Application Note

Environmental

The Determination of 40 Per- and Polyfluoroalkyl Substances in Biosolids

Using dual-phase Agilent Bond Elut blended PFAS WAX/Carbon S solid phase extraction cartridges, followed by post extraction matrix reduction with Agilent Captiva EMR PFAS Food II cartridges

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Abstract

This study evaluates a modified EPA Method 1633 sample preparation workflow for the quantitation of per- and polyfluoroalkyl substances (PFAS) in biosolids using NIST SRM 2781 sewage sludge as a matrix analog. Dual-phase Agilent Bond Elut blended PFAS WAX/Carbon S solid phase extraction (SPE) cartridges containing 200 mg PFAS weak anion exchange (WAX) and 50 mg Carbon S demonstrated greater permeability than layered cartridges of the same bed mass and were less susceptible to clogging from fine matrix particulates. Further matrix reduction was achieved by replacing the syringe filtration step outlined in EPA Method 1633 with a post extraction cleanup using Agilent Captiva Enhanced Matrix Removal (EMR) PFAS Food II cartridges. Recovery accuracy and precision were assessed across 40 PFAS targets, with and without EMR cleanup. EMR-treated extracts showed improved recovery consistency, with several outliers in non-EMR extracts attributed to coelution interferences and signal suppression. These findings demonstrate that combining blended SPE sorbents with EMR cleanup enhances PFAS quantitation in biosolids and supports broader adoption of this approach for complex solid matrices.

Introduction

The U.S. Environmental Protection Agency (EPA) developed Method 1633[1] to standardize sample preparation and analytical procedures for detecting PFAS across a broad range of environmental matrices, including wastewater, surface water, groundwater, landfill leachate, soil, fish and shellfish tissue, and biosolids. Biosolids, derived from treated sewage sludge, are regulated under 40 CFR Part 503[2] and are commonly applied as fertilizers or soil amendments in accordance with EPA guidelines.[3] These materials are compositionally complex, containing organic matter of approximately 24 to 42% protein, 7 to 18% carbohydrate, and 1 to 14% lipid. Inorganic constituents account for roughly 23 to 45% of the total mass and may include elevated concentrations of heavy metals.[4]

For this study, NIST SRM 2781[5] domestic sewage sludge was selected as a biosolids matrix analog, as it is the only commercially available certified reference material for sewage sludge[6], and includes reference values for six PFAS compounds. The sample's compositional complexity presents analytical challenges when applying EPA Method 1633. High organic content can interfere with LC/MS/MS analysis by introducing isobaric interferences or suppressing electrospray ionization of target ions, potentially leading to failure of method quality control criteria. Additionally, the sample's fine particle size (≤ 74 µm, 200 mesh) can contribute to clogging of SPE media.

To address these particulate and chemical interferences, a novel sample preparation approach was investigated. A dual-phase SPE cartridge containing a single layer of blended WAX and Carbon S sorbents was used for the initial extraction, following the EPA Method 1633 protocol for solid samples. Compared to traditional dual-layer cartridges, the blended format offers improved permeability and reduced susceptibility to clogging. Following WAX extraction, the eluate was passed through a matrix-reduction cartridge to further minimize chemical interferences prior to LC/MS/MS analysis.

The matrix-reduction cartridges selected for this study were Captiva EMR PFAS Food II cartridges, which utilize a mixed-mode passthrough cleanup approach. This design effectively removes complex matrix co-extractives while maintaining the acceptable recoveries of PFAS analytes. In another study, an alternative workflow combining QuEChERS extraction with EMR cleanup using Captiva EMR PFAS Food II demonstrated acceptable quantitation performance for all 40 PFAS analytes listed in EPA Method 1633 while adhering to the method's stringent quality control criteria.[7]

Although this approach offers both effective performance and a streamlined workflow, it deviates from the standard sample preparation protocol outlined in EPA Method 1633. The method developed in this study remains consistent with EPA procedure, with one key modification: replacing the syringe filtration step with EMR cleanup.

In this study, 0.1 g samples of SRM 2781 were extracted in accordance with EPA Method 1633. The flow characteristics and permeability of SPE cartridges were evaluated by comparing layered and blended formats. Following SPE extraction, target recovery accuracy and precision were assessed with and without matrix reduction using Captiva EMR PFAS Food II cartridges. The passthrough cleanup significantly improved both accuracy and precision. Overall, the combination of blended sorbents and post extraction cleanup presents an effective strategy for overcoming analytical challenges in complex biosolids matrices.

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, U.S.). Reagent water was prepared using a Milli-Q 7003 purification system from MilliporeSigma (Burlington, MA, U.S.).

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. Biosolid 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 mol formic acid in water and methanol to minimize potential contamination and ensure complete sample recovery.

Sample

The NIST SRM 2781domestic sludge was purchased from MilliporeSigma (Burlington, MA, U.S.).

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.

