Achieving low parts-per-quadrillion detection limits for PFAS analysis in drinking water

A collaborative exploration of instrument sensitivity and sample preparation for ultra-trace analysis of PFAS on the SCIEX 7500 system

Thep Phomsopha[1], Simon Roberts[2], Craig M. Butt[2], Karl Oetjen[2], Matt Noestheden[2], Sam Lodge[3], Andrew N. Patterson[1]
[1]Eurofins Environment Testing West Sacramento; [2]SCIEX, USA; [3]Phenomenex

This technical note describes the collaboration between SCIEX and Eurofins Environment Testing to address the 2022 US EPA drinking water health advisory levels (HALs) for 4 per- and polyfluoroalkyl substances (PFAS).[1] Sample preparation and instrumental methods were developed to achieve low parts-per-quadrillion (ppq) HALs—which is pg/L equivalent—for perfluorooctanoic acid (PFOA, 4 ppq), perfluorooctane sulfonic acid (PFOS, 20 ppq), perfluorobutane sulfonic acid (PFBS, 2 ppb) and hexafluoropropylene oxide dimer acid (HFPO-DA, or GenX, 10 ppt). The high sensitivity of the SCIEX 7500 system allowed for a simplified sample preparation procedure, reducing PFAS contamination. Method extraction spikes showed excellent recovery at 4 ppq for PFOA, PFBS and HFPO-DA, and at 20 ppq for PFOS.

PFAS are widely detected in drinking water and have been recognized as global environmental contaminants for over 20 years. The US EPA drinking water HALs for PFAS are not enforceable regulatory limits but indicate the levels below which adverse health effects are not anticipated (considering lifetime exposure).[2] The low ppq levels for PFOA and PFOS in the 2022 EPA drinking water HALs demand an unprecedented level of cleanliness and instrumental sensitivity which ultimately necessitates newer, robust analysis techniques.

Key benefits of the method for ultra-trace level PFAS analysis

- Extensive PFAS contamination reduction by using a positive-pressure, HEPA-filtered, clean room for sample preparation
- Modified SPE sample preparation, requiring only 25x concentration and eliminating sample blow down and reconstitution
- Elevated sensitivity of the SCIEX 7500 system to reach low ppq (pg/L) detection levels
- Excellent recovery of extracted spikes at 4 ppq (pg/L) for PFOA, PFBS and HFPO-DA, and at 20 ppq (pg/L) for PFOS

