

## Application News

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

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

William Lipps<sup>1</sup>, Dominika Gruszecka<sup>1</sup>, Toshiya Matsubara<sup>1</sup>

<sup>1</sup> Shimadzu Scientific Instruments, Inc.

#### User Benefits

- ◆ Validated method for 30 PFAS in Egg 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 eggs 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	0.0055
Perfluorooctanoic acid	PFOA	335-67-1	0.0055
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	0.0055

Perfluorotridecanoic acid	PFTrDA	72629-94-8	0.0055
Perfluorotetradecanoic acid	PFTeDA	376-06-7	0.055
Perfluorobutanesulfonic acid	PFBS	375-73-5	0.0055
Perfluoropentanesulfonic acid	PFPeS	2706-91-4	0.0055
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	0.0055
Perfluorodecanesulfonic acid	PFDS	335-77-3	0.0055
Perfluoroundecanesulfonic acid	PFUnDS	749786-16-1	0.055
Perfluorododecanesulfonic acid	PFDoS	79780-39-5	0.055
Perfluorotridecanesulfonic acid	PFTrDS	791563-89-8	0.055
Perfluorooctanesulfonamide	PFOSA	754-91-6	0.55
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9CI-PF3ONS	756426-58-1	0.0055
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11CI-PF3OUdS	763051-92-9	0.0055
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	0.0055
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	0.55
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2 FTS	27619-97-2	0.0055
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	0.0055

## ■ Sample Preparation and Analysis Conditions

Large brown eggs were purchased locally. Samples were prepared cracking and removing the whole egg from the shell and grinding with dry ice for 30 seconds at 4000 rpm. The ground material was placed in a beaker. Test portions were 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 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 whole egg samples spiked in triplicate at 0.0055, 0.055, 0.55 and 5.5 ng/g. Since standards were extracted in an egg 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 a QuEChERS packet was added. The sample was shaken 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. 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).

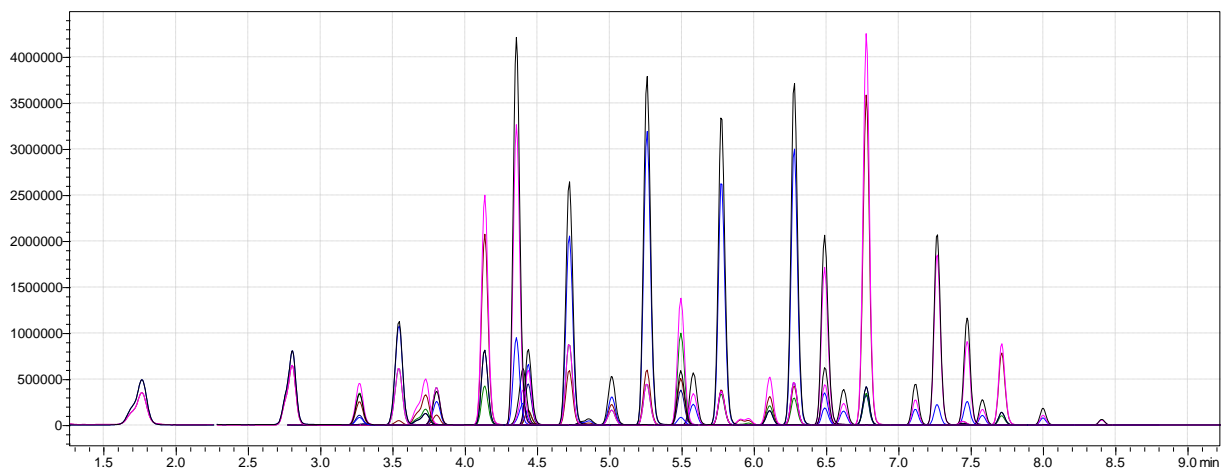


Figure 2: Chromatogram of 0.55 ng/g PFAS in an egg matrix with separation of all peaks in nine minutes

**MRM chromatogram**

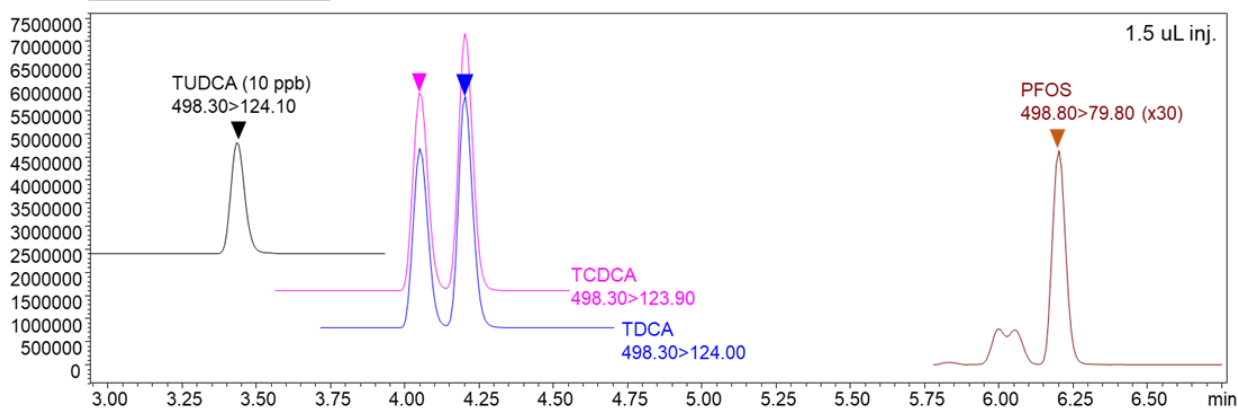


Figure 3: Separation of PFOS from Cholic Acids and baseline resolution between PFOS branched and linear isomers

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

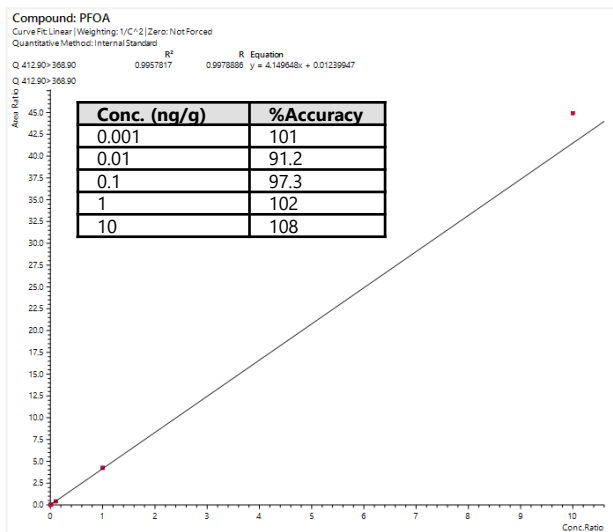


Figure 4: PFHxS Calibration Curve

