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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 Milk by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS/MS)

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User Benefits

- Validated method for 30 PFAS in Milk 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 milk 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 three 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	0.01
Perfluoropentanoic acid	PFPeA	2706-90-3	0.01
Perfluorohexanoic acid	PFHxA	307-24-4	0.01
Perfluoroheptanoic acid	PFHpA	375-85-9	0.01
Perfluorooctanoic acid	PFOA	335-67-1	0.01
Perfluorononanoic acid	PFNA	375-95-1	0.01
Perfluorodecanoic acid	PFDA	335-76-2	0.01
Perfluoroundecanoic acid	PFUnA	2058-94-8	0.01
Perfluorododecanoic acid	PFDoA	307-55-1	0.01

Perfluorotridecanoic acid	PFTrDA	72629-94-8	0.01
Perfluorotetradecanoic acid	PFTeDA	376-06-7	0.01
Perfluorobutanesulfonic acid	PFBS	375-73-5	0.01
Perfluoropentansulfonic acid	PFPeS	2706-91-4	0.01
Perfluorohexanesulfonic acid	PFHxS	355-46-4	0.01
Perfluoroheptanesulfonic acid	PFHpS	375-92-8	0.01
Perfluorooctanesulfonic acid	PFOS	1763-23-1	0.01
Perfluorononanesulfonic acid	PFNS	68259-12-1	0.01
Perfluorodecanesulfonic acid	PFDS	335-77-3	0.01
Perfluoroundecanesulfonic acid	PFUnDS	749786-16-1	0.01
Perfluorododecanesulfonic acid	PFDoS	79780-39-5	0.01
Perfluorotridecanesulfonic acid	PFTrDS	791563-89-8	0.01
Perfluorooctanesulfonamide	PFOSA	754-91-6	0.01
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1	0.01
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9	0.01
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	0.01
4,8-Dioxa-3H-perfluorononanoic acid	DONA	919005-14-4	0.01
1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	4:2 FTS	757124-72-4	0.01
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2 FTS	27619-97-2	0.01
1H,1H, 2H, 2H-Perfluorodecane sulfonic acid	8:2 FTS	39108-34-4	0.01
1H,1H, 2H, 2H-Perfluorododecane sulfonic acid	10:2 FTS	120226-60-0	0.01

■ Sample Preparation and Analysis Conditions

Whole milk (3.7 % fat) was purchased and sampled directly from the carton. Test portions were spiked in triplicate at three 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 test portions spiked with 0.005, 0.01, 0.05, 0.10, 0.50, and 1.0 ng/g of each target analyte. Quantitation was carried out on additional whole milk samples spiked in triplicate at 0.01, 0.10 and 1.0 ng/g. Since standards were extracted in milk 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 150 µL of formic acid and 10 mL of acetonitrile was added. The samples were shaken by hand for 10 seconds and a QuEChERS packet was added. The sample was shaken again for 5 minutes and then centrifuged for 5 minutes at 4000 rpm. An aliquot of the acetonitrile layer was transferred to a tube to which additional QuEChERS reagent was added. The sample was shaken for 10 seconds by hand, for 5 minutes on a shaker, and then centrifuged for 5 minutes at 4000 rpm. An aliquot was removed to another tube and concentrated to less than 1 ml under nitrogen, reconstituted to 1 ml in a methanol-water mixture, transferred to a 1.5 mL tube and centrifuged for 10 minutes at 15,000 rpm. The supernatant was transferred to an LC vial for analysis.

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).

