Application Note

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Analysis of Per and Polyfluoroalkyl Substances in Edible Fish Tissue Using Agilent Captiva EMR–Lipid and LC/MS/MS

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Abstract

This application note presents the development and evaluation of a multicomponent method for the analysis of 25 per- and polyfluoroalkyl substances (PFAS) in fish muscle tissue. The method incorporates a solvent extraction followed by a pass-through lipid removal step using the Agilent Captiva Enhanced Matrix Removal–Lipid (EMR–Lipid) and Quantitative Analysis by LC/MS/MS. The average surrogate recovery for 140 extractions across 25 fish species was 105% with an average relative standard deviation (RSD) of 14%.

Introduction

PFAS are a group of more than 5,000 synthetic organofluorine chemicals that were first developed in the 1940s.[1] The chemical characteristics that have led to their extensive use as surfactants and coatings in a wide range of commercial applications include resistance to heat, water, oil, grease, and stains. Commercial applications of PFAS include cosmetics, food packaging, nonstick cookware, firefighting foams, electronic devices, aircraft, vehicles, and various textiles (such as carpets, leather products, furniture, clothing, surgical gowns, and so on). The chemical structure of PFAS molecules includes a chain of strong carbon-fluorine bonds, making them resistant to environmental degradation, and thus these chemicals tend to be pervasive, persistent, and environmentally stable. Contaminated water and food, including seafood, are considered the main exposure routes for humans to PFAS.

Analysis of fish tissue extracts can be challenging due to the presence of matrix interferences such as fats and lipids. The Captiva EMR–Lipid pass-through cleanup technique efficiently removes major lipid classes without analyte loss. Removal of lipid interferences assures minimal matrix ionization effects and improves method reliability and ruggedness.

The objective of this study was to demonstrate the use of Captiva EMR–Lipid cleanup for PFAS extracts of edible fish tissue, collected from a large urban estuary, followed by LC/MS/MS analysis.

Experimental

Sample collection

Fish were collected using hook and line, baited traps, and nets from 24 locations within and next to a large urban estuarine bay in Tampa Bay, FL, USA in 2020 and 2021. Each fish was placed in a polypropylene bag (ULINE, Pleasant Prairie, WI, USA) and stored in coolers on bagged ice for transport back to the laboratory. A total of 140 individual fish (n = 140) from 25 species were analyzed for 25 target PFAS.

Chemicals and reagents

All solvents and reagents–acetonitrile (ACN), acetic acid, ammonium acetate, formic acid, and methanol–were LC/MS or ACS grade, purchased from Fisher Scientific (Waltham, MA, USA). Millipore purified water (18 µΩ, Bedford, MA, USA) was filtered with a 0.22 µm membrane filter. Individual native compounds (chemical purities of >98%), and mass-labelled internal and surrogate standards (chemical purities of >98% and isotopic purities of ≥99%) were purchased through Wellington Laboratories (Guelf, ON, Canada).

Sample extraction

As shown in Figures 1 and 2, muscle tissues (2 g) from individual fish were placed into 50 mL polypropylene test tubes. Surrogate standards (SS) at 100 pg/g were added to the tissues and allowed to sit at room temperature for 15 minutes. A ceramic homogenizer (5/16 × 5/8 in) and 2 mL of purified water with 1% formic acid (v/v) was then added and homogenized for 2 minutes at 1,500 rpm, followed by 8 mL of cold ACN with 2% formic acid (v/v) and homogenized for an extra 5 minutes (1,500 rpm), and then centrifuged for 5 minutes at 5,000 rpm. An aliquot of 2.4 mL of the extract supernatant was then transferred to a 3 mL Captiva EMR–Lipid cartridge (part number 5190-1003) and allowed to elute via gravity into a 15 mL polypropylene test tube until eluate droplets were no longer observed. A 600 µL volume of 80/20 ACN/water mixture was added to the cartridge and again allowed to elute by gravity. Then, vacuum was applied (–6 to –9 in Hg) to force the remaining eluant into the collection tube. The extract was mixed and then a 500 µL aliquot of the clean extract was transferred to a polypropylene autosampler vial. Internal standards (45 pg/mL) were added, and 300 µL of water was added for a final extract volume of 800 µL.

