Application Note

Environmental

Determination of 40 PFAS in Biosolids Following EPA Method 1633 Quality Control Guidance

Using Agilent Captiva EMR PFAS Food II passthrough cleanup and LC/MS/MS detection

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Abstract

This application note describes the development and validation of a multiresidue method for analyzing 40 per- and polyfluoroalkyl substances (PFAS) in biosolids. The method uses QuEChERS extraction followed by enhanced matrix removal (EMR) mixed-mode passthrough cleanup using Agilent Captiva EMR PFAS Food II cartridges, and subsequent LC/MS/MS detection. It features streamlined and efficient sample preparation, sensitive LC/MS/MS analysis, and reliable quantitation based on neat standard calibration curves. Validation was performed in accordance with U.S. Environmental Protection Agency (EPA) Method 1633 quality control guidance for quantitative analysis of PFAS in biosolids, meeting all specified acceptance criteria requirements.

Introduction

The determination of PFAS residues in biosolids is a critical approach for monitoring and regulating environmental PFAS contamination. In 2021, the U.S. EPA published Method 1633 for the quantitative analysis of 40 PFAS compounds in aqueous, solid, biosolid, and tissue samples using LC/MS/MS.[1] The method involves extraction of biosolid samples with basic methanol (MeOH), followed by matrix cleanup using carbon-based materials and weak anion exchange (WAX) solid phase extraction (SPE). Quantitation is based on the use of isotopically labeled internal standards (ISTDs), enabling accurate and reliable quantitation through neat calibration standard curves. Two sets of ISTDs were used: extracted internal standards (EIS) and nonextracted internal standards (NIS). The EIS set, comprising 24 isotopically labeled PFAS compounds, was spiked into the sample matrix prior to extraction to track analytes throughout the sample preparation process. The NIS set, consisting of seven isotopically labeled compounds, was added to the final extract to assess matrix effects and calculate EIS recovery.

The original matrix cleanup process was further streamlined by using a dual-phase SPE cartridge containing both carbon and WAX sorbents—either stacked or blended. This approach effectively consolidated two cleanup steps into one, reducing the risk of contamination, enhancing method reproducibility, and saving time. The improved method for PFAS in biosolids was demonstrated using dual-phase Bond Elut PFAS Carbon S/WAX cartridges.[2]

While EPA Method 1633 offers a robust and reliable approach for aqueous sample preparation in PFAS analysis, its application to complex matrices, such as biosolids, presents notable challenges. The multiple extraction steps using MeOH and acetonitrile (ACN) are time-consuming. Additionally, the subsequent dispersive SPE cleanup with carbon material is difficult to perform, often resulting in high variability, analyte loss, and increased contamination risk. The WAX SPE cleanup is also insufficient for matrix removal, leading to unacceptable quantitation results and issues with instrument durability and robustness. Although the modified method using dual-phase carbon/WAX SPE cartridges provides improvements over the original workflow, it does not fully resolve all issues. The crude extract still requires a solvent exchange—from a high-organic to a high-aqueous medium—prior to SPE loading. Moreover, the standard SPE workflow—conditioning, equilibration, loading, washing, and elution—adds further complexity. Combined, the solvent switch and SPE procedure make the overall method highly time-consuming and labor-intensive. In addition, the sample extract after SPE is still not clean enough, and an additional matrix cleanup step, such as EMR passthrough cleanup, is thus necessary.[2]

QuEChERS extraction has been reported as an effective approach for PFAS analysis in food sample preparation.[3] This method significantly reduces extraction time while using less than 50% of organic solvent, yet still maintains high extraction efficiency. The EMR mixed-mode passthrough cleanup integrates seamlessly with the QuEChERS workflow, offering a simple and effective solution for matrix removal from crude sample extracts. A sample preparation workflow combining QuEChERS extraction with passthrough cleanup using Agilent Captiva EMR PFAS Food II cartridges was successfully demonstrated for the analysis of 40 PFAS compounds in biological tissue, yielding acceptable quantitation results.[4] Given its efficiency and regulatory compliance, this workflow shows strong potential for extension to other solid environmental matrices, such as biosolids.

The objective of this study was to apply the QuEChERS-EMR approach for the analysis of 40 PFAS analytes in biosolids and to validate the approach in accordance with EPA Method 1633 quality control (QC) guidance for quantitative analysis of PFAS in biosolids. Detection and quantitation were performed using the Agilent 1290 Infinity II LC system and Agilent 6495D triple quadrupole LC/MS (LC/TQ).

Experimental

Chemicals and reagents

Native PFAS, isotopically labeled EIS, and NIS stock solutions were obtained from Wellington Laboratories (Guelph, Ontario, Canada). The concentrations of individual compounds in these stock solutions vary and are detailed in Appendix Table 1 from a previous application note.[5] MeOH, ACN, and isopropyl alcohol (IPA) were purchased from VWR (Radnor, PA, USA). Acetic acid (AA) and ammonium acetate (NH_4OAc) were procured from MilliporeSigma (Burlington, MA, USA).

Solutions and standards

Native PFAS, EIS, and NIS spiking solutions were prepared by diluting the corresponding stock solutions with MeOH. The concentration of native PFAS targets in the standard spiking I was ≥ 25 ng/mL in MeOH, and standard spiking II was ≥ 0.5 ng/mL in MeOH. EIS compounds were prepared in two spiking solutions: EIS spiking I at ≥ 5 ng/mL and EIS spiking II at ≥ 25 ng/mL in MeOH. NIS compounds were spiked at ≥ 5 ng/mL in MeOH. These concentrations reflect the lowest concentration compounds in the stock solutions; other compounds in the mixture were present at proportionally higher concentrations based on their respective stock solution levels.

