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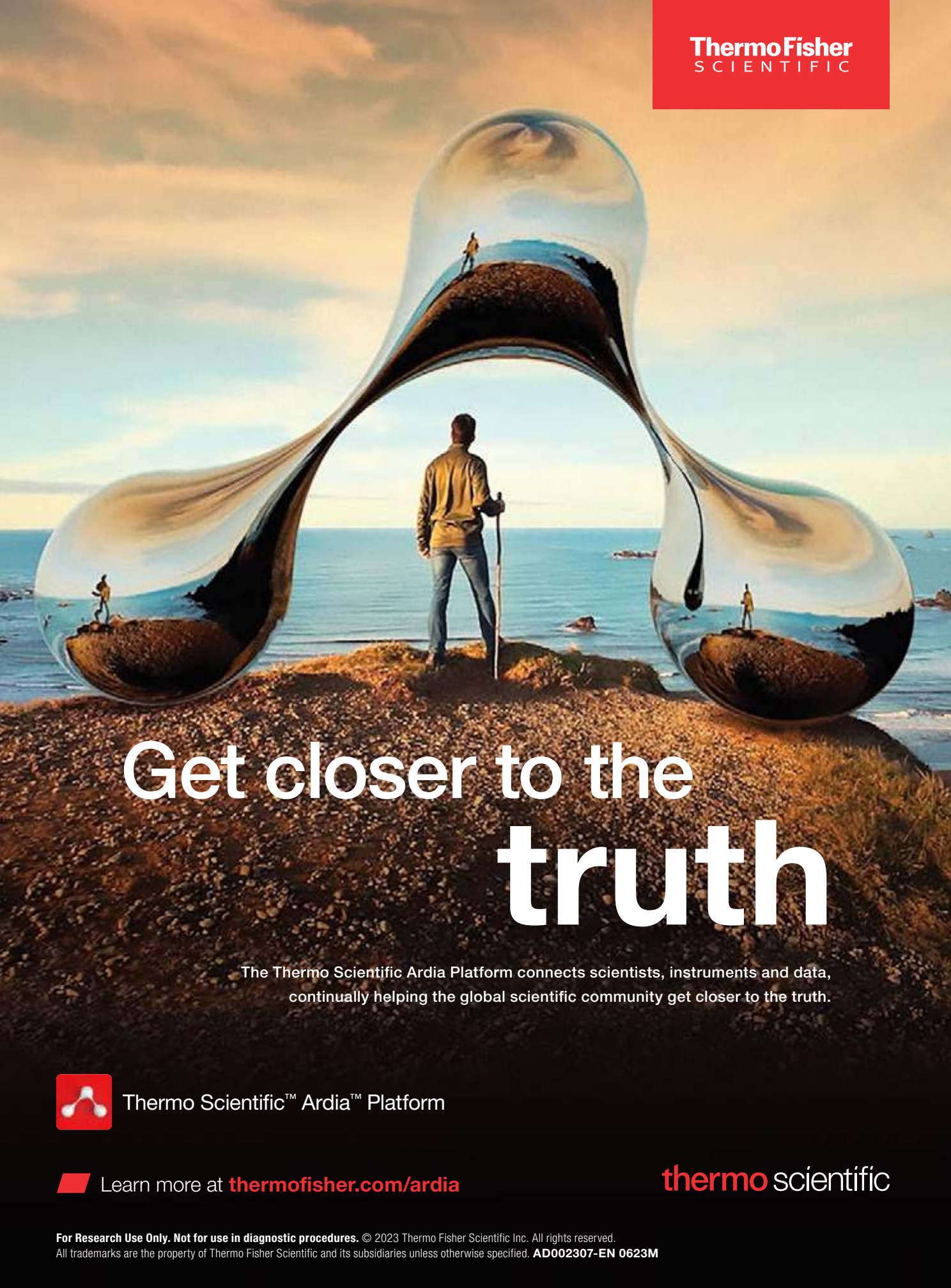

September 2023

HOT TOPICS IN PFAS

A supplement to *LCGC North America*

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Cover Image: Green Wind - stock.adobe.com | Sampling and Analysis for Investigating Airborne PFAS Transmission

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Measurement of PFAS in Complex Consumer Product Matrices Using Negative Reagent Ions

By Leslie P. Silva[1], Stefan Swift[2], Patrik Španěl[2], and Vaughan S. Langford[1]
[1] Syft Technologies, Ltd.
[2] J. Heyrovský Institute of Physical Chemistry

PFAS are widely used in consumer products to improve durability and provide various properties, including non-stick or stain coatings and water resistance (Kenny Newberry, https://axial.acs.org/earth-space-and-environmental-chemistry/phasing-out-pfas-in-consumer-products). Understanding their presence in products is important because these molecules not only persist in the environment but are potentially toxic and persist in the bodies of humans and animals. Testing for the presence of low-concentration PFAS impurities using direct measurement techniques can be challenging due to many consumer products containing high concentrations of volatiles, resulting in reagent ion depletion and saturation effects (Perkins *et al.* https://doi.org/10.3390/analytica4030024). This leads to a loss of sensitivity for the PFAS contaminants.

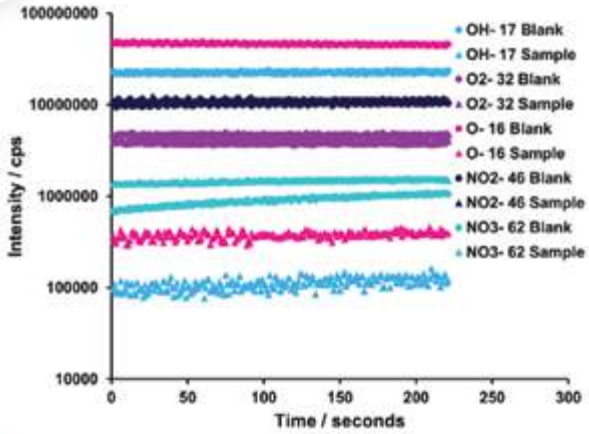
Selected ion flow tube mass spectrometry (SIFT-MS) is a direct MS technique that utilizes eight reagent ions simultaneously to analyze whole air. This is advantageous in complex mixtures like personal care and household products, as each reagent ion offers unique selectivity through orthogonal ionization properties, potentially mitigating matrix effects caused by the more abundant volatiles present in these products. While PFAS react with the eight reagent ions available in SIFT-MS, three reagent ions are insensitive to the more abundant components in these products: O_2^-, NO_2^- and NO_3^-. This application note demonstrates selective, high-sensitivity quantitation of two fluorinated species even in products with complex volatile matrices, such as shaving cream, fabric softener, deodorant, and dishwasher rinse aid.

Standards were prepared for pentafluoropropionic acid (PFPA) and heptafluorobutyric acid (HFBA) into Nalophan bags filled with zero air. Full scans from m/z 10 – 400 were run on a Syft Tracer™ instrument to identify product ions and determine their respective branching ratios for all reagent ions. Consumer products suspected of having PFAS were decanted into 250 mL glass bottles. Samples were heated to 40 °C for 10 minutes, and the sample bottle septum was pierced directly with the instrument inlet. Zero air flowed into the bottle to equalize the pressure during sampling. Full scans were run on each sample and post-processed to identify and quantify PFPA and HFBA.

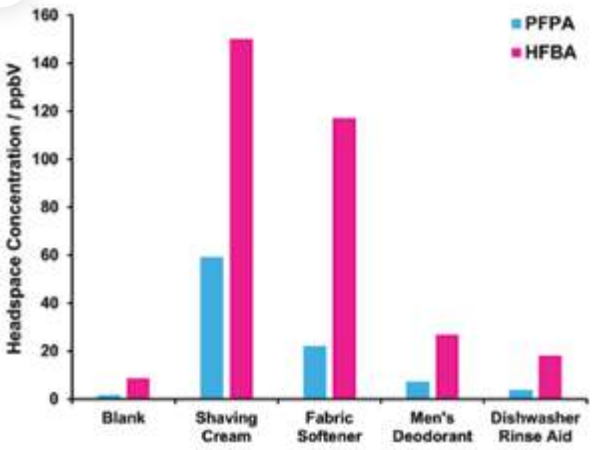
PFPA and HFBA were measured in four consumer products using the five negative reagent ions (OH^-, O_2^-, NO_2^-, NO_3^-, O^-). Figure 1(a) displays the reagent ion signals for the negative reagent ions in a blank and in the fabric softener sample. Saturation was observed for O^- and OH^- (Figure 1(a) – light blue and pink traces) and they were excluded from the concentration calculations. Reagent ion signals for O_2^-, NO_2^- and NO_3^- (teal, navy and purple) remain high in these complex matrices, enabling the remaining product ions for PFPA and HFBA to be averaged for determination of the headspace concentration. The concentrations obtained for the products are shown in Figure 1(b).

Figure 1. (a) Comparison of reagent ion levels in a blank (circles) vs fabric softener (triangles). (b) Measured levels of PFPA and HFBA in four consumer products.

(a)



(b)



SIFT-MS successfully quantified PFPA and HFBA in consumer products at low levels, even when the overall matrix VOC concentration was high. The eight reagent ions in Syft Tracer™ provide enhanced selectivity, because three negative reagent ions can measure low concentrations of PFAS without any ion suppression effects. This prevents the need for sample dilution or pre-concentration of this important class of toxic VOCs, facilitating rapid sample screening.

Per- and Polyfluoroalkyl Substances (PFAS) or Interference? Using High-Resolution Mass Spectrometry as an Investigative Tool in Food Analysis

Brian Ng, Christine M. Fisher, Susan Genualdi, Wendy Young, Elsie Peprah, Ann M. Knolhoff, and Lowri deJager

Accurate quantification of per- and polyfluoroalkyl substances (PFAS) in food is necessary to understand potential dietary exposure. While beneficial for most applications, quantification of PFAS using low-resolution triple quadrupole instruments can be complicated by the presence of co-eluted interferences, which can result in false positives or inaccurate PFAS concentrations. This article discusses the use of high-resolution mass spectrometry (HRMS) to distinguish between matrix interferences and PFAS compounds in a variety of food matrices, and incorporation of these interferences into routine triple quadrupole methods for monitoring.

Due to the extensive use of per- and polyfluoroalkyl substances (PFAS) in consumer products and industrial applications since the 1950s, these compounds have been detected in the environment, humans, and food. Therefore, the accurate quantification and determination of PFAS in foods is important to better understand potential dietary exposure.

Since the identification of taurodeoxycholic acids as an interference with perfluorooctane sulfonic acid (PFOS) in human serum (1), interferences continue to be identified with individual PFAS in samples including environmental, biological, and food matrices. As analytical methods continue to expand with additional analytes and matrices, the detection of interferences and false positives has also increased.

Routine low-resolution triple quadrupole analysis continues to be the primary analytical technique for the quantification of PFAS in food and environmental samples due to its lower cost, ease of use, and typically lower detection limits compared to high-resolution mass spectrometry (HRMS). However, the unit mass resolution of triple quadrupole methods is insufficient to differentiate PFAS compounds from potential interferences within ± 1 Dalton (Da) of the target precursor mass and tandem mass spectrometry (MS/MS) transition mass. This can lead to false positives or over-estimation of PFAS concentrations. This is particularly challenging for complex matrices, such as foods, and for short-chain PFAS compounds with only one transition ion, such as perfluorobutanoic acid (PFBA) and perfluoropentanoic acid (PFPeA). This challenge is exacerbated when the only available transition is not unique or diagnostic (for example, loss of CO_2 from a compound containing a carboxyl group). These shorter chain analytes are known to uptake into plants and can transport to the leafy portions (2), which makes their accurate quantification in plant and agricultural products challenging. In addition, recent publications have identified PFPeA interferences in chocolate-containing foods and shellfish (3,4), and PFBA interferences from fatty acids in biological and environmental matrices (4,5).

