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

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1. Overview

A novel sample preparation technique for PFAS determination in fish was evaluated. A variety of SFE parameters including cosolvent concentrations and additives were tested and results were confirmed by LCMS/MS analysis,

2. Introduction

Polyfluoroalkyl 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 wildlife and fish, 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 was evaluated as an alternative sample preparation technique for the recovery of 18 PFAS compounds from fish tissue.

3. Methods

For this study, 0.5 grams of freeze-dried fish tissue was milled and mixed with 1 packet of Miyazaki Hydro-Protect and placed into a 5 mL vessel for extraction. After extraction, the sample was dried down under nitrogen and reconstituted with 1 mL of methanol. The sample was centrifuged and 1 µL was injected for LCMS/MS analysis. During the method development experiments, fish samples were spiked with commercially available PFAS standards before extraction. Figure 2 shows a representative chromatogram of the 18 PFAS analyzed.

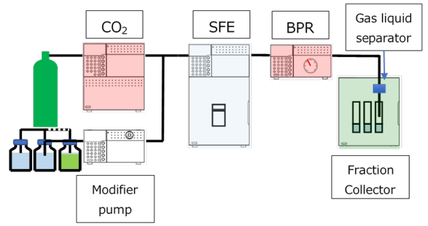


Figure 1. System Configuration of Nexera UC offline SFE system

Table 1. SFE and LC/MS/MS method conditions

SFE conditions	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

Column:	Shim-pack GIST C18 100mm×2.1mm, 2.7 um
PFAS delay column:	Shim-pack XR-ODSII 75 x 3 mm
Mobile phase A:	10mM Ammonium acetate
Mobile phase B:	Methanol
Flow rate	0.5 mL/min
Time program	B conc: 20% (0 min) - 90% (1-11min)
Injection vol	1 µl
Column temperature	35 ℃
ESI mode	Negative

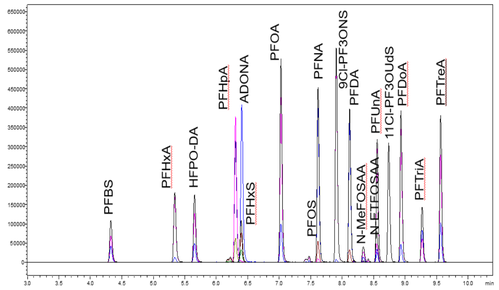


Figure 2. LCMS/MS chromatogram of PFAS standard (50 pg each on column)

4. Results

4-1. Method development for PFAS

A number of modifier concentrations and additives were evaluated for the recovery of the PFAS compounds. Optimum extraction conditions were obtained using 20% methanol without the need for additives. All compounds showed good recovery with these conditions. A matrix matched calibration curve was generated with concentrations from 0.5 to 100ng/g spiked to a fish tissue sample found to be free from PFAS contamination. Reproducibility (n=3) was also tested at varying concentration levels. Finally, three fish samples (two wild caught and one farm raised) with unknown PFAS concentrations were tested with the above method.

4-2. Extraction Recovery for PFAS

Table 2. SFE recoveries of a 50 pg PFAS spike

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

4-3. Matrix Matched Calibration Curve for PFAS

Table 3. Matrix matched calibration curve results (ng/g)

	Lowest Cal point (LOQ)	Highest Cal point	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

4-4. Reproducibility for PFAS

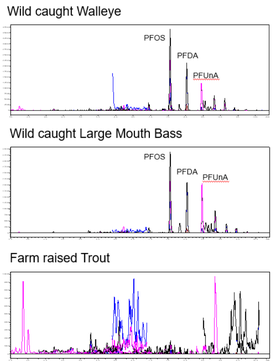
Table 4. %RSD results (n=3) for varying spiked concentrations

Compound	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

Table 2 shows the recovery results for the 18 PFAS compounds that were tested with this method. Recovery results ranged from 94.6% to 105.9%. A matrix matched calibration curve was prepared by spiking fish tissue before extraction with concentrations ranging from 0.5 ng/g to 100 ng/g. Table 3 shows the linearity results and quantitation limits obtained: r2 of 0.999 was obtained for all compounds and quantitation limits ranged from 0.5 to 2 ng/g. Table 4 shows reproducibility results obtained from spiking fish tissue with three different concentration levels (2, 20, and 100 ng/g); experiment was run in triplicates. At 2 ng/g, %RSD was less than 25%, except for PFTriA (27%) and N-MeFOSAA (45%). %RSDs at 20 and 100 ng/g were less than 12% for all compounds evaluated. These results demonstrate the reproducibility of SFE as a sample preparation technique.

4-5. Quantitative Analysis of wild caught and farm-raised fish samples

Three real world fish samples with unknown PFAS concentrations were run with the developed method. Results are presented in Figure 3 below. High concentrations of perfluorooctanesulfonic acid (PFOS), perfluorodecanoic acid (PFDA), and perfluroundecanoic acid (PFUnA) were found in wild caught walleye and large mouth bass. The farm raised trout sample showed no PFAS at quantifiable levels with this method..



Compound	Walleye	Large Mouth Bass	Farm raised Trout
PFBS	1	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	1.4	n.d.
PFNA	2.4	1.1	n.d.
PFOS	51.7	77.3	n.d.
9Cl-PF3ONS	1	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	n.d.
PFDoA	2.8	4.5	n.d.
PFTriA	4.1	7.3	n.d.
PFTreA	1.4	2.3	n.d.

Figure 3. PFAS concentrations (ng/g) found in fish samples

5. Conclusions

A novel method using supercritical fluid extraction for the extraction of PFAS compounds from fish tissue was evaluated and provided excellent results for recovery, linearity, and reproducibility. The results presented in this poster demonstrate the suitability of SFE as a sample preparation technique for PFAS analysis.

Wild caught fish samples contained high levels of several PFAS substances.

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.