

## Application News

LCMSTM-8060NX High Performance Liquid Chromatograph Mass Spectrometer  
NexeraTM series High Performance Liquid Chromatograph

### Determination of 30 PFAS in Fish Oil by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS/MS)

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#### User Benefits

- ◆ Validated method for 30 PFAS in fish oil meeting all criteria of AOAC SMPR 2023.003
- ◆ High precision, excellent recovery, low Limit of Quantification (LOQ)
- ◆ Simple and rapid extraction

#### Introduction

Per- and polyfluorinated substances (PFAS) are a diverse group of man-made chemicals used in numerous products since the 1950s. PFAS can enter the food supply by contact in environmentally contaminated areas, during food processing, or exposure to packaging. Because PFAS have been linked to serious health effects, accurate methodology is needed. In this application news, we describe a single laboratory validation study with a rapid extraction of low concentrations of 30 PFAS in fish oil followed by analysis using the Shimadzu Nexera Liquid Chromatograph coupled to a Shimadzu LCMS-8060NX triple quadrupole mass spectrometer (Figure 1).

We optimized the chromatography and instrument operating parameters to achieve excellent peak shape, separation, and sensitivity. Sensitivity was improved for 4 PFAS, PFOA, PFHxA, PFNA, and PFOS.

In this study, we spiked samples at five concentrations in triplicate. For greater accuracy, standards were matrix-matched and extracted and spikes were quantified using the isotope dilution technique. Recovery and precision were compared to the requirements of AOAC SMPR 2023.003. In addition, we determined the Limit of Quantitation (LOQ) as the lowest concentration meeting accuracy and precision, ion ratio, retention time, and signal-to-noise ratio criteria of the qualifier ion. All recovery, precision, and LOQ's met the acceptance criteria of the SMPR. The target analytes, their acronym, chemical abstract number, and experimentally determined LOQ are shown in Table 1.



**Figure 1:** Nexera™ and LCMS™-8060NX. The ion focus design improves signal intensity with higher gas flows and higher effective temperatures.

**Table 1:** PFAS Analytes, Acronyms, CAS No. and Method LOQ

Analyte name	Acronym	CAS No.	LOQ (ppb)
Perfluorobutanoic acid	PFBA	375-22-4	0.25
Perfluoropentanoic acid	PFPeA	2706-90-3	0.25
Perfluorohexanoic acid	PFHxA	307-24-4	0.25
Perfluoroheptanoic acid	PFHpA	375-85-9	0.25
Perfluorooctanoic acid	PFOA	335-67-1	0.25
Perfluorononanoic acid	PFNA	375-95-1	0.25
Perfluorodecanoic acid	PFDA	335-76-2	0.25
Perfluoroundecanoic acid	PFUnA	2058-94-8	0.25
Perfluorododecanoic acid	PFDoA	307-55-1	0.25

Perfluorotridecanoic acid	PFTrDA	72629-94-8	0.25
Perfluorotetradecanoic acid	PFTeDA	376-06-7	0.25
Perfluorobutanesulfonic acid	PFBS	375-73-5	0.25
Perfluoropentanesulfonic acid	PFPeS	2706-91-4	0.25
Perfluorohexanesulfonic acid	PFHxS	355-46-4	0.25
Perfluoroheptanesulfonic acid	PFHpS	375-92-8	0.25
Perfluorooctanesulfonic acid	PFOS	1763-23-1	0.25
Perfluorononanesulfonic acid	PFNS	68259-12-1	0.25
Perfluorodecanesulfonic acid	PFDS	335-77-3	0.25
Perfluoroundecanesulfonic acid	PFUnDS	749786-16-1	0.25
Perfluorododecanesulfonic acid	PFDoS	79780-39-5	0.25
Perfluorotridecanesulfonic acid	PFTrDS	791563-89-8	0.25
Perfluorooctanesulfonamide	PFOSA	754-91-6	0.25
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9CI-PF3ONS	756426-58-1	0.25
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11CI-PF3OUdS	763051-92-9	0.25
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	0.25
4,8-Dioxa-3H-perfluorononanoic acid	DONA	919005-14-4	0.25
1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	4:2 FTS	757124-72-4	0.25
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2 FTS	27619-97-2	0.25
1H,1H, 2H, 2H-Perfluorodecane sulfonic acid	8:2 FTS	39108-34-4	0.25
1H,1H, 2H, 2H-Perfluorododecane sulfonic acid	10:2 FTS	120226-60-0	0.5

