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Application News

LCMS™-8060NX High Performance Liquid Chromatograph Mass Spectrometer
Nexera™ series High Performance Liquid Chromatograph

Determination of 30 PFAS in Protein Powder by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS/MS)

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User Benefits

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

■ 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 protein powder using the QuEChERS technique 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, PFHxS, PFNA, and PFOS.

In this study, we spiked samples at four 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	0.055
Perfluoropentanoic acid	PFPeA	2706-90-3	0.055
Perfluorohexanoic acid	PFHxA	307-24-4	0.055
Perfluoroheptanoic acid	PFHpA	375-85-9	0.055
Perfluorooctanoic acid	PFOA	335-67-1	0.055
Perfluorononanoic acid	PFNA	375-95-1	0.0055
Perfluorodecanoic acid	PFDA	335-76-2	0.0055
Perfluoroundecanoic acid	PFUnA	2058-94-8	0.0055
Perfluorododecanoic acid	PFDoA	307-55-1	0.055

Perfluorotridecanoic acid	PFTrDA	72629-94-8	0.055
Perfluorotetradecanoic acid	PFTeDA	376-06-7	0.055
Perfluorobutanesulfonic acid	PFBS	375-73-5	0.055
Perfluoropentansulfonic acid	PFPeS	2706-91-4	0.055
Perfluorohexanesulfonic acid	PFHxS	355-46-4	0.0055
Perfluoroheptanesulfonic acid	PFHpS	375-92-8	0.0055
Perfluorooctanesulfonic acid	PFOS	1763-23-1	0.055
Perfluorononanesulfonic acid	PFNS	68259-12-1	0.055
Perfluorodecanesulfonic acid	PFDS	335-77-3	0.055
Perfluoroundecanesulfonic acid	PFUnDS	749786-16-1	0.055
Perfluorododecanesulfonic acid	PFDoS	79780-39-5	0.055
Perfluorotridecanesulfonic acid	PFTrDS	791563-89-8	0.55
Perfluorooctanesulfonamide	PFOSA	754-91-6	0.55
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1	0.0055
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9	0.0055
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	0.55
4,8-Dioxa-3H-perfluorononanoic acid	DONA	919005-14-4	0.0055
1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	4:2 FTS	757124-72-4	0.055
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2 FTS	27619-97-2	0.055
1H,1H, 2H, 2H-Perfluorodecane sulfonic acid	8:2 FTS	39108-34-4	0.0055
1H,1H, 2H, 2H-Perfluorododecane sulfonic acid	10:2 FTS	120226-60-0	0.055

■ Sample Preparation and Analysis Conditions

Organic, plant-based protein powder was purchased locally. Test portions were weighed directly from the store packaging and spiked in triplicate at four 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 protein powder test portions spiked with concentrations of 0.001, 0.01, 0.1, 1.0 and 10.0 ng/g. Quantitation was carried out on additional protein powder samples spiked in triplicate at 0.0055, 0.055, 0.55 and 5.5 ng/g. Since standards were extracted in a protein powder 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, 10 mL of water and 10 mL of acetonitrile was added. The samples were shaken for 1 minute and a QuEChERS packet was added. The sample was shaken again 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 13 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. For greater sensitivity, the extract was concentrated to dryness and dissolved in 0.4 mL of a 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).