Matrix-spiked 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 calibration standards in the final 5 mL extract (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

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	0.10	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

SPE permeability and clogging studies

Permeability and clogging assessments of the SPE cartridges listed in Table 3 were conducted using an Agilent VacElut SPS 24 manifold (Table 7) equipped with stopcock valves. To evaluate permeability, each cartridge was first primed with 5 mL of MilliQ water at 23 °C, drawn through under vacuum (8 inHg) until the meniscus reached the top frit. This step ensured proper wetting of the sorbent bed. Subsequently, an additional 5 mL water was added, and flow was initiated by opening the stopcock while simultaneously starting a stopwatch. The time taken for the water to reach the top frit was recorded, after which the flow was halted. This procedure was repeated in 5 mL increments until a total of 50 mL had passed through each cartridge. The length and diameter of the packed beds within the cartridges were measured using calipers to the nearest 0.01 mm.

For clogging studies, 0.1 g of biosolid was extracted in accordance with EPA Method 1633, as illustrated in Figure 1. Flow measurements were conducted using the same procedure as the permeability tests, but under a slightly reduced vacuum pressure of 5 inHg.

Table 3. Cartridges used for permeability and clogging studies.

Product Description	Part Number
Agilent Bond Elut PFAS WAX SPE cartridge, 150 mg, 6 mL	5610-2150
Agilent Bond Elut blended PFAS WAX/Carbon S SPE cartridge, 200/50 mg, 6 mL	5610-2245
Layered commercial benchmark WAX/GCB, 200/50 mg, 6 mL	NA
Agilent Bond Elut layered PFAS WAX/Carbon S SPE cartridge, 200/50 mg, 6 mL	5610-2238
Agilent Bond Elut layered PFAS WAX/Carbon S SPE cartridge, 500/50 mg, 6 mL	5610-2239
Agilent Bond Elut layered Carbon S/PFAS WAX SPE cartridge, 50/200 mg, 6 mL	5610-2241
Layered commercial benchmark GCB/WAX 50/200 mg, 6 mL	NA

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 PFAS analysis 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) was 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 4, 5, and 6, respectively. The hybrid multisampler was operated in classic flow-through mode with extended inner and outer wash enabled.

Table 4. 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 (part number 5062-8100)
Guard Column	Agilent ZORBAX RRHD Eclipse Plus C18 column, 2.1 × 50 mm, 1.8 µm (part number 959757-902)
Analytical Column	Agilent ZORBAX RRHD Eclipse Plus C18 column, 2.1 × 100 mm, 1.8 µm (part number 959758-902)

Table 5. 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 6. 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 for extraction studies

The PFAS-suitable consumables and supplies used for the PFAS extraction and analysis are listed in Table 7.

Table 7. Agilent PFAS-suitable supplies and consumables.

Product Description	Part Number
Bond Elut blended PFAS WAX/Carbon S SPE cartridge, 200/50 mg, 6 mL	5610-2245
Captiva EMR PFAS Food II cartridge, 750 mg, 6 mL	5610-2232
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 tubes, 60 mL	12131012
SPE adapters	12131001
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 except for 4:2, 6:2, and 8:2 FTS in which a quadratic model was used. 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.

Sample preparation

Sample preparation closely followed the extraction procedure for solid matrices outlined in EPA Method 1633, with modifications to accommodate dual-phase cartridges and EMR cleanup, as illustrated in Figure 1. Samples were prepared in triplicate. The moisture content of the biosolid sample was not measured prior to analysis, and no dry weight correction was applied to the 0.1 g sample mass when reporting PFAS concentrations.

To evaluate the resilience of the SPE cartridges to particulate contamination, the SPE tubes were intentionally left unpacked with glass wool. Additionally, the final eluate filtration step using a nylon membrane was replaced with the Captiva EMR cleanup step. Samples were analyzed before and after the Captiva EMR cleanup to assess the performance of the matrix reduction.

