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An Automated Sample Extraction and Cleanup Workflow Prior to LC-MS/MS for Analysis of PFAS in Solids and Fish Tissues for EPA 1633

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EPA 1633 – A method for multiple complex matrices

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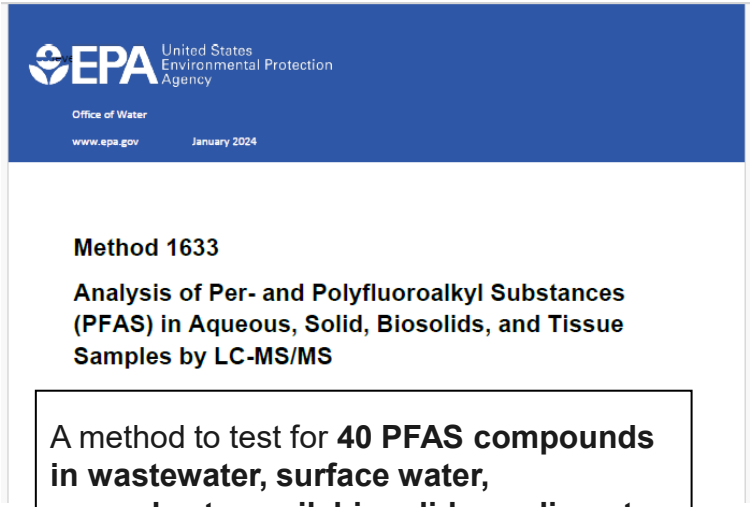

A method to test for **40 PFAS compounds in wastewater, surface water, groundwater, soil, biosolids, sediment, landfill leachate, and fish tissue.**

Intended to be used as follows with Regulations in development in US:

- Military sites
- Clean Water Act Compliance (wastewater discharge permits)
- Superfund sites
- General remediation and investigation programs

Goal of this work:

1. Automate the sample extraction and SPE clean up for solid samples covered by EPA 1633 (soil, sediment, biosolids and fish tissue)

The Method

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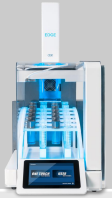
Step	EPA 1633 as written	Automated Method
Sample Extraction	Soils – 3x extraction with basic methanol • 65 minutes of shaking, 30 minutes of centrifugation Tissues – 3x extraction with basic methanol and acetonitrile • 16+ hours of shaking, 30 minutes of centrifugation	**Automated sample extraction** • Sample extraction step reduced to < 10 minutes per sample 
SPE cleanup	Manual extraction using negative pressure manifold • Time consuming and ties up scientist • Batch of 10 samples (50 mL each) takes ~2 hours	**Automated SPE system** • Allows scientists to spend time on other responsibilities • Batch of 8 samples takes ~1 hour 
SPE cartridge	Graphitized Carbon Black (GCB) clean-up followed by Weak Anion Exchange (WAX) SPE	**Dual phase SPE cartridge containing WAX and GCB sorbents** • Allows sample extraction and clean-up steps to be fully automated 
LC-MS/MS analysis	Tandem quadrupole analysis for sensitivity and selectivity	**High sensitivity tandem quad mass spectrometer** 

Automated Solvent Extraction – CEM EDGE

- Pressurized Fluid Extraction
- Sequential – automates 12 samples per rack
- Filtration step included
- Reusable Q-Cup sample cell
- Applicable to many matrix types
- Safe

Automating SPE with the Promochrom SPE-03 System

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MOD-004

SPE-03

Main Specifications	✓ 8 samples in parallel ✓ Automatic bottle rinsing ✓ Clean PFAS background
Key advantages	✓ High efficiency ✓ Flexible with sample and column types ✓ Handles challenging matrices ✓ Compact and simple design

Sample preparation to analysis workflow

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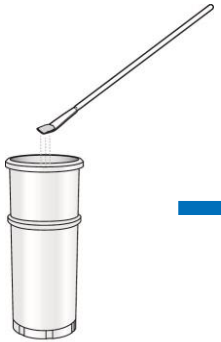

