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Supercritical Fluid Extraction: A Solution for the Extraction of PFAS in Environmental Samples

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Acknowledgments

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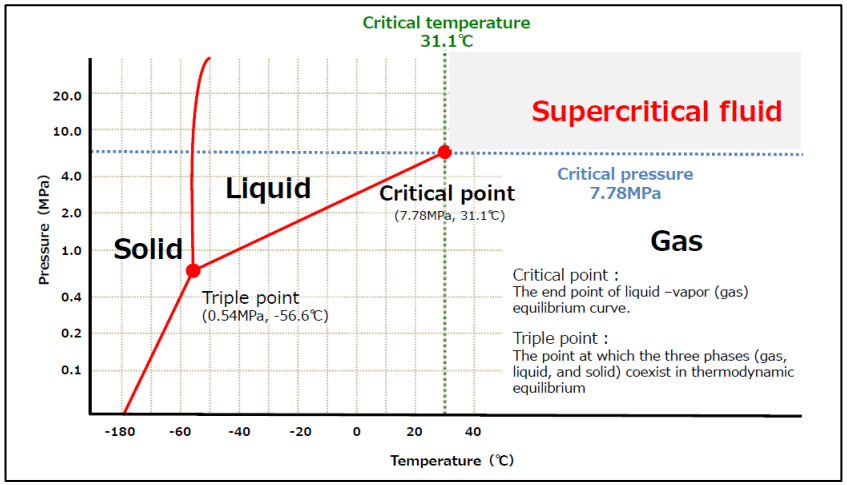
In today's presentation

1. Supercritical Fluid Extraction
2. Method development for PFAS in fish
3. Results – method performance
4. Results – samples
5. Conclusions
6. Q&A

Supercritical Fluids

Supercritical CO_2 is a fluid state of carbon dioxide where it is held at or above its critical temperature (31.1 °C) and critical pressure (73.8 bar)



- ❑ Low viscosity of the mobile phase
- ❑ Applicable for Extraction (SFE) and LC separations (SFC)
- ❑ Superior to LC for chiral separations
- ❑ Green technique as less organic solvents are used

Supercritical Fluid Configurations

Supercritical **F**luid **E**xtraction(SFE) pretreatment system
:**Off-line SFE**

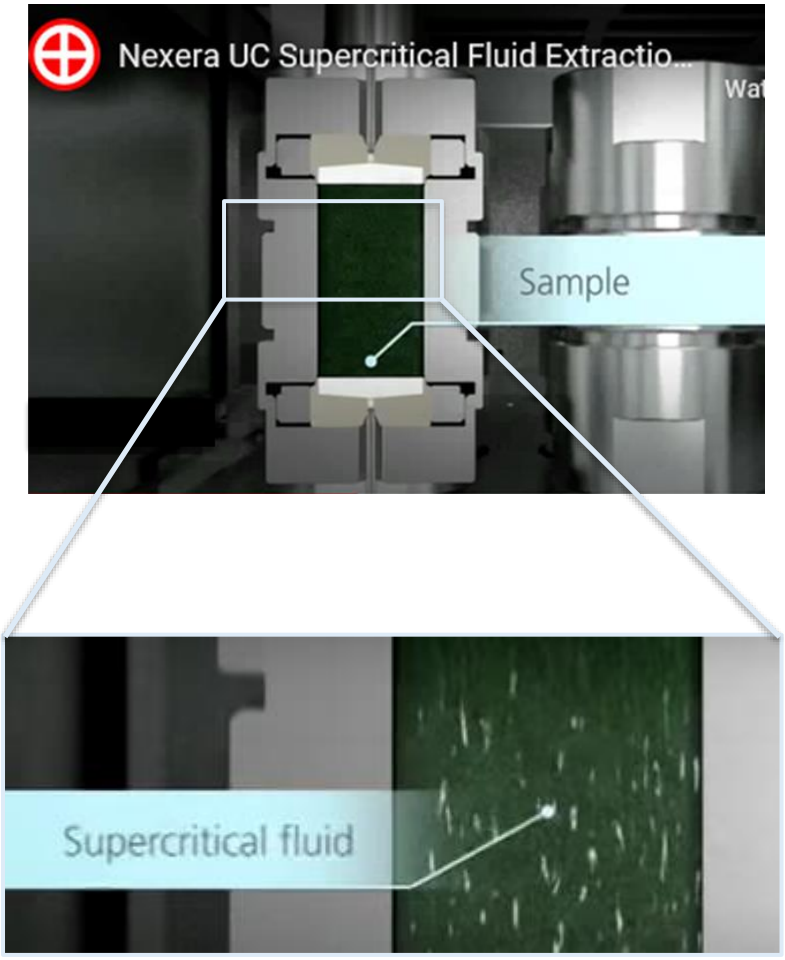
Supercritical **F**luid **C**hromatograph (SFC)
:**SFC(-MS)**