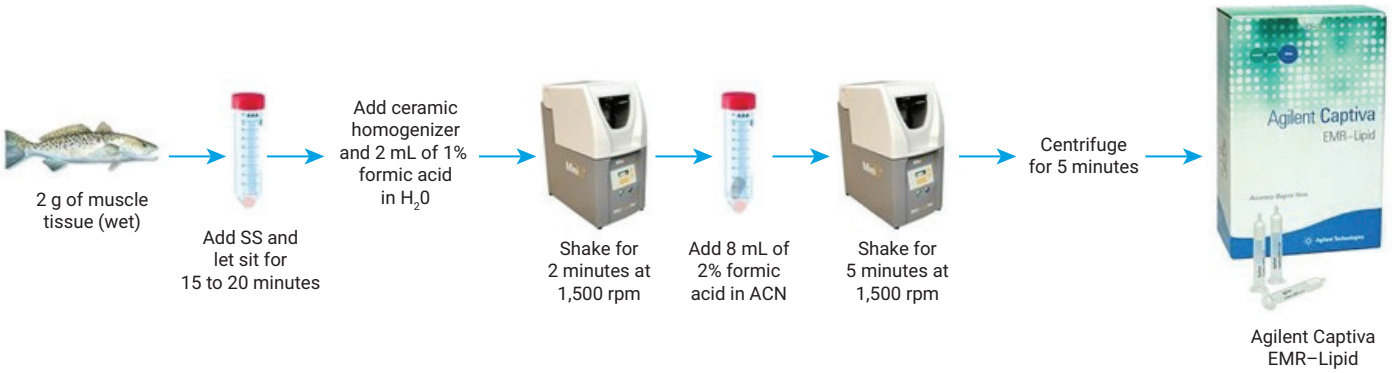

Figure 1. Sample preparation schema.

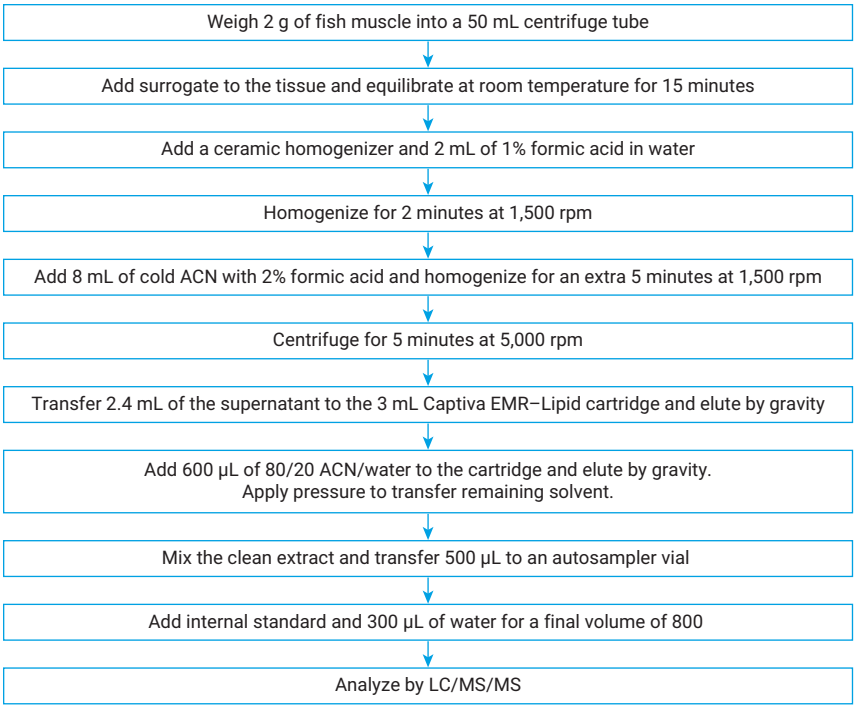

Figure 2. Sample preparation workflow diagram.

Instrumentation

An Agilent 1290 Infinity II LC was modified before analysis to reduce system background contamination. Plumbing considerations followed recommended guidelines by Anumol *et al.*[2] Solvent lines were replaced with PEEK tubing, the pump seals were replaced with PTFE-free seals, and an inline filter and delay column were added. The Agilent 6470B triple quadrupole LC/MS with the Agilent Jet Stream ESI source was used for detection. The 6470B triple quadrupole LC/MS was operated in negative electrospray ionization mode with dynamic multiple reaction monitoring (dMRM). The LC and MS method parameters are listed in Tables 1 and 2, respectively. Figure 3 shows selected extracted ion chromatograms for PFAS found in crevalle jack muscle extracts. The concentrations of PFOA, PFHxS, PFNA, PFHpS, PFOS, and PFUnDA were 53.5, 280, 443, 80.4, 23,700, and 1,102 pg/g, respectively.