The standard PFAS spiking I and II, EIS spiking I, and NIS spiking solutions were used to prepare neat calibration standards at the following concentrations for native PFAS targets: 0.008, 0.025, 0.05, 0.1, 0.2, 0.5, 1.0, 2.5, 5.0, and 10.0 ng/mL in ACN containing 1% AA. EIS and NIS compounds were spiked at 0.1 ng/mL. As with the spiking solutions, these calibration levels correspond to the lowest concentration compounds, with others proportionally higher. After preparation, all calibration standards were diluted with 10% water, mixed thoroughly, and used for LC/MS/MS injection. This dilution step mimics the additional 10% dilution introduced during sample cleanup using EMR cartridges.

All standards were stored at 4 °C and used within two weeks. Prior to use, all spiking solutions were brought up to room temperature and vortexed thoroughly. This is critical to ensure the concentration consistency of spiking solutions. For routine calibration curve generation, aliquots of the calibration solutions were transferred to vials with polypropylene (PP) inserts for instrument injection.

The ACN with 1% AA extraction solvent was prepared by adding 10 mL of glacial AA to 990 mL of ACN and stored at room temperature. LC mobile phase A was 5 mM NH_4OAc in water, and mobile phase B was ACN. Needle wash solvents included IPA, water, and ACN.

Equipment and material

The study was performed using an Agilent 1290 Infinity II LC system consisting of an Agilent 1290 Infinity II High Speed Pump (G7120A), an Agilent 1290 Infinity II Multisampler (G7167B), and an Agilent 1290 Infinity II Multicolumn Thermostat (G7116B). The LC system was coupled to an Agilent 6495D LC/TQ (G6495D) equipped with an Agilent Jet Stream iFunnel electrospray ion source. Agilent MassHunter Workstation software was used for data acquisition and analysis.

Other equipment used for sample preparation included:

- Centra CL3R centrifuge (Thermo IEC, MA, USA)
- Geno/Grinder (Metuchen, NJ, USA)
- Multi Reax test tube shaker (Heidolph, Schwabach, Germany)
- Pipettes and repeater (Eppendorf, NY, USA)
- Agilent positive pressure manifold 48 processor (PPM-48; part number 5191-4101)
- Ultrasonic cleaning bath (VWR, PA, USA)

The 1290 Infinity II LC system was modified using an Agilent InfinityLab PFC-free HPLC conversion kit (part number 5004-0006), including an InfinityLab PFC delay column, 4.6 × 30 mm (part number 5062-8100). Chromatographic separation was performed using an Agilent ZORBAX RRHD Eclipse Plus C18 column, 95Å, 2.1 × 100 mm, 1.8 µm (part number 959758-902), and an Agilent ZORBAX RRHD Eclipse Plus C18 column, 2.1 × 5 mm, 1.8 µm, 1,200 bar pressure limit, UHPLC Guard (part number 821725-901).

Other Agilent consumables used included:

- Bond Elut QuEChERS EN extraction kit, EN 15662 method, buffered salts, ceramic homogenizers (part number 5982-5650CH)
- Captiva EMR PFAS Food II cartridges, 6 mL cartridges, 750 mg (part number 5610-2232)
- Polypropylene (PP) snap caps and vials, 1 mL (part numbers 5182-0567 and 5182-0542)
- PP screw cap vials and caps, 2 mL (part numbers 5191-8121 and 5191-8151)
- Tubes and caps, 50 mL, 50/pk (part number 5610-2049)
- Tubes and caps, 15 mL, 100/pk (part number 5610-2039)

All the consumables used in the study were tested and verified for acceptable PFAS cleanliness.

LC/MS/MS instrument conditions

An identical instrument method was applied in this study. See the previous application note[5] for detailed method conditions.

Sample preparation procedure

Domestic sludge standard reference material NIST 2781 was used in this study and obtained from Sigma Aldrich (Saint Louis, MO, US). Samples were stored at room temperature until use.

For each sample, 0.5 g of biosolid powder was weighed into a 50 mL PP tube. Native PFAS and EIS standards were spiked into all prespiked QC samples, while EIS was spiked into matrix blanks (MBs). For procedure blanks (PBs), only 0.5 mL of water was used with EIS being spiked.

Table 1 summarizes the spiking details for prespiked samples. Due to a 20-fold dilution introduced during sample preparation, final extract concentrations were corrected accordingly. To ensure accurate quantitation using neat calibration standards prepared in solvent, it was critical to maintain theoretical EIS and NIS concentrations equivalent to those in the calibration standards—0.1 ng/mL in the final ACN extract. Spiking concentrations for native PFAS and EIS are listed in Table 3. After spiking, all biosolid samples were vortexed for 2 to 3 minutes and allowed to equilibrate for 30 minutes prior to extraction. The detailed extraction procedure is illustrated in Figure 1.

Following sample extraction and cleanup, an aliquot of 1.111 mL of the sample eluate was transferred into a 2 mL PP vial, corresponding to 1 mL of sample extract in ACN. To this vial, an aliquot of 20 µL of NIS spiking solution (5 ng/mL) was added. The vial was capped and vortexed for 10 to 20 seconds to ensure thorough mixing. This step adjusted the NIS concentration in the sample to match that of the calibration curve standards. All prepared samples were then ready for injection into the LC/TQ system for analysis.

