

Overcoming Typical Challenges for PFAS Analysis to Meet Evolving Regulatory Requirements

Introduction

Per- and polyfluoroalkyl substances (PFAS) represent a group of thousands of anthropogenic compounds that have been produced and widely used in industrial applications and consumer products since the 1950s. Some of the major industry sectors using PFAS include aerospace and defense, automotive, aviation, food contact materials, textiles, leather and apparel, construction and household products, electronics, firefighting, food processing, and medical supplies.

These compounds have unique physical and chemical characteristics: they all contain carbon-fluorine bonds (among the strongest chemical bonds in organic chemistry), which means they are highly stable and resistant to degradation and are known to persist in the environment longer than any other artificial substance. This, along with their ubiquitous use, has led to the accumulation of PFAS in the environment, with growing concern of human exposure to these chemicals.¹⁻³

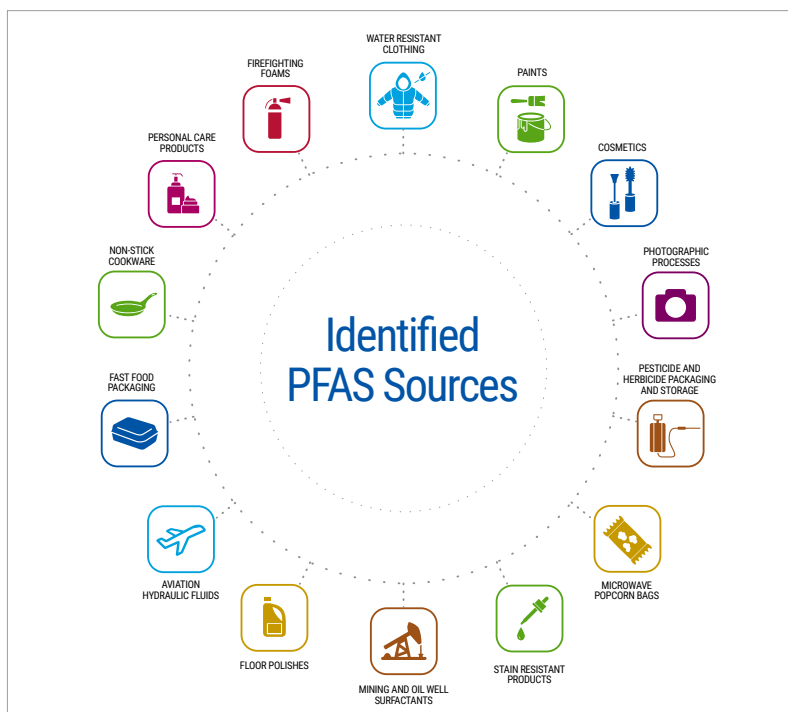


Figure 1: Sources of PFAS compounds.

Most PFAS are also easily transported in the environment, covering long distances from the source of their release. Many PFAS are found in human and animal blood and are present at low levels in a variety of food products and in the environment all across the globe. PFAS are found in soil and different water resources, including drinking, surface, ground-, and wastewater.¹⁻⁶

Among PFAS, perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are the most prevalent in the environment and so are included in many advisory guidelines.

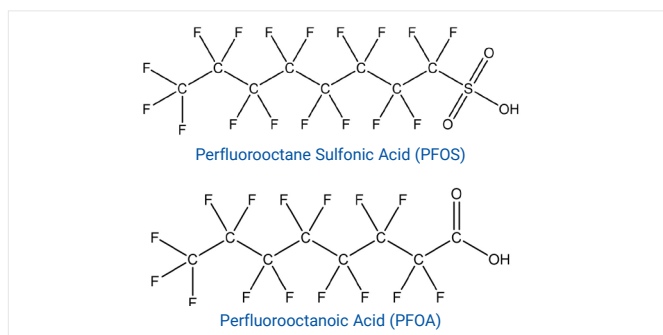


Figure 2. PFOA and PFOS chemical structures.

This article gives a non-exhaustive overview of the most known PFAS regulations, exploring the typical challenges encountered in their LC/MS/MS analysis, with practical examples on how to address them.