Table 1. LC conditions.

Parameter	Value
LC	Agilent 1290 Infinity II LC
Analytical Column	Agilent ZORBAX RRHD Eclipse Plus C18 column, 2.1 × 100 mm, 1.8 µm (p/n 959758-902) with Agilent 1290 Infinity II inline filter 0.3 µm (p/n 5067-6189)
Delay Column	Agilent ZORBAX Eclipse Plus 95 Å C18 column, 4.6 × 50 mm, 3.5 µm (p/n 959943-902)
Column Temperature	50 °C
Injection Volume	20 µL
Mobile Phase	**A)** 20 mM ammonium acetate in 95/5 water/ACN **B)** 10 mM ammonium acetate in 95/5 ACN/water
Gradient	**Time (min) / % A / % B / Flow (mL/min)** 0 / 100 / 0 / 0.300 6 / 70 / 30 / 0.300 9 / 50 / 50 / 0.300 16 / 15 / 85 / 0.300 17 / 0 / 100 / 0.300 20 / 0 / 100 / 0.300 21 / 100 / 0 / 0.300 31 / 100 / 0 / 0.300

Table 2. MS conditions.

Parameter	Value
MS	Agilent 6470B triple quadrupole LC/MS with Agilent Jet Stream ESI source
Source Parameters	
Polarity	Negative
Drying Gas	230 °C, 4 L/min
Sheath Gas	250 °C, 12 L/min
Nebulizer Gas	15 psi
Capillary Voltage	2,500 V
Nozzle Voltage	0 V