## ■ Sample Preparation and Analysis Conditions

Fish oil soft gels were purchased locally. Samples were prepared by cutting the tip of the capsule with scissors and squeezing the oil out into a 50 ml tube. Test portions were spiked in triplicate at five different concentrations with 30 native PFAS (Table 1) and 16 isotopically labeled internal standards. Calibration curves for use in the quantitative analysis were prepared using 10-gram fish oil test portions spiked with concentrations of 0.1, 0.5, 1.5, 5.0 and 15.0 ng/g. Quantitation was carried out on additional fish oil samples spiked in triplicate at 0.25, 0.5, 1.0, 5.0, and 10 ng/g. Since standards were extracted in a fish oil matrix and carried through the same procedure, the final concentration of each PFAS in the sample can be calculated directly from the curve.

10-gram portions were weighed, spiked with target analytes and internal standards, and 10 mL of acetonitrile was added. The samples were vortexed for 1 minute and then centrifuged for 5 minutes at 4000 rpm. An aliquot of the acetonitrile layer was transferred to a tube and diluted 5 times with PFAS-free reagent water. The sample was then passed through a weak anion exchange (WAX) Solid Phase Extraction (SPE) cartridge and the PFAS were eluted with basic methanol-water mixture.

A volume suitable to obtain the required sensitivity of the extract was injected onto a UHPLC system (Shimadzu Nexera). Adequate separation of all compounds was achieved in nine minutes. (Figure 2 chromatogram shows the separation of all peaks).

For this study, Shimadzu evaluated 1984 different instrument settings, and 6 different column and gradient combinations, to achieve excellent peak shape and resolution between peaks, as well as to maximize the signal-to-noise ratio of PFOA, PFHxS, PFNA, and PFOS. Mass spectrometry was performed on a Shimadzu LCMS-8060NX with heated electrospray ionization operated in negative mode. Specific compound MRM transitions and associated internal standards are listed in Table 2. Chromatography was adjusted to provide sufficient separation of PFOA from potential cholic acid interferences, and to provide baseline resolution of branched and linear isomers (Figure 3).

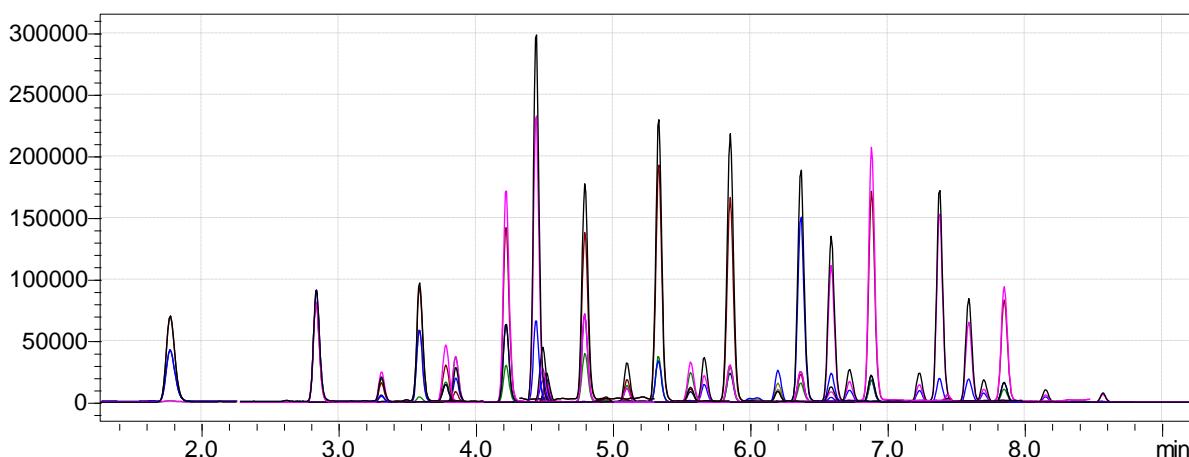


Figure 2: Chromatogram of 0.5 ng/g PFAS in a fish oil matrix with separation of all peaks in nine minutes

