Understanding PFAS: Analysis and Implications

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Per- and polyfluoroalkyl substances (PFAS) are a large group of man-made chemicals that have been used in a wide range of industrial applications and consumer products since the 1940s [1,2]. Known for their persistence in the environment and human body - hence their nickname 'forever chemicals' - PFAS pose significant concerns for public health and environmental safety [1-3]. PFAS comprise a diverse class of thousands of chemicals characterised by the fully (per) or partly (poly) fluorinated carbon chain connected to different functional groups. The carbon-fluorine bond is one of the strongest in organic chemistry, contributing to this class of chemical's remarkable stability. This stability is advantageous for applications requiring resistance to heat, water, and oil [1-2]. Consequently, PFAS such as the synthetic fluoropolymer of tetrafluoroethylene - polytetrafluoroethylene (PTFE) have been used in products such as non-stick cookware and other industrial applications. Dupont created the Teflon™ brand name of PTFE, meanwhile it has become synonymous and it is worth noting that it's use is strictly regulated and requires licensing. Moreover, other PFAS compounds and industrial uses includes and is not limited to: water-repellent clothing, stain-resistant fabrics, food packaging, and firefighting foams [1-2].

PFAS contamination is widespread and persistent [2], accumulating in the environment and in living organisms [2-6]. The chemicals can be found in soil, air, water, and in the blood of humans and wildlife globally [2-8]. Health studies have linked PFAS exposure to various adverse outcomes, including thyroid disease, elevated cholesterol levels, weakened immune response, and an increased risk of some cancers [7-9]. Given their persistence, once PFAS enter the environment, they are difficult to remove. It is thus important that these chemicals can be monitored and appropriate remediation applied to contaminated environments [10]. PFAS have become a focal point for regulatory agencies worldwide due to their persistence, bioaccumulation, and potential adverse health effects. The regulatory landscape for PFAS is complex, involving multiple challenges and considerations. This article examines the key regulatory issues associated with PFAS and looks at the analytical challenges that face the chromatographer in analysing these compounds.

One of the primary regulatory challenges is the lack of uniform standards for PFAS across different jurisdictions. While some countries and regions have established regulations, the standards often vary significantly. For instance:

- In the United States, the Environmental Protection Agency (EPA) has issued legally enforceable levels for six PFAS, including PFOA and PFOS at maximum contaminant levels of 4.0 parts per trillion (ppt), but does not have enforceable federal limits for other PFAS in drinking water [11].
- The European Union has set limits for PFAS in drinking water, with the Drinking Water Directive establishing a sum limit of 0.5 µg/L for all PFAS, set on 12 January 2021 [12].
- Different U.S. states have their own regulations, with OEHHA California publishing a public health goal of 0.007 ppt for PFOA and 1 ppt for PFOS in April, 2024, limits much lower than the federal advisories [13].

This disparity complicates compliance for industries operating in multiple regions and creates confusion regarding safe levels of exposure [14]. The legislation is continually evolving in response to scientific findings, that has led manufacturers and researchers to continuously improve their analytical workflows with regards to the limits of sensitivity and resolution. Furthermore, to identify, minimise and/or avoid PFAS interferences to the sample analysis where possible, e.g., not being able to use mobile phase modifiers that can be classified as PFAS such as trifluoroacetic acid (TFA) – typically used for ion pairing attributes [15].

As new PFAS are continually developed, as is our greater understanding of the dangers associated with them, regulators face the challenge of keeping pace with substances whose health and environmental impacts are not yet fully understood [10, 14]. This piecemeal approach can lead to the substitution of regulated PFAS with unregulated alternatives that may pose similar risks. Regulating PFAS is further complicated by scientific uncertainties. The health effects of many PFAS are not well characterised, and there is ongoing research to determine safe exposure levels. Risk assessment methodologies vary, and long-term epidemiological studies are needed to fully understand the implications of chronic low-level exposure.

PFAS contamination is widespread, affecting water supplies, soil, and air. Identifying and managing contaminated sites is a significant regulatory challenge. The cost of remediation is high, and there is often uncertainty about the most effective methods for removing PFAS from the environment. Liability for contamination is another contentious issue [3]. Determining responsibility for cleanup costs can lead to extensive legal battles, especially in cases involving historical contamination by manufacturers and users of PFAS. Effective regulation requires robust monitoring and enforcement mechanisms. Monitoring PFAS in the environment is technically challenging due to their low concentrations and the need for sophisticated analytical methods [10]. Ensuring compliance with regulations requires significant resources, and enforcement actions can be hindered by limitations in detection and measurement capabilities.

