMS data processing workflows were applied to reduce dataset complexity and retain only the most analytically relevant molecular features. Distinct high-resolution mass spectrometry (HRMS) fingerprints were observed across various types of reusable plastic bags, as well as following different physical treatments applied to reusable sports bottles. In particular, scratching and washing of plastic bags led to a notable increase in contaminant release into food simulants, as revealed by the intensification of specific LC-MS peaks. The microplastics generated during these weathering processes were found to leach the same chemical constituents as those present in the original bulk polymer, reinforcing that microplastics serve as vectors for chemical contaminants. Similarly, mechanical stress such as squeezing and washing of sports bottles resulted in variations in the intensity of selected molecular features, suggesting an alteration of contaminant migration dynamics. Multiple contaminants of emerging concern (CECs) were structurally characterized through MS/MS fragmentation and library-matching approaches, enabling the identification of potentially hazardous compounds migrating into food-relevant matrices. To complement the chemical analysis, microplastics were also characterized using scanning electron microscopy (SEM) and Raman imaging techniques, enabling direct visualization and confirmation of their presence in food simulants. These imaging methods provided valuable insights into the physical degradation of the plastics and the migration potential of resulting microplastic fragments. This novel study demonstrates that consumer weathering can significantly enhance the release of chemical contaminants through the vector function of microplastics. This highlights the importance of considering real-world usage patterns when evaluating the safety of reusable plastic

food-contact materials. Future work will focus on deepening the understanding of the relationship between the properties of microplastics and the dynamics of chemical migration into food systems with MS applications.

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OP-10
Pomegranate juice authentication by flow-injection nano-LC-ESI mass spectrometry

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Abstract

Pomegranate juice, valued for its health benefits, is becoming a target for economically motivated adulteration with cheaper alternatives like apple, pear, or grape juices. Conventional authentication methods often fail due to limitations in sensitivity or specificity. This study presents a rapid, high-throughput method combining flow-injection mass spectrometry (FI-MS) with machine learning (ML) for detecting adulteration in pomegranate juice. Classification models were developed using various ML algorithms to detect adulterated pomegranate juice samples, and subsequently applied to commercial products for validation. This approach demonstrates potential for accurate and efficient juice authentication, protecting consumers and producers from fraudulent products and ensuring product integrity in the marketplace.

Pure fresh fruit juices (15 pomegranate, 15 grape, 14 pear, and 13 apple) were prepared in-lab using a blender. Pure pomegranate samples were mixed with each other pure juices individually at concentrations ranging from 1% to 40% to mimic adulterated products. Quality control (QC) samples were prepared by mixing equal-volume of all juices. Samples were diluted, centrifuged and filtered before being injected into the nano-LC-ESI-Orbitrap via an autosampler. Sixteen commercial samples were purchased from local stores and analysis in the same procedure. The injection volume, flow rate, ionization source voltage and dilution factor of samples were optimized using the QC sample. Support Vector Machine (SVM), k-Nearest Neighbours (kNN), Random Forest (RF) algorithm were employed to classify and semi-quantify the samples. The optimized nano-LC-MS method enabled a sensitive detection of 8355 reproducible MS features (i.e., relative standard deviation <30%) in QC samples.

Preliminary testing on the performance of each ML algorithms was conducted by building cross-validation (CV) models to classify pure juices. The overall accuracy of SVM, kNN and RF models were 73.33, 80.00, 100%, respectively, indicating the high suitability of RF for this dataset. Thus, RF is selected for the following model construction.

To enable the differentiation of samples both by fruit species and level of adulteration, a hierarchical classification strategy was adopted. Specifically, the first model categorized samples into 7 classes: each pure juice is one class (i.e., 4 classes in total), and each type of adulterated pomegranate is one class (i.e., 3 classes in total). The optimized RF model yielded 100%, 94.95% and 90.00% accuracy in calibration, cross-validation and external validation, respectively. Identified mixture samples undergo a second layer analysis for the categorization of its concentration level (i.e., 1%, 5%, 10%, 20%, 30% or 40%). The RF model achieved 100%, 75.00%, 94.44% accuracy in calibration, cross-validation and external validation, respectively.

To examine if the models constructed using lab-prepared juice samples are applicable to commercial products, 16 juice samples including pure juice and mixed juice were purchased. Only the first model for fruit species identification was applied to commercial juices, since the lack of concentration information of mixed juice prevent the downstream validation of the semi-quantification model. Based on preliminary examination, all pure pomegranate products were classified correctly, while all mixed juices and juices containing no pomegranate were classified into the groups with species labeled on the products. This confirmed the potential of this nano-LC-MS coupled with machine learning approach in authenticating commercial pomegranate juice products.

A high-throughput nano-LC-MS method, optimized RF models and a hierarchical analytical workflow were integrated to simultaneous authenticate pomegranate juice by fruit species and adulteration levels for the first time.

OP-11

To Automate or Not to Automate: Optimizing Parameters in Liquid Chromatography–Triple Quadrupole Mass Spectrometry for Pesticides

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The use of pesticides involves a complex balance between their benefits and potential environmental risks. However, their widespread use has raised concerns about their adverse ecological impacts and potential human health effects. Pesticides and their degradation products can easily be transported into various environmental compartments. Therefore, effective screening for pesticides is essential to prevent and mitigate contamination. Target analysis using liquid chromatography with tandem mass spectrometry (LC-MS/MS) has become the method of choice for environmental analysis of pesticides because of its high selectivity and sensitivity. In developing LC-MS/MS methods optimization of important parameters, such as selection of precursor ions, product ions, fragmentor voltages, and collision energies for each analyte is a critical first step that will influence sensitivity of the method. This optimization process is often performed using automated software that is built in with the instrument and is unique to each instrument brand. However, it is important to check the reliability of these automated workflows by comparing conditions obtained through manual optimization for compounds that tends to fragment, or form adducts such that the precursor and fragment ions observed depend on the chemical environment.

In this study, we optimized MS conditions for six fluorinated pesticides — isoxaflutole, fomesafen, fipronil, pyroxasulfone, oxathiapiprolin, and cyflufenamid — using both automated and manual optimization methods. The computerized optimization was performed using Agilent MassHunter Optimizer software, which injected 50 ppb pesticide standards directly into the system without the use of a column. The fragmentor voltages and collision energies were scanned within the ranges of 70–240 V and 10–100 eV, respectively. For manual optimization, we used the Agilent MassHunter Workstation Data Acquisition software. Precursor ions were identified in MS2 scan mode across a mass range from 100 m/z to 100 m/z above the molecular weight of the analyte. The most abundant precursor ion was selected for further optimization of the fragmentor voltage and for the identification of product ions. Additionally, product ions were determined using a collision energy range of 10–100 eV.

Comparison of the values obtained from automated and manual optimizations methods showed the same values for cyflufenamid, fipronil, fomesafen, and oxathiapiprolin in terms of precursor ions, quantifier ions (Q, the most abundant product ion), and qualifier ions (q, the second most abundant ion). For isoxaflutole, automatic and manual optimization selected the same precursor ion but different quantifier and qualifier ions. On the other hand, automated optimization for pyroxasulfone selected a precursor ion corresponding to [M+K]+ instead of [M+H]+ adduct, resulting in different quantifier and qualifier ions between the two approaches. When comparing the intensities obtained from both optimization methods, all compounds except for pyroxasulfone exhibited higher intensities during manual optimization. Regarding the quantifier to qualifier ratio (Q/q), all six compounds demonstrate approximately the same Q/q ratio. For the fragmentor voltages, all six compounds show lower fragmentor energies for precursor ions in manual optimization. In automatic optimization software, a ramp of fragmentor voltages and collision energies is utilized, whereas in manual optimization, these two parameters are adjusted manually to achieve the maximum abundances. The primary challenge lies in minimizing optimization time while maintaining high analytical performance. However, when dealing with hundreds of compounds, it is impractical to optimize each analyte manually. To achieve the best possible performance, manual optimization may require a considerable amount of time to fine-tune the parameters for the compounds of interest. Furthermore, MS parameters, even among instruments of the same model, can vary significantly. Consequently, parameters that are effective on one instrument may not yield the same results on another, potentially reducing sensitivity and affecting detection limits. Therefore, it is recommended that when certain analytes do not have good signal intensities to achieve the desired limit of detection, manual optimization should be performed to improve sensitivity of the LC-MS/MS method.

