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# SepScience

October 2023 | issue 2

PREMIER LEARNING FOR ANALYTICAL SCIENTISTS


## Unraveling the Analytical Challenges

IMPROVING PROCESSES

### Advances in PFAS Workflows

The latest manufacturer solutions

ANALYTICAL TECHNIQUES

### Measuring Atmospheric PFAS

Separations and selective targeting

NEW FINDINGS

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## Features

# EDITOR'S NOTE

Welcome to Separation Science's October issue, "PFAS: Unraveling the Analytical Challenges." This issue takes a timely dive into the intricate world of per- and polyfluoroalkyl substances (PFAS) – a group of synthetic chemicals whose ubiquity and persistence have sparked global attention and concern.

In an area rife with regulatory uncertainty and heightened scrutiny, determining optimal workflows for PFAS analysis is paramount. We join Dr. Tarun Anumol, a recognized authority in the field who offers an expansive perspective on decoding the complexity of PFAS workflows. We explore many of the challenges scientists face in this area and examine how manufacturers are providing solutions.

Given the widespread distribution of these compounds, understanding atmospheric PFAS is pivotal. "Separations and Selectivity for Measurements of Atmospheric PFAS" underscores the intricate nuances of effectively measuring PFAS in both gaseous and particulate phases.

With interest in this field piqued, researchers are investigating PFAS levels in a range of materials. We delve into the world of fast food packaging and discover how sustainable packaging could be resulting in increased PFAS exposure. Another area of concern is PFAS in pharmaceuticals, with relevant regulations evolving across the globe. On page 20, we review the history of PFAS in pharmaceuticals and consider associated regulations and testing requirements.

Whether you are just entering the realm of PFAS or a seasoned researcher, our compilation of webinars and symposiums promises to enhance your knowledge base and inspire curiosity. For those looking to dive deeper into PFAS analysis, our curated roundup of eBooks and compendiums can be perused at your own pace.

Of course, gaining insight through expert discussion is one of the most constructive ways to learn about PFAS analytical methods. We provide an executive summary of a recent forum where esteemed PFAS experts converged to dissect challenges and provide insights.

The Separation Science team is dedicated to delivering premier learning for analytical scientists. We welcome your questions and feedback about our editorial coverage—please reach out to me at acichocki@sepscience.com.

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# Latest advances in GC/MS air analysis workflows: from PFAS to utilizing hydrogen carrier gas

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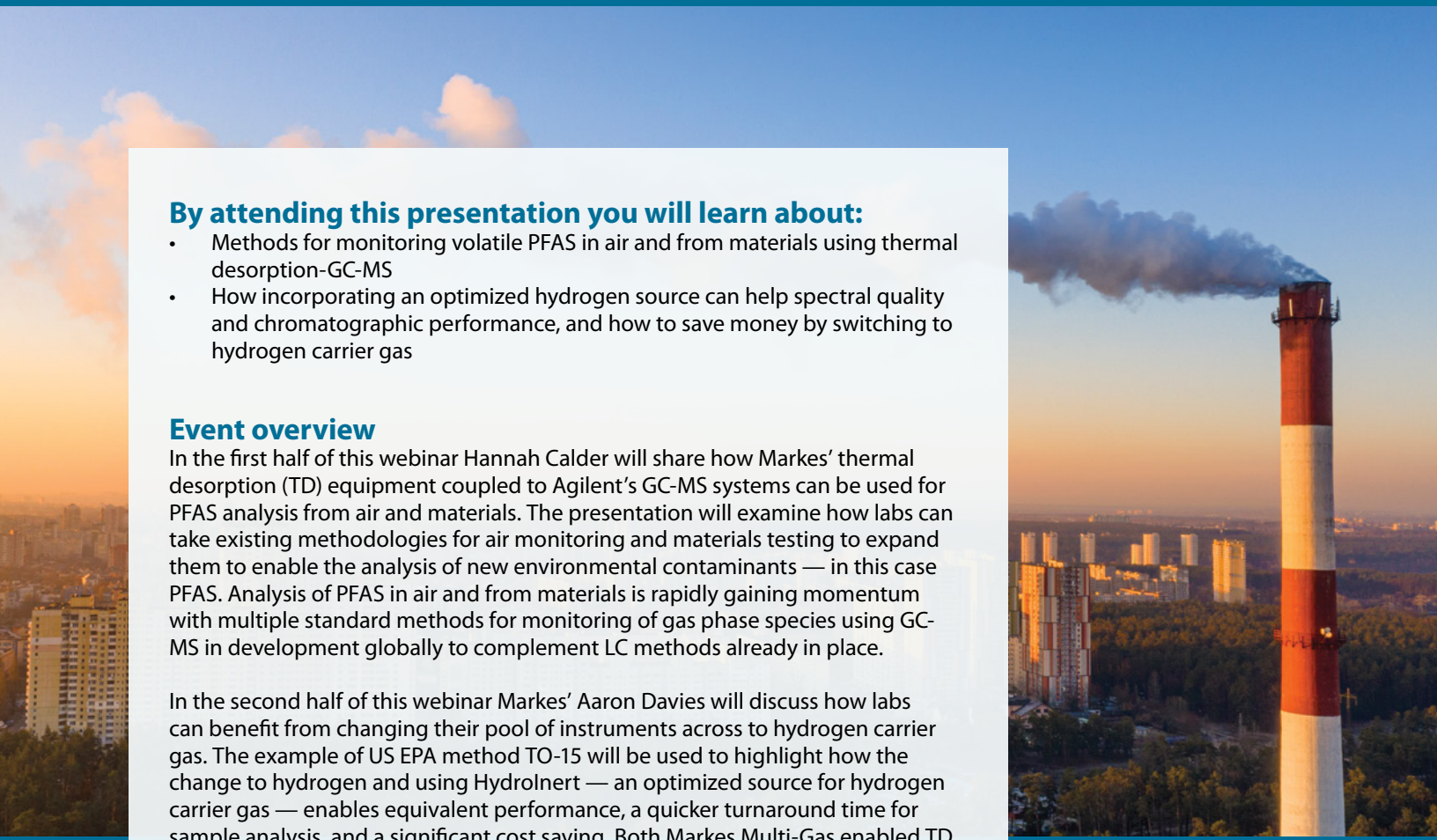

## By attending this presentation you will learn about:

- Methods for monitoring volatile PFAS in air and from materials using thermal desorption-GC-MS
- How incorporating an optimized hydrogen source can help spectral quality and chromatographic performance, and how to save money by switching to hydrogen carrier gas

## Event overview

In the first half of this webinar Hannah Calder will share how Markes' thermal desorption (TD) equipment coupled to Agilent's GC-MS systems can be used for PFAS analysis from air and materials. The presentation will examine how labs can take existing methodologies for air monitoring and materials testing to expand them to enable the analysis of new environmental contaminants — in this case PFAS. Analysis of PFAS in air and from materials is rapidly gaining momentum with multiple standard methods for monitoring of gas phase species using GC-MS in development globally to complement LC methods already in place.

In the second half of this webinar Markes' Aaron Davies will discuss how labs can benefit from changing their pool of instruments across to hydrogen carrier gas. The example of US EPA method TO-15 will be used to highlight how the change to hydrogen and using HydroInert — an optimized source for hydrogen carrier gas — enables equivalent performance, a quicker turnaround time for sample analysis, and a significant cost saving. Both Markes Multi-Gas enabled TD instruments and Agilent GC/MS systems are certified safe for use with hydrogen carrier gas. When coupled with the new HydroInert source, mass spectral fidelity is retained and users can continue using existing helium based mass spectral libraries and quantitative methods.

**Presenters:**

**Hannah Calder**
*Environmental Air Market Development Manager, Markes International*

**Aaron Davies**
*Commercial Marketing Manager for Thermal Desorption, Markes International*

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# On-line and on guard for clean water

## Address new stringent PFAS controls with Shimadzu's on-line SPE LC-MS/MS method for drinking water analysis

**by Shimadzu**

Per- and polyfluoroalkyl substances (PFAS) are a family of synthetic chemicals known for their unique properties, including water and oil repellency and heat resistance. They have been widely used in various products such as nonstick cookware, food packaging, and firefighting foams. However, PFAS are soluble in water and persistent, leading to global contamination in the environment, food, and even in humans and wildlife.

The most hazardous PFAS compounds have faced global and EU-level restrictions for over a decade. And by January 2024, the European Union (EU) Commission is set to adopt Directive EU 2020/2184, a new set of technical guidelines for analyzing PFAS in drinking water. This new legislation specifies a limit of 0.1 µg/L for a group of 20 PFAS of highest concern. The cumulative maximum concentration for all PFAS compounds will be set at 0.5 µg/L of water.

To monitor and meet these regulatory requirements, updated analytical methods are crucial. A new application note by Anja Grüning from Shimadzu Europa describes a means to facilitate routine PFAS analysis in drinking water laboratories using a Shimadzu LCMS-8060NX triple-quadrupole mass spectrometer paired with a Nexera X3 UHPLC system (Figure 1). It presents the analysis of 44 types of PFAS compounds and 22 internal standards using an on-line solid-phase extraction (SPE) approach that minimizes sample preparation steps.

▲ **Figure 1.** The Shimadzu LCMS-8060NX coupled to a Nexera X3 system

## METHODS AND MATERIALS

Forty-four PFAS standards and one IS-mixture (ISO 21675-LSS) were procured from suppliers then diluted with methanol to create individual stock solutions, each with a final concentration of 1 ng/µL per compound. Subsequently, further dilutions of this stock mixture were prepared and spiked into Evian water to generate calibration samples for the analysis of drinking water. The calibration samples covered a concentration range from 0.5 ng/L to 100 ng/L. Bottled Evian water was selected as the matrix for the drinking water analysis, as no noticeable PFAS were detected in the blank samples. To maintain consistency, all samples (excluding blanks) were spiked with IS to achieve a final concentration of 20 ng/L.

Traditional SPE involves performing extraction and purification steps separately from the analytical chromatographic instrument. On-line SPE, however, uses compact cartridges placed within the eluent flow path, allowing for direct elution onto the HPLC column. In this application note, 1 mL of sample is injected directly on a SPE-trap column, with no further sample preparation required.