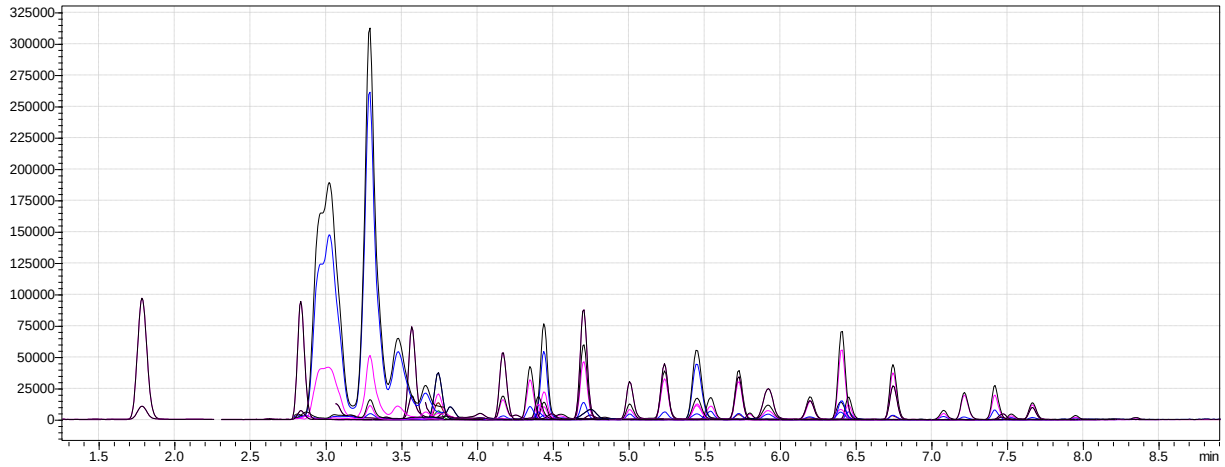


Figure 2: Chromatogram of 0.055 ng/g PFAS in a protein powder matrix with separation of all peaks in nine minutes. The chromatogram shows other co-eluting peaks between 2.7 and 3.6 minutes, but they did not interfere.

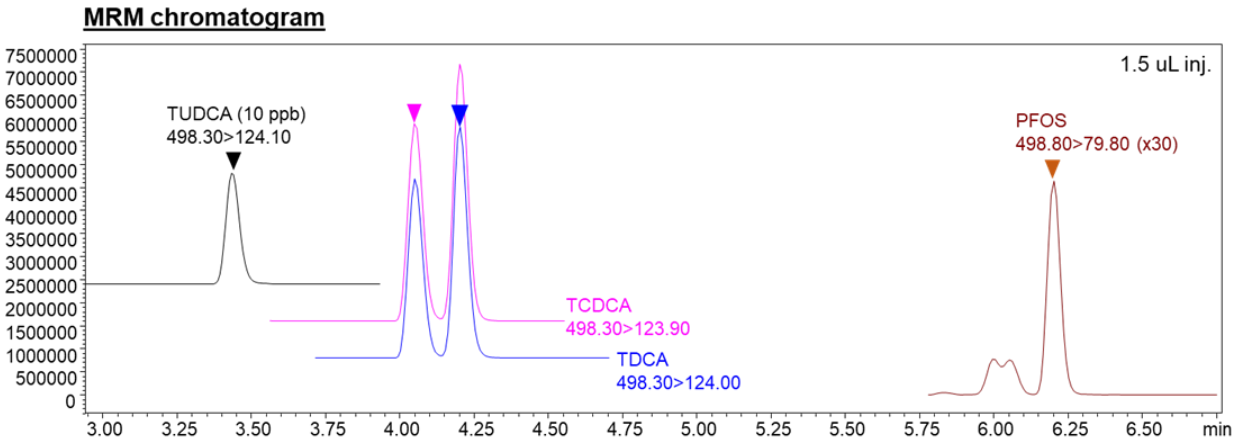


Figure 3: Separation of PFOS from Cholic Acids and baseline resolution between PFOS branched and linear isomers