On-line SFE-SFC(-MS)

Supercritical Fluid Extraction (SFE)

What is it?

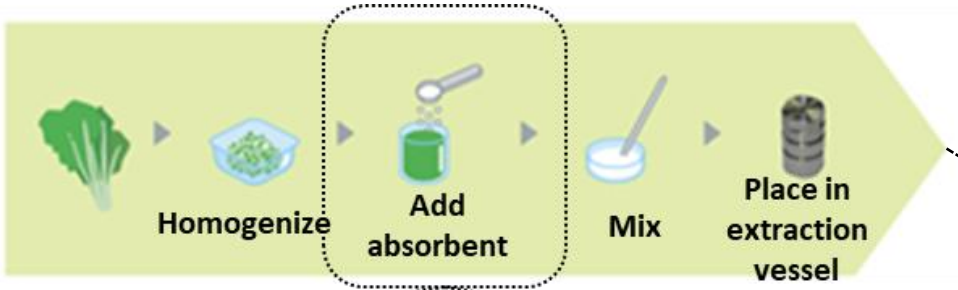
- SFE is a process that separates a component(s) from a matrix.
- Typically CO_2 is used to extract components from a solid matrix.
- Cosolvents like methanol and ethanol may be needed to help extract more polar components



Benefits

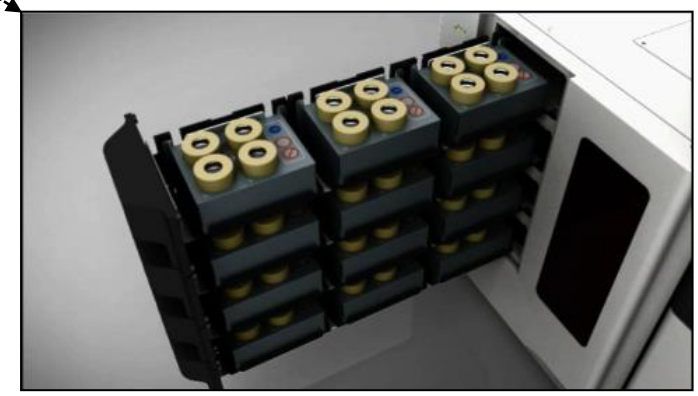
1) **Automation:**
   - reduces analyst errors and improves reproducibility
2) **Green technique:**
   - reduces organic solvent use and total volume of waste generated
3) **Selectivity:**
   - pressures and temperatures can be varied to allow step-wise extraction
4) **Speed:**
   - supercritical fluid has a faster diffusion into matrix than liquids, reducing extraction times

SFE vs conventional extraction



Automated sample preparation reduces analyst time and effort

Sorbent for dehydrating samples with high water content



Up 48 samples can be automatically extracted and analyzed by using Rack Changer

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Off-line SFE for “legacy” contaminants

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Off-line supercritical fluid extraction/gas chromatography-mass spectrometry analysis of pesticides in fish

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Analytical Conditions

Extraction Conditions

Vessel Temp:	50 °C
System Pressure:	30.0 MPa
Flow rate:	1.0 mL/min 100% CO2
Static Extraction time:	25 min
Dynamic Extraction Time:	30 min
Trap Column:	Shimadzu C18, 4.6 x 50 mm, 5um
Column Temp:	trap 20 °C, elute 50 °C
Column Rinse:	Hexane, 2 mL/min

