Application note | 002750

Environmental

Novel semi-automated method for the analysis of per- and polyfluoroalkyl substances (PFAS) in soil samples

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Keywords

US EPA Method 1633A, AutoTrace 280 PFAS, EXTREVA ASE, Vanquish Flex Binary UHPLC, TSQ Altis triple quadrupole mass spectrometer, Chromeleon CDS

Application goal

Modify the Thermo Scientific™ EXTREVA™ ASE™ Accelerated Solvent Extractor to meet the performance requirements of US EPA Method 1633A PFAS analysis in soil.

Application benefits

- **Increased efficiency:** The method streamlines multiple steps of US EPA Method 1633A by automating extraction and clean-up processes, significantly increasing throughput and reducing the potential for human error and contamination.
- **Reduced manual labor:** By eliminating the need for extract evaporation and filtration steps, the method minimizes manual handling, thereby reducing labor and the risk of introducing errors.
- **Enhanced accuracy and reliability:** The use of the EXTREVA ASE and Thermo Scientific™ Dionex™ AutoTrace™ 280 PFAS instruments, which exhibit low-to-no PFAS background, ensures that the method meets the stringent performance requirements of US EPA Method 1633A, providing reliable and precise PFAS analysis of soil samples.

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Introduction

PFAS, a group of synthetic chemicals comprising more than 12,000 species, have a ubiquitous presence in our ecosystem due to their extensive use since the 1940s. These chemicals are characterized by their persistence in the environment, their ability to bioaccumulate, and their ease of transport through soil and water. Exposure to even low levels of PFAS can adversely impact human health.[1] Consequently, regulatory agencies are working to restrict and remediate PFAS contamination in drinking water, soils, fish tissue, and biosolids. The United States Environmental Protection Agency (US EPA) has recently published the US EPA Method 1633A for PFAS analysis in solid and semi-solid samples.[2] US EPA Method 1633A involves several intricate steps, including sample preparation, sample extraction, extract enrichment via evaporation, extract reconstitution, pH adjustment, extract clean-up by solid phase extraction (SPE), and extract filtration prior to liquid chromatography-mass spectrometry (LC-MS) analysis. Typically, these procedures are performed manually, which can be labor-intensive and time-consuming. Additionally, several sample and extraction handling steps can introduce human errors and potential PFAS contamination. Reducing sample and extract handling is imperative, as US EPA Method 1633A requires PFAS analyses in low parts-per-billion (ppb) to parts-per-trillion (ppt) levels in samples. To enhance automation and reduce sample handling and manual labor, a modified gas-assisted dynamic accelerated solvent extraction (GA-dASE) system, known as the Thermo Scientific EXTREVA ASE Accelerated Solvent Extractor,[3] was employed for the automated extraction of soil samples. Additionally, the Thermo Scientific Dionex AutoTrace 280 PFAS System was utilized for semi-automated SPE clean-up. This innovative approach also eliminates the need for extract evaporation and filtration steps, thereby streamlining the workflow and significantly reducing sample handling. Consequently, this modification not only increases throughput but also meets the stringent method performance criteria established by US EPA Method 1633A.

Experimental

Materials

PFAS backgrounds from instruments, chemicals, and general lab supplies are a huge problem in trace-level PFAS analysis. To accomplish trace-level analysis of PFAS, instruments are often upgraded to minimize fluorinated components. The EXTREVA ASE system was upgraded for PFAS analysis with the EXTREVA ASE PFAS upgrade kit (Table 1). Specifically, PEEK tubing was used from the solvent bottle to the solvent mixer inlet, and stainless steel (SS) tubing was used from the solvent mixer inlet to the solvent pump. As demonstrated previously in AN73883, the Dionex AutoTrace 280 PFAS system has low PFAS background.[4] PFAS upgrade kits were used in the Thermo Scientific™ Vanquish Flex™ Binary UHPLC System to make it amenable for PFAS analysis (Table 1).

For soil sample analysis, clean loamy soil was utilized for spike samples as well as method and matrix blanks. Certified reference material (CRM) from ERA, Waters was employed to assess high concentration recoveries and conduct carry-over tests. Throughout this study, PFAS-free or minimum PFAS solvents, chemicals, and consumables were used to minimize the background PFAS contamination (Table 1). This approach ensured that the analytical results were accurate and not influenced by external PFAS sources.

In this study, the calibration solutions were prepared using mixtures from Wellington Laboratories following the seven levels outlined in EPA Method 1633A. The calibration standards, spanning from 0.05 to 62.5 ng/mL, were created from seven distinct mixes, each containing specific PFAS with concentrations ranging from 1000 to 20000 ng/mL. Any calibration standard or spike stock solution made from these mixes thus were not uniform in concentrations at any particular level (see Table S1). For simplicity, any spiked concentration or calibration standard level mentioned in this study refers to the concentration of perfluorononanoic acid (PFNA). Concentrations of other PFAS are relative to PFNA and can be found in Table S1. For a complete list of common PFAS compound acronyms used throughout this application note, please refer to Table 1 of EPA Method 1633A.[2]

Sample extraction

Analyte extraction was performed using the EXTREVA ASE system, which employs gas-assisted dynamic solvent extraction under high pressure and temperature. This advanced technology allows for the automated extraction of four samples in parallel. SS cells (10 mL) were prepared by placing a Thermo Scientific™ Dionex™ Cellulose Filter (P/N 068093) at the bottom. Sample aliquots were then mixed with an appropriate amount of diatomaceous earth (DE) and transferred into the SS cells. For spiked samples, the required spike amount was directly added to the sample. Subsequently, 25 µL of extraction internal standard solution (EIS, MPFAC-HIF-ES from Wellington Laboratories) was added to each sample. The cells were sealed with another cellulose filter and capped with a lid. A solvent mixture of 80% methanol and 20% acetonitrile was used for the extraction of PFAS from soil samples. Detailed extraction parameters for PFAS extraction are provided in Table 2.

