Extraction of 40 PFAS Compounds from Soil and Tissue Following EPA Method 1633

Abstract

Per- and polyfluoroalkyl substances (PFAS) are a class of manmade chemicals used in various industries due to their favorable properties for goods such as nonstick cookware and firefighting foam. Their stability and widespread use have contributed to their accumulation in the environment, and the lack of remediation techniques for their removal has allowed for their bioaccumulation in humans and animals. PFAS have been shown to cause health issues in humans, such as cancer, endocrine disruption, and infertility. Thus, monitoring environmental solid samples, such as soil and tissue, is critical. The EDGE PFAS™ is an automated solvent extraction system designed for the extraction of PFAS from solid samples. In this study, the EDGE PFAS was used to extract 40 spiked PFAS compounds from soil and tissue following EPA Method 1633.[1] The automated extraction was less than 10 minutes per sample and yielded acceptable recoveries and RSDs without carryover in the system. The EDGE PFAS is an ideal option for laboratories that want to automate their PFAS extractions of solid samples.

Introduction

There are currently thousands of PFAS compounds that have been used extensively across many industries. Due to their exceptional durability and bioaccumulation, they have earned the moniker of forever compounds. PFAS possess a chain of linked carbon atoms with fluorine atoms branching off of the main chain. The presence of the strong carbon-fluorine bond contributes to the stability of these compounds. Due to their ubiquity, PFAS have leached into the environment through production and waste streams, making their way into water sources. From these water sources, PFAS can rapidly spread, contaminating soil and biological tissue. Furthermore, these compounds have been found to bioaccumulate in animals and humans, and exposure in humans has been shown to cause adverse health outcomes. Thus, the assessment of the levels of PFAS in the environment is important to the health and safety of humans.

The Environmental Protection Agency (EPA) have provided EPA Method 1633 for analysis of PFAS, including soil and tissue sample types. The extraction method for the solid samples detailed in this method is a long manual process. Since the method is performance-based, the extraction can be modified as long as quality control requirements are met. The EDGE PFAS system can be used to extract both the soil and tissue samples in less than 10 minutes, automating the solvent addition, extraction, and filtering of the extract. This allows for a rapid, efficient, and simple extraction of PFAS from these solid environmental samples.

In this work, the EDGE PFAS was utilized to effectively extract PFAS from soil and tissue samples with acceptable recoveries and RSD values. Animal tissues are difficult matrices to extract and add complexity to both the sample preparation and the analysis. With the EDGE PFAS system, one simple method can be applied to many different difficult sample types.

Materials and Methods

Reagents and Samples

Soil reference material, Soil 2022-110, was purchased from North American Proficiency Testing (NAPT), ground chicken was purchased from a local grocery retailer. The chicken was used as a representative matrix for tissue samples as described in EPA Method 1633. The majority of the reagents: HPLC-grade methanol, HPLC-grade water, potassium hydroxide, formic acid, and acetic acid, were purchased from MilliporeSigma. Ammonium hydroxide was purchased from Honeywell. Native Replacement PFAS Solution (PFAC-MXF), Native Perfluoroalkyl Ether Carboxylic Acids and Sulfonates Solution (PFAC-MXG), Native PFAS Solution (PFAC-MXH), Native-N-Me/EtFOSA Solution (PFAC-MXJ), Native X:3 Fluorotelomer Carboxylic Acid Solution (PFAC-MXJ), and Mass-Labelled PFAS Extraction Standard Solution (MPFAC-HIF-ES) were purchased from Wellington Laboratories. The cleanup material, graphitized carbon black, was purchased from Restek Corporation, and Oasis® WAX for PFAS analysis 6 cc vac cartridge was purchased from Waters Corporation.

EDGE Sample Preparation

Each Q-Cup® was rinsed with methanol and allowed to dry prior to use. Q-Cups were prepared with the Q-Disc® PFAS, followed by weighing 5 g of soil or 2 g of ground chicken into each Q-Cup. Each sample was spiked with native PFAS at the concentrations listed in **Table 1** (page 2). The extracted internal standards (EIS) were spiked at the concentrations listed in EPA Method 1633. Each sample was prepared in quadruplicate. All Q-Cups, along with polypropylene centrifuge tubes, were loaded into an EDGE PFAS rack and extracted on the EDGE PFAS system using the method listed.

