Determination of PFAS in Aqueous Environmental Samples by US EPA 1633 (4th draft) Using an Automated FREESTYLE XANA-PFAS SPE Tabletop System

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Keywords

- Sample Preparation
- LC-MS/MS
- High Throughput Lab Automation
- PFAS

Abstract

Water quality is of the utmost importance, and recently, the importance of analyzing aqueous environmental samples for emerging contaminants has been brought to light. Among the emerging compounds of interest are per- and polyfluorinated alkyl substances (PFAS), which have been found to be persistent environmental contaminants derived from various industries. For example, perfluorooctane sulfonate (PFOS) has been used in several industries, including the semiconductor and photographic industries, in some firefighting foams, and hydraulic fluids used in the aviation industry. Modern analytical laboratories specializing in these types of analyses are looking to automation to help increase sample throughput while ensuring the high quality of the resulting data.

The following application note shows how the FREESTYLE XANA-PFAS Tabletop robotic system can fully automate the preparation of aqueous samples for LC-MS/MS analysis according to the SPE-based method described in US EPA 1633 (4th draft) Method [1]. The resulting extracts are introduced into an LC-MS/MS instrument for detection and quantification.

Introduction

The US EPA 1633 (4th draft) method requires solid phase extraction (SPE) prior to liquid chromatography-tandem mass spectrometry (LC-MS/MS). This method uses SPE cartridges containing a weak anion exchanger, mixed-mode polymeric sorbent, and a dispersive clean-up step using graphitized carbon black. The EluCLEAN PFAS – WAX/GCB SPE column contains 150 mg of a weak anion exchanger, mixed-mode polymeric sorbent mixed with 10 mg of graphitized carbon black. This cartridge provides equivalent recoveries and low standard deviations for all PFAS compounds determined using this method, allowing it to replace the two-step dispersive clean-up step and WAX SPE cartridge used in US EPA 1633 (4th draft).

Implementation of fully automated parallel sample preparation allows multiple samples to be processed at the same time [2], providing high sample throughput with low demand for personnel resources. The FREESTYLE XANA-PFAS Tabletop sampler was designed specifically for PFAS determination. It contains no fluorine-containing polymers such as PTFE in the flow path, thus solving the significant issue of high blank values present in other systems. In this study, no measurable blank values were seen from the system.

Experimental

Materials

A mixed standard containing native branched and linear perfluorinated compounds listed in the US EPA method 1633 (4th draft) document was purchased from Wellington Laboratories (part number EPA-1633STK). A mixed standard containing the 24-isotope dilution internal standards listed within the US EPA method 1633 (4th draft) document was purchased from Wellington Laboratories (part number MPFAC-HIF-ES).

Two hundred and fifty (250) milliliters of water were added to polypropylene bottles. The appropriate volume of the primary dilution standard was added to create each replicate sample used for method evaluation, resulting in the concentrations shown in Table 1.

Table 1: Concentrations of PFAS compounds used during the study.

Compounds	Conc. [ng]
11Cl-PF3OUdS, 9Cl-PF3ONS, ADONA, HFPO-DA, NFDHA, PFEESA, PFMB, PFMPA, PFPeA	2
PFBA, 4:2FTS, 6:2FTS, 8:2FTS	4
N-MeFOSE, N-EtFOSE	10
5:3 FTCA, 7:3 FTCA	20
FBSA-I, PFECHS, FHXSA-I, P37DMOA, FOUEA, 6:2 diPAP, 8:2 diPAP	1.67
PFHxDA, PFODA	0.84
L-PFUdS, L-PFTrDS	3.35
6:2 PAP, 8:8PAP, PFDPA	16.75
All other PFAS	1

Acetic acid was added to the samples to achieve a pH of 4-7 and the samples were mixed. The method was designed to comply with the SPE procedure described in the US EPA 1633 (4th draft) method.

All other reagents and solvents used were reagent grade.

Instrumentation

All automated solid phase extractions were performed using an LCTech FREESTYLE XANA-PFAS Tabletop robotic sampler, as shown in Figure 1. All analyses were performed using a Thermo Scientific Vanquish Flex UHPLC with an Accucore RP-MS (2.1 x 100 mm, 2.6 µm) analytical column and Agilent ZORBAX Eclipse plus C18 (4.6 x 50 mm, 3.5 µm) Delay Column. The UHPLC system also included tubing modifications to minimize the PFAS background from the LC system by substituting all critical parts of the LC system made from materials that contain organic fluorine compounds. A Thermo Scientific TSQ Quantis Triple Quadrupole Mass Spectrometer was used for detection.