Analysis was performed within 15 minutes using multiple reaction monitoring (MRM) acquisition to characterize PFAS compounds. At least two transitions for each compound were recorded where available. Analytical conditions are listed in Table 1. The optimized MRM transitions are available in the full application note.

Given that PFAS may exist in reagents, glassware, pipettes, tubing, degassers, and various components of the LC-MS/MS instrument, the use of a solvent delay column becomes imperative. In this method, compact C18 columns are strategically positioned between the mixer and the autosampler, as well as between the mixer and the valve, to mitigate potential PFAS contamination and separate it from sample-derived PFAS.

Long-chain PFAS are more likely to adsorb to surfaces due to factors including their larger molecular size and extended environmental stability compared to shorter-chain complexes. To reduce PFAS adsorption effects, this method uses LabTotal Vials with PP-caps and aluminum septa on vial surfaces.

| | |
|---|---|
| Mass Spectrometer | : LCMS-8060NX |
| Ionization | : Electrospray Ionization (ESI), negative Interface |
| Voltage | : -1 kV |
| Focus Voltage | : -2 kV |
| Heating Gas | : 15 L/min |
| DL Temp. | : 150 ˚C |
| Interface Temp. | : 300 ˚C |
| Nebulizing Gas | : 3 L/min |
| Drying Gas | : 3 L/min |
| Heat Block | : 400 ˚C |
| Dwell-/Pause-time | : 4 (3 for IS) / 1 msec |
| CID | : 270 kPa |
| UHPLC | : Nexera X3 |
| Pump A (Analytical) | : 2 mM ammonium acetate in $H_2O$ |
| Pump B (Analytical) | : 10 mM ammonium acetate in Methanol |
| Pump C (Trap) | : $H_2O$ + modifier (sample loading) |
| Pump D (Trap) | : Methanol (washing of SPE and delay column) |
| Analytical Column | : Shim-pack Scepter 1.9 µm, C18-120, 2.1 x 50 mm |
| Delay Column | : Shim-pack GIST HP 3 µm, C18-AQ, 3 x 30 mm |
| Trap Column | : Evolute Express ABN on-line SPE cartridge |
| Injection Volume | : 1000 µL |
| Cooler Temperature | : 15 ˚C |
| Column Oven | : 50 ˚C |

**Table 1.** Analytical conditions for characterizing PFAS compounds in drinking water

## RESULTS

Calibration curves were constructed using weighted linear regression, with data points weighted based on the inverse of their concentration (1/conc).

- The method's linear range spans from 0.5 ng/L to 100 ng/L for most PFAS compounds;
- For some PFAS, such as PFNS (a specific PFAS compound), the linear range is from 0.5 ng/L to 50 ng/L;
- An $R^2$ of at least 0.99 suggests a high degree of linearity and accuracy for all PFAS compounds within their respective concentration ranges;
- The lowest calibration point (0.5 ng/mL) can be determined in 77.3% of all PFAS.

Exemplary calibration curves and MRM chromatograms at 1 ng/L are available in the full application note.

The study involved analyzing control samples three times, each at concentrations of 5 ng/L and 25 ng/L, to assess the consistency of analytical results. In most cases, the variability in the measurements, represented as the percentage relative standard deviation, was less than 20% for over 95% of the compounds and quality control samples.

## CONCLUSIONS

Shimadzu's on-line SPE LC-MS/MS method for drinking water analysis offers an efficient solution for monitoring PFAS compounds, including those targeted by the EU Directive EU 2020/2184. By utilizing an innovative approach that minimizes sample preparation steps, this method simplifies routine PFAS analysis in drinking water laboratories. With a focus on precision and accuracy, it covers 44 PFAS compounds and 22 internal standards. The study demonstrates the effectiveness of this method through robust calibration curves, high linearity, and consistent analytical results, ensuring compliance with the evolving regulatory landscape.

# Advances in PFAS Workflow Solutions

We delve into the world of PFAS workflows, as seen through the lens of PFAS expert Dr. Tarun Anumol

**by Aimee Cichocki**

Effective workflow development is key in any analytical laboratory. Using the right tools and technologies ensures that scientists are able to achieve suitably accurate and repeatable results while meeting throughput goals and other objectives. As the analysis of per- and polyfluoroalkyl substances (PFAS) continues to be an area of focus in analytical laboratories across the globe—with no sign of going away anytime soon—there is an increasingly intense spotlight on the workflows used for this type of testing.

At the workflow development stage, laboratory personnel must consider each component, from consumables to instruments to software. As with other types of analysis, a broad range of factors are at play, including sensitivity, contamination potential, and sustainability, to name a few.

Dr. Tarun Anumol, Global Environmental Market Director at Agilent Technologies, has vast experience working on various components of PFAS workflow development.

Through this in-depth interview, he elucidates the nuances and challenges of PFAS analysis, emphasizing its importance and the technological strides made to support scientists in their pursuits.

## Could you describe how PFAS analysis has changed over time and the importance of some of the different testing methods?

PFAS testing has changed in quite a few ways. One of the ways to look at it is through the lens of the lab analysts and the major challenges they are facing. One of the issues is that PFAS have become so ubiquitous, and we're realizing this is not just limited to an environmental problem, with PFAS being present only in water, soil, and air. We're finding PFAS in many consumer products and materials, for example, even in medical devices. That said, another challenge with PFAS is the scope. Depending on the definition of PFAS, we expect that anywhere from around 5,000 to a million PFAS have been produced, and many of them have been used in commerce. We are currently only looking at about one or two percent of those from a regulatory perspective. As we conduct more research, we continuously add new compounds to test for, broadening the analytical scope, which is a big challenge for analytical chemists in the lab. The third major challenge around PFAS testing, and where much of the evolution is happening, relates to how we address the background contamination. What's interesting from an environmental perspective is the levels that we're looking at for PFAS are about 1,000 to 10,000 times lower than previously regulated contaminants that we've analyzed, such as pesticides and semi-volatile organic compounds. As such, the sensitivity required is a lot higher. But that also means you need to have much cleaner backgrounds in your lab and ensure your analysis is robust and reliable in those low concentrations. The fourth challenge, and again where PFAS analysis is evolving, is continually changing regulations. New analytical methods are under development for various matrices in different countries and regions. These come with new regulations, which can also mean different accreditations and audits. So, PFAS analysis is constantly evolving considering all these factors, and labs have to be aware and on top of them to be at the forefront of producing the highest quality PFAS data.

## What is the importance of having streamlined and efficient workflows for PFAS detection?

What is interesting with the PFAS workflow is you really need to consider the entire process, including sample collection, sample preparation, extraction, instrumental analysis, data processing, and data reporting, to be successful. Unlike for many other analytes, the instrumental analysis for PFAS is not the most difficult or challenging part. These compounds love to be ionized, especially on LCMS instruments. The bigger challenges for the lab are around making sure you're not introducing PFAS as background, because of how low we are looking to measure them and the latent presence of

***"Using the right tools and technologies ensures that scientists are able to achieve suitably accurate and repeatable results while meeting throughput goals and other objectives."***

PFAS everywhere. PFAS are often present in common lab materials, such as laboratory glassware or tools, sample preparation equipment, and consumables, and they're even present in lab surroundings. Thankfully, there are specific products and tools that are QC'd for PFAS. In response to these challenges, Agilent and other vendors have characterized their LCMS systems to ensure they are not introducing fluoropolymers or other PFAS. Considering the entire workflow is more critical in PFAS than in many other analyses, and you really need to limit the potential for contamination at each stage.

## How large of an issue are consumables in PFAS workflows?

PFAS analysis requires very high sensitivity, which typically means you need more sample preparation, more cleanup, and more extraction or concentration. That would typically lead us to think we need more tools. But many of our traditional lab consumables come into contact with fluoropolymers, which can be a source of PFAS contaminants. Analysts need to watch out for some of the most basic items normally used in labs. For example, vial caps often have PTFE, a fluoropolymer that can add PFAS contamination into your sample. In other cases, PFAS may not be involved in the manufacturing process, but they can appear as contaminants in the raw materials. This means you can have varying backgrounds from batch to batch of consumables. To address this, Agilent has produced a line of PFAS products including vials, caps, filters, and SPE cartridges that go through QC to check for the presence of any PFAS. But while much of the onus is on consumables manufacturers, the end user also has a responsibility to follow best practices, including performing QA and QC, and following SOPs that outline steps such as checking and recording batch and lot numbers. Most laboratories do this, but the importance has been heightened with PFAS

because of the increased contamination potential that we've seen. We're also measuring these compounds at much lower levels than some of the other regulated analytes we look at, further increasing the potential for contamination.

## You mentioned the issue of background PFAS. Can you discuss some of the other contributors to consider?

PFAS are found in so many products and in our natural environment. For example, we used to spray products that contain PFAS on carpets to prevent liquids seeping in, and some of these carpets might be found within laboratories. Another example is air conditioning filters. Many of these use fluoropolymer filters, such as PTFE filters, which can potentially cause PFAS contamination. Many cosmetics can also contain trace levels of PFAS. Your clothes might even be coated with fluorinated compounds—for example, the water-resistant coating on raincoats often contains PFAS. While it's not impossible to limit background PFAS, these considerations highlight the importance of good laboratory practice and having robust QA and QC, both on raw materials and throughout the analytical workflow.

## When it comes to improving workflow efficiency, how can software help to improve the data review process?

The secret that's not so secret is that most analytical chemists spend about 60 to 70% of their time using software to review, process, and evaluate data. The hardware has come so far that you no longer have to spend hours manually tuning your instruments, and maintenance is significantly lower and simpler. There are three critical factors to consider when selecting software for PFAS workflows. One is it needs to be intuitive so that it doesn't require a mass spectrometry expert and a novice can use it effectively. It's also important to reduce the amount of cross training and expertise required. Ideally, software across different instrumentation (such as GC, GC/MS, ICP/MS) should be similar so that personnel can easily operate multiple instruments. Finally, chemists want to minimize the amount of time they spend on manual evaluation of data, such as re-evaluation of peaks and manual integration. In one sample of PFAS, you're typically analyzing somewhere between 20 and 60 different components. In a batch of 100 samples, that's 6,000 individual peaks that a lab chemist might have to look at. Ideally, they shouldn't have to review peaks at all. This is really where the benefit of the new software comes in, including making use of certain tools like AI and machine learning to learn how peak integration is performed. There are also special functions such as "review by exception," where the software only flags chromatograms that are potential outliers based on certain criteria input by the user. These tools reduce manual review time and significantly increase lab throughput.

