Extraction of 30 PFAS Compounds from Clams and Bacon

Abstract

The presence of Per- and Polyfluoroalkyl Substances (PFAS) in food has become an increasing concern due to their persistence and bioaccumulation. As regulations tighten with decreasing action limits, the need for a harmonized and accurate method for PFAS determination is critical. PFAS solvent extraction from food is inherently difficult due to contamination risks, the low concentrations at which these compounds occur, and the complexity of food matrices. Traditional extraction methods are often manual, time-consuming, and inefficient. This study evaluates the EDGE PFAS™ system, an automated solvent extraction system, for extracting PFAS from clams and bacon. This method provides a streamlined process with efficient extraction, minimal sample handling, and high recovery rates with excellent reproducibility. The EDGE PFAS™ system offers a rapid, simple, and effective solution for PFAS testing in food, supporting regulatory compliance and food safety efforts.

Introduction

As concern for PFAS in solid samples continues to grow, new methodologies that are applicable for these sample types are emerging. With EPA 1633A[1], ASTM D8535-23[2], and FDA method C-010.03[3], there is good direction on how to approach the challenges of solid samples for PFAS analysis. Common to all methodology for PFAS in solid samples is a solvent extraction step, however the specifics do vary. In general, the solvent extraction methods for solid samples are long, manual processes. AOAC has published Standard Method Performance Requirements (SMPRs®) for PFAS in produce, beverages, dairy products, eggs, seafood, meat products, and feed: SMPR 2023.003[4]. The SMPR outlines the necessary recovery and repeatability standards.

The samples analyzed, both clams and bacon, are included in the target matrix categories for SMPR 2023.003. Clams could also be considered tissue samples, covered under EPA 1633A. Methodologies may differ when considering these samples as food or environmental, particularly in the solvent extraction step, where different solvents may be used. Here, a simple, two-cycle extraction, using methanol as the solvent, demonstrates proof of concept of the extraction efficiency of the EDGE PFAS system. This method can easily be adapted to use the solvents described in EPA 1633A, ASTM D8535-23 or FDA method C-010.03.

The EDGE PFAS system can extract solid samples in less than 15 minutes, automating the solvent addition, extraction, and filtration of the extract. This enables rapid, efficient, and simple extraction of PFAS from these samples. Utilizing this automated solvent exaction process to extract clams and bacon resulted in acceptable recoveries and RSD values in accordance with AOAC SMPR 2023.003. The EDGE PFAS system offers a versatile solution for laboratories, applying one simple method to varying challenging sample types.

Materials and Methods

Reagents and Samples

Fresh clams and bacon were purchased from a local grocery retailer. The HPLC-grade methanol and HPLC-grade isopropanol were purchased from Sigma Aldrich. Native PFAS Precision and Recovery Standard Solution (PFAC30PAR), Sodium Perfluoro-1-Undecanesulfonate (L-PFUdS), Sodium Perfluoro-1-Dodecanesulfonate (L-PFDoS), Sodium Perfluoro-1-Tridecanesulfonate (L-PFTrDS), and Sodium 1H,1H,2H,2H-perfluorododecane sulfonate (10:2 FTS) were purchased from Wellington Laboratories. The eCleanUP™, a proprietary sorbent, was purchased from CEM Corporation.

Sample Preparation

The IKA A11 basic analytical mill was used to mill the samples. The fresh clams were removed from their shells prior to milling. Both fresh clams and bacon had been frozen prior to 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 the addition of 1 g of eCleanUP and then 2 g of milled fresh clams or bacon into each. A spiking solution containing 100 ng/mL of each of the 30 native PFAS was prepared. Half of the samples were spiked with 100 µl of the prepared spiking standard prior to extraction while the other half were spiked at the same concentration post-extraction. Each sample was prepared in triplicate. 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 on page 2.

EDGE PFAS Method for PFAS from Food

Q-Disc: Q-Disc PFAS

Cycle 1
Extraction Solvent: Methanol
Top Add: 10 mL
Temperature: 65 °C
Hold Time: 03:00 (mm:ss)

Cycle 2
Extraction Solvent: Methanol
Top Add: 10 mL
Temperature: 65 °C
Hold Time: 03:00 (mm:ss)

Wash 1
Wash Solvent: Isopropanol
Wash Volume: 10 mL
Temperature: --
Hold Time: --:--

Wash 2
Wash Solvent: Methanol
Wash Volume: 10 mL
Temperature: 65 °C
Hold Time: 00:30 (mm:ss)

Wash 3
Wash Solvent: Methanol
Wash Volume: 10 mL
Temperature: --
Hold Time: --:--

Analysis

Separation and analysis was performed by Waters Corporation using an ACQUITY™ Premier BSM FTN System with a PFAS kit and a Xevo™ TQ Absolute MS. 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 1**. The source parameters used to monitor the MRM transitions of each compound are in **Table 2**.