OP-12

Identification of Known and Novel PFAS in Aqueous Film-Forming Foam (AFFF) by Non-Targeted Screening with High-Resolution Mass Spectrometry and Pattern Analysis

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Per- and polyfluoroalkyl substances (PFAS) are a group of industrial compounds that have been identified as a class of environmental contaminants of high concern due to their negative health effects and widespread presence in the environment. Current targeted methods focus on a short list of legacy and emerging PFAS and are therefore not comprehensive in measuring all potential PFAS contamination in environmental samples. Non-targeted analysis (NTA) with high-resolution mass spectrometry (HRMS) is a promising technique for the discovery and identification of new PFAS. A major challenge with NTA is the vast amount of data produced and finding the molecules of interest among thousands of detected peaks in complex samples.

The sample analyzed in this study was aqueous film-forming foams (AFFF) (NIST Reference Material 8690). The sample was diluted 200x with methanol prior to injection. A 22-minute LC gradient was employed on a 100 mm C18 analytical column using an LC that was modified to control for PFAS background contamination. As PFAS may be present at low levels in this complex sample, data independent acquisition was utilized to yield as much information as possible from a single injection. Data collection was done with ion mobility enabled data-independent acquisition on a SELECT SERIES™ Cyclic™ IMS (Waters Corporation). Data processing utilized the waters_connect™ software package including the UNIFI™ and PATTERN Analysis Applications.

Detected peaks in the AFFF sample were first compared to an internal PFAS HRMS library for identification in the UNIFI application. Identifications were based on metrics of accurate mass, retention time error, diagnostic fragments and measured collision cross section (CCS). The reference material does contain several known PFAS and those along with a few additional compounds were identified with this initial suspect screening.

For the discovery of new PFAS not in the library, an additional data reduction tool, the PATTERN Analysis application, can be used to discriminate compounds which are most likely to be PFAS. The tool takes advantage of the idiosyncratic properties of compound classes such as PFAS, that separate them from naturally occurring compounds. Flagging of detected compounds is based on class-specific product ions and neutral losses, mass defect, 12C/13C isotope ratio, trendlines on Kendrick plots and the distinctive relationships between both m/z and CCS. IMS adds an additional advantage as PFAS have lower CCS values compared to other compound classes of similar m/z. The application also can search for sequencies of the compounds with a repeating mass difference. Given that many PFAS are a part of homologous series with repeating CF2, this functionality was used to look for sequences in which subsequent m/z values differ by 49.9968 (CF2). The sequence detection tool revealed several series of PFAS that were not reported in the reference material information sheet. Of particular interest, there was a new series of zwitterionic PFAS tentatively identified that is not in the EPA CompTox Database of >14,000 PFAS.

Novelty: Identification of new PFAS series with an innovative and user-friendly software tool for complex HRMS data sets.

OP-14

Role of Mass Spectrometry in Achieving “One Health” with “Zero Casualty”: Lessons Learned from Decades of Research on Emerging Contaminants and Forever Chemicals

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Industrialization, technological advancements, and increasing global population have resulted in widespread environmental pollution, which has wide-ranging impacts on the health of people, animals and ecosystems. To prevent unintended consequences of future technological innovations, a “One Health” perspective that recognizes the interconnectedness of human health, animal health, and the environment, must be adopted. One Health issues include environmental pollution, antimicrobial resistance, water scarcity, and food insecurity, among other things. For years, industrialization and agricultural practices treat land as a disposable commodity instead of life’s source and sustenance, resulting in significant changes in pollution patterns, introducing new contaminants into the environment, such as “emerging contaminants” and “forever chemicals” that are now found globally, from our drinking water supplies to remote areas in the Arctic regions. Despite efforts on environmental protection and regulations, new hazardous environmental pollutants continue to emerge, pointing to gaps in our ability to anticipate the toxicological effects of chemicals, detect them in a timely manner, and prevent them from polluting the environment before irreversible damage occurs. Notably, the advancement in our knowledge on emerging contaminants has been driven by the introduction of highly sensitive and powerful analytical instrumentation that allowed trace quantification and identification of contaminants in complex environmental matrices. High efficiency chromatographic separations coupled to high-resolution mass spectrometers have increased our understanding and awareness of the presence and fate of emerging contaminants in the environment, and the complex ecological consequences that they pose on biological systems. In this presentation, an overview of important research milestones in the area of emerging contaminants, focusing on the fate and treatment of pharmaceuticals and forever chemicals in water resources will be presented. As we shape the future through innovations, we should aim to have “zero casualty” from industrial, agricultural, and municipal wastes through interdisciplinary collaboration and educational reforms to effectively address the rising environmental and health crises.

OP-15

Chemical Characterization of "Non-Traditional" Sources of PFAS Through the Use of High-Resolution Ion Mobility Separation Coupled to High-Resolution Mass Spectrometry

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Poly/per-fluorinated aliphatic substances (PFAS) are of increasing concern in both the environment and food, and the understanding of their chemical complexity is strategically important. According to published literature, known PFAS chemicals, quantified on a targeted basis using available reference standards, have been shown to account for only 20-30% of total organic fluorine in "traditional source" samples. The remaining 70-80% is derived from other PFAS that are either commercial chemicals for which no reference standards are available, or downstream degradation/metabolism products of commercial chemicals with novel structures. Additionally, it is arguable if the target compound lists associated with regulatory methods even apply to "non-traditional" PFAS sources (non-AFFF, etc.).

This presentation will describe an analytical approach utilizing both liquid- and gas-chromatographic separations coupled to a high-resolution ion-mobility spectrometer in series with a high-resolution time-of-flight mass spectrometer. This multidimensional, non-targeted separation dramatically improves identification of PFAS compounds. In addition, a post-acquisition data reduction strategy will be discussed which effectively enables more automated characterization of non-targeted PFAS, either as native or degradation/transformation compounds. This becomes especially beneficial in "non-traditional" source contamination or samples where degradation products are of growing interest. A comprehensive approach will be demonstrated on samples obtained from a variety of sources, including surface waters, sediments and biota to demonstrate the utility of this approach as compared to more conventional instrumental analyses.

Novelty: Non-targeted PFAS characterization employing a multidimensional analytical approach coupled with data reduction tools towards a comprehensive understanding of PFAS source, transport and fate.

OP-16
Quantitation of ultrashort- and short-chain PFAS in beverages by a direct injection LC-MS/MS method

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SCIEX[1], Phenomenex[2], York University[3]

Abstract

While PFAS detection in beverages has received less attention than food matrices, emerging data indicate the prevalence of ultrashort- and short-chain perfluoroalkyl acids (PFAAs) in beverages.1-4 Ultrashort- and short-chain PFAAs represent a subset of PFAS with a chain length of 1–6 perfluorinated carbons. Given their water solubility and documented phytoaccumulation5, these chemicals presumably accumulate in aquatic environments and terrestrial plant tissues, resulting in exposure through drinking water and plant-derived beverages. Trifluoroacetic acid (TFA) has been reported at concentrations of 10s–100s µg/L in fruit juices2 and wine3, compared to the ng/L levels typically observed for other PFAAs. Here, a direct injection LC-MS/MS method enabled the quantitation of ultrashort- and short-chain PFAAs at ng/L to µg/L concentrations in beverages.

Assorted water and beverages were sourced from Toronto, Ontario. The water samples were spiked with isotopically-labeled internal standards and directly injected without dilution. For all other beverages, 10 mL of each sample was first centrifuged, followed by diluting 1 mL of the supernatant with varying volumes of water, depending on the sample. Further in-vial dilution was performed with only water to maintain a 100% aqueous vial composition. Sample extracts were chromatographically separated using a mixed-mode column comprised of C18- and ionic-based retention. Instrumental analysis was performed using a triple quadrupole system in negative electrospray ionization mode.

