OVERCOMING THE CHALLENGES OF REDUCING BACKGROUND INTERFERENCE FOR LC/MS/MS TRACE PFAS ANALYSIS

Per- and polyfluoroalkyl substances (PFASs) represent a large group of thousands of anthropogenic compounds that have been produced and widely used in many sectors including automotive, food processing and packaging, textiles, construction and household products, electronics, firefighting, and medical articles.

These compounds have unique physical and chemical characteristics: they all contain carbon-fluorine bonds (among the strongest chemical bonds in organic chemistry), that means they are highly stable and resistant to degradation, and they are known to persist in the environment longer than any other man-made substance. This, along with their ubiquitous use, have led to the accumulation of PFAS in the environment, with growing concern of human exposure to these chemicals.[1-6]

The optimization of analytical methods for identification and quantification of PFASs is essential for risk assessment. Because of its high sensitivity, selectivity and robustness, the most widely used analytical method for PFAS detection is based on Liquid Chromatography coupled with Mass Spectrometry technique (LC/MS/MS). Coupling SPE with LC/MS/MS has been one of the most popular approaches to PFAS analysis in aqueous samples, and has been employed in EPA Method 537.1, as well as ISO 25101.[7,8]

But the key challenge of measuring ppt levels of PFAS is that these compounds are ubiquitous throughout the environment and accumulate everywhere, including the 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 a sample measurement. Even the use of glass sample containers can generate additional challenges, the glass in fact adsorbs PFAS compounds.

Reducing PFAS background

To reduce background contamination and reach accurate ultra-trace levels, every step of the analytical protocol must be free of PFAS materials: from sample collection to sample preparation and analysis.

To start, purchase high quality mobile phases (LCMS grade solvents). Additionally, instead of utilizing conventional glass vials with PTFE-lined septa, polyethylene vials and caps are necessary to reduce the possibility of contamination. The HPLC pump, autosampler, and SPE system all contain PFAS components that require mitigation as well.

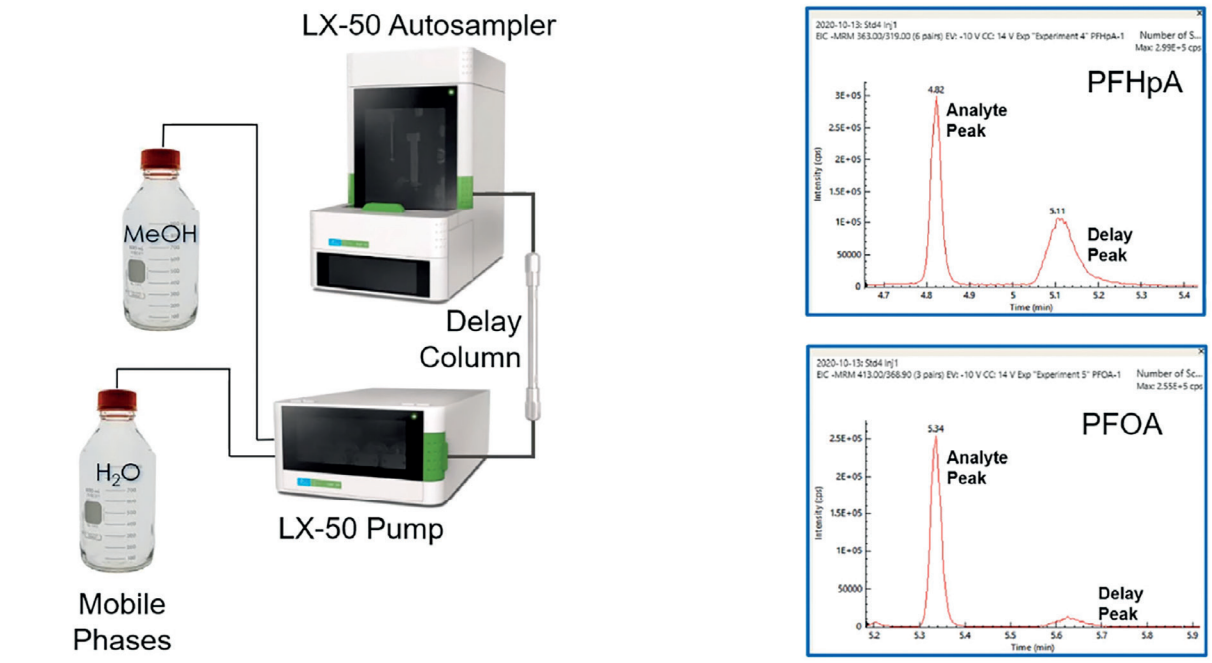

Figure 1. Reducing background from pump and mobile phases.