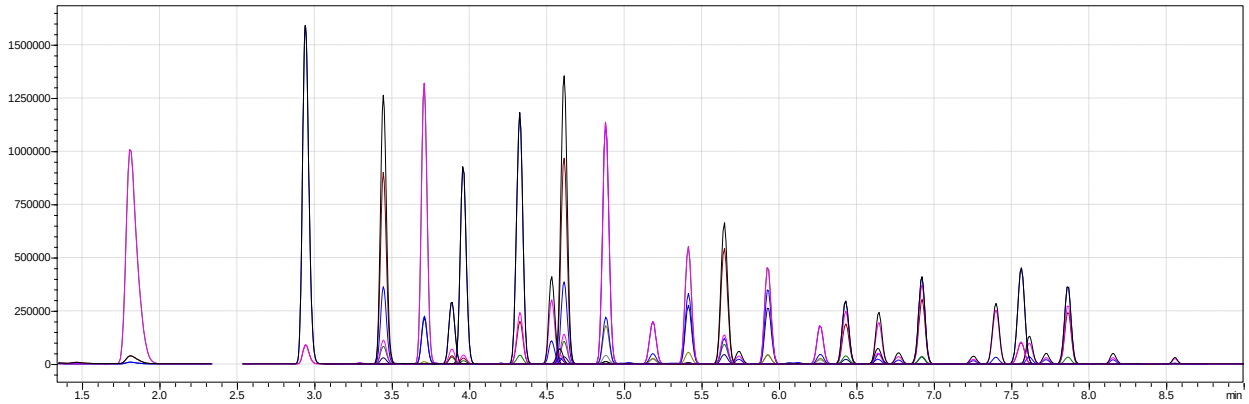


Figure 2: Chromatogram of 0.1 ng/g PFAS in a milk matrix with separation of all peaks in nine minutes

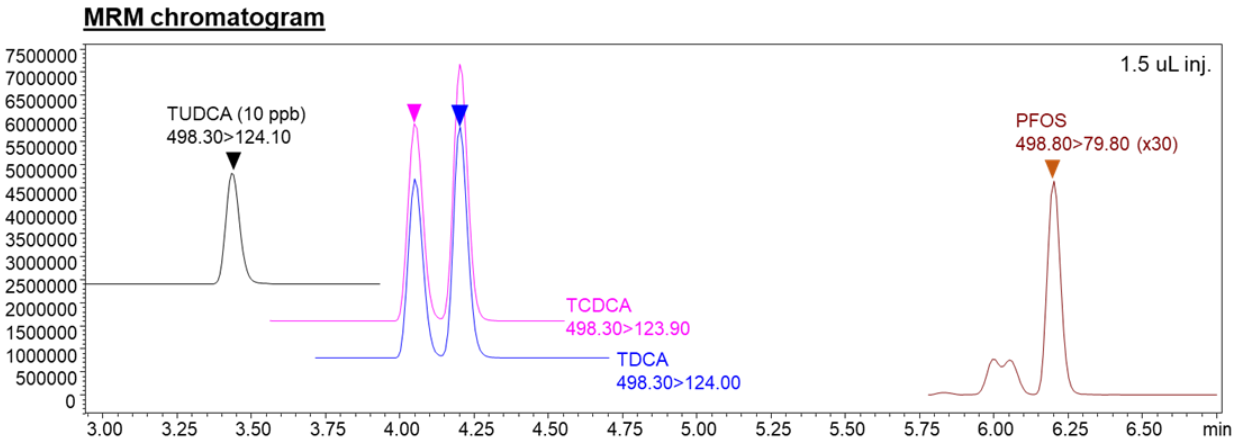


Figure 3: Separation of PFOS from Cholic Acids and baseline resolution between PFOS branched and linear isomers

Table 2: MRM Transitions and Internal Standard Associations

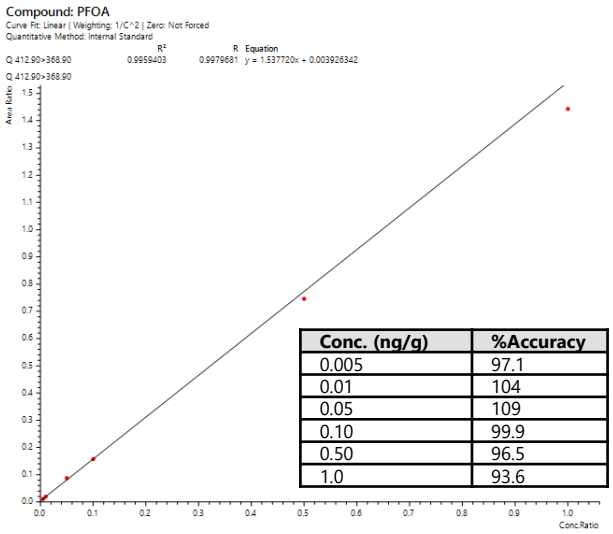
Analyte	Quantitation Ion	Qualifier Ion	Internal Standard
PFBA	212.9 > 168.8		$^{13}C_4$-PFBA
PFPeA	262.9 > 218.8		$^{13}C_5$-PFPeA
PFHxA	313 > 268.8	313 > 118.9	$^{13}C_5$-PFHxA
PFHpA	362.9 > 318.9	362.9 > 168.8	$^{13}C_4$-PFHpA
PFOA	412.9 > 368.9	412.9 > 168.8	$^{13}C_8$-PFOA
PFNA	462.9 > 418.5	462.9 > 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.

