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Application News

No. HPLC-037

High Performance Liquid Chromatography

Analysis of PFAS Compounds in Fish Tissue Using Offline Supercritical Fluid Extraction and LC-MS/MS

■ Introduction

Per and Poly-fluoroalkyl substances (PFAS) are synthetic compounds that are found in a wide range of industrial and consumer products. Due to the strong nature of the carbon-fluorine bond, these compounds are resistant to degradation and have been found to accumulate in fish, wildlife and multiple environmental samples (ex. water, soil…), posing a significant health risk to humans. Current sample preparation techniques for PFAS analysis are laborious and not easily automated. In this study, supercritical fluid extraction (SFE) was evaluated as an alternative sample preparation technique for the extraction of eighteen PFAS compounds from fish tissue, as a preconcentration step prior to their analysis by LC-MS/MS.

■ Experimental Approach

For this study, the Shimadzu Nexera UC offline SFE system (configuration shown in Figure 1) was employed. 0.5 grams of freeze-dried fish tissue was milled and mixed with 1 packet (1 gram) of Miyazaki Hydro-Protect and placed into a 5 mL extraction vessel for extraction.

Optimized extraction conditions to maximize PFAS recoveries are shown in Table 1. After extraction, the sample was dried down under nitrogen and reconstituted with 1 mL of methanol. The sample was centrifuged and the supernatant was transferred to an LC vial. 1 uL of the supernatant was injected for LC-MS/MS analysis. Table 2 shows the LC-MS/MS conditions used for the Shimadzu LCMS-8050 for this study; a representative chromatogram is included in Figure 2.

Table 1: SFE optimized method conditions

Item	Value
Mobile phase	CO_2/MeOH
Modifier concentration	20% MeOH
Flow rate	5 mL/min
Vessel temperature	60 ℃
Extraction cycles	3
Back pressure	20 MPa
Extraction time	45 minutes

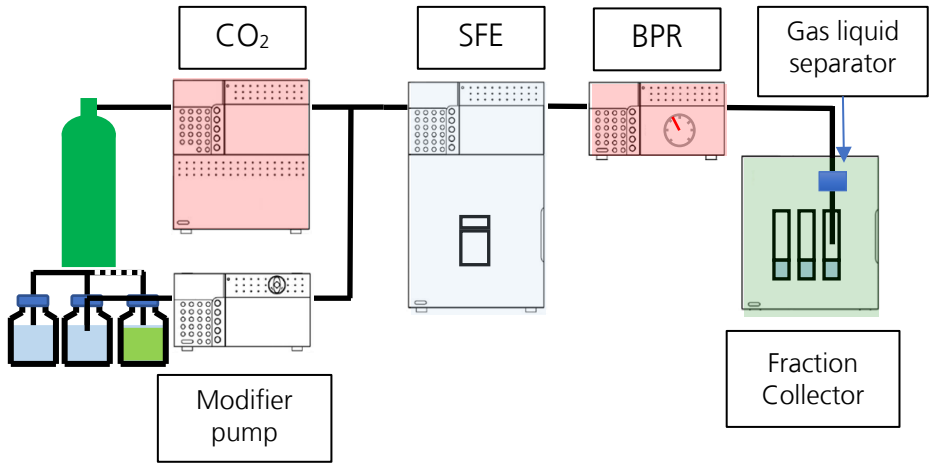


Figure 1: System Configuration of offline SFE system for direct collection method.
CO2: CO2 pump; SFE: Supercritical Fluid Extraction Module; BPR: Back pressure regulator

Table 2: LC-MS/MS method conditions used in Shimadzu LCMS-8050

Item	Value
Column	Shim-pack GIST C18 2.7 um 100 x 2.1 mm
Delay column	XR-ODSII 3 x 75 mm
Mobile phase	A: 10 mM ammonium acetate in H_2O; B: MeOH
Flow rate	0.5 mL/min
Gradient	0 min: 20% B 9 min: 90% B 11 min: 90% B 11.5 min: 20% B 15 min: 20% B
Oven temperature	35 °C
Injection volume	1 µL
Ionization mode	ESI (-)