Figure 1: FREESTYLE XANA-PFAS Tabletop robotic sampler used to automate the US EPA 1633 (4th draft) method.

Automated Solid Phase Extraction Procedure

1. The user places the 250 mL water samples into the FREESTYLE XANA-PFAS Tabletop sampler rack as shown in Figure 2, along with corresponding 6 mL, EluCLEAN WAX/GCB SPE cartridges and 50 mL polypropylene centrifuge vials in their respective positions.
2. The FREESTYLE XANA-PFAS Tabletop sampler conditions the SPE cartridge using 15 mL of 1% ammonium hydroxide in methanol.
3. The FREESTYLE XANA-PFAS Tabletop sampler conditions the SPE cartridge using 5 mL of 0.3 M formic acid in water.
4. The FREESTYLE XANA-PFAS Tabletop sampler loads the entire water sample onto the SPE cartridge using a flow rate of 5 mL/min.
5. The FREESTYLE XANA-PFAS Tabletop sampler rinses the empty sample bottle once using 5 mL of water and loads the rinsate onto the SPE cartridge.
6. The FREESTYLE XANA-PFAS Tabletop sampler rinses the empty sample bottle once using 5 mL of (1:1) 0.1 M formic acid in water-methanol and loads the rinsate onto the SPE cartridge.
7. The FREESTYLE XANA-PFAS sampler dries the SPE cartridge for at least 15 minutes using nitrogen.
8. The FREESTYLE XANA-PFAS sampler rinses the empty sample bottle once using 5 mL of 1% ammonium hydroxide in methanol, loads the rinsate onto the SPE cartridge, and collects the eluate into a 50 mL polypropylene centrifuge tube.
9. The user adds 20 µL of concentrated acetic acid and 10 µL of the non-extracted internal standard mix to the final extract, mixes well, and filters through a nylon syringe filter (25 mm, 0.2 mm) into a 15 mL polypropylene vial.
10. The final extract is evaporated to 500 µL – 1 mL using the D-EVA rotational vacuum evaporator [3] (temperature: 45 °C, vacuum: 20 mbar) and then transferred into a polypropylene autosampler vial and placed on the LC-MS/MS system.

Figure 2: Loading of the FREESTYLE XANA-PFAS Tabletop robotic sampler for automated PFAS SPE extractions.

LC Method Parameters

Pump	Gradient Flow rate = 0.5 mL
Mobile Phase	A – 20 mM ammonium acetate in water, with 2% methanol and 0.1% acetic acid B – 20 mM ammonium acetate in methanol with 2% water and 0.1% acetic acid
Run time	25 minutes
Injection volume	5.0 µL
Column Temperature	45 °C

Mass Spectrometer Parameters

Operation	Electrospray negative mode
Spray voltage	2500 V
Sheath gas	50 Arb
Aux gas	10
CID gas	2 mTorr
Ion transfer tube temp.	325 °C
Vaporizer temp.	300 °C
Gas flow (N_2)	9 L/min
Q1 resolution	0.7 FWHM
Q3 resolution	1.2 FWHM
Cycle time	0.5 seconds
Chromatographic peak width	6 seconds

Results and Discussion

Representative mass chromatograms for both LOQ and blank samples following their automated extraction using the FREESTYLE XANA PFAS Tabletop robotic sampler are presented in Figure 3.

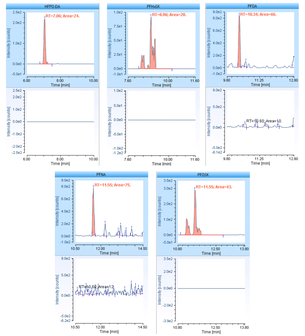

Figure 3: Overlay mass chromatogram comparisons of resulting of LOQ sample (upper) vs. blank sample (lower) processed using the FREESTYLE XANA-PFAS Tabletop robotic sampler for A). HFPO, B). PFHxS, C). PFOA, D). PFNA, and E). PFOS.

These results show the proposed PFAS compounds of the latest National Primary Drinking Water Regulation can be determined with high sensitivity and no background interference from the system. In fact, no responses were observed in blank samples at the retention times of all PFAS compounds which demonstrates that the FREESTYLE XANA PFAS Tabletop sampler is free from blind values when screened for the PFAS analytes found in US EPA 1633 (4th draft) method.