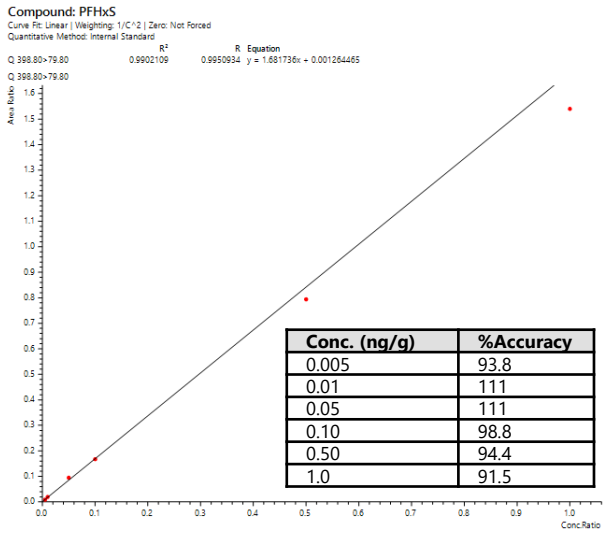
Exact labeled analogs were used as isotope dilution standards, except in a few cases where we found that $^{13}C_2$ suffered matrix interferences. In these cases, another non-interfering isotope was chosen. The analytes tested and the associated calibration isotopes are shown in Table 3.

Figure 3: PFOA Calibration Curve



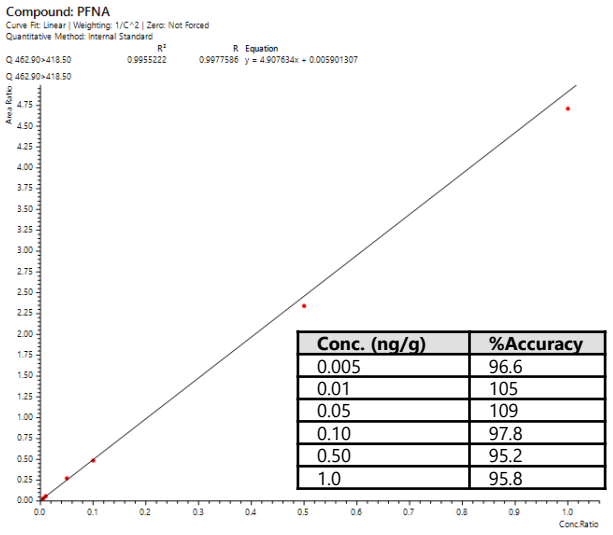
Conc. (ng/g)	%Accuracy
0.005	97.1
0.01	104
0.05	109
0.10	99.9
0.50	96.5
1.0	93.6

Figure 4: PFHxS Calibration Curve



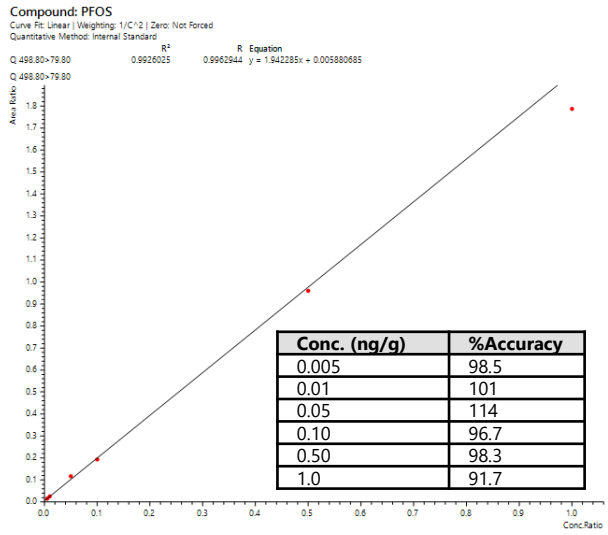
Conc. (ng/g)	%Accuracy
0.005	93.8
0.01	111
0.05	111
0.10	98.8
0.50	94.4
1.0	91.5