In EPA Method 1633A, approximately 30 mL of extract goes through the evaporation step to bring the extract volume to about 10 mL followed by dilution to 40-50 mL with deionized water (DI). Thus, not only is the evaporation step lengthy but also redundant. In addition, there is a high risk of losing neutral and volatile PFAS during evaporation.

Table 1. Materials and supplies for PFAS analysis

Instruments	Catalog #	Description
EXTREVA ASE accelerated solvent extractor with evaporation	B51004598	Automated extraction and evaporation system
Dionex AutoTrace 280 PFAS	22136-60101	SPE clean-up system
Thermo Scientific™ TSQ Altis™ Mass Spectrometer	TSQ02-10002	Triple quadrupole mass spectrometer
Vanquish split sampler FT	VF-A10-A	HPLC system
Standards		
Wellington	MPFAC-HIF-IS	Labeled PFC internal standard
Wellington	MPFAC-HIF-ES	Labeled PFC extraction standard
Wellington	PFAC-MXF	Native replacement PFAC standard
Wellington	PFAC-MXG	Perfluoroether acid/sulfonate standard
Wellington	PFAC-MXH	Native PFC standard mix
Wellington	PFAC-MXI	Native FOSA/FOSE standard mix
Wellington	PFAC-MXJ	Native propanoic acid mix
ERA Waters	603	Certified reference soil
Solvents		
Fisher Scientific	A458-1	UHPLC methanol (case of 6)
Fisher Scientific	A955-4	UHPLC acetonitrile
Chemicals		
Fisher Scientific	A113-10X1AMP	Acetic acid LC-MS grade
Fisher Scientific	A117-10X1AMP	Formic acid
Fisher Scientific	A470-500	Ammonium hydroxide
Consumables		
Thermo Fisher Scientific	6ASV9-2P	12X32 mm amber Target DP ID vial
Thermo Fisher Scientific	62819	Dionex ASE Prep DE Diatomaceous Earth
Phenomenex	63110	Phenomenex™ Strata™ PFAS (GCB/WAX) CS0-9214
Fisher Scientific	02-707-410	Pipette tips
Parts		
Thermo Fisher Scientific	B51004603	EXTREVA ASE PFAS upgrade kit
Thermo Fisher Scientific	80100-62144	PFAS upgrade Kit (Vanquish Flex Binary system)
Thermo Fisher Scientific	68087	Thermo Scientific™ Dionex™ ASE™ 150/350 Stainless Steel Extraction Cells
Thermo Fisher Scientific	68075	Aluminum funnel for 5,10, and 22 mL cells
Thermo Fisher Scientific	22184-62238	GC vial coupler
Thermo Fisher Scientific	22184-62239	Vial seal, FFKM
Thermo Fisher Scientific	22184-62237	GC vial light guide
Thermo Fisher Scientific	22184-62244	Evaporation adapter, 250 mL vial
Thermo Fisher Scientific	22184-62236	Concentration flask assembly, 250 mL

Table 2. EXTREVA ASE extraction parameters for PFAS in solid sample

Extraction parameters	
Cell type	Stainless steel
Cell size	10 mL
Oven temperature	60°C
Purge time	30 s
Nitrogen flow (gas assisted extraction)	10 mL/min per channel
Cell fill volume	50%
Solvent flow rate	1.0 mL/min
Extraction solvent	Acetonitrile:methanol (20:80 v/v%)
Extraction volume	≈22 mL
Pre-run rinse	10 mL, Acetonitrile:methanol (20:80 v/v%)
Extraction time (4 samples in parallel)	15 min

To address these issues, the EXTREVA ASE-based workflow offers a more efficient alternative by eliminating the evaporation step. Instead, samples undergo further concentration during SPE, which minimizes the risk of PFAS loss and streamlines the sample preparation process.

Sample cleanup

In this study, the EXTREVA ASE-based workflow integrates what were previously two distinct steps in EPA Method 1633A—Graphitized Carbon Black (GCB) clean-up and Weak Anion Exchange (WAX) clean-up—into a single step. This integration leverages the bimodal nature of the GCB/WAX SPE cartridge, streamlining the process. These cartridges, together with the Dionex AutoTrace 280 PFAS system, provide a semi-automated way to combine both clean up steps. The solvents used for the conditioning and elution steps are the same as in US EPA Method 1633A, but since carbon was packed inside the cartridges, filtration was not necessary. In this EXTREVA ASE-based workflow, the extract (≈22 mL) from the modified EXTREVA ASE system was diluted to 120 mL with reagent water to reach the recommended methanol composition of 14–20%, and it was passed through the GCB/WAX cartridges (Table 3). Samples were eluted with 5 mL of 1% methanolic ammonium hydroxide in collection tubes that contained 25 µL of non-extracted internal standard (NIS). Finally, 25 µL of acetic acid was added to 5 mL extract before an aliquot of 300 µL was transferred to a polypropylene insert autosampler vial for LC-MS/MS analysis.