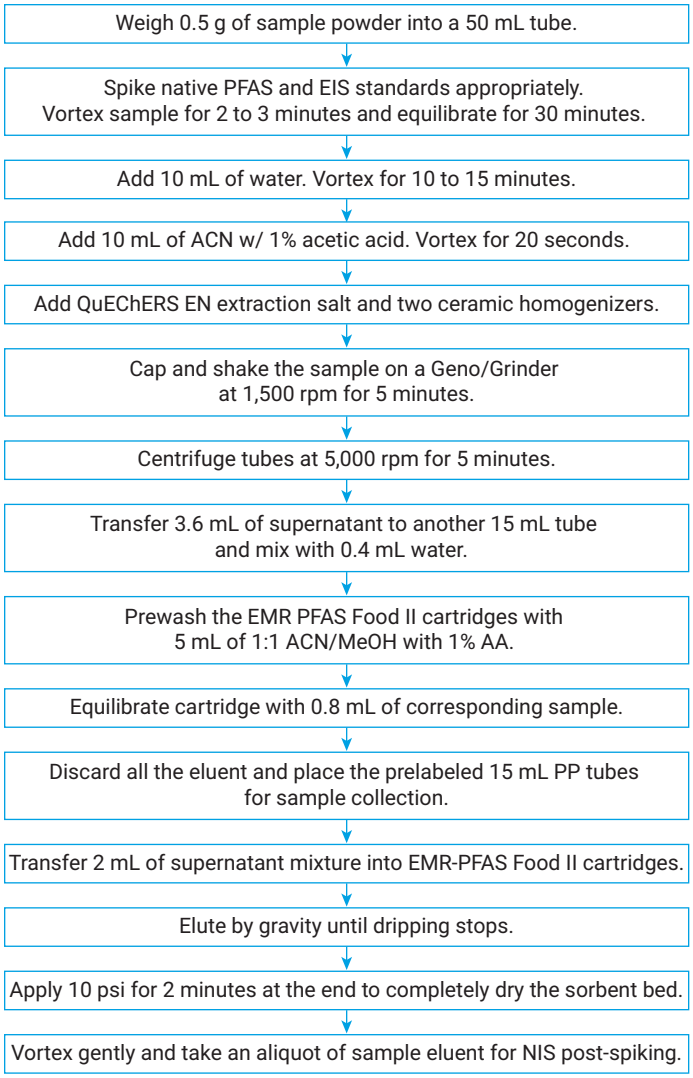

Figure 1. Sample preparation procedure using QuEChERS extraction followed by EMR mixed-mode passthrough cleanup on the Agilent Captiva EMR PFAS Food II cartridge.

Table 1. Spiking of prespiked QC samples with native PFAS targets and EIS compounds.

Samples	Native PFAS Target Spiking				EIS Compound Spiking				Replicates (N)
	Spiking Solution and Conc. (ng/mL)	Spiking Volume (µL)	Conc. in Sample (µg/kg)	Conc. in Sample Extract (ng/mL)	Spiking Solution and Conc. (ng/mL)	Spiking Volume (µL)	Conc. in Sample (µg/kg)	Conc. in Sample Extract (ng/mL)	
MB	NA	NA	NA	NA	EIS spiking solution II (25 ng/mL)	40	2	0.1	7
QC-LOQ	PFAS spiking solution (25 ng/mL)	3.2	0.16	0.008		40	2	0.1	6
QC-Low (12.5x LOQ)		40	2	0.1		40	2	0.1	6
QC-Mid (125x LOQ)	PFAS stock solution (250 ng/mL)	40	20	1		40	2	0.1	6

NA = not applicable

Results and discussion

LC/TQ instrument method

The LC/MS/MS detection method used in this study was directly adopted from previous work.[3] Chromatograms presented in earlier studies[3] demonstrated improved peak distribution and separation across the retention time window, along with baseline separation for critical PFOS isomers and isobaric cholic acids potentially coming from matrix. These enhancements contributed to more reliable quantitation.

Sample preparation procedure

The sample preparation workflow–QuEChERS extraction followed by EMR mixed-mode passthrough cleanup with Captiva EMR PFAS Food II cartridges–significantly streamlined the overall process. The ACN-based solvent extraction, combined with salt partition, enhanced extraction efficiency and matrix cleanup by retaining polar co-extractives in the aqueous phase. Compared to the extended extraction procedure in EPA Method 1633, the QuEChERS approach shortened the process time without compromising extraction efficiency. Additionally, solvent use was reduced from a total volume of 25 mL to 10 mL per sample, and the drying step prior to SPE loading was eliminated.

The EMR mixed-mode passthrough cleanup further simplified matrix removal process, offering efficient and selective cleanup. This approach replaced the traditional SPE workflow—consisting of conditioning, equilibrating, loading, washing, and eluting—with a prewashing, equilibrating, and loading passthrough procedure.

Originally developed for PFAS analysis in food matrices, the QuEChERS-EMR workflow demonstrated strong potential for application to environmental solid matrices, such as biosolids and soil/sediment. In a comparison study, an analyst required only 2 to 3 hours to prepare a batch of 10 to 15 samples using the QuEChERS-EMR method, whereas the traditional SPE-based method took 5 to 7 hours for the same sample quantity. This represents time savings of over 50%. Solvent and consumable use were also reduced by 50% or more, contributing to improved laboratory productivity and cost efficiency.

Quantitation method

The quantitation approach used in EPA Method 1633 is based on the use of both isotopic EIS and NIS compounds. EIS compounds are used for target quantitation, while NIS compounds are used for EIS recovery calculations. The use of EIS and NIS provides a much more accurate quantitation of native PFAS analytes, as well as simultaneous method-critical performance assessment, including recovery and matrix effect, in the same batch.