High-resolution mass spectrometry (HRMS), often using orbital ion trap or time-of-flight (TOF) instruments, can be used to differentiate PFAS compounds from matrix interferents that differ in mass by ± 0.0001 to 0.0010 Da (less than a ±10 ppm mass difference). Therefore, PFAS analytes, such as PFBA and PFPeA, can be identified using the accurate mass of the precursor and product ions for PFBA (*m/z* 212.9792 to *m/z* 168.9894) and PFPeA (*m/z* 262.9760 to *m/z* 218.9862). For this article, due to the order of magnitude difference in mass accuracy between instruments, Da and ppm will be used to describe the mass accuracy of triple quadrupole and HRMS instruments, respectively.

This article describes an approach that can be used to differentiate matrix interferences from true positive PFAS detections using HRMS and tandem mass spectrometry (MS/MS). The approach is demonstrated using a matrix interferent for PFPeA that is commonly found in chocolate; however, this procedure can be broadly applied to diverse matrices and other PFAS compounds with suspected interferents.

Determination of Matrix Interferences Using HRMS

When there is a suspected matrix interference based on the triple quadrupole data (such as positive detections for PFBA and PFPeA, retention time shifts relative to corresponding labeled internal standards, or unexpectedly high concentrations), the sample can be analyzed using liquid chromatography (LC)–HRMS with the same or a similar LC method as used for the

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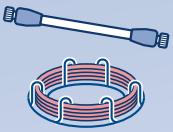
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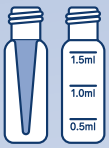

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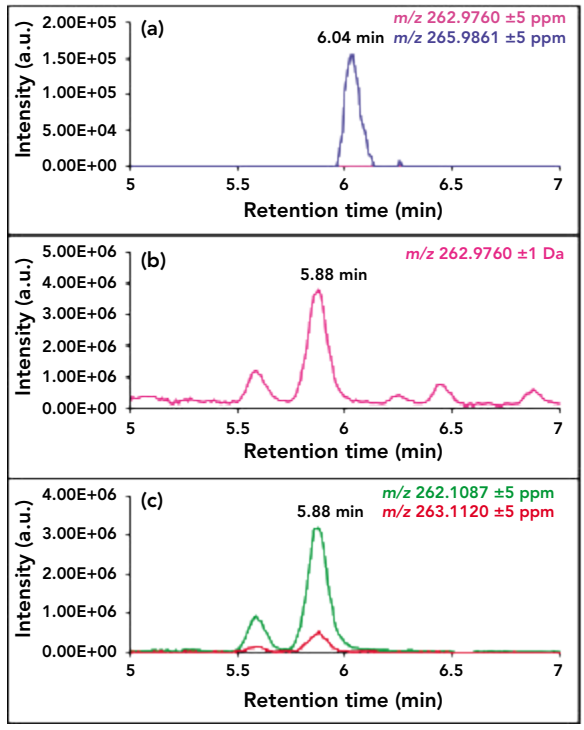

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FIGURE 1: LC–HRMS EICs generated from a chocolate chip cookie sample for (a) PFPeA (pink; *m/z* 262.9760 ± 5 ppm) and the labeled PFPeA surrogate (blue; *m/z* 265.9861 ± 5 ppm); (b) PFPeA *m/z* 262.9760 ± 1 Da; and (c) the suspected interferent at *m/z* 262.1087 ± 5 ppm (green) and its associated ^{13}C isotope (red; *m/z* 263.1120 ± 5 ppm).

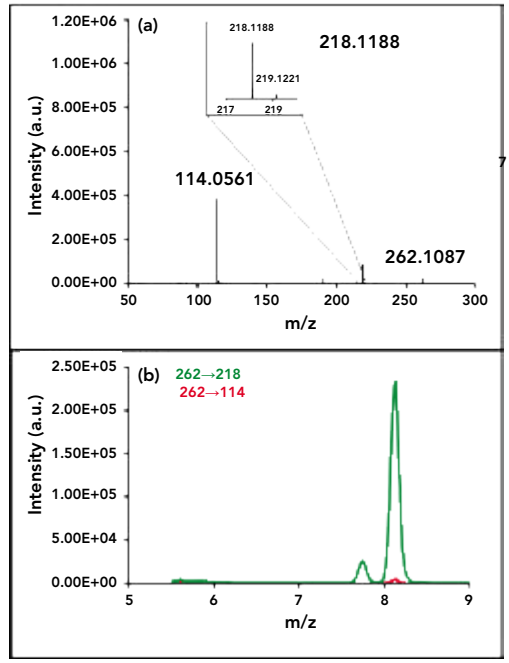

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FIGURE 2: (a) The HRMS-MS/MS spectrum for the suspected interferent at *m/z* 262.1087; and (b) EICs for the *m/z* 262 to *m/z* 218 (green) and *m/z* 262 to *m/z* 114 (red) transitions of the suspected interference on the triple quadrupole instrument.

triple quadrupole method. To illustrate the process for accurately identifying interferents with HRMS, an example is shown from a chocolate chip cookie with a suspected PFBA interferent analyzed with both triple quadrupole (ABSciex 6500 Plus QTRAP hybrid quadrupole/linear ion trap) and HRMS (Thermo Q-Exactive Orbitrap) instrumentation. The full scan mass spectral data is examined first by generating extracted ion chromatograms (EICs) for the accurate masses of the suspected PFAS compounds and any relevant surrogate compounds with a mass range of ±1 Da to mimic the triple quadrupole mass accuracy and ±5 ppm to utilize accurate mass. Figure 1a shows the EICs for PFPeA (pink; *m/z* 262.9760 ± 5 ppm) and the labeled PFPeA surrogate (blue; *m/z* 265.9861 ± 5 ppm); the latter was spiked into the chocolate chip cookie sample. There is no peak for PFPeA at 6.04 min (as expected based on the labeled PFPeA retention time). Figure 1b shows an EIC for a mass range of ±1 Da of PFPeA (*m/z* 261.9760 to *m/z* 263.9760) which can be used to directly compare the HRMS results to the triple quadrupole data. The data show that the peak at 5.88 min is likely a matrix interferent which caused a false positive detection on the triple quadrupole instrument due to the retention time proximity to the standard (shown in Figures 1a and 1b). The accurate mass of the suspected interferent at 5.88 min was determined to be *m/z* 263.1120. Interestingly, this is actually the ^{13}C isotope of *m/z* 262.1087. Figure 1c shows the extracted ion chromatograms (EICs) for the exact masses of the ^{12}C (*m/z* 262.1087 ± 5 ppm) and ^{13}C (*m/z* 263.1120 ± 5 ppm) isotopes, where the elution profiles align well with each other and the EIC of the suspected triple quadrupole interferent in Figure 1b.

Once the mass-to-charge of the suspected interferent is found, MS/MS data is examined to verify that the interferent precursor generates a product ion within ±1 Da of the expected transition mass (for example, *m/z* 262.9760 → *m/z* 218.9862 for PFPeA) in the MS/MS spectrum.

• Continued on Page 16

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Tackling PFAS Complexity with HRMS and Bioanalytical Techniques

Carrie McDonough and Wesley Scott

Per- and polyfluoroalkyl substances (PFASs) are highly persistent anthropogenic compounds that are widespread in the environment. There are thousands of PFASs, yet few neat standards exist for unequivocal identification, quantification, or toxicity assays. The bioanalytical study of complex commercial PFAS mixtures is an innovative route to better understand novel PFAS exposure and toxicity. Here, we highlight efforts using high-resolution mass spectrometry (HRMS) and exposure-relevant mixtures to prioritize PFASs based on their potential to accumulate in living organisms.

Per- and polyfluoroalkyl substances (PFASs) are unlike many legacy hydrophobic persistent organic pollutants (POPs) in that fatty tissues are not their main reservoir in the body. Many PFASs are anions at physiological pH, and they have similar structures to endogenous fatty acids. Fatty acids are transported throughout the body by the transporter protein serum albumin, and so are PFASs. Although the bioaccumulation of legacy POPs can be predicted by hydrophobicity alone, PFAS toxicokinetics in the human body are related to affinity for human serum albumin (HSA) and renal transporters (organic anion transporters, or OATs), as well as affinity for the phospholipids that make up cellular membranes. These complex interactions have necessitated the development of more sophisticated models to predict and understand PFAS toxicokinetics (1), as well as multiple in vitro and in vivo experimental approaches to ground truth them (2).

Analytical Challenges to Comprehending PFAS Body Burden

The observation that "trace amounts of organic fluorocompounds derived from commercial products" were present in blood from the general population was first made in the 1970s using nuclear magnetic resonance (NMR) (3). Since then, our ability to identify and measure PFASs in biological samples has advanced significantly. Presently, liquid chromatography with tandem mass spectrometry (LC–MS/MS) is recognized as the go-to method for sensitive and accurate measurement of targeted PFASs, with detection limits of 0.1–1 ng/mL, depending on the analyte. Using these methods has revealed that PFASs are present in the blood of virtually every human on Earth, as well as most wildlife. Although the levels of PFASs are often low (near detection limits), they have been found to be elevated in countless communities with PFAS-impacted drinking water and in occupationally exposed individuals. The health consequences of these exposures are concerning, including immunosuppression, several types of cancer, increased risk of obesity, and liver disease.

LC–MS/MS has revealed that perfluoroalkyl acids (PFAAs), such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), are widespread in human blood. The coupling of this technique with integrative organofluorine measurement techniques like total organofluorine combustion ion chromatography (TOF-CIC) has also highlighted how little of the total organofluorine body burden is accounted for by these targeted LC–MS/MS methods. A recent study monitoring the blood of the general Swedish population found that an average of 60% and 41% of total organofluorine was unexplained by targeted LC–MS/MS in females and males, respectively (4). The data raised the following question: what compounds make up the rest of this organofluorine? Are they fluorinated pharmaceuticals, novel PFASs with no analytical standards, or ultra-short-chain or neutral volatile PFASs not caught by traditional reverse-phase LC (RPLC)?

There are thousands of PFASs with diverse properties that pose a considerable challenge for analytical chemists attempting to characterize all PFASs contributing the unidentified organofluorine burden in a biological sample. In addition, some PFASs (referred to as "precursors" or "pre-PFAAs") are labile under certain conditions, transforming in the environment or in vivo to form perfluorinated end products that are highly stable. Many PFAS precursors eventually form common perfluoroalkyl acids, where original precursor functionality and structure have been lost. This complicates the understanding of source contribution or environmental forensics because it is impossible to know which precursor was initially present. This also precludes an understanding of the full health impacts because direct exposure to a distinct precursor

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is expected to have different toxicological effects than exposure to the final product PFAA (5).

We know that humans are chronically exposed to a complex mixture of PFASs, including poorly understood pre-PFAAs that are widespread in commercial products. Because of studies combining LC–MS/MS and integrative techniques like TOF-CIC, we also know that we are not capturing total organofluorine with targeted LC–MS/MS techniques. Without confirming molecular identification, it is impossible to fully understand the predominant exposure sources and the associated health risks.

Bioanalytical Strategies for Prioritizing Novel PFASs

The use of complex, exposure-relevant mixtures enables the study of novel PFASs present in these mixtures when there are no neat standards. This allows for the prioritization of novel PFASs based on their bioaccumulation or biotransformation to known toxic PFAAs. Yeung and Mabury demonstrated the utility of such methods in 2013 by conducting detailed analyses of rainbow trout tissues after exposure to two distinct aqueous film-forming foams (AFFFs) (6). AFFFs are used to fight fuel fires, and they were widely used as part of fire training activities by first responders and military personnel. Prolonged use of AFFFs during training or simulation exercises have left large impacted fire training areas where the soils, groundwater (7), and surrounding coastal environments (8) will remain contaminated for decades to centuries to come (9). Yeung and Mabury noted that not only did targeted PFASs measurable by LC–MS/MS accumulate in the AFFF-exposed fish, but the fish also contained significant amounts of unknown organofluorine.