Analytical Conditions

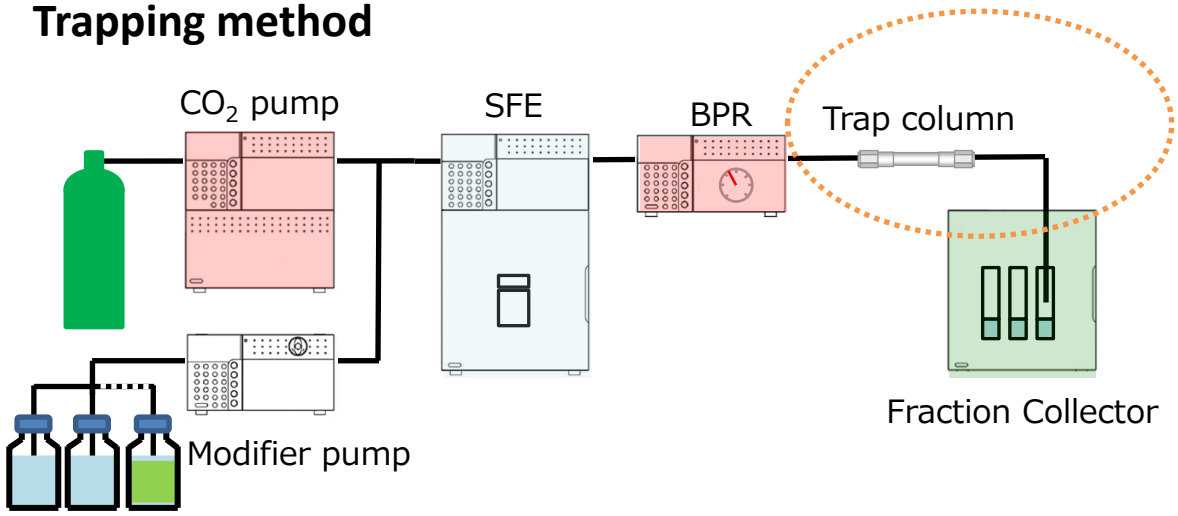
GC-MS:	GCMS-TQ8040 (Shimadzu)
Sampler:	AOC-20i/s (Shimadzu)
Column:	SH-Rxi-5MS (30m x 0.25mmI.D., df=0.25µm, Shimadzu)
IF Temp.:	290 °C IS Temp.: 230 °C Event Time: 0.3sec
Inj. Temp.:	275 °C
Flow Control:	Linear Velocity, 43.5cm/sec
Inj. Mode:	Splitless (High Press. Inj., 250kPa, 1.5min)
Oven Temp.:	50 °C (0.5min), 28 °C /min to 265 °C, 3 °C /min to 285 °C, 25 °C /min to 330 °C (1min)
Inj. Volume:	1 µL

Pesticide	Walleye “post-spiked” extract, ppb	Walleye “pre-spiked” extract with corn oil(*), ppb	%Recovery
2,4,5,6-tetrachloro-m-Xylene	48.39	45.28	93.6
alpha-BHC	*49.83*	*40.07*	*80.4*
gamma-BHC	51.46	40.46	78.6
Chlordene	52.2	44.35	85
Heptachlor	*47.63*	*39.07*	*82*
Aldrin	53.21	41.77	78.5
Heptachlor epoxide	54.12	44.14	81.6
trans-Chlordane	49.83	43.21	86.7
cis-Chlordane	52.5	44.99	85.7
trans-Nonachlor	52.47	43.71	83.3
4,4'-DDE	51.25	45.98	89.7
o,p'-DDD	52.44	42.71	81.5
Dieldrin	53.53	44.09	82.4
Endrin	53.36	44.36	83.1
4,4'-DDD	61.15	41.64	68.1
cis-Nonachlor	53.65	41.34	77
4,4'-DDT	*44.41*	*40.88*	*92.1*
Methoxychlor	*46.1*	*45.14*	*97.9*
Mirex	50.74	38.79	76.4
Decachlorobiphenyl	41.74	41.09	98.5