## How does software impact other areas of the workflow?

Aside from the back-end data processing, we also need to consider the front-end data acquisition software. One example that resonates with most chemists is starting a run at the end of the day, going home, and coming back the next day to find out only a few samples ran because of an issue with the instrument. Or perhaps the whole batch ran, but one of the samples was a "hot sample" that contained a very high concentration of PFAS and contaminated the next few samples, so now you need to run everything again. To address these issues, some software providers are incorporating automated feedback loops at the data acquisition stage. When the software determines the concentration or response in a particular sample is above the response of the highest calibration curve, it can automatically pause the worklist and notify the user. Or it can even run blanks until the concentration comes down. Software can address other issues, too. For example, if solvents are running low, instead of continuing to run samples without solvent, the software can recognize the problem, pause the worklist, and notify the user. These tools help to reduce the number of re-runs, increasing efficiency. They also help guide novices who may not consider certain factors. One more area to acknowledge is the environmental sustainability component. Analysts, particularly those working in environmental laboratories, want to minimize sample waste, reduce the amount of organic solvent requiring disposal, and avoid using power unnecessarily. A more efficient workflow, assisted by modern software, can help with all of these factors.

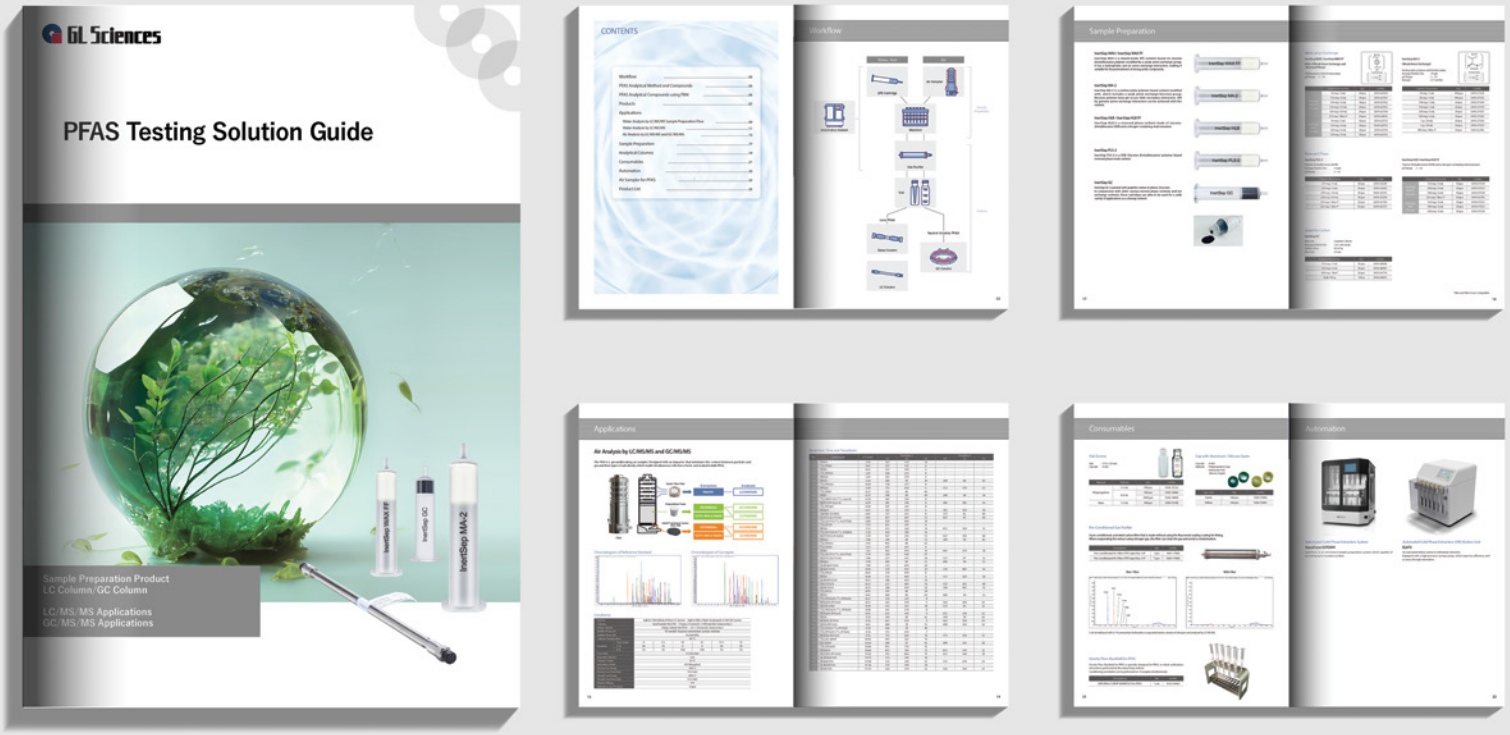
## Do you have any final thoughts to share on the topic of PFAS analysis?

My favorite saying within PFAS analysis is that you can't manage what you can't measure. We really need to find the right strategies to measure PFAS in the environment. Only once we've determined the levels, occurrence, and prevalence can we decide on the appropriate remediation strategies. While no one wants to get into environmental situations such as this, particularly where pervasive contaminants are involved, it's positive that the issue has come to light and authorities are taking mitigating steps. This is an important time in analytical chemistry, and we'll likely be conducting PFAS analysis testing for the next several decades at least.

***Aimee Cichocki*** *is the managing editor for Separation Science. She can be reached at acichocki@sepscience.com.*

GL Sciences

Separation Sciences for All



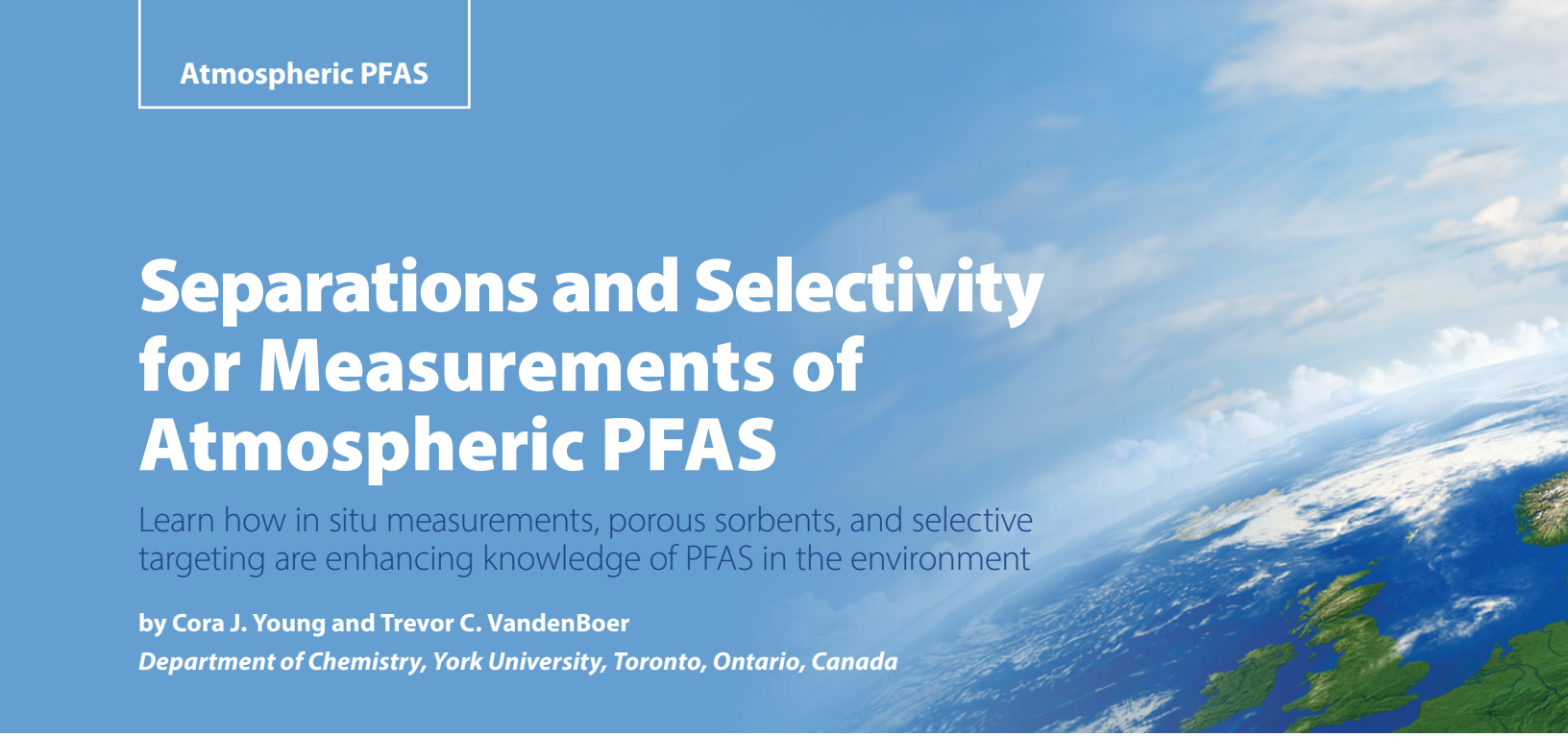

Per- and polyfluoroalkyl substances (PFAS) have garnered significant attention due to their widespread presence and potential environmental and health risks. GL Sciences recognizes the importance of accurate and efficient PFAS measurement. To address this, we have created a comprehensive PFAS Testing Solution Guide to assist in PFAS analysis and monitoring.

Besides the application of liquid chromatography-tandem mass spectrometry (LC-MS/MS) and solid-phase extraction for ionic PFAS analysis, we also introduce a revolutionary sampler for collecting neutral PFAS in the air. We offer new technologies that involve gas chromatography-tandem mass spectrometry (GC-MS/MS).

Furthermore, this guide provides best practices for sample preparation products to prevent contamination, a crucial aspect of PFAS analysis. We also remain at the forefront of innovation to discuss new trends and advancements in PFAS measurement technology.

Through the provision of this guide, our goal is to contribute to the effective monitoring and control of PFAS contamination by assisting analysts in their work. We firmly believe that taking a proactive approach to PFAS analysis is essential to safeguarding the environment and public health.