Table 3. SPE clean-up method. Sample path has 10 mL void volume.

Process 6 samples using the following steps:
1. Condition cartridge with 10.0 mL of 1% NH_4OH in methanol into solvent waste
2. Condition cartridge with 5.0 mL of 1% NH_4OH in methanol into solvent waste
3. Condition cartridge with 5.0 mL of 0.3M Formic acid into solvent waste
4. Pause and alert operator, resume when CONTINUE is pressed
5. Load 70.0 mL of sample onto cartridge
6. Pause and alert operator, resume when CONTINUE is pressed
7. Load 70.0 mL of sample onto cartridge
8. Pause and alert operator, resume when CONTINUE is pressed
9. Rinse sample bottle with 5 mL DI. Load 15.0 mL of sample onto cartridge
10. Pause and alert operator, resume when CONTINUE is pressed
11. Rinse sample bottle with 5 mL DI. Load 15.0 mL of sample onto cartridge
12. Pause and alert operator, resume when CONTINUE is pressed
13. Rinse sample bottle with 5 mL 1:1 Methanol: 0.1M Formic Acid. Load 15.0 mL of sample onto cartridge
14. Pause and alert operator, resume when CONTINUE is pressed
15. Dry Cartridge with gas for 0.8 minutes
16. Pause and alert operator, resume when CONTINUE is pressed
17. Manually rinse sample bottle with 5 mL 1% NH_4OH in methanol to collect
18. End

LC-MS method

Samples were analyzed by LC-MS/MS for quantitation using a Vanquish Flex Binary UHPLC system coupled with a TSQ Altis triple quadrupole mass spectrometer.[5] The UHPLC system was configured with a PFAS upgrade kit to replace wetted Teflon™ surfaces with PEEK lines and fittings. Additionally, the kit included a Hypersil GOLD C18 delay column installed between the pump and analytical column to stagger the retention time of any PFAS found in the system or mobile phases. The system was further modified with a strong solvent loop installed between the autosampler and the analytical column to preserve peak shape while injecting larger volumes of high organic samples on the Acclaim RSLC 120 C18 analytical column. LC parameters and gradient program can be found in Table 4.

The TSQ Altis mass spectrometer was equipped with a heated electrospray ionization (HESI) source and operated in negative ionization mode. The spray voltage was set to -1,000 V. The sheath gas, auxiliary gas, and sweep gas were set to 50, 12, and 0.5 arbitrary units, respectively. The ion transfer tube temperature was set to 225°C, and the vaporizer temperature was set to 300°C. The mass spectrometer was operated in SRM mode. The chromatographic peak width was set to 5 sec, and the chromatographic filter was enabled. Cycle time was enabled and set to 12.5 points per peak. The Q1 and Q3 resolutions for every transition at full width at half maximum were set to 0.7 Da. Argon was used as the collision gas at a pressure of 2.5 mTorr.

Table 4: LC parameters with gradient program

Mobile phases:
Aqueous Phase (A): 2% acetonitrile, 0.1% acetic acid, and 2 mM ammonium acetate in water
Organic Phase (B): 2% water, 0.1% acetic acid, and 2 mM ammonium acetate in acetonitrile
Injection Volume: 5 µL
Column Oven Temperature: 40 °C
Autosampler Temperature: 20 °C
Flow Rate: 0.4 mL/min
Gradient Program:
Time 0 min: 10% B
Time 1 min: 30% B
Time 5 min: 46% B
Time 10 min: 76% B
Time 10.5 min: 86% B (isocratic for 0.8 min)
Time 11.3 min: 86% B
Time 11.4 min: 10% B
End at 14 min

MS/MS method

The ionization source and MS conditions for the TSQ Altis mass spectrometer were adapted from Thermo Fisher Scientific Application Note AN002771 and can be found in Table 5.[5]

Table 5. Ionization source and MS conditions for the TSQ Altis mass spectrometer. Gases are presented in arbitrary units (au).

Source conditions	
Spray voltage	-1,000 V
Sheath gas	50 au
Aux gas	12 au
Sweep gas	0.5 au
Ion transfer tube temperature	225 °C
Vaporizer temperature	300 °C
MS conditions	
Chromatographic peak width	5 s
Chromatographic filter	On
Use cycle time	On
Points per peak	12.5
Q1 resolution (FWHM)	0.7 Da
Q3 resolution (FWHM)	0.7 Da
CID gas	2.5 mTorr, Argon
Source fragmentation	0 V

Results and discussion

Instrument sensitivity and linearity

The TSQ Altis mass spectrometer was mass-calibrated in negative mode. Instrument sensitivity was evaluated by analyzing the lowest calibration standard (0.05 ng/g) and assessing the signal-to-noise ratio (S/N) for each analyte. To determine the sensitivity of the mass spectrometer, S/N ≥ 3:1 for analytes with the quantification and confirmation ions, or S/N ≥ 10:1 if the analytes possessed only a quantification ion in their multiple reaction monitoring (MRM) profile was used. At the lowest calibration standard concentration (0.05 ng/g), all analytes had S/N ratios ≥ 10:1, confirming sufficient instrument sensitivity (Table 6).