**MRM chromatogram**

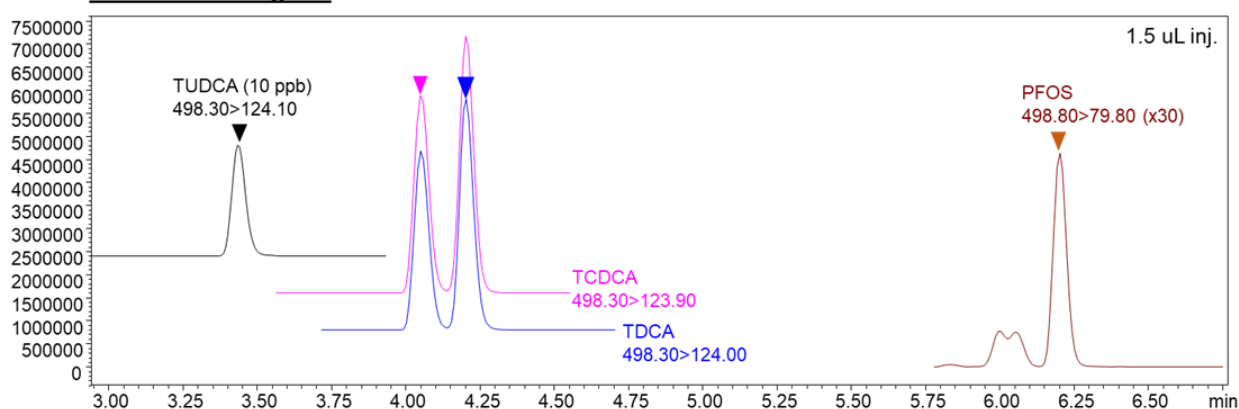


Figure 3: Separation of PFOS from Cholic Acids and baseline resolution between PFOS branched and linear isomers