To combat interference from these sources, a delay column may be installed in the flow path between the pump and the autosampler, as shown in Figure 1.

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 (see chromatograms in Figure 1) allowing clear separation of PFAS contaminants from the analytes of interest, enabling more authentic measurements of PFAS in the sample.

In many cases, the HPLC autosampler contains fluoropolymer tubing which will introduce PFAS contamination upon sample injection. It is recommended to replace all tubing with high performance polyether ether-ketone (PEEK).

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

Figure 2. PerkinElmer QSight® 220 LC/MS/MS Triple Quadrupole system.

a. Instrument calibration range is the actual concentration range of calibration standards used to determine calibration curves.
b. Method calibration range is determined by multiplying the instrument calibration range by 1/250 to account for the SPE sample preparation/concentration.
c. R^2 values are the average of triplicate calibration curves.

Table 1. Instrument and method calibration ranges and linearity (R^2) for eight-point calibration curves of all EPA Method 537.1 analytes and surrogates.

Compound	Instrument Calibration Range (ng/L)[a]	Method Calibration Range (ng/L)[b]	R^2 [c]
PFBS	16.4 - 26287	0.07 - 105.1	0.9994
PFHxA	5.5 - 29703	0.02 - 118.8	0.9987
$^{13}C_2$-PFHxA	4.6 - 24752	0.02 - 99.0	0.9989
$^{13}C_3$-HFPO-DA	67.5 - 24752	0.27 - 99.0	0.9992
HFPO-DA	18.5 - 29703	0.07 - 118.8	0.9985
PFHpA	5.5 - 29703	0.02 - 118.8	0.9984
PFHxS	5.2 - 28218	0.02 - 112.9	0.9998
ADONA	5.2 - 28218	0.02 - 112.9	0.9990
PFOA	5.5 - 29703	0.02 - 118.8	0.9998
PFOS	5.3 - 28515	0.02 - 114.1	0.9974
PFNA	18.5 - 29703	0.07 - 118.8	0.9993
9Cl-PF3ONS	5.1 - 27772	0.02 - 111.1	0.9998
PFDA	81.0 - 29703	0.32 - 118.8	0.9990
$^{13}C_2$-PFDA	4.6 - 24752	0.02 - 99.0	0.9988
NMeFOSAA	5.5 - 29703	0.02 - 118.8	0.9998
PFUnA	18.5 - 29703	0.07 - 118.8	0.9968
NEtFOSAA	5.5 - 29703	0.02 - 118.8	0.9968
d5-NEtFOSAA	18.3 - 99010	0.07 - 396.0	0.9962
11Cl-PF3OUdS	5.2 - 28069	0.02 - 112.3	0.9997
PFDoA	18.5 - 29703	0.07 - 118.8	0.9963
PFTrDA	5.5 - 29703	0.02 - 118.8	0.9959
PFTA	5.5 - 29703	0.02 - 118.8	0.9967

a. Instrument LOD/LOQ was determined using the signal-to-noise ratio (S/N) of the peak from the lowest detectable calibration standard (5-18 ng/L) and extrapolating to the concentration at which the S/N = 3 or 10 for LOD or LOQ, respectively. This is an estimate to demonstrate expected LOD/LOQ and can vary from lab to lab.

b. Method LOD/LOQ is calculated by multiplying the Instrument LOD/LOQ by 1/250 to account for the 250 to 1 sample concentration from the SPE extraction. LOD/LOQ cannot be used as MRLs but provide an estimate of instrument sensitivity.

