Application Note

Environmental

Determination of Per- and Polyfluoroalkyl Substances in Soil and Sediment

Using blended Agilent Bond Elut PFAS WAX/Carbon S SPE cartridges for U.S. EPA Method 1633

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Abstract

U.S. Environmental Protection Agency (EPA) Method 1633 provides a standardized protocol for the determination of per- and polyfluoroalkyl substances (PFAS) in environmental matrices. This application note evaluates a modified sample preparation approach using blended dual-phase solid-phase extraction (SPE) cartridges containing Agilent Bond Elut PFAS WAX and Carbon S sorbents for the extraction of 40 PFAS from soil and sediment samples. The method adheres to the performance-based criteria of Method 1633 and meets all quality control requirements including matrix spike recoveries, relative percent differences, and internal standard recoveries. These findings support the use of blended dual-phase SPE cartridges as a viable alternative for PFAS extraction in solid environmental matrices.

Introduction

U.S. EPA Method 1633 was developed to standardize the determination of PFAS in aqueous, solid, and tissue samples.[1] The method was validated through multilaboratory studies using a two-step extraction process using polymeric weak anion exchange (WAX) SPE followed by matrix reduction with loose carbon. Since its release, many laboratories have adopted a simplified one-step approach by combining WAX and carbon sorbents into a single dual-phase cartridge with layered sorbents. This dual-phase SPE streamlines sample preparation by integrating analyte extraction and matrix reduction into a single step.

As a performance-based method, EPA 1633 allows procedural modifications provided they comply with 40 CFR Part 136.6, which mandates documentation and adherence to quality acceptance criteria. An alternative to layering is blending the sorbents, which simplifies cartridge design and eliminates the need for a separating frit. Blended sorbents are applicable to all matrix types specified in the method.

An earlier application note comparing blended versus layered sorbents demonstrated equivalent performance for PFAS extraction from aqueous environmental samples, meeting the quality control requirements of EPA Method 1633.[2] In this application note, Agilent blended dual-phase PFAS WAX/Carbon S SPE cartridges, 200/50 mg, 6 mL, were evaluated for the extraction of 40 PFAS from soil and sediment samples following the solid sample procedure in EPA Method 1633. Results show that all quality control criteria were met using the blended dual-phase cartridges.

Experimental

Chemicals and reagents

Native PFAS standards and isotopically labeled analogs were purchased as kits from Wellington Laboratories, Inc. (Guelph, ON, Canada). Agilent InfinityLab methanol (MeOH) for LC/MS (part number 5191-5111) and InfinityLab acetonitrile (ACN) (part number 5191-5101) were used to prepare the reagents and mobile phase. Reagent-grade acetic acid, ammonium acetate, formic acid, isopropanol (IPA), and ammonium hydroxide were from Sigma-Aldrich (St Louis, MO, USA). Reagent water was prepared using a Milli-Q 7003 purification system from MilliporeSigma (Burlington, MA, USA).

Solutions

All solutions used in the analysis were prepared in accordance with U.S. EPA Method 1633. Calibration standards were prepared in a solvent mixture consisting of 4% reagent water, 1% ammonium hydroxide, and 0.625% acetic acid in methanol. Soil samples were extracted using 0.3% (v:v) ammonium hydroxide in methanol. Prior to sample loading, SPE cartridges were conditioned with 0.3 M formic acid in water and subsequently eluted with 1% ammonium hydroxide in methanol. Sample containers were rinsed with a 1:1 solution of 0.1 M formic acid in water and methanol to minimize potential contamination and ensure complete sample recovery.

Samples

Topsoil was obtained from a local home gardening retailer, and sediment samples were collected from a nearby river in Wilmington, DE, USA.

Standards and spiking solutions

All solutions required for sample extraction and standard preparation were prepared according to the protocols outlined in U.S. EPA Method 1633. Table 1 lists the nominal calibration concentrations for native PFAS analytes, extracted internal standards (EIS), and nonextracted internal standards (NIS). For analytes available as salts, nominal concentrations were converted to their corresponding acid forms to ensure consistency in reporting.