Table 2: MRM Transitions and Internal Standard Associations

Analyte	Quantitation Ion	Qualifier Ion	Internal Standard
PFBA	212.9 > 168.8		<sup>13</sup> C <sub>4</sub> -PFBA
PFPeA	262.9 > 218.8		<sup>13</sup> C <sub>5</sub> -PFPeA
PFHxA	313 > 268.8	313 > 118.9	<sup>13</sup> C <sub>5</sub> -PFHxA
PFHpA	362.9 > 318.9	362.9 > 168.8	<sup>13</sup> C <sub>4</sub> -PFHpA
PFOA	412.9 > 368.9	412.9 > 168.8	<sup>13</sup> C <sub>8</sub> -PFOA
PFNA	462.9 > 418.5	462.9 > 218.6	<sup>13</sup> C <sub>9</sub> -PFNA
PFDA	512.9 > 468.95	512.9 > 268.55	<sup>13</sup> C <sub>6</sub> -PFDA
PFUnA	562.9 > 518.95	562.9 > 269	<sup>13</sup> C <sub>7</sub> -PFUnA
PFDoA	612.9 > 268.6	612.9 > 168.6	<sup>13</sup> C <sub>7</sub> -PFUnA
PFTTrDA	663 > 618.6	663 > 168.5	<sup>13</sup> C <sub>7</sub> -PFUnA
PFTeDA	713 > 669	713 > 168.5	<sup>13</sup> C <sub>7</sub> -PFUnA
PFBS	298.8 > 79.8	298.8 > 98.9	<sup>13</sup> C <sub>3</sub> -PFBS
PFPeS	348.8 > 79.8	348.8 > 98.9	<sup>13</sup> C <sub>4</sub> -PFHpA
PFHxS	398.8 > 79.8	398.8 > 98.9	<sup>13</sup> C <sub>3</sub> -PFHxS
PFHpS	448.8 > 79.8	448.8 > 99	<sup>13</sup> C <sub>6</sub> -PFDA
PFOS	498.8 > 79.8	498.8 > 98.9	<sup>13</sup> C <sub>8</sub> -PFOS
PFNS	548.9 > 79.8	548.8 > 99	<sup>13</sup> C <sub>7</sub> -PFUnA
PFDS	598.8 > 99	598.8 > 79.8	<sup>13</sup> C <sub>7</sub> -PFUnA
PFUnDS	649 > 99	649 > 80	<sup>13</sup> C <sub>8</sub> -PFOS
PFDoS	699 > 80	699 > 99	<sup>13</sup> C <sub>2</sub> -PFDoA
PFTTrDS	749 > 80	749 > 279.6	<sup>13</sup> C <sub>7</sub> -PFUnA
PFOSA	498 > 78	498 > 477.95	<sup>13</sup> C <sub>8</sub> -PFOSA
9Cl-PF3ONS	530.8 > 350.8	532.9 > 352.95	<sup>13</sup> C <sub>8</sub> -PFOS
11Cl-PF3OUdS	631 > 451	632.9 > 452.95	<sup>13</sup> C <sub>7</sub> -PFUnA
HFPO-DA	284.8 > 168.8	284.8 > 118.9	<sup>13</sup> C <sub>3</sub> -HFPO-DA
DONA	376.9 > 250.8	376.9 > 84.9	<sup>13</sup> C <sub>4</sub> -PFHpA
4:2 FTS	326.9 > 80.9	326.9 > 306.8	<sup>13</sup> C <sub>2</sub> -4:2 FTS
6:2 FTS	426.9 > 80.8	426.9 > 406.9	<sup>13</sup> C <sub>2</sub> -6:2 FTS
8:2 FTS	526.9 > 506.45	526.9 > 80.55	<sup>13</sup> C <sub>3</sub> -PFHxS
10:2 FTS	626.9 > 606.7	626.9 > 80.9	<sup>13</sup> C <sub>7</sub> -PFUnA

## Quantitative Analysis

Calibration standards were processed the same as samples. A linear model not forced through zero isotopic dilution calibration in matrix-matched standards provided the best fit and best recoveries of analytes. Residuals of each point in the curve were  $\pm 25\%$  of the expected value. Calibration curves for PFOA, PFHxS, PFNA, and PFOS are shown in Figures 3 – 6 respectively. Branched and linear isomers of PFHxS and PFOS were integrated together.