Several recent studies have demonstrated the usefulness of similar techniques incorporating high-resolution mass spectrometry (HRMS) and in vitro or in vivo exposures to complex AFFF mixtures for investigating the bioaccumulation and toxicity of PFAS-containing AFFFs. Yang and associates (10) used size-exclusion column co-elution and HRMS to measure the binding potential of PFASs to human liver fatty acid binding protein (*h*L-FABP) and identify novel PFASs in a commercial AFFF mixture and in AFFF-impacted waters that are *h*L-FABP ligands. They identified novel substituted perfluoroalkyl sulfonates (PFSAs) including unsaturated, ketone-, hydrogen-, chlorine-, and oxygen-substituted PFSAs, as *h*L-FABP ligands. Li and colleagues (11) combined HRMS and equilibrium dialysis to identify AFFF-associated PFASs with high bioaccumulation potential. They tentatively identified several of the novel substituted PFSAs also found by Yang and associates that were likely noncovalently bound to HSA, as well as at least one novel AFFF-associated sulfonamide precursor (N-dimethylammoniopropyl perfluorohexane sulfonamide) that appeared to have some potential for covalently binding to HSA.

Recent in vivo treatments using AFFF have also been used for determining bioaccumulation potential and biotransformation products of novel PFASs. Our work applying HRMS to analyze serum from mice dosed with a field-collected predominantly electrochemically fluorinated AFFF (the same AFFF used by Li and colleagues in HSA binding studies) also identified several novel substituted PFSAs accumulating in blood serum after a brief 6 d depuration, confirming that these compounds highlighted by in vitro studies were indeed accumulating in AFFF-exposed living organisms (12). We also identified a series of novel bis-sulfonamides in post-depuration mouse serum, though these compounds were neither detected in the original AFFF used in our dosing study, nor have they been recognized as a potential component of AFFF in other HRMS studies. Potential explanations for the presence of these bis-sulfonamides in AFFF-dosed mouse serum where these compounds may have been present in the AFFF at a low level and were not detectable until their accumulation in mouse serum enhanced their relative abundance in comparison with other AFFF components. This highlights their bioaccumulation potential, or they formed as metabolic products of other sulfonamide-based AFFF components. The discovery of these compounds in an in vivo AFFF-dosed model highlights the usefulness of such methods for prioritizing identification of novel biologically relevant PFASs that may be hidden in complex mixtures prior to exposure.

Our later work using HRMS to characterize PFAS mixtures in human blood serum from an AFFF-impacted community in El Paso County, Colorado, further highlights the utility of these laboratory-based complex mixture studies to inform and direct human biomonitoring. Among the approximately 200 residents whose blood was analyzed in this study, unsaturated PFOS (UPFOS), a compound tentatively identified with high bioaccumulation potential in our mouse experiments, was detected in 85% of residents, and it was also found in raw drinking water (13). The spatial trends in semi-quantitative abundance suggested this compound originated from the same AFFF source as other PFASs in El Paso County drinking water, and the widespread presence of UPFOS in human serum echoed our findings of high bioaccumulation potential in AFFF-exposed mice.

Similar methods have recently been applied to prioritize novel compounds in complex PFAS-containing environmental samples. Bangma and associates (14) treated mice with industrially impacted surface water from Bladen County, North Carolina, a site previously impacted by Chemours' Fayetteville Works where elevated detections of several novel ether PFASs were found. Their results corroborated recently characterized human serum profiles from the same area (15), highlighting PFO5DA, Nafion Byproduct 2, and HydroEVE as compound needing prioritization for toxicological testing.

Future Outlook for PFAS Analysis in Biological Matrices

Despite the application of many advanced analytical techniques and the work of numerous investigators worldwide, the significant portion of

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unidentified organofluorine remaining in the biosphere presents a significant challenge in the analytical community. Although techniques combining HRMS with in vitro or in vivo prioritization of complex, exposure-relevant dosing mixtures hold promise, there are still many uncertainties inherent in these approaches. For example, these studies may be influenced by saturation of binding sites, competitive binding amongst individual PFASs, or both. Such saturation and competition are likely not representative of real exposure scenarios occurring at lower concentrations, but elevated dosages are needed to facilitate identification of novel compounds. Additionally, measuring PFASs requires an expensive, time-intensive methodology such as LC–HRMS, and the uncovering of novel PFASs will likely require additional method development and even more advanced analytical instrumentation to capture sub-classes like the ultra-short-chain and neutral volatile PFASs. Despite these many challenges, continual progress is being made to understand total PFAS body burden, including advances in sample preparation, instrumentation, and in silico techniques to broaden the fraction of total PFASs that are encompassed. Additionally, efforts to harmonize naming conventions, provide machine-readable structures for novel PFASs (16), and communicate confidence of novel PFAS identifications in a consistent way (17), as well as the growing number of PFASs identified and entered into spectral databases, are accelerating discovery and understanding of the biological relevance of novel PFASs.

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• Continued from Page 10

For example, Figure 2a shows the MS/MS spectrum for the suspected PFPeA interferent at *m/z* 262.1087 in the cookie. The most abundant fragment in the MS/MS spectrum is *m/z* 218.1188, which has a ^{13}C isotope at *m/z* 219.1221. This fragment is within the ±1 Da window of the PFPeA fragment mass at *m/z* 218.9862, further indicating this compound is likely the false positive being detected on the triple quadrupole instrument.

At this point, attempts can be made to identify the interferent. If the structure of the interferent is known, additional sample preparation steps can be developed to remove it. As mentioned previously, taurodeoxycholic acid has been identified as a common component in biological and food matrices which interferes with the main transition used to quantify PFOS (*m/z* 499 → *m/z* 80). This compound can be removed from samples with an additional cleanup step utilizing graphitized black carbon during sample preparation (6). However, identifying interferents and developing additional sample preparation procedures for each can require significant time and resources, which may not be feasible in every case. Because interferences will continue to arise as more PFAS compounds are monitored and additional foods are tested, there is a need for a rapid approach to limit these interferences.

One way to address this challenge is by leveraging additional product ions in the MS/MS spectrum of the interferent generated on the HRMS instrument. In the chocolate chip cookie example, the suspected interferent generated a product ion of *m/z* 114.0561 as shown in Figure 2a. Because this product ion is not present for PFPeA, the *m/z* 262 to *m/z* 114 transition can be added to the triple quadrupole method to confirm the presence of this interference (Figure 2b). This interferent was also confirmed by Bangma and co-authors in their investigation of PFPeA in cocoa mix (4). As more interferents are identified and documented, these additional transitions can be added to triple quadrupole methods to rapidly distinguish them from PFAS compounds in the same analysis, without the need for additional sample preparation development/procedures. This is also extremely valuable for labs that may not have access to HRMS instrumentation.

Conclusion

The approach described here is broadly applicable to diverse PFAS compounds and matrices, making it amenable to the rapid pace at which new PFAS interferences are being found. In addition to the PFPeA in the chocolate chip cookie example, this approach has been successfully applied to distinguish interferences from PFBA in deer meat samples and from 4:2 fluorotelomer sulfonic acid (4:2 FTS) in corn snaplage using a different HRMS instrument (ABSciex 7600 ZenoTOF). Given the challenges associated with interferences in complex matrices, the use of HRMS is a powerful tool for PFAS confirmation and for investigation of interferences.

HRMS is capable of distinguishing matrix interferents from targeted PFAS compounds in complex matrices based on mass differences within ± 0.0001-0.0010 Da (for example, ±5 to 10 ppm), reducing the risk of false positives and over-estimation. In addition, the full MS/MS spectrum of interferents can be leveraged to establish alternate transitions to distinguish PFAS and interferent compounds in the same analytical data acquisition on triple quadrupole mass spectrometers, without requiring interferent identification and additional sample preparation procedures. This approach ensures that PFAS amounts reported in foods are accurate when used for the calculation of dietary exposure estimates.

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A Multiphase Sampling and Analytical Approach for Investigating Airborne PFAS Transmission

Steve Suh, David Kennedy, Reika Takahara, Manabu Takayanagi, Hiroshi Hayashida, and Zhonghua Shen

The authors evaluated a sampling and analytical system to measure differing modes of atmospheric per- and polyfluorinated substances (PFAS) transmission. The sampling was conducted with the FM4 sampling module, which features particulate collection followed by polyurethane foam sorption followed by activated carbon disc adsorption, a configuration designed to capture PFAS-laden particulate matter (one-micron to ten-microns), as well as aerosol, volatile, and ionic PFAS species in a single sampling event. Individual sampling media fractions were subsequently analyzed by gas chromatography coupled with tandem quadrupole mass spectrometry (GC–MS/MS) and liquid chromatography coupled with tandem quadrupole mass spectrometry (LC–MS/MS) for 33 ionic and 20 neutral PFAS species. A multi-day field sampling event conducted in an outdoor urban environment demonstrated that the system was able to capture and differentiate a number of airborne PFAS species. The study showed that simultaneous, multi-phase sample collection coupled with GC–MS/MS and LC–MS/MS analysis can be a useful approach to further elucidate the mode and manner of atmospheric PFAS transmission.

Investigations of new environmental per- and polyfluorinated substances (PFAS), contaminant sources, and dispersion modes continue to expand. Early studies targeted a small number of PFAS analytes (primarily long chain carboxylic and sulfonic acids) and largely focused on water-borne dispersion arising from PFAS manufacturing and industrial applications such as fire suppression foams (1). However, improved analytical methods based on tandem mass spectrometry have greatly increased the understanding of environmental PFAS contamination. More recently, focus has shifted to environmental PFAS compounds that display a much wider range of functionalities with more diverse chemical properties (2). This has expanded the view of how PFAS are dispersed in the environment.

Some non-ionic PFAS (particularly fluorotelomers) possess significant volatility and still others (both ionic and non-ionic) can be adsorbed upon airborne particulates (3). Highly polar ionic PFAS can become concentrated (up to 62,000-fold (4)) at the air-water interface of small bubbles, and then be dispersed as aerosols upon bursting. This phenomenon underlies the assertion that sea spray aerosol transport is responsible for the wide distribution of PFAS in remote arctic regions (5). Airborne transport also introduces the potential for dispersion of PFAS from aerated waste management facilities (6) and from indoor use of PFAS-containing stain repellents and anti-fogging agents (7).

These multiple modes of dispersion and potential sources have increased interest in airborne PFAS transmission but have also made it more difficult to study the phenomenon. PFAS analytical chemistry has made great strides, but fewer advances are seen in PFAS air sample collection. Notably, in 2021, the U.S. Environmental Protection Agency (EPA) introduced Other Test Method 45 (OTM-45), which features a multimedia air sampling configuration for measuring selected PFAS from stationary sources (8). However, the sampling apparatus is quite complex and difficult to run and is not amenable to high throughput PFAS analysis. A further advance has been reported by Wu and co-authors (9), which features a novel, integrated sampler design which can simultaneously collect both particulate and vaporous contaminants from ambient air, and has improved applicability for PFAS sampling from stationary sources.

In this paper, we present field testing results from the deployment of a commercially available sampling device of the same design. The FM4 sampler simultaneously collects different airborne PFAS fractions (particulate, vapor, and ionic) on separate sorption media (quartz fiber filter, polyurethane foam and activated carbon fiber disc) for subsequent analysis. The analytical techniques employed (10) feature gas chromatography coupled with tandem quadrupole mass spectrometry (GC–MS/MS) for neutral PFAS analytes and liquid chromatography coupled with tandem quadrupole mass spectrometry (LC–MS/MS) for ionic PFAS.