Table 1. Calibration level concentrations.

Compounds	Level Concentration (ng/mL)							
	1	2	3	4	5	6	7	8
Native PFAS								
PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrDA, PFTeDA, PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFDoS, PFOSA, NMeFOSA, NEtFOSA, NMeFOSAA, NEtFOSSA	0.02	0.05	0.13	0.25	0.50	1.0	2.0	2.5
PFPeA, PFMPA, NFDHA, PFMBA, PFEESA	0.04	0.10	0.25	0.50	1.0	2.0	4.0	5.0
PFBA, 4:2FTS, 6:2FTS, 8:2FTS, HFPO-DA, ADONA, 9Cl-PF3ONS, 11CL-PF3OUdS	0.08	0.20	0.50	1.0	2.0	4.0	8.0	10.0
NMeFOSE, NEtFOSE	0.20	0.50	1.25	2.50	5.0	10.0	20.0	25.0
3:3FTCA	0.25	0.63	1.25	2.5	5.0	10.0	12.5	--
5:3FTCA, 7:3FTCA	0.50	1.25	3.13	6.25	12.5	25.0	50.0	62.5
EIS								
$^{13}C_2$-PFDoA, $^{13}C_2$-PFTeDA, $^{13}C_6$-PFDA, $^{13}C_7$-PFUnA, $^{13}C_9$-PFNA	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
$^{13}C_3$-PFBS, $^{13}C_3$-PFHxS, $^{13}C_4$-PFHpA, $^{13}C_5$-PFHxA, $^{13}C_8$-PFOA, $^{13}C_8$-PFOS, $^{13}C_8$-PFOSA, D_3-NMeFOSA, D_5-NEtFOSA	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
$^{13}C_2$-4:2FTS, $^{13}C_2$-6:2FTS, $^{13}C_2$-8:2FTS, $^{13}C_5$-PFPeA, D_3-NMeFOSAA, D_5-NEtFOSAA	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
$^{13}C_3$-HFPO-DA, $^{13}C_4$-PFBA	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
D_7-MeFOSE, D_9-EtFOSE	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
NIS								
$^{13}C_5$-PFNA, $^{13}C_2$-PFDA	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
$^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{18}O_2$-PFHxS, $^{13}C_4$-PFOS	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
$^{13}C_3$-PFBA	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40

For soil and sediment extractions, matrix spikes (MS) and matrix spike duplicate (MSD) samples were prepared at midlevel concentrations, as listed in Table 2. The spiking concentrations for the isotopically labeled EIS and NIS were selected to match the concentrations present in the calibration standards (Table 1).

Table 2. Native PFAS spiking concentrations.

Compounds	Spike Concentration (ng/g)
PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrDA, PFTeDA, PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFDoS, PFOSA, NMeFOSA, NEtFOSA, NMeFOSAA, NEtFOSSA	0.25
PFPeA, PFMPA, NFDHA, PFMBA, PFEESA	0.50
PFBA, 4:2FTS, 6:2FTS, 8:2FTS, HFPO-DA, ADONA, 9Cl-PF3ONS, 11CL-PF3OUdS	1.0
NMeFOSE, NEtFOSE	2.5
3:3FTCA	1.25
5:3FTCA, 7:3FTCA	6.3

Instrumentation and method

Sample analysis was performed using an Agilent Infinity II LC system consisting of an Agilent 1290 Infinity II high-speed pump (G7120A), an Agilent 1260 Infinity II hybrid multisampler (G7167C), and an Agilent 1290 Infinity II multicolumn thermostat (G7116B). The LC system was modified for PFAS analysis using the Agilent InfinityLab PFC-free HPLC conversion kit (part number 5004-0006). The LC system was coupled to an Agilent 6475A triple quadrupole LC/MS equipped with an Agilent Jet Stream Electrospray ion source. Agilent MassHunter Workstation software, version 12.1 update 3 and analysis version 12.1 update 2, were used for data acquisition. The Agilent extended PFAS MRM Database for LC/TQ (G1736AA) was used for optimized MRM settings. The optimized LC, hybrid multisampler, and ion source conditions are listed in Tables 3, 4, and 5, respectively. The hybrid multisampler was operated in classic flow-through mode with extended inner and outer wash enabled.