For PFAS targets that exist as isomeric mixtures, the method requires summation of all isomer peaks within a defined retention time window for integration and quantitation. This requirement was addressed using spectrum summation, which integrates all relevant peaks collectively. This strategy was applied to all targets with linear and branched isomers, including PFOA, PFNA, PFOSA, N-MeFOSA, N-EtFOSA, N-MeFOSAA, N-EtFOSAA, N-MeFOSE, N-EtFOSE, PHFxS, and PFOS.

For FTS targets, M+2 isotopically labeled FTS compounds were commonly used as EIS. However, during quantitation across a broad dynamic range, all FTS targets exhibited quadratic calibration curves, indicating non-linearity. This behavior was attributed to the natural sulfur +2 isotope contribution from native FTS analytes, which interfered with the EIS transitions and resulted in inconsistent EIS responses.[6] To mitigate this issue, less-abundant MRM transitions were selected for all three FTS EIS compounds, specifically using *m/z* 81 as the product ion. This adjustment enabled linear calibration curves for all FTS targets, significantly improving quantitation accuracy. Figure 2 illustrates the calibration curves for 4:2 FTS using $^{13}C_2$-4:2 FTS as the EIS compound for quantitation, comparing different quantifier MRM transitions. The results confirmed that using the 329.0 → 81.0 transition as the EIS quantifier yielded markedly improved linearity for 4:2 FTS across a 1250-fold dynamic range, spanning concentrations from 0.032 to 40 µg/kg in biosolid matrix.

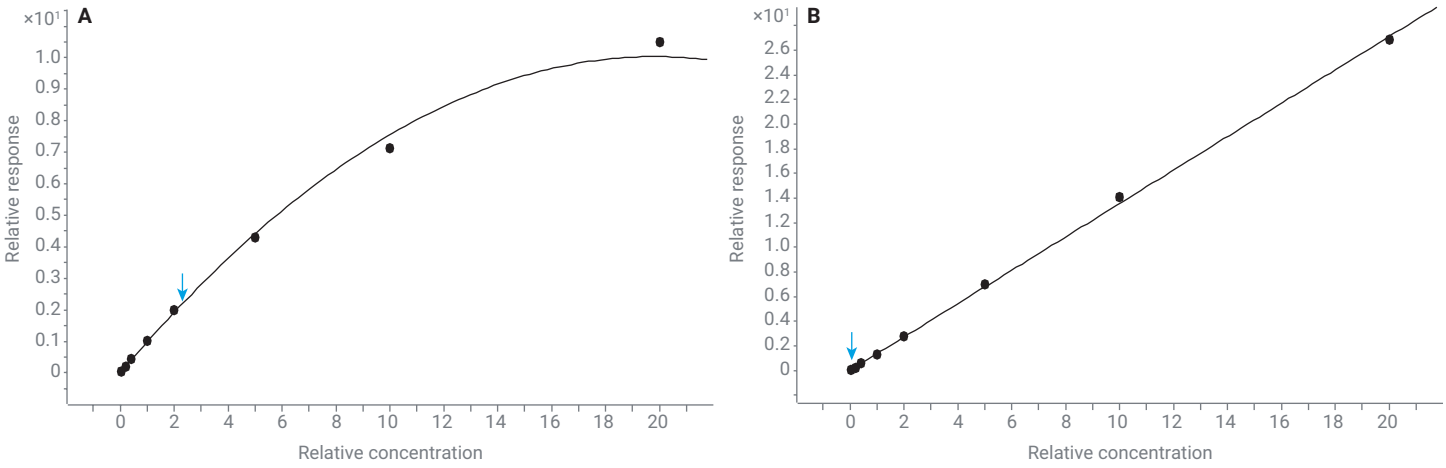

Figure 2. Calibration curves of 4:2 FTS using $^{13}C_2$-4:2 FTS as the assigned EIS, with different quantifiers. (A) EIS quantifier as 329.0 → 309.0, (B) EIS quantifier as 329.0 → 81.0.

Biosolid matrix

Biosolid is a complex and heterogeneous matrix composed of nutrients, organic matter, and inorganic contaminants. They contain macronutrients such as nitrogen (N) and phosphorus (P), primarily derived from human waste, along with essential micronutrients for plant growth, including manganese (Mn), copper (Cu), and zinc (Zn). The matrix also includes biological components like lipids and cellulose, inorganic substances, and potentially hazard contaminants such as pathogens, pharmaceuticals, pesticides, and "forever chemicals"—PFAS. Through treatment processes, the organic matter in sewage sludge is stabilized and pathogen levels are reduced, transforming it into biosolids suitable for use as a soil amendment in agricultural and environmental applications.[7-9]

Biosolids present an extremely complex matrix, posing significant challenges for reliable PFAS quantitation. Their heterogeneous composition—including nutrients, organic and inorganic matter, microbial residues, and various contaminants—requires robust and highly efficient sample preparation to ensure accurate analysis.

To address these challenges, the combination of QuEChERS extraction and EMR mixed-mode matrix-targeted passthrough cleanup offers substantial advantages in both matrix removal and PFAS extraction efficiency. However, due to the significant matrix complexity, a reduced sample size was necessary. In accordance with EPA Method 1633, the recommended maximum sample size for biosolid extraction is 0.5 g.[1] Preliminary method development confirmed the suitability of this sample size, which introduced a 20-fold dilution factor during sample preparation. This 0.5 g sample size was also adopted to the validated method in this study.