Mitigating the Adverse Impact of PFAS Exposure

With increasing awareness and scientific evidence of the environmental and health impacts of PFAS, governmental agencies have acknowledged the need for continuous monitoring of their levels in a variety of samples. Regulations have been put in place to surveil PFAS exposure in environmental matrices. Among them are:

- **U.S. EPA Method 537.1** for the determination of 18 PFAS in drinking water. The LC/MS/MS method is based on isotopic internal standards with reversed-phase solid-phase extraction (SPE) sample preparation.⁹
- **U.S. EPA Method 8327**, designed to measure a group of 24 PFAS compounds in ground-, surface, and wastewater samples, uses LC/MS/MS with external calibration.¹⁰
- **U.S. EPA Method 533** addresses some shorter chain and more polar PFAS compounds. It contains a list of 25 PFAS compounds (C4-C12) comprising the majority of those in 537.1, with the addition of some polar fluorotelomers and ether carboxylic acids. The LC/MS/MS method uses isotopic dilution and ion exchange SPE sample preparation.¹¹
- **U.S. EPA Draft Method 1633** was introduced in 2021 and is for use in the Clean Water Act (CWA). It is a composite method for a broad range of PFAS in multiple matrices. The method encompasses 40 targeted PFAS compounds in various matrices, including aqueous, solids, biosolids, and tissues.¹²

- **ISO 25101:2009** is utilized for the determination of the PFOS and PFOA in unfiltered samples of drinking, ground-, and surface water by coupling SPE with LC/MS/MS.¹³
- **Directive (EU) 2020/2184** is the EU Drinking Water Directive, which includes maximum limits for total PFAS of 0.5 µg/L. For the sum of the 20 PFAS of most concern, the maximum limit is 0.1 µg/L, which requires a limit of detection (LOD) of 30 ng/L for the sum and 1.5 ng/L for individual compounds. Environmentally relevant PFAS should be determined not only in clean drinking water but also in ground-, surface, and wastewater, which means that interfering compounds and matrices need to be separated.^{14,15}

For a method showing sensitivity and robustness for the analysis of the additional 20 PFAS compounds, refer to the application note, "Analysis of PFAS in Drinking Water by Large Volume Direct Injection Following the EU Drinking Water Directive 2020/2184."

- On December 30, 2022, the Chinese Ministry of Ecology and Environment made public the List of New Pollutants for Priority Management (2023), which was due to take effect March 1, 2023. This list includes PFOS and PFOA.

The optimization of analytical methods for identification and quantification of PFAS is essential for risk assessment. Because of its high sensitivity, selectivity, and robustness, the most widely used analytical method of PFAS detection is based on LC/MS/MS.

Liquid chromatography allows for the separation of the various PFAS of interest. Triple quadrupole mass spectrometry systems, with two quadrupoles and a collision cell, allow the filtering of specific mass transitions for each analyte, adding an extra layer of selectivity to the method. Mass spectrometers provide exact masses and resolve peaks with small mass differences, which makes them suitable for simultaneous detection of thousands of substances.

While LC/MS/MS is a very sensitive detection method on its own, there is still the need for sample preconcentration to detect PFAS levels present in the environment. The choice of sample preparation methods has implications for selectivity and sensitivity and the possibilities of identifying the substances in the sample. Sample handling and processing can significantly alter the molecular composition of the samples and the results of the analysis. Among sample preparation techniques, solvent extraction and solid-phase extraction are widely used to extract emerging contaminants from aqueous samples and have been employed in EPA Method 537.1, as well as ISO 25101.

The examples of applications described below were performed with the PerkinElmer QSight® LC/MS/MS System (Figure 3).



Figure 3. PerkinElmer QSiight 220 LC/MS/MS triple quadrupole system.

Typical Challenges for PFAS Analysis

Considering their ubiquity and large number of potential sources of contamination, PFAS present some challenges for analytical laboratories. These include:

- The risk of contamination during the analytical workflow
- The runtime needed to reach the detection and quantification limits imposed by the regulations
- Lab downtime caused by matrix-induced ion source contamination

While analytical labs need to meet evolving regulatory requirements for a list of compounds that is constantly expanding, they also need to maintain their high level of operational efficiency.

Minimizing Sources of Contamination

The key challenge of measuring parts-per-trillion levels of PFAS is that these compounds are ubiquitous throughout the environment and accumulate everywhere, including laboratory equipment and accessories. In fact, many of the components used in liquid chromatographs, mass spectrometers, and solid-phase extraction systems are made of polytetrafluoroethylene (PTFE) or PTFE copolymers, which leach PFAS compounds and cause background interference during sample measurement. Even the use of glass sample containers can generate additional challenges, because glass adsorbs PFAS compounds. Special care is required, and alternate materials must be used throughout the laboratory to reduce the absorption of these chemicals.

To reach accurate ultratrace levels, every step of the analytical protocol must be free of PFAS, from sample collection to sample preparation, analysis, and measurement. Table 1 summarizes the necessary steps for reducing background contamination during sample preparation and analysis.