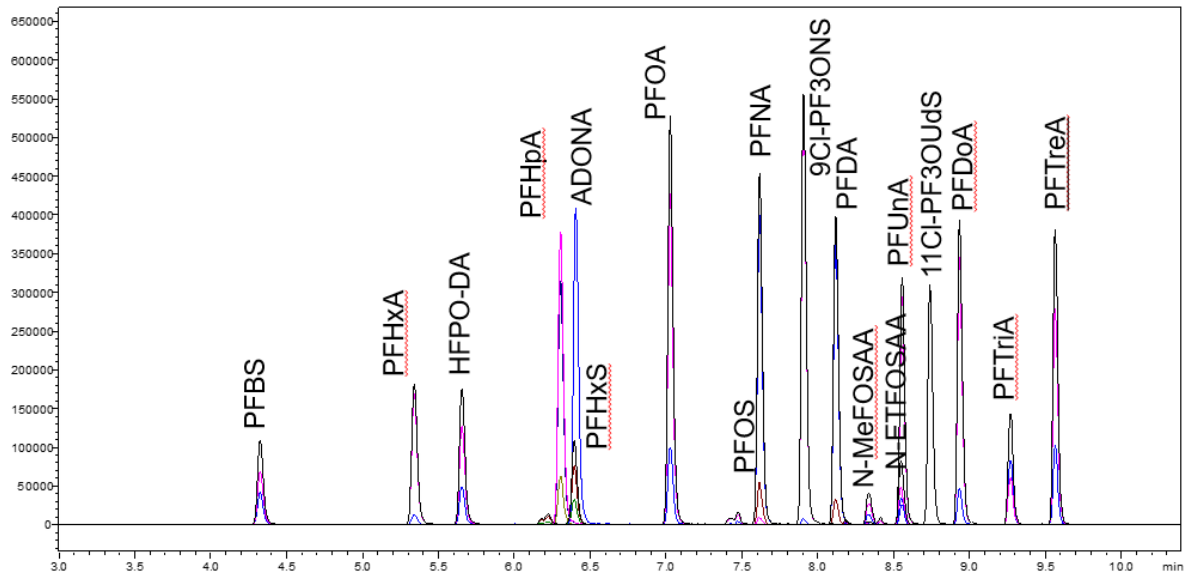


Figure 2: LC-MS/MS chromatogram of target PFAS in a commercial standard diluted in MeOH (50 pg each on column)

■ Results and Discussion

Recovery, Linearity, Reproducibility

A set of experiments to identify the combination of CO_2's modifier and additives that maximized the extraction efficiency of 18 PFAS was first conducted in this work. While 100% CO_2 can be effective in extracting nonpolar compounds, the addition of a cosolvent is often required in SFE to extract more polar compounds. Optimum extraction conditions were found to be 20% methanol without the need for additives. The 18 targets from this study showed recoveries over 95% with these conditions, as shown in Table 3.

Linearity of a matrix matched calibration curve, to minimize the impact from coextracted matrix components, was evaluated. Concentrations from 0.5 to 100 ng/g were spiked to a freeze-dried farm-raised trout fish tissue sample found to be free from PFAS contamination.

Linearity results are shown in Table 4 along with the determined limit of quantitation for each compound; r^2 for all compounds was >0.9995 except for N-MeFOSAA (r^2: 0.9994). Linearity results show accurate determinations for PFAS compounds can be obtained regardless of concentration levels.

Reproducibility results for supercritical fluid extractions were determined at three PFAS concentration levels: 2 ng/g, 20 ng/g and 100 ng/g. Extractions were performed in triplicated samples. Table 5 summarizes the variability of the extraction at each of the concentrations evaluated. %RSDs at 20 and 100 ng/g were less than 12% for all compounds evaluated. At 2 ng/g, %RSD was less than 25%, except for PFTriA (27%) and N-MeFOSAA (45%). These results demonstrate the reproducibility of SFE as a sample preparation technique.

Table 3: % recovery of target PFAS

Compound	% recovery
PFBS	98.7
PFHxA	105.9
HFPO-DA	97.4
PFHxS	102.7
PFHpA	100.5
ADONA	100.7
PFOA	104.2
PFNA	101.9
PFOS	98.1
9Cl-PF3ONS	100.5
PFDA	99.9
N-MeFOSAA	102.2
N-EtFOSAA	97.6
PFUnA	94.6
11Cl-PF3OUds	102.2
PFDoA	96.3
PFTriA	99.8
PFTreA	97.2

Quantitative analysis of fish samples

Three fish samples with unknown PFAS concentrations were then evaluated with this method. The samples were wild caught Walleye, wild caught Large Mouth Bass, and farm raised Trout. Figure 3 shows the LC-MS/MS chromatogram of an extracted sample from each fish's type. Table 6 shows the concentration of PFAS determined in each type of fish. The wild caught Walleye and Large Mouth Bass were found to contain the largest amounts of PFOS, PFDA, and PFUnA. No PFAS compounds were detected above the LOQ in the farm raised Trout sample.