The domestic sludge standard reference material (SRM), NIST 2781, pretreated as dry powder for complete moisture removal, is intended for evaluating analytical methods for PFAS and other target analytes in sludge.[10] The SRM sample was screened using the developed method and showed significant positive detection of PFAS analytes in this study. Due to the limited availability of other treated biosolid matrix samples, NIST 2781 was subsequently used in spiking experiments for method validation.

Method validation

The developed method was validated for the quantitative determination of 40 PFAS targets in the biosolid matrix. Method calibration was performed using neat standards prepared in the extraction solvent—ACN with 1% AA. Due to varying concentrations of PFAS compounds in the stock mix solution, the calibration dynamic ranges differed among analytes and are summarized in Table 2. All calibration curves were fitted using linear regression with $1/x^2$ weighting, yielding $R^2 > 0.99$ or relative standard error (RSE) < 20%.

Method accuracy and precision were evaluated using three levels of prespiked QC samples. For each level, six replicates were prepared to assess both accuracy and precision. Additionally, seven replicates of MBs were prepared and analyzed. All PFAS compounds positively detected in the MBs were confirmed based on retention time and qualifier ions and were used to correct for matrix background in the calculation of spiking recoveries.

Method detection limit (MDL) was then calculated based on a published U.S. EPA procedure[11], specifically the following Equation 1 for six replicates with 99% confidence.

Equation 1. $MDL = SD_{MBspiking} \times 3.314$

Where $SD_{MBspiking}$ is the standard deviation (SD) of MBs or prespiked LOQ samples. For analytes with positive detections in matrix blanks exceeding 30% of the LOQ spiking level, the SD of seven MBs was applied. For few analytes with no detection or with positive detection below 30% of the spiking LOQ level in the MBs, the SD was derived from six replicates of LOQ samples.

The method's validated LOQ was determined based on the lowest prespiking concentration that met acceptable criteria for accuracy, precision, and selectivity. In cases where positive detections in MBs interfered with the experimental LOQ spike, the LOQ was instead calculated using Equation 2.

Equation 2. $LOQ_{cal} = SD_{MB} \times 10$

Table 2 presents the method MDL and LOQ results obtained using the QuEChERS-EMR approach. According to EPA Method 1633, MDLs and LOQs for biosolids were not determined during the multi-laboratory validation study. Due to the smaller sample sizes typically used for biosolids, their MDLs and LOQs are estimated to be approximately 10 times higher than those for solid samples.[1] Based on the reported MDL and LOQ values for solids, the corresponding estimated values for biosolids are also included in Table 5. The results demonstrate that the method achieved MDLs and LOQs lower than the estimated values (that is, 10-fold of the solid sample results from EPA Method 1633) for most analytes, except for N-MeFOSAA, PFOS, N-EtFOSAA, PFDS, N-MeFOSE. The elevated values for these compounds are attributed to the significantly high concentrations detected in the matrix blank.

Table 2. Comparison of QuEChERS-EMR method quantification results with estimated EPA 1633 results.[1]