The data presented below illustrate how the analysis of PFAS components captured on the various sampling media can differentiate chemically diverse PFAS species and suggest potential atmospheric transport modes. Results are presented from multiday field sampling events, which show distinctly different atmospheric PFAS compositions and sug-

gest further opportunities for the application of this approach.

Experimental: Consumables

Standards and Reagents

Target PFAS analytes and internal standards were obtained from Wellington Laboratories, Cambridge Isotope Laboratories, Biosynth Carbosynth, and Sigma-Aldrich, and are presented in Table I along with chromatographic retention times and MS/MS transition parameters. LC–MS grade methanol and acetonitrile were obtained from Kanto Chemical Co., ethyl acetate and ammonium acetate were obtained from Fujifilm Wako Pure Chemical Corporation, and dichloromethane was obtained from Kishida Chemical Co., Ltd.

FM4 Sorption Media

Quartz fiber filter (QFF) media (47 mm) of three classes (>10 µm, 10–2.5 µm and 2.5–1.0 µm), Polyurethane foam (PUF) sorption media (47 mm x 50 mm) and activated carbon fiber discs (GAIAC-47 mm) were obtained from GL Sciences, Inc.

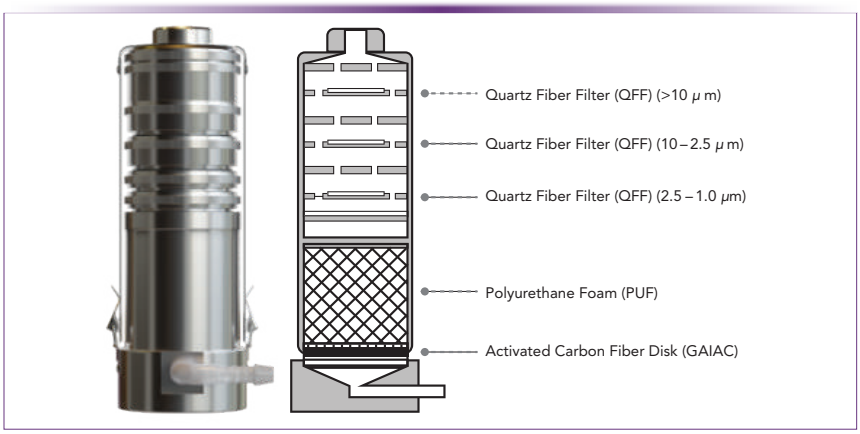

FIGURE 1: Schematic diagram of the FM4 sampler.

Chromatographic Columns

LC column: 1.9 µm, 2.1 mm I.D. x 100 mm InertSustain AQ C18 and GC column: 0.25 mm i.d. x 30 m, df = 0.25 µm InertCap Pure-WAX were both obtained from GL Sciences, Inc.

Experimental - Instrumentation

Air Sampling Device

The FM4 air sampling devices (10) were acquired from GL Sciences, Inc. and were operated at a flow rate of 20 L/min. A schematic diagram is presented in Figure 1.

LC–MS/MS Instrumentation

The instrumentation parameters for the LC–MS/MS target analytes were

TABLE I: Analytes and surrogates; retention times and MS/MS parameters
(a): LC–MS/MS analytes and surrogates

No.	Analyte	RT (min)	Transition 1			Transition 2		
			Q1	Q3	CE	Q1	Q3	CE
1	PFPrA	2.46	163	119	10	-	-	-
2	$^{13}C_4$-PFBA	4.61	217	172	6	-	-	-
3	PFBA	4.61	213	219	6	-	-	-
4	$^{13}C_5$-PFPeA	5.41	268	223	4	-	-	-
5	PFPeA	5.41	263	219	6	-	-	-
6	PFPrS	5.50	249	99	30	249	80	45
7	$^{13}C_3$-PFHxA	6.04	318	273	4	-	-	-
8	PFHxA	6.04	313	269	6	313	119	22
9	$^{13}C_3$-PFBS	6.27	302	80	40	-	-	-
10	PFBS	6.27	299	80	40	299	99	34
11	$^{13}C_3$-HFPO-DA ($^{13}C_3$-GenX)	6.29	287	169	4	-	-	-
12	HFPO-DA (GenX)	6.29	285	169	4	285	185	16
13	13C4-PFHpA	6.60	367	322	8	-	-	-
14	PFHpA	6.61	363	319	6	363	169	18
15	ADONA (DONA)	6.81	377	251	8	377	85	40
16	6:2 FTSA	6.85	427	407	23	427	81	44
17	$^{13}C_2$-6:2 FTSA	6.86	429	409	24	-	-	-
18	$^{13}C_8$-PFOA	7.12	421	376	8	-	-	-
19	PFOA	7.12	413	369	10	413	169	15
20	$^{13}C_2$-8:2 FTUCA	7.29	459	394	16	-	-	-
21	8:2 FTUCA	7.29	457	393	12	457	343	44
22	PFHxS	7.40	399	80	53	399	99	45
23	$^{13}C_3$-PFHxS	7.43	402	80	65	-	-	-
24	$^{13}C_9$-PFNA	7.61	472	427	8	-	-	-
25	PFNA	7.61	463	419	10	463	219	18
26	$^{13}C_2$-8:2 FTSA	7.79	529	509	27	-	-	-
27	8:2 FTSA	7.80	527	507	28	527	81	55
28	PFHpS	7.94	449	80	55	449	99	51
29	d_3-N-Me-FOSAA	7.99	573	419	20	-	-	-
30	N-MeFOSAA	8.00	570	419	20	570	483	16
31	$^{13}C_6$-PFDA	8.07	519	474	8	-	-	-
32	PFDA	8.08	513	469	6	513	269	18
33	d_5-N-EtFOSAA	8.21	589	419	20	-	-	-
34	10:2 FTUCA	8.21	557	493	16	557	243	44
35	N-EtFOSAA	8.23	584	419	20	584	483	16
36	$^{13}C_8$PFOS	8.41	507	80	58	-	-	-
37	PFOS	8.41	499	80	60	499	99	55
38	$^{13}C_7$-PFUnDA ($^{13}C_7$-PFUnA)	8.51	570	525	8	-	-	-
39	PFUnDA (PFUnA)	8.51	563	519	7	563	269	16
40	^{9}Cl-PF_3ONS	8.78	531	351	28	531	83	32

Continued on Page 21

developed with an Agilent 1260 Infinity Prime LC coupled with an Agilent Ultivo Triple Quadrupole LC–MS/MS, and are presented in Table II. The analytical methodology followed ISO 21675:2019.

GC–MS/MS Instrumentation

The instrumentation parameters for the GC–MS/MS target analytes were developed with an Agilent 8890/7010B Triple Quadrupole GC–MS/MS, and are presented in Table IIb. The analytical methodology followed the procedures described more fully in reference (10).

Preparation and Extraction of Sampling Media

The sampler and media were pre-cleaned to remove PFAS artifacts. QFF media were baked for 3 h at 350 °C to remove volatile contaminants. The PUF filters and GAIAC discs were sequentially cleaned with water, methanol, ethyl acetate and dichloromethane, and dried in a vacuum oven. Metal components of the FM4 sampler were cleaned by sonication in 50% water:ethanol.

For LC–MS/MS analysis, QFF media were extracted and vortexed three times with 4 mL of methanol and the combined 12 mL of extract was then reduced in volume to 1 mL by nitrogen gas evaporation at 40 °C. PUF media and GAIAC disks were each extracted three times with 10 mL of 1:1 dichloromethane:ethyl acetate and the combined 30 mL of extract was then reduced in volume to 1 mL by nitrogen gas evaporation at 35 °C and directly analyzed by GC–MS/MS. The remaining extract was then evaporated to near dryness with nitrogen gas at 40 °C and taken up in 1 mL of methanol for LC–MS/MS analysis.

Results and Discussion

Method Chromatographic Performance

Typical MRM chromatograms are displayed in Figure 2. Chromatograms for the 33 LC–MS/MS target analytes (Figure 2a) are displayed separately from the 24 stable isotope surrogates (Figure 2b) to better show the distribution of the surrogates amongst the analytes. The number aside each peak

corresponds to the analyte numbering system in Tables Ia and Ib. Chromatograms for the 20 GC–MS/MS target analytes and 8 surrogates are displayed together in Figure 2c. The numbers aside each peak correspond to the analyte numbering system in Table Ib. Note that the neutral PFAS analytes N-MeFOSA and N-EtFOSA are analyzed by both GC–MS/MS and LC–MS/MS, but FOSA (PFOSA) is analyzed by LC–MS/MS.

TABLE I (CONTINUED): Analytes and surrogates; retention times and MS/MS parameters (a): LC–MS/MS analytes and surrogates

No.	Analyte	RT (min)	Transition 1			Transition 2		
			Q1	Q3	CE	Q1	Q3	CE
41	$^{13}C_2$-PFDoDA ($^{13}C_2$-PFDoA)	8.94	615	570	8	-	-	-
42	PFDoDA (PFDoA)	8.95	613	569	9	613	319	22
43	PFDS	9.26	599	80	65	599	99	60
44	PFTrDA (PFTrA)	9.36	663	619	9	663	169	29
45	FOSA (PFOSA)	9.61	498	78	75	498	169	30
46	$^{13}C_8$-FOSA ($^{13}C_8$-PFOSA)	9.74	506	78	49	-	-	-
47	$^{13}C_2$-PFTeDA ($^{13}C_2$-PFTeA)	9.76	715	670	7	-	-	-
48	PFTeDA (PFTeA)	9.76	713	669	10	713	169	33
49	$^{13}C_4$-8:2 diPAP	10.42	993	545	19	-	-	-
50	8:2 diPAP	10.43	989	97	45	989	543	28
51	$^{13}C_2$-PFHxDA	10.46	815	770	12	-	-	-
52	PFHxDA	10.46	813	769	12	813	219	32
53	PFOcDA (PFODA)	11.02	913	869	11	913	169	39
54	d_3-N-MeFOSA	11.03	515	169	30	-	-	-
55	N-MeFOSA	11.04	512	169	27	512	219	23
56	d_5-N-EtFOSA	11.36	531	169	30	-	-	-
57	N-EtFOSA	11.37	526	219	23	526	169	27

Method Quantification

Target PFAS analytes were quantified by external calibration. Calibration curves for LC–MS/MS analytes were generated at 2, 19, 50, 200, 1000, and 5000 pg/L and for GC–MS/MS at 25, 50, 100, 250, 500, 1000, 2000, 5000, 20,000, and 50,000 pg/L. All linear regression points differed by less than 20% from known value. The limit of quantification was calculated as the smallest concentration of the standard on the calibration curve that could be accurately measured within ±20% of its theoretical value at a signal-to-noise ratio of 10 or greater. To verify the stability of the instrument, a mixed standard solution adjusted to 1000 pg/mL for LC–MS/MS and 5 ng/mL for GC–MS/MS was measured for each analysis batch. If the concentration of the mixed standard solution was not within ±20% of the corresponding theoretical value, a new calibration curve was prepared. The material blank (procedural blank) and material recovery (procedural recovery) were analyzed for every batch of samples. The target compounds can also be quantified by the internal standard method, and loss in the sampling and extraction processes can thereby be corrected to a limited extent. However, in the field sampling data presented below the results were not so corrected, owing to the limited number of commercially available isotopically labeled internal standards.