Chromatographic optimization resulted in reproducible retention times (RTs, <1 %CV) and excellent retention (>4 min) for the early-eluting TFA and perfluoromethane sulfonic acid (PFMS) from the interference-prone void region. Instrumental dynamic range was leveraged to enable dilutions and smaller injections to reduce the matrix load on the column. The in-vial limits of quantitation (LOQs) ranged from 0.5 ng/L to 50 ng/L. Overall, acceptable accuracies within ±30% and %CV <30% were achieved at the LOQ level, while accuracies within ±25% and %CV <20% were achieved at other levels for most analytes. Beverage spikes were performed in green tea, apple juice, sake and white wine. Overall, most of the target PFAS compounds exhibited excellent recovery (80–120%) and precision (%CV < 20%). The method applicability was tested in assorted beverages. Overall, TFA accounted for the majority of the PFAS detected in the samples. Concentrations of TFA spanned several orders of magnitude depending on the matrix, ranging from ng/L levels in drinking water to tens and hundreds of µg/L levels in juice and wine, respectively. PFMS was the second most prevalent PFAA, primarily detected in juice, followed by PFBA. Detections of TFA, PFBA and PFMS were confirmed using their qualifier transitions. Optimization on the SCIEX 7500 system enabled the use of the F- fragment (m/z 19) as the qualifier transition of TFA, PFPrA, PFBA and PFPeA. Given the historical precedence of monitoring PFBA and PFPeA with only the M-44 transition (loss of CO2), the ability to use this second qualifier transition greatly simplifies their detection by removing the need for orthogonal confirmation using high-resolution mass spectrometry or a second chromatographic gradient. Interlaboratory comparison of TFA levels measured independently by LC-MS/MS and IC-MS demonstrated relatively good inter-method accuracy (8–31%) in 4 selected beverage samples.

Novelty: This work features a simple direct injection LC-MS/MS method for the analysis of ultrashort- and short-chain PFAS in complex beverage matrices.

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OP-17
PFAS in the future: Challenges for Baby Food

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Abstract

This application note presents the development and validation of a multiresidue method for the analysis of per- and polyfluoroalkyl substances (PFAS) in baby food. The method uses QuEChERS extraction, followed by Enhanced Matrix Removal (EMR) mixed-mode passthrough cleanup using the Agilent Captiva EMR PFAS Food I cartridge, then LC/MS/MS detection. The method features simplified and efficient sample preparation, sensitive LC/MS/MS detection, and reliable quantitation using neat standard calibration curves.

The Captiva EMR PFAS Food I cartridges were validated for matrix removal, PFAS recovery, and repeatability. The method included calibration, LOQ determination, and precision testing. Due to ultralow LOQ needs for key PFAS (e.g., PFOA, PFOS), seven QC levels were prespiked in baby food samples, with replicates for accuracy. Matrix blanks were also used to assess background interference. PFAS were spiked at levels from 0.001 to 0.2 µg/kg, with higher concentrations for PFBA and PFPeA. Internal standards were added at 0.1 µg/kg across all samples to ensure reliable quantitation.

A simplified, rapid, and reliable method using QuEChERS extraction followed by EMR mixed-mode passthrough cleanup with the Agilent Captiva EMR PFAS Food I cartridge and LC/MS/MS detection was developed and validated for 30 PFAS targets in baby food. The novel cleanup method demonstrated significant improvement on traditional dSPE cleanup in terms of matrix removal, PFAS recovery, and sample volume recovery over the traditional dSPE cleanup. It also features a simplified sample cleanup method, saving time and effort, and thus improves overall lab productivity. A 500x calibration curve dynamic range with correlation coefficient $R2 > 0.99$ for all 30 PFAS targets were validated. Validated LOQ for the 30 PFAS of interest were calculated. The entire method was validated with acceptance performance that meets the requirements described in AOAC SMPR 2023.003.

Novelty: Usage of the novel Captiva EMR PFAS Food I cartridge to be able to meet the AOAC performance requirements on the 30 PFAS for baby food analysis.

Acknowledgments and References : Limian Zhao, Matthew Giardina and Emily Parry from Agilent Technologies.

OP-18

High-throughput protein target mapping enables accelerated bioactivity discovery for environmental chemicals

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Identification of physical protein targets for toxicants is the bottleneck for toxicological research. Here, we established an ultrahigh-throughput affinity-selection mass spectrometry (AS-MS) platform to systematically identify protein targets of prioritized chemical contaminants. After benchmarking the platform, we screened 50 human proteins against 481 prioritized chemicals, including 446 ToxCast chemicals and 35 per-and polyfluoroalkyl substances (PFAS). Among 24,050 interactions assessed, we discovered 35 novel interactions involving 14 proteins, with fatty acid-binding proteins (FABPs) emerging as the most ligandable protein family. Given this, we selected FABPs for further validation, which revealed a distinct PFAS binding pattern: legacy PFAS selectively bound to FABP1, whereas replacement compounds, PFECAs, unexpectedly interacted with all FABPs. X-ray crystallography further revealed that the ether group enhances molecular flexibility of alternative PFAS, to accommodate the binding pockets of FABPs. Our findings demonstrate that AS-MS is a robust platform for the discovery of novel protein targets on the proteome-wide level.

OP-19
PFAS and fluorotelomer biomarkers in Canadian birds: Quantitative Targeted and Suspect Screening

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Abstract

Advances in non-targeted analysis (NTA) methods allow for their application in the study of complex environmental matrices including biological tissues.[1] While individual or small groups of legacy and known contaminants are the focus of existing regulatory frameworks, undetected, unmeasured and/or unmonitored substances (including transformation products [TPs] and alternative or replacement chemicals) represent a significant unknown for wildlife exposure.[2] Per- and polyfluoroalkyl substances (PFAS) are a class of thousands of chemicals used extensively in industrial and commercial products as lubricants and surfactants. Current targeted methods are largely limited to 20-30 PFAS. Multiple Canadian environmental monitoring programs rely on sentinel bird species for minimally-invasive egg collections each Spring. Wildlife exposure to many precursor (e.g., fluorotelomers) and TPs (e.g., metabolites) is poorly understood.

This study's objective was to compare PFAS profiles across three sentinel bird species with varying dietary behaviours or trophic positions. Eggs were collected in Spring 2024 and 2025 from multiple nesting sites in Southwestern Ontario. Egg extracts were quantified via UPLC-QqQ-MS/MS followed by UPLC-Orbitrap-data-dependent-acquisition-(DDA) based suspect screening (SS). DDA-Top7 was performed with dynamic exclusion equal to approximately the half-width (~3s) of each chromatographic peak and a 1 *m/z* quadrupole isolation window. An iterative exclusion approach was used following 4 repeated injections. Compound Discoverer was used for feature screening and identification with existing mass lists and mzVault MS^2 databases from both in-house collected spectra and *in silico* fragmentation spectra. Multiple candidate filters were used including CF_2-normalized mass defects.

∑PFAS concentrations in European starling (EUST) eggs collected in 2023 from urban and nonurban nest box sites across Canada were not statistically different ($p > 0.05$) between sites (< 151 ng/g ww) except for eggs collected from the Brantford (ON) landfill (BRLF) (mean: 1048 ng/g ww),[3] consistent with historical Canadian EUST monitoring data.[4] 2024 EUST collections in Southwestern ON were functionally identical for a given site. Emerging PFAS such as GenX, F-53B, ADONA, and two side-chain fluorinated polymers were undetectable in EUST eggs. Multiple fluorotelomer alcohol (FTOH) TPs were identified during SS including 6:2, 8:2, 10:2, and 12:2 FTOH-sulfate. FTOH-sulfates were confirmed by retention time, and isotopic and MS^2 pattern via in-house synthesized individual standards. 6:2, 10:2, and 12:2 homologues were only detected in BRLF eggs. 8:2 FTOH-sulfate was detectable at all sites. FTOH-sulfates are presumably biomarkers of FTOH exposure, although FTOHs themselves were undetectable. SS also identified several fluorotelomer sulfonates, the 6:2 polyfluoroalkyl phosphate diester, and branched isomers of several perfluoroalkyl acids (PFAAs). ∑PFAS concentrations were

greatest in BRLF tree swallow (TRES) eggs (mean: 467 ng/g ww) compared to other sites (< 139 ng/g ww). While no breeding colony of herring gulls (HERGs) is present in Brantford, levels were generally similar or greater in HERG eggs collected from the nearby Hamilton Harbour colony. Low levels of PFECHS and F-53B components are measured in both TRES and HERG eggs, and FTOH-sulfates are expected to be detectable in BRLF TRES eggs. The majority of PFAA body burden in HERGs is known to be transferred to their offspring *in ovo* (~80%).[5] Although limited sample sizes per site make inter-species comparisons difficult, relative distributions did not differ significantly between sites within a given species. Reducing sample preparation steps to minimize losses to the examinable chemical space, such as during solid-phase extraction, is required.