Table 1. Steps to reduce PFAS contamination.

Source of Contamination	Mitigation
Mobile Phases	<ul style="list-style-type: none"> ▪ Purchase LC/MS-grade solvents ▪ Use a delay column
PFAS Parts and Tubing in HPLC Pump	Use a delay column
PFAS Tubing in HPLC Autosampler	Replace with PEEK tubing
Vials and PTFE Lined Caps	Use only polyethylene vials and caps
PFAS Tubing in SPE Apparatus	Replace with polyethylene tubing

Mobile Phase and HPLC Pump

High-quality mobile phases and blank runs are important aspects of PFOS analysis. Instead of using conventional glass vials with PTFE-lined septa, polyethylene vials and caps can be used to reduce the possibility of contamination. The HPLC pump, autosampler, and SPE system all contain PFAS components that require mitigation as well. The HPLC system pump has PTFE parts that can leach PFAS compounds, and contamination is likely in all but the highest grades of reagents.

To combat interference from these sources, a delay column can be installed in the flow path between the pump and the autosampler. The delay column captures PFAS contaminants coming from the mobile phase, the solvent lines, or the pump before they reach the autosampler. As a result, the captured compounds elute via the gradient later than the analyte peak in the sample, enabling more authentic measurements of PFAS in the sample.

Autosampler

In many cases, the HPLC autosampler contains fluoropolymer tubing that introduces contamination on injection of the sample. It is recommended to replace all tubing with high-performance polyetheretherketone (PEEK) tubing to eliminate the possibility of PFAS contamination during sample injection. For convenience, PerkinElmer offers a kit specifically developed to replace the tubing in their autosamplers for PFAS applications.

Solid-Phase Extraction (SPE) System

SPE extraction configurations normally include an abundance of fluoropolymers. The tubing connecting sample bottles to the SPE cartridges can be a significant source of PFAS contamination. Replacement of all transfer tubing with linear low-density polyethylene (LLDPE) or PEEK tubing is necessary to avoid PFAS leaching. In addition, some of the valving on the manifold may be constructed of PTFE; substitution with polyethylene stopcocks is recommended. Finally, sample collection during SPE extraction should employ polyethylene centrifuge tubes.

After proper mitigation of all possible PFAS sources, the LC/MS/MS system will be ready to analyze PFAS at low parts for trillion levels.

For the detailed workflow description and the complete set of results obtained with this approach please refer to the article, [“Analysis of Perfluoroalkyl and Polyfluoroalkyl Substances in Drinking Water: Validation Studies of EPA Method 537.1 Using the QSight 220 UHPLC/MS/MS.”](#)

Optimizing the Runtime

There are thousands of PFAS, and the broad range of their chemistries makes it difficult to establish a simple method for testing them, even in drinking water. As regulations continue to lower limits, it becomes more challenging to develop and validate efficient analytical methods. The lowest-concentration minimum reporting levels for current EPA methods are down to or below single-digit parts per trillion.

In a recent study, PerkinElmer scientists optimized the EPA Method 533 and EPA Method 537.1 on the QSight 220 LC/MS/MS system.

EPA Method 537.1 describes a chromatographic run that takes approximately 37 minutes to separate the 18 analytes, surrogates, and internal standards. For the same method, PerkinElmer scientists were able to achieve a runtime of about 10 minutes. This represents a significant time saving while maintaining excellent chromatographic resolution and excellent separation of the linear and branched isomers. An example of their separation is shown in Figure 1.

In terms of instrument sensitivity, the limits of quantitation (LOQ) and limits of detection (LOD) confirm that the QSight 220 LC/MS/MS system is highly capable of performing the method successfully. With the 250-to-1 sample concentration from the SPE extraction step, the limits were well below the current

requirements for all compounds – even those at extremely low levels. The detailed extraction procedure, analytical method, and results are presented in the application note, [“Analysis of Perfluoroalkyl and Polyfluoroalkyl Substances in Drinking Water: Validation Studies of EPA Method 537.1 Using the QSight 220 UHPLC/MS/MS.”](#)

Reducing Lab Downtime

Samples are often complex dirty matrices, which can impact the ion source and increase the need for cleaning, causing lab downtime.

Matrix effect is the effect on an analytical method caused by all other components of the sample except the specific compound to be quantified. Several approaches have been investigated to improve reproducibility and robustness of LC/MS/MS methods that are subjected to matrix effect.