Table 3. LC conditions.

Parameter	Value
Column Temperature	50 ± 5 °C
Flow Rate	0.400 mL/min
Mobile Phases	A) 5 mM ammonium acetate in 95:5 water:ACN B) ACN
Gradient	Time (min) %A %B 0.00 98.00 2.00 0.20 98.00 2.00 11.00 0.00 100.00 13.00 0.00 100.00 13.10 98.00 2.00
Delay Column	Agilent InfinityLab PFC Delay Column 4.6 × 30 mm (p/n 5062-8100)
Guard Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm (p/n 959757-902)
Analytical Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959758-902)

Table 4. Hybrid multisampler conditions.

Parameter	Setting			
Injection Volume	2 µL			
Draw Speed	200 µL/min			
Eject Speed	200 µL/min			
Wait Time After Draw	3.0 s			
Wash Steps	**Step**	**Task**	**Solvent**	**Duration/ Volume**
	1	Inner wash	1:1 IPA:ACN	150 µL
	2	Inner wash	Mobile Phase B	150 µL
	3	Seat wash	1:1 IPA:ACN	150 µL
	4	Seat wash	Mobile Phase B	150 µL
	5	Reconditioning	Mobile Phase A	–
	Draw sample			
	1	Outer wash	1:1 IPA:ACN	10 s
	2	Outer wash	Mobile Phase B	5 s
	Injection			

Table 5. Ion source conditions.

Parameter	Setting
Polarity	Negative
Gas Temperature	230 °C
Gas Flow	8 L/min
Sheath Gas Flow	10.0 L/min
Nebulizer Pressure	15 psi
Sheath Gas Temperature	355 °C
Capillary Voltage	2,500 V
Nozzle Voltage	0

Supplies and consumables

The PFAS-suitable consumables and supplies used for the PFAS extraction and analysis are listed in Table 6.

Table 6. PFAS-suitable supplies and consumables.

Agilent Consumables and Supplies	Agilent Part Number
Blended PFAS WAX/Carbon S SPE Cartridge, 200/50 mg, 6 mL	5610-2245
Polypropylene autosampler screw top vials, 2 mL	5191-8121
Polypropylene/silicone septa screw cap, 9 mm	5191-8151
Centrifuge tubes and caps, 50 mL	5610-2039
Centrifuge tubes and caps, 15 mL	5610-2039
Empty SPE cartridges, 60 mL	12131012
SPE adapters	12131001
Glass wool, silanized	8500-1572
Captiva premium nylon syringe filter, 25 mm diameter, 0.2 µm pore size	5190-5092
Vac Elut SPS 24 manifold with collection rack for 10 × 75 mm test tubes	12234003
Collection rack and funnel set for 12 or 15 mL conical tubes, for Vac Elut SPS 24 manifold	12234027
Vac Elut polypropylene stopcock valves	12234520

Calibration and quantitation

Quantitation was performed using stable-isotope dilution methodology, where the responses and concentrations of native PFAS compounds were measured relative to those of the EIS. The EIS responses and concentrations were, in turn, measured relative to the NIS. Isotopically labeled reference compounds used for native PFAS and EIS matched those listed in Table 10 of EPA Method 1633. Calibration curves were constructed using a 1/x weighted linear least squares regression model, constrained to include the origin (0,0), for all analytes. For PFAS compounds with branched isomers, individual isomer responses were summed to yield a total response. PFAS standards supplied as salts were corrected to reflect the acid form concentrations. The limit of quantitation (LOQ) was defined as the concentration of the lowest calibrator, as shown in Table 1.