Novelty: A modern SS approach was used to more comprehensively profile PFAS levels, including TPs, in three bird species from (or near) the same nesting sites within the same breeding period.

Acknowledgments and References

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OP-20

Per and polyfluoroalkyl substances (PFAS) in Aquatic Ecosystems and Marine Mammals: Detection, Fate, and Impacts

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PFAS are chemicals extensively used in various industrial and consumer products due to their unique physical properties1,2. Their occurrence is reported in every environmental compartment, as well as in food materials. These chemicals are highly persistent, and their toxicity and potential for bioaccumulation pose significant environmental and health concerns 1-4. We investigated the presence of PFAS in river water samples from South India, with a specific focus on five major rivers in Kerala, India. A total of 43 individual PFAS compounds were identified across these five river sites. For all sites, we observed a 100% detection frequency for the Perfluorosulfonic Acids (PFSAs) group, which includes compounds such as PFHxS and PFOS. Similarly, the Perfluorocarboxylic Acids (PFCAs) group, including PFBA, also showed a 100% detection frequency. Additionally, C4 to C9 PFCAs and C4 to C8 PFSAs had detection frequencies of 50% or higher. The primary source of PFAS found in river water is believed to be attributed largely to industrial discharges, as well as municipal solid and liquid waste management practices. Industrial activities, such as those involving manufacturing processes that use PFAS compounds, contribute significantly to this contamination.

In addition, we investigate the occurrence of PFAS in the liver, muscle, and brain tissues of various marine mammals, including the harbor seal, gray seal, harp seal, harbor porpoise, white-sided dolphin, white-beaked dolphin, and True's beaked whale from the St. Lawrence Estuary and Gulf, Quebec, Canada. PFOS was found to be the predominant compound in all marine mammal species, and several long-chain PFCAs, such as PFNA, PFDA, PFUnA, PFTrDA, and PFHxDA, were detected at a frequency of 100% in both muscle and liver samples. Additionally, PFDoA and 7:3 fluorotelomer carboxylic acid (7:3 acid) also showed a detection frequency of 100% in liver samples. In brain samples, a total of 34 PFAS compounds were detected, with the geometric mean concentrations ranging from 0.02 ng/g (wet weight) to 41 ng/g (wet weight). For individual long-chain C9 to C13 PFCAs, the detection frequencies ranged from 77% to 95%. In contrast, the detection frequencies for shorter-chain C3 to C6 PFCAs, specifically PFHxA, PFBA, and PFPrA, were much lower, ranging from 2.5% to 5%. In a comparative analysis of PFAS distribution in liver, muscle, and brain tissues, correlation analysis revealed that concentrations in these tissues are positively correlated with each other. This study highlights concerns regarding the impact of PFAS on marine aquatic systems and potential neurocognitive impacts on their brain functions.

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OP-21

Analysis of Individual Chain Length Congeners of Polychlorinated Alkanes in Lipid-Rich Samples by Liquid Versus Gas Chromatography with High-Resolution Mass Spectrometry

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Chlorinated paraffins (CPs) are industrial chemicals and used as flame retardants, plasticizers and high temperature lubricants.[1,2] Globally, CP production increased up to 13 Mt in 2020.[3] CP mixtures are complex with thousands of homologues, congeners and isomers. Main components of CPs are polychlorinated alkanes (PCAs). Analysis in (especially) complex lipid-rich environmental samples is limited due to (LC-MS and GC-MS based) analytical challenges and the lack of available of individual PCA standards.[4,5] An absence of reliable PCA analysis methods has resulted in high variation and low data comparability among studies. The PCA homologue group defining short-chain CPs (SCCPs, C10–C13) dominate reports in samples as compared to there being much less for medium-chain (MCCPs, C14–C17) and long-chain CPs (LCCPs, ≥ C18).

Our study compared two recently developed LC- and GC-HRMS based methods to determine individual carbon chain length PCA congeners (C8ClX up to C26ClX) by application to lipid-rich biological test samples. In both cases the internal standard was 1,5,5,6,6,10-13C10-hexachlorodecane. Test samples were adipose tissue from polar bears were collected in 2015 to 2024 from different locations in Arctic Canada.[6,7] For the GC-HRMS based method, sample extraction used a novel freeze filtration lipid removal approach.[8] The cleaned-up fraction analysis of PCAs were either by use of a Waters direct injection full-scan APCI-QTOF-MS SYNAPT G2S HRMS instrument or a GC(1610)-Orbitrap Exploris GC240 HRMS (ThermoFisher) instrument. Where applicable, data processing was based on a deconvolution and quantification procedure.[9]

Robust and sensitive, LC- and GC-HRMS based analysis methods were developed for quantitation of individual chain length congeners PCAs (e.g. C9ClX to C20ClX) in lipid-rich test samples. For the GC-HRMS based method, samples were extracted and then subjected to freeze filtration (> 95 % lipid removal), cleaned up by a multilayer SPE cartridge followed by PCA analysis. Matrix effects (ME) showed an enhancement effect for PCA quantitative analyses by GC but was rectified by using olive oil as an analyte protectant. For the GC-HRMS based method, method limits of quantification for PCAs ranged from 1.1 to 17.5 ng/g. Mean recoveries of C9ClX to C20ClX homologues ranged from 56 to 120 % and 79 to 160 % in 50 ng/g and 10 ng/g fortified sunflower oil, respectively, and with relative standard deviations < 11 % and < 19 %, respectively.

Reliable GC-HRMS based method (for C9ClX to C20ClX) applicability was demonstrated as C9ClX to C18ClX homologues were detectable whereas C19ClX and C20ClX were not in all polar bear adipose samples (collected in 2023-2024). For Western Hudson Bay (WHB) bears, ΣPCA concentrations were as high as 48.2 ng/g wet weight. Comparable results for polar bear adipose samples were found using a comparable LC-HRMS based analysis method.[7] For example, The Southern Hudson Bay bears showed the highest ΣPCAs in 2015 and averaging 200 ng/g wet weight, and C14ClX to C17ClX were predominant at 44% of ΣPCAs) followed by C10ClX to C13ClX (30% of ΣPCAs). WHB bears had ΣPCA concentrations in the range of 80 ng/g wet weight. To our knowledge, these carbon chain homologue groups currently represent the most specific level at which PCAs have been reported by either LC- or GC-HRMS based analysis methods.

Novelty: Robust and sensitive, LC- and GC-HRMS based analysis methods were developed and demonstrated comparable quantitation of individual chain length PCAs (e.g. C8ClX to C26ClX) in lipid-rich test samples of polar bear adipose.