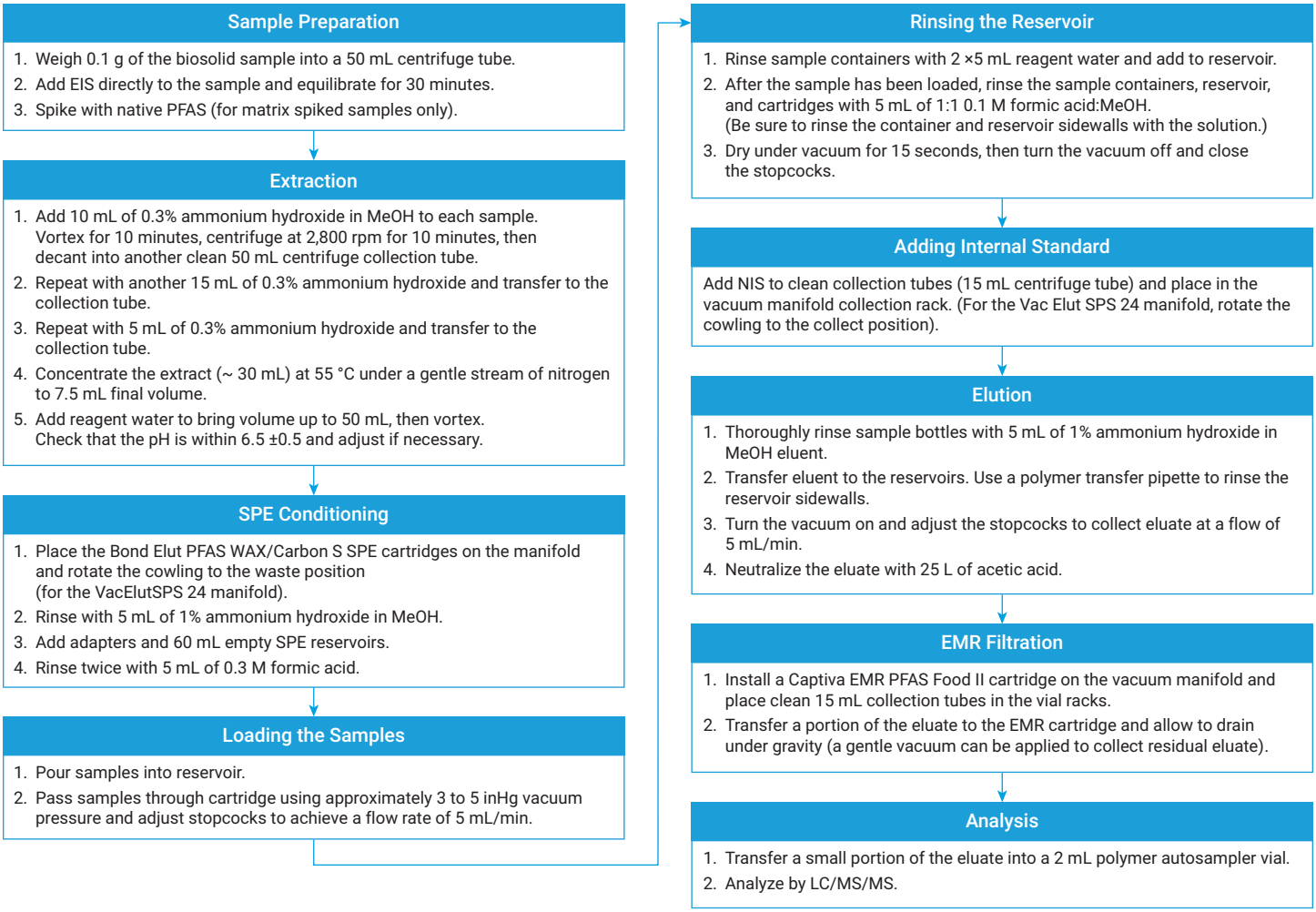

Figure 1. Sample preparation procedure.

Results and discussion

Permeability studies

To determine flow rates, incremental 5 mL volumes of water were passed through each cartridge, and the corresponding times were recorded. Flow rates were calculated from the slope of the resulting curves showing volume versus time (Figure 2). The Agilent Bond Elut PFAS WAX, 150 mg cartridge and Agilent Bond Elut PFAS WAX/Carbon S, 500/50 mg cartridge served as controls. As expected, the 150 mg cartridge exhibited the highest flow rate, while the PFAS WAX/Carbon S, 500/50 mg cartridge showed the lowest.

Notably, the dual-phase Agilent Bond Elut blended PFAS WAX/Carbon S, 200/50 mg cartridge demonstrated a flow rate closer to that of the single-phase 150 mg bed, outperforming the dual-phase Agilent Bond Elut layered PFAS WAX/Carbon S, 200/50 mg configuration. This suggests that blending sorbents can enhance permeability compared to layered arrangements, even when the total sorbent mass remains constant.