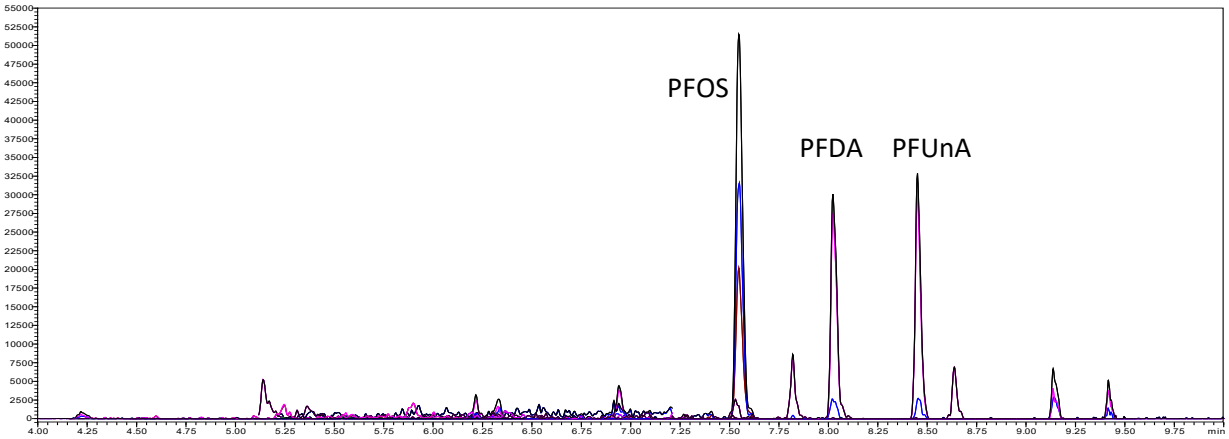
Table 4: Linearity of PFAS compounds spiked onto fish tissue

	Lowest Calibration Standard (LOQ) ng/g spiked on fish	Highest Calibration Standard ng/g spiked on fish	Linearity (R^2)
PFBS	0.5	100	0.9999
PFHxA	0.5	100	0.9995
HFPO-DA	1	100	0.9997
PFHpA	1	100	0.9996
PFHxS	0.5	100	0.9999
ADONA	0.5	100	0.9997
PFOA	0.5	100	0.9997
PFNA	0.5	100	0.9997
PFOS	2	100	0.9999
9Cl-PF3ONS	1	100	0.9995
PFDA	0.5	100	0.9998
N-MeFOSAA	2	100	0.9994
N-ETFOSAA	1	100	0.9999
PFUnA	1	100	0.9997
11Cl-PF3OUdS	0.5	100	0.9999
PFDoA	1	100	0.9996
PFTriA	2	100	0.9997
PFTreA	1	100	0.9995

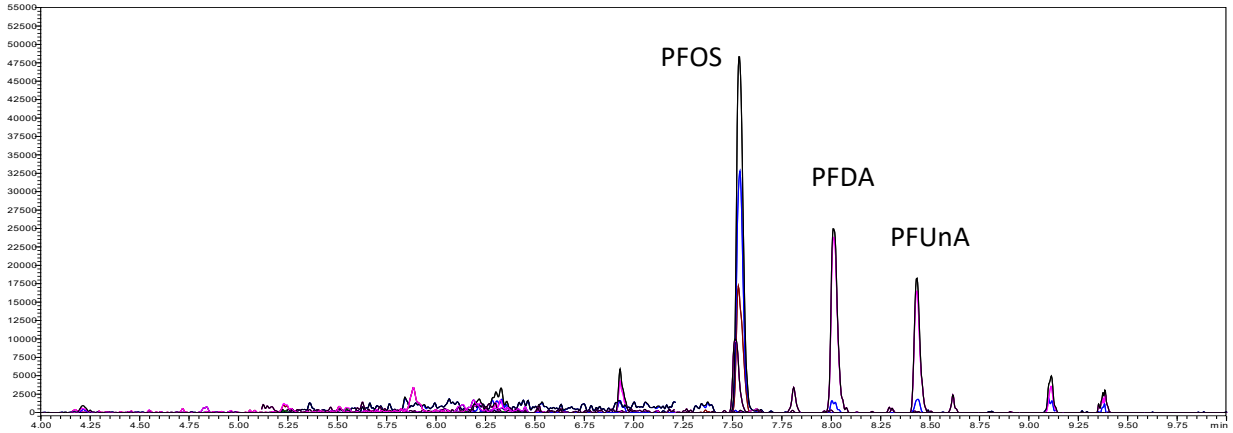
Table 5: Reproducibility of PFAS SFE extractions (n=3)