Figure 3: PFOA Calibration Curve

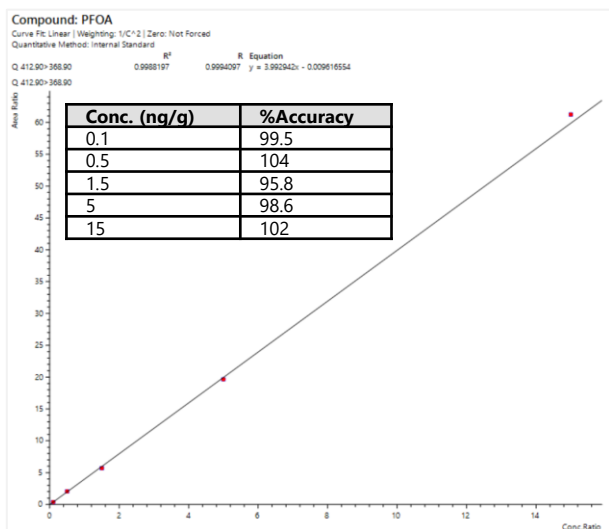


Figure 4: PFHxS Calibration Curve

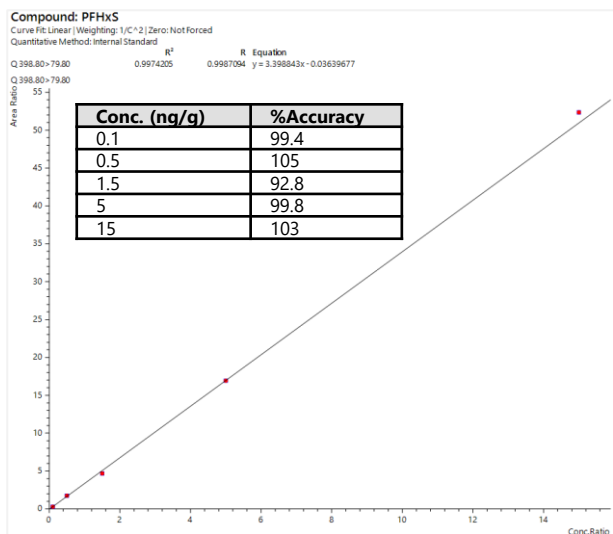


Figure 5: PFNA Calibration Curve

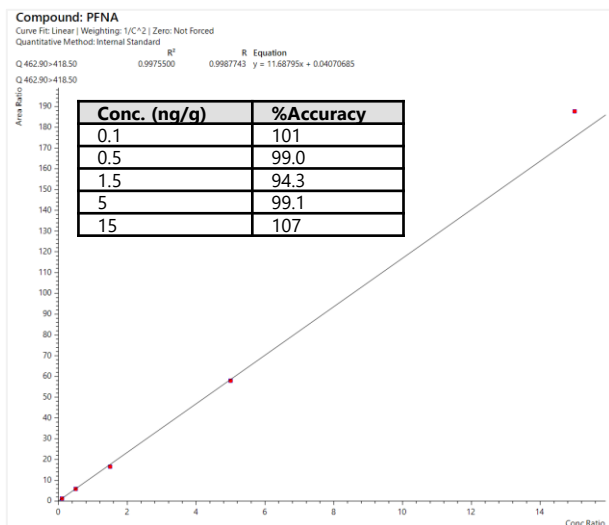
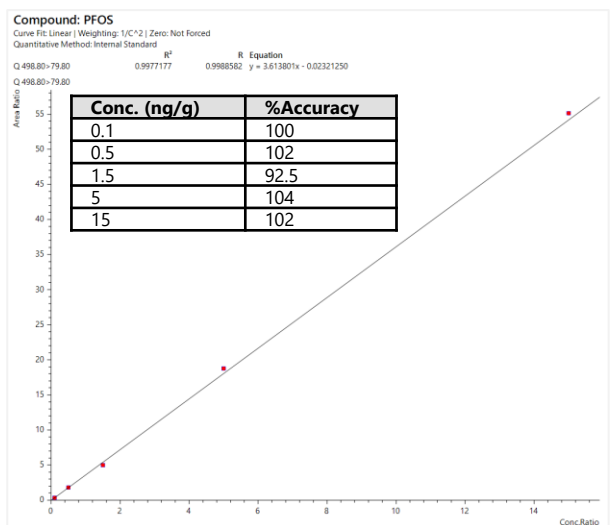


Figure 6: PFOS Calibration Curve



Blank matrixes and five different concentrations ranging from below the SMPR required LOQ to 20 times the estimated LOQ were analyzed in triplicate. Recovery and repeatability for each analyte at each concentration are given in Table 3. The LOQ for each analyte was estimated by spiking at concentrations at, or below, the required LOQs listed in SMPR-2023\_003. The spiked samples were analyzed in triplicate and the mean and repeatability standard deviation were calculated. Then, the standard deviation was divided by the mean and multiplied by 100% to calculate the repeatability percent relative standard deviation (RSD).

The LOQs for all matrixes and compounds were determined using an Excel worksheet that compared each of the requirements of the SMPR including retention time, recovery, repeatability,  $S/N > 3$  for the qualifier ion and an ion ratio of  $\pm 30\%$ . PFBA, PFPeA, and PFOSA LOQ were set at the minimum concentration, meeting recovery and repeatability requirements and a  $S/N > 10$ . The lowest concentration to meet all the requirements of the SMPR was set as the LOQ. Figure 7 shows examples of the LOQ spike for PFHxS, PFNA, PFOA, and PFOS and their corresponding internal standards.