In this method, the calibration range spans from 0.05 ng/g to 62.5 ng/g and includes a total of nine concentration points. Instrument linearity was verified by calculating the relative standard error (RSE) in accordance with EPA Method 1633A. The RSE% met the US EPA Method 1633A criteria of ≤20% for all target and extracted internal standard (EIS) compounds (Figure 1).

Table 6. S/N ratio of PFAS native compounds relative to 0.05 ng/g

Compound	S/N Peak-to-peak	Compound	S/N Peak-to-peak
PFBA	22	PFDA	420
PFMPA	208	5:3FTCA	612
PFPeA	148	PFOS	5151
PFMBA	513	PFUdA	209
4:2 FTS	432	9Cl-PF3ONS	3027
NFDHA	157	PFNS	1030
PFHxA	163	PFDoA	391
PFBS	272	N-MeFOSAA	147
HFPO-DA_CO2	499	7:3FTCA	753
PFEESA	1561	PFDS	644
PFHpA	176	PFTrDA	249
3:3FTCA	101	N-EtFOSAA	600
PFPeS	629	11Cl-PF3OUdS	3415
ADONA	1623	PFOSA	6578
6:2 FTS	1128	PFTeDA	898
PFOA	238	PFDoS	1032
PFHxS	3532	N-MeFOSE	583
PFNA	371	N-MeFOSA	375
8:2 FTS	761	N-EtFOSE	499
PFHpS	842	N-EtFOSA	1297

EXTREVA ASE PFAS blank

Like any new instrument, the EXTREVA ASE system requires rinsing with solvents (approximately 2-3 L of 1:1 methanol:acetonitrile) to reduce the PFAS background below the EPA Method 1633A Detection Limit (MDL). For this instrument blank study, empty SS cells (n=8) were extracted with an extract volume of 22 mL and evaporated to 1 mL using the EXTREVA ASE system. The 1 mL extract was then diluted to 5 mL with a diluent to achieve a methanol concentration between 13% and 20%. Subsequently, 25 µL each of NIS and concentrated acetic acid were added. A 5 µL aliquot was measured using the TSQ Altis mass spectrometer. Figure 2 shows the PFAS background of the EXTREVA ASE system. The highest concentration was found for PFHxA at 0.04 ng/g (average 0.01 ng/g), which is below the Limit of Quantitation (LOQ) of 0.05 ng/g and the US EPA 1633A method detection limit (MDL) of 0.06 ng/g. Other PFAS compounds detected, but also below the LOQ, include PFHpA, PFOA, PFBS, and N-MeFOSA.