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OP-22
PFAS in the Environment: Challenging Matrices, and Analytical Innovations

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Abstract

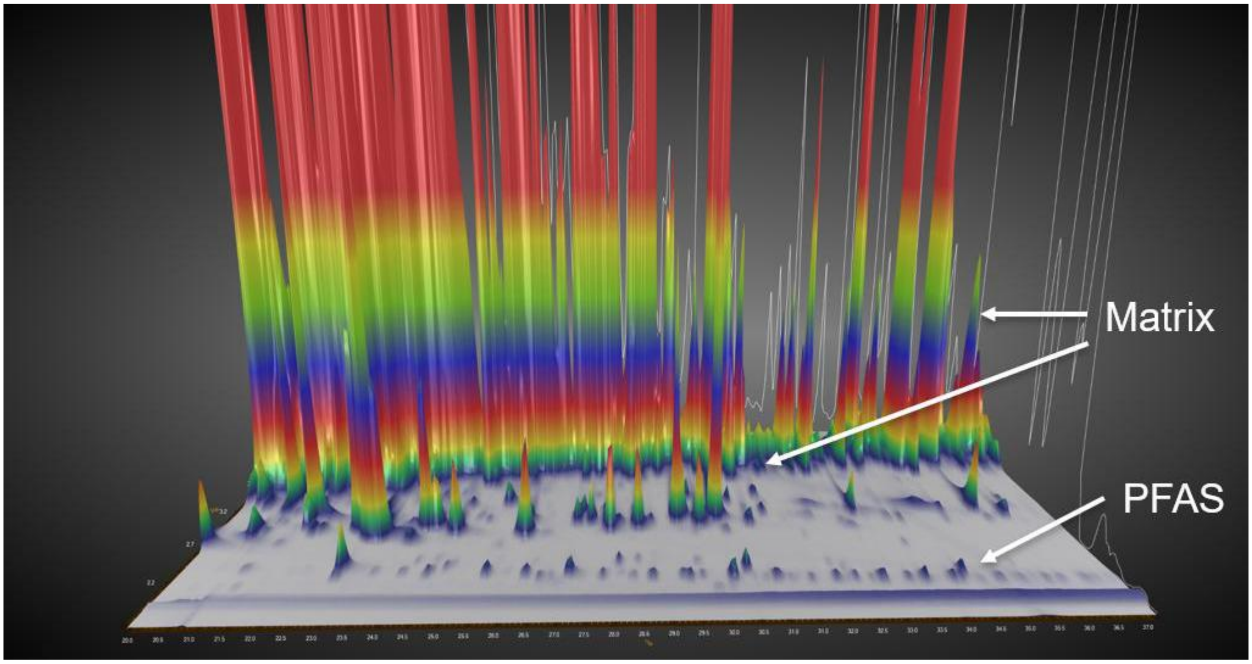
Per- and polyfluoroalkyl substances (PFAS) have become a major global concern due to their persistence in the environment and potential risks to human health. Although PFAS are most often associated with contaminated water and soils, their presence in air and consumer goods is receiving increasing attention. Key sources and monitoring scenarios include direct industrial emissions, background contamination in ambient air, and products and materials that we wear and use every day. Measuring PFAS in environmental samples is technically challenging: concentrations are extremely low, the chemical family is vast and diverse, and matrix complexity make their detection particularly challenging.

Significant progress has now been made with the introduction of US EPA OTM-50 for source emissions, ASTM D8591 and D8560 for materials and indoor air, and the establishment of CEN TC264 WG48 in Europe to address both ambient and source emissions of PFAS. We demonstrate a flow modulated TD-GCxGC-ToF MS method was used to better separate and detect trace volatile PFAS species from landfill soil gas and water retardant fabrics previously worn by people. Furthermore, we briefly introduce an OTM 50 compliant method using a Cannister based TD-GC-Q and TD-GC-QQQ for the analysis of PFAS in air.

This talk will give a brief mention of new sample preparation strategies and challenges for compliance with OTM 50 and similar airborne PFAS methods. This will highlight the workflows and method development strategies, including matrix management and background reduction, that has been critical to optimising sampling and analytical techniques for PFAS air monitoring. In greater focus, this talk will demonstrate how multidimension gas chromatography, in combination with fast acquisition time of flight mass spectrometry can separate and identify unknown volatile PFAS species from otherwise overwhelming background signal from dirty samples. Results indicate that even when tandem mass spectrometry such as QQQ is combined with standard 1D GC methods, background matrix signal still overloads various portions of the instrument resulting in heightened MLOD's and missed identification of untargeted and unknown PFAS species. This was demonstrated with landfill gas samples collected on thermal desorption tubes. While analytically relevant PFAS peaks gave peak heights of 10^5, the landfill gas background overwhelmed this exceeding 10^9 peak heights in the same analytical run. By using a second-dimension column that had little affinity for PFAS species, background compounds could instead be retained and therefore separated, leaving an efficient, straight band of perfluorinated compounds at the front of the 2D chromatogram. Because ToF mass spectrometry was used for detection, exact mass data was then used to assist with NIST database matching of otherwise unknown PFAS species.

Ultimately, this same landfill gas method was used to separate, identify and quantify the concentration of various fluorotelomer alcohols desorbed from water repellant fabrics. MLOQ's were found to be as low as 3 pg spiked on TD tubes with real concentrations in fabric found to range between 30 to 500 pg/g.

Novelty: Integration of 2D GC with high speed ToF for separation of trace PFAS from overloading background signal.



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OP-23
Detective Stories in Mass Spectrometry – It's Not So Elementary My Dear Watson!

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Abstract

Most practitioners of mass spectrometry do not need to think about the structures of the ions formed in a mass spectrometer that give rise to the mass spectral signals, or the gas phase reactions that lead to them. They leave that to nerds like me. Ion chemistry is a rich field populated by a wide variety of stable ion structures and reactions that have no neutral counterpart. Exploring these reactions and ion structures requires a combination of methodologies that include tandem mass spectrometry, more advanced spectroscopies and computational chemistry. In my talk, I will present the ion structure varieties that have been deduced and how, and take the audience through some examples from our own work that demonstrate that when it comes to ion chemistry, it is definitely not so elementary! We will cover examples from tandem mass spectrometry, imaging photoelectron photoion coincidence spectroscopy, infrared multiphoton photodissociation spectroscopy and theory, and especially how these can be used in concert to explore ion reactions.

OP-24

Simultaneous detection of two adulteration practices for plant-based milk alternatives using flow-injection mass spectrometry and machine learning

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Abstract

Plant-based milk alternatives (PBMA) have become popular substitutions for animal milk products given their well-characterized health benefits and reduced environmental impacts than their animal counterpart. PBMA produced with different plant sources can be drastically different in their consumer acceptability, production cost and nutritional values, creating opportunities for fraudsters to replace premium PBMA with lower quality counterparts. Besides, liquid form products are susceptible to dilution to further reduce production cost. To protect consumers and genuine industries, rapid authentication methods that can simultaneously authenticate plant species and concentration of PBMA are urgently needed. Flow injection-mass spectrometry (FI-MS), by-passing the chromatographic separation, is a rapid, high-throughput and sensitive technique highly suitable for authenticity analysis.

A total of 304 PBMA samples involving 8 types of plant species at 3 different concentrations (original and 2 and 2.5 times diluted) were prepared for each of almond, cashew, hazelnut, macadamia, oat, peanut, pistachio, and walnut) were prepared in lab with a food blender. The 3 different concentrations were labeled as undiluted and diluted (2 and 2.5 times diluted). To ensure the optimal analytical performance, ionization voltage, sample pretreatment procedures were optimized using a QC sample prepared by mixing equal volume of PBMA samples of different plant species. Three machine learning (ML) algorithms – support vector machine (SVM), k-nearest neighbours (kNN), random forest (RF) – were evaluated to classify samples based on plant species and concentrations.