Weigh Sample into Q-Cup

Place in EDGE & press play

Dry and dilute sample to prep for SPE

SPE cleanup on SPE-03

Analyze on Xevo TQ Absolute

EDGE PFAS Methods

Soils

Cycle	Solvent	Top Add (mL)	Temp (°C)	Hold Time (min)	Rinse (mL)
1	0.3% ammonium hydroxide in MeOH	15	65	3:00	---
2	0.3% ammonium hydroxide in MeOH	10	65	3:00	5

Tissue

Cycle	Solvent	Top Add (mL)	Temp (°C)	Hold Time (min)	Rinse (mL)
1	0.05 M KOH in MeOH	10	65	3:00	---
2	Acetonitrile	10	65	3:00	---
3	0.05 M KOH in MeOH	0	---	---	5

- Automated system wash steps after each sample extraction completed

SPE Method – Automated on SPE-03

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1.
- Pack SPE cartridge with glass wool to half height of barrel
- Condition SPE cartridges
  - 15 mL 1% (v/v) ammonium hydroxide in methanol
  - 5 mL 0.3 M formic acid

2.
- Load sample at 5 mL/min
- Wash cartridge with 10 mL of reagent water, ensuring to rinse reservoir with this solution
- Wash with 5 mL of 1:1 0.1M formic acid:methanol, ensuring to rinse reservoir with this solution
- Dry cartridge for 15 seconds

3.
- Place collection tubes in manifold
- Rinse bottle with 5 mL 1% (v/v) ammonium hydroxide in methanol. Transfer to cartridge and elute
- Add 25 µL acetic acid to each sample
- Spike each sample with Non-Extracted Internal Standard (MPFAC-HIF-IS from Wellington)

Dual Layer SPE Cartridge with GCB and WAX

Instrument Methods

Source Parameters

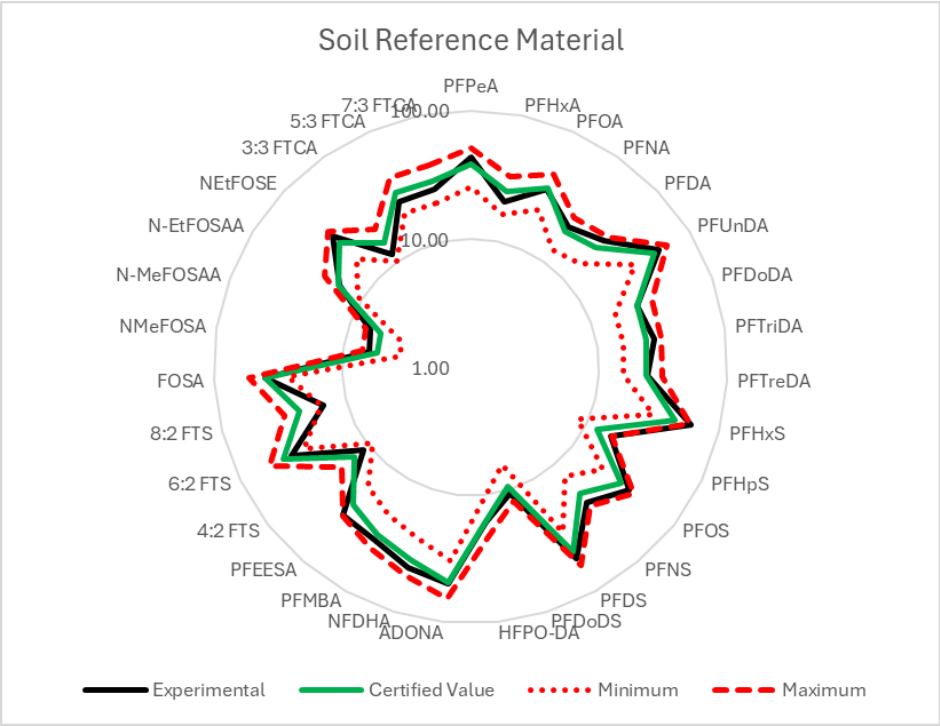
- Instrument: Xevo TQ Absolute MS
- Ion Mode: ESI-
- Capillary Voltage: 0.5 kV
- Desolvation Temperature: 350°C
- Desolvation Flow: 900 L/hr
- Cone Flow: 150 L/hr