Cartridge blank determination

Cartridge blank determinations were performed to evaluate potential residues in the sorbent blend, cartridge tubes, and frits that could interfere with PFAS extraction recoveries. Cartridges were rinsed under vacuum with 5 mL of 1% ammonium hydroxide in methanol, and the rinseate was collected. To neutralize the solution, 25 µL of acetic acid was added, followed by vortexing for 1 minute. EIS and NIS were then added, and the solution was vortexed for an additional minute before analysis by LC/MS/MS.

Sample preparation

Sample preparation closely followed the extraction procedure for solid matrices outlined in EPA Method 1633, with modifications for dual-phase cartridges, as illustrated in Figure 1.

For topsoil samples, the moisture content was approximately 37%, requiring an 8 g sample to achieve a 5 g dry weight equivalent. For sediment samples, with a moisture content of approximately 24%, a 6.6 g sample was used to achieve the same dry weight.

Sample Preparation

1. Weigh 5 g (dry weight) of the solid sample into a 50 mL centrifuge tube.
2. Add EIS directly to the sample and equilibrate for 30 minutes.
3. Spike with native PFAS for MS and MSD samples, if required.

Extraction

1. Add 10 mL of 0.3% ammonium hydroxide in MeOH to each sample. Vortex for 10 minutes, centrifuge at 2,800 rpm for 10 minutes, then decant into another clean 50 mL centrifuge collection tube.
2. Repeat with another 15 mL of 0.3% ammonium hydroxide and transfer to the collection tube.
3. Repeat with 5 mL of 0.3% ammonium hydroxide and transfer to the collection tube.
4. Concentrate the extract (~30 mL) at 55 °C under a gentle stream of nitrogen to 7.5 mL final volume.
5. Add reagent water to bring volume up to 50 mL, then vortex. Check that the pH is within 6.5 ± 0.5 and adjust if necessary.

SPE Conditioning

1. Loosely pack glass wool to half the height of the Agilent PFAS WAX/Carbon S SPE cartridges and place on the manifold (For the Agilent Vac Elut SPS 24, rotate the cowling to the waste position).
2. Rinse with 5 mL of 1% ammonium hydroxide in MeOH.
3. Add adapters and 60 mL empty SPE reservoirs.
4. Rinse twice with 5 mL of 0.3 M formic acid.

Loading the Samples

1. Pour samples into reservoir.
2. Pass samples through cartridge using approximately 3 to 5 in Hg vacuum pressure and adjust stopcocks to achieve a flow rate of 5 mL/min.

Rinsing the Reservoir

1. Rinse sample containers with 2 × 5 mL reagent water and add to reservoir.
2. After the sample has been loaded, rinse the sample containers, reservoir, and cartridges with 5 mL of 1:1 0.1 M formic acid:MeOH. (Be sure to rinse the container and reservoir sidewalls with the solution.)
3. Dry under vacuum for 15 seconds, then turn the vacuum off and close the stopcocks.

Adding Internal Standard

Add NIS to clean collection tubes (15 mL centrifuge tube) and place in the vacuum manifold collection rack (For the Vac Elut SPS 24, rotate the cowling to the collect position).

Elution

1. Thoroughly rinse sample bottles with 5 mL of 1% ammonium hydroxide in MeOH eluent.
2. Transfer eluent to the reservoirs. Use a polymer transfer pipette to rinse the reservoir sidewalls.
3. Turn the vacuum on and adjust the stopcocks to collect eluate at a flow of 5 mL/min.
4. Neutralize the eluate with 25 µL of acetic acid.

Filtration

1. Install an Agilent Captiva premium nylon syringe filter on a 5 mL polymer syringe.
2. Decant sample supernatant into the syringe barrel.
3. Filter samples into another clean 15 mL centrifuge tube.