Using the optimal sample pretreatment method and FI-MS parameters, a few thousands of features were detected in positive ionization mode with high reproducibility (i.e., relative standard deviation of QC <30%). PBMA samples were separated into calibration dataset (60-70%) and test dataset (30-40%) for establishing and validating classification models. Initially, a single classification model to identify the 8 plant species and dilution adulteration (i.e., 16 classes in total) was constructed using the three ML algorithms. RF model demonstrated an overall better performance than the other two algorithms, with calibration accuracy of 100%, cross-validation (CV) 76.4% and test 88.5%. The substantial decrease in accuracies in CV and validation indicate a severe model overfitting. To improve the overall classification accuracy, a hierarchical classification strategy was then adopted for this multi-class classification task. Two types of hierarchical classification workflow (i.e., plant species identification followed by dilution identification, or the reversed order) were individually evaluated. After thoroughly comparing the performance of different classification models, an optimal workflow was determined. Briefly, the first model using RF achieved 100% accuracy across calibration, CV and validation to identify dilution practice, followed by two other models designed for plant species identification for the undiluted samples (i.e., RF with 100% accuracy in calibration and validation, and 99.4% accuracy in CV) and diluted samples (i.e.,

RF with 100% accuracy in calibration and validation, and 88.5% accuracy in CV), individually. The performance for the diluted samples is expected to be improved by increasing the number of diluted samples in model construction and evaluation. Overall, the hierarchical classification approach achieved a reliable simultaneous detection of two fraudulent practices (i.e., plant species substitution and dilution).

Novelty: By integrating high-throughput FI-MS, machine learning algorithms and hierarchical classification strategy, this is the first study to simultaneously authenticate plant species and concentration of PBMA products.

OP-25
Building Robust LC-MS/MS Workflows: The Power of Thoughtful Sample Preparation

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Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS) is indispensable for detecting trace contaminants in complex matrices. However, reliable results begin with thoughtful sample preparation. Understanding key principles, including sample stability, pH effects, solvent selection, and method limitations, is essential for building robust, reproducible workflows.

The presentation explores how strategic sample preparation is critical to successful LC-MS/MS applications. Case studies include pesticide and mycotoxin analysis in hemp, detection of illegal additives and residues in wine, and proof-of-concept studies for PFAS in food and biosolids. Robustness testing in fetal bovine serum further highlights the importance of method resilience in biological matrices.

While modern platforms can mitigate matrix effects and enhance throughput, their effectiveness depends on fit-for-purpose sample preparation. By combining sound fundamentals with streamlined workflows, high-quality data can be achieved, reducing cost, improving efficiency, and minimizing environmental impact.

Poster Presentations

PP-01

Development of a general protocol for non-targeted analysis of PFAS in drinking water: QA/QC for reproducible identification

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Abstract

Per- and poly-fluoroalkyl substances (PFAS) comprise thousands of synthetic chemicals that are linked to numerous adverse human health effects. Current monitoring programs in Canada tend to focus on the most commonly found and researched PFAS, however, those characterized in exposure assessments still represent a relatively low proportion of the total registered for commercial use. Non-target analysis (NTA) is an emerging tool used to identify and prioritize chemicals but unlike targeted analysis, there are still no clearly established processes for NTA method development and validation. Since identification of unknown PFAS without any reference standards represents a challenge, the aim of this study was to develop and evaluate an NTA protocol that could provide reproducible results regarding the identification of PFAS in water samples.

Sample preparation was carried out via solid-phase extraction with HLB cartridges and using a robust QC protocol (e.g., use of internal standards) in order to ensure reliable data. QC samples were prepared by spiking 41 known PFAS standards. Source and drinking water samples were collected during the winter season from 12 water treatment plants across Canada. Extracts were analyzed using a ThermoFisher Q-Exactive LC-Orbitrap-MS in negative mode. Data analysis to identify PFAS was performed using the software tools FluoroMatch and Compound Discoverer. Each data acquisition sequence (consisting of calibration standards, blanks, QC samples, and water samples) was analyzed three (3) times using each of the software tools. A retention time model was developed to aid in improving the identification process of new PFAS.

Fluoromatch, Compound Discoverer, and TraceFinder software tools were used to validate the NTA workflow by examining the identification rate of 41 known PFAS and 16 known labelled PFAS standards. Using TraceFinder, all but two (2) PFAS were identified in calibration standards and water QC spiked samples; all labelled internal standards were also consistently identified using this software.

Fluoromatch and Compound Discoverer exhibited very similar true positive rates (87.8% for Fluoromatch and 88.3% for Compound Discoverer), but both had certain disadvantages. . Using FluoroMatch, isotopic standards must be identified by manually searching the data, which is not sufficiently compliant with the proposed QA/QC protocol of this study. With Compound Discoverer some incorrect annotation of chemical features were observed; more specifically, chromatographic peaks indicated the presence of noise rather than chemical compounds. Furthermore, regarding molecular features for which formulas are provided but not annotated from databases, it is challenging to propose molecular structures using Compound Discoverer since there can be hundreds of possible structures from one single formula. In contrast, Fluoromatch most often proposes one possible structure from one single molecular feature.

Using the developed NTA workflow, eighty-eight (88) features were identified in drinking and source water samples based on Fluoromatch with level 3 confidence. Compound Discoverer allowed for the identification of 64 PFAS in water samples with level 3 confidence. Using the proposed retention time model, six (6) PFAS were identified with level 2 confidence.

Novelty: Identification of new PFAS including 1-hydro-pentadecafluoroheptane and 7H-perfluorooctane sulfonic acid. Development of a general protocol for the analysis of PFAS to ensure reproducible data.

PP-02

Trend-guided Prioritization of Chemical Features in Non-Targeted Analysis: A Case Study of Tire-derived Transformation Products

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Abstract

High-resolution mass spectrometry-based non-targeted analysis (NTA) detects thousands of chemical features per sample, requiring effective strategies to prioritize environmentally relevant compounds [1]. Trend-guided prioritization links changes in peak area (i.e., signal abundance) to environmental processes [2,3], helping identify transformation products (TPs). Tire particles, a complex source of organic contaminants, release a wide range of known and unknown chemicals. In this study, we apply trend-guided prioritization to investigate oxidative transformations of tire-derived compounds using laboratory-simulated ozonation. By comparing temporal trends in the chemical leaching process before and after ozone exposure, we assess polar or semi-polar TPs formed and mobilized into water. This approach supports structural elucidation and prioritization of contaminants of emerging concern, including their transformation products.

Experiments were conducted in triplicate using 1.00 g of tire particles and 10 mL of LC-MS grade water. Two experiments were performed: (I) tire particles were in contact with water on a mixer for various time lengths up to 48 h to mimic contaminant release during stormwater or snowmelt events. (II) Dry particles were exposed to ozone (5 mg/h generation rate, 1 L/min flow rate) for 0–4 h, equivalent to approximately 1 day to 4 months during summer, and subsequently leached in water for 24 h. All samples were spiked with internal standards and extracted using solid-phase extraction. Analysis was conducted using liquid chromatography coupled to high-resolution Orbitrap mass spectrometry. A trend-based prioritization approach was then applied to identify features exhibiting time-dependent behavior, supporting the detection and prioritization of transformation products derived from tire-associated chemicals.

Detected chemical features were prioritized through a multistep workflow combining statistical filtering and temporal trend analysis. A total of 14,409 features were initially detected, of which 13,309 exhibited peak areas above those in procedural blanks. Among these, 4,404 features showed statistically significant temporal trends ($p < 0.05$), and 199 features demonstrated strong second-degree polynomial fits over the leaching period ($R^2 > 0.9$). Polynomial coefficients (a, b, c) were used to categorize the temporal trend shapes, grouping features into ($a > 0$) or ($a < 0$) patterns and further ranking them by abundance within each category. Following this ranking, molecular formulas were assigned when possible, and selected features were confirmed using analytical standards. One notable example is 1-cyclohexylurea, which was the third most abundant feature in the $a < 0$ group, showing an increasing trend over time. It is proposed as a transformation product of 1,3-dicyclohexylurea. Both compounds were confirmed in urban surface water samples. 1,3-Dicyclohexylurea has previously been reported in the literature, with tire wear identified as a potential source [4,5]. A plausible transformation mechanism involves oxidative cleavage of the N–C bond, forming the mono-substituted degradation product. The increased abundance of 1-cyclohexylurea following ozone exposure suggests a role for atmospheric aging, while its presence in untreated leachates indicates that aqueous-phase transformation may also occur. Additionally, 2-cyclohexylbenzoic acid was identified as the eighth most abundant feature within the $a < 0$ group. Its structural similarity to 1-cyclohexylurea suggests the presence of a broader class of cyclohexylated transformation products, underscoring the effectiveness of this prioritization strategy in identifying structurally

related compounds. In parallel, hierarchical clustering of peak area time-series was performed. The optimal number of clusters was set to three based on silhouette analysis to balance cluster resolution with interpretability. Both 1-cyclohexylurea and 1,3-dicyclohexylurea were grouped into the first cluster.