	%RSD		
	100 ng/g	20 ng/g	2 ng/g
PFBS	2.3	7.9	21.7
PFHxA	4.9	4.1	15.6
HFPO-DA	3.9	4.4	9.9
PFHxS	4.2	4.4	19.9
PFHpA	2.6	4.9	2.4
ADONA	3.9	3.2	13.2
PFOA	2.9	3.1	13.1
PFNA	3.5	3.6	18.1
PFOS	4.1	3.9	22.1
9Cl-PF3ONS	2.5	1.3	3.6
PFDA	1.6	7.4	20.9
N-MeFOSAA	9.5	9.6	44.7
N-EtFOSAA	8.4	6.2	10.7
PFUnA	2.3	2.8	18.4
11Cl-PF3OUds	4.1	4.9	7.8
PFDoA	4.7	5.8	15.9
PFTriA	4.4	11.6	26.8
PFTreA	2.3	3.6	11.5

(a) Wild caught Large Mouth Bass



(b) Wild caught Walleye



(c) Farm raised Trout

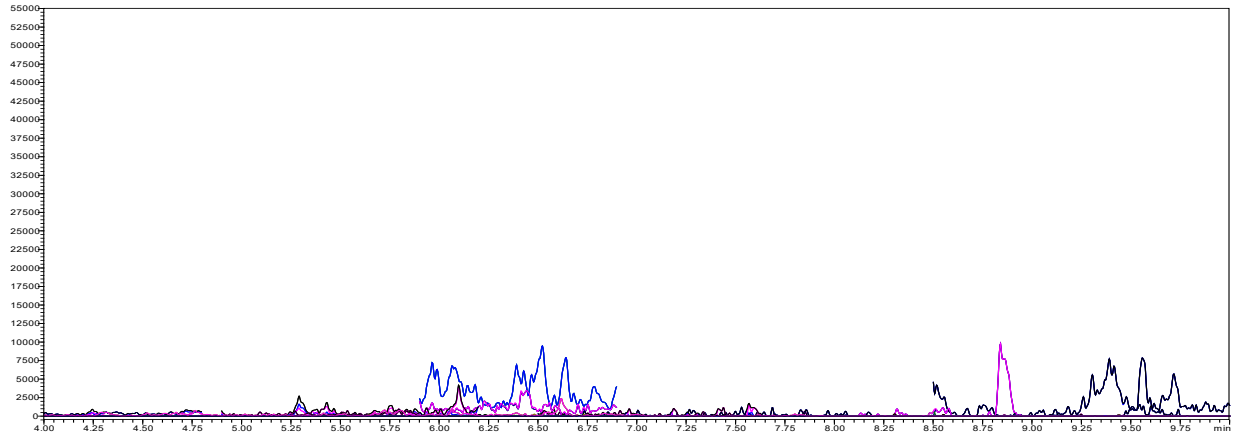


Figure 3: SFE extracted sample chromatograms from (a) Wild caught Large Mouth Bass, (b) Wild caught Walleye, and (c) Farm raised Trout

Table 6: Concentration of 18 PFAS in unknown fish samples

	Walleye ng/g	Large Mouth Bass ng/g	Farm raised Trout ng/g
PFBS	1.0	1.6	n.d.
PFHxA	n.d.	n.d.	n.d.
HFPO-DA	n.d.	n.d.	n.d.
PFHxS	n.d.	n.d.	n.d.
PFHpA	n.d.	n.d.	n.d.
ADONA	n.d.	n.d.	n.d.
PFOA	1.0	1.4	n.d.
PFNA	2.4	1.1	n.d.
PFOS	51.7	77.3	n.d.
9Cl-PF3ONS	1.0	2.7	n.d.
PFDA	6.7	10.5	n.d.
N-MeFOSAA	n.d.	n.d.	n.d.
MN-MeFOSAA	n.d.	n.d.	n.d.
N-EtFOSAA	n.d.	n.d.	n.d.
PFUnA	5.7	14.2	n.d.
11Cl-PF3OUds	0.7	3.0	n.d.
PFDoA	2.8	4.5	n.d.
PFTriA	4.1	7.3	n.d.
PFTreA	1.4	2.3	n.d.

■ Conclusion

A novel supercritical fluid extraction method, using the Shimadzu Nexera UC offline SFE system, for the extraction of PFAS compounds from fish tissue was evaluated and provided excellent results for recovery, linearity, and reproducibility. The results summarized here demonstrate the suitability of SFE as a sample preparation technique for PFAS analysis.

This sample preparation technique can be automated to allow the processing of up to 48 samples per batch to help reduce manual labor in testing laboratories.

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