Analysis

1. Transfer a small portion of the eluate into a 2 mL polymer autosampler vial.
2. Analyze by LC/MS/MS.

Figure 1. Sample preparation procedure.

Results and discussion

Cartridge blank determination

Three replicate cartridge washes were analyzed to assess the presence of any interfering PFAS residues originating from the sorbent blend, cartridge tubes, or frits. Each cartridge was rinsed with 5 mL of 1% ammonium hydroxide in methanol under vacuum, and the rinseate was collected for analysis. As shown in Figure 2, the green dashed line represents the LOQ, while the red dashed line indicates half the LOQ for each native PFAS. All measured residues in the replicates were below the half LOQ threshold, demonstrating a high level of cleanliness in the SPE cartridges.

Soil and sediment analysis

Topsoil and river sediment samples were analyzed in duplicate. Table 7 lists the native PFAS compounds detected above the LOQ, along with the relative percent deviation (RPD) between replicates. For most compounds, RPD values were well below 30%, indicating good reproducibility. Exceptions included 6:2 FTS and PFTrDA in the topsoil sample, which exhibited slightly elevated RPDs above 30%. Compounds detected in only one of the two replicates were present at concentrations near the LOQ; in these cases, one replicate fell just below the LOQ and was therefore not reported.

Table 7. Concentration of native PFAS determination in soil and sediment samples.

Topsoil				River Sediment			
Target	Sample 1 (ng/g)	Sample 2 (ng/g)	RPD (%)	Target	Sample 1 (ng/g)	Sample 2 (ng/g)	RPD (%)
PFBA	0.097	0.088	10%	6:2FTS	1.34	1.58	18%
PFPeA	0.082	0.089	8%	PFOA	0.039	0.036	8%
PFHxA	0.128	0.113	12%	PFOS	0.081	0.068	18%
PFBS	0.055	0.054	2%	PFUnDA	0.066	0.070	6%
PFHpA	0.057	0.061	7%	PFNS	0.032	< LOQ	--
6:2FTS	0.981	0.714	31%	PFDoA	0.030	0.029	2%
PFOA	0.222	0.203	9%	PFTrDA	0.042	0.048	13%
PFNA	0.172	0.173	0%				
PFDA	0.087	0.090	4%				
PFOS	0.315	0.352	11%				
PFUnDA	0.085	0.084	1%				
PFDoDA	0.037	0.036	5%				
PFTrDA	0.022	0.030	31%				
PFTeDA	0.021	< LOQ	--				