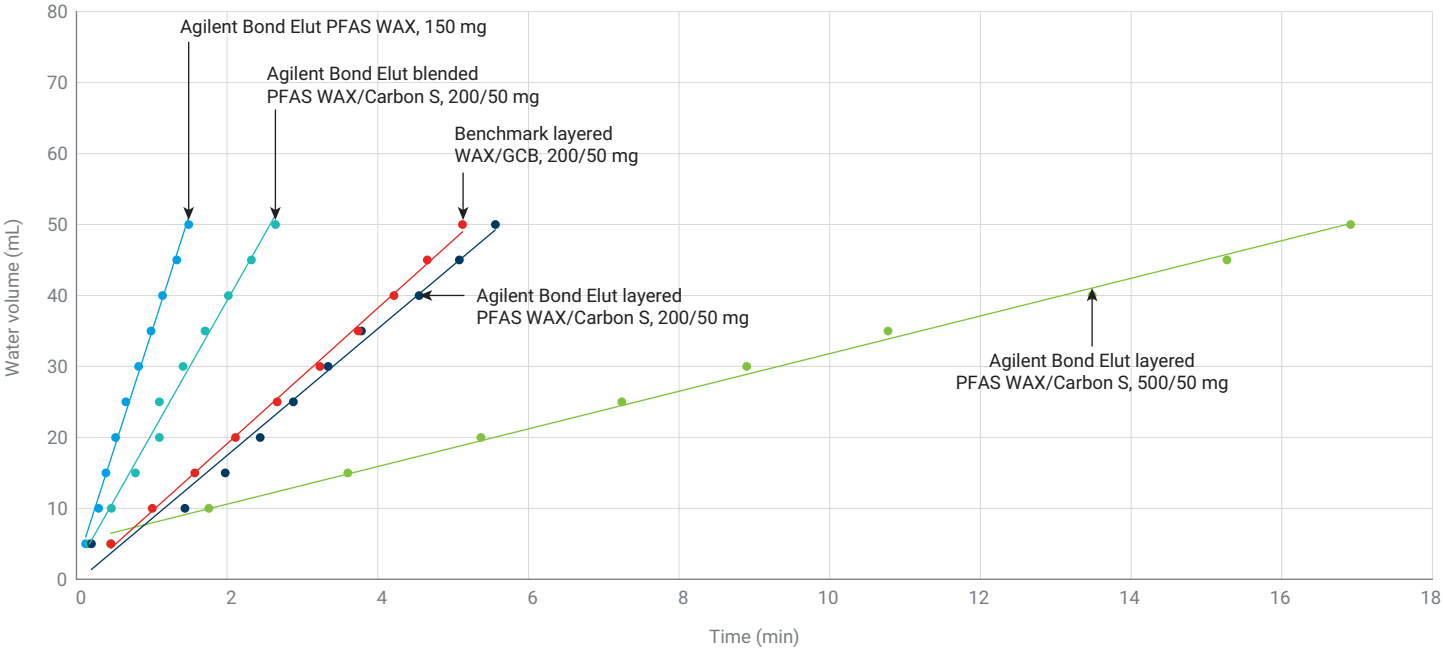

Figure 2. Water flow rate measurements for SPE cartridges with different bed masses and configurations.

Clogging studies

To evaluate the clogging resistance of different cartridge formats, experiments similar to the permeability tests were conducted using extracted matrix. In these studies, 0.1 g of SRM 2781 was extracted following EPA Method 1633. After evaporation and dilution, the final extract volume was 50 mL, consisting of approximately 20% MeOH and 80% water. Incremental 5 mL portions were applied sequentially, with the time required to pass each portion recorded.

Figure 3 presents the results for three cartridges: the dual-phase Agilent Bond Elut blended PFAS WAX/Carbon S, 200/50 mg cartridge; the dual-phase Agilent Bond Elut layered Carbon S/PFAS WAX, 50/200 mg cartridge; and a benchmark GCB/ WAX, 50/200 mg cartridge. (In the layered formats, carbon was positioned on top, as is typical for processing solid samples.) The blended Bond Elut cartridge maintained a higher flow rate than both layered configurations. In contrast, the benchmark cartridge exhibited nonlinear flow behavior after approximately 20 mL of extract was loaded, indicating clogging within the packed bed.

SRM 2781 accuracy and precision

The NIST SRM 2781 certificate of analysis provides reference mass fraction values for six PFAS compounds, each accompanied by a stated measurement uncertainty. These certified values were compared to concentrations obtained using EPA Method 1633, applied to dual-phase blended PFAS WAX/Carbon S, 200/50 mg cartridges—with and without post extraction matrix reduction using Captiva EMR PFAS Food II cartridges (Table 8). The reported concentrations in Table 8 represent the average of three replicate measurements, with uncertainties calculated at the 95% confidence level.

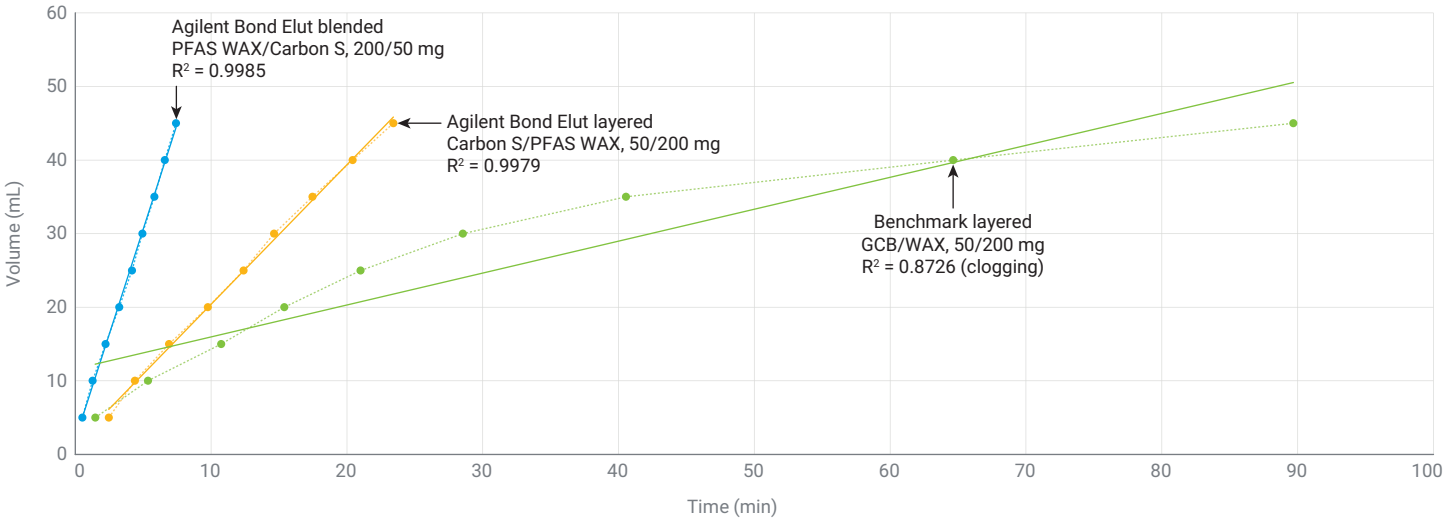

Figure 3. Matrix extract flow rate measurements for SPE cartridges with different packing configurations.