In LC/MS analysis, ions that are formed in an ion source are normally sampled into a mass spectrometer through a small aperture or capillary, followed by devices such as an ion funnel or ion guide. Axial electric fields are often applied to transfer ions through multiple pumping stages before reaching the mass analyzer at high vacuum. During this transfer, ions, neutrals, and solvated charged species can deposit on inner surfaces, contaminating the path of the ions' migration to high vacuum, causing signal fluctuation, instability, and drift. In addition, transporting ions under influence of the axial electric field in a collision-rich environment causes ion scattering and discrimination between high- and low-mass ions so that the mass spectrometer requires tuning to set different lens parameters for high and low masses.

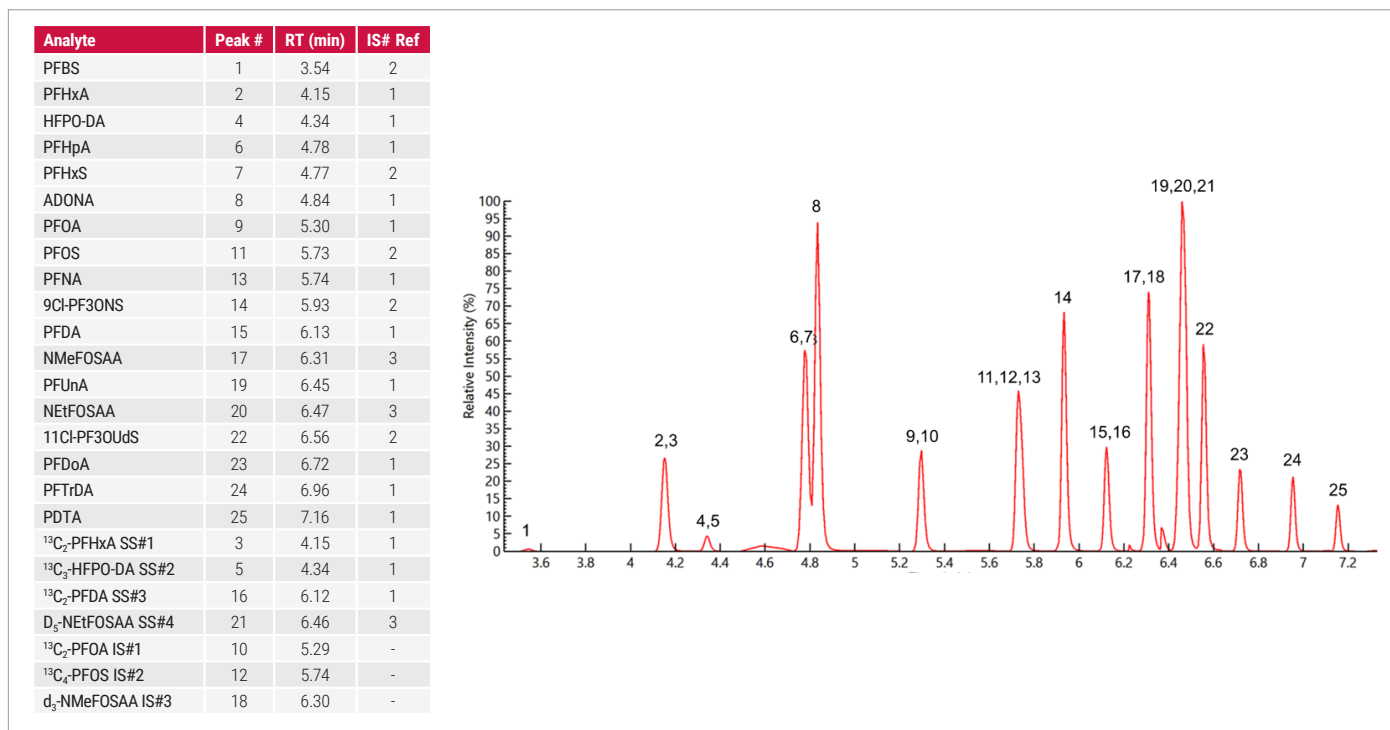


Figure 1: Sources of PFAS compounds.

To mitigate that, PerkinElmer has introduced QSight StayClean™ hot surface–induced desolvation (HSID™) technology, in which ions and solvated charged species are entrained in a hot laminar flow of sampling gas to be transported to a vacuum region. The laminar flow gas shields ions and solvated species from striking the HSID walls, acting as a constant cleaning agent. Additionally, solvated species gain energy from the hot gas and become desolvated. This enables enhanced uniform response across the entire mass range with no lens optimization. Most mass spectrometers today have an orthogonal sampling introduction to reduce contamination. Because of its proximity to the ion source, the interface can still be exposed to contamination as described above. Through the combination of the coaxial flow ionization source and laminar flow ion guide technologies, long-term signal stability and reproducibility are achieved and frequent cleaning is no longer required.