**Table 3:** Recovery and repeatability for each analyte at each spike concentration

Analyte	Spike conc. (ppb)	Average conc. (ppb)	Standard Deviation	%RSD	Average Recovery%
PFBA	Blank	ND			
	0.25	0.254	6.17	6.07	101.7
	0.5	0.434	7.21	8.31	86.8
	1.0	1.017	3.90	3.83	101.7
	5.0	5.338	7.41	6.94	106.8
	10	10.414	3.17	3.04	104.1
PFPeA	Blank	ND			
	0.25	0.249	2.87	2.88	99.7
	0.5	0.447	5.37	6.00	89.5
	1.0	1.025	2.20	2.15	102.5
	5.0	5.247	8.12	7.74	104.9
	10	10.238	2.98	2.91	102.4
PFHxA	Blank	0.008			
	0.25	0.264	4.76	4.50	105.6
	0.5	0.483	4.25	4.40	96.6
	1.0	1.103	1.17	1.06	110.3
	5.0	5.537	6.70	6.05	110.7
	10	10.836	5.22	4.81	108.4
PFHpA	Blank	ND			
	0.25	0.266	3.07	2.87	106.7
	0.5	0.482	4.35	4.51	96.4
	1.0	1.089	2.63	2.42	108.9
	5.0	5.556	8.68	7.81	111.1
	10	10.898	4.01	3.68	109.0
PFOA	Blank	0.009			
	0.25	0.270	3.15	2.92	108.0
	0.5	0.470	2.78	2.96	94.0
	1.0	1.022	1.14	1.11	102.2
	5.0	5.292	5.17	4.88	105.8
	10	10.508	1.59	1.51	105.0
PFNA	Blank	ND			
	0.25	0.261	1.91	1.83	104.3
	0.5	0.473	2.11	2.23	94.7
	1.0	1.054	2.91	2.76	105.4
	5.0	5.453	3.67	3.36	109.0
	10	10.879	1.39	1.27	108.8
PFDA	Blank	0.006			
	0.25	0.265	6.96	6.57	105.9
	0.5	0.491	3.98	4.05	98.2
	1.0	1.054	0.81	0.77	105.4
	5.0	5.568	3.46	3.11	111.4
	10	10.804	1.37	1.26	108.1
PFUnA	Blank	0.010			
	0.25	0.259	6.56	6.34	103.4
	0.5	0.529	4.65	4.39	105.9
	1.0	1.040	3.08	2.96	104.0
	5.0	5.453	4.99	4.58	109.0
	10	11.149	1.21	1.08	111.5
PFDoA	Blank	0.011			
	0.25	0.252	6.11	6.06	100.8
	0.5	0.552	2.93	2.65	110.5
	1.0	1.076	7.91	7.35	107.6
	5.0	5.845	4.88	4.17	116.9
	10	11.709	1.20	1.02	117.1
PFTrDA	Blank	0.019			
	0.25	0.235	18.57	19.79	93.8
	0.5	0.544	9.18	8.43	108.9
	1.0	1.064	1.30	1.22	106.4
	5.0	5.459	4.67	4.27	109.2
	10	10.772	1.80	1.67	107.8
PFTeDA	Blank	0.028			
	0.25	0.202	19.65	24.26	81.0
	0.5	0.472	2.76	2.93	94.3
	1.0	0.994	7.50	7.54	99.4
	5.0	5.695	7.96	6.99	113.9
	10	11.144	2.97	2.67	111.4
PFBS	Blank	ND			
	0.25	0.240	3.96	4.13	96.0
	0.5	0.467	6.35	6.79	93.5
	1.0	0.920	0.46	0.50	92.0
	5.0	4.573	4.12	4.51	91.4
	10	9.796	2.10	2.15	98.0