From this study, we demonstrate that supervised and unsupervised trend-based NTA prioritization approaches combined with laboratory experiments can help identify transformation products from thousands of detected chemical features, enabling the identification of environmentally relevant tire-derived contaminants.

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PP-03

Targeted and non-targeted analyses of reaction products formed during remediation of toluene by heat-activated peroxydisulfate in groundwater

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Abstract

Groundwater is a vital freshwater resource, and its contamination poses a significant threat to ecosystems and communities. Petroleum hydrocarbons, including priority pollutants such as benzene, toluene, ethylbenzene, and xylenes, are common groundwater contaminants. *In situ* chemical oxidation (ISCO) is a remediation technology where oxidants like peroxydisulfate (PDS) are injected into the subsurface to degrade contaminants into benign products. To date, column studies have assessed ISCO performance by monitoring only PDS consumption and contaminant removal and none have thoroughly evaluated the formation of reaction products. Some oxidation intermediates may be toxic and can form precipitates. The toxic precipitated products would continue to pose risks if they are not removed, and precipitates in the subsurface may cause pore blockage and reduce ISCO efficiency by limiting oxidant-contaminant contact.

In this presentation, we investigate the removal of toluene NAPL using heat-activated PDS in 60-cm glass columns packed with silica sand. Two PDS concentrations were tested: a low dose (5 mM) and a high dose (50 mM). PDS, toluene, and major intermediates were monitored at multiple points along the column and at the effluent. Results showed that toluene was removed by both oxidation as well as dissolution. Though toluene removal was much higher in the high dose columns, it also resulted in greater accumulation of reaction products. Concentrations of benzaldehyde, the major byproduct, were about an order of magnitude higher in the high dose columns than in the low dose columns, with no benzaldehyde formation in the control columns. Visual observations and microscopy also showed greater formation of precipitated byproducts in high dose columns.

To characterize reaction products, we used a combination of GC-MS and LC-HR-MS analyses, liquid-liquid and solid phase extraction of large volumes of effluents with different solvents, and solid-liquid extraction of sand. Targeted and non-targeted GC-MS and LC-HR-MS analyses of effluents detected many C_xH_y, $C_xH_yO_z$, and $S_wC_xH_yO_z$ (organo-sulfur) compounds. GC-MS analysis of sand extracts also detected $S_wC_xH_y$ (organo-sulfides) and elemental sulfur for the first time. XPS of sand further indicated the presence of reduced sulfur species. No transformation products were detected in controls.

Novelty: The comprehensive analysis in this column study on toluene oxidation by PDS enabled detection of many reaction products for the first time, including dimers and organo-sulfur dimers.

PP-04

Leveraging high-resolution mass spectrometry (HRMS) for resolving PFAS interferences and quantitation in food matrices

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Abstract

Since the introduction of regulated maximum levels (MLs) for PFOS, PFOA, PFNA and PFHxS in food, the European Reference Laboratory (EURL) and AOAC International have established performance criteria and target limits of quantitation (LOQs) as guidance for food testing. Nominal mass triple quadrupole systems are predominantly used for quantifying PFAS in food due to their high sensitivity, but newer accurate mass systems are closing the sensitivity disparity with nominal mass systems. High-resolution mass spectrometry (HRMS) is critical for confirming low-level detection of PFAS, particularly in the presence of co-eluting interferences and for analytes with a single MS/MS transition, such as PFBA and PFPeA. This work demonstrates an HRMS method for the simultaneous quantitation and identification of PFAS in food.

Food (salmon, clam, egg and corn silage feed) were extracted using a QuEChERS method that had been developed for PFAS analysis in food.1 The extracts were cleaned up using dispersive SPE, followed by additional SPE and ENVI-carb clean-up. LC-MS/MS analysis was performed on a high-end quadrupole time-of-flight (QTOF) mass spectrometer. Quantitation was performed using solvent-based calibration standards. Method performance was evaluated by replicate matrix spikes in egg, salmon and clam, each of which was spiked at the target LOQs specified by the EURL POP guidance and AOAC SMPR.

Identifying both known and unknown interferences is challenged by the diversity of food matrices and classes of PFAS. These interferences may be present as co-eluting chromatographic peaks and contribute to erroneously high levels, which can result in false positives or introduce quantitative biases. Individual monitoring of secondary transitions for the target analyte or the interference (if known) can help confirm their presence. Other solutions include time-consuming modifications to the LC method or sample preparation to either resolve or remove these interferences. Here, the increased specificity from HRMS enabled the use of narrow extraction windows to isolate the accurate masses of the target PFAS, mass-resolving out co-eluting interferences observed in the food matrix. This was especially useful for providing secondary confirmation of analytes lacking another transition such as PFBA and PFPeA. The high sensitivity of the QTOF system enabled a small injection volume of 3 µL and the use of solvent-based calibration to minimize the matrix load on the instrument. The in-vial LOQs ranged from 0.01 to 0.2 ng/mL based on a single replicate injection of the calibration curve. Back-calculation of the solvent LOQs through the sample preparation steps resulted in an approximation of the in-sample LOQs at 0.02–0.4 ng/g. While further experiments are necessary to validate these LOQs in matrix, they highlight the sensitivity potential of the QTOF system to meet the EU MLs for PFOS, PFOA, PFNA and PFHxS and the target LOQs listed in the EU POP guidance and AOAC SMPR. Matrix spikes in EU-regulated food matrices demonstrated acceptable recovery and repeatability for most of the target PFAS, meeting the criteria set in the EURL POPs guidance document and AOAC SMPR.

Novelty: This work features an LC-HRMS method for the simultaneous quantitation and identification of PFAS in food. HRMS selectivity enabled more confident PFAS quantitation by mass-resolving co-eluting interferences in food matrices.

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PP-05

Reaction Mechanisms for Fast Pyrolysis of Polystyrene by Using a Pyroprobe Integrated With an Ion Trap Mass Spectrometer

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The escalating problems associated with the management of plastic waste, particularly polystyrene, necessitate the development of innovative waste management and recycling strategies. Fast pyrolysis of polystyrene is an attractive option because it offers an environmentally friendly alternative by breaking polystyrene into valuable products. Fast pyrolysis involves rapid heating in the absence of oxygen to generate value-added solid, liquid (main product), and gaseous products. In this presentation, tandem mass spectrometry coupled with an in-source fast pyrolysis device is introduced as a powerful approach for structural characterization of the pyrolysis products of polystyrene. Quantum chemical calculations based on density functional theory (DFT) were used to delineate reaction pathways and mechanisms. All reactions were identified to involve radical mechanisms, such as β-scission and hydrogen atom abstraction.

The fast pyrolysis experiments were conducted using a commercial CDS Analytical Pyroprobe 5200 that uses a resistively heated platinum ribbon. A small amount of sample was loaded, and it adhered to the ribbon via electrostatic attractions. The probe was placed inside an atmospheric pressure chemical ionization (APCI) source of a linear quadrupole ion trap (LQIT). APCI in the positive-ion mode and tandem mass spectrometry experiments (MSn) based on collision-activated dissociation (CAD) were employed to elucidate the structures of the primary pyrolysis products. Relevant ionized model compounds and molecular orbital calculations were used to guide the data analysis. Variations in the relative abundances of the pyrolysis products with varying conditions, such as the final temperature, heating rate, final temperature holding time etc. were also studied.

The study utilized commercial and waste polystyrene as feedstocks. The volatile pyrolysis products were analyzed via tandem mass spectrometry experiments based on CAD. Initial experiments indicated no significant compositional differences between the primary pyrolysis products of commercial and waste polystyrene, suggesting that impurities in waste polystyrene had negligible effects on the product distribution. Styrene (protonated of m/z 105), methylstyrene (protonated of m/z 119), and various dimers such as 2,4-diphenyl-2,4-hexadiene (protonated of m/z 235) and trimers, like 1,3,5-triphenyl-1,5-hexadiene (protonated of m/z 311) were identified as the major primary products by comparing their (+) APCI CAD tandem mass spectra with those of relevant ionized model compounds.