Table 2: MRM Transitions and Internal Standard Associations

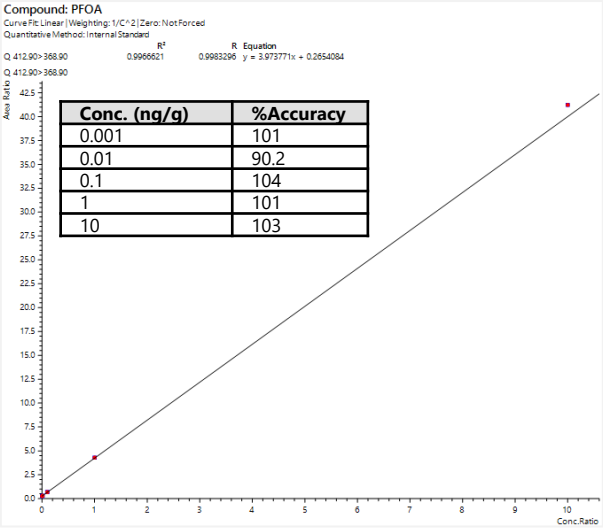
Analyte	Quantitation Ion	Qualifier Ion	Internal Standard
PFBA	212.9 > 168.8		$^{13}C_4$-PFBA
PFPeA	262.9 > 218.8		$^{13}C_5$-PFPeA
PFHxA	313 > 268.8	313 > 118.9	$^{13}C_5$-PFHxA
PFHpA	362.9 > 318.9	362.9 > 168.8	$^{13}C_4$-PFHpA
PFOA	412.9 > 368.9	412.9 > 168.8	$^{13}C_8$-PFOA
PFNA	462.9 > 418.5	462.9 > 218.6	$^{13}C_9$-PFNA
PFDA	512.9 > 468.95	512.9 > 268.55	$^{13}C_6$-PFDA
PFUnA	562.9 > 518.95	562.9 > 269	$^{13}C_7$-PFUnA
PFDoA	612.9 > 268.6	612.9 > 168.6	$^{13}C_7$-PFUnA
PFTrDA	663 > 618.6	663 > 168.5	$^{13}C_7$-PFUnA
PFTeDA	713 > 669	713 > 168.5	$^{13}C_7$-PFUnA
PFBS	298.8 > 79.8	298.8 > 98.9	$^{13}C_3$-PFBS
PFPeS	348.8 > 79.8	348.8 > 98.9	$^{13}C_4$-PFHpA
PFHxS	398.8 > 79.8	398.8 > 98.9	$^{13}C_3$-PFHxS
PFHpS	448.8 > 79.8	448.8 > 99	$^{13}C_6$-PFDA
PFOS	498.8 > 79.8	498.8 > 98.9	$^{13}C_8$-PFOS
PFNS	548.9 > 79.8	548.8 > 99	$^{13}C_7$-PFUnA
PFDS	598.8 > 99	598.8 > 79.8	$^{13}C_7$-PFUnA
PFUnDS	649 > 99	649 > 80	$^{13}C_8$-PFOS
PFDoS	699 > 80	699 > 99	$^{13}C_2$-PFDoA
PFTrDS	749 > 80	749 > 279.6	$^{13}C_7$-PFUnA
PFOSA	498 > 78	498 > 477.95	$^{13}C_8$-PFOSA
9Cl-PF3ONS	530.8 > 350.8	532.9 > 352.95	$^{13}C_8$-PFOS
11Cl-PF3OUdS	631 > 451	632.9 > 452.95	$^{13}C_7$-PFUnA
HFPO-DA	284.8 > 168.8	284.8 > 118.9	$^{13}C_3$-HFPO-DA
DONA	376.9 > 250.8	376.9 > 84.9	$^{13}C_4$-PFHpA
4:2 FTS	326.9 > 80.9	326.9 > 306.8	$^{13}C_2$-4:2 FTS
6:2 FTS	426.9 > 80.8	426.9 > 406.9	$^{13}C_2$-6:2FTS
8:2 FTS	526.9 > 506.45	526.9 > 80.55	$^{13}C_3$-PFHxS
10:2 FTS	626.9 > 606.7	626.9 > 80.9	$^{13}C_7$-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 ±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.

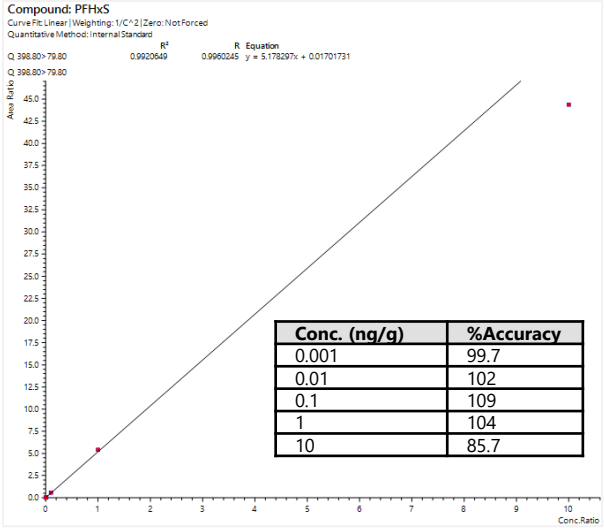
Exact labeled analogs were used as isotope dilution standards, except in a few cases where we found that $^{13}C_2$ suffered matrix interferences. In these cases, another non-interfering isotope was chosen. The analytes tested and the associated calibration isotopes are shown in Table 3.