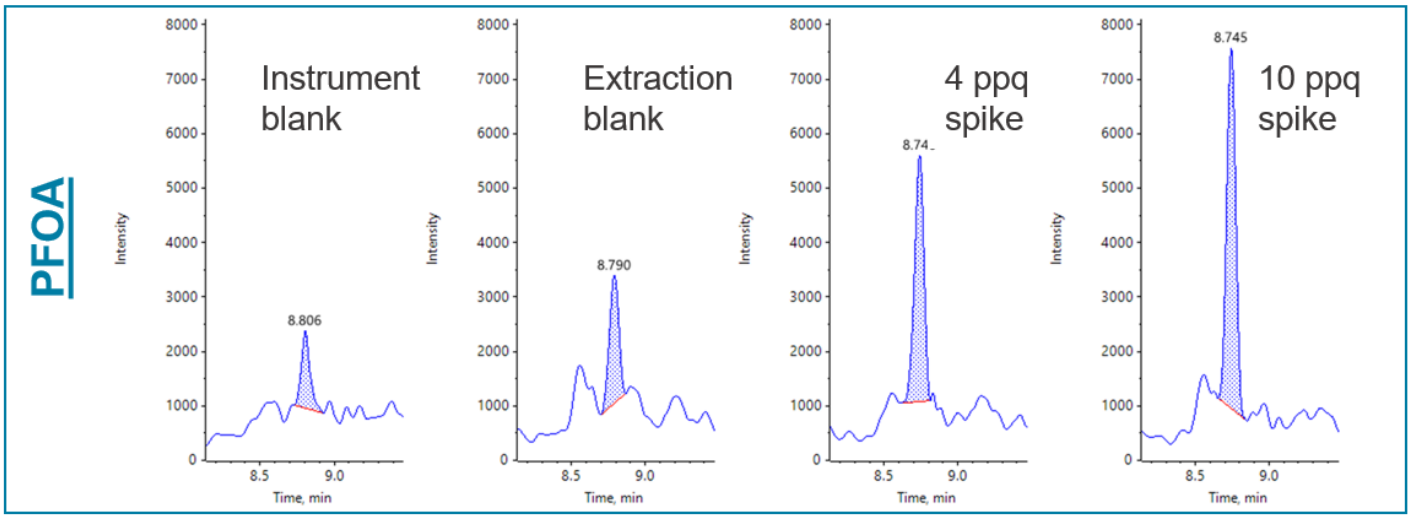

Figure 1. Extraction spikes and blanks for PFOA. Chromatogram shows MRM XIC of m/z 413.0>169.0 transition. Instrument blank, extraction blank, 4 ppq (pg/L) and 10 ppq (pg/L) extraction spikes are shown.

Methods

Chemicals and standards: HPLC grade methanol and ammonium hydroxide (certified ACS Plus) were obtained from Fisher Scientific, and water was supplied by a Milli-Q lab water system (Sigma Millipore). Native and mass-labeled standards were purchased from Wellington Laboratories (Guelph, ON).

Contamination minimization: Extensive measures were performed to minimize PFAS contamination during all sample preparation and analysis stages. Specifically, pipette tips, solvent bottles, SPE manifold components, and collection tubes were thoroughly rinsed with methanol. In addition, sample extractions were performed in a positive pressure, HEPA-filtered clean room specifically built for ultra-trace level analysis of environmental contaminants. During sample preparation, the final blow down and reconstitution steps were omitted, as these have been shown to result in PFAS contamination. Finally, the LC system was modified to replace the accessible fluoropolymer tubing with PEEK and include a delay column to reduce PFAS contamination from the pumps.

Sample preparation: A series of blanks and 4, 10, and 20 ppq recovery spike samples (n=2) were prepared in 250 mL of Milli-Q water. The sample extraction followed a modified EPA Method 533 procedure, and the solid phase extraction (SPE) cartridges were the Phenonmenex Strata™ X-AW (500 mg). Isotope dilution standards were spiked prior to extraction (final concentration = 10 pg/mL). The final SPE eluent was adjusted to 10 mL of 80:20 methanol/water (0.1% ammonium hydroxide) and an aliquot was transferred to a polypropylene vial for analysis.

Chromatography: The LC system was a SCIEX ExionLC system which had been modified to remove the fluoropolymer tubing. A delay column was used to minimize any remaining PFAS contamination from the pumps. The analytical column was the Gemini C18 (3 µm, 100 x 3 mm, Phenomenex, P/N: 00D-4439-Y0) with a Zorbax Diol guard cartridge (6 µm, 12.5 x 4.6 mm, P/N: 820950-911). The delay column was the Luna Omega PS C18 (5 µm, 50 x 4.6 mm, Phenomenex, P/N: 00B-4753-E0). The mobile phases were water and methanol (modified with 5mM and 2mM ammonium acetate, respectively) with a flow rate of 0.7 mL/min. The column oven was 45ºC, and the injection volume was 100 µL. Gradient conditions are shown in Table 1.

Mass spectrometry: Analysis was performed using the SCIEX 7500 system with an OptiFlow Pro ion source and ESI probe in negative ion mode. The source and MS parameters were set to optimize the response of PFOA and are listed in Table 2. Data were collected using the Scheduled MRM algorithm using compound-specific and source and gas optimized parameters.

Data processing: SCIEX OS software 3.0 was used for data processing. Analyte responses were normalized to their corresponding mass-labeled standard. Calibration curves were weighted as 1/x, using the linear calibration curve without forcing through zero.

Table 1. LC gradient profile used for the analysis of HFPO-DA, PFBS, PFOA and PFOS.

Time (min)	Flow rate (mL/min)	A (%)	B (%)
0	*0.7*	*45*	*55*
1	*0.7*	*45*	*55*
5	*0.7*	*35*	*65*
8	*0.7*	*5*	*95*
8.5	*0.7*	*1*	*99*
12.95	*0.7*	*1*	*99*
13	*0.7*	*90*	*10*
15	*0.7*	*90*	*10*

Table 2. Source, gas, and temperature conditions.