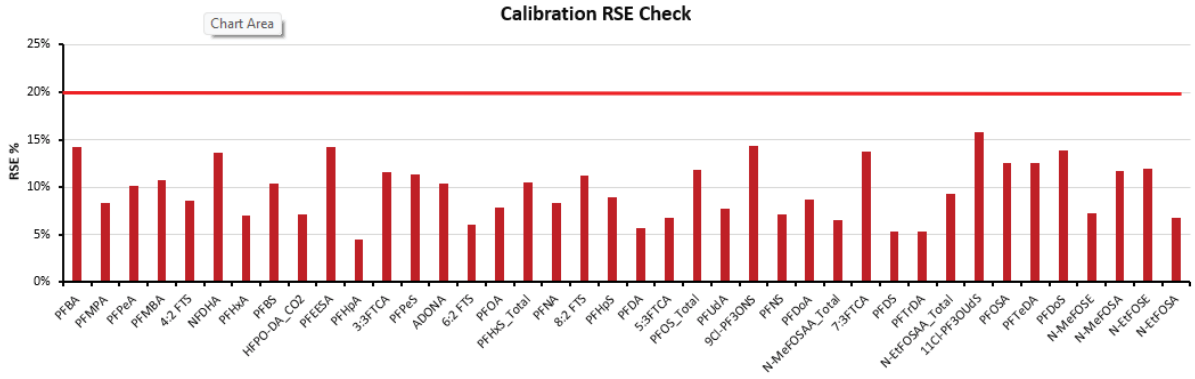

Figure 1. RSE (%) of the calibrations curve

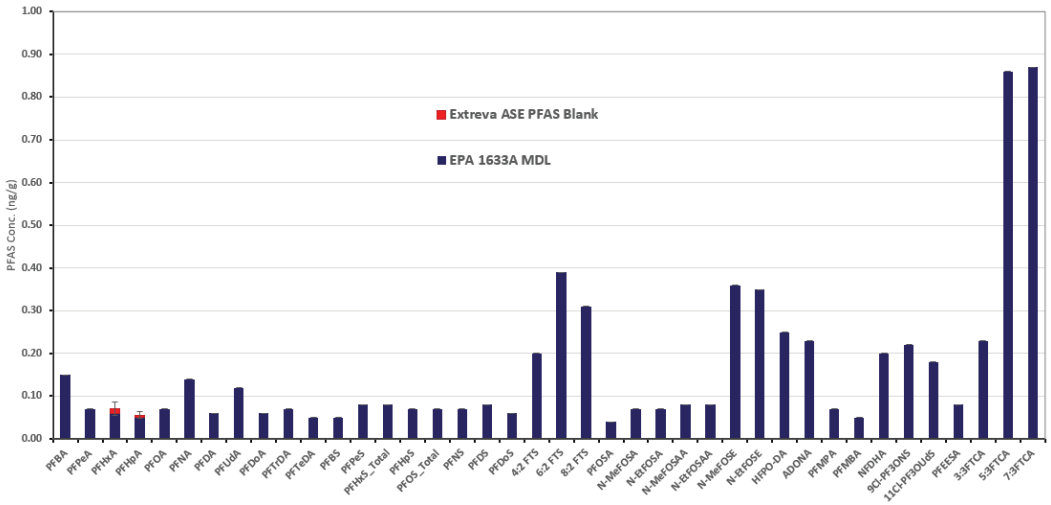

Figure 2. EXTREVA ASE system PFAS background concentration (ng/g) plotted against US EPA Method 1633A MDL. PFAS background concentrations are well below US EPA Method 1633A MDL. Error bars shown for n=8 samples.

Method blank

A method blank is a quality control sample used in analytical chemistry to monitor potential contamination during the analytical process. PFAS background contamination can originate from instrument components, sample handling, chemicals, supplies, and other sources in the laboratory. To minimize PFAS background in the workflow, instruments and reusable consumables must be rinsed multiple times with Optima™ grade methanol. Thus, it was essential to quantify the total PFAS background of the EXTREVA ASE workflow, which includes sample preparation, extraction, clean-up, and analysis. For this study, criteria were established such that PFAS concentrations in method blanks must be less than the MDL specified by US EPA Method 1633A. Clean loamy soil was used to determine the method blanks (n=8), as it represents the matrix of a real soil sample.

The concentrations of PFAS compounds in the method blanks are very low, below the US EPA 1633A MDL for all target analytes (Figure 3). Notably, 6:2 FTS exhibits the highest concentration in the method blanks at 0.08 ng/g, which is still well below the US EPA 1633A MDL. Identifying the source of 6:2 FTS is challenging, making it difficult to eliminate from the workflow. The US EPA 1633A multilab validation data also highlights the “most frequent detection” of 6:2 FTS in method blanks. Additionally, other PFAS compounds detected in the method blanks, though still below the US EPA Method 1633A MDL, include PFHxA, PFHpA, and PFOS.

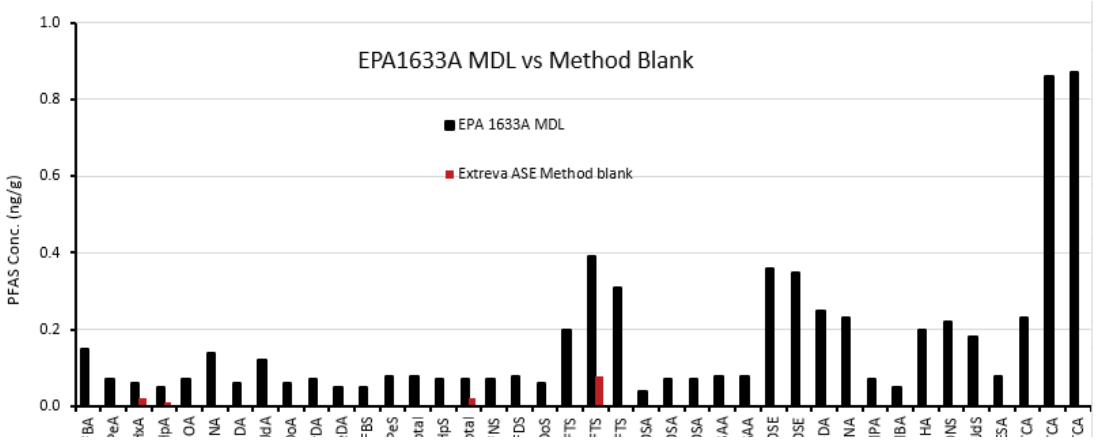

Figure 3. PFAS concentration (ng/g) in EXTREVA ASE method blanks compared to US EPA 1633AMDL

MDL determination

The US EPA recommends that each laboratory conduct a study to determine its own MDL, aiming to achieve results comparable to those specified in US EPA Method 1633A. Following the EPA's guidelines, spike-based MDLs (MDLs) were calculated by spiking seven replicates of 5 g of clean loamy soil with PFAS standards, and blank-based MDLs (MDLb) were determined from the method/matrix blanks by selecting the highest concentrations found among several blank replicates (n=8).[6] The final MDL concentrations for this method were established by selecting the higher concentration between MDLs and MDLb. Notably, PFOA was detected in some blanks, resulting in a slightly higher MDL for this method compared to the US EPA Method 1633A MDL (as shown in Table 9 of US EPA Method 1633).[2] Overall, this method demonstrated similar or lower MDL compared to the MDL outlined in US EPA Method 1633A (Figure 4).