Two different SPE columns were examined during this study. The EluCLEAN PFAS – WAX/GCB SPE column can be used as an exact equivalent to the SPE cartridge plus dispersive graphitized carbon black designated within the US EPA 1633 (4th draft) method). This cartridge contains a weak anion exchanger, mixed-mode polymeric sorbent with a pKa above 8, and optimized parameters for PFAS enrichment. The EluCLEAN PFAS – Universal SPE column is a unique combination phase that improves the recovery rates of PFAS analytes through numerous interactions. This sorbent blend can be used with a wide variety of matrices and is available in a highly pigmented version (HP) with an improved matrix recovery for highly pigmented matrices.

The precision of the automated method was evaluated for all PFAS compounds using four replicate extracted laboratory-fortified blank samples having PFAS compound concentrations listed in Table 1. Figure 4 shows that the precision for all PFAS compounds extracted using the EluCLEAN PFAS – WAX/GCB SPE cartridge was found to be within the acceptance limit found within the US EPA method 1633 (4th draft) of being less than 20% RSD. Figure 5 shows that the precision of less than 20% RSD obtained for all PFAS compounds extracted using the EluCLEAN PFAS – Universal SPE cartridge was within the acceptance limit stated in US EPA method 1633 (4th draft).

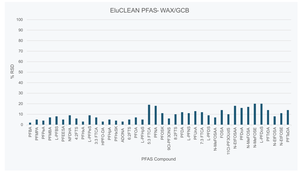

Figure 4: Results of Initial Demonstration of Precision (IDP) using the EluCLEAN PFAS – WAX/GCB cartridge.

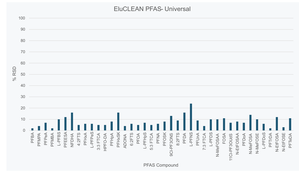

Figure 5: Results of Initial Demonstration of Precision (IDP) using the EluCLEAN PFAS – Universal cartridge.

TTo meet the acceptance criteria for the initial demonstration of accuracy (IDA) of the US EPA 1633 (4th draft) method, the average recovery of the replicate values of the laboratory-fortified blank samples used to establish precision must fall within ±30% of the true value. Figure 6 shows that the % recoveries for all PFAS compounds met this acceptance criteria when the EluCLEAN PFAS – WAX/GCB cartridge was used within the automated SPE procedure. Figure 7 shows that the % recoveries for all PFAS compounds also met this acceptance criteria when the EluCLEAN PFAS – Universal cartridge was used within the automated SPE procedure..

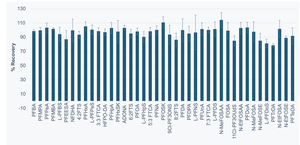

Figure 6: Results of Initial Demonstration of Accuracy (IDA) using the EluCLEAN PFAS – WAX/GCB cartridge.

Figure 7: Results of Initial Demonstration of Accuracy (IDA) using the EluCLEAN PFAS – Universal cartridge.

Conclusions

As a result of this study, we were able to show:

- All PFAS compounds found within the US EPA 1633 (4th draft) method can be successfully extracted from aqueous samples using an automated large-volume solid phase extraction method and determined using LC-MS/MS.
- This method was readily automated using the FREESTYLE XANA-PFAS Tabletop sampler.
- The background obtained using the FREESTYLE XANA-PFAS Tabletop sampler was free from background contamination.
- For all PFAS compounds, % recoveries were within 70% to 130%, meeting the US EPA 1633 (4th draft) method acceptance criteria.
- The precision of the automated SPE method was less than 20% RSD for all PFAS compounds determined, which meets the acceptance criteria of the US EPA 1633 (4th draft) method.
- Both the EluCLEAN PFAS – WAX/GCB and the EluCLEAN PFAS – Universal SPE cartridges were found to provide acceptable results according to the US EPA 1633 (4th draft) method, enabling a single SPE cartridge to be used for clean-up and enrichment.

References

[1] United States Environmental Protection Agency, 4th Draft Method 1633*: Analysis of Per- and Polyfluorinated Alkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS, https://www.epa.gov/system/files/documents/2022-12/3rd%20Draft%20Method%201633%20December%202022%2012-20-22_508.pdf, accessed July 2023. *Finalized for the Aqueous Matrices: Wastewater, Surface Water, and Groundwater.

[2] LCTech Application Note No. AN0033, Determination of Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) in Water with FREESTYLE XANA and LC-MS/MS, 2022.

[3] LCTech Application Note No. AN0045, D-EVA – Automated EVAporation of PFAS compliant to US-EPA 537.1, 2021.