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Achieving Low Parts-per-Quadrillion Detection Limits for PFAS Analysis in Drinking Water

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Introduction

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). 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). 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.

Sample Preparation

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.

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 Strata™-X-AW (500 mg/12 mL, Part No.: 8B-S038-HCH). Isotope dilution standards were spiked prior to extraction (final concentration = 10 pg/mL). The final SPE eluent was adjusted to 10 mL of Methanol / Water / Ammonium Hydroxide (80:20:0.1, v/v/v) and an aliquot was transferred to a polypropylene vial for analysis.

LC Conditions

Column: Gemini™ 3 µm C18
Dimensions: 100 x 3.0 mm
Part No.: 00D-4439-Y0
Guard Cartridge: ZORBAX® 6 µm Diol, 12.5 x 4.6 mm
Delay Column: Luna™ Omega 5 µm PS C18
Delay Column Dimensions: 50 x 4.6 mm
Delay Column Part No.: 00B-4753-E0
Mobile Phase: A: 5 mM Ammonium Acetate in Water
B: 2 mM Ammonium Acetate in Methanol

Gradient:

Time (min)	%B
0	55
1	55
5	65
8	95
8.5	99
12.95	99
13	10
15	10

Flow Rate: 0.7 mL/min
Injection Volume: 100 µL
Temperature: 45 °C
LC System: SCIEX® ExionLC™
Detection: MS/MS
Detector: SCIEX 7500, with OptiFlow® Pro Ion Source

MS/MS Conditions

Polarity: Negative
Source Temperature: 410 °C
GS1: 35
GS2: 70
CUR: 45
CAD: 10
ISV: -1500 V

Results and Discussion

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.

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 1**). 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.

Figure 1. Extraction Spikes and Blanks for PFOA. Chromatogram Shows MRM XIC of m/z 413.0>169.0 Transition.