Figure 3: PFOA Calibration Curve



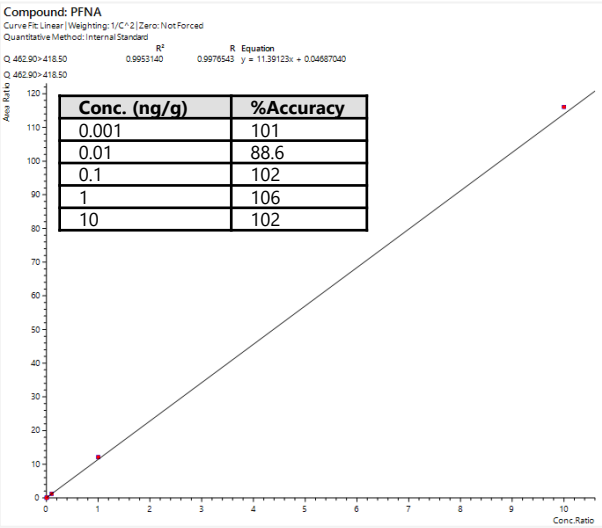
Conc. (ng/g)	%Accuracy
0.001	101
0.01	90.2
0.1	104
1	101
10	103

Figure 4: PFHxS Calibration Curve



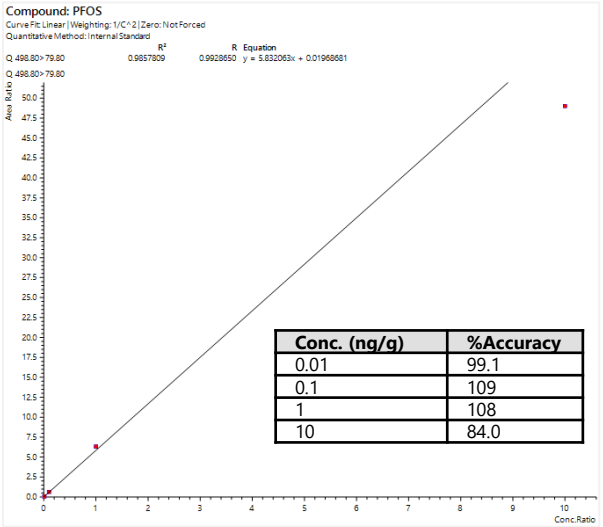
Conc. (ng/g)	%Accuracy
0.001	99.7
0.01	102
0.1	109
1	104
10	85.7