Table 2. Instrument sensitivity (LOQ & LOD) for all target analytes in EPA Method 537.1.

Analyte	Instrument (ng/L)[a]		Method (ng/L)[b]	
	LOD	LOQ	LOD	LOQ
PFBS	2.00	6.68	0.008	0.027
PFHxA	2.31	7.70	0.009	0.031
HFPO-DA	6.70	22.35	0.027	0.089
PFHpA	2.10	6.99	0.008	0.028
PFHxS	0.38	1.28	0.002	0.005
ADONA	0.24	0.79	0.001	0.003
PFOA	2.57	8.56	0.010	0.034
PFOS	0.92	3.07	0.004	0.012
PFNA	2.52	8.40	0.010	0.034
9Cl-PF3ONS	0.60	2.00	0.002	0.008
PFDA	2.17	7.24	0.009	0.029
NMeFOSAA	0.29	0.96	0.001	0.004
PFUnA	3.50	11.67	0.014	0.047
NEtFOSAA	0.25	0.85	0.001	0.003
11Cl-PF3OUdS	0.44	1.48	0.002	0.006
PFDoA	2.02	6.73	0.008	0.027
PFTrDA	1.55	5.16	0.006	0.021
PFTA	4.29	14.30	0.017	0.057

may be constructed of PTFE; substitution with polyethylene stopcocks is recommended. Finally, sample collection during SPE extraction should employ polyethylene centrifuge tubes.

Example: Validation study using EPA 537.1

A recent study validated PerkinElmer's PFAS mitigative steps by employing EPA Method 533 [9] and EPA Method 537.1 on a QSight® 220 LC/MS/MS system (Figure 2). The method involved fortification with surrogates to monitor the extraction efficiency.

250 mL drinking water sample was collected in a polyethylene bottle. The sample was concentrated by SPE using a polystyrenedivinylbenzene (SDVB) stationary phase. In this step, the sample was loaded onto the SPE tube and eluted with methanol. The extract was evaporated to dryness under nitrogen and reconstituted in 1 mL of 96% methanol. This concentrated the sample by a factor of 250, thereby enabling quantification of the low levels necessary for the analysis. Internal standards were added after reconstitution of the sample.

Subsequently, 10 µL of sample was injected onto a C18 column in the LC/MS/MS instrument. The mass spectrometer was used in Multiple Reaction Monitoring (MRM) mode. Retention times for the calibration standards enabled identification of the compounds and the MRM transitions, for both quantifier and qualifier ions.

Separation

EPA Method 537.1 describes a chromatographic technique that takes approximately 37 minutes to separate the 18 analytes, surrogates, and internal- standards. However, improvements to the chromatographic method made by PerkinElmer scientists achieved a run time of about 10 minutes. This represented significant time savings while maintaining excellent chromatographic resolution, and excellent separation of the linear and branched isomers. An example of their separation is shown in Figure 3.

Calibration

Calibration curves were run for all 18 analytes and the surrogate standards, encompassing the range necessary to include the lower limits of detection (LOD) from EPA regulations. The full method ranged from 0.02 ppt to 120 ppt. As demonstrated in Table 1, excellent linearity was observed, with all correlation coefficient (R^2) values for the calibration curves of 0.99 or better.

Sensitivity

In terms of instrument sensitivity, the limits of quantitation (LOQ) and limits of detection (LOD) were estimated based on signal-to-noise ratios. Data reported in Table 2 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.

Experiments were conducted to define the method detection limits of all target analytes for EPA Method 537.1. The lowest concentration minimum reporting limits (LCMRLs) as well as the experimental minimum reporting limits (MRLs) were also determined. Results are tabulated in Table 3. Experimental MRLs are at acceptable levels to meet the current requirements for all the targeted PFAS compounds.

Recovery

Recovery studies were completed for all 18 analytes by spiking fortified laboratory field blanks at four different levels, ranging from 0.3 ppt up to 80 ppt. Figure 4 shows the recoveries for each analyte at each of the four concentrations. EPA Method 537.1 requires recoveries between 70% to 130% of the known spiking level. The developed method using the QSight® 220 LC/MS/MS met requirements for recovery across all four concentrations evaluated.