There have been various policy and legislative initiatives aimed at addressing PFAS contamination:

- In the U.S., the PFAS Action Act aims to designate PFAS as hazardous substances under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), also known as the Superfund law. This would facilitate the cleanup of contaminated sites and hold polluters accountable. [16]
- The European Chemicals Agency (ECHA) is working towards restricting the manufacture, use, and sale of PFAS under the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulation [17].

These initiatives reflect growing recognition of the need for comprehensive regulatory frameworks but also highlight the challenges of implementing and enforcing such measures. Regulating PFAS has significant implications for industries that manufacture or use these chemicals. Compliance with stricter regulations can entail substantial costs for monitoring, reporting, and implementing alternative substances or technologies. The economic impact on industries, particularly in sectors like manufacturing, textiles, and firefighting, needs careful consideration to balance environmental protection with economic sustainability. The use of PFAS in so many areas has resulted in a relatively high level of background contamination, since so many materials contain it, from tissues to lab-coats. This makes the analysis of PFAS incredibly challenging as it requires the separation of background PFAS from sample PFAS.

With all of the interest in PFAS, it is evident that methods are required for the analysis of these potentially carcinogenic compounds. Using regulatory guidance, EPA 537.1 [18] and similar methods, the following 18 compounds were highlighted for separation and detection along with suitable isotopically labelled internal standards.

Table 1. List of test compounds and internal standards utilised

1. PFBS	8. ADONA	15. PFDA	22. 11Cl-PF3OUdS
2. PFHxA	9. PFOA	16. $^{13}C_2$-PFDA	23. PFDoA
3. $^{13}C_2$PFHxA	10. $^{13}C_2$-PFOA	17. NMeFOSAA	24. PFTrDA
4. HFPO-DA	11. PFOS	18. d_3-NMeFOSAA	25. PFTA
5. $^{13}C_3$-HFPO-DA	12. $^{13}C_4$-PFOS	19. PFUnA	
6. PFHpA	13. PFNA	20. NEtFOSAA	
7. PFHxS	14. 9Cl-PF3ONS	21. d_5-NEtFOSAA	

Calibration standard with PFAS standards, IS and surrogates standards at 500 ng/L (in sample concentration of 2 ng/L, after 250x sample pre-concentration during sample preparation specified in EPA 537.1).

The separation and detection were performed using an Avantor® ACE® Excel® 3 C18, 100 x 2.1 mm, with the mobile phase A: 5 mM ammonium acetate in H_2O and B: methanol (MeOH).

The flow rate was set to 0.4 mL/min, and the column thermostat set at 40 °C. *Table 2* details the gradient conditions employed. A PFAS delay column (Avantor® ACE® PFAS Delay Column, 50 x 2.1 mm) was employed, and installed prior to the injector loop. [19]

Table 2. Gradient conditions employed in the separation of 18 PFAS compounds

Time (mins)	% B
0	5
0.1	20
8.5	95
10.5	95
10.6	5

A SCIEX QTRAP® 6500+ system was used to detect the compounds of interest, running in negative ESI mode with a source temperature of 450°C and an Ionspray™ source voltage of -4500 V.

Figure 1 shows an example chromatogram obtained by injecting a 1 µL sample at a concentration of 1000 ng/L [19].

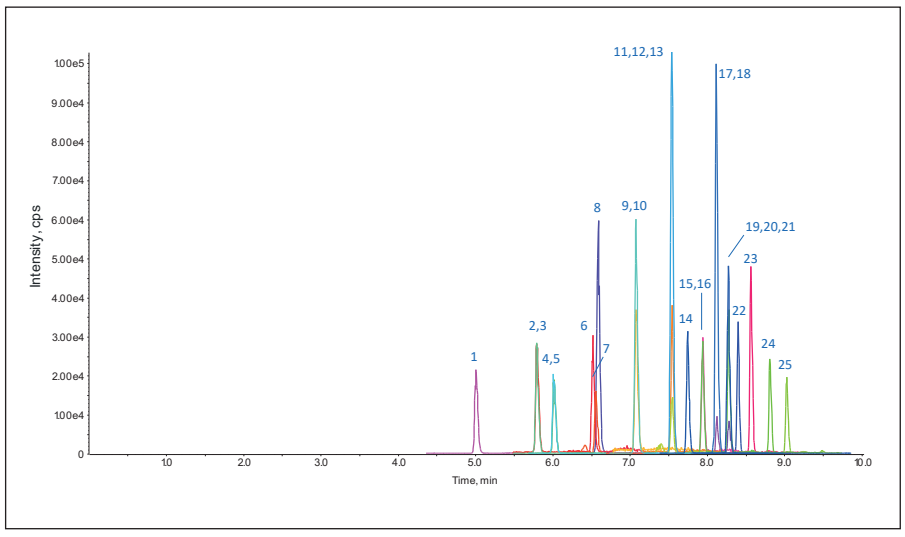

Figure 1. Example chromatogram obtain with 18 PFAS compounds and selected stable isotope labelled internal standards