Table 8. SRM 2781 reference mass fractions and measured concentrations.

Analyte	Reference Value (µg/kg)	Concentration (µg/kg) Without EMR Cleanup	Error (%)	Concentration (µg/kg) With EMR Cleanup	Error (%)
PFHxA	13 ± 2	12.5 ± 1.9	4	13.5 ± 1.0	4
PFHpA	7.96 ± 1.5	8.81 ± 2.32	11	8.43 ± 2.42	6
PFOA	28.5 ± 3.3	33.1 ± 4.9	16	33.7 ± 4.5	18
PFHxS	9.39 ± 1.76	30.79 ± 15.59	228	10.38 ± 3.69	11
PFOS	225 ± 41	148 ± 5	34	241 ± 91	7
PFOSA	6.31 ± 0.97	6.02 ± 0.75	5	6.29 ± 0.55	0.3

Significant differences in both accuracy and precision were observed between measurements conducted with and without the Captiva EMR post extraction cleanup. This effect was most pronounced for PFHxS. Without EMR cleanup, the average measured concentration was 30.79 ± 15.59 µg/kg, which is substantially higher than the certified reference value of 9.39 ± 1.75 µg/kg. In contrast, the use of Captiva EMR cleanup yielded a concentration of 10.38 ± 3.69 µg/kg, closely aligning with the reference. The discrepancy in the uncleaned measurement was attributed to a substantial coeluting interferent. Figure 4 illustrates the extracted ion chromatograms for the MRM transitions of PFHxS, comparing results with and without EMR treatment. The EMR cleanup effectively removed the interferent, enabling accurate and precise quantification.

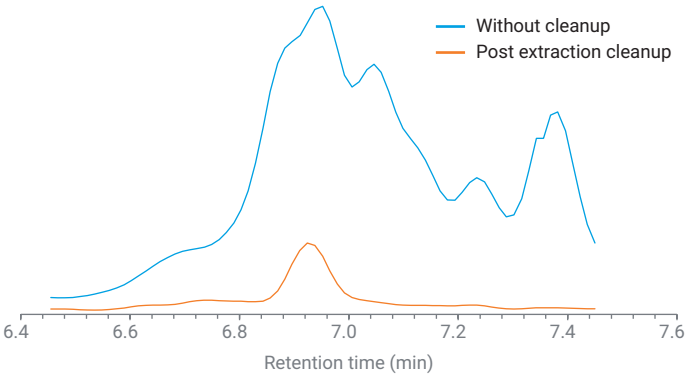

Figure 4. Extracted MRM chromatograms for PFHxS with EMR PFAS Food II post extraction cleanup (orange) and without cleanup (blue).

The measurement of PFOS concentration without EMR cleanup was biased lower relative to the certified reference value. Specifically, the concentration measured without EMR was 148 ± 5 µg/kg, compared to the reference value of 225 ± 41 µg/kg. This discrepancy was attributed to interference affecting the MRM transition of the EIS, $^{13}C_8$-PFOS, which is used for quantifying the native PFOS peak. The interference artificially elevated the signal of the EIS, resulting in an underestimation of the PFOS concentration. As illustrated in Figure 5, the Captiva EMR cartridge effectively removed the interfering species, enabling more accurate quantitation. With EMR cleanup, the measured PFOS concentration was 241 ± 91 µg/kg, closely aligning with the reference value.

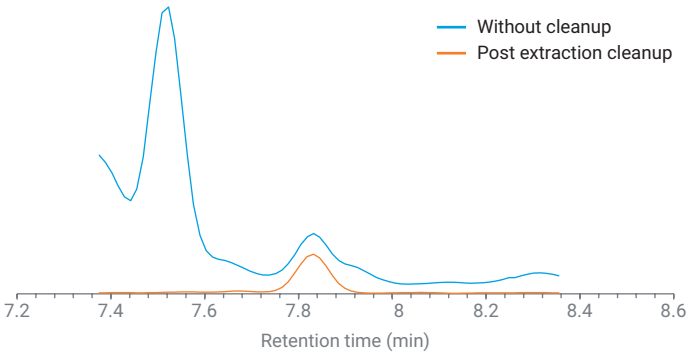

Figure 5. Extracted MRM chromatograms for $^{13}C_8$-PFOS EIS with EMR PFAS Food II post extraction cleanup (orange) and without cleanup (blue).

Sample analysis

The average concentrations of PFAS targets in the biosolid sample, with and without EMR cleanup, are presented in Table 9. For most compounds, the relative percent differences (RPDs) between the two treatments fall within the 30% threshold required for matrix duplicates.[8] However, in addition to the previously noted interferences affecting PFHxS and PFOS, RPDs exceeding 30% were observed for PFPeS, PFHpS, PFNS, and NMeFOSA. These discrepancies were attributed to interferences that impacted either the target analyte signals or those of their corresponding EIS. For PFBS, 5:3 FTCA, and PFTeDA, EMR cleaning altered the reporting being either above or below LOQ.

Table 9. Average PFAS target concentrations with and without EMR cleanup.