Method quantification limits (MQLs) were developed for all 49 target analytes. The MQLs in the blank test of each material and the measurement of real samples are presented in Table III. The MQLs from the blank tests are expressed as pg (total quantity). The MQLs from the field measurements are expressed as pg/m^3 (volumetric mass), based upon a 72-h sampling event with the sampler operating at a flow rate of 20 L/min. Volumetric mass MQLs for the 48-h and 24-h sampling events are higher by factors of 2 and 3 respectively, owing to the reduced volume of air processed. The LC–MS/MS field sampling MQLs were generated on an Agilent 1269 Infinity II LC interfaced with a Sciex Triple Quad 4500 MS/MS system and, for GC–MS/MS, on a Shimadzu GCMS-TQ8050 system. Both LC–MS/MS and GC–MS/MS MQLs followed the analytical methodology and instrumentation parameters previously described in the Experimental section.

Sampling Method Performance Characterization

Material recovery tests were performed by directly spiking native and surrogate standards onto each sorption matrix to establish the efficiency of the analytical extraction process. Native analyte recoveries for most analytes and media were between 70% and 120%. Exceptions were the FTUCAs and FDIAs where recoveries ranged from 33% to 75% (average of 50%) with the longer chain analytes showing higher recoveries.

Media blank tests (n = 5) performed on pre-treated media showed no mean extractable quantities greater than 20 pg for most analytes with the exception of PFHxA in PUF and GAIAC (60 pg and 82 pg, respectively) and BPFB in GAIAC (51 pg).

Travel blank tests (n = 2) were performed with fully prepared FM4 samplers transported to the sampling site and returned to the laboratory without air collection. All field travel blanks were generally clean and showed analyte levels consistent with the media blanks. The only exception was 6:2 FTOH which was below the MQL in the media blank, but appeared in the travel blanks at a mean level of 73 pg in the GAIAC media, suggesting an external contamination source.

Finally, recovery tests were performed to evaluate the loss of target analytes during the field sampling process. A surrogate standard mixture

TABLE I (CONTINUED): Analytes and surrogates; retention times and MS/MS parameters (b): GC–MS/MS analytes and surrogates

No.	Analyte	RT (min)	Transition 1			Transition 2		
			Q1	Q3	CE	Q1	Q3	CE
1	BTFBB	5.00	292	213	26	294	213	18
2	6:2 FTI	5.13	474	263	28	327	181	16
3	BPFB	5.60	248	167	24	248	117	22
4	8:2 FTI	6.53	574	427	8	547	313	20
5	d_4-4:2 FTOH	6.83	199	130	6	248	130	8
6	4:2 FTOH	6.90	196	127	10	196	77	26
7	PFDoI	7.25	219	69	28	169	69	16
8	$^{13}C_2$-d_2-6:2 FTOH	7.82	298	129	6	248	130	8
9	6:2 FTOH	7.86	296	77	26	344	95	24
10	4:3 FTOH	7.99	195	175	8	195	95	24
11	10:2 FTI	8.13	527	481	8	527	145	10
12	6:3 FTOH	8.90	295	275	8	295	181	24
13	$^{13}C_2$-d_2-8:2 FTOH	8.99	409	69	60	448	129	4
14	8:2 FTOH	9.02	396	127	12	131	69	22
15	PFBuDiI	9.05	327	181	8	327	69	60
16	PFHxDiI	9.75	177	127	28	281	181	22
17	8:3 FTOH	9.98	395	95	12	131	69	20
18	$^{13}C_2$-d_2-10:2 FTOH	10.25	515	96	19	495	69	60
19	10:2 FTOH	10.28	505	669	60	131	69	60
20	PFODiI	10.38	527	127	14	381	69	60
21	d_5-N-EtFOSA	14.57	450	430	12	450	380	18
22	N-EtFOSA	14.61	448	69	60	131	69	28
23	d_3-N-MeFOSA	15.16	433	114	28	433	413	16
24	N-MeFOSA	15.19	448	428	12	131	69	28
25	d_7-N-MeFOSE	16.57	465	415	14	530	465	16
26	N-MeFOSE	16.59	526	462	18	462	93	28
27	d_9-N-EtFOSE	16.66	449	428	14	449	378	20
28	N-EtFOSE	16.69	540	69	54	540	448	20

was spiked directly onto the sampling media and ambient air was sampled for 24 h, 48 h, and 72 h (*n*= 6 for each). Recoveries were similar to the material recovery tests (70% to 120%), showing that PFAS retained by the media are not lost during the sampling process. The exceptions were the short chain PFCAs and FOSAs where recoveries ranged from 31% to 68% (average of 52%). These results are approximately 30% to 50% lower than in the material recovery tests, showing that such analytes could be partially lost during sampling and suggesting that field sampling results could be biased low. The longer chain PFDAs, however, did not exhibit reduced recovery. Although these recovery results were adequate for the scoping objectives of this study, future research studies using PFAS-fortified aerosol might be better served by quantifying specific analyte recoveries.

Field Sampling Results

Several field sampling events were conducted to evaluate the ability of the sampling device and the associated analytical protocol to characterize atmospheric PFAS composition. The sampling site was located in a mixed rural and residential area of Tsukuba city, Ibaraki Prefecture, Japan, approximately 35 miles northeast of Tokyo. There is no heavy industry in the immediate area and there are no known PFAS-contaminated sites nearby.

Sampling was conducted in a covered, open-air structure. Three FM4 samplers (*n* = 3) were operated simultaneously at a flow rate of 20 liters per min for a period of 24 h. The samplers were then shipped to the laboratory in Tsukuba city, where the sorption media were removed and analyzed per the prescribed protocol. Twenty-five days later, the same three samplers (cleaned and replenished) were returned to the Tsukuba sampling site and a duplicate 24-hour sampling and analytical event was conducted. Comparisons of the two 24-h sampling events are presented in Figure 3a and Figure 3b. The duplicate sampling and analytical procedures were then repeated as a 48-h event and finally as a 72-h event. However, only the results for the 24-h sampling events are presented here, as these data adequately illustrate the utility of procedure.

Note that the southwest wind direction on Day 1 (Figure 3a) placed the sampling site downwind from the highly populated and industrialized Tokyo-Yokohama area, whereas on Day 2 (Figure 3b), with a northwest wind, the site was downwind from the less urbanized Tochigi prefecture. A comparison of the colored composition wheels from the two sampling events, shows that the general atmospheric PFAS composition and specific PFAS concentrations from the two events were markedly different.

Examining the individual analyte concentrations, the atmospheric composition on Day 1 appears equally weighted toward fluorotelomer alcohols (FTOHs) and perfluorocarboxylic acids (PFCAs), whereas PFCAs predominate on Day 2. After removing the Day 1 contribution from 6:2 FTOH (a previously recognized travel blank contributor), the Day 1 data still show a

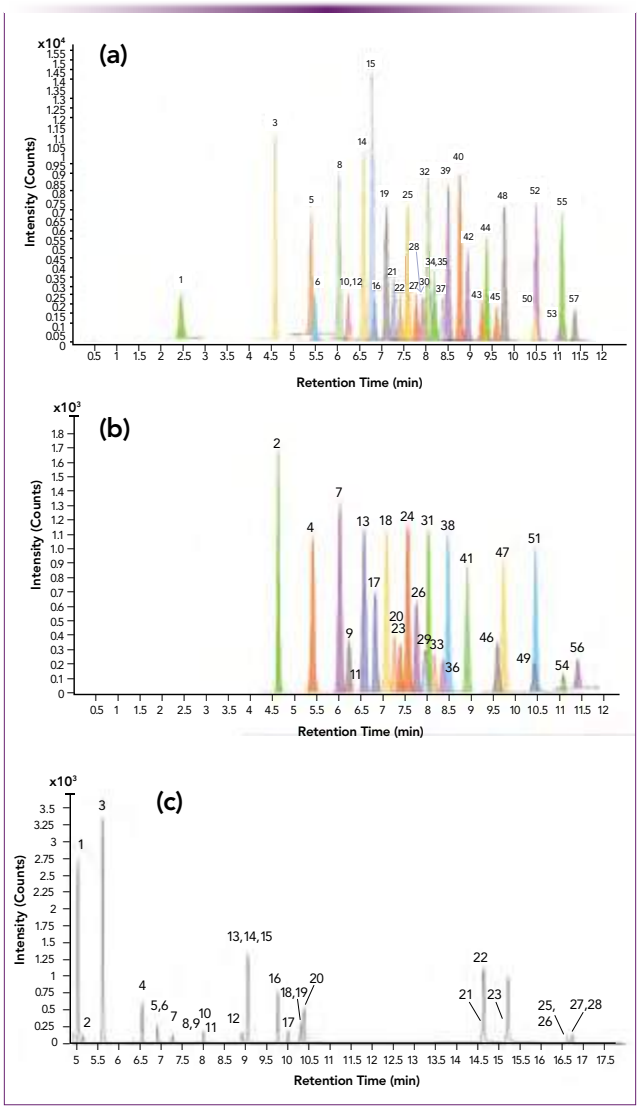

FIGURE 2: LC–MS/MS and GC–MS/MS chromatograms. (a) LC–MS/MS target analyte chromatograms, (b) LC–MS/MS surrogate chromatograms, and (c) GC–MS/MS analyte and surrogate chromatograms.

much higher contribution from FTOHs, owing to the presence of 8:2 FTOH.

Also noticeable are the distinctly different PFCA patterns in the two sampling events. Day 2 shows a generally descending pattern of PFCA concentrations from short chain (PFPrA) to long chain (PFUnDA) in all media, whereas the Day 1 PFCA contribution predominantly comes from PFPrA in PUF and ACFF, but not in QFF. Additionally, the Day 2 descending PFCA pattern is quite pronounced on the QFF media wherein ionic PFAS are presumably sorbed on 1–10-micron particles. Surprisingly, no PFPrA was seen in the QFF media on Day 1, whereas it predominates the QFF results on Day 2. However, the descending pattern continues on the PUF and ACFF media suggesting that the bulk of the nonvolatile ionic PFAS are present in aerosol particles (less than 1 micron) which are partially entrained in the PUF media, but more fully captured by the ACFF.

Other compositional differences are noticed, such as the 3X higher level of PFBS and the 5X higher level of PFBA on Day 2. Also of interest is the presence of 8:2 FTOH on Day 1, which was undetected on Day 2, as well as the presence of 6:2 FTI, owing to the unusual chemical nature of this fluorotelomer analyte. One could continue to tease out differences from the two 24-h sampling events, but the primary conclusion is that two distinctively different atmospheric PFAS compositions were sampled.

The same can be said of the data from the 24-h and 72-h sampling events which are not presented here but which show equally intriguing differences. For example, the 72-h Day 2 event displays the same distinctive descending PFCA pattern seen in the 24-h Day 2 event. However, this pattern is completely absent in the other 72-h event and both 48-h events. In addition, compared with the 24-h event, PFCA concentrations in the 72-h events are 2–3 times higher in the PUF and ACFF fractions, suggesting that the particulate:aerosol ratios were significantly different for the two events.