Figure 5: PFNA Calibration Curve



Conc. (ng/g)	%Accuracy
0.005	96.6
0.01	105
0.05	109
0.10	97.8
0.50	95.2
1.0	95.8

Figure 6: PFOS Calibration Curve



Conc. (ng/g)	%Accuracy
0.005	98.5
0.01	101
0.05	114
0.10	96.7
0.50	98.3
1.0	91.7

Blank matrixes and three different concentrations ranging from the SMPR required LOQ to 100 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	0.000			
	0.01	0.012	8.20	7.02	116.8
	0.1	0.103	2.10	2.02	103.5
	1	0.973	0.57	0.58	97.3
PFPeA	Blank	0.000			
	0.01	0.011	2.63	2.45	107.1
	0.1	0.099	0.66	0.66	99.3
	1	0.947	0.38	0.40	94.7
PFHxA	Blank	ND			
	0.01	0.010	1.74	1.67	104.4
	0.1	0.096	0.93	0.97	96.1
	1	0.947	0.10	0.11	94.7
PFHpA	Blank	0.001			
	0.01	0.011	7.89	6.79	116.2
	0.1	0.099	0.45	0.45	99.4
	1	0.968	1.50	1.55	96.8
PFOA	Blank	ND			
	0.01	0.010	11.84	11.35	104.3
	0.1	0.098	2.89	2.93	98.5
	1	0.977	1.00	1.02	97.7
PFNA	Blank	0.000			
	0.01	0.011	0.96	0.86	112.2
	0.1	0.100	0.92	0.93	99.5
	1	0.976	0.61	0.62	97.6
PFDA	Blank	0.001			
	0.01	0.011	2.91	2.59	112.7
	0.1	0.104	0.42	0.40	104.2
	1	0.994	2.71	2.73	99.4
PFUnA	Blank	0.000			
	0.01	0.011	1.20	1.08	111.5
	0.1	0.101	0.84	0.83	100.9
	1	0.980	0.72	0.74	98.0
PFDoA	Blank	0.000			
	0.01	0.012	13.65	11.65	117.1
	0.1	0.101	1.54	1.52	101.3
	1	0.951	1.47	1.55	95.1
PFTrDA	Blank	0.000			
	0.01	0.011	2.40	2.24	106.9
	0.1	0.101	1.60	1.59	100.9
	1	0.994	0.68	0.68	99.4
PFTeDA	Blank	0.000			
	0.01	0.011	4.14	3.80	108.8
	0.1	0.098	0.67	0.68	98.2
	1	0.974	3.40	3.49	97.4
PFBS	Blank	ND			
	0.01	0.012	8.49	7.19	118.1
	0.1	0.102	1.65	1.62	101.6
	1	0.958	0.25	0.26	95.8
PFPeS	Blank	ND			
	0.01	0.010	11.26	11.41	98.7
	0.1	0.102	1.05	1.03	101.9
	1	0.953	1.62	1.70	95.3
PFHxS	Blank	0.000			
	0.01	0.010	10.50	10.76	97.6
	0.1	0.099	3.88	3.89	99.6
	1	0.956	1.36	1.42	95.6
PFHpS	Blank	ND			
	0.01	0.010	7.26	6.85	106.0
	0.1	0.105	2.27	2.17	104.4
	1	0.983	2.10	2.14	98.3