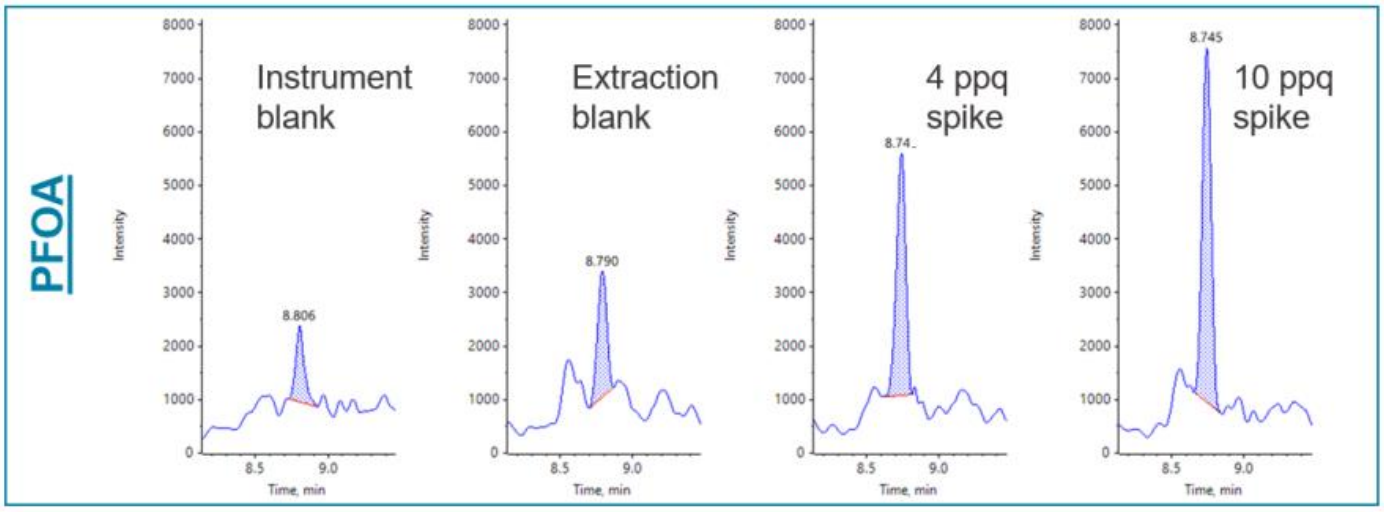
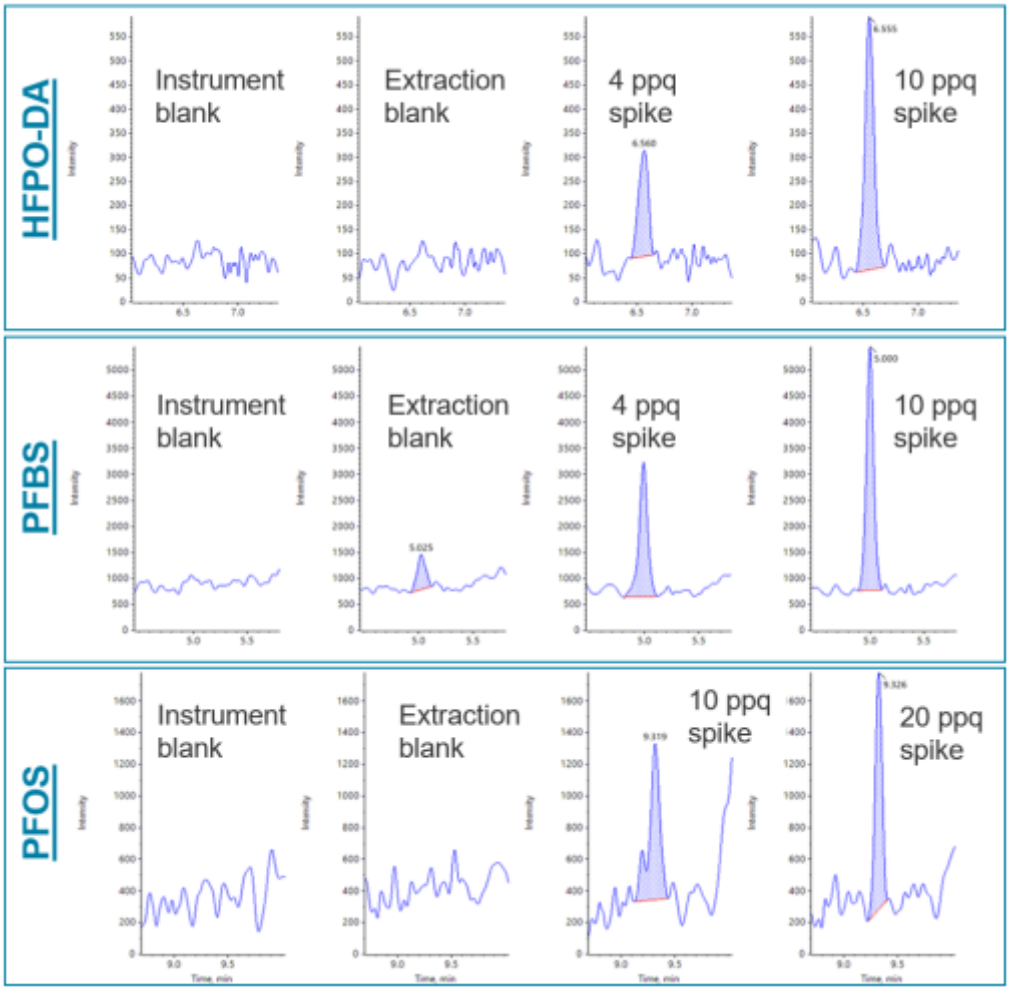


Table 1. Recovery Results for 4, 1, and 20 ppq (pg/L) Extraction Spikes (n=2).

	4 ppq Spike		10 ppq Spike		20 ppq Spike	
Compound	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.



Conclusion

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: 1) minimal PFAS contamination using a clean room, modified sample preparation, and extensive cleaning, 2) simplified sample preparation to increase throughput and reduce contamination by eliminating sample blow down and reconstitution, and 3) the sensitivity of the SCIEX® 7500 system to achieve low ppq (pg/L) detection levels.