**Get the PFAS Testing Solution Guide**



# Separations and Selectivity for Measurements of Atmospheric PFAS

Learn how in situ measurements, porous sorbents, and selective targeting are enhancing knowledge of PFAS in the environment

**by Cora J. Young and Trevor C. VandenBoer**

***Department of Chemistry, York University, Toronto, Ontario, Canada***

Fluorination of organic molecules leads to unique physical and chemical properties, which are exploited in numerous commercial applications. Poly- and perfluorinated molecules tend to have low polarizability, which leads to increased volatility compared to non-fluorinated analogues. Thus, poly- and perfluoroalkyl substances (PFAS) are more likely to partition to the gas phase and thereby enter the atmosphere. The presence of PFAS in the atmosphere can have important implications. For example, long-range transport occurs much faster in the atmosphere than in other Earth spheres, enhancing the potential for global distribution and contamination.[1] In addition, inhalation can be a source of human exposure to PFAS.[2] In the atmosphere, PFAS can be present in both gaseous and particulate (solids or liquid suspended in gas) phases.[3] Measurements of atmospheric PFAS in the context of outdoor and indoor air, as well as in laboratory experiments, are needed to better understand PFAS environmental sources, fate, and impacts.

***"Poly- and perfluoroalkyl substances (PFAS) are more likely to partition to the gas phase and thereby enter the atmosphere."***

## Measurement of PFAS in atmospheric samples

Atmospheric deposition is a common method to assess the atmospheric loading of ionizable PFAS.[4] This is a long-established method for collecting ionizable atmospheric species.[5] It is advantageous because it provides an aqueous sample, which allows standard analytical methods for quantification of PFAS to be applied. An additional benefit of this approach is that PFAS present in atmospheric deposition samples are representative of the transfer from the atmosphere to the terrestrial/aquatic environment, which is of direct interest for ecosystem burden and global mobility research.

While these deposition samples are useful, the measurement of PFAS in the atmosphere in either gaseous or particulate form is important for species that do not readily deposit. Measurements of both phases come with a co-benefit of understanding the atmospheric chemical processing of PFAS. A challenge in atmospheric sampling is the separation of ultra-trace to trace target PFAS (sub-ppqv to pptv mixing ratios, or fg/$m^3$ to ng/$m^3$ mass loadings) from a high volume of atmospheric matrix. Based on this current range of anticipated atmospheric loadings, an effective separation is typically attempted in one of two ways by researchers: i) in situ measurement, where the separation and measurement are performed in real time, or ii) selective collection of target analytes paired with offline quantitative analysis.

Atmospheric chemists typically prefer the first approach, as it provides fast measurements that are on the same timescales as atmospheric processes, allowing for a more complete understanding of atmospheric fate and transport. One method that has measured atmospheric PFAS in situ uses iodide chemical ionization mass spectrometry.[6,7] In this approach, an atmospheric sample is pulled through a pressure-controlled ion-molecule reaction chamber, and PFAS forms ionized adducts selectively with iodide. For example, the interaction of the 4:2 fluorotelomer alcohol to form a charged adduct that can be detected in real time by a mass spectrometer by this ionization scheme is:

$$C_4F_9CH_2CH_2OH + I^- \rightarrow [C_4F_9CH_2CH_2OH \cdot I]^-$$

So far, this direct atmospheric sampling approach has been limited to laboratory measurements.

In contrast, the majority of atmospheric measurements of PFAS have relied on the collection of target analytes with offline quantitative analysis, such as LC-MS-MS for ionizable PFAS and GC-MS for neutral PFAS. Like many atmospheric pollutants, PFAS can be found in the gas phase, aerosol phase, or both.[3] Aerosols can be collected for offline PFAS analysis through standard collection techniques such as filtration[3] and impaction.[8] However, analytical determination biases must be considered.

The most common method for the collection of gaseous PFAS is on a sorbent under flow (active sampling) or through diffusion (passive sampling). In either approach, the sorbent targeting PFAS is subject to competitive processes with other components of the atmospheric matrix that may be present at much higher levels.[9] Typical sorbents are one or both of polyurethane foam (PUF) and polystyrene-divinylbenzene (XAD) porous resin. The sorbent(s) are used to separate PFAS from the atmosphere, extracted using solvent, concentrated through solvent evaporation, and then analyzed by GC-MS or LC-MS-MS. Air sampling methods with PUF and XAD are common for semi-volatile persistent organic pollutants (POPs) such as polychlorinated biphenyls. This was the basis for the first method to collect multiple PFAS from the atmosphere.[10] In that active sampling method, large volumes (hundreds of $m^3$) of the atmosphere containing gaseous PFAS were collected on PUF and XAD sorbents under high flow rates (several $m^3$/hr). Many PFAS have much higher volatility than POPs, which means this method only collects relatively low-volatility PFAS efficiently. Method recoveries decreasing with increasing PFAS volatility have been reported,[11] with losses of the more volatile PFAS in the analytical suite due to both the sampling and sample preparation steps.[12] While losses can be quantified using internal standards,[12] recoveries <50% for important PFAS, such as 4:2 and 6:2 fluorotelomer alcohols,[13,14] limit the ability of these methods to detect PFAS at atmospherically relevant levels. Despite the drawbacks, this method is commonly used, especially in monitoring programs where PFAS is targeted opportunistically alongside other POPs[15] because the equipment is widely available, standard procedures are in place, site personnel are trained, and the equipment is already deployed in the desired regions. Several other passive and active sampling methods based on PUF and XAD have subsequently been developed. Similar volatility-dependent losses have been reported for some of these active methods,[3] indicating that these challenges with measuring volatile PFAS are common across active sampling methods designed with these sorbents. Since all active sampling methods are intended to be exhaustive, and passive sampling techniques are equilibrium-based, passive samplers based on PUF, XAD, or both will not be able to capture highly volatile PFAS effectively.[16]

***"Long-range transport occurs much faster in the atmosphere than in other Earth spheres, enhancing the potential for global distribution and contamination."***

Sampling of gaseous PFAS has, in some cases, undergone more selective targeting based on the unique physical properties of particular sub-classes of compounds. Some of the first measurements of gaseous acidic PFAS used

carbonate-coated annular denuders.[17] This is a standard method for selective collection of gas phase inorganic acids (such as nitric acid or hydrochloric acid) resulting from fossil fuel combustion.[18] So long as the reactive capacity of the coating is not exceeded, the acidic analytes are accumulated quantitatively and irreversibly. This approach has been used in a few studies focused on ultra-short chain perfluorocarboxylic acids,[19,20] with denuders typically extracted into aqueous solution for offline analysis. Thermal desorption (TD) sampling has also effectively targeted several classes of neutral PFAS.[9,21,22] The TD technique uses highly porous materials (such as Tenax® and activated carbon) to exploit adsorptive surface interactions to trap analytes. Sorbent properties can be selected based on the volatility of the targets, including the use of sorbent mixtures to expand the analyte volatility range. The complex mixture inherent to atmospheric samples is then ideally fully extracted, which has been shown for some PFAS sub-classes. Still, high quality internal standards remain recommended to correct for or mitigate any method biases (such as GC inlet liner matrix effects).[22] This is because the collected analytes are typically introduced to a GC directly upon being thermally displaced from the sorbent in a reversed gas flow, sometimes simultaneously subjected to cryotrapping to facilitate focused injection volumes. By quantitatively transferring all trapped molecules into the instrument, TD can achieve similar method detection limits to the more commonly used active sampling techniques described above in much smaller sampling volumes (<1 $m^3$).[14] Recent innovations in TD have expanded the suite of PFAS target analytes from atmospheric samples[21] and are expected to continue to do so.

## Ongoing challenges in measuring atmospheric PFAS

Determining whether PFAS are present as gases or in particles is critical for understanding their atmospheric fate and transport.[23] This requires the separation of gaseous and particulate PFAS during sampling. Acidic PFAS, as terminal degradation products, can be subject to sampling artifacts during separations of these two atmospheric phases, particularly if the particles are sampled prior to the gases, for example, with a filter followed by a sorbent. Gaseous acidic PFAS can sorb to filter substrates such as quartz fiber, leading to a "blow on" artifact.[24] Meanwhile, particulate acidic PFAS could repartition or be displaced by atmospheric gaseous acids (such as hydrochloric acid), leading to a "blow off" effect[25] (Figure 1). As with other atmospheric acids, when sampling acidic PFAS, the gases should be collected prior to the particles, and characterization of the propensity of analytes to blow off of particles should be made in order to ascertain if additional collection media are required to trap liberated analytes following the filter.[23]

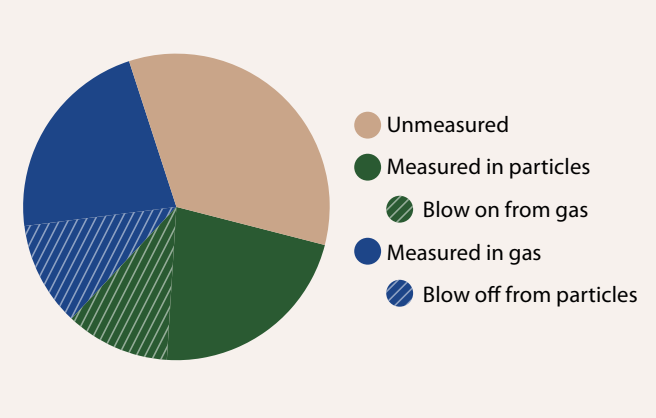



**Figure 1.** Potential distribution of atmospheric PFAS measured using a typical sampling method with particles collected prior to gases (not to scale).

Lastly, the measurement of total organic fluorine (TOF) has increased our understanding of PFAS in the condensed phase.[26] However, TOF methods developed to date require a condensed phase sample. Application of current TOF methods to the atmosphere requires a collection step to transfer gaseous and particulate analytes to the condensed phase. This is more challenging for the most volatile PFAS for which there are currently no robust atmospheric collection methods. Because there is selectivity inherent in any collection method that separates PFAS analytes from air, samples subjected to offline TOF analysis should be anticipated to be inherently incomplete (Figure 1). Thus, the current suite of separation methods developed for past PFAS compound sub-classes in the atmosphere cannot and should not be expected to be effective for use to give a true measure of TOF in the atmosphere. If in situ methods are developed, there is a much higher chance of obtaining such a measurement and capturing the atmospheric chemistry drivers that dictate the sources and fate of atmospheric PFAS.