	Concentration (µg/kg)				Concentration (µg/kg)		
Analyte	Without EMR Cleanup	With EMR Cleanup	RPD	Analyte	Without EMR Cleanup	With EMR Cleanup	RPD
PFBA	5.7	6.6	10.5	PFHpS	2.6	1.2	73.3
PFMPA	< LOQ	< LOQ	–	8:2FTS	5.2	4.8	7.4
3-3 FTCA	< LOQ	< LOQ	–	PFDA	5.3	5.0	6.1
PFPeA	6.2	7.8	22.8	NMeFOSAA	45.9	58.9	24.7
PFMBA	< LOQ	< LOQ	–	PFOS	147.8	240.6	47.8
4:2FTS	< LOQ	< LOQ	–	NEtFOSAA	408.7	399.9	2.2
NFDHA	< LOQ	< LOQ	–	PFUnA	2.9	2.7	7.2
PFHxA	12.5	13.5	8.0	9Cl-PF3ONS	< LOQ	< LOQ	–
PFBS	< LOQ	2.4	–	PFNS	6.5	13.7	70.7
HFPO-DA	< LOQ	< LOQ	–	PFDoA	2.3	2.5	7.3
PFEESA	< LOQ	< LOQ	–	PFDS	258.2	214.4	18.5
5-3 FTCA	22.1	< LOQ	–	PFTrDA	1.0	1.1	6.7
PFHpA	8.8	8.4	4.3	11Cl-PF3OUdS	< LOQ	< LOQ	–
PFPeS	0.9	1.7	67.0	PFTeDA	0.8	< LOQ	–
ADONA	< LOQ	< LOQ	–	PFOSA	6.0	6.3	4.3
6:2FTS	19.9	21.5	7.6	PFDoS	< LOQ	< LOQ	–
PFOA	33.1	33.7	1.9	NMeFOSE	337.9	381.5	12.1
PFHxS	30.8	10.4	99.2	NMeFOSA	5.8	3.7	42.8
PFNA	2.9	3.0	4.2	NEtFOSE	127.7	134.0	4.8
7-3 FTCA	26.1	27.5	5.2	NEtFOSA	7.3	9.2	23.1

Figure 6 displays the extracted MRM chromatograms for PFPeS, PFHpS, $^{13}C_8$-PFOS, and D_3-NMeFOSA, comparing results with and without EMR cleanup. For PFPeS and PFHpS, substantial coeluting interferences obscure the target ions, leading to either underestimation or overestimation of their concentrations. In the case of PFNS, its associated EIS ($^{13}C_8$-PFOS) elutes adjacent to a prominent interferent, complicating accurate peak integration. For NMeFOSA, the response of its EIS (D_3-NMeFOSA) is notably suppressed in the extract without EMR cleanup, resulting in an inflated calculated concentration. Although no direct interference is observed within the MRM window, the diminished signal suggests coelution was occurring.