Table 1. 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 2. 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

The fresh clams contained a high amount of moisture. To ensure the entire sample remained inside the Q-Cup and did not seep through the Q-Disc prior to extraction, eCleanUP, a proprietary sorbent, was added to the bottom of each Q-Cup. To maintain consistency between the two sample types, eCleanUP was used with all samples.

Both clams and bacon were extracted using the same EDGE PFAS method, which generated a filtered extract upon run completion. These extracts were then diluted and analyzed. The efficiency of the extraction method was assessed by comparing extracts spiked prior to extraction with those spiked post-extraction. This simple approach allows for a clear understanding of the performance of the EDGE PFAS system. The percent recoveries and RSD values for the 30 spiked PFAS are reported in **Table 3** (Page 4). These 30 spiked PFAS are the target analytes of SMPR-2023.003, which establishes performance criteria for recovery and repeatability of these analytes. PFOS, PFOA, PFHxS, and PFNA are regulated in the EU, thus their percent recovery should be within 80-120% with repeatability less than or equal to 20%. For the non-regulated PFAS, the percent recovery should be within 65-135% with repeatability less than or equal to 25%. The performance criteria of SMPR-2023.003 were met for all 30 PFAS extracted from clams and bacon using the EDGE PFAS system.

This study focused on the solvent extraction efficiency of the EDGE PFAS system. Modifications to this method, such as solvent, solvent volume, and number of cycles can be made, based on the needs of the lab. However, changes to the temperature and hold time are not recommended, as these parameters have been optimized for the extraction of PFAS. To further highlight the efficiency of the solvent extraction, no cleanup was performed on the extracts prior to analysis. When extracting complex sample types, it is advisable to include a cleanup step. In this study, matrix effects did not compromise the results. Furthermore, a cleanup step helps protect analytical equipment. In-cell cleanup can be performed on the EDGE PFAS system.

Conclusion

PFAS continue to pose significant environmental contamination challenges and, as the scope of required testing increases, our understanding deepens. Their migration throughout the ecosystem has led to PFAS contamination being detected in nearly every corner of the globe and in all manner of solid samples. As analysis methods increase in sensitivity, there is a growing need for simpler and quicker extraction methods to contend with the increasing sample throughput. In this study, the EDGE PFAS system was shown to extract PFAS from clams and bacon. The automated extraction method proved to be rapid, simple, and efficient, yielding acceptable recoveries and RSD values.

References

[1] United States Environmental Protection Agency. Method 1633, Revision A Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LCMS/MS, December, 2024. https://www.epa.gov/system/files/documents/2024-12/method-1633a-december-5-2024-508-compliant.pdf (accessed May 20, 2025).

[2] ASTM International. Designation: D8535-23, Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Soil/Biosolid Matrices by Solvent Extraction, Filtering, and Followed by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS), 2023. https://store.astm.org/d8535-23.html (accessed May 20, 2025).

[3] U.S. Food and Drug Administration: Method Number C-010.03, Determination of 30 Per and Polyfluoroalkyl Substances (PFAS) in Food and Feed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), 2024. https://www.fda.gov/media/131510/download (accessed May 20, 2025).

[4] AOAC: SMPR® 2023.003, Standard Method Performance Requirements (SMPRs) for Per- and Polyfluoroalkyl Substances (PFAS) in Produce, Beverages, Dairy Products, Eggs, Seafood, Meat Products, and Feed, 2023. https://www.aoac.org/wp-content/uploads/2023/11/SMPR-2023_003-1.pdf (accessed May 20, 2025).

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Table 3. Average % Recovery and % RSD (n=3) for 30 PFAS in Clams and Bacon

	Clams		Bacon	
Compound	**% Recovery**	**% RSD**	**% Recovery**	**% RSD**
PFBA	89	5.3	122	20
PFPeA	95	5.7	110	27
PFHxA	86	10	102	14
PFHpA	93	3.6	111	7.4
PFOA	115	5.0	97	3.3
PFNA	111	2.3	106	4.4
PFDA	106	11	104	7.7
PFUnA	101	3.8	106	8.0
PFDoA	102	8.4	103	11
PFTrDA	100	3.6	105	3.9
PFTeDA	104	4.6	96	1.3
PFBS	95	9.6	95	18
PFPeS	104	1.8	89	11
PFHxS	100	4.4	99	5.2
PFHpS	87	5.6	104	4.5
PFOS	87	15	104	8.6
PFNS	94	6.5	108	11
PFDS	98	9.8	114	5.2
PFUnDS	91	9.0	118	9.0
PFDoS	96	7.1	120	8.5
PFTrDS	91	9.0	120	6.5
PFOSA	100	1.0	111	6.2
9Cl-PF3ONS	106	8.1	110	4.1
11Cl-PF3OUdS	93	6.0	110	4.8
HFPO-DA	92	6.9	85	2.0
DONA	127	9.6	94	2.6
4:2 FTS	76	11	91	12
6:2 FTS	105	1.8	103	6.3
8:2 FTS	91	1.4	79	7.5
10:2 FTS	84	14	122	8.6

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