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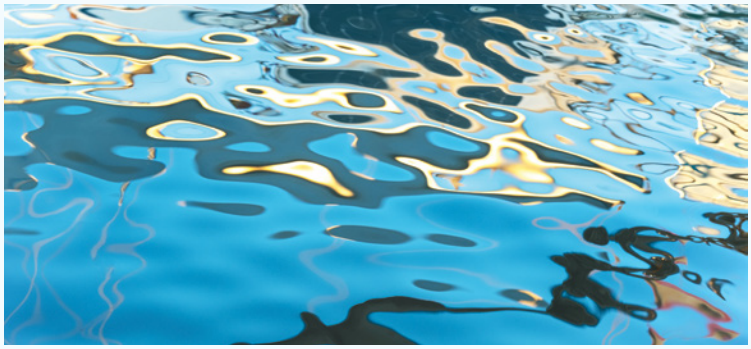
## PFAS Analysis Applications eBook



Studies have linked PFAS to reproductive and developmental issues, depressed immune response, liver and kidney problems, and cancers. Researchers studying these compounds of concern examine such matrices as drinking water, food packaging, foodstuffs, soil, blood, and tissue samples and require a wide range of analytical sampling and analysis support. Separation Science, in collaboration with PerkinElmer, offers its 'PFAS Analysis' eBook featuring the latest applications and methods, together with a case study exploring analytical tools for PFAS detection.

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## PFAS Solution Guide

PFAS have been manufactured and used in a variety of industries around the globe since the 1940s. Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) have been the most extensively produced and studied of these chemicals.

The United States Environmental Protection Agency (USEPA) has issued drinking water health advisories for two PFAS: PFOA and PFOS at 70 ng/L. As the research surrounding these problematic, lifelong chemicals becomes more expansive, the need for sensitive and specific extraction methods becomes more vital for accurate detection and quantitation.

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# Unwrapping Fast Food's PFAS Problem

Swapping single-use plastics for paper-based fast food packaging can create inadvertent pathways for public PFAS exposure, finds a new study.

**by Adam Dickie**

The past few years have seen increasing regulation targeting plastic packaging, including the EU's ban on single-use cutlery, plates, and straws in 2021, followed by similar legislation in Canada in 2022. In the fast food industry, many manufacturers have responded by introducing more sustainable packaging from paper and other plant-based sources.

However, a recent analytical study of paper-based containers and wrappers from Canadian fast food restaurants suggests that these 'greener' options may have undesirable consequences. The results indicate that polymeric PFAS coatings used to repel fat and water from paper can eventually transform into compounds of toxicological concern.

"Our broader – and ambitious – goal is to identify all major sources of PFAS around us," explains Marta Venier from the O'Neill School of Public and Environmental Affairs at Indiana University, one of the study's main authors. "We knew that fast food packaging has a need for grease repellency to keep the containers intact, so we saw a possible connection to our research."

## Analyzing fluorine concentration

After collaborators from Miriam Diamond's group at the University of Toronto, Canada, collected food packaging samples including 'compostable' fiber bowls, burger wrappers, popcorn bags, and dessert covers, the researchers used particle-induced gamma-ray emission spectroscopy (PIGE) performed by Graham Peaslee's group at Notre Dame University to screen for total fluorine content. PIGE is a nuclear excitation technique that enables non-destructive testing for fluorine contaminants.

The PIGE analysis revealed significant differences in fluorine levels across various types of fast food packaging. Approximately half of the samples had no detectable fluorine. However, certain materials, including donut and pastry bags, contained fluorine at concentrations ranging from 10,000 to 30,000 µg F/m$^2$. Meanwhile, molded fiber bowls were found to contain fluorine at a concentration of 1,000,000 µg F/m$^2$. This higher concentration underscores the greater structural demands placed on these bowls.

***"Our broader – and ambitious – goal is to identify all major sources of PFAS around us. I think we'll be busy for quite some time"***

"The advantage of PIGE is that it gives you a really quick answer if fluorine is there or not, and it requires little sample preparation," says Venier. "We then take the resulting few with high fluorine content and put them through secondary analysis."

The team selected eight products for targeted analysis based on their high fluorine concentrations. Following solvent extraction, they analyzed the targets using LC-MS/MS with an ultrahigh performance LC coupled to a triple-quadrupole MS, and with GC-MS in the positive chemical ionization mode. Among the 55 PFAS compounds originally targeted, two short-chain species appeared most frequently: 6:2 fluorotelomer alcohol (6:2 FTOH) and 6:2 fluorotelomer methacrylate (FTMAc) at levels ranging from 300 to 5,700 ng/g.

A recent study suggests that previous research may have underestimated the health risks associated with 6:2 FTOH. Furthermore, the authors believe this is the first instance of FTMAcs reported in food packaging.

## Elusive PFAS

Venier and her team noticed a significant disparity after completing the targeted analysis – the total fluorine concentrations derived from PIGE measurements were 100 to 5,000 times higher than results obtained through gas and liquid chromatography.

"In general, there could be both organic and inorganic fluorine associated with PFAS. In the case of bowls, when you detect fluorine, it's clearly organic," says Venier. "With organic PFAS there's the issue of fluorinated precursors that we can't get out through regular extractions. One way to close the gap is through a hydrolysis treatment."

Using assays based on strong alkaline treatments, the team successfully retrieved a portion of the fluorine balance, with recoveries reaching as high as 30% in certain instances. Nevertheless, some PFAS components remained elusive. Eventually, the demands of a meticulous reviewer led them to a partial explanation.

"We had samples that we sealed in the lab for two years, and we decided to re-extract them to get the data the reviewer was asking for," recalls Venier. "When we saw from the results that the composition had changed, we began to look at the volatility of some of these chemicals."

The PFAS concentrations in the re-extracted samples were notably lower than the initial measurements, showing reductions of up to 85%. The new contaminant levels ranged from 130 to 2,430 ng/g. Since the concentrations of less mobile ionic PFAS contaminants remained consistent, the researchers hypothesize that some of the mass losses might have occurred due to the volatilization of certain FTOH and FTMAc compounds.

The findings from the team's targeted analysis suggest that consumers might encounter PFAS concentration levels in their fast food wrappers that exceed conventional exposure thresholds. Furthermore, these results highlight previously overlooked implications for indoor air quality.

"Our next work is looking at PFAS from other products, including building materials, as we're trying to map out all major sources in the environment," Venier remarks. "I think we'll be busy for quite some time."

***Adam Dickie*** *is a science writer for Separation Science. He can be reached at adickie@sepscience.com.*

# PFAS in Pharmaceuticals: An Evolving Regulatory Landscape

Examine the regulatory crossroads resulting from the ubiquitous presence of PFAS in widely prescribed drugs.

**by Aimee Cichocki**

PFAS in pharmaceuticals is an inherently complex area to navigate. Drugs with chemical structures that fall under some recent definitions of PFAS have been developed and prescribed since the 1950s. Broadly prescribed drugs, Prozac and Lipitor, are examples of those defined as PFAS under the most inclusive definitions.

Balancing the continued production of life-altering medicines while attempting to adhere to mandates limiting the production and distribution of pervasive environmental contaminants results in a challenging regulatory path. In turn, affected organizations such as pharmaceutical companies and analytical testing laboratories face an uncertain future in ensuring testing methods and results meet the changing standards.

## A background on fluoro-pharmaceuticals

Fluorine-containing pharmaceuticals (also called fluoro-pharmaceuticals) have been in circulation for around 70 years, with the first, a corticosteroid called fludrocortisone (marketed as Florinef), introduced in 1954. Following the success of fluorinated corticosteroids, numerous fluoro-pharmaceuticals were developed in the 1980s and 1990s, including fluoroquinolones, such as ciprofloxacin, norfloxacin, and levofloxacin, and popular cholesterol drug atorvastatin (Lipitor). Authors of a 2022 study compiled a database of 340 fluoro-pharmaceuticals and found that the percentage of fluoro-pharmaceuticals among the total number of registered synthetics increased from 34% to 43% between 2015 and 2019.

"Several reasons contribute to the rising development of fluoro-pharmaceuticals," explains Leo Yeung, Associate Professor in Chemistry at the Man-Technology-Environment (MTM) Research Centre of Örebro University, Sweden. "Most chemicals contain hydrogen or a C-H bond. Replacing a hydrogen atom with a fluorine atom may not alter the parent compound's structure significantly, as fluorine is the second smallest atom after hydrogen. Additionally, the C–F bond is one of the strongest bonds, enhancing the metabolic stability of fluoro-pharmaceuticals. Fluorine, the most electronegative element, induces bond polarization, potentially altering the lipophilicity/hydrophilicity balance of a particular compound. These and other properties contribute to fluorine having possible impacts on the absorption, distribution, metabolism, and excretion (ADME) of pharmaceuticals."

## Pharmaceutical PFAS definitions

Depending on the definition used, not all fluoro-pharmaceuticals will fall under the class of PFAS. According to a recent Organisation for Economic Co-operation and Development (OECD) definition: "PFASs are defined as fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it), i.e. with a few noted exceptions, any chemical with at least a perfluorinated methyl group ($-CF_3$) or a perfluorinated methylene group ($-CF_2-$) is a PFAS."

"When we look at the definition of PFAS from Buck et al. and OECD 2018, PFAS are referring to highly fluorinated synthetic chemicals," says Yeung. "With the update of the definition of OECD in 2021, it will cover fluorinated substances. Different definitions will give different interpretations of the number and percentage of organofluorine pharmaceuticals as well as organofluorine compounds/PFAS."

Indeed, a 2022 study examined nine definitions of PFAS and used these to screen 260 organofluorine drugs. It found that some of the broadest PFAS definitions included widely prescribed pharmaceuticals such as fluoxetine (Prozac) and Lipitor.

## PFAS regulations and testing for the pharmaceutical industry

The regulatory landscape around PFAS is in its relative infancy, with current regulations mainly focused on drinking water. Examples include the latest EU drinking water directive and US regulations pertaining to the Safe Drinking Water Act (SDWA). But significant changes are anticipated in the near future. For example, the European Chemicals Agency (ECHA) published a PFAS restriction proposal in early 2023, with a six-month consultation period ending in September 2023. Meanwhile, the Government of Canada released its Risk Management Scope for Per- and Polyfluoroalkyl Substances (PFAS) in May 2023, and in the US, the Environmental Protection Agency (EPA) is taking regulatory steps to address PFAS.