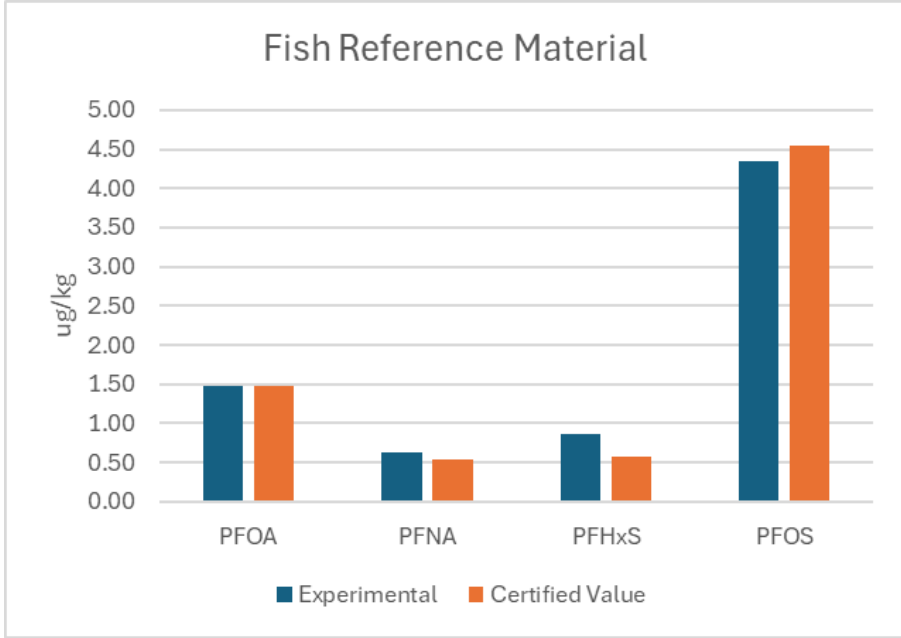
LC Method

- Instrument: ACQUITY™ Premier BSM FTN System with PFAS Kit
- Column: ACQUITY Premier **BEH™ C18** Column 2.1mm x **50 mm**, 1.7 µm
- Isolator Column: Atlantis™ Premier **BEH C18 AX** Column 2.1mm x 50 mm, 5.0 µm
- Mobile Phase A: Water + 2 mM ammonium acetate
- Mobile Phase B: **Acetonitrile** + 2 mM ammonium acetate
- Injection Volume: **2 uL**
- **Gradient:**

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

Certified Reference Material Analysis





- Results were well within the certified values for both reference materials demonstrating the method is accurate and reliable