Target	RT (min)	Quantification Reference IS	Calc. Range (µg/kg)	Detected in MB (µg/kg)	Calc. MDL (µg/kg)	Estimated MDL by EPA 1633* (µg/kg)	Calc. LOQ or Exper. LOQ (µg/kg)	Estimated LOQ Range by EPA 1633* (µg/kg)
PFBA	3.29	$^{13}C_4$-PFBA	0.64–800	6.43	0.04	1.5	1.40	6.4–16
PFMPA	3.81	$^{13}C_4$-PFBA	0.32–400	ND	0.02	0.7	0.32	3.2–8
3:3 FTCA	3.99	$^{13}C_5$-PFPeA	0.8–1000	ND	0.29	2.3	10.0	8.0–50
PFPeA	4.36	$^{13}C_5$-PFPeA	0.32–400	6.21	0.33	0.7	1.05	3.2–8
PFMBA	4.61	$^{13}C_5$-PFPeA	0.32–400	0.21	0.01	0.5	0.03	3.2–8
4:2 FTS	4.92	$^{13}C_2$-4:2 FTS	0.64–800	ND	0.11	2	0.64	6.4–15
NFDHA	5.43	$^{13}C_5$-PFHxA	0.32–400	0.19	0.02	2	0.05	3.2–8
PFHxA	5.29	$^{13}C_5$-PFHxA	0.16–200	11.48	0.36	0.6	1.13	1.6–4
PFBS	5.43	$^{13}C_3$-PFBS	0.16–200	0.36	0.12	0.5	0.39	1.6–4
HFPO-DA	5.72	$^{13}C_2$-HFPO-DA	0.64–800	0.68	0.03	2.5	0.11	6.4–16
5:3 FTCA	6.02	$^{13}C_4$-PFHpA	4–5,000	19.82	1.47	8.6	4.69	40–100
PFEESA	6.05	$^{13}C_4$-PFHpA	0.32–400	0.21	0.01	0.8	0.03	3.2–7
PFHpA	6.57	$^{13}C_4$-PFHpA	0.16–200	7.00	0.45	0.5	1.44	1.6–4
PFPeS	6.89	$^{13}C_4$-PFHpA	0.16–200	0.31	0.04	0.8	0.14	1.6–4
ADONA	7.10	$^{13}C_8$-PFOA	0.64–800	0.40	0.01	2.3	0.03	6.4–15
6:2 FTS	7.41	$^{13}C_2$-6:2 FTS	0.64–800	3.87	0.26	3.9	0.83	6.4–15
PFOA Isomers	7.80	$^{13}C_8$-PFOA	0.16–200	28.11	0.68	0.7	2.17	1.6–4
PFHxS Isomers	8.22	$^{13}C_3$-PFHxS	0.16–200	7.07	0.70	0.6	2.23	1.6–4
7:3 FTCA	9.11	$^{13}C_3$-PFHxS	4–5,000	16.80	0.87	8.7	2.77	40–100
PFNA Isomers	9.27	$^{13}C_9$-PFNA	0.16–200	2.68	0.38	1.4	1.22	1.6–13
PFHpS	10.12	$^{13}C_9$-PFNA	0.16–200	0.88	0.30	0.7	0.96	1.6–4
8:2 FTS	10.34	$^{13}C_2$-8:2 FTS	0.64–800	4.41	0.30	3.1	0.95	6.4–15
PFDA	10.94	$^{13}C_6$-PFDA	0.16–200	4.33	0.18	0.6	0.57	1.6–4
N-MeFOSAA Isomers	10.93	D_3-N-MeFOSAA	0.16–200	48.26	1.78	0.8	5.66	1.6–4
PFOS Isomers	11.20	$^{13}C_8$-PFOS	0.16–200	197.52	16.10	0.7	51.28	1.6–4
N-EtFOSAA Isomers	11.20	D_5-N-EtFOSAA	0.16–200	356.12	42.80	0.8	136.31	1.6–4
PFUnA	11.66	$^{13}C_7$-PFUdA	0.16–200	2.42	0.16	1.2	0.51	1.6–5
9Cl-PF3ONS	11.81	$^{13}C_7$-PFUdA	0.64–800	0.33	0.01	2.2	0.03	6.4–15
PFNS	11.94	$^{13}C_7$-PFUdA	0.16–200	11.00	1.12	0.7	3.56	1.6–4
PFDoA	12.16	$^{13}C_2$-PFDoA	0.16–200	2.19	0.07	0.6	0.21	1.6–4
PFDS	12.41	$^{13}C_2$-PFDoA	0.16–200	121.07	10.46	0.8	33.31	1.6–4
PFTrDA	12.58	$^{13}C_2$-PFDoA	0.16–200	0.81	0.12	0.7	0.37	1.6–4
11Cl-PF3OUdS	12.72	$^{13}C_8$-PFOS	0.64–800	ND	0.04	1.8	0.11	6.4–15
PFOSA Isomers	12.73	$^{13}C_8$-PFOSA	0.16–200	5.74	0.21	0.4	0.67	1.6–4
PFTeDA	12.97	$^{13}C_2$-PFTeDA	0.16–200	1.36	0.38	0.5	1.22	1.6–4
PFDoS	13.18	$^{13}C_8$-PFOS	0.16–200	0.16	0.07	0.6	0.23	1.6–4
N-MeFOSE Isomers	13.98	D_7-N-MeFOSE	1.6–2,000	311.36	7.17	3.6	22.82	16–40
N-MeFOSA Isomers	14.05	D_3-N-MeFOSA	0.16–200	3.54	0.37	0.7	1.19	1.6–4
N-EtFOSE Isomers	14.26	D_9-N-EtFOSE	1.6–2,000	87.44	3.28	3.5	10.46	16–40
N-EtFOSA Isomers	14.36	D_5-N-EtFOSA	0.16–200	7.01	0.49	0.7	1.55	1.6–4

* The estimated MDL values and LOQ ranges by EPA Method 1633 were based on 10-fold of the reported pooled values and ranges for solids.[1]
Data in red indicate the results higher than EPA Method 1633 published levels due to significant positive detection in matrix.

EIS and NIS compound recoveries were evaluated using all matrix samples from the validation batch. A total of 25 samples were used to calculate average recoveries. Figure 3 illustrates the average recoveries and RSDs of EIS and NIS compounds in the biosolid matrix samples prepared using the QuEChERS-EMR approach. The center green band with red dotted lines represents the EPA Method 1633 recovery acceptance range for EIS and NIS compounds in biosolids. The purple line displays the recoveries of EIS compounds (24, shown on the left) and NIS compounds (seven, shown on the right) obtained using the developed method. The lower light blue band with red dotted lines indicates the RSD acceptance range, while the purple columns show the RSD results achieved in this study. All acceptance ranges are based on EPA Method 1633 QC criteria.

Since all EIS and NIS compounds are isotopically labeled ISTDs, they are inherently free from any natural occurrence in the MB. The recovery results for prespiked EIS compounds indicate that the method offers excellent analyte recovery through the extraction and matrix cleanup processes. Additionally, the recoveries for postspiked NIS compounds confirm that acceptable matrix effects were achieved through the entire process. Both EIS and NIS compounds demonstrated acceptable recoveries within acceptance criteria. Compared to the NIS recoveries, which typically range around 100%[4], the lower recoveries observed in biosolids (70 to 80%) indicate increased matrix suppression due to the more complex nature of the biosolid matrix.