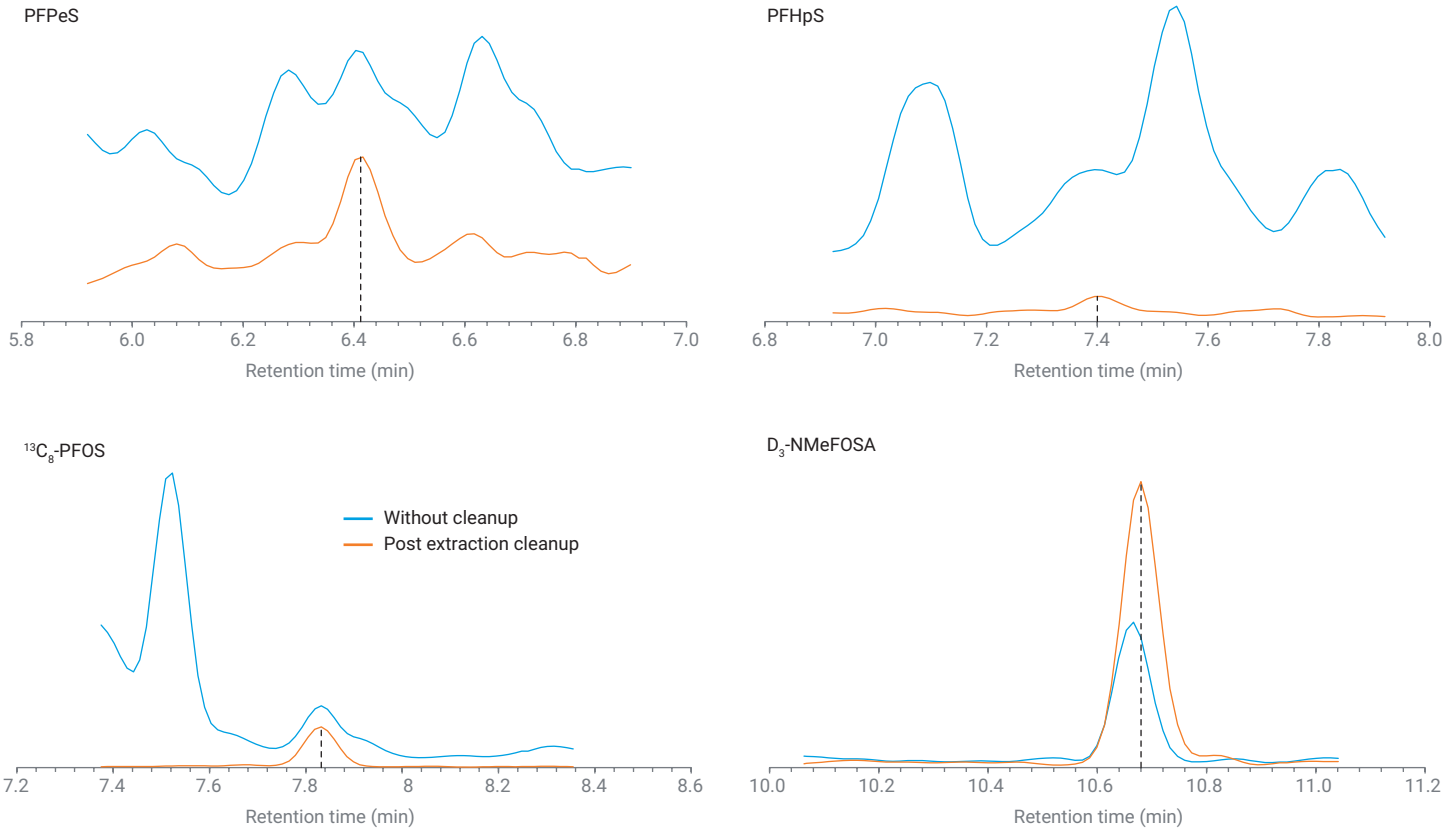

Figure 6. Extracted MRM chromatograms with EMR PFAS Food II post extraction cleanup (orange) and without cleanup (blue).

Matrix spikes

Three replicate biosolid matrix spikes were prepared at midlevel concentrations (Table 2) and extracted following EPA Method 1633 solids procedure, both with and without EMR cleanup. Figure 7 presents the average background subtracted percent recoveries and associated precisions expressed as relative standard deviations (RSDs) for the target compounds, excluding NMeFOSAA, PFOS, NEtFOSAA, and PFDS. The endogenous concentrations (Table 9) of those excluded targets exceeded the spike level by a factor of 10 or more and were omitted to avoid misleading recovery results caused by heteroscedasticity and amplified uncertainty from background subtraction. For reference, Figure 7 includes the ongoing precision and recovery (OPR) levels acceptance limits as listed in Table 7 of EPA Method 1633 and matrix spike reproducibility limit of 30% as listed in the Department of Defense data validation guidelines.[8]

Several outliers in Figure 7 were observed in the extracts without EMR cleanup, all of which can be attributed to matrix interferences. Notably, PFHxS recovery and precision were impacted by significant coeluting interferences (Figure 4), necessitating manual chromatographic peak integration and resulting in increased variability in response area measurements. For PFNS and NMeFOSA, integration of their respective EISs also introduced greater variability, as previously noted (Figure 6). In contrast, no isobaric interferences were observed within the MRM windows for PFOSA and NEtFOSA; however, their lower recoveries may be attributed to undetected coeluting interferences causing signal suppression not visible at the monitored *m/z* transitions.