While the implications of proposed regulations on the pharmaceutical industry remain unclear, laboratories can prepare to some extent based on what is known so far. Of course, the type of testing will depend on which regulations must be followed. "For example, in the new EU drinking water directive, two parametric values have been given: PFAS Total and Sum of PFAS," advises Yeung.

"'PFAS Total' means the totality of per- and polyfluoroalkyl substances with the parametric value of 0.5 µg/L. 'Sum of PFAS' means the sum of per- and polyfluoroalkyl substances considered a concern as regards water intended for human consumption listed in point 3 of Part B of Annex III—these contain a perfluoroalkyl moiety with three or more carbons ($-C_nF_{2n}-$, n ≥ 3) or a perfluoroalkylether moiety with two or more carbons ($-C_nF_{2n}OC_mF_{2m}-$, n and m ≥ 1) with the parametric value of 0.1 µg/L" explains Yeung. "Sum of PFAS is relatively straightforward to test as most of the listed compounds have been studied, and analytical methods (such as US EPA 533, ISO 21675:2019, and prEN 17892) and reference standards are available."

"As for PFAS Total, it is not yet decided what method can be used to capture the totality of PFAS because the method should be able to capture 'old' PFAS as well as 'new' PFAS," adds Yeung. "Several research articles have been published to show the capability of measuring PFAS Total using methods such as adsorbable organofluorine (AOF)-combustion ion chromatography, extractable organofluorine (EOF)-combustion ion chromatography, particle-induced gamma-ray emission spectrometry (PIGE), continuum source molecular absorption spectrometry (CS-MAS), and $^{19}F$ NMR." Yeung notes that a technical guideline on the EU drinking water directive will be developed and published in 2024 to give more information. "We don't yet know how 'new' compounds will be developed," adds Yeung. "Analytical capability should cover small polar compounds to large compounds, as well as those well ionized to those non-ionizable compounds. This is challenging, but it may stimulate more innovative development from this aspect."

As regulations around PFAS evolve, there is a pressing need for pharmaceutical companies and testing laboratories to stay informed and adapt. With regulatory bodies worldwide ramping up their efforts to address PFAS concerns, the next few years promise to be transformative for the pharmaceutical sector. Only through collaboration, continued research, and a commitment to both human health and environmental stewardship can the industry navigate this intricate and shifting terrain.

***Aimee Cichocki*** *is the managing editor for Separation Science. She can be reached at acichocki@sepscience.com.*

# PFAS Insights: On-Demand Learning

## Critical Insights in the Detection of PFAS in Our Environment

Per- and polyfluoroalkyl substances (PFAS) are used in a wide variety of products, including surfactants, fire-fighting foams, nonstick cookware coatings, lubricants, and coatings for food packaging. Because of the widespread use and pervasive nature of PFAS, testing is of great importance to mitigate potential risks to our health and the environment.

Separation Science, in conjunction with PerkinElmer, offers an on-demand presentation demonstrating solutions to optimize PFAS analysis workflows. By viewing this presentation, you will gain a detailed overview of PFAS LC/MS/MS methods, including direct injection and SPE methods fitting to various global regulations; learn how the LX50 QSight LC/MS/MS system can deliver reliable and comprehensive results for PFAS contaminants; understand how to overcome limitations related to system contamination; and discover tips and tricks for method optimization and sample preparation, specifically for PFAS.

**Presenter: Derek Mattern (Subject Matter Expert - LC/MS/MS, PerkinElmer)**

Dr. Derek Mattern has been at PerkinElmer since the beginning of 2018. He started working as a local field application specialist in Germany, focusing on the QSight® LC/MS/MS system. Since the beginning of 2020, he has become a subject matter expert covering the EMEAI region, giving application support for PerkinElmer's LC/MS/MS instruments. He received his PhD from the Friedrich Schiller University in Jena, Germany, researching natural products in various microorganisms. Before his graduate studies, he was a Fulbright scholar at the Max Planck Institute for Chemical Ecology in Germany, and he completed his undergraduate degree in the USA.

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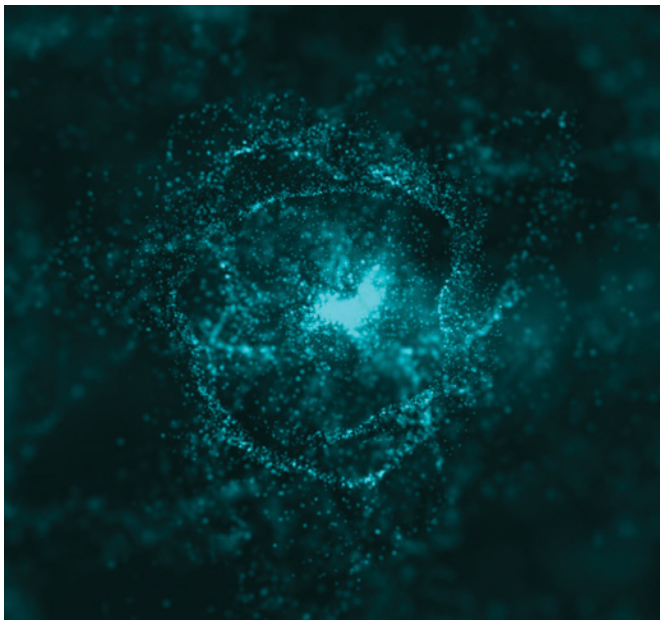
## Global PFAS Testing Virtual Symposium: Advances in Testing and Occurrence



With thousands of PFAS commercially produced, the need for robust and reliable testing and analysis of these compounds is critical. This on-demand symposium, produced in collaboration with Agilent, comprises presentations from experts in the field of PFAS analysis. It covers a variety of topics to improve PFAS testing for widescale monitoring and improved data quality in a number of different matrices.

View presentations in this symposium to learn about: the latest advances in the quantification of legacy and novel PFAS; developing and accreditation for routine and regulatory PFAS methods, including EPA and EU guidelines; novel analytical techniques to identify new PFAS; and testing methods optimized for water, air, commercial products, textiles, soil, and others.

### Symposium Presentations:

**Optimizing Water, Soil, and Serum Sample Extraction for PFAS Analysis**
Presented by Dr. Bradley Clarke (Senior Lecturer in Environmental Science and Analytical Chemistry, University of Melbourne, Australia)

**Optimization and Application of Analytical Methods for Assessing PFAS Treatment and Toxicity**
Presented by Dr. Arjun Venkatesan (Associate Director, Center for Clean Water Technology, Stony Brook University, USA)

**PFAS Analysis: Application in the Water Works of Berlin, Germany**
Presented by Frederik Zietzschmann (Laboratory of Berliner Wasserbetriebe, Germany)

**Perspectives and Challenges of Commercial Environmental PFAS Testing in the USA**
Presented by Stephen Somerville (Technical Director – PFAS, Pace Analytical, USA)

**Novel Analytical Tools for Per- and Poly-fluoroalkyl Compounds and ISO21675**
Presented by Dr. Nobuyoshi Yamashita (Chief Senior Research Scientist at the Research Institute for Environmental Management, National Institute of Advanced Industrial Science and Technology [AIST], Japan)

**Uncovering Xenobiotics in Nontargeted Analyses Using Ion Mobility Spectrometry, Mass Defect Analysis, and Machine Learning**
Presented by Erin S. Baker, Ph.D (Associate Professor, North Carolina State University, USA)

**VIEW THE SYMPOSIUM PRESENTATIONS**

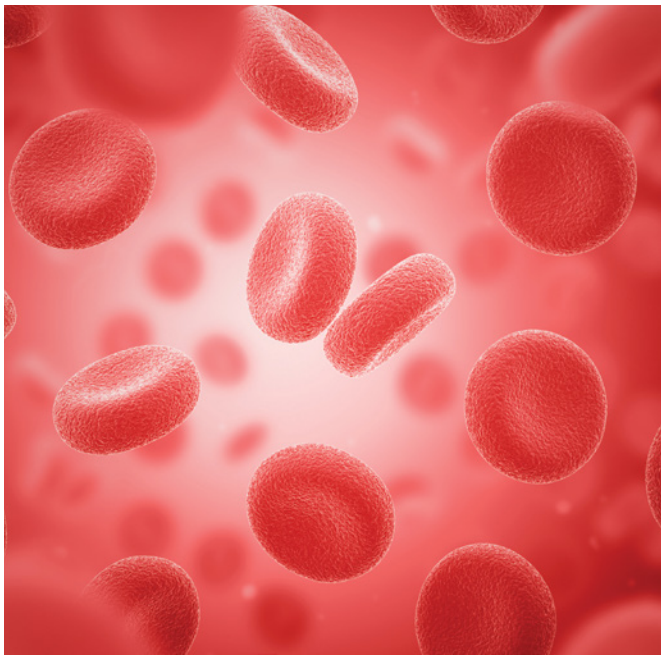
Agilent

## Quantitation of Selected PFAS in Human Whole-Blood Utilizing VAMS (Volumetric Absorptive Micro Sampling) and Latest-Generation Triple Quadrupole Instrumentation

Historically, PFAS blood testing has been performed on the serum component of blood. Serum is a less complex matrix to work with compared to whole blood, and many PFAS partition towards the serum. The downside is that a blood draw and processing are required, which adds cost and complexity to an already expensive test. Interested PFAS test candidates may be located in a remote area or unable to secure a mobile phlebotomist, making the blood draw a deciding factor in having a test performed. Additionally, pediatric PFAS tests are not uncommon, and a heel or finger prick is less stressful for all parties than a blood draw.

Eurofins has pioneered an approach that utilizes a field-deployed, self-administered blood collection device paired with very sensitive instrumentation to achieve biologically relevant detection limits for PFAS. Analyte lists with both long and short-chained PFAS will allow

researchers snapshots into PFAS with short half-lives while still supporting legacy PFAS on the CDC NHANES list. Hopefully, a better understanding of PFAS body burden will be realized with a whole-blood approach as it is understood that some PFAS, such as perfluorooctane sulfonamide (FOSA), partition towards whole blood rather than serum.