Reaction mechanisms involving radical-mediated pathways, such as β-scission, intramolecular hydrogen atom transfer, and hydrogen atom abstraction were proposed based on density functional theory (DFT) calculations. The primary findings highlight the predominance of dimers over monomers and trimers, with 2,4-diphenyl-2,4-hexadiene (protonated of m/z 235) being the most abundant product. Operating conditions, including temperature, heating rate, and residence time, were found to significantly influence the product distribution. For instance, higher temperatures favored the formation of dimers, while faster heating rates promoted the cracking of larger molecules into monomers.

These findings provide molecular-level insights into polystyrene fast pyrolysis, paving the way for optimized reactor designs and more efficient recycling strategies. The study underscores the importance of integrating advanced analytical techniques and computational methods to unravel complex reaction pathways in polymer pyrolysis.

Novelty: This study integrates tandem mass spectrometry coupled with an in-source fast pyrolysis device and DFT calculations to elucidate primary polystyrene pyrolysis products and radical-driven reaction mechanisms.

PP-06
Tandem Amadori-Heyns Rearrangement: A novel route to non-enzymatic de-glycation

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Abstract

During thermal processing of food, reducing sugars react with free amino acids or with amino acid side chains of proteins and generate complex mixtures of molecules[1] known as advanced glycation end-products (AGEs) or Maillard reaction products (MRPs). The molecules derived from free amino acid reaction with sugars contribute to flavor and color development in processed food and those generated from proteins may pose considerable health risks. There is increasing evidence that consumption of so-called ultra-processed foods (UPF) that are rich in AGEs is correlated with chronic diseases[2]. Efforts to mitigate the AGE load of processed foods can be directed towards developing natural food additives that can chemically reverse the glycation during food processing and mitigate health risk factors associated with thermal processing.

Amadori products were prepared through ball milling[3] in stainless steel grinding jars with 2 stainless steel balls. The jars were seated in the Retsch Mixer Mill and milled at a frequency of 30 Hz for 30 min. The de-glycating amino acids were added to the grinding jar at the end of the reaction and milling was continued under the same conditions. Around 10 mg of the reaction mixture was dissolved in aqueous methanol solution and analyzed by ESI/QqTOF/MS/MS. The analysis was performed on a Bruker Maxis Impact quadrupole time-of-flight mass spectrometer operated in negative ionization mode. Tandem mass spectrometry (MS/MS) was conducted in multiple reaction monitoring (MRM) mode with collision energy of 15 eV.

Hypothesis: Amadori rearrangement products being keto sugars, can react with free amino acids during thermal processing and undergo subsequent Heyns rearrangement, a process we termed "tandem Amadori Heyns rearrangement". The resulting ene-diamine intermediate can exist in equilibrium with the Schiff bases of the initial Amadori product and with that of the newly formed Heyns compound, due to the eneamine-imine tautomerism. This equilibrium can provide the opportunity to hydrolyze the Schiff bases of the Amadori product and in effect de-glycate the amino acid, transferring the sugar moiety from the protein to the free amino acid. However, with cysteine, serine or threonine as the free amino acids, the glycated protein can also undergo side-chain assisted de-glycation where the sulfur or the oxygen atoms of their side chains can initiate intramolecular nucleophilic substitution reaction at the imine carbon of the Schiff base and promote the formation of stable six-membered 1,4-thiazine or 1,4-oxazine derivatives, these intermediates can readily eliminate the amino acid on the protein moiety and form stable thiazinium or oxazinium ions, that can be detected by HRMS.

The concept of de-glycation was tested using Amadori product of histidine, followed by its further reaction in the presence of selected free amino acids such as cysteine. Analysis of the data by q-TOF-MS/MS has indicated the formation of glycated histidine at m/z 316.1130 in the milled glucose histidine model as the major product and during the subsequent de-glycation step with free amino acids, the intensity of this peak was significantly reduced on the expense of the newly formed glycated amino acid peak, in addition to the formation of tandem Amadori Heyns rearrangement product at m/z 419.1206. Reduction in the intensity of histidine Amadori peak and concomitant increase in the intensity of the glycated cysteine may indicate that the sugar moiety was transferred from Amadori product of histidine to the added amino acid cysteine to form a new glycated amino acid as characterized by MS/MS fragmentation pattern[4]. Among the added amino acids, cysteine, threonine and serine were found to be the most efficient amino acids.

Novelty: Enzymatic de-glycation, an important natural defense mechanism against impact of biological glycation, has been studied extensively, however, the concept of non-enzymatic de-glycation at the Amadori stage, relevant for the prevention of AGE formation in processed foods, is a new approach.

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PP-07
Non-targeted analysis of endocrine-disrupting chemicals in ovarian follicular fluid via new diagnostic fragmentation evidence and mass spectral library matching

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Endocrine-disrupting chemicals (EDCs) are emerging contaminants that interfere with hormone actions in the endocrine system. Such chemicals are widely found in the environment and food supply, entering human bodies through inhalation, ingestion, and dermal absorption. Human exposure to EDCs can lead to a series of health concerns.[1] In particular, recent studies have demonstrated the connections between increased EDC concentrations in ovarian follicular fluid (fluid surrounding developing ova) and decreased human fertility.[2] However, most existing studies focus on a limited number of prevalent EDCs, which potentially overlooks the chemicals and metabolites that are not known or prioritized. Here, to address the gap, we developed a new non-targeted analysis (NTA) workflow based on liquid chromatography–high resolution mass spectrometry (LC–HRMS).

In this study, we first optimized the chemical extraction and sample clean-up procedures for follicular fluid samples, with key steps including enzymatic hydrolysis, protein precipitation and lipid removal. Next, we characterized the fragmentation patterns of parabens – a group of common EDCs used as preservatives,[3] and compared the different data acquisition modes: data-dependent acquisition (DDA) and data-independent acquisition (DIA). Lastly, we applied the new NTA workflow to real follicular fluid samples to identify unknown chemicals. In addition to paraben identification through extracting the newly identified diagnostic fragmentation ions, we expanded our chemical coverage through mass spectral library searches using commercially available software. A new in-house retention time prediction model was also developed to increase the identification confidence of the unknowns.

By taking advantage of the higher-energy collisional dissociation (HCD) in the orbitrap mass spectrometer, we first identified up to 17 characteristic product ions for parabens and their metabolites. Compared to conventional spectral matching via online databases and in silico fragmentation algorithms, paraben precursor ion prioritization through such diagnostic fragment ion extraction achieved more accurate compound identification at concentrations as low as 1 ng/mL. However, the application of diagnostic evidence is still limited to specific groups of chemicals. Thus, to broaden compound coverage beyond known fragmentation patterns, we supplemented our analysis with mass spectral library searches using Compound Discoverer software. Method validation was performed by spiking over 60 EDCs into a follicular fluid sample. Results showed that, at spiking levels of 1, 5 and 25 ng/mL, the true positive identification rates were 41%, 70% and 83%, respectively.

As a proof-of-concept application, we then applied the new NTA workflow to a pooled follicular fluid sample collected from a population group undergoing fertility treatment. Our results confirmed the presence of parabens, mono-phthalates and UV filters in the sample with level 1 confidence. This NTA work not only revealed the distribution of exogenous substances in human biological samples, but it also implied the possible metabolic processes occurring in human body. Specifically, beyond parent compound identification, we detected major metabolites formed via hydrolysis, hydroxylation, sulfation, and amino acid conjugation. These findings highlight the utility of our workflow in characterizing EDCs in biological samples. Given the public health relevance of EDCs as emerging contaminants, potential correlations between chemical exposure and reproductive health implications are an area for future study.

Novelty: This work is the first detailed NTA investigation of EDCs in follicular fluid. Our new paraben annotation approach by mass spectral ‘fingerprints’ extraction enables rapid and accurate chemical identification.

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