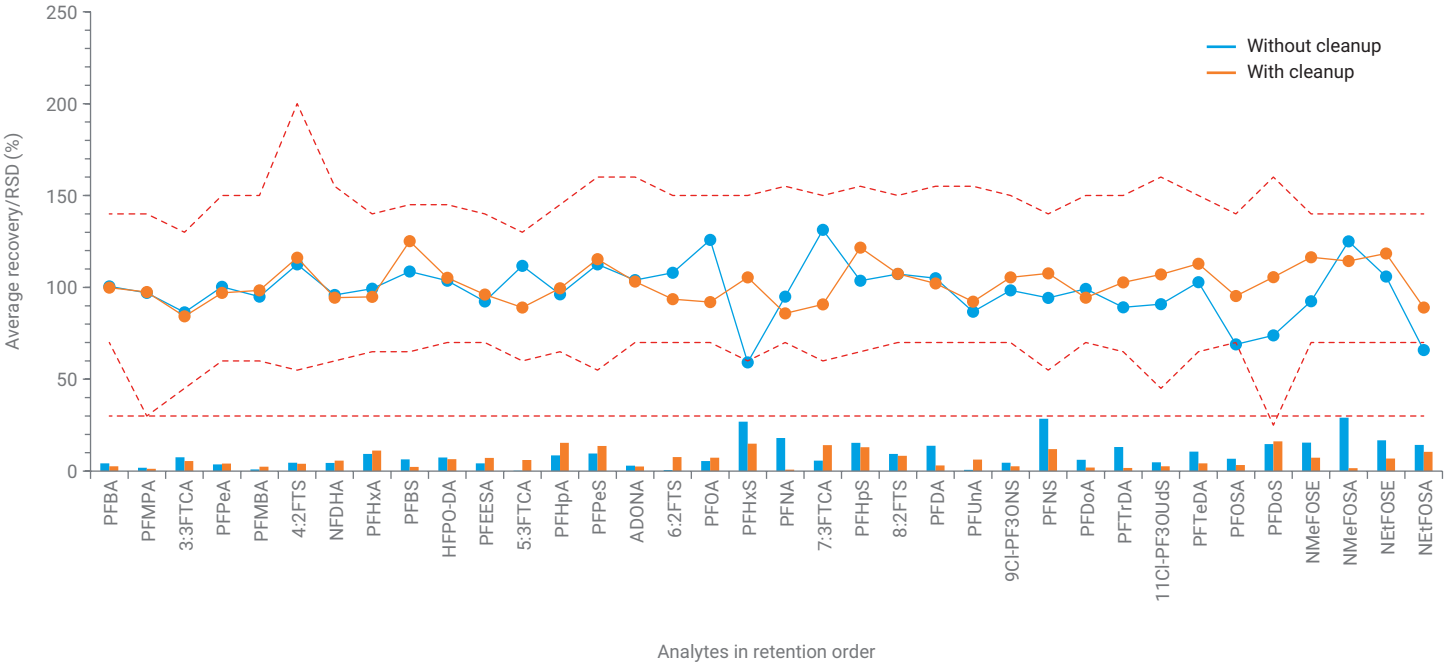

Figure 7. Average matrix spike recoveries and RSDs for samples prepared with EMR cleanup (orange line/bars) and without EMR cleanup (blue line/bars). OPR accuracy limits and ≤ 30% RSD limit indicated as hashed red lines.

EIS and NIS recoveries

Figure 8 shows the recoveries of EIS and NIS for extracts prepared with and without EMR cleanup, based on three replicate extractions. The figure includes the recovery acceptance limits for biosolid matrices as specified in Table 8 of EPA Method 1633. The use of EMR cleanup improved both accuracy and precision of EIS recovery, yielding an average of 92% ± 3% compared to 89% ± 6% (95% confidence, n = 72). As previously shown (Figure 5), coeluting matrix interferences with $^{13}C_8$ PFOS led to inflated recovery values that exceeded the acceptance limit. When comparing datasets, a downward trend in recovery was observed with increasing retention time from D_5-NEtFOSAA to D_5-NEtFOSA in samples without EMR cleanup. This pattern suggests that EMR cleanup effectively mitigates the impact of more hydrophobic matrix interferences.

Conclusion

This study demonstrates that combining the Agilent Bond Elut blended PFAS WAX/Carbon S SPE cartridges with Agilent Captiva EMR PFAS Food II cleanup improves PFAS quantitation in biosolids by reducing matrix interferences and enhancing recovery accuracy and precision. The modified workflow offers a practical solution for overcoming analytical challenges in complex solid matrices and supports broader application of EPA Method 1633 in environmental monitoring.

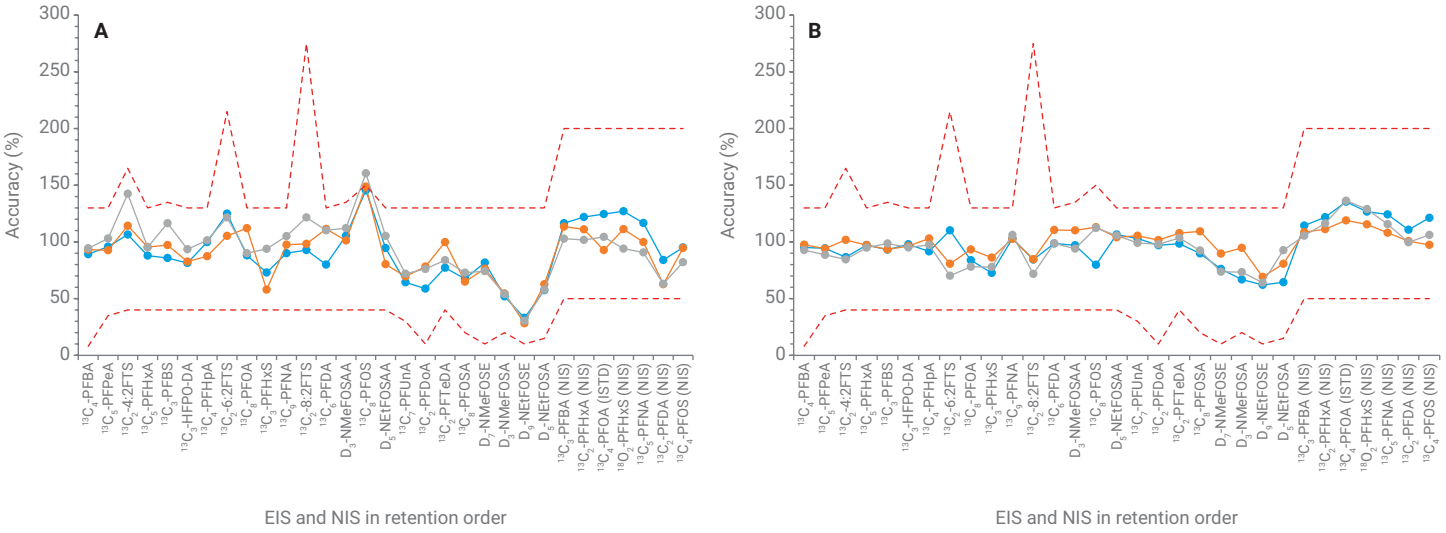

Figure 8. EIS and NIS recoveries for biosolid extracts (A) without EMR cleanup and (B) with EMR cleanup, with recovery acceptance limits (hashed red lines).

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