Table 1. Spiked Concentrations of the Native PFAS Compounds

2.5 ng/mL	5 ng/mL	10 ng/mL	25 ng/mL	50 ng/mL
PFHxA	PFPeA	PFBA	NMeFOSE	5:3FTCA
PFHpA	HFPO-DA	4:2FTS	NEtFOSE	7:3FTCA
PFOA	ADONA	6:2FTS		
PFNA	PFMPA	8:2FTS		
PFDA	PFMBA	3:3FTCA		
PFUnA	NFDHA			
PFDoA	9Cl-PF3ONS			
PFTrDA	11Cl-PF3OUdS			
PFTeDA	PFEESA			
PFBS				
PFPeS				
PFHxS				
PFHpS				
PFOS				
PFNS				
PFDS				
PFDoS				
PFOSA				
NMeFOSA				
NEtFOSA				
NMeFOSAA				
NEtFOSAA				

EDGE PFAS Method for PFAS from Soil and Tissue

Q-Disc: Q-Disc PFAS

Cycle 1
Extraction Solvent: 0.3% ammonium hydroxide in methanol (soil) or 0.05 M KOH in methanol (tissue)
Top Add: 15 mL
Rinse: 0 mL
Temperature: 65 °C
Hold Time: 03:00 (mm:ss)

Cycle 2
Extraction Solvent: 0.3% ammonium hydroxide in methanol (soil) or 0.05 M KOH in methanol (tissue)
Top Add: 10 mL
Rinse: 5 mL
Temperature: 65 °C
Hold Time: 03:00 (mm:ss)

Wash 1
Wash Solvent: extraction solvent
Wash Volume: 15 mL
Temperature: 65 °C
Hold Time: 00:15 (mm:ss)

Wash 2
Wash Solvent: extraction solvent
Wash Volume: 15 mL
Temperature: ---
Hold Time: --:-- (mm:ss)

Post Extraction Cleanup

Samples were concentrated under nitrogen at 55 °C to 7 mL and reconstituted up to 50 mL with HPLC grade water. The pH of the samples was adjusted with 50% formic acid or 30% ammonium hydroxide to pH 6.5 +/- 0.5. The samples then underwent loose graphitized carbon black and WAX SPE cleanup, according to EPA Method 1633.

Analysis

Analysis was done by Waters Corporation using an ACQUITY™ Premier System attached to a Xevo™ TQ Absolute. The LC system was modified with the Waters PFAS Analysis Kit. The compounds were separated using an ACQUITY Premier BEH C18 column (2.1 mm x 50 mm, 1.7 µm). A 2 µl injection was used, and the mobile phases were 2 mM ammonium acetate in water (A) and 2 mM ammonium acetate in acetonitrile (B). The gradient used is indicated in **Table 2**. The source parameters used to monitor the MRM transitions of each compound are in **Table 3**.

Table 2. UPLC Gradient Used for Separation

Time (min)	Flow (mL/min)	%A	%B
0	0.3	95	5
0.5	0.3	75	25
3	0.3	50	50
6.5	0.3	15	85
7	0.3	5	95
8.5	0.3	5	95
9	0.3	95	5
11	0.3	95	5

Table 3. Source Parameters Used

Parameter	Value
Ion mode	ESI-
Source temp	100 °C
Capillary Voltage	0.5 kV
Desolvation Temperature	350 °C
Desolvation Flow	900 L/hr
Cone Flow	150 L/hr

Results

Samples for both the soil and chicken were extracted with a simple and rapid automated extraction method; the same parameters were used for both sample types, with exception of the extraction solvent. The extraction solvents were chosen based on EPA Method 1633. For both sample types, extraction took less than 10 minutes, including solvent addition, extraction, and filtration. The same clean up and analysis procedures were applied to all samples. Acceptable % recovery and % RSD values were achieved for all 40 native PFAS compounds in both sample types, as seen in **Table 4**. Also, acceptable % recovery and % RSD were achieved for the extracted internal standards in both sample types, as seen in **Table 5** (page 4). Using traditional extraction techniques, three long cycles are generally required to efficiently extract both soil and tissue samples, with tissue samples taking longer than 16 hours. Utilizing the EDGE PFAS, only two short cycles of 3 minutes each were needed to achieve acceptable recoveries for both soil and chicken samples.

The soil samples exhibited slightly higher recovery values and tighter % RSDs when compared to those for chicken; all were in the acceptable range. This small difference may be attributed to the higher fat content of the chicken sample and other contaminants present that interfered with analysis. Tissue samples are known to be a challenging matrix to extract. Being able to use a rapid, simple, and efficient automated extraction for these challenging samples will greatly help environmental PFAS labs that are dealing with an influx of PFAS samples.

Conclusion

PFAS are an ongoing issue for environmental contamination and, as the scope of required testing increases, the more we learn. Their migration throughout the ecosystem has led to PFAS contamination being discovered nearly in all corners of the globe and in all manner of living beings. As analysis methods increase in sensitivity, simpler and quicker extraction methods are also needed to contend with the increasing sample throughput required. In this study, we have shown the use of the EDGE PFAS to extract spiked soil and tissue samples. Acceptable recoveries and RSD values were achieved with a rapid, simple, and efficient automated extraction method.