Figure 5: PFNA Calibration Curve



Conc. (ng/g)	%Accuracy
0.001	101
0.01	88.6
0.1	102
1	106
10	102

Figure 6: PFOS Calibration Curve



Conc. (ng/g)	%Accuracy
0.01	99.1
0.1	109
1	108
10	84.0

Blank matrixes and at least three different concentrations ranging from below the SMPR required LOQ to approximately 70 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 matrices 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 ±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			
	0.055	0.071	6.83	5.31	128.7
	0.55	0.599	2.41	2.22	108.9
	5.5	6.077	0.70	0.64	110.5
PFPeA	Blank	ND			
	0.055	0.061	3.70	3.36	110.2
	0.55	0.598	0.29	0.27	108.6
	5.5	6.275	2.88	2.53	114.1
PFHxA	Blank	ND			
	0.055	0.049	2.50	2.81	89.1
	0.55	0.546	2.08	2.10	99.2
	5.5	5.758	1.32	1.26	104.7
PFHpA	Blank	0.005			
	0.055	0.057	1.46	1.41	103.3
	0.55	0.541	4.25	4.32	98.4
	5.5	5.339	2.40	2.47	97.1
PFOA	Blank	0.000			
	0.055	0.060	4.83	4.40	109.7
	0.55	0.614	2.34	2.10	111.6
	5.5	5.962	2.80	2.58	108.4
PFNA	Blank	ND			
	0.0055	0.006	10.70	10.53	101.7
	0.055	0.060	0.67	0.61	109.2
	0.55	0.604	2.45	2.23	109.8
	5.5	6.158	2.84	2.54	111.9
PFDA	Blank	ND			
	0.0055	0.005	4.86	5.09	95.6
	0.055	0.058	1.52	1.45	104.8
	0.55	0.589	3.72	3.48	107.1
	5.5	5.996	3.88	3.55	109.0
PFUnA	Blank	ND			
	0.0055	0.007	21.51	18.99	113.3
	0.055	0.059	6.09	5.73	106.3
	0.55	0.595	2.56	2.36	108.2
	5.5	5.655	4.91	4.78	102.8
PFDoA	Blank	ND			
	0.055	0.060	10.10	9.34	108.2
	0.55	0.597	6.61	6.09	108.6
	5.5	5.748	7.59	7.26	104.5
PFTrDA	Blank	ND			
	0.0055	0.004	12.72	17.77	71.6
	0.055	0.052	2.20	2.30	95.5
	0.55	0.561	2.15	2.10	102.0
	5.5	5.583	3.40	3.35	101.5
PFTeDA	Blank	0.000			
	0.0055	0.005	4.51	4.94	91.2
	0.055	0.049	5.97	6.69	89.3
	0.55	0.568	1.33	1.29	103.3
	5.5	5.432	4.41	4.46	98.8
PFBS	Blank	ND			
	0.055	0.062	11.70	10.33	113.2
	0.55	0.620	7.16	6.35	112.7
	5.5	5.952	6.67	6.16	108.2
PFPeS	Blank	0.001			
	0.055	0.063	3.61	3.14	114.8
	0.55	0.602	6.16	5.62	109.5
	5.5	6.168	0.86	0.77	112.2
PFHxS	Blank	ND			
	0.0055	0.006	6.85	6.02	113.8
	0.055	0.059	3.86	3.60	107.1
	0.55	0.587	1.17	1.10	106.7
	5.5	5.486	2.16	2.17	99.7
PFHpS	Blank	0.001			
	0.0055	0.006	15.90	14.87	106.9
	0.055	0.058	2.05	1.94	105.6
	0.55	0.563	3.63	3.55	102.3
	5.5	6.008	7.58	6.94	109.2

PFOS	Blank	ND			
	0.055	0.057	17.30	16.79	103.0
	0.55	0.603	4.60	4.20	109.6
	5.5	5.325	2.41	2.49	96.8
PFNS	Blank	ND			
	0.055	0.054	4.15	4.24	98.0
	0.55	0.610	8.84	7.97	110.9
	5.5	6.174	5.02	4.48	112.3
PFDS	Blank	0.003			
	0.055	0.056	18.37	18.08	101.6
	0.55	0.532	3.72	3.85	96.6
	5.5	5.808	6.93	6.56	105.6
PFUnDS	Blank	0.001			
	0.0055	0.005	10.13	12.93	78.4
	0.055	0.056	3.07	3.03	101.4
	0.55	0.561	3.32	3.25	101.9
	5.5	4.688	3.45	4.04	85.3
PFDoS	Blank	0.003			
	0.0055	0.006	7.39	6.50	113.7
	0.055	0.058	4.98	4.75	104.8
	0.55	0.523	3.60	3.79	95.0
	5.5	4.703	1.74	2.04	85.5
PFTrDS	Blank	ND			
	0.55	0.695	2.08	1.65	126.4
	5.5	6.531	6.31	5.32	118.8
PFOSA	Blank	ND			
	0.055	0.053	7.62	7.91	96.3
	0.55	0.614	3.36	3.01	111.7
	5.5	5.397	2.50	2.55	98.1
9Cl-PF3ONS	Blank	0.000			
	0.0055	0.006	3.21	2.76	116.4
	0.055	0.063	2.40	2.10	114.3
	0.55	0.607	3.84	3.48	110.3
	5.5	5.687	1.52	1.47	103.4
11Cl-PF3OUdS	Blank	ND			
	0.0055	0.004	9.37	12.62	74.2
	0.055	0.060	7.15	6.56	109.0
	0.55	0.557	0.90	0.89	101.3
	5.5	5.777	3.99	3.80	105.0
HFPO-DA	Blank	0.030			
	0.55	0.663	8.31	6.89	120.5
	5.5	6.199	8.09	7.18	112.7
DONA	Blank	ND			
	0.0055	0.005	5.20	5.91	88.0
	0.055	0.060	3.83	3.54	108.2
	0.55	0.546	4.27	4.30	99.3
	5.5	5.671	1.33	1.29	103.1
4:2 FTS	Blank	0.012			
	0.055	0.054	5.74	5.85	98.0
	0.55	0.589	2.02	1.89	107.1
	5.5	5.963	5.17	4.77	108.4
6:2 FTS	Blank	0.004			
	0.055	0.071	15.56	12.10	128.6
	0.55	0.582	4.10	3.87	105.9
	5.5	5.729	4.83	4.64	104.1
8:2 FTS	Blank	0.000			
	0.0055	0.005	7.43	7.54	98.6
	0.055	0.055	7.09	7.10	99.8
	0.55	0.553	5.34	5.32	100.5
	5.5	5.004	4.06	4.46	91.0
10:2 FTS	Blank	ND			
	0.055	0.055	10.56	10.61	99.5
	0.55	0.673	8.99	7.35	122.3
	5.5	5.601	11.11	10.91	101.8