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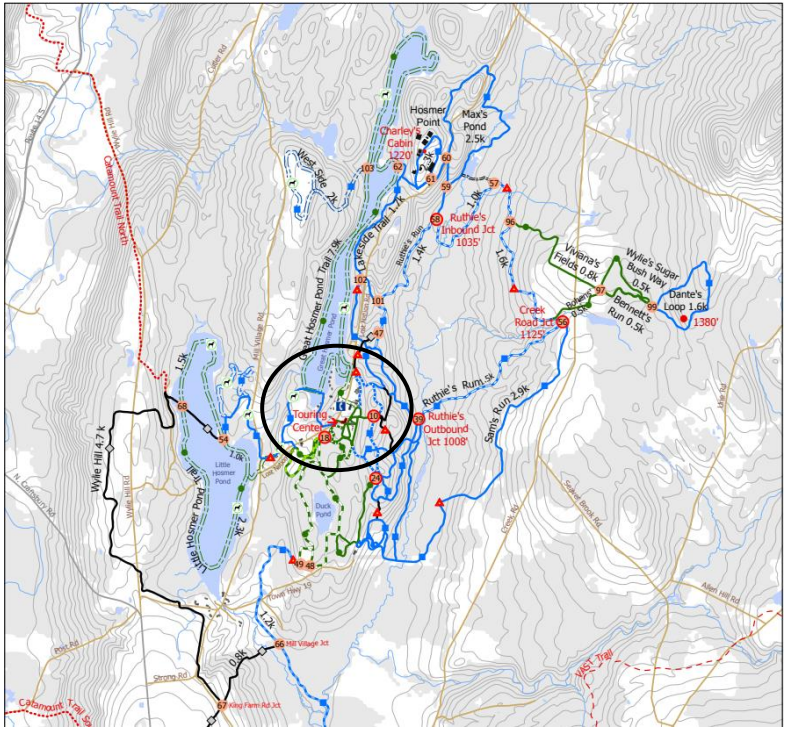
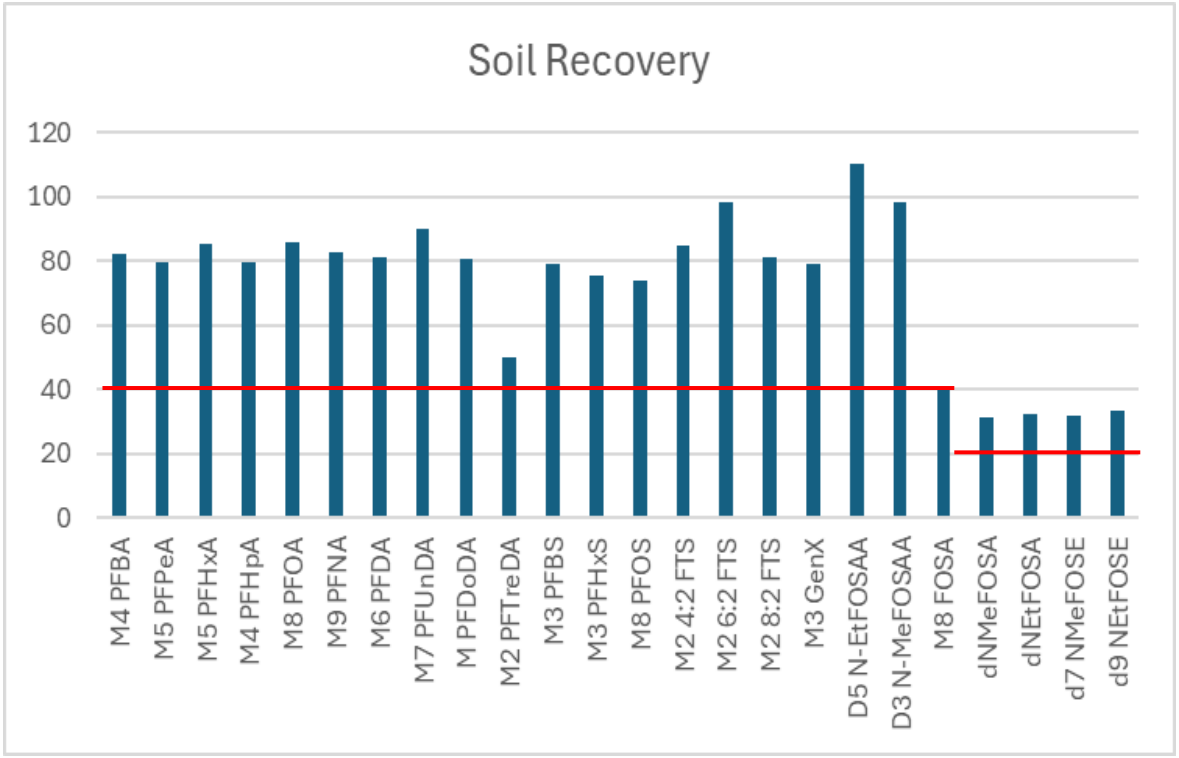
Alpine Ski Racing Hill