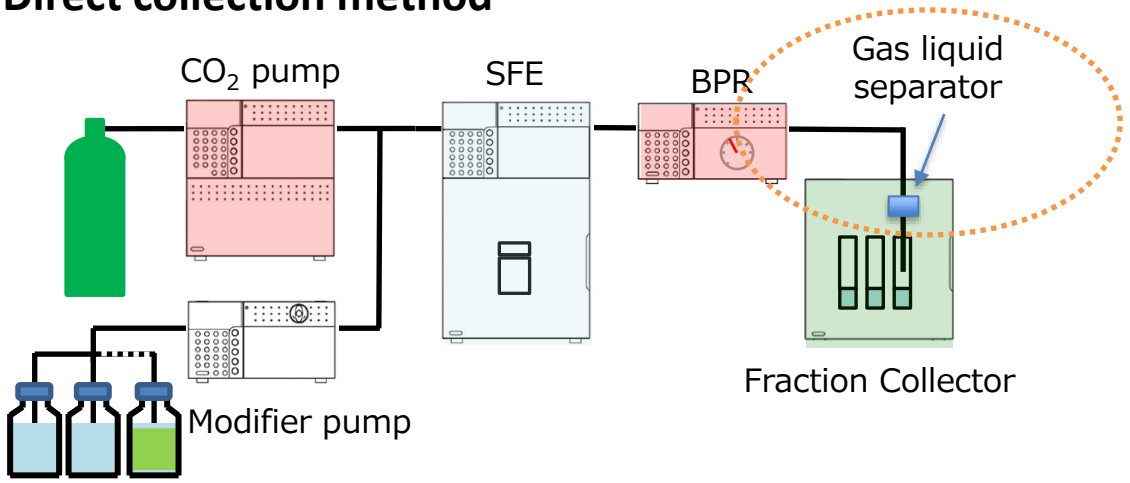
(*) oil added to improve recoveries from low fat samples

SFE Instrument configuration

Trapping method



Direct collection method



Experimental workflow

Grind samples
with the mixer mill

Weigh 0.5 g of sample

Mix with Hydro-protect (1g)
*Add surrogates

Transfer to the vessel

Extraction

Collection

Add ground sample

Dry down with N2

Reconstitution with
1 mL of MeOH

Centrifuge/Filtration

Transfer to LCMS vials and
analyze (1µL)

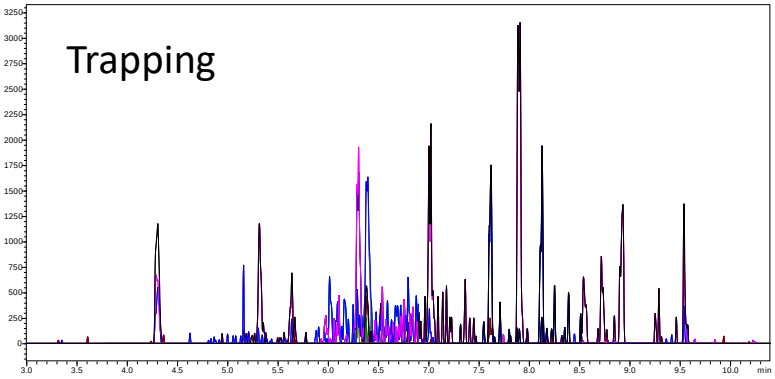
Instrument conditions

Nexera UC offline SFE	Value
Trap column	None
Mobile phase	A: CO2 B: MeOH
Modifier concentration	20%
Flow rate	5 mL/min.
Time program	46 min (combination of 3 static and dynamic cycles)
Vessel temperature	60 ℃
BPR pressure	20 MPa

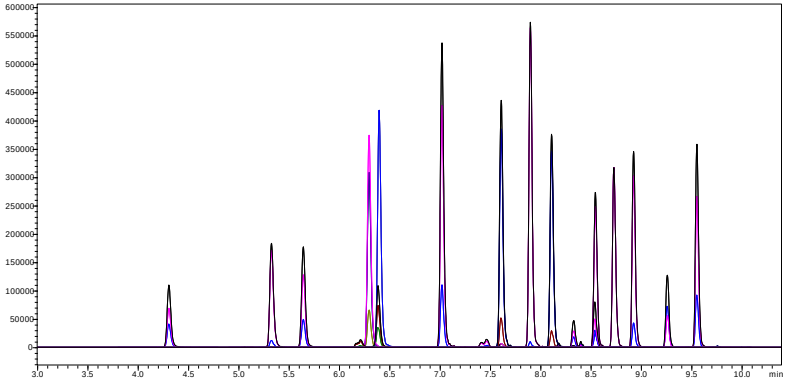
LCMS-8050	Value
Column	Shim-pack GIST C18 2.7 um 100 x 2.1 mm XR-ODSII 3 x 75 mm for delay column
Mobile phase	A: 10 mM ammonium acetate in H2O B: MeOH
Flow rate	0.5 mL/min.
Gradient program	0 min: 20 %B; 9 min: 90 %B; 11 min: 90 %B; 11.5 min: 20 %B; stop 15 min
Oven temperature	35 ℃
Injection volume	1 µL