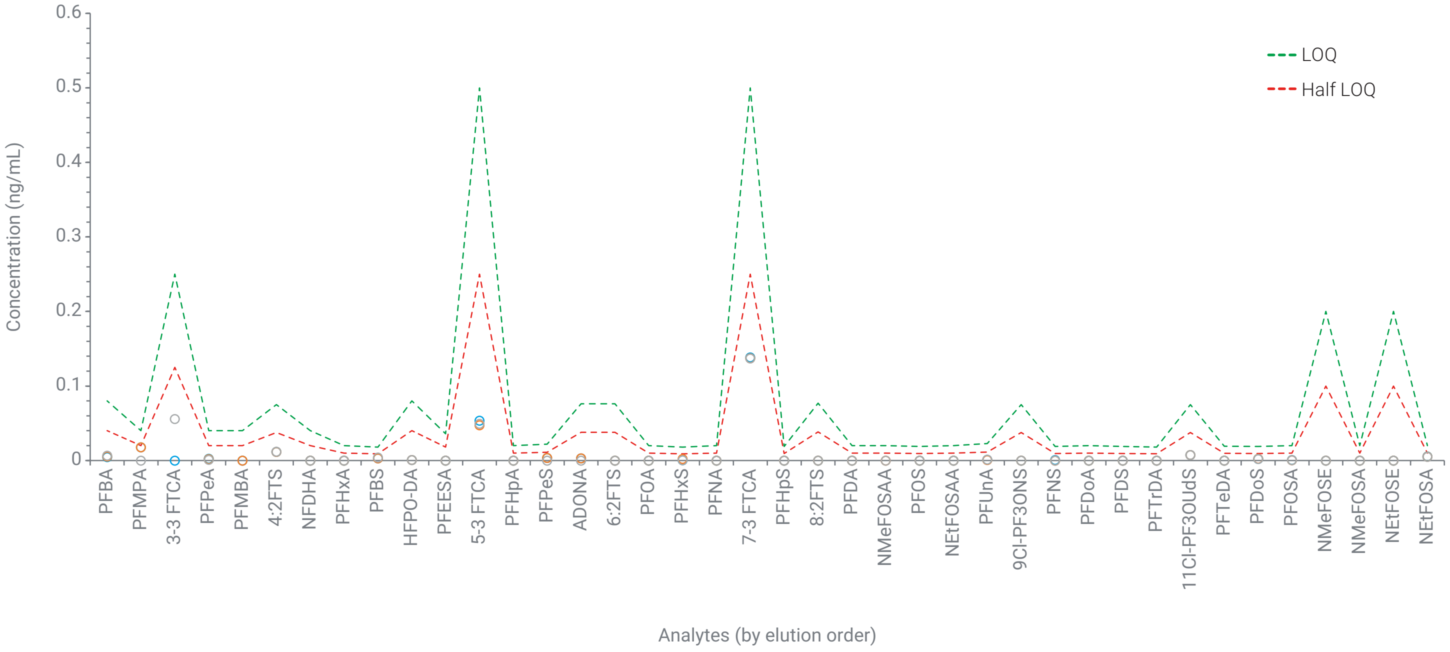

Figure 2. Cartridge blank analysis with three replicates represented by blue, orange, and gray circles. The LOQ for each native PFAS is indicated by the green hashed line and half LOQ by the red hashed line.

Matrix spikes

MS and MSD samples were prepared and analyzed for both topsoil and river sediment at midlevel concentrations, as listed in Table 2. Recoveries and RPDs for native PFAS in these samples are presented in Figure 3. The shaded regions in the figure represent the ongoing precision and recovery (OPR) limits for native PFAS in solid matrices, as specified in Table 7 of EPA Method 1633, along with the ≤ 30% RPD threshold defined in the Department of Defense Data Validation Guidelines, Module 6.3 All MS and MSD results demonstrated native PFAS recoveries and reproducibility within the established OPR and RPD limits, confirming the reliability of the method for both matrix types.