ND = average results less than zero

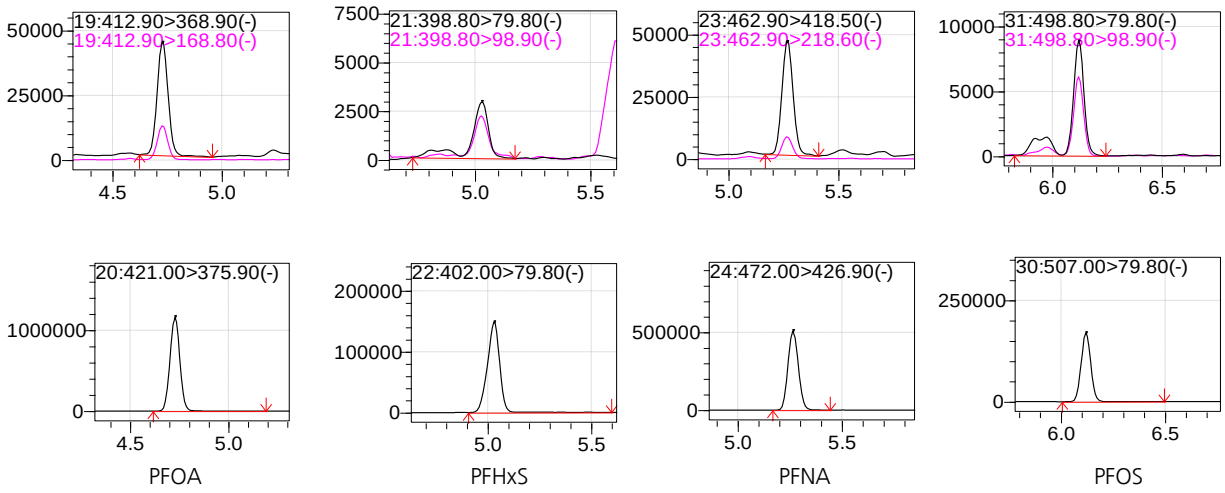


Figure 7: LOQ Peaks with Internal Standards

■ 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 protein powder 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

ULTRA FAST MASS SPECTROMETRY

LCMS-8045RX LCMS-8050RX LCMS-8060RX LCMS-2020 LCMS-2050 Q-TOF LCMS-9030/9050

Founded in 1875, Shimadzu Corporation, a leader in the development of advanced technologies, has a distinguished history of innovation built on the foundation of contributing to society through science and technology. Established in 1975, Shimadzu Scientific Instruments (SSI), the American subsidiary of Shimadzu Corporation, provides a comprehensive range of analytical solutions to laboratories throughout North, Central, and parts of South America. SSI maintains a network of ten regional offices strategically located across the United States, with experienced technical specialists, service and sales engineers situated throughout the country, as well as applications laboratories on both coasts.

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