Separation Science, in conjunction with Sciex, produced an on-demand presentation that provides an effective approach for studying PFAS in human whole blood and discusses the challenges of PFAS analysis in blood serum and how to overcome them. This presentation is ideal for: exposomics laboratory scientists and QA/QC managers working in the environmental and clinical research areas; those involved in method development, interested in implementing and optimizing PFAS or any other environmental contaminant applications; and those interested in learning about the findings surrounding PFAS.

**Presenter: Andrew Patterson (Technical Director, Eurofins Environment Testing America - Specialty Services Division)**
Andrew brings 20 years of experience in the environmental laboratory industry with a focus on HRGC/HRMS analyses and LC-MS/MS analyses. These approaches have focused on PCBs, dioxins, brominated flame-retardants, PFAS, and emerging contaminants. Prior to joining Eurofins, Andrew was the Technical Director for Vista Analytical Laboratory in Northern California, where he developed all aspects of PFAS capability within the lab. Before his time at Vista, Andrew worked in both the HRMS and LCMS laboratories at Alta Analytical with a focus on implementing performance-based EPA methods. Andrew holds a Bachelor of Science in Microbiology from Cal Poly San Luis Obispo, California, USA.

**VIEW THE ON-DEMAND PRESENTATION**

SCIEX
The Power of Precision

## Practical Challenges in PFAS Extraction for Water Samples and How to Address Them

Analyzing PFAS in water samples down to sub-ppt quantitative levels is not a trivial exercise, especially as analysts discover how ubiquitous these chemicals are in our environment. Achieving analytical results that represent what is in the sample and not the surrounding environment requires special handling techniques and precautions, careful selection of solvents and reagents, and configuration or modification of sample preparation equipment and LCTQ instruments.

Separation Science, in collaboration with Biotage, offers an on-demand presentation covering PFAS and some of the specific problems encountered with their analysis, along with practical solutions to implement during EPA and ISO methods investigation. The session includes a review of what PFAS are, their pervasiveness, and the importance of monitoring. This is followed by a discussion of some of the PFAS-specific problems encountered and the practical solutions

implemented during investigations into US EPA method 537.1, US EPA method 533, and ISO Method 21675 over the last few years. This practical, experience-based presentation is useful for anyone involved in or performing method development for PFAS in aqueous matrices using a range of methods.

**Presenter: Bill Jones (Director of Chemical R&D, Biotage LLC)**
Bill has been with Biotage for 19 years. Here, he has been involved with the development, design, and testing of SPE disks and membrane drying consumables, along with the development of automated evaporation and separation instrumentation and working with the EPA to incorporate the technology developed into EPA methods.

**VIEW THE ON-DEMAND PRESENTATION**

Biotage®

## How to Optimize Your PFAS Air Sampling Method Using Thermal Desorption

Research surrounding the health effects of PFAS continues to grow, along with the investigation of exposure pathways. PFAS in air are monitored globally, but regulatory changes have prompted a requirement for this to become routine. This has highlighted some challenges when performing PFAS monitoring at ultra-low concentration levels, including the need for pre-concentration of large volumes of air and for PFAS-free blanks.

Thermal desorption (TD) has long been considered the gold-standard technique in environmental air monitoring for volatile organic compounds. The PFAS community is now turning to the technique for monitoring key species in air, and for undertaking research projects to understand the extent of the PFAS species present in the environment.

This presentation, produced by Separation Science in conjunction with Markes International, discusses the advantages that TD offers, how to optimize a system for targeted and untargeted PFAS analysis, and how to overcome challenges posed by low-level PFAS monitoring. By viewing this on-demand presentation, you will learn how to optimize thermal desorption for monitoring PFAS in stack gases and ambient and indoor air, which sampling techniques should be employed for different types of PFAS and breakdown products, and how to manage challenges associated with the sampling and analysis of PFAS in air.

Users new to the topic of PFAS monitoring will increase their understanding of the options available for sampling and analysis. TD users will gain information on how instrumentation could be used for expanding monitoring activities. This presentation is ideal for researchers interested in PFAS, laboratory and project managers working with PFAS, analysts who want to monitor PFAS destruction efficiency, and environmental consultants.

**Presenter: Helen Martin (Thermal Desorption Business Unit Manager, Markes International**)
Helen oversees research and development, application development, and product marketing for the Markes International TD instrument range. She specializes in the sampling and analysis of vapor-phase organic compounds from air, water, soil, and materials.

**Presenter: Hannah Calder (Environmental Air Market Development Manager, Markes International)**
Hannah specializes in the application of thermal desorption in environmental air monitoring. Hannah joined Markes International's global team of technical experts in 2013 following her Master's degree in Chemistry obtained from Cardiff University.

**VIEW THE ON-DEMAND PRESENTATION**

MARKES international

Watch Presentation ▶

# Why is PFAS testing so challenging?

This summary relates to a recent expert discussion forum event produced by Separation Science, in collaboration with Thermo Fisher Scientific. Separation Science would like to thank all the experts for their time and knowledge in the execution of this event.

Detecting per- and polyfluoroalkyl substances (PFAS) in the environment is key to their remediation. PFAS contamination, often in the form of perfluorooctanoic acid (PFOA) or perfluorooctane sulfonate (PFOS), can be determined by one of several regulatory methods. These include global ASTM regulations, the Stockholm Convention on persistent organic pollutants (POPs), the EU Drinking Water Directive, US EPA Methods 533 and 537, US EPA Draft Method 1633 for the determination of PFAS in drinking water, and US EPA OTM-45 to test for PFAS in air and emissions.

In the increasingly regulated area of environmental testing, determining the best workflow for PFAS analysis can be challenging. In this discussion forum, a panel of PFAS experts answered some key questions related to this topic, including those submitted by analytical scientists working in this field.

## SAMPLE STABILITY AND CONTAMINATION

The discussion began with some initial thoughts on sample stability. "We set a method up for 48 PFAS compounds following EPA 1633, and undertook a stability trial in surface water that produced results in accordance with those specified," explains John Quick, Principal Scientist at ALS Environmental, UK. "The majority of compounds are stable when stored at 5 °C for 30 days, with a few exceptions being sulfidoaminoethanol compounds which tend to convert to their corresponding acetic acids very quickly after about 5 days, and 6:2 FTAB where we noticed some degradation between 14–30 days."

In terms of storage and stability handling, recommendations appear to depend on the actual PFAS in question, with a few outliers requiring freezing, but for most, 5 °C for up to 30 days is adequate. "I have colleagues who have frozen samples for many years, without any detrimental effects, so most of these compounds are fairly stable - these polyfluoroalkyl substances are the ones that need to be monitored in terms of potential transformation," adds Chris Higgins, Environmental Scientist at the Colorado School of Mines.

PFAS compounds are found in trace levels as impurities in a variety of products and commonly used laboratory items, so how can laboratories reduce or eliminate these sources of background PFAS to ensure accuracy of results? Higgins, who has been working with these types of compounds for the last 20 years, suggests that there are two key issues to consider for this persistent problem. "The first thing to be cognizant of is what comes in contact with your sample as these are meaningfully going to contribute background," he explains. "Second, more often than not in my experience, is contamination. Pay attention to the chemical background you see in your blanks because that will often be indicative of where your contamination is coming from."

## ANALYTICAL TECHNIQUES

The discussion moved on to particular analytical techniques for analyzing extremely low levels of PFAS,

required from a regulatory perspective, beginning with a look at an alternative to LC-MS/MS: combustion ion chromatography (C-IC). Combustion ion chromatography measures total halogens (fluorine, chlorine, bromine, iodine) and sulfur in difficult to analyze solid, gas, and liquid samples. "EPA Method 1621 describes the CIC method very clearly—it's a screening method for essentially low parts per billion (ppb) concentrations," begins Ed George, Senior Applications Scientist at Thermo Fisher Scientific. "In essence, what you're trying to achieve with the method is to understand what's going on at a given location. Perhaps you're looking at general PFAS contamination at a particular site—you use the C-IC method to determine total fluoride to identify hot spots and then with targeted and non-targeted analysis you can understand how much the PFAS contamination is contributing to that total fluoride number. Not all fluoride is necessarily from PFAS, but with a history and understanding of the site a better calculation can be made. C-IC is not a perfect technique, but it works fairly well for screening and is a good complementary technique to the targeted and non-targeted analysis."

One fact that is known about the more sensitive total organic fluorine proxies is that they often require an extraction or absorption step to isolate them at least from water. It is important for researchers who use these approaches to be aware that they are selecting for compounds that only absorb to those media.

"We still lack a true total organic fluorine measurement technique accurate down to low ppb, let alone parts per trillion (ppt) levels," suggested Lee Ferguson, Associate Professor of Environmental Science and Engineering at Duke University, North Carolina. "This is an area of active research, especially for instrument manufacturers. There's a real challenge and a great opportunity here to make significant advancements." The conversation then moved to the comparative use of both liquid chromatography (LC) and gas chromatography (GC) in the comprehensive analysis of PFAS, particularly in air samples—is analysis using both techniques required? In addition, is the total oxidizable precursor (TOP) assay in air samples practical? TOP assay is a method which oxidatively converts precursor compounds of PFAS into measurable perfluorinated alkyl acids (PFAA).

With comprehensive air analysis it is important to know about all of the perfluoroalkyl substances present, and various approaches are required for their sampling and analysis. "These start with small molecules, such as ozone-depleting substances which require grab analysis with canisters via established methods," explains Vladimir Nikiforov, from NILU-Norwegian Institute for Air Research. "Next, there are larger perfluorocarbons and similar molecules which are very inert—the so called 'tracer gases' or PFTBA (perfluorotributylamine). Then there are more polar substances such as acrylates, FOSA/FOSE, and related compounds, and these can be sampled on thermal desorption tubes, SPE sorbents, and there are established methods in this area."

***"Pay attention to the chemical background you see in your blanks because that will often be indicative of where your contamination is coming from."***

"Carboxylic acids are present mainly in the particle phase in the air, so this is where filters would be used," continues Nikiforov. "And finally, there are perfluorosulfonic acids, such as PFOS, that exist only in the form of anions. Accordingly, for the analysis of the most volatile substances, it would be GC/MS with cryofocusing. For substances like PFTBA it would also be GC because of a lack of ionization with LC. For FOSA/FOSE, both GC and LC can be used, with or without derivatization. For carboxylic acids, LC/MS is usually used, but we have recently shown, in cooperation with both Thermo Fisher Scientific and Markes International, that perfluorocarboxylic acids can be analyzed by GC/MS. Finally, for PFA and other sulfonates, LC is almost exclusively used."