Analyte recovery from spiked clean loamy soil

Seven replicates of 5 g of clean loamy soil were spiked with 0.2 ng/g of native standards and processed in three separate batches on three different days. The samples were analyzed using the TSQ Altis mass spectrometer. Analyte recoveries from the spiked samples (n=7) were compared to the lower and upper limits of the US EPA Method 1633A requirements for IPR (solid).[2] While average recoveries of most analytes (37 out of 40) ranged from 80% to 120%, three PFAS compounds, PFBA, PFOS, and 3:3 FTCA, had recoveries of 126%, 132%, and 69%, respectively. However, these recoveries still fall within the US EPA's acceptable limits (Figure 5). For all 40 compounds except PFDoS, the RSD% is below 20%, with PFDoS having an RSD% of 22%, which is well within the EPA's requirement of 40% for this compound.

In terms of surrogate (EIS) recoveries, 19 out of 23 EIS compounds have recoveries between 70% and 110%, while four compounds have lower recoveries of up to 55% but still fall within the US EPA's acceptable limits (Figure 6). The RSD% for the recoveries of all EIS compounds (n=7) is ≤15%.

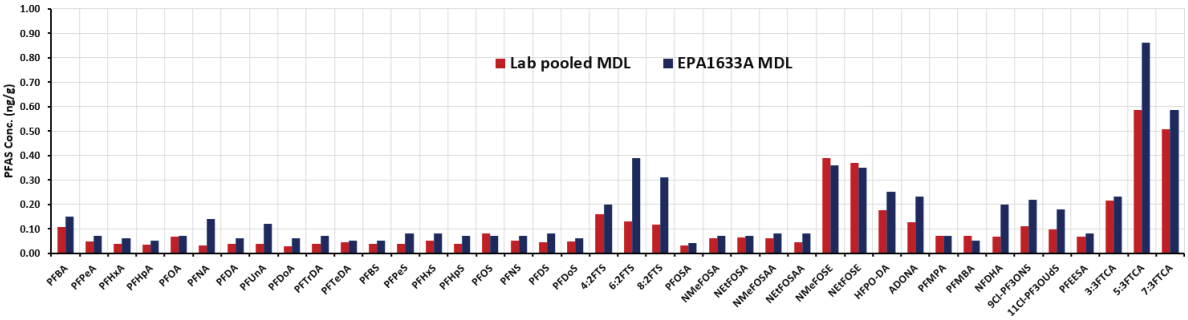

Figure 4. Lab MDL (ng/g) compared to US EPA Method 1633A MDL

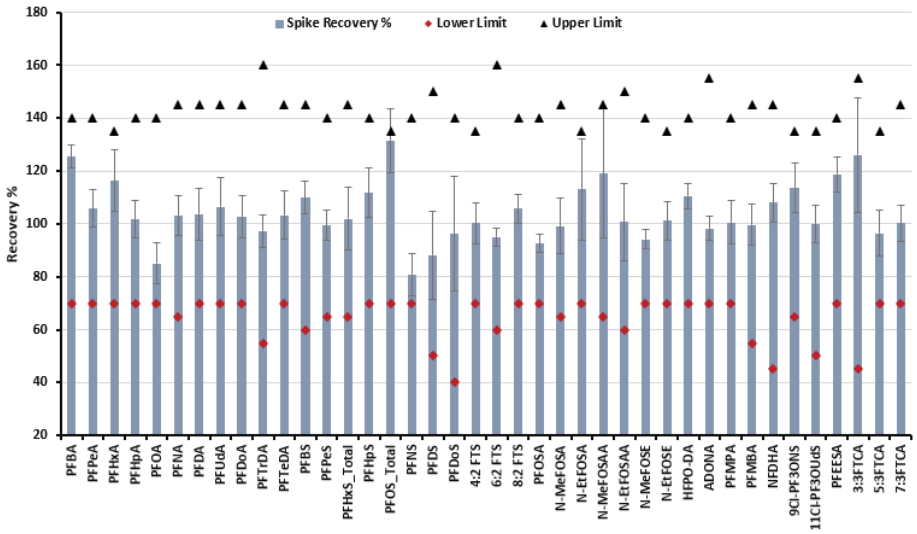

Figure 5. Analyte recoveries from spiked soil samples (n=7) compared to upper/lower limit of US EPA Method 1633A for initial precision and recovery (IPR) study.