Nordic (Cross Country) Trails



Recovery of extracted internal standards from soil



- Highly contaminated soils from ski racing sites were tested – unable to perform sample fortification of natives
  - Soil sample composition varied
- Red line indicates the average EPA 1633 minimum recovery guideline for each extracted internal standard
- Recovery in soil samples easily met the guidance criteria

PFAS detected in Soil – Alpine Racing

- Soil taken from ski slope where ski wax is commonly used
- 24 PFAS detected across all soil samples tested
- Concentrations reported as ng/g in sample (ppb)
- Ski wax preparation area had largest number of detections and contributed most of concentration
  - PFCAs dominate, but mostly even contribution from C6-C13

*PFBA was removed from heat map due to background contamination

Down Slope →

Soil (ng/kg)

	Wax Station	Start Line	Mid Slope	Finish Line
PFBA	35	1358	765	407
PFPeA	61	520	213	123
PFHxA	143	182	135	92
PFHpA	153	187	99	106
PFOA	248	236	134	124
PFNA	183	171	17	7
PFDA	284	102	51	23
PFUnDA	218	55	22	5
PFDoDA	189	63	36	31
PFTriDA	152	45	4	11
PFTreDA	231	48	10	ND
PFBS	13	14	5	8
PFPeS	ND	ND	ND	ND
PFHxS	BLoQ	BLoQ	9	ND
PFHpS	ND	ND	ND	ND
PFOS	45	76	62	18
PFNS	ND	ND	ND	ND
PFDS	ND	18	38	ND
PFDoDS	ND	ND	ND	ND
6:2 FTS	BLoQ	ND	4	ND
NMeFOSA	16	16	26	ND
NEtFOSA	6	8	25	ND
NMeFOSE	14	20	32	60
NEtFOSE	ND	BLoQ	83	ND
Total PFAS	1990	3116	1770	1013

Highest Concentration

Lowest Concentration

PFAS detected in Soil – Cross Country Racing

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Soil (ng/kg)