Method development

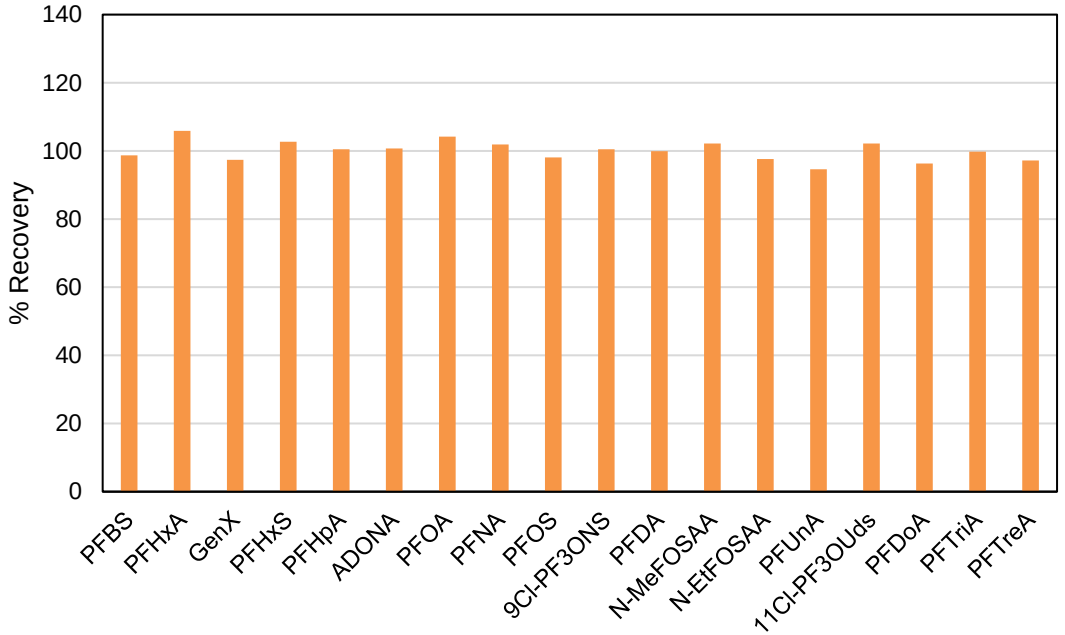
Trapping vs direct collection



Direct collection

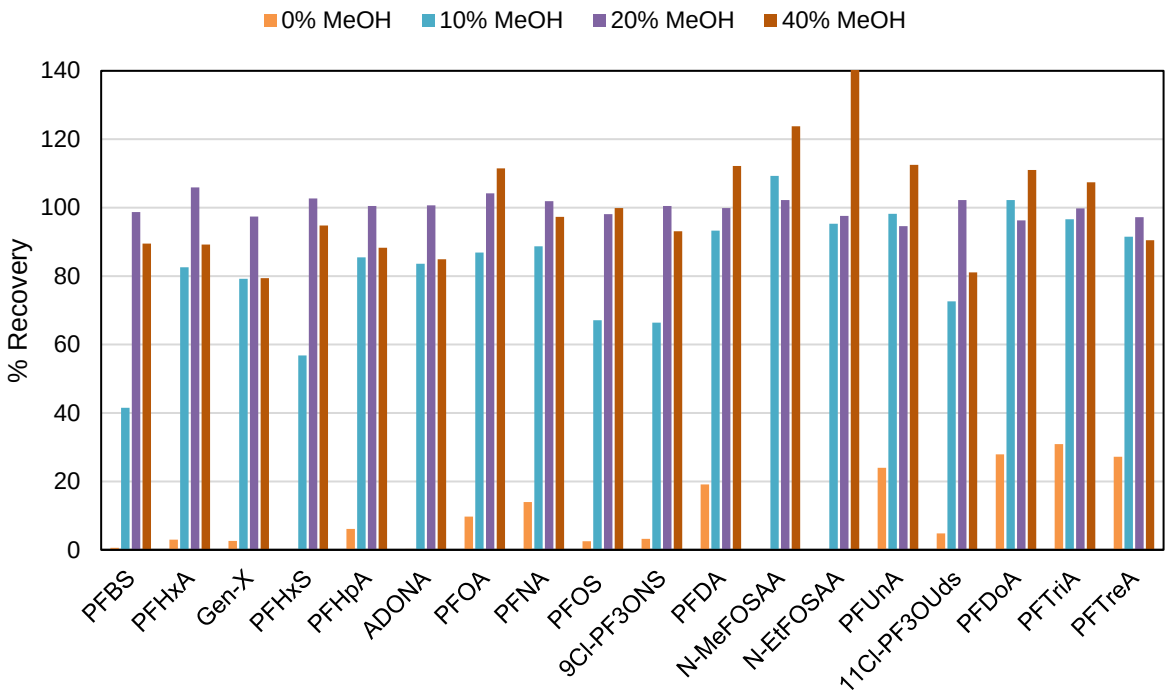


- ✓ Acceptable recoveries (>95%) for all target compounds with direct collection (spike: 50 ng on Hydroprotect)



Method Development

Modifier and additive concentration



Modifiers:

- *0% methanol*
- *10% methanol*
- *20% methanol*
- *40% methanol*

Additives:

- *none*
- *0.1% formic acid*
- *10 mM ammonium acetate*
- *5% H2O*

(Spike: 50 ng on Hydroprotect)

✓ Best recoveries (>95%) for all targeted compounds with 20% methanol as modifier.
✓ Recoveries were within same range for the additives tested →no additive was selected.