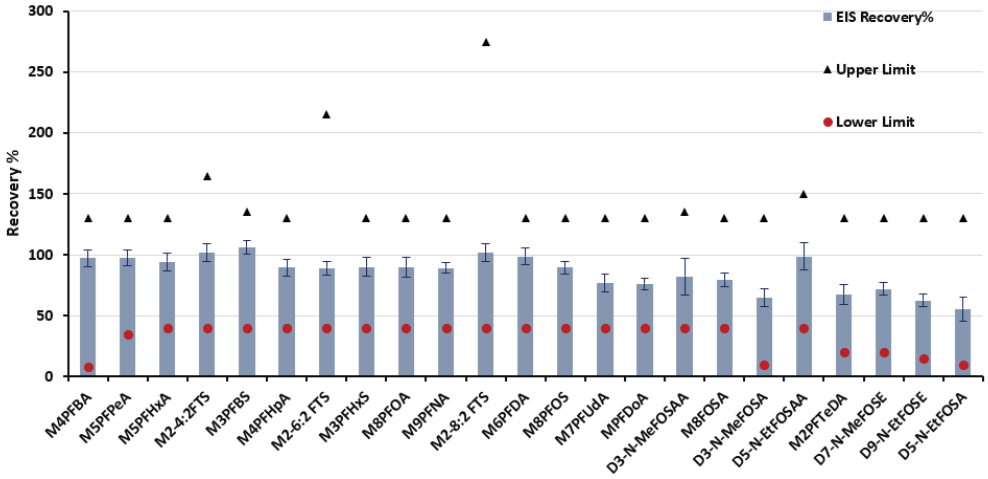

Figure 6. EIS recoveries in spike soil samples compared to US EPA 1633A upper/lower limits

Analyte recovery in CRM and carry-over

CRMs are the gold standard for validating analytical methods. CRM #603 (ERA) is a pre-homogenized soil sample containing 40 PFAS compounds listed in US EPA Method 1633A, with concentrations ranging from <5 ppb to 46 ppb. This CRM is ideal for testing the efficiency of our method in extracting soil samples with high PFAS concentrations. Additionally, it helps determine any carry-over after running the sample on the modified EXTREVA ASE system. A single rinse with 40 mL of 4:1 Methanol:ACN was performed followed by blank cell extraction. The extracts were then analyzed on the TSQ Altis mass spectrometer to detect carryover.

Regarding analyte recoveries, 29 out of 31 reported compounds had recoveries between 73% and 98%. The recoveries for 3:3 FTCA and 7:3 FTCA were slightly lower at 65% and 70%, respectively, but still within the recovery limits specified by US EPA Method 1633A (Figure 7). The RSD% for all compounds was ≤ 20%.

In the carry-over samples, which were the blank cell extracts following the CRM run, only 6:2 FTS was detected at concentrations of 0.07 ± 0.018 ng/g. These levels are significantly below the EPA MDL of 0.39 ng/g (Figure 8), indicating minimal carry-over. Additionally, in the blank QC sample associated with this batch, we detected 6:2 FTS at 0.08 ng/g. These findings suggest that the single rinse with 40 mL of 4:1 Methanol:ACN was effective in cleaning the modified EXTREVA ASE system after processing a high-concentration sample (up to 46 ppb).

The detection of 6:2 FTS in both the carry-over and blank QC samples aligns with the frequent occurrence of this compound in method blanks, as noted in the US EPA multilab validation data. Despite this, the concentrations observed were well below the acceptable limits, demonstrating the robustness of the cleaning procedure. Therefore, it can be concluded that the single rinse protocol is sufficient to prevent significant carry-over, ensuring the integrity of subsequent analyses. This efficiency is crucial for maintaining the accuracy and reliability of PFAS measurements in environmental samples.

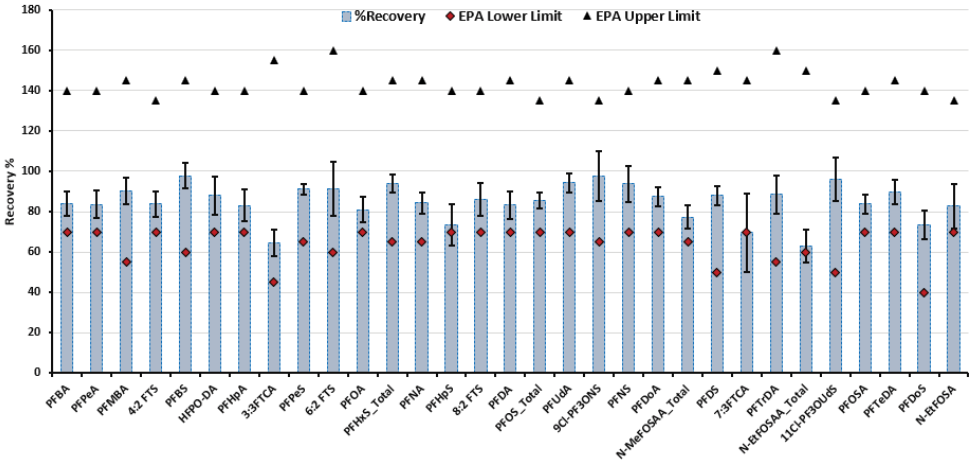

Figure 7. Recovery % of the PFAS analytes from the CRM #603 (ERA) compared to the US EPA 1633A IPR recovery requirements.