CONCLUSION

LC/MS/MS analysis of PFAS at ultra-trace levels requires mitigation to both liquid chromatograph and mass

Analyte	Peak #	RT (min)	IS# Ref
PFBS	1	3.54	2
PFHxA	2	4.15	1
HFPO-DA	4	4.34	1
PFHpA	6	4.78	1
PFHxS	7	4.77	2
ADONA	8	4.84	1
PFOA	9	5.30	1
PFOS	11	5.73	2
PFNA	13	5.74	1
9Cl-PF3ONS	14	5.93	2
PFDA	15	6.13	1
NMeFOSAA	17	6.31	3
PFUnA	19	6.45	1
NEtFOSAA	20	6.47	3
11Cl-PF3OUdS	22	6.56	2
PFDoA	23	6.72	1
PFTrDA	24	6.96	1
PDTA	25	7.16	1
$^{13}C_2$-PFHxA SS#1	3	4.15	1
$^{13}C_3$-HFPO-DA SS#2	5	4.34	1
$^{13}C_2$-PFDA SS#3	16	6.12	1
d_5-NEtFOSAA SS#4	21	6.46	3
$^{13}C_2$-PFOA IS#1	10	5.29	-
$^{13}C_4$-PFOS IS#2	12	5.74	-
d_3-NMeFOSAA IS#3	18	6.30	-

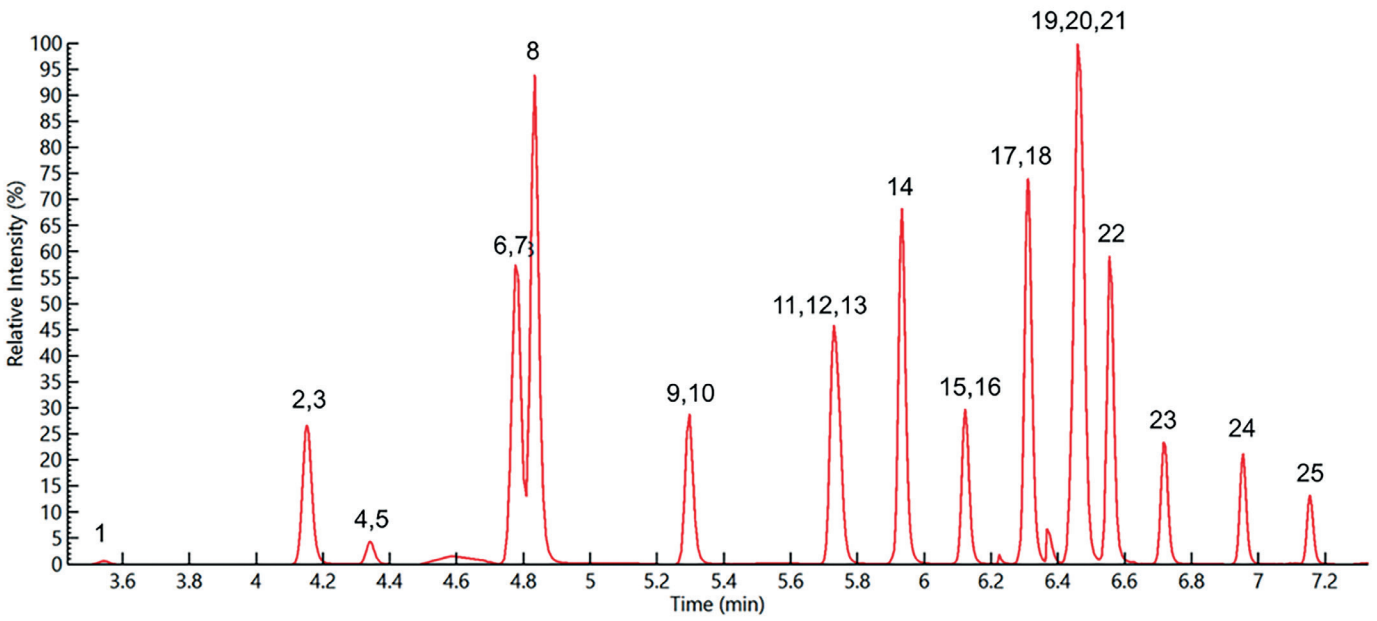

Figure 3. TIC of an 80 ng/L extracted fortified laboratory field blank sample containing all method analytes, surrogates, and internal standards.