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Method Performance

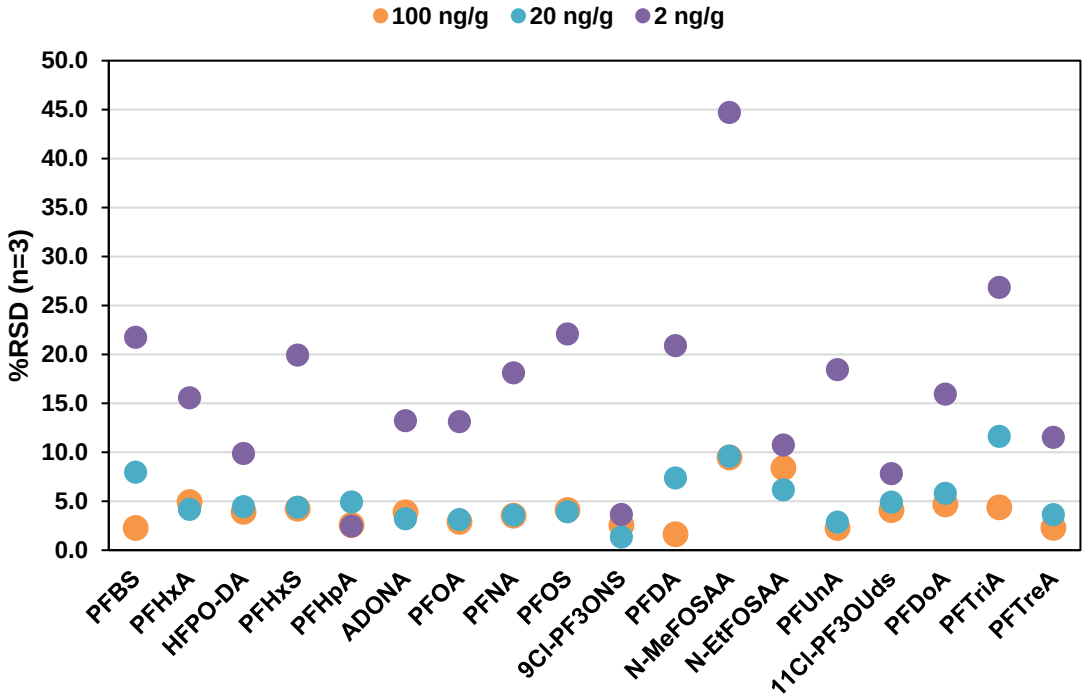
Calibration – Matrix matched

	Lowest Cal point (LOQ) ng/g	Highest Cal point ng/g	Linearity (R^2)
PFBS	0.5	100	0.9999
PFHxA	0.5	100	0.9995
HFPO-DA	1.0	100	0.9997
PFHpA	1.0	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	1.0	100	0.9995
PFDA	0.5	100	0.9998
N-MeFOSAA	2.0	100	0.9994
N-ETFOSAA	1.0	100	0.9999
PFUnA	1.0	100	0.9997
11Cl-PF3OUdS	0.5	100	0.9999
PFDoA	1.0	100	0.9996
PFTriA	2.0	100	0.9997
PFTreA	1.0	100	0.9995

✓ Good linearity was observed in the 0.50 to 100 ng/g range with matrix matched calibration. PFAS spiked on 0.5 g of fish and 1 g of Hydroprotect. Unspiked fish analyzed as blank.