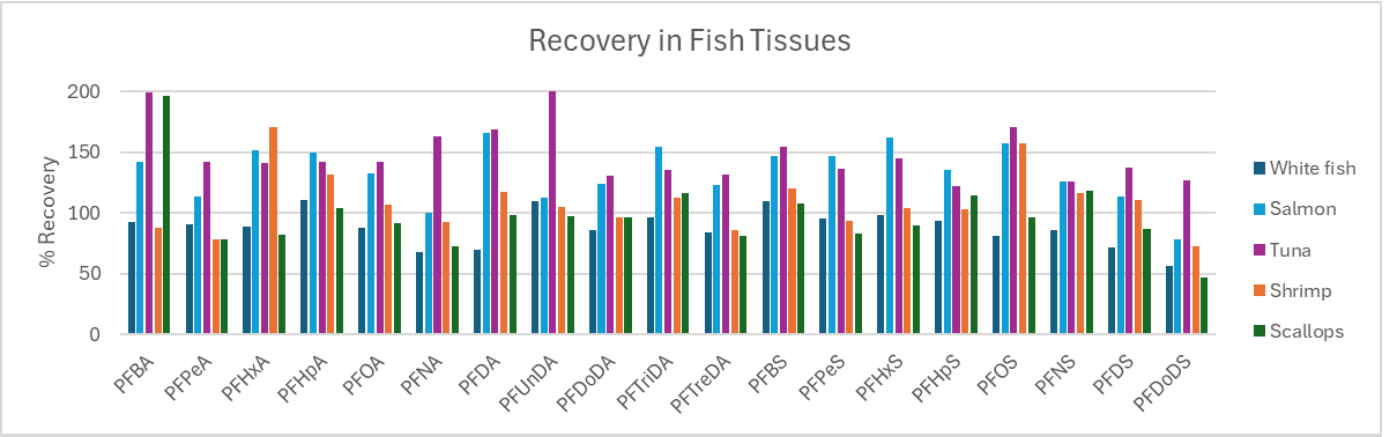
	Race Course Near Pond 1	Pond 1 Sediment	Pond 2 Sediment	Start/Finish Line	Garden Near Pond 2	Garden Near Waxing	Ski Wax Test Hill Top	Ski Wax Test Hill Bottom	Race Course Location 1	Race Course Location 2	Non-Race Trail	Parking Lot Waxing Area
PFBA	296	1337	1884	201	280	167	98	123	118	102	782	159
PFPeA	89	307	581	25	318	157	51	140	41	27	363	98
PFHxA	12	ND	15	33	259	141	71	126	ND	ND	ND	17
PFHpA	40	23	54	50	197	113	43	146	ND	9	50	62
PFOA	45	34	73	97	786	378	82	271	47	7	79	145
PFNA	39	10	14	87	435	311	47	328	92	41	43	150
PFDA	66	15	20	117	1056	261	78	1839	315	54	59	583
PFUnDA	33	28	22	66	272	104	46	991	126	46	51	230
PFDoDA	19	83	ND	46	358	65	55	1262	89	17	16	450
PFTriDA	21	247	10	39	133	49	25	945	94	26	22	221
PFTreDA	25	693	13	57	224	49	58	1554	138	34	26	796
PFBS	9	ND	ND	58	6	ND	ND	ND	ND	ND	ND	ND
PFHxS	17	ND	9	ND	21	9	15	ND	ND	ND	ND	10
PFHpS	16	ND	ND	ND	19	7	11	ND	ND	ND	ND	8
PFOS	131	ND	9	186	3533	1048	276	454	60	29	183	27
PFNS	12	ND	ND	ND	11	ND	14	ND	ND	ND	ND	13
FOSA	ND	ND	ND	ND	37	9	11	ND	ND	ND	ND	21
N-MeFOSAA	31	17	ND	31	31	16	57	10	ND	17	ND	ND
N-EtFOSAA	22	ND	ND	23	68	23	31	ND	ND	7	ND	10
NMeFOSE	ND	27	25	ND	13	14	9	18	15	41	11	32
NEtFOSE	ND	ND	8	ND	39	10	ND	14	ND	6	ND	ND
4:2 FTS	33	ND	ND	ND	16	ND	ND	ND	ND	ND	ND	ND
6:2 FTS	ND	178	174	ND	ND	ND	ND	ND	ND	ND	ND	ND
8:2 FTS	68	ND	ND	93	38	42	ND	53	ND	ND	44	310
5:3 FTCA	9	ND	20	12	14	ND	ND	10	16	12	8	7
7:3 FTCA	ND	11	15	14	26	ND	ND	111	16	ND	11	91
Total PFAS	1034	3010	2946	1233	8190	2972	1078	8396	1167	474	1749	3440

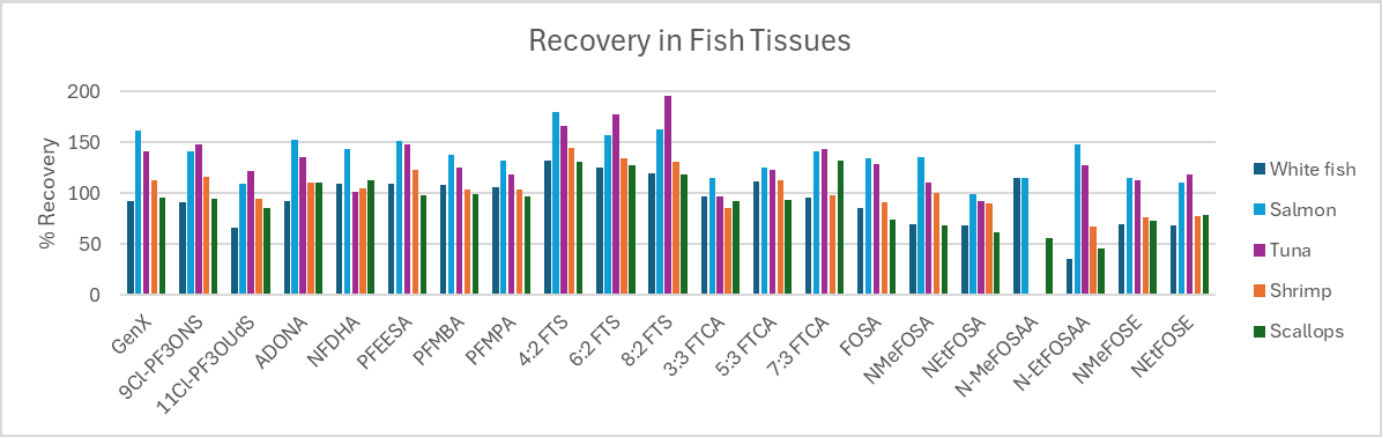
- Soil taken from the surrounding areas where ski wax is commonly used
- 26 PFAS detected across all soil samples tested
- Concentrations reported as ng/g in sample (ppb)
- High concentrations of PFAS were detected in all soil samples
  - Contamination from waxes is widespread at this site
  - PFCAs and PFSAs both commonly present
- Ski wax preparation and testing area and the on-site garden had the highest amounts of PFAS detected.