PFOS	Blank	ND			
	0.01	0.010	6.44	6.54	98.4
	0.1	0.099	2.57	2.59	99.2
	1	0.961	1.32	1.37	96.1
PFNS	Blank	ND			
	0.01	0.010	10.24	10.20	100.4
	0.1	0.102	2.86	2.81	101.8
	1	0.992	2.58	2.60	99.2
PFDS	Blank	ND			
	0.01	0.009	3.74	3.95	94.8
	0.1	0.103	6.13	5.95	103.0
	1	1.015	1.22	1.20	101.5
PFUnDS	Blank	ND			
	0.01	0.011	2.19	2.01	109.3
	0.1	0.105	4.78	4.56	105.0
	1	0.988	3.49	3.54	98.8
PFDoS	Blank	ND			
	0.01	0.010	8.88	9.15	97.0
	0.1	0.101	2.24	2.22	100.6
	1	0.963	2.27	2.36	96.3
PFTrDS	Blank	ND			
	0.01	0.010	5.12	4.83	106.0
	0.1	0.099	3.30	3.32	99.4
	1	0.990	2.08	2.10	99.0
PFOSA	Blank	ND			
	0.01	0.011	3.59	3.36	106.8
	0.1	0.099	1.83	1.85	99.0
	1	0.950	0.55	0.58	95.0
9Cl-PF3ONS	Blank	0.000			
	0.01	0.010	4.42	4.31	102.5
	0.1	0.103	0.96	0.94	102.5
	1	0.971	3.15	3.25	97.1
11Cl-PF3OUdS	Blank	ND			
	0.01	0.009	5.32	5.61	94.8
	0.1	0.101	1.10	1.09	100.8
	1	0.992	1.22	1.23	99.2
HFPO-DA	Blank	ND			
	0.01	0.010	7.16	7.19	99.6
	0.1	0.099	2.17	2.18	99.3
	1	0.932	0.80	0.86	93.2
DONA	Blank	0.000			
	0.01	0.010	0.80	0.77	103.9
	0.1	0.099	0.42	0.42	98.9
	1	0.961	2.12	2.21	96.1
4:2 FTS	Blank	ND			
	0.01	0.011	4.97	4.63	107.5
	0.1	0.102	2.75	2.69	102.1
	1	0.989	1.28	1.29	98.9
6:2 FTS	Blank	0.001			
	0.01	0.012	11.91	10.39	114.6
	0.1	0.106	5.06	4.80	105.5
	1	0.984	1.40	1.43	98.4
8:2 FTS	Blank	0.001			
	0.01	0.012	8.01	6.86	116.7
	0.1	0.097	1.53	1.57	97.1
	1	0.888	0.23	0.26	88.8
10:2 FTS	Blank	0.000			
	0.01	0.011	10.94	10.09	108.4
	0.1	0.095	3.27	3.43	95.4
	1	0.862	1.22	1.42	86.2

ND = average results less than zero

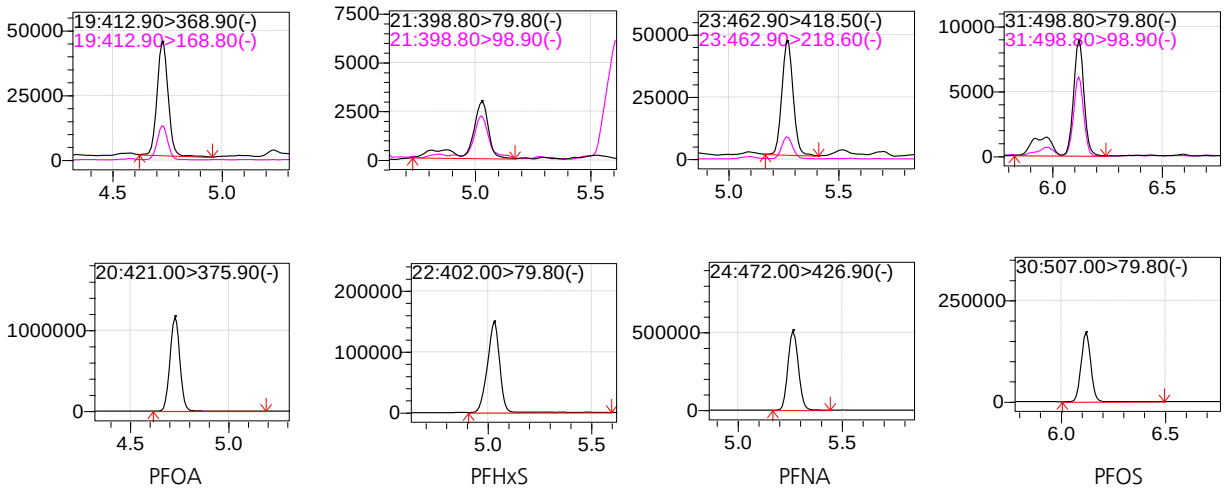


Figure 7: LOQ Peaks with Internal Standards

■ 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 high fat milk 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

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