a. Experimental DL was determined from ten LFB replicates fortified at ~4.0 ng/L measured over three days and calculated according to section 9.2.8 in EPA Method 537.1 rev 2.0

b. Reference DL values from EPA Method 537.1 rev 2.0 (Table 5) determined from seven LFB replicates fortified at 4.0 ng/L measured over three days and calculated according to section 9.2.8

c. Experimental LCMRLs were determined from ten replicates each at five fortification levels ranging from ~0.2 – 80 ng/L using the EPA LCMRL Calculator.[11]

d. Reference LCMRL values from EPA Method 537.1 rev 2.0 (Table 5).

e. Experimental MRLs were determined from seven LFBs fortified at concentrations ranging from ~0.2 to 4.0 ng/L according to section 9.2.6 of EPA Method 537.1 rev 2.0 using the Half Range prediction interval method with confirmed upper and lower Prediction Interval Results (PIR) ≤150% and ≥50%, respectively.

Table 3. Method detection limits and lowest concentration minimum reporting limits and minimum reporting levels determined experimentally on the QSight® LC/MS/MS system and compared to reference values report in EPA Method 537.1.

Analyte	Experimental DL (ng/L)[a]	EPA 537.1 DL (ng/L)[b]	Experimental LCMRL (ng/L)[c]	EPA 537.1 LCMRL (ng/L)[d]	Experimental MRL (ng/L)[e]
PFBS	1.1	6.3	0.72	1.8	1.4
PFHxA	1.5	1.7	0.93	1.0	0.30
HFPO-DA	1.5	4.3	0.57	1.9	1.6
PFHpA	1.6	0.63	0.10	0.71	1.6
PFHxS	1.2	2.4	0.60	1.4	0.29
ADONA	1.4	0.55	ND	0.88	0.28
PFOA	1.3	0.82	0.34	0.53	0.30
PFOS	1.4	2.7	1.0	1.1	0.29
PFNA	1.6	0.83	0.50	0.70	1.6
9Cl-PF3ONS	1.1	1.8	0.68	1.4	1.5
PFDA	1.1	3.3	0.40	1.6	0.30
NMeFOSAA	1.2	4.3	0.22	2.4	0.30
PFUnA	1.3	5.2	0.30	1.6	1.6
NEtFOSAA	1.2	4.8	0.73	2.8	1.6
11Cl-PF3OUdS	0.66	1.5	0.39	1.5	0.28
PFDoA	1.2	1.3	0.19	1.2	0.30
PFTrDA	1.0	0.53	0.82	0.72	4.0
PFTA	0.86	1.2	1.5	1.1	4.0

spectrometer to eliminate the leaching of fluorochemicals from components within the systems. Manual SPE configurations also require mitigative steps to eliminate any components constructed of PTFE to minimize or eliminate any PFAS contamination. PerkinElmer offers kits and knowhow to streamline remediation. The use of high-grade reagents and PFAS-free laboratory accessories are also critical. By implementing steps to remove or reduce background contamination and appropriate sample preparation, PerkinElmer's highly sensitive QSight® 220 LC/MS/MS system has proven to be extremely capable of meeting the challenging demands of low-level PFAS analysis in drinking water. Validation studies demonstrated that the instrument easily meets stringent requirements of EPA 537.1 and 533 regulations for all targeted analytes.