Fish tissue samples

- Seven fish and mollusk tissues sourced from local grocery store
  - Mix of wild caught and farm raised
- Fish tissues tested were fortified with 40 PFAS compounds
- Tissue homogenized using a blender before sampling

Recovery of native PFAS in fortified fish tissues





- Overall recoveries for the fortified PFAS were good
- Fish high in fatty acid content (salmon and tuna) experienced some enhancement effects resulting in high recovery
- Had some difficulties with NMeFOSAA and NEtFOSAA
- Method needs some more work for the more complex tissue matrices, but very promising

PFAS detected in Fish

	Farm Raised shrimp	Wild Caught shrimp	Wild Caught Scallops	Wild Caught Cod	Wild Caught Salmon	Farm Raised Tilapia	Wild Caught Tuna
PFBA	13.3	1.15	0.99	1.65	1.70	0.97	1.11
PFPeA	13.1	0.42	1.29	0.60	0.97	0.23	0.19
PFHxA	1.17	0.12	0.12	0.14	0.08	--	--
PFHpA	0.89	0.07	0.04	--	0.08	--	--
PFOA	**1.18**	0.12	0.07	0.16	0.11	0.04	0.03
PFNA	0.40	0.20	0.12	0.25	0.15	--	0.06
PFDA	0.19	0.13	0.08	0.39	--	--	--
PFUnDA	0.20	0.05	--	0.29	0.16	--	--
PFDoDA	0.10	--	--	--	--	--	--
PFTreDA	BLoQ	BLoQ	--	--	--	--	--
PFOS	--	0.48	--	--	0.19	--	--
PFEESA	0.08	--	--	--	--	--	--
PFMBA	0.07		--	--	--	--	--
6:2 FTS	--	BLoQ	--	--	--	--	--
ADONA	0.11	--	--	--	--	--	--
FOSA	BLoQ	--	BLoQ	BLoQ	BLoQ	--	BLoQ
EU sum of PFOA, PFOS, PFNA, PFHxS	1.58	0.80	0.19	0.41	0.46	0.04	0.09

- Concentrations reported in ng/g
- PFCAs were the majority of the PFAS detected in all fish samples
- Farm raised shrimp contained more total PFAS than the wild caught (Key West) shrimp
- Farm raised shrimp contained a PFOA level above EU guidelines:

	Fish Muscle	Crustaceans and Molluscs
PFOS	2.0	3.0
PFOA	0.2	0.7
PFNA	0.5	1.0
PFHxS	0.2	1.5
Sum	2.00	5.0

Conclusions

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- The extraction of soils and fish tissues was automated from sample extraction through SPE cleanup
- Time of sample preparation was significantly reduced
  - Soils – 4-5 hours down to 2-3 hours
  - Tissues – 2 days of prep down to 2-3 hours
- Efficiency of laboratory scientists is greatly increased
- Automated sample preparation produced results equivalent to manually preparing samples and were all within EPA guidance
- Accurate results were easily obtained for soil and fish certified reference materials
- Authentic soil and fish samples were analyzed using this workflow and PFAS were detected in samples

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