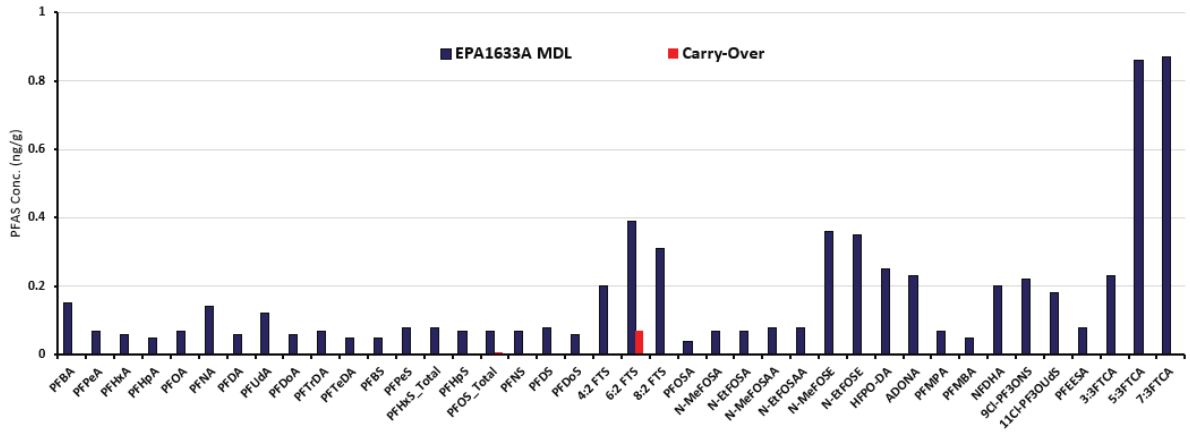

Figure 8. Carry-over in blank samples after running a high concentration CRM sample

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Conclusion

The method described for analyzing PFAS has demonstrated high efficiency and reliability in both low- and high-concentration samples. Specifically, the method has achieved excellent analyte recoveries at concentrations as low as 0.2 ng/g and as high as 46 ng/g, with RSD often below 15%. This indicates a robust performance across a wide range of concentrations, ensuring precise and accurate quantification of PFAS.

An important aspect of this method is its automation of several critical steps in the analytical workflow, including extraction and sample clean-up. Automation significantly reduces both the turnaround time and the manual labor required for the analysis. These efficiencies not only enhance throughput, but also minimize the potential for human error, thereby improving the overall reliability and reproducibility of the results. The reduction in time and labor underscores the method's practical advantages in a high-throughput laboratory setting, where efficiency and accuracy are paramount.

In summary, the method not only meets the analytical performance criteria for US EPA Method 1633A but also offers significant operational benefits. The combination of high-recovery rates, low RSD, and substantial reductions in turnaround time and manual labor make this method a valuable tool for laboratories tasked with PFAS analysis.

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6. MDL determination: Definition and Procedure for the Determination of the Method Detection Limit, Revision 2; December 2016

Supplemental data

Table S1: Concentrations of PFAS in calibration standards

Analyte	0.05 ppb Cal	0.1 ppb Cal	0.2 ppb Cal	0.5 ppb Cal	1.25 ppb Cal	2.5 ppb cal	5 ppb Cal	12.5 ppb Cal	62.5 ppb Cal
PFBA	0.20	0.40	0.80	2.00	5.00	10.0	20.0	50.0	250.0
PFPeA	0.10	0.20	0.40	1.00	2.50	5.0	10.0	25.0	125.0
PFHxA	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
PFHpA	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
PFOA	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
PFNA	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
PFDA	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
PFUdA	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
PFDoA	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
PFTrDA	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
PFTeDA	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
PFBS	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
PFPeS	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
PFHxS_Total	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
PFHpS	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
PFOS_Total	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
PFNS	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
PFDS	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
PFDoS	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
4:2 FTS	0.20	0.40	0.80	2.00	5.00	10.0	20.0	50.0	250.0
6:2 FTS	0.20	0.40	0.80	2.00	5.00	10.0	20.0	50.0	250.0
8:2 FTS	0.20	0.40	0.80	2.00	5.00	10.0	20.0	50.0	250.0
PFOSA	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
N-MeFOSA	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
N-EtFOSA	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
N-MeFOSAA	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
N-EtFOSAA	0.05	0.10	0.20	0.50	1.25	2.5	5.0	12.5	62.5
N-MeFOSE	0.50	1.00	2.00	5.00	12.50	25.0	50.0	125.0	625.0
N-EtFOSE	0.50	1.00	2.00	5.00	12.50	25.0	50.0	125.0	625.0
HFPO-DA	0.20	0.40	0.80	2.00	5.00	10.0	20.0	50.0	250.0
ADONA	0.20	0.40	0.80	2.00	5.00	10.0	20.0	50.0	250.0
PFMPA	0.10	0.20	0.40	1.00	2.50	5.0	10.0	25.0	125.0
PFMBA	0.10	0.20	0.40	1.00	2.50	5.0	10.0	25.0	125.0
NFDHA	0.10	0.20	0.40	1.00	2.50	5.0	10.0	25.0	125.0
9Cl-PF3ONS	0.20	0.40	0.80	2.00	5.00	10.0	20.0	50.0	250.0
11Cl-PF3OUdS	0.20	0.40	0.80	2.00	5.00	10.0	20.0	50.0	250.0
PFEESA	0.10	0.20	0.40	1.00	2.50	5.0	10.0	25.0	125.0
3:3FTCA	0.25	0.50	1.00	2.50	6.25	12.5	25.0	62.5	312.5
5:3FTCA	1.25	2.50	5.00	12.50	31.25	62.5	125.0	312.5	1562.5
7:3FTCA	1.25	2.50	5.00	12.50	31.25	62.5	125.0	312.5	1562.5