Parameter	Value
Curtain gas (CUR)	*45 psi*
Collision gas (CAD)	*10*
IonSpray Voltage (ISV)	*-1500 V*
Temperature (TEM)	*410 ºC*
Nebulizer gas (GS1)	*35 psi*
Heater gas (GS2)	*70 psi*

Blank evaluation

The extensive contamination mitigation steps during the sample preparation and instrumental analysis resulted in blank levels that were below the 4 ppq spike level, allowing for accurate and unbiased quantification. Specifically, two blanks were analyzed to ensure data quality, the instrument blank and the extraction blank. The instrument blank primarily represents contamination from internal standards and the LC system, whereas the extraction blank represents contamination from the sample preparation procedure. The instrument blanks did not contain any of the analyte peaks except for a minor PFOA peak (m/z 413>169 transition only) which was <25% of the 4 ppq spike. These results indicate negligible PFAS contamination originating from the LC system and the mass-labeled standards. Extraction blanks did not show any detectable PFOS and only minor traces of PFBS (0.5-1.2 ppq). HFPO-DA contamination was limited to 1 out of 4 extraction blanks, in which 14 ppq of HFPO-DA was detected and no peaks were found in the remaining blanks. PFOA was consistently detected in the extraction blanks at an average of 1.9 ppq (range: 0.5-2.7 ppq), which represented 35% of the calculated 4 ppq spike concentration.

Initial experiments following the EPA Method 533 protocol resulted in highly variable blank contamination that was >20 ppq. Each consumable, reagent and apparatus used in the entire workflow was cleaned and tested to achieve minimal background and contamination. While the final method required modifications from the EPA Method 533 extraction procedure, it resulted in significantly lower blank levels and ultimately eliminated all but ~2 ppq of PFOA contamination. Future work will systematically evaluate all stages of the sample preparation process to determine the critical steps in reducing contamination.

Extraction recovery achieves EPA drinking water health advisory levels

Results for the method extraction spikes (n=2) into 250 mL of laboratory water showed excellent recovery and precision at 4 ppq for PFOA, PFBS and HFPO-DA, and at 20 ppq for PFOS (Table 3). Chromatograms for the instrument blanks, extraction blanks and extraction spikes are shown in Figures 1 and 3. Specifically, the mean extraction recoveries at the 4 ppq spike were: 79.3% for HFPO-DA (%CV = 6.2%), 139% for PFBS (%CV = 19.5%) and 138% for PFOA (%CV = 0.8%). The mean recovery for PFOS at the 20 ppq spike level was 113% (%CV = 0.9%). These results indicate that the method can achieve the low ppq EPA drinking water health advisory levels for PFOA and PFOS and is several orders of magnitude lower than the levels for HFPO-DA and PFBS.

Regarding PFOA, the average recovered concentration of the 4 ppq spiked samples was 5.5 ppq. The increased recovery of these samples is presumably due to contributions by lab contamination, as shown by the 1.9 ppq average PFOA concentration in the extraction blanks. Subtracting the blank PFOA level resulted in an average of 90% recovery for the 4 ppq spike.

Table 3. Recovery results for 4, 10 and 20 ppg (pg/L) extraction spikes (n=2).

Compound	4 ppq spike		10 ppq spike		20 ppq spike	
	Mean recovery (%)	%CV	Mean recovery (%)	%CV	Mean recovery (%)	%CV
HFPO-DA	*79.3*	*6.2*	*92.5*	*5.2*	*97.8*	*3.1*
PFBS	*139*	*19.5*	*110*	*8.7*	*105*	*5.7*
PFOA	*138*	*0.8*	*104*	*4.7*	*103*	*3.2*
PFOS	*nd*	*-*	*nd*	*-*	*113*	*9.8*

** Since the PFBS and PFOS were purchased as their potassium and sodium salts, the actual PFBS spikes concentrations were 3.5, 8.8 and 17.7 ppq and the actual PFOS spike concentrations were 3.7, 9.3 and 18.6 ppq.*