Moreover, the 72-h Day 1 concentration of 8.2 FTOH (80 pg/m^3) is quite similar to the 24-h Day 1 event but is 3–4X higher than the 72-h Day 2 event and for both 48-h events. Notably, HFPO-DA appears in both the 48-h Day 1 and Day 2 events at concentrations of 25 pg/m^3 and 70 pg/m^3, respectively, but was not observed at all in the 24-h and 72-h events. Finally, a visual com-

TABLE II: Instrumentation parameters (a): LC–MS/MS parameters

System	Agilent 1260 Infinity II Prime LC coupled with Agilent Ultivo Triple Quadrupole MS/MS						
LC Column	InertSustain AQ-C18: 1.9 um, 2.1mm I.D. x 100 mm (GL Sciences, Inc.)						
Delay Column	Delay Column for PFAS: 3.0 mm I.D. x 30 mm (GL Sciences, Inc.)						
Mobile Phase (A)	10 mmol/L aqueous ammonium acetate						
Mobile phase (B)	Acetonitrile						
Column Temp.	40 °C						
Gradient	Time (min)	0	1.5	10.0	11.0	11.1	15.0
	A%	90	70	0	0	90	90
	B%	10	30	100	100	10	10
Flow Rate	0.3 mL/min						
Injection Volume	2uL						
Ionization Mode	AJS (Negative)						
Monitoring Mode	MRM						

TABLE II: Instrumentation parameters (b): GC–MS/MS parameters

System	Agilent 8890/7010B Triple Quadrupole GC/MS			
GC Column	InertCap Pure-WAX (GL Sciences, Inc.), 0.25 mm I.D. x 30 m, df = 0.25 um			
Injection	Splitless			
Injection Volume	2 uL			
Injection Temp.	200 °C			
Carrier Gas/Flow	Helium / 1.2 mL/min			
Column Gradient	Rate (°C/min)	0	10	20
	Temp. (°C)	40	200	250
	Hold (min)	2	0	20
Ion Source Temp.	320 °C			
Ionization Mode	EI			
Monitoring Mode	MRM			

parison of all six color composition wheels shows that the 48-h Day 2 event sampled a distinctly different atmospheric PFAS composition compared to the other five events.

Changes in meteorological conditions (such as wind direction) and the potential contribution of transitory sources certainly played a role in the varying atmospheric PFAS analyte contributions and their measured concentrations. However, this study was not designed to definitively identify the causes of these variations; that should be the subject of further investigation. Rather, the primary purpose of this study was to demonstrate the variable complexity of atmospheric PFAS contamination and to highlight the utility of the multistage sampling and analytical approach for investigating potential sources, impacts and potential remediations.

Conclusions

This paper described the characterization and field testing of a multistage field sampling device combined with LC–MS/MS and GC–MS/MS analysis for the determination of 54 PFAS analytes in air at the low

TABLE III: Field sampling method quantification limits

Analyte	MQL		Analyte	MQL		Analyte	MQL	
	(pg)	(pg/m^3)		(pg)	(pg/m^3)		(pg)	(pg/m^3)
PFEtS	2	0.02	PFTeDA	2	0.02	4:2 FTOH	250	2.89
PFPrS	10	0.07	PFHxDA	2	0.02	4:3 FTOH	5,000	57.87
PFBS	2	0.02	PFOcDA	2	0.02	6:2 FTOH	250	5.79
PFHxS	2	0.02	6:2 FTSA	2	0.07	6:3 FTOH	2,000	11.57
PFOS	2	0.02	8:2 FTSA	2	0.07	8:2 FTOH	250	11.57
PFDS	10	0.07	8:2 FTUCA	10	0.07	8:3 FTOH	500	11.57
PFPrA	200	1.45	10:2 FTUCA	2	0.02	10:2 FTOH	1,000	5.79
PFBA	50	0.35	8:2diPAP	2	0.07	6:2 FTI	25	0.29
PFPeA	10	0.07	HFPO-DA	10	0.07	8:2 FTI	50	0.29
PFHxA	2	0.02	N-MeFOSAA	2	0.02	10:2 FTI	2,000	23.15
PFHpA	2	0.02	N-EtFOSAA	2	0.07	PFDoI	1,000	11.57
PFOA	2	0.02	FOSA	2	0.02	PFBuDiI	100	0.58
PFNA	2	0.02	N-MeFOSA	2,000	23.15	PFHxDiI	250	2.89
PFDA	10	0.07	N-EtFOSA	500	2.89	PFODiI	1,000	11.57
PFUnDA	10	0.07	N-MeFOSE	250	2.89	BTFBB	25	0.29
PFDoDA	2	0.02	N-EtFOSE	100	0.58	BPFB	50	0.29
PFTrDA	2	0.07						

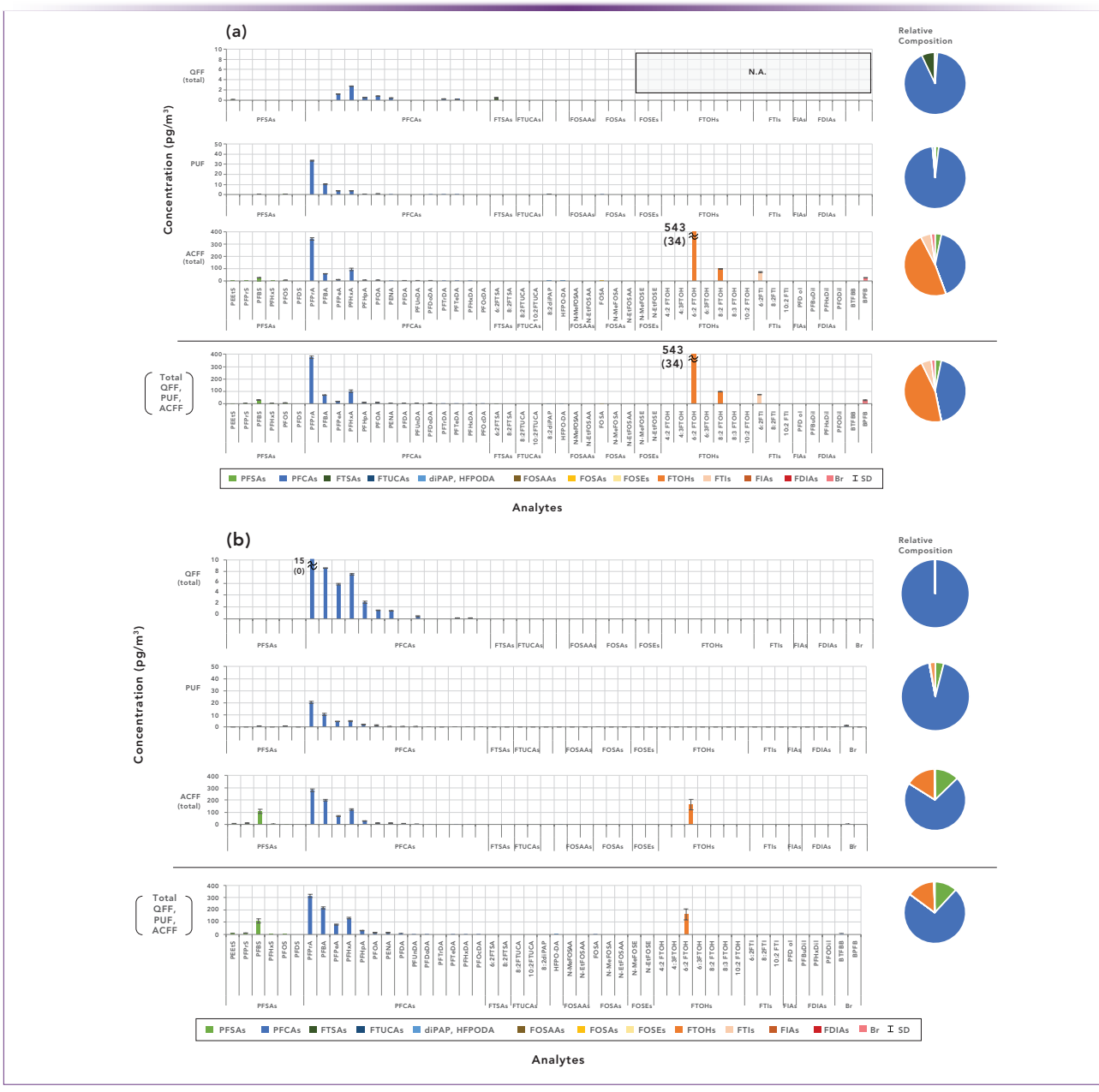

FIGURE 3: Comparison of the two 24-h sampling events. (a) PFAS analyses from the first sampling event (n = 3) with weather: southwest wind; mostly clear/sometimes foggy; 10 to 24 ° C (16 °C average); (b) PFAS analyses from the second sampling event (n = 3) with weather: northwest wind; clear; -3 to 11 °C (3 °C average).

pc/m^3 level. Field-testing experiments demonstrated the ability of the approach to quantitatively characterize local atmospheric PFAS composition and to differentiate the PFAS compositions from different sampling events. This sampling and analytical approach should have application for the investigation of airborne PFAS sources and transmission modes and for the evaluation of potential mitigation and remediation approaches.

Acknowledgments

The FM4 sampling module was developed by Futamura Chemical Co., Ltd. under a joint research agreement with National Institute of Advanced Science and Technology (AIST). Part of this research was performed by the Environment Research and Technology Development Fund (1G-2102) of the Environmental Restoration and Conservation Agency of Japan.

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New Technologies That Accelerate Persistent Organic Pollutant (POP) Sample Preparation

Chris Shevlin and Rahmat Ullah

Despite best efforts in 2004 to ban their use, persistent organic pollutants (POPs) remain prevalent across the globe, including in soil. To protect human health, agricultural and environmental soil require careful investigation, but preparing samples for gas chromatography-mass spectrometry (GC–MS) analysis is time-consuming, and mostly done manually. Accelerated solvent extraction (ASE) has been the preferred preparation method for the past few decades, and while we have seen advancements, the method remains manual. Now, new technology offers parallel sample processing, combined extraction and evaporation, and automation—leading to faster analysis, reduced risk of error, and freed-up time for personnel.

Persistent organic pollutants (POPs) are still a cause of safety concern. After significant efforts from the Stockholm Convention on POPs to reduce and eliminate these compounds in 2004 (1), many are now banned. However, their persistence means they can remain in soil for a long time. From soil, POPs can make their way into the food chain, causing adverse effects. Accurate POP quantitation is therefore essential to protect human health.

Proper sample preparation is critical for easier gas chromatography-mass spectrometry (GC–MS) analysis. Cleaner samples with fewer compounds reduce competitive ionization and enhance the signal, allowing analysis to better meet reproductivity, recovery, and sensitivity requirements. In addition, clean samples greatly reduce instrument maintenance, as dirty samples can foul up equipment and cause ion suppression, leading to signal loss.

To prepare samples for analysis, the compounds need to be removed from solid (or semi-solid) matrices and taken up into a liquid for injection. However, the workflow is complex, consisting of three steps: extraction, cleanup, and evaporation (Figure 1).

Two-thirds of all processing time is spent preparing the samples, which is still mostly manual. Despite many advances in preparation, it remains resource-intensive, requiring personnel to do laborious and tedious processes. It is also error-prone: over 80% of all laboratory errors occur during sample preparation. These drawbacks have profound influence on the overall workflows:

- **Sample throughput is low,** and turnaround times are long
- **Increased errors drive up costs for re-running samples,** and bring risks to accreditation
- **Staff retention and recruitment is challenging,** contributing to high turnover and limiting productivity

Accelerated solvent extraction (ASE), a high-temperature and high-pressure technique, offers fast and effective sample extraction. Due to its strengths, ASE is often the technique of choice, but relatively few changes have been made over recent years, offering space for improvement. ASE is, for example, a manual process that requires subsequent cleanup and solvent evaporation to concentrate samples for instrumental analysis. Alternatively, manual processes like Soxhlet can be used; however, it uses large volumes of solvent and takes a lot of time, which a chemist must solely dedicate to the process, which drives up costs.

Now, parallel extraction protocols with automated sample preparation exist as alternatives to manual preparation. Additionally, both extraction and evaporation can be combined in an automated system to save time and drive productivity:

- **Freed-up time for personnel:** Combining tasks increases walk-away time during sample preparation
- **Reduced errors:** There is less user intervention required, and no spills occur
- **Accurate workflows:** Automation allows end-point detection to stop the process at the desired level

New systems feature a gas-assisted extraction mode, which saves on both solvent use and extraction time. After collection, the needles can be rinsed to reduce carryover. Evaporation is then performed in the still-sealed vessels—under gentle vacuum, to avoid the loss of semi-volatile compounds—and a nitrogen (N_2) stream is blown through the same collection needle. Samples that are less volatile can also be heated to aid evaporation.