Highlights

There are currently over 7,000 PFAS-related compounds, and many more derivative compounds are expected to be created in the future. Important developments dealing with new PFAS challenges include:

Final Toxicity Assessments for GenX and Additional PFAS

The EPA has stated that the toxicity assessments for two PFAS, hexafluoropropylene oxide dimer acid and its ammonium salt – also referred to as GenX chemicals¹⁷ – will soon be published. GenX chemicals are considered extremely persistent in drinking water and have known impacts on human health, including reproductive and immunological toxicities, and on the environment. In addition to the assessments for GenX PFAS, the EPA Office of Research and Development is also developing toxicity assessments for five other PFAS, including PFBA, PFHxA, PFHxS, PFNA, and PFDA.¹⁸

Identifying PFAS Categories

Part of the difficulty in gathering information on PFAS is that it is such a large and diverse class of compounds. In response to this complexity, the EPA is planning to reclassify PFAS compounds into smaller categories based on parameters such as chemical structure, physical and chemical properties, and toxicological properties. The EPA has outlined two approaches to categorizing PFAS:

- Utilize toxicity and toxicokinetic data to develop PFAS categories for further hazard assessment and to inform hazard or risk-based decisions.
- Develop PFAS categories based on removal technologies using existing understanding of treatment, remediation, destruction, disposal, control, and mitigation principles.

These approaches will help identify missing elements in the EPA's understanding of PFAS from hazard assessments and removal technology perspectives, and further assist the EPA's prioritization for future actions.

The EU Drinking Water Directive establishes a new group-limit value for PFAS of 0.5 µg/L, in addition to limits for 16 individual PFAS of 0.1 µg/L in drinking water. Such measures can be supported by cost-effective and targeted monitoring of PFAS in the environment to provide early warning of pollution.¹⁶

Limit Usage and Production

In June 2019, the European Council of Ministers highlighted the widespread occurrence of PFAS in the environment, in products, and in people and called for an action plan to eliminate all nonessential uses of PFAS. The move toward zero pollution requires that product lifecycles are made safer from the start, based on the concept of “safe and circular by design.” This approach offers opportunities to protect the health of European citizens and environments while driving innovation for safer chemicals.

Innovations in Method Development Addressing New PFAS Categories

As the future of PFAS testing moves toward expanding PFAS toxicity assessments, optimizing methods, and identifying additional PFAS categories, it will be even more critical that researchers develop innovations within method development for enhanced PFAS analysis.

Governmental agencies are planning to develop additional targeted methods for detecting and measuring specific PFAS and nontargeted methods for identifying known PFAS in the environment.

Additional method development will be utilized for total PFAS analyses that measure the amount of PFAS in environmental samples without identifying specific PFAS.

Conclusions

Overcoming PFAS analytical challenges is critical for a comprehensive understanding of PFAS toxicities and environmental impacts. Properly developed and validated analytical methods allow researchers to increase throughput and decrease sources of contamination, while reducing runtimes. All these aspects are crucial for an analytical laboratory to succeed.

The threat of PFAS contamination is a global concern, so it is paramount that regulatory authorities, analytical technology manufacturers, and water treatment authorities combine efforts to develop the necessary legislation and innovations to facilitate necessary PFAS research and mitigation.

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Additional Resources

- **PFAS Analysis Interactive Brochure**
<https://www.perkinelmer.com/libraries/bro-pfas-solutions-final>
- **Case Study:** PFAS Monitoring: How One Scientist is Thinking Ahead for the Environment
<https://www.perkinelmer.com/libraries/cst-developing-methods-for-pfas-monitoring>
- **Infographic:** PFAS Health Concerns in Air, Water and Soil
<https://www.perkinelmer.com/library/pfas-health-concerns-in-air-water-and-soil.html>
- **Webinar:** Microextraction Approaches for Environmental Monitoring of Emerging Contaminants
<https://www.perkinelmer.com/library/microextraction-approaches-for-environmental-monitoring-of-emerging-contaminants.html>
- **Webinar:** Water Contaminants: Analysis of PFAS Using LC/MS/MS Technology
<https://www.perkinelmer.com/library/water-contaminants-analysis-of-pfas-using-lc-ms-ms-technology.html>
- **Webinar:** What's in your Water? Monitoring of PFAS in Drinking Water According to EPA Method 537.1
<https://www.perkinelmer.com/library/what-is-in-your-water-monitoring-of-pfas-in-drinking-water.html>