Gemini™ Ordering Information

3 µm Microbore, Minibore and Midbore™ Columns (mm)										SecurityGuard™ Cartridges (mm)
Phases	**50 x 1.0**	**20 x 2.0**	**30 x 2.0**	**50 x 2.0**	**100 x 2.0**	**150 x 2.0**	**50 x 3.0**	**100 x 3.0**	**150 x 3.0**	**4 x 2.0*/10pk**
C18	00B-4439-A0	00M-4439-B0	00A-4439-B0	00B-4439-B0	00D-4439-B0	00F-4439-B0	00B-4439-Y0	00D-4439-Y0	00F-4439-Y0	AJ0-7596
C6-Phenyl	—	—	—	00B-4443-B0	00D-4443-B0	00F-4443-B0	00B-4443-Y0	00D-4443-Y0	00F-4443-Y0	AJ0-7914
NX-C18	00B-4453-A0	00M-4453-B0	00A-4453-B0	00B-4453-B0	00D-4453-B0	00F-4453-B0	00B-4453-Y0	00D-4453-Y0	00F-4453-Y0	AJ0-8367

for ID: 2.0 – 3.0 mm

Luna™ Omega Ordering Information

5 µm Analytical Columns (mm)					SecurityGuard Cartridges (mm)
Phases	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**4 x 3.0*/10pk**
Polar C18	00B-4754-E0	00D-4754-E0	00F-4754-E0	00G-4754-E0	AJ0-7601
PS C18	00B-4753-E0	00D-4753-E0	00F-4753-E0	00G-4753-E0	AJ0-7606
C18	00B-4785-E0	00D-4785-E0	00F-4785-E0	00G-4785-E0	AJ0-7612

for ID: 2.0 – 3.0 mm

* SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

PFAS CRM Native Standards. All analytes at the same concentration in acid form for easy calculation and dilution.

Product	Part	Volume	Concentration
EPA 533 mix	AL0-101838	1 mL	2 µg/mL in Methanol
EPA 537.1 mix	AL0-101839	1mL	2 µg/mL in Methanol
EPA 533 + 537.1 mix	AL0-101840	1 mL	2 µg/mL in Methanol

Strata™-X-AW Ordering Information

Strata			
Format	**Sorbent Mass**	**Part Number**	**Unit**
Tube			
	100 mg	8B-S038-EBJ	3 mL (50/box)
	100 mg	8B-S038-ECH	6 mL (30/box)
	200 mg	8B-S038-FBJ	3 mL (50/box)
	200 mg	8B-S038-FCH	6 mL (30/box)
	500 mg	8B-S038-HBJ	3 mL (50/box)
	500 mg	8B-S038-HCH	6 mL (30/box)
Giga™ Tube			
	500 mg	8B-S038-HDG	12 mL (20/box)
	1 g	8B-S038-JDG	12 mL (20/box)
	1 g	8B-S038-JEG	20 mL (20/box)
	5 g	8B-S038-LFF	60 mL (16/box)

Strata tubes available in multiple sizes. Contact Phenomenex for details.

Other Recommended Products for Your PFAS Methods

Description	Part No.
Luna™ Omega Column 3 µm PS C18 50 x 3 mm	00B-4758-Y0
Kinetex™ EVO Column 5 µm C18 100 x 2.1 mm	00D-4633-AN
Strata PFAS (WAX/GCB) SPE 200 mg, /50 mg, /6mL tubes, 30/pk	CS0-9207
Strata SDB-L 500 mg/6mL tubes, 30/pk	8B-S014-HCH
Verex™ Vial, 9 mm Screw, PP, 1.7 mL, 1000/pk	AR0-39P0-13
Verex Vial, 9 mm Screw, PP, 300 µL, 1000/pk	AR0-39P2-13
Verex Vial, 9 mm Screw, PP, 700 µL, 1000/pk	AR0-39P1-13
Vial Cap Verex Cert+ Cap (one piece), 9 mm, PE w/ Starburst pre-Slit, 2mL, 1000/pk	AR0-89P6-13-C

Columns and vials available in multiple sizes. Contact Phenomenex for details.

Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/Chat.

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Have questions or want more details on implementing this method? We would love to help!
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