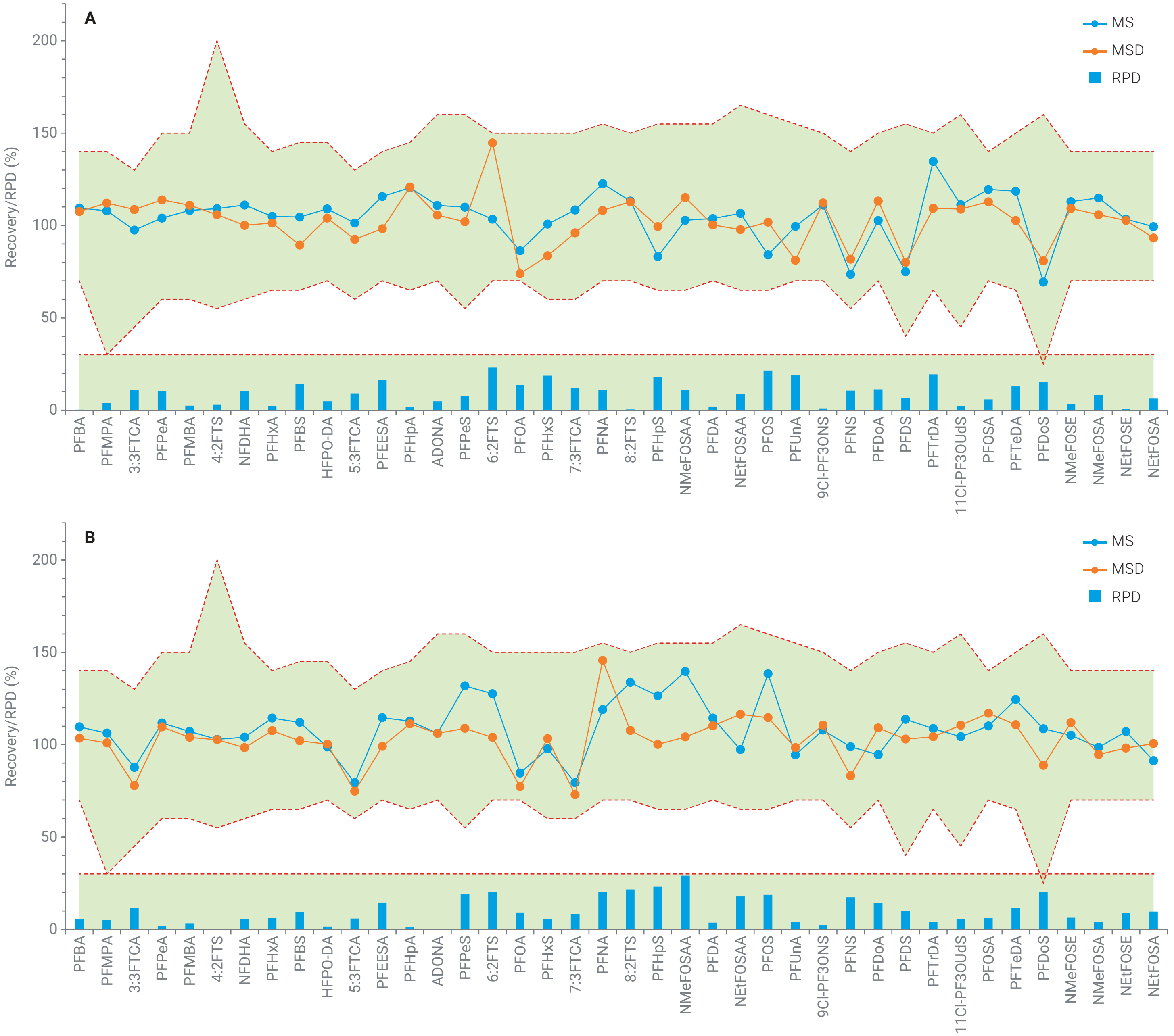

Figure 3. Topsoil (A) native PFAS spike recoveries of MS (blue circles), MSD (orange circles), and RPD (blue bars). River sediment (B) native PFAS spike recoveries of MS (blue circles), MSD (orange circles) and RPD (blue bars). Green areas indicate OPR and RPD acceptance limits.

EIS and NIS recoveries

EPA Method 1633 requires reporting of EIS and NIS recoveries for all field samples. Figure 4 presents the recoveries of both EIS and NIS for duplicate topsoil and sediment samples, as well as for the MS and MSD samples for each matrix.

The shaded region in the figure represents the acceptance interval specified in Table 8 of Method 1633. All EIS and NIS recoveries fall within this acceptance range, confirming method performance across sample types.