So, how effective are combined and automated extraction and evaporation systems for POP purification (Figure 2)? Here, we explore their suitability for preparing organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and polyaromatic hydrocarbons (PAHs) in soils.

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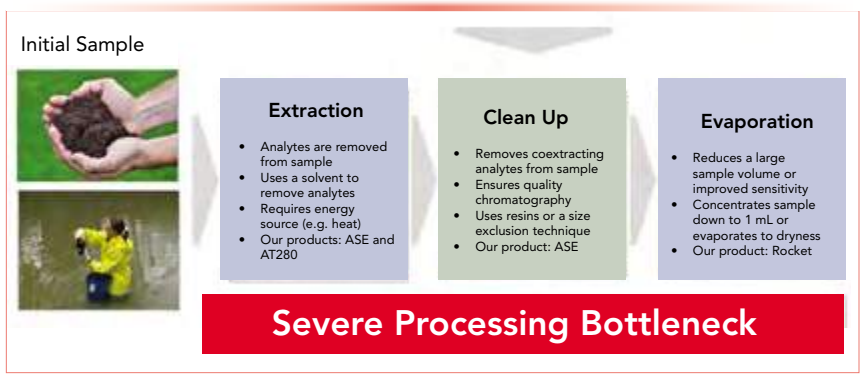

FIGURE 1: The POP sample preparation workflow.

TABLE I: Extraction and concentration (evaporation) conditions for the studies using an ASE system.

Extraction	
Parameter	Value
Cell type	Stainless steel
Cell size	10 mL and 100 mL
Oven temperature	100 °C
Purge time	45 s (10 mL cell); 180 s (100 mL cell)
Nitrogen flow (gas assisted extraction)	10 mL/min per channel
Cell fill volume	50%
Solvent flow rate	1.1 mL/min (10 mL cell); 0.75 mL/min (100 mL cell)
Extraction solvent	As above
Extraction volume	~26 mL (10 mL cell); ~70 mL (100 mL cell)
Pre-run rinse	10 mL, solvents as above
Concentration	
Mode	Fixed volume
Collection bottle	100 mL vial assembly
Final fixed volume	1 mL
Rinse solvent	1.6 mL, solvents as above
Evaporation temperature	40 °C
Nitrogen flow rate	50 mL/min per channel
Vacuum	8 psi (414 torr/551 mbar)

Materials and Methods

OCP Sample Setup

Pesticides and surrogate standards were mixed and diluted with hexane to produce stock solutions. The stock solution was diluted to obtain calibration standards with concentrations of 0.01, 0.02, 0.05, 0.1 and 0.2 µg/mL. Pentachloronitrobenzene (2 µg/mL) was then added to each standard.

PCB Sample Setup

PCB Congener Mix and surrogate standard (2,4,5,6-tetrachloro-m-xylene) were mixed and diluted with hexane to form stock solutions (1 µg/mL). The stock solution was diluted to make calibration standards of 0.005, 0.02, 0.05, 0.10, 0.20, and 0.3 µg/mL. An internal standard solution of decachlorobiphenyl (10 µg/mL, 20 µL) was then added to each calibration standard.

PAH Sample Setup

PAHs and surrogate standards were mixed and diluted with acetone-methylene chloride in a 1:1 ratio. The resultant stock solutions were diluted to make calibration standards with concentrations of 0.1, 0.2, 0.5, 0.75, 1.0, and 2.0 µg/mL. An internal standard solution of naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12, and perylene-d12 (20 µg/mL, 20 µL) was then added to each calibration standard.

General Setup

For each study, clean loam soil (2 g) was mixed in a glass beaker with diatomaceous earth (2 g, Dionex ASE Prep DE dispersant). This mixture was poured into an extraction cell and spiked with the appropriate standard, as detailed above. All the samples then underwent ASE using the Thermo Fisher Scientific EXTREVA ASE accelerated solvent extractor, as per the conditions outlined in Table I. The solvents used were acetone-hexane (1:1) for OCPs, hexane for PCBs, and acetone-methylene chloride (1:1) for PAHs.

Results and Discussion

Newer systems combine sample extraction and evaporation in a single automated platform, greatly reducing the need for human intervention. Extraction and concentration of multiple samples is performed in parallel, increasing throughput. As an added bonus, these systems also reduce the volume of solvent used for extraction, making the workflow more cost-effective and environmentally friendly.

OCP Analysis

OCPs, synthetic chlorinated hydrocarbon derivates, were once widely used to protect crops, livestock, buildings, and households from insect damage. But OCPs persist, bioaccumulate, and biomagnify, leading to their restriction and banning in many locations globally, including the United States and the European Union. In addition to their harmful properties, OCPs can be analytically challenging; they are semi-volatile, meaning samples must be carefully cleaned for accurate quantitation.

A recent study examined 20 different OCP analytes in spiked soil matrices (2). Compared to single extraction instruments and serial processing, an automated instrument combining extraction and evaporation gave higher throughput for OCP samples, especially in conjunction with parallel processing. Recovery studies were per-

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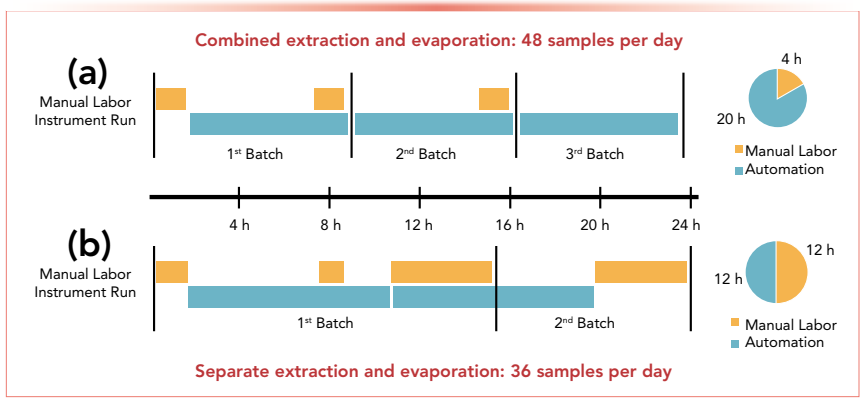

FIGURE 2: Schematic comparing (a) combined, and (b) separate extraction and evaporation systems, showing daily sample throughput and time required for manual labor and automation.

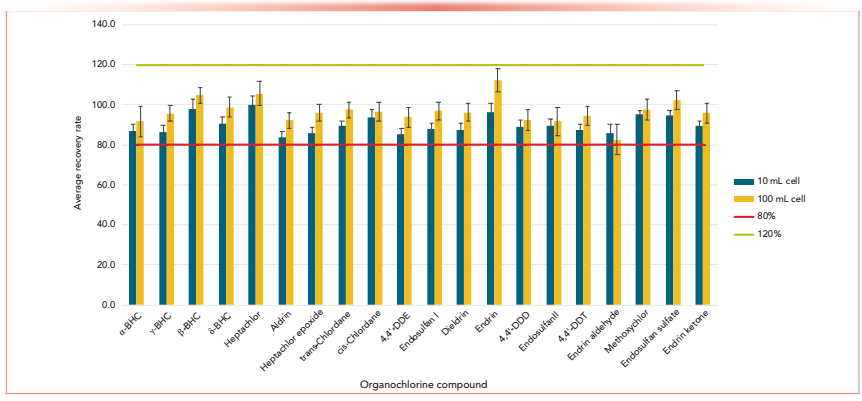

FIGURE 3: Average recovery rates of OCP analytes for the 25 µg/kg spike level, with the 80% and 120% limits shown in red and green, respectively.

formed, with all OCP recoveries falling between 80% and 115% for both 10 mL and 100 mL cells (Figure 3), meeting the recommended acceptance criteria from the U.S. Environmental Protection Agency (EPA).

Further analysis and studies demonstrated the strength of automated and combined extraction and evaporation. Significantly, the relative standard deviation (RSD) was below 7.9% for all standards, demonstrating reproducibility of the method using the system. Carryover studies were also performed—even from high spike samples, very little carryover was detected (0.00–0.43%), ensuring result accuracy and integrity.

PCB Analysis

PCBs are a class of chlorinated hydrocarbons that were widely produced and used until their manufacture was banned in 1979. These resilient, non-flammable, and stable compounds also benefit from high boiling points, making them suitable for a plethora of applications from hydraulic equipment to paints. But PCBs also bioaccumulate and biomagnify, and, given their toxicity, pose a significant threat to human health.

TABLE II: The average recoveries for the 100 µg/kg spike level. Method 1 uses a solvent flow rate of 1.6 mL/min; method 2 uses a flow solvent rate of 0.35 mL/min.

Compound	10 mL – Method 1		10 mL – Method 2		100 mL	
	Average Recovery % (*n* =12)	RSD %	Average Recovery % (*n* =12)	RSD %	Average Recovery % (*n* =12)	RSD %
PCB 18	82.8	7.4	79.2	3.8	77.0	2.6
PCB 31	88.2	7.3	81.4	4.9	80.4	2.8
PCB 52	90.4	7.8	82.5	3.7	82.0	2.5
PCB 44	93.7	6.3	87.5	4.3	82.4	2.8
PCB 66	91.6	6.2	87.6	3.1	87.7	2.6
PCB 101	92.6	5.1	86.3	5.0	86.5	2.6
PCB 87	94.3	5.2	87.1	2.6	88.7	2.6
PCB 110	95.5	5.2	86.8	2.9	86.9	2.2
PCB 151	92.9	4.0	84.6	3.3	88.9	1.9
PCB 153	91.4	3.6	90.7	6.2	92.8	2.2
PCB 141	95.6	3.0	90.7	4.0	93.4	2.3
PCB 138	99.4	4.7	94.6	4.4	94.8	2.3
PCB 187	95.4	6.8	94.8	3.5	92.9	2.5
PCB 183	94.9	6.5	89.2	4.2	92.8	2.6
PCB 180	97.4	4.6	93.7	4.4	101	4.7
PCB 170	92.7	8.1	98.0	4.4	99.8	2.0
PCB 206	85.8	8.7	93.7	4.3	97.0	2.1

Aiming to simplify PCB analysis, a study used an automated protocol combining extraction and evaporation for sample preparation (3). PCB analytes in soil samples were quantified, with two sample sizes tested: 2 g of soil in a 10 mL cell, and 20 g of soil in a 100 mL cell.

PCB recoveries were between 77.0% and 100.9%, also meeting EPA acceptance criteria (5). Notably, even highly volatile compounds such as PCB18 were extracted with high efficiency. The RSD was also determined and found to be below 20% for all compounds, demonstrating that the extraction and evaporation protocol showed good reproducibility (Table II). Further, proficiency testing (PT) samples were purchased and run, with all PCBs detected within PT published acceptance ranges.

Researchers optimized the workflow further, offering additional efficiencies using the method. Most significantly, they reduced the flow rate from 1.6 mL/min to 0.35 mL/min (Table II, method 2), finding it only minimally affected recovery rates but slashed solvent consumption by half.

PAH Analysis

PAHs are persistent environmental contaminants that arise from the incomplete combustion of organic materials such as coal, oil, petrol, and wood. Hundreds of PAHs are known, but only 16 have been designated as high priority pollutants by the EPA for toxicity, carcinogenicity, and mutagenicity. Also, as PAHs bind to particulate matter, they can be transported long distances away from their origin, further intensifying the need for accurate concentration determination.

Recently, a study used new ASE methodology to determine the 16 PAHs in soil (4). All the recovery rates of the PAH analytes were between 77.5% and 106.6%, well within EPA requirements. The method was also highly reproducible, with an RSD of below 20% for all compounds investigated.