Regarding the application of TOP analysis, perfluorocarbons would not convert to measurable acids and so would not be determined in this way. Likewise, more volatile substances would volatilize and escape the reaction vessel so additional information could be acquired, but it would not be total.

## LINEAR AND BRANCH ISOMERS

It is important to recognize that there are many different PFASs that have both linear and branch isomers, and many of the chemicals made by electrochemical fluorination (ECF) as a synthetic process have branched isomers. When analyzing PFAS with C18 columns and an earlier than expected peak appears, is it really a PFAS? If it is a transition for an ECF-derived chemical, which are many of the sulfonates and precursors for persulfonates, then there is a good chance what you are seeing is a branched isomer. Depending on the regulatory context, quantification of the peak may be required.

## MEET THE EXPERTS

**Lee Ferguson** is an Associate Professor of Environmental Science and Engineering at Duke University in Durham, NC, USA. He received B.S. degrees from the University of South Carolina in Chemistry and Marine Science in 1997 before earning a Ph.D. in Coastal Oceanography at State University of New York – Stony Brook in 2002. His postdoctoral research was conducted in the area of proteomics at the Pacific Northwest National Laboratory in Richland, WA, USA. Before joining Duke, Dr. Ferguson was an Assistant and Associate Professor of Chemistry at the University of South Carolina.

**John Quick** is the Principal Scientist at ALS Environmental in Coventry. John's background is in chromatography and over the last years John and his team have developed methods to analyze all the compounds included in the CIP program (A UK version of the Water Framework Directive) with detection levels not seen by any other commercial laboratory in the past.

**Vladimir Nikiforov** graduated from St. Petersburg State University in 1986, got a degree in 1990 (Synthesis of fluoroketones for extraction of anions) and continued with the same university doing research and teaching in Synthetic organic, Physical organic, Environmental chemistry until 2010. From 2010 to 2014 he was a head of laboratory of migration of POPs in the Center of Ecological Safety of the Russian Academy of Sciences and then joined NILU-Norwegian Institute for Air Research. His current research interests include PFAS and other organofluorines, non-target and suspect screening, QSAR, development of analytical methods for new pollutants, and microplastics in all matrices and in air samples in particular.

**Chris Higgins** is an Environmental Chemist at the Colorado School of Mines. Dr. Higgins received his A.B. in Chemistry from Harvard University, and graduate degrees in Civil and Environmental Engineering from Stanford University. He joined Mines in 2009, attaining the title of University Distinguished Professor in 2022. His research focuses on the movement of contaminants in the environment. In particular, he studies chemical fate and transport in atural and engineered systems, with a focus on poly- and perfluoroalkyl substances (PFASs). Dr. Higgins has authored more than 135 peer-reviewed publications. His research has been supported by NSF, NIH, EPA, USDA, and the DoD.

**Ed George** worked for 15 years in the environmental laboratory industry, holding positions of Lab Technician, Lab Manager, and R&D Manager, responsible for developing novel sample preparation and analytical methods in soil and water on GC/MS/MS and LC-MS/MS platforms. He participated in several collaborative method development projects with the USEPA. In 2014, he joined Thermo Fisher Scientific and is currently a Senior Applications Scientist. He has worked closely with key collaborators on food safety and environmental projects in universities and industry and has recently developed workflow applications for pesticide and veterinary drug residues in food on both LC-MS/MS and high-resolution MS platforms.

"If you're using triple quadrupole analysis there is well documented bias; which MRM you're using will result in a different concentration. Consequently, there's some hesitancy to integrate those peaks for that very reason," comments Higgins. "This is one reason why I particularly value high-resolution mass spectrometry work because we're typically quantifying from the parent ion itself, and you don't have that bias of the fragmentation from MRM that's been chosen to quantify the branched isomers."

"There are some issues with potential contamination from other compounds," adds Ferguson. "Bile salts, for example, can interfere with some of the peaks for PFOS that are some of the branched peaks. This is another argument in favor of using high-resolution mass spectrometry as a quantitation technique for some of these compounds. Any time that you're dealing with really low levels of trace contaminants and you can take advantage of high mass accuracy and high resolution to minimize potential interference, it is recommended."

Quick agrees with Higgins and Ferguson. "In our lab, we compared the relative responses of the branched and linear PFOS, both by high-resolution MS, and they were broadly very similar. There's a definite advantage of high-resolution MS for the quantification of those particular compounds."

## NON-TARGETED SCREENING

The discussion moved on to non-targeted analysis and how laboratories can efficiently and effectively analyze samples for non-targeted and suspect PFAS screening. In terms of laboratory requirements there are two primary hardware issues. First, there is very high accuracy mass measurements— the more accurately mass can be measured the better the definition of the elemental composition, which then helps define the subsequent structure annotation. Second is fast and high-resolution data-dependent MS/ MS acquisition. "If you can put these two things together and avoid the temptation to run very short LC gradients, you're stretching out your ability to acquire MS/MS spectra for as many of these compounds as fast as possible," comments Ferguson. "Because we're usually working down in the ppt range, approaches such as online solid phase extraction coupled to high-resolution MS are really important for doing non-targeted PFAS analysis."

From a workflow perspective, much progress has been made in the last 4-5 years for data that can be better used for annotation of PFAS structures. Key tools include compound databases, and the EPA has made good progress in compiling some of these from multiple sources; open source platforms for processes such as predicting biotransformation products for polyfluorinated compounds; and the use of in silico MS/ MS tools to predict tandem MS spectra.

"If you can pull these things together into a software package that allows annotation in at least a semi-automated way you can make a good start at structure annotation for suspect PFAS," added Ferguson. "For true unknown identification there are still challenges, and it's difficult to do in an automated way. Here, we're dealing with structures that we don't have in databases, and we're relying on both expert annotation by expert mass spectrometrists as well as software tools that allow us to do multistage mass spectrometry. In our laboratory, at least when we're dealing with true unknowns, we've used approaches such as MS3 or MS4 data acquisition to attempt mapping of fragment trees for unknown compounds. In addition, it's best to have authentic standards, but again this is a challenge when working with PFAS."

"New standards are being made all the time," adds Higgins. "But unfortunately they are not keeping track with the pace of new PFAS being observed in the environment. The reality is that there are thousands of these compounds, and at any one inspection site there could be hundreds, the vast majority of which will be detected by suspect screening. With standards not being available we need to start thinking about how we're going to communicate with confidence the identity of these compounds."

***"This is another argument in favor of using high-resolution mass spectrometry as a quantitation technique for some of these compounds."***

Will there be a bigger role for GC/MS in non-targeted workflow analysis? Nikiforov thinks not. "I believe in the coming years, LC/MS will be the key approach. However, even LC/MS will have challenges in some instances, for example, with carboxylic acids, which don't produce molecular ions, particularly at low concentrations. Here derivatization can help, after which GC analysis could be used."

"For some of the more volatile PFAS compounds such as fluorotelomer alcohols, high-resolution GC/MS does have some significant advantages, especially in complex mixtures," highlights Ferguson. "Although it can be very difficult to perform, real non-targeted analysis with GC/

EIMS systems, in combination with improving spectral libraries, will help us find unknown compounds. Some recent examples have been PFAS in paints and some volatile PFAS in firefighting foams. We shouldn't get so focused on the polar and ionic PFAS that we forget all the volatile and semivolatile fluorocarbon materials. It becomes a real issue for human exposure in the indoor environment, as well as occupational exposure."

"I also think that GC is probably coming more into the equation with PFAS analysis," agrees Quick. "Especially with regards to the EU Water Framework Directive, which now has fluorotelomer alcohols in its target list of 24 PFAS."

***"If we are talking wish lists, for me a fully automated evaluation of spectra to make identifications with confidence, without the need for an expert."***

## CONFIDENCE WITHOUT STANDARDS?

The discussion moved onto perspectives on the reporting of PFAS discovered in non-targeted analysis workflows for which there are no available reference standards. "It's an interesting challenge for laboratories, with a lot of questions," comments George. "What's the best approach for porting the samples? How do we annotate? How do we report? When I worked at the EPA we used the term tentatively identified compounds, which we grouped together and tried our best to identify. Now we have to consider, from our non-targeted and suspect screening, the best way to report back to the customer, in a way that's understandable by the customer—this is still a real challenge in the PFAS world."

"I think that it's important when reporting non-targeted suspect screening data to a non-expert, a customer who isn't a mass spectrometrist, that those data come with some descriptive metrics on confidence," adds Ferguson. "I also strongly urge that we don't use the term identification in non-targeted analysis, and annotation is a much better term. Unless we're actually confirming with authentic standards or a technique like NMR, it's difficult to say a compound has been identified. As more practitioners start to perform non-targeted and suspect screening work on environmental samples this will become increasingly important, because as reports get out into the literature, propagation of error becomes a real possibility if it's not done correctly."

"Could we have some kind of a CRM for this?" asks George. "It's not an easy approach, but it could be a way to test a lab's quality control and how well screening procedures work. It might help give confidence in the analysis performed in the lab. It can be checked against some kind of a standard and the non-target and/or suspect screening procedure."

## LOOKING FORWARD

The discussion wrapped up with panelists outlining what they would like to see in the future of PFAS analysis.

"Ultra-sensitive total PFAS measurements would be amazing," begins Ferguson. "And then also faster and faster data-dependent MS/MS for nontargeted identification and annotation for PFAS. Those would be my two top wish list items."

"I would prefer a universal GC column that would handle very nonpolar and very polar volatile organofluorines," adds Nikiforov. "Mass spectrometers are very good now and I have a feeling that GC is not a match for MS."

"For me it's standards," says Quick. "Better availability of standards, especially labeled standards that we can use as surrogates to compensate for matrix suppression, will make my life a lot easier."

"If we are talking wish lists, for me a fully automated evaluation of spectra to make identifications with confidence, without the need for an expert," adds Higgins. "We're going to be doing suspect analysis for a long time, and my grad students in particular would be grateful for such software."

"Making it easier to process data for non-targeted analysis and suspect screening," concludes George. "The report that you generate is really important for the customer to fully understand its contents and have full confidence in it."

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