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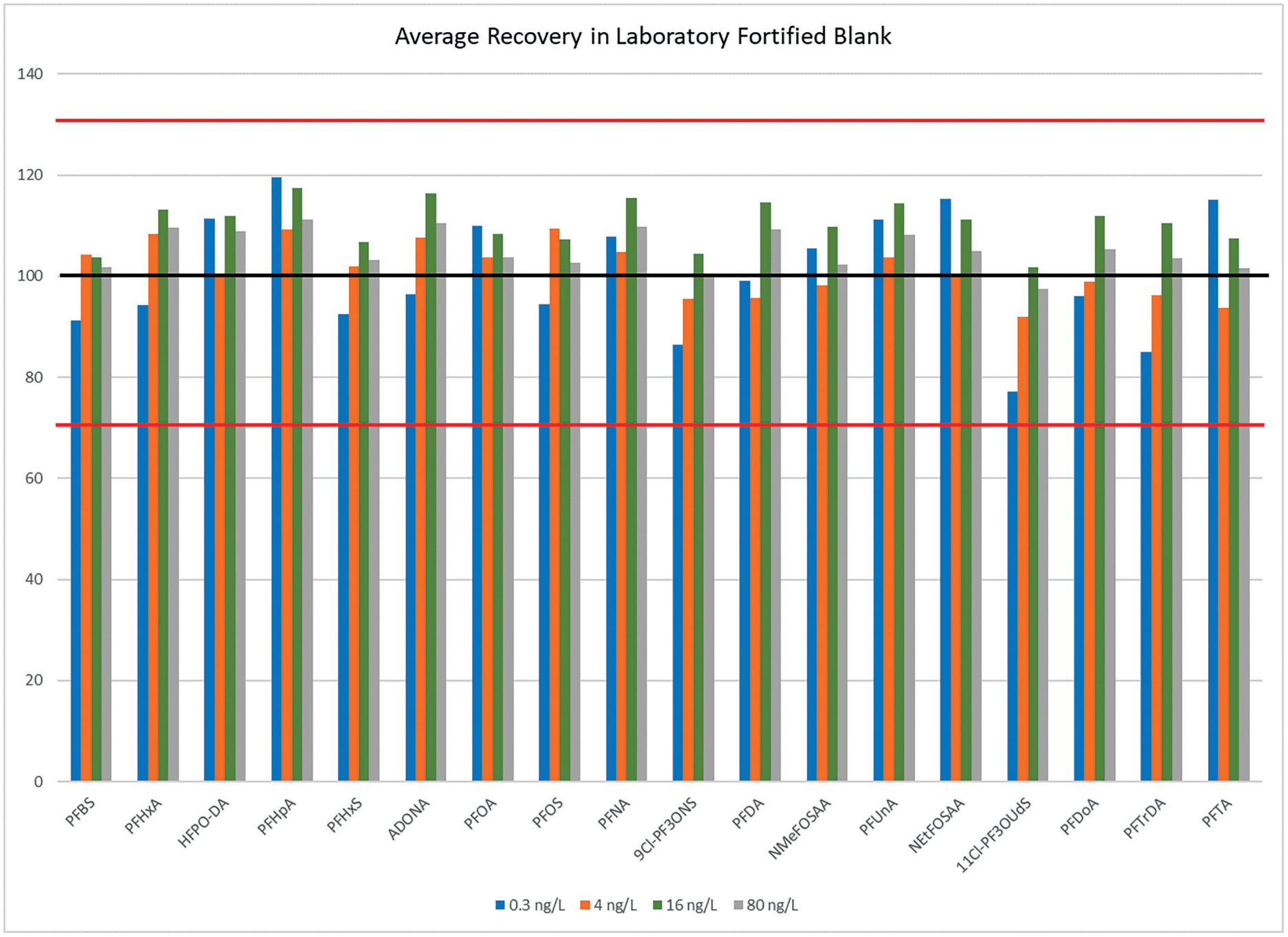

Figure 4. PFAS recovery precision & accuracy summary.

Authors

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Jamie Foss is the Liquid Chromatography Product Manager for PerkinElmer. Prior to this role, he was a Sr. Application Scientist developing strategic applications in support PerkinElmer's LC and LC-MS product portfolio. He has developed a wide variety of applications across food, environmental, industrial, forensics and cannabis. Prior to joining PerkinElmer in 2016, he spent 4 years as a forensic chemist for the State of Maine where he focused on the analysis of controlled substances and clandestine laboratory investigation.

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