PFPeS	Blank	ND			
	0.25	0.276	7.43	6.72	110.5
	0.5	0.471	3.21	3.41	94.1
	1.0	1.079	4.42	4.10	107.9
	5.0	5.282	2.68	2.53	105.6
	10	11.338	4.10	3.62	113.4
PFHxS	Blank	0.013			
	0.25	0.262	4.08	3.89	104.9
	0.5	0.482	1.97	2.04	96.4
	1.0	0.941	1.99	2.12	94.1
	5.0	4.740	4.58	4.83	94.8
	10	9.739	0.49	0.51	97.4
PFHpS	Blank	ND			
	0.25	0.266	3.21	3.02	106.3
	0.5	0.464	5.18	5.59	92.8
	1.0	1.066	2.45	2.30	106.6
	5.0	5.037	0.35	0.35	100.7
	10	11.747	3.06	2.61	117.5
PFOS	Blank	0.008			
	0.25	0.242	5.29	5.46	96.8
	0.5	0.464	3.63	3.90	92.9
	1.0	0.922	3.99	4.33	92.2
	5.0	4.614	4.50	4.88	92.3
	10	9.836	2.56	2.60	98.3
PFNS	Blank	ND			
	0.25	0.256	5.54	5.41	102.4
	0.5	0.488	4.03	4.14	97.5
	1.0	1.011	3.78	3.74	101.1
	5.0	4.918	0.85	0.86	98.4
	10	11.269	1.63	1.44	112.7
PFDS	Blank	0.004			
	0.25	0.261	2.69	2.58	104.2
	0.5	0.480	7.88	8.21	96.0
	1.0	0.986	3.78	3.84	98.6
	5.0	4.884	2.71	2.77	97.7
	10	11.074	2.04	1.84	110.8
PFUnDS	Blank	0.038			
	0.25	0.229	11.65	12.74	91.4
	0.5	0.471	3.69	3.92	94.2
	1.0	0.936	5.08	5.43	93.6
	5.0	4.868	5.07	5.21	97.3
	10	9.633	2.03	2.10	96.3
PFDoS	Blank	0.014			
	0.25	0.240	17.49	18.23	95.9
	0.5	0.463	3.94	4.26	92.5
	1.0	1.008	8.40	8.34	100.8
	5.0	5.083	3.24	3.19	101.7
	10	10.273	3.84	3.74	102.7
PFTrDS	Blank	0.013			
	0.25	0.217	10.40	11.99	86.8
	0.5	0.449	2.72	3.03	89.9
	1.0	1.017	8.78	8.64	101.7
	5.0	5.471	5.86	5.36	109.4
	10	11.963	3.60	3.01	119.6
PFOSA	Blank	ND			
	0.25	0.254	5.16	5.08	101.7
	0.5	0.497	7.54	7.59	99.4
	1.0	0.998	6.46	6.48	99.8
	5.0	5.054	3.82	3.78	101.1
	10	10.768	3.10	2.88	107.7
9CI-PF3ONS	Blank	0.007			
	0.25	0.261	4.09	3.91	104.5
	0.5	0.500	1.76	1.76	100.1
	1.0	0.976	3.38	3.46	97.6
	5.0	4.989	5.16	5.17	99.8
	10	10.804	1.33	1.23	108.0
11CI-PF3OUdS	Blank	0.004			
	0.25	0.276	0.75	0.68	110.5
	0.5	0.496	3.36	3.39	99.3
	1.0	1.036	9.19	8.87	103.6
	5.0	5.483	4.11	3.75	109.7
	10	12.079	4.51	3.73	120.8