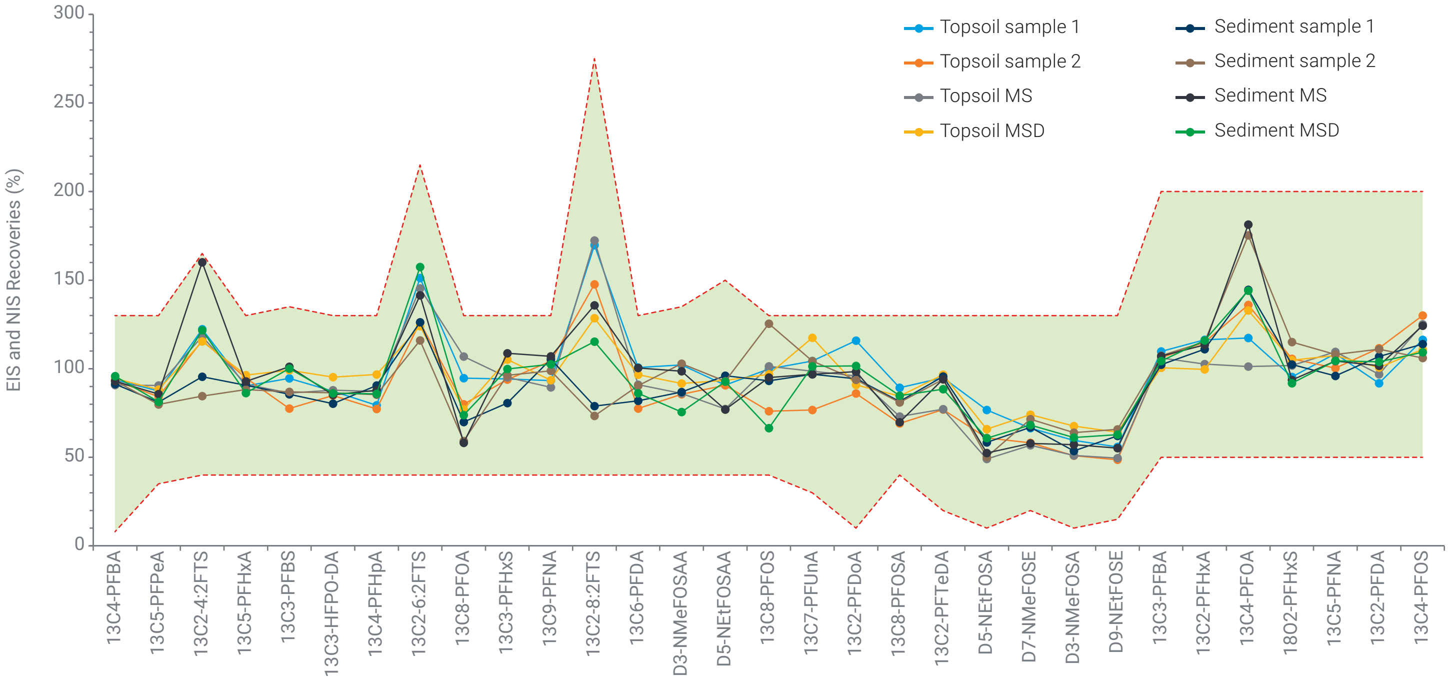

Figure 4. EIS and NIS recovery for topsoil sample 1 (light blue), topsoil sample 2 (orange), topsoil MS (gray), topsoil MSD (yellow), sediment sample 1 (dark blue), sediment sample 2 (brown), sediment MS (black), sediment MSD (dark green).

Conclusion

The use of blended dual-phase Agilent Bond Elut PFAS WAX and Carbon S SPE cartridges offers a streamlined and effective alternative to layered sorbent configurations for PFAS extraction from soil and sediment samples. All method performance criteria outlined in EPA Method 1633 were met, including acceptable recoveries and reproducibility for matrix spikes and internal standards. This approach simplifies sample preparation while maintaining analytical integrity, supporting its implementation in routine environmental monitoring workflows.

References

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3. U.S. Department of Defense. *Data Validation Guidelines Module 6: Data Validation Procedure for Per- and Polyfluoroalkyl Substances Analysis by QSM Table B-24*. Environmental Data Quality Workgroup, October 2022. https://www.denix.osd.mil/edqw/denix-files/sites/43/2023/02/Module-6-Data-Validation-Guidelines-1633-PFAS-Final-1.pdf

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DE-008952

Printed in the USA, August 22, 2025
5994-8290EN

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