To further determine the accuracy and precision of the approach, the team performed carryover studies. Less than 0.5% carryover was determined for all analytes, demonstrating the efficacy of rinsing between extractions.

TABLE III: The average recoveries of certified soil samples for all 16 PAH analytes.

PAH Compound	Certified Value (µg/kg)	Acceptance Range (µg/kg)	Average recovery and RSD (10 mL cell, *n* =12)	
			Avg (*n* =12) (µg/kg)	RSD (*n* =12)
Naphthalene	494 ± 38	164 to 824	362	6.76
Acenaphthylene	630 ± 38	328 to 933	490	1.58
Acenaphthene	651 ± 64	141 to 1162	502	1.25
Fluorene	157 ± 19	10.7 to 202	140	3.07
Phenanthrene	290 ± 26	65.2 to 516	283	0.58
Anthracene	612 ± 51	173 to 1051	447	2.76
Fluoranthene	333 ± 25	119 to 547	349	0.95
Pyrene	202 ± 20	35.7 to 369	240	2.21
Benzo[a]anthracene	329 ± 20	158 to 500	404	1.22
Chrysene	146 ± 12	49.8 to 241	168	4.45
Benzo[b]fluoranthene	69.9 ± 4.5	32.6 to 107	79	1.74
Benzo[k]fluoranthene	266 ± 21	95.0 to 437	251	1.41
Benzo[a]pyrene	223 ± 17	83.5 to 363	206	4.34
Indeno[1,2,3-cd] fluoranthene	88.3 ± 8.3	19.5 to 158	106	6.50
Dibenz[a,h]anthracene	193 ± 16	74.4 to 312	230	1.95
Benzo[ghi]perylene	224 ± 22	44.3 to 404	274	1.49

Finally, 12 replicates on certified PT samples all gave RSDs of below 7%, falling within the acceptance range of the accompanying certificate (Table III).

Conclusion

Fast and effective sample preparation is crucial to maintain the highest level of quality in POP analysis, but traditional, manual procedures currently make it time-consuming, resource-intensive and error prone. What's more, inefficient processes can be laborious and demotivating for analysts, leading to recruitment and retention issues.

Now, sample preparation doesn't need to be a bottleneck in the analytical workflow. Automating sample preparation and combining extraction and evaporation steps significantly improves the process by making it faster and more cost-effective. In addition, a protocol that runs samples in parallel can improve capabilities further by dramatically increasing sample throughput.

The advanced and automated ASE methods that combine extraction and evaporation enable highly efficient sample preparation for OCBs, PCBs, and PAHs. Three studies demonstrated that the method met the recovery requirements outlined by the EPA for each class of analyte, and all samples had RSDs below 20%, demonstrating the reproducibility of the approach. Additionally, a rinse between extractions can effectively minimize carryover, with less than 0.5% carryover of the analytes detected.

Beyond accuracy and precision in POP analyte determination, automated parallel extraction and evaporation systems also improve sample throughput, while reducing error and hands-on time. Such an automated workflow can improve POP analysis for many laboratories, and result in increased personnel productivity, happiness, and retention.

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Analytical Techniques in Exploring the Persistent Global PFAS Problem

Simon Hird, Warren Potts, and Ken Rosnack

Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals with unique properties. They are used in a wide range of different industries because they repel water and oil, and they are heat-resistant. However, PFAS contamination has become a widespread global concern because of their ubiquitous occurrence, persistence in the environment and living organisms, and potential health risks. Governments around the world are continuously introducing and updating regulatory limits for PFAS in drinking water, other types of water, biota, and, most recently, food. Analyzing such a wide range of sample types for PFAS requires high sensitivity and selectivity, typically provided by methods based on advanced liquid chromatography–tandem quadrupole mass spectrometry (LC–MS/MS) instrumentation. However, one critical aspect to sampling and analyzing PFAS accurately is to avoid PFAS contamination during these processes. This paper emphasizes the significance of robust and reliable PFAS analysis to support efforts to mitigate the global PFAS crisis. Collaboration, research, and advanced analytical solutions are essential to safeguard food, water, and the environment, thusly reducing the impact of PFAS on public health and the environment.

Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals with a myriad of industrial and cosmetic uses, and they contain unique properties that enable them to repel oil and water as well as being resistant to heat. Common uses include firefighting foams, water-resistant and non-stick coatings, waterproof mascara, floor polishes, and oil-resistant coatings for paper products approved for food contact.

Currently, there are thousands of known PFAS with more than 600 permitted for use in the United States alone. Despite decades of research on PFAS, fundamental barriers remain to addressing worldwide contamination by these chemicals and their associated impacts on environmental quality and health. What we do know is that three of the most researched PFAS compounds (perfluorooctanoic acid [PFOA], perfluorooctane sulfonic acid [PFOS], and perfluorohexane sulfonate [PFHxS]) have shown adverse health effects in people (1–3).

- They are highly mobile, ubiquitous, persistent, and toxic environmental contaminants that accumulate in humans, animals, and the environment.
- They are characterized by their high chemical stability, enormous structural diversity, and consistent presence in both the scientific literature and mass media.
- The basic structures of PFAS consist of a carbon chain with substituted fluorine atoms replacing hydrogen atoms on the chain.
- Different categories of PFAS can be grouped based upon the different substituents and functional groups within or terminal to the chain.

Dietary intake is considered as one of the main human exposure pathways of these chemicals. Once they enter the human body, PFAS do not metabolize and instead can accumulate in tissues as mentioned previously.

Regulatory Framework Around the Globe

As advisory and regulatory limits continue to be created and updated to protect public health and the environment, detection requirements for PFAS have been getting more demanding. For example, on June 15th, 2023, the U.S. Environmental Protection Agency (EPA) tightened its lifetime health advisory levels in drinking water for PFOA and PFOS. The proposed interim level for PFOA is 0.004 ng/L, or four parts per quadrillion (ppq), currently the lowest recommended level. As an analogy, think of trying to identify four specific ants out of all the estimated one quadrillion ants on the planet! Since then, in March 2023, the U.S. EPA proposed establishing legally enforceable levels for six PFAS (PFOA, PFOS, perfluorononanoic acid [PFNA], hexafluoropropylene oxide dimer acid [HFPO-DA], PFHxS, and perfluorodecane sulfonic acid [PFBS]) in drinking water as part of the National Primary Drinking Water Regulation (4). The proposed maximum contaminant levels (MCLs) for PFOA and PFOS are 4.0 ppt while the remaining are based on a "Hazard Index".

Other established drinking water maximum levels of PFOA and PFOS are 80 and 40 ng/L in China (5),

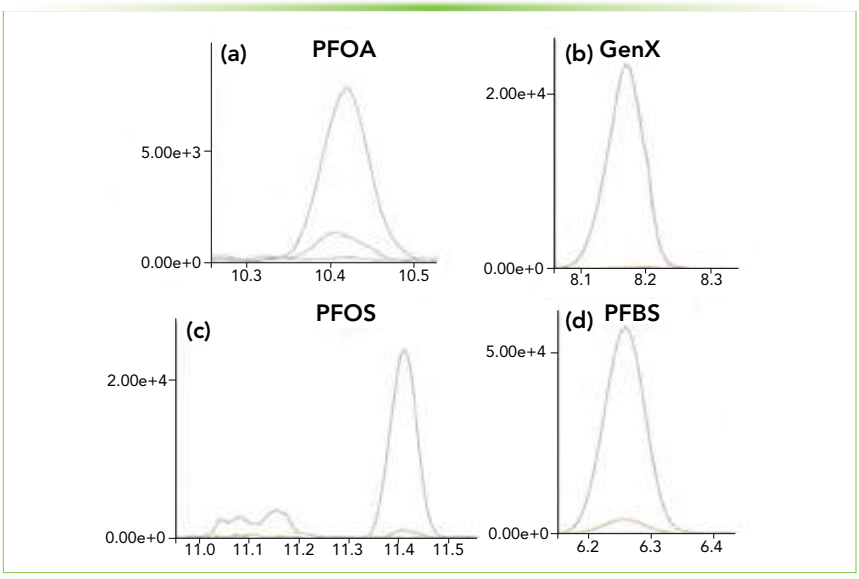

FIGURE 1: Overlaid chromatograms are shown for solvent blanks (green), extraction blanks (orange), and spiked extracts (blue) for (a) PFOA at 0.004 ng/L; (b) GenX at 0.02 ng/L; (c) PFOS at 0.02 ng/L; and (d) PFBS at 0.02 ng/L. Axis labels are time (min) for *x*-axis and intensity for *y*-axis.

respectively, whereas those in Australia are 560 and 70 ng/L, respectively (6). As stated in the *Official Journal of the European Union*, "By 12 January 2024, the Commission shall establish technical guidelines regarding methods of analysis for monitoring of per- and polyfluoroalkyl substances under the parameters 'PFAS Total' [totality of all individual PFAS should not exceed 500 ng/L] and 'Sum of PFAS' [sum of PFAS in Part B of Annex II should not exceed 100 ng/L], including detection limits, parametric values and frequency of sampling" (7).

The first regulatory limits for some PFAS in some foods of animal origin have recently been agreed to in Europe alongside recommended indicative limits in many other foods. Commission Regulation (EU) 2022/2388 (8) that amended Regulation (EC) No 1881/2006 was enacted on January 1st, 2023, and it set mandatory individual maximum levels for a limited number of PFAS (PFOA, PFOS, perfluorononanoic acid [PFNA] and perfluorohexane sulfonic acid [PFHxS]), together with a maximum level for the sum of those PFAS, in various foods of animal origin. The regulation was restricted to food of animal origin because this group was thought to be the most important contributor to human exposure to PFAS. Maximum levels, depending on the commodity, range from 0.2 to 50 µg/kg. As you can see, where maximum or advisory limits exist for water and food, the actual specific PFAS, as well as the maximum limits themselves included in the legislation, varies significantly between or even within regions.

Quantifying These Persistent Pollutants

Reliable analytical methods are needed to check compliance with regulatory limits and to determine exposure. For routine monitoring and research purposes, detection of PFAS to the ng/L, ng/kg, or part-per-trillion (ppt) level requires the use of liquid chromatography–tandem quadrupole mass spectrometry (LC–MS/MS). Although avoiding sample preparation may improve laboratory throughput, as well as reduce potential sources of contamination, a sample preconcentration step can be used for that ultimate method sensitivity.

With so many common sources (9) of PFAS contamination in our everyday environment, it can be difficult to routinely achieve such low-level analysis. Although complete avoidance of PFAS is impossible, steps can be taken to minimize background contributions. In the field, caution should be taken to avoid Teflon-containing materials such as waterproof clothing, plastic clipboards, and chemical ice packs. In the laboratory, items to avoid include sticky notes, certain glass disposable pipettes, vial caps with Teflon seals, and low density polyethylene (LDPE) containers. Detergents and personal care products used in the laboratory, used to launder laboratory coats or worn by scientists should also be considered possible contamination sources. It is recommended that all laboratory supplies be checked for PFAS contamination before use in the analysis.

Conclusion

Although not all countries have testing requirements in place, a good number of proactive steps are being taken by others. In response to growing evidence of the harmful effects of PFAS, many changes and regulations have been proposed around the world. The impact of PFAS continues to be researched and our understanding improved. Waters' PFAS solution – including quality controlled sample preparation, flexible data management tools, PFAS specific training, along with certified reference materials and proficiency testing – is helping scientists determine PFAS at very low levels and study their persistence and toxicity, as governments work to develop plans to remove them from our environment. Through customer collaboration, we continue to refine and improve our solutions that enable scientists and innovators around the world to ensure the safety of food and water. It is part of our overall commitment to leave the world better than we found it.

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