Method Performance

Extraction efficiency and reproducibility

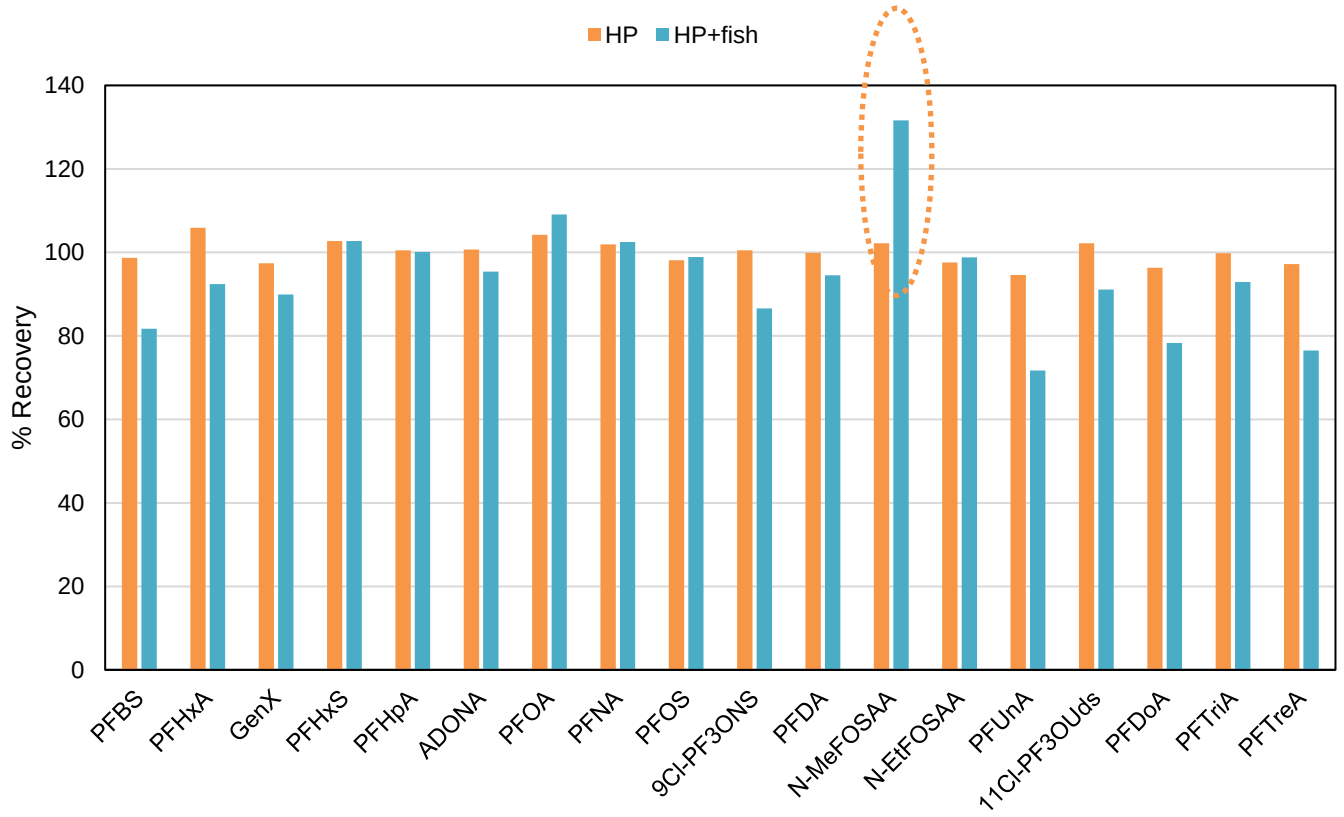


✓ Good extraction efficiency (94-116%) and reproducibility were obtained.