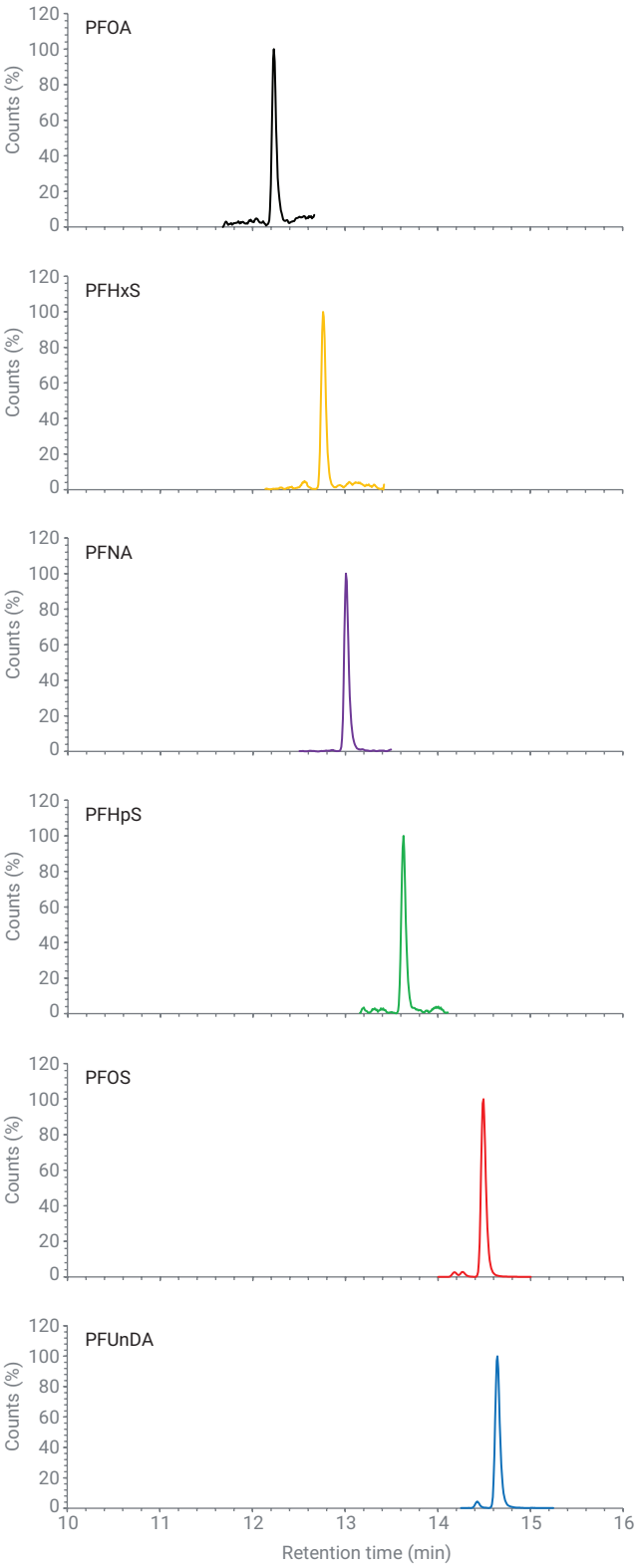

Figure 3. Example chromatogram of select PFAS detected in field caught fish muscle (crevalle jack). Concentrations in this sample for the five depicted PFAS ranged from 53.5 pg/g w/w for PFOA to 23.7 ng/g w/w for PFOS.

Quantitation

A total of 25 native PFAS compounds were selected for the study. Also, four isotopically labeled PFAS were selected as surrogates and 15 PFAS as internal standards. All standards were purchased from Wellington Laboratories (Guelf, ON, Canada). Appendix A lists the compounds, retention times, MRM transitions, and optimized dissociation voltages.

Each PFAS was quantified using a quadratic equation of a 5-point calibration curve using 10-fold dilutions (0.1, 1, 10, 100, and 1,000 ng/L). The target responses were normalized to that of the internal standard responses added to the samples and calibrants at the same concentration (45 pg/mL). To ensure the highest data quality throughout the study, a quality assurance/quality control program followed the ASTM and EPA methodology.[3–5]

Results and discussion

Matrix spike accuracy and precision

To validate the extraction procedure, duplicate fish tissue samples were spiked with the 25 native PFAS targets at a concentration of 100 pg/g and extracted; the results are shown in Figure 4. For the two samples, average recoveries for each target were within 70 to 130% with an average recovery of 102% and an average range of ±15%.

Surrogate recovery accuracy and precision

To ensure data quality in the study, the extraction procedure accuracy and precision were evaluated. The average recovery and relative standard deviation (RSD) were calculated for the surrogates spiked into edible fish tissue for the whole sample set, which encompassed 140 extractions across 25 fish species and the method blanks (spiked reagent water). The accuracy and precision results are listed in Tables 3 and 4 for the fish tissue and method blanks respectively. The method performance was outstanding, with average recoveries in samples ranging from 101 to 114% and RSDs from 12 to 16%, with an overall average recovery of 105 ±14% RSD for the fish tissue (Table 3). The overall surrogate recoveries in method blanks ranged from 71 to 129% with a mean recovery of 106 ±15% RSD (Table 4). These results were well within the typical acceptance criteria of 70 to 130% accuracy and less than 30% RSD indicated a robust and reliable extraction and analysis procedure.

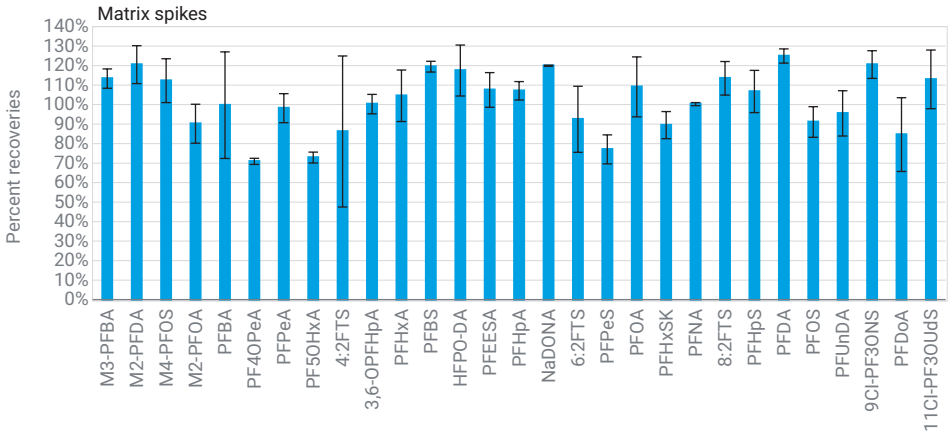

Figure 4. Mean recoveries of the four isotopically labeled and 25 native PFAS added to two fish muscle samples spiked before extraction. Error bars represent the range of recoveries of the two fish extracts.

Table 3. Surrogate accuracy and precision in fish samples (n = 140).