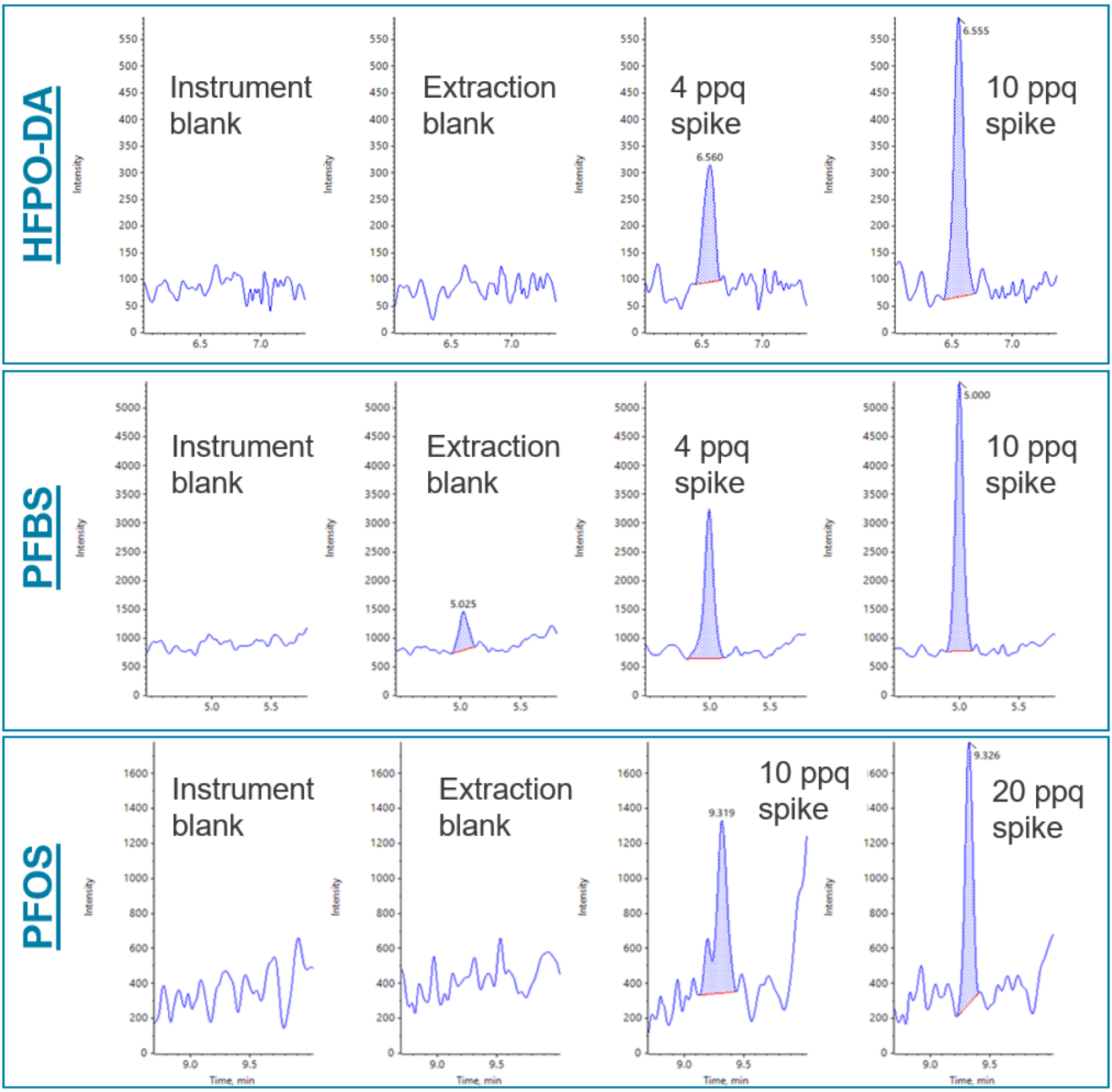

Figure 2. XIC chromatograms for HFPO-DA (m/z 285.0>169.0), PFBS (m/z 298.9>80.0) and PFOS (m/z 499>99.0) quantifier MRM transitions from method extraction samples. Instrument blank, extraction blank, and two spike levels are shown.

Conclusions

This collaborative technical note demonstrated the ability to meet the ultra-trace levels of detection required for the 2022 EPA drinking water health advisory levels for PFAS. Future experiments will include an MDL study at the 4 and 20 ppq (pg/L) spiking levels for PFOA and PFOS, respectively.

The method showed:

- Minimal PFAS contamination using a clean room, modified sample preparation and extensive cleaning
- Simplified sample preparation to increase throughput and reduce contamination by eliminating sample blow down and reconstitution
- The sensitivity of the SCIEX 7500 system to achieve low ppq (pg/L) detection levels

References

1. United States Environmental Protection Agency. “EPA announces new drinking water health advisories for PFAS chemicals, $1 billion in bipartisan infrastructure law funding to strengthen health protections”. June 15, 2022. https://www.epa.gov/newsreleases/epa-announces-new-drinking-water-health-advisories-pfas-chemicals-1-billion-bipartisan
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500 Old Connecticut Path | Framingham, MA 01701 USA
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