Table 4. Average % Recovery Values and % RSD (n=4) for 40 Native PFAS in Soil and Chicken

	Soil		Chicken	
Compound	**% Recovery**	**% RSD**	**% Recovery**	**%RSD**
PFBA	115	7.59	99.2	14.3
PFPeA	114	5.73	99.2	11.6
PFHxA	110	4.55	99	11.9
PFHpA	115	4.16	101	9.07
PFOA	115	7.59	98.3	9.38
PFNA	119	3.9	98.2	9.62
PFDA	118	9.27	99.5	8.63
PFUnA	111	6.84	95.4	11.2
PFDoA	118	7.68	102	8.57
PFTrDA	110	6.82	101	9.28
PFTeDA	115	6.87	98	9.67
PFBS	110	1.14	91.3	17.7
PFPeS	109	11.7	84.4	12.1
PFHxS	79.8	10.9	72.1	10.6
PFHpS	110	7.85	94	13.4
PFOS	92	17	71.9	10.2
PFNS	120	5.23	105	10.2
PFDS	112	7.3	93.6	10.2
PFDoS	102	10.2	85.4	9.5
4:2FTS	118	8.72	107	10.7
6:2FTS	115	5.71	99.3	11.9
8:2FTS	115	9.74	104	7.58
PFOSA	116	9.1	100	9.65
NMeFOSA	90.4	7.24	77.2	8.5
NEtFOSA	90.6	7.52	78	9.2
NMeFOSAA	93.9	9.42	77.8	8.16
NEtFOSAA	94.9	10.9	74.2	10.9
NMeFOSE	94.7	7.32	80.6	8.48
NEtFOSE	90.3	6	78.8	9.04
HFPO-DA	85.7	3.89	74.5	11.6
ADONA	83.7	8.14	78.1	11.5
PFMPA	93.1	8.02	83.6	10
PFMBA	93.9	4.97	83.4	13
NFDHA	90	5.45	79.2	15.1
9Cl-PF3ONS	90.6	5.6	95.6	7.05
11Cl-PF3OUdS	85.7	6.17	91.2	7.98
PFEESA	89.1	3.55	80.3	14.2
3:3FTCA	64.1	3.98	93.5	12.7
5:3FTCA	78.1	7.12	95.4	13.9
7:3FTCA	93.2	7.64	121	11.1

Table 5. Average % Recovery Values and % RSD (n=4) for the Extracted Internal Standards in Soil and Chicken

	Soil		Chicken	
Compound	**% Recovery**	**% RSD**	**% Recovery**	**%RSD**
$^{13}C_4$-PFBA	83.08	4.13	82.93	11.18
$^{13}C_5$-PFPeA	86.83	4.68	85.28	13.48
$^{13}C_5$-PFHxA	90.10	7.10	90.50	12.61
$^{13}C_4$-PFHpA	88.65	5.70	92.68	9.64
$^{13}C_8$-PFOA	88.53	7.86	102.33	6.62
$^{13}C_9$-PFNA	87.73	4.72	109.20	5.13
$^{13}C_6$-PFDA	86.43	10.59	106.13	4.27
$^{13}C_7$-PFUnA	95.90	9.03	115.40	9.06
$^{13}C_2$-PFDoA	97.88	10.96	120.18	6.27
$^{13}C_2$-PFTeDA	93.35	9.60	120.20	6.47
$^{13}C_3$-PFBS	82.75	6.12	84.98	7.99
$^{13}C_3$-PFHxS	88.30	6.60	98.85	7.94
$^{13}C_8$-PFOS	88.83	8.69	107.38	4.43
$^{13}C_2$-4:2FTS	67.98	7.59	74.05	6.84
$^{13}C_2$-6:2FTS	76.70	3.68	88.83	8.30
$^{13}C_2$-8:2FTS	109.30	2.40	148.23	7.39
$^{13}C_8$-PFOSA	75.68	7.16	95.43	3.70
D_3-NMeFOSA	60.33	3.96	84.75	5.13
D_5-NEtFOSA	62.40	3.67	85.80	3.46
D_3-NMeFOSAA	77.55	9.58	117.03	6.22
D_5-NEtFOSAA	91.00	14.23	154.08	4.83
D_7-NMeFOSE	64.40	7.44	87.85	3.39
D_9-NEtFOSE	69.80	6.17	84.60	4.57
$^{13}C_3$-HFPO-DA	88.03	2.78	89.25	8.39

Reference

[1] United States Environmental Protection Agency. Method 1633 Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS, Revision 1, January, 2024. https://www.epa.gov/system/files/documents/2024-01/method-1633-final-for-web-posting.pdf (accessed February 6, 2024).

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