Surrogate	Average Accuracy (%)	RSD (%)
$^{13}C_3$-PFBA	101	15
$^{13}C_2$-PFDA	114	16
$^{13}C_4$-PFOS	113	13
$^{13}C_2$-PFOA	92	12

Table 4. Surrogate accuracy and precision in method blanks (n = 13).

Surrogate	Average Accuracy (%)	RSD (%)
$^{13}C_3$-PFBA	107	16
$^{13}C_2$-PFDA	107	16
$^{13}C_4$-PFOS	111	11
$^{13}C_2$-PFOA	100	17

Conclusion

This application note demonstrates the development and implementation of a rugged extraction and analysis method for the determination of 25 PFAS in a large-scale study comprising 25 fish species using the Agilent Captiva EMR–Lipid and LC/MS/MS. For more information regarding the implementation of this method, please see the publication by Pulster *et al.*[6]

References

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Appendix

Appendix A

Table 5. Compound retention times, MRM transitions, and voltages.

Compound	Retention Time (min)	Internal Standard	Precursor Ion	Product Ion	Fragmentor (V)	Collision Energy (V)	Collision Cell Accelerator (V)
Target Analytes							
PFBA	6.169	M4-PFBA	213	169	72	8	2
PF4OPeA	7.691	M5-PFPeA	229	85	50	16	2
PFPeA	9.214	M5-PFPeA	263	219	72	4	2
PF5OHxA	9.748	M2-4:2FTS	279	85	80	8	5
4:2FTS	10.288	M2-4:2FTS	327	307	150	20	2
3,6-OPFHpA	10.562	M5-PFHxA	201	85	120	4	5
PFHxA	10.700	M5-PFHxA	313	269	72	8	2
PFBS	10.962	M3-PFBS	299	80	154	36	2
HFPO-DA	11.089	M3-HFPO-DA	285	169	135	8	4
PFEESA	11.479	M4-PFHpA	315	135	124	28	5
PFHpA	11.621	M4-PFHpA	363	319	72	8	2
NaDONA	11.914	M3-HFPO-DA	377	251	50	8	5
6:2FTS	12.020	M2-6:2FTS	427	407	135	18	4
PFPeS	12.007	M2-6:2FTS	349	80	135	20	4
PFOA	12.391	M8-PFOA	413	369	72	8	2
PFHxS	12.880	M3-PFHxS	399	80	156	56	2
PFNA	13.134	M9-PFNA	463	419	72	8	2
8:2FTS	13.467	M2-8:2FTS	527	507	200	30	4
PFHpS	13.699	M6-PFDA	449	80	148	50	2
PFDA	13.897	M6-PFDA	513	469	72	12	2
PFOS	14.502	M8-PFOS	499	80	135	50	4
PFUnDA	14.638	M7-PFUdA	563	519	100	12	2
9Cl-PF3ONS	14.975	M7-PFUdA	531	351	150	28	3
PFDoA	15.239	M7-PFUdA	613	569	100	8	2
11Cl-PF3OUdS	16.641	M7-PFUdA	631	451	150	36	2

Compound	Retention Time (min)	Internal Standard	Precursor Ion	Product Ion	Fragmentor (V)	Collision Energy (V)	Collision Cell Accelerator (V)
Surrogate Standards							
M3-PFBA	6.169	M4-PFBA	216	172	72	8	2
M2-PFOA	12.391	M8-PFOA	415	370	72	8	2
M2-PFDA	13.896	M6-PFDA	515	470	72	8	2
M4-PFOS	14.501	M8-PFOS	503	99	135	54	4
Internal Standards							
M4-PFBA	6.164		217	172	72	8	2
M5-PFPeA	9.214		268	223	72	4	2
M2-4:2FTS	10.287		329	309	150	24	2
M5-PFHxA	10.699		318	273	72	8	2
M3-PFBS	10.961		302	80	130	44	2
M3-HFPO-DA	11.080		287	169	135	4	5
M4-PFHpA	11.620		367	322	72	8	2
M2-6:2FTS	12.019		429	409	150	28	2
M8-PFOA	12.390		421	376	72	8	2
M3-PFHxS	12.879		402	80	156	48	2
M9-PFNA	13.133		472	427	72	8	2
M2-8:2FTS	13.466		529	509	200	28	4
M6-PFDA	13.896		519	474	72	8	2
M8-PFOS	14.500		507	80	148	54	2
M7-PFUdA	14.637		570	525	73	5	4

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DE86273469

Printed in the USA, September 13, 2022
5994-5227EN