When developing the method it was noted that there was a significant amount of background contamination, which subsequently led to an intensive investigation to systematically determine the source and levels of contamination. A variety of possible sources were identified. Initially, the sample vials and caps were the focus, with a simple solvent extraction being applied to determine if there were any extractable PFAS present. Each possible source and alternatives were extracted using 300 µL of methanol. The resulting solution was injected as a 10 µL aliquot. For the early eluting compounds this resulted in some poor peak shapes which is the combined effect of injection of a larger volume of strongly eluotropic diluent, weakly retained analytes being more susceptible to system dispersion, as well as being present at low concentration levels and close to the instrument's limit of detection. Due to the use of the qualifying extracted ion chromatograms, this facilitated the identification/qualification of contaminants without having to introduce another possible contamination source e.g. from a pre-concentration step and the use of a blow down evaporator.

The data, given in *Table 2*, is not quantitative, but the peak areas do indicate that there are detectable levels of PFAS. In this case two septa, both based on a polyimide silicone material compared to the polypropylene material used in the other septa, were identified as having ADONA (3H-perfluoro-3-[(3-methoxy-propoxy)propanoic acid]) present at detectable levels.

Table 3. Extraction of trace residuals present in caps, septum or vials demonstrates that some vial caps do contain PFAS components

	Blank run					
Vial Manufacturer	N/A	1	2	3	3	1
Vial material	N/A	PP	PP	PP	PP	PP
Cap Manufacturer	N/A	1	2	3	1	3
Septum material	N/A	PP	PP	PI/Si	PI/Si	PP
ADONA	×	×	×	✓14930	✓2341	×
Other PFAS (18 cpds)	×	×	×	×	×	×

Additional to the testing of the vials and caps, the analysis also looked at other possible sources of contamination as highlighted in *Figure 2* [20]. The extraction process employed was also qualitative for this set of experiments and involved taking a small proportion of the sample and inserting into a test tube, adding 2 mL of methanol and shaking for a short period of time and then injecting 10 µL of the resulting solution into the LC-MS using the method specified previously. It can be seen that there are a range of PFAS compounds that have been identified. The profiles are different for the different sample types. It is interesting to note that the two sources of paper tissue that were being analysed were the same brand but bought on different dates, suggesting that the manufacturing process may involve variable amounts of PFAS material that is added due to the recycling of the paper, or the possibility of the production line having contamination issues.

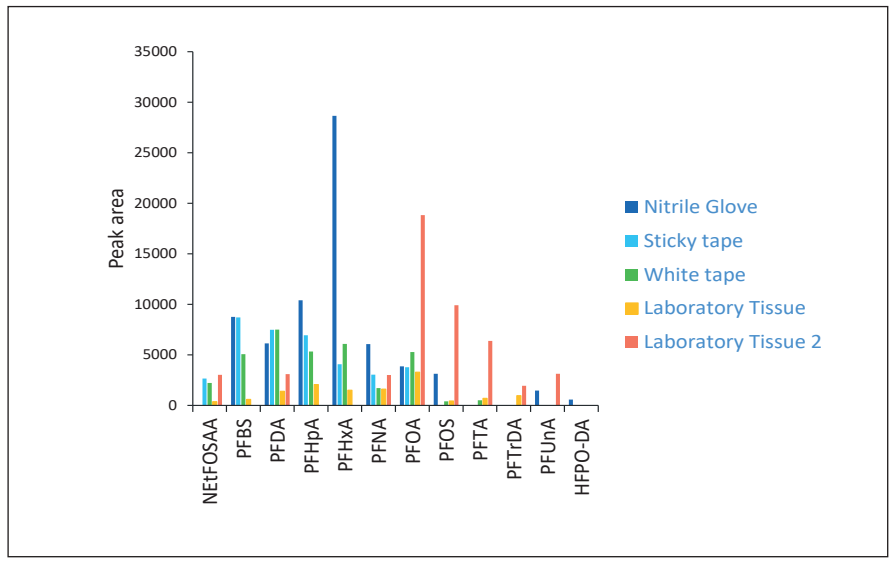

Figure 2. Extraction of trace residual PFAS present in a variety of common laboratory consuables

As well as looking for possible contamination within general laboratory and its consumables, PFAS can also be found in solvents and in tubing associated with the instrumentation [21]. PFAS originating from the mobile phases or components on the HPLC system prior to the autosampler will also result in chromatographic peaks, since it will build up at the front of the column during the low elutropic phase of the gradient elution, and when the gradient reaches a critical concentration, the PFAS components will start to elute, resulting in inaccurate concentration determination of the individual PFAS.