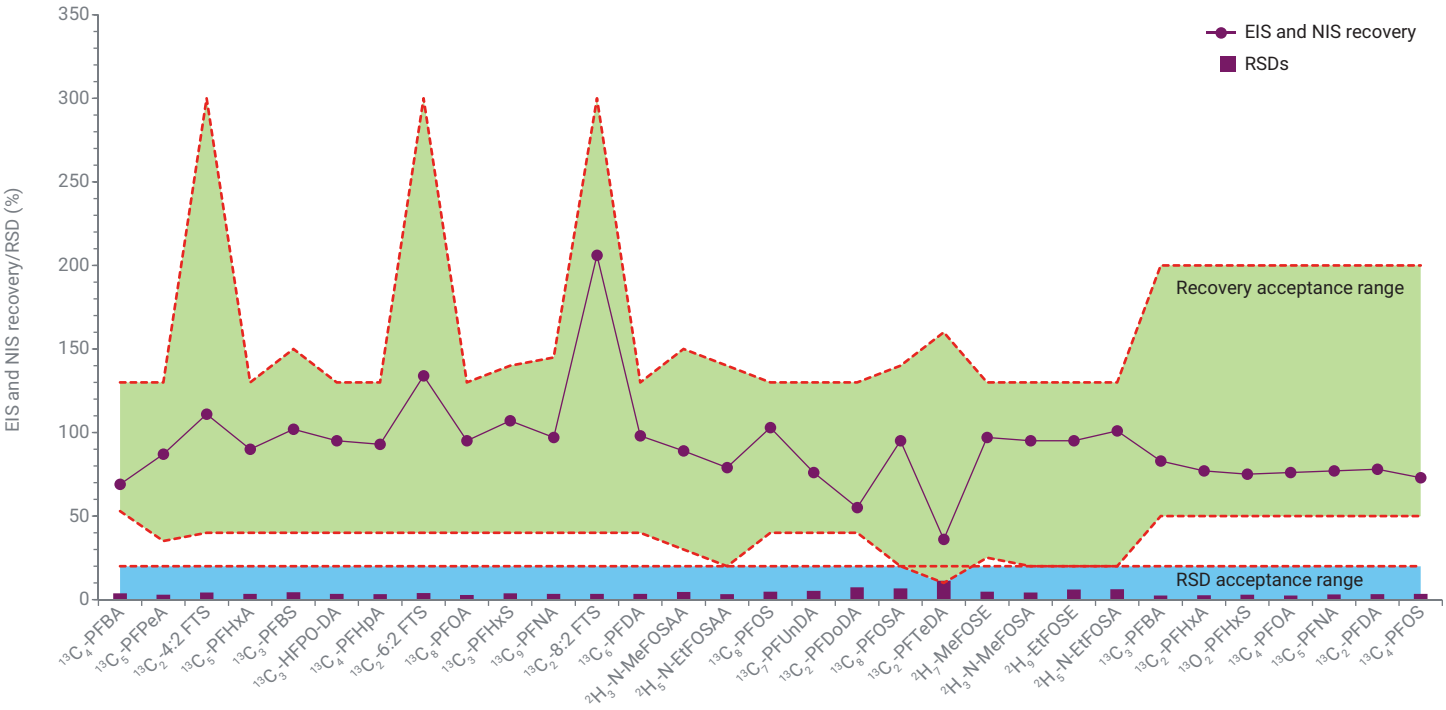

Figure 3. Average recoveries of EIS and NIS compounds in the biosolids validation batch using the QuEChERS-EMR method. The purple line in the middle exhibits the EIS and NIS recovery, while the columns at the bottom present the RSDs.

Figure 4 presents the quantitation accuracy and precision results for 40 PFAS targets in biosolids, based on two levels of prespiked QC samples. The center green band with red dotted lines represents the recovery acceptance range for native PFAS analytes in solid and biosolid matrices. The dark green and purple lines display the recoveries of native PFAS analytes prespiked at low and mid-levels, respectively, as obtained using the developed method. The lower light blue band with a red dotted line indicates the RSD acceptance range for native PFAS compounds, while the dark green and purple columns show the RSDs achieved at low and mid-levels, respectively, as obtained using the developed method. All acceptance ranges are based on EPA Method 1633.

Results from the LOQ-level prespiked samples are excluded from the figure, as the results of over 70% of analytes are not reportable due to significant positive detections in the biosolid matrix blank. For the two higher spiking levels, most of the PFAS analytes demonstrated acceptable quantitation accuracy. However, significantly elevated background levels in the matrix blank led to quantitation accuracy failure for NEtFOSAA, PFOS, and PFDS at the low-spiking level, and for PFDS at mid-spiking level. All analytes at both spiking levels presented with acceptable quantitation precision, with RSDs below 10%.