%RSDs at 100 ng/g: ≤ 5% (except N-MeFOSA & N-EtFOSA: <10%)
%RSD at 20 ng/g: ≤ 10% (except PFTriA: 12%)
%RSD at 2 ng/g: from 2% to 27% (except N-MeFOSA: 44%)

Method Performance

Matrix Effect

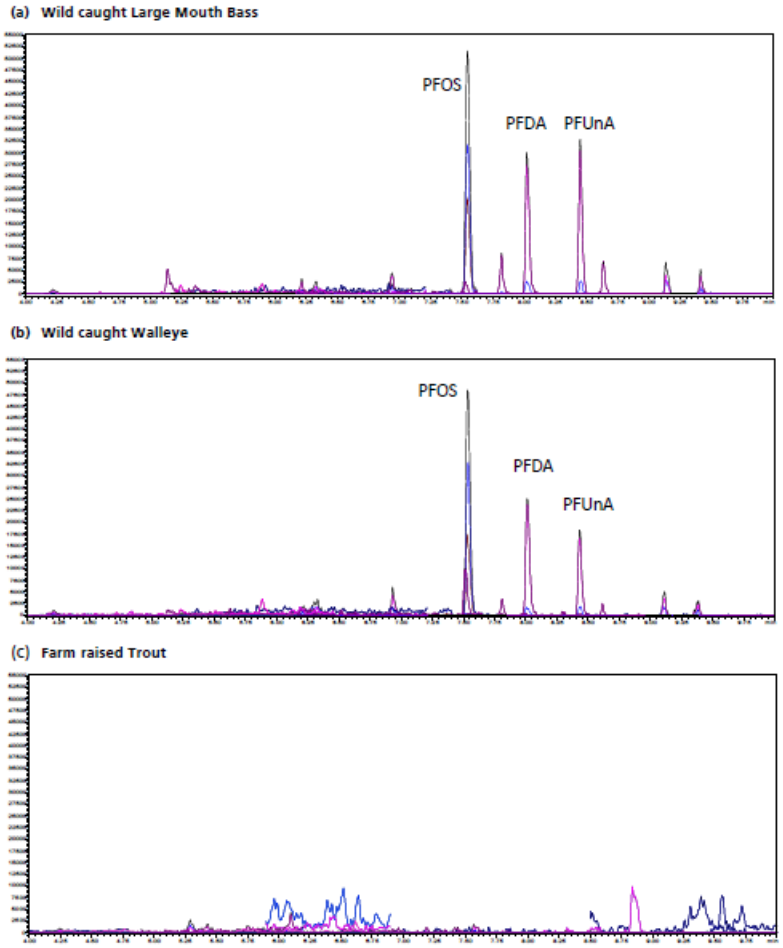


✓ No severe matrix effect observed. Recoveries for all target compounds except N-MeFOSAA were within 70-130%

Spike: 50 ng on 1 g of Hydroprotect without or with 0.5 g of fish. Unspiked fish analyzed as blank.

Sample analysis

- ✓ PFAS compounds were detected in Walley and Large Mouth Bass, but not in the farm raised Trout sample.



	Walleye (ng/g)	Large Mouth Bass (ng/g)	Trout (ng/g)
PFBS	1.00	1.62	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	1.00	1.43	n.d.
PFNA	2.37	1.13	n.d.
PFOS	51.65	77.34	n.d.
9Cl-PF3ONS	0.98	2.68	n.d.
PFDA	6.68	10.52	n.d.
N-MeFOSAA	n.d.	n.d.	n.d.
N-EtFOSAA	n.d.	n.d.	n.d.
PFUnA	5.65	14.24	n.d.
11Cl-PF3OUds	0.68	2.97	n.d.
PFDoA	2.79	4.48	n.d.
PFTriA	4.12	7.28	n.d.
PFTreA	1.39	2.33	n.d.

Conclusions

- The suitability of SFE as a sample preparation technique for PFAS analysis was demonstrated.
  - *SFE provided excellent results for recovery, linearity, and reproducibility.*
  - *Current extraction time could be shorten (experiments to confirm this are pending).*
- Wild caught fish samples contained several target PFAS above the limit of quantification.
- SFE can be automated, resulting in increased lab productivity.

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