The approach to help alleviate this is to use a delay column which is placed in line before the injector, thus any PFAS components that are in the system will build up on the delay column preferentially before eluting onto the main analytical system. Once the gradient composition reaches a critical amount the PFAS components that have been retained on the delay column will be eluted onto the analytical column. The delay column is chosen so that it will introduce a retention time difference between the system derived PFAS and sample derived PFAS components. *Figure 3* shows an example chromatogram; in this scenario the system is contaminated with a range of PFAS. It can be seen in the chromatogram that there are two peaks present. The first peak relates to the PFAS in the sample and the second broader peak relates to the PFAS in the system. The poor shape of the chromatography is caused by the PFAS reagent going through two columns and also the build-up effect that the PFAS has as more solvent is passed through the column. The separation of the system PFAS components and the sample derived components means that accurate quantification of analytes within the sample becomes feasible. However, it should be stated that this does not preclude inaccurate quantification of the PFAS levels due to other sources of contamination within the sample itself, and as has already been shown, PFAS is ubiquitous within a laboratory environment. If this approach was not employed, then the system PFAS would elute at the same time as the sample PFAS, due to the nature of the focussing effect when using gradient chromatography. This would obviously result in the inaccurate quantification of the amount of the individual analytes in the sample. As mentioned previously, the ubiquitous nature of PFAS means that system contamination can come from a variety of sources from the solvents to the tubing, whereas sample contamination can come from a myriad of sources contained within workflow materials & consumables, sample prep workflows etc.

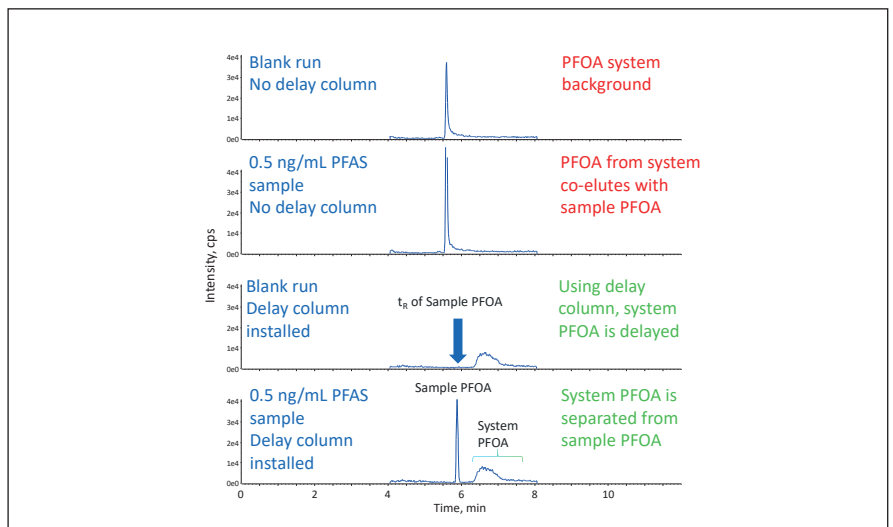

Figure 3. Examples of how the PFAS delay column effectively separates system PFAS from sample PFAS

Conclusion

The unique nature of per- and polyfluoroalkyl substances in providing inert materials that have high thermal insulation properties and high levels of hydrophobicity have resulted in them becoming ubiquitous across society and within the analytical laboratory. This study has highlighted that PFAS can be found in a variety of sources from nitrile gloves to paper tissues and even in some septa used in sample vials. It is also feasible for background PFAS compounds to originate from the HPLC system, specifically PEEK tubing, however the use of a delay column and carefully chosen method conditions will ensure that any contamination of the HPLC system or solvents can be separated from sample PFAS components meaning that accurate quantification can be achieved.

Analytical science plays an important role in modern society, ensuring that we live in a world that is safe, and potentially identifying risks that are not obvious. It is however extremely important that as scientists we do not lose the relationship with the context in our bid to develop ever more sensitive assays. It is important to understand that the data we produce is tightly associated with the total workflow (process and reagents) that we employ and that assumptions about the cleanliness/purity of these components may result in erroneous/misinterpretations of the resulting data. As scientists we must understand the purpose/aim of what we do ('why') is of equal importance to understanding the scientific workflow/strategy and its limitations ('what' and 'how').

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