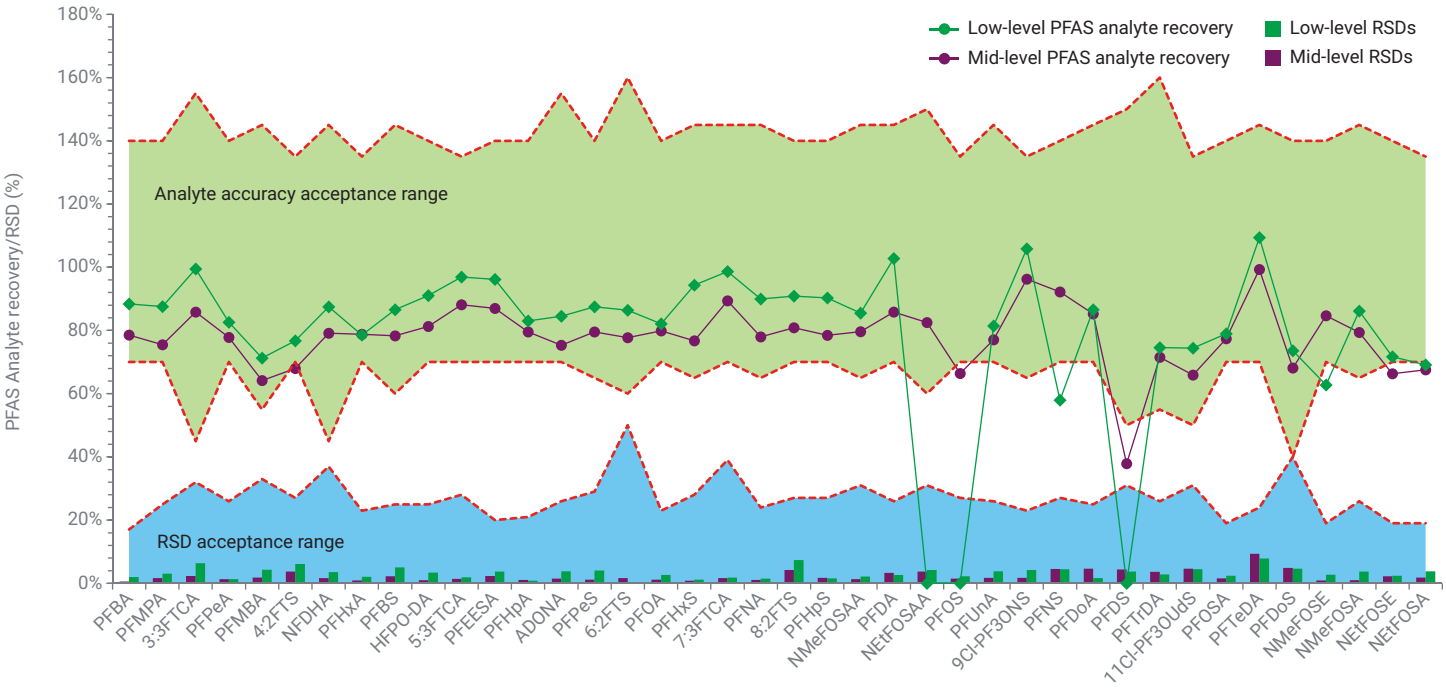

Figure 4. Quantitation accuracy (recovery%) and precision (RSD%) results for analysis of 40 PFAS targets in biosolids at two spiking levels using the QuEChERS-EMR method. Lines indicate the native PFAS analytes recoveries and columns represent the RSDs. Dark green represents the results of low-level, and purple indicates the results of mid-level.

Method proficiency test

For the NIST 2781 Standard Reference Material, its certificate of analysis (COA) provides noncertified mass fractions for selected PFAS contaminants present in the sample.[10] Additionally, PFAS concentrations in the NIST 2781 sample were reported in a previous study.[11] PFAS compounds listed in the COA for the NIST 2781 sample, along with their detections in this study and previous reported results, are summarized in Table 3. Other detected PFAS compounds in the NIST 2781 sample are listed in Table 2.

The results demonstrate that, for all detected PFAS listed in the COA, concentrations fall within the reported COA ranges when using the QuEChERS-EMR method. The approach showed excellent reproducibility, with RSD below 10% for all six PFAS compounds across seven replicates of testing. Compared to previously reported results, four compounds—PFHxA, PFHpA, PFOA, and PFOS—exhibited comparable quantitation levels within the reported range, with RSDs under 10%. However, PFHxS and PFOSA, when analyzed using the previously reported method, showed significant deviations and poor reproducibility, suggesting inadequate method performance for quantifying these two compounds.

Conclusion

A simplified, rapid, and reliable method was developed and validated for the quantitation of 40 PFAS targets in biosolid/sledge using QuEChERS extraction, followed by Agilent Captiva EMR PFAS Food II passthrough cleanup and analysis by LC/MS/MS under EPA Method 1633 guidelines. Compared to traditional EPA 1633 SPE-based sample preparation approaches, the new method offers significant advantages: it reduces preparation time by over 50%, lowers solvent consumption by approximately 80%, and minimizes the use of consumables.

Validation following EPA Method 1633 criteria confirmed acceptable recoveries for both EIS and NIS compounds, as well as acceptable quantitation results for native PFAS analytes. The method also achieved MDL and LOQ levels lower than the estimated LOQs derived from reported solid sample levels and EPA Method 1633 guidance, except few exceptions due to significant positive detection in matrix. A subsequent proficiency test on selected PFAS analytes in the NIST 2781 Standard Reference Material demonstrated acceptable quantitation with excellent reproducibility. Overall, this approach offers an efficient, cost-effective, and high-performance alternative to conventional sample preparation approaches for PFAS analysis in biosolid/sludge matrices.

The use of EMR mixed-mode passthrough cleanup effectively enhances biosolid matrix reduction, which is critical for ensuring analytical method performance and reliable PFAS quantification in biosolids. When compliance with the SPE-based EPA Method 1633 is required, adding an EMR cleanup step after SPE provides an effective improvement to the original method, helping ensure successful analysis.[2] For situations with more flexibility in method selection, the QuEChERS-EMR approach offers a streamlined sample preparation workflow that saves time and cost without compromising PFAS quantification performance.

Table 3. Proficiency test results and comparison. All concentrations reported in µg/kg.

Analytes	NIST 2781 Non-Certified Conc.[10]	Detection in the Study (n = 7)			Reference Report[11] (n = 6)		
		Ave. Conc.	RSD	Diff.	Ave. Conc.	RSD%	Diff. (%)
PFHxA	13.0 ± 2.0	11.48	1.1%	–11.7%	12.45	4.6%	–4.20%
PFHpA	7.96 ± 1.5	7.00	2.4%	–12.1%	7.64	4.2%	–4.00%
PFOA	28.5 ± 3.3	28.11	0.9%	–1.4%	27.17	2.3%	–4.70%
PFHxS	9.39 ± 1.76	7.07	3.6%	–24.7%	3.37	109.7%	–64.1%
PFOS	225 ± 41	197.52	3.0%	–12.2%	244.3	4.6%	8.60%
PFOSA	6.31 ± 0.97	5.97	1.3%	–5.4%	1.85	49.9%	–70.70%

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