HFPO-DA	Blank	0.010			
	0.25	0.251	4.55	4.53	100.4
	0.5	0.469	7.34	7.81	93.9
	1.0	0.876	3.07	3.50	87.6
	5.0	4.538	4.21	4.63	90.8
	10	9.461	3.98	4.21	94.6
DONA	Blank	ND			
	0.25	0.244	3.92	4.01	97.7
	0.5	0.466	4.58	4.92	93.1
	1.0	1.026	1.17	1.14	102.6
	5.0	5.390	9.33	8.65	107.8
	10	10.210	3.70	3.62	102.1
4:2 FTS	Blank	ND			
	0.25	0.250	10.10	10.10	99.9
	0.5	0.480	2.93	3.05	96.0
	1.0	1.107	7.81	7.05	110.7
	5.0	5.443	9.61	8.83	108.9
	10	10.788	7.37	6.83	107.9
6:2 FTS	Blank	ND			
	0.25	0.267	12.51	11.70	106.9
	0.5	0.511	13.05	12.78	102.1
	1.0	1.079	9.92	9.19	107.9
	5.0	5.342	8.95	8.38	106.8
	10	10.826	9.54	8.81	108.3
8:2 FTS	Blank	0.009			
	0.25	0.225	9.23	10.25	90.1
	0.5	0.483	0.38	0.39	96.6
	1.0	1.016	0.85	0.84	101.6
	5.0	5.945	10.53	8.86	118.9
	10	10.140	3.49	3.44	101.4
10:2 FTS	Blank	0.003			
	0.5	0.478	10.34	10.82	95.6
	1.0	1.060	8.61	8.13	106.0
	5.0	5.939	9.65	8.13	118.8
	10	11.502	2.70	2.35	115.0

ND = average results less than zero

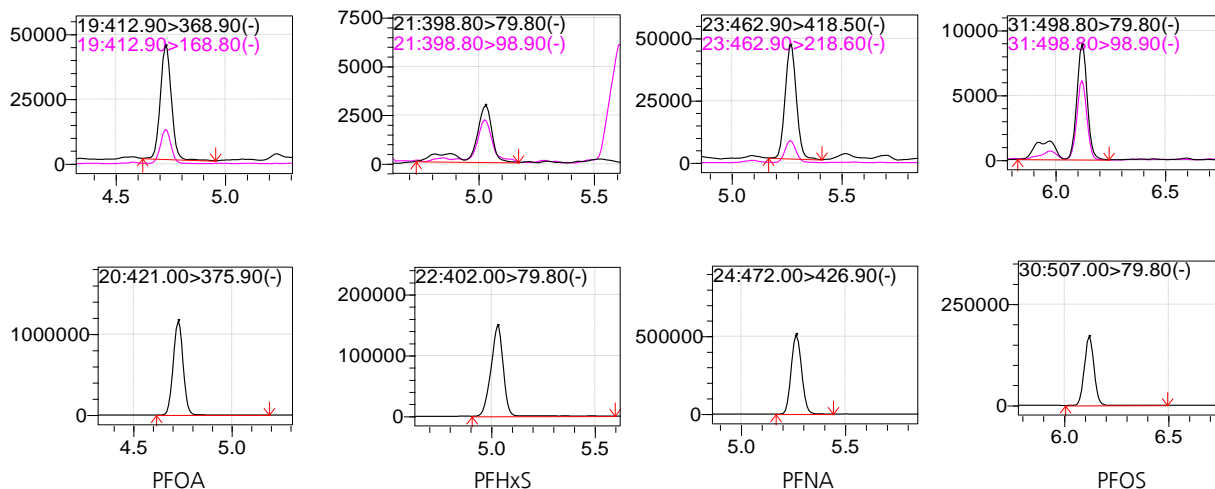


Figure 7: LOQ Peaks with Internal Standards

## Conclusion

The Shimadzu LCMS-8060NX Triple Quadrupole Mass Spectrometer coupled with a Shimadzu Nexera Liquid Chromatograph was used in a single laboratory study to measure 30 PFAS compounds in a fish oil matrix and compared to criteria set by AOAC SMPR 2023.003. Chromatography conditions and the mass spectrometer were optimized to achieve excellent separation of all analytes, baseline resolution between linear and branched isomers, and a two-minute separation between PFOS and potentially interfering cholic acids.

Precision and recovery and the experimentally determined LOQ are well within the requirements of the SMPR.

## Reference

- 1) AOAC SMPR 2023.003

# UAFMS

ULTRA FAST MASS SPECTROMETRY



LCMS-8045RX

LCMS-8050RX

LCMS-8060RX

LCMS-2020

LCMS-2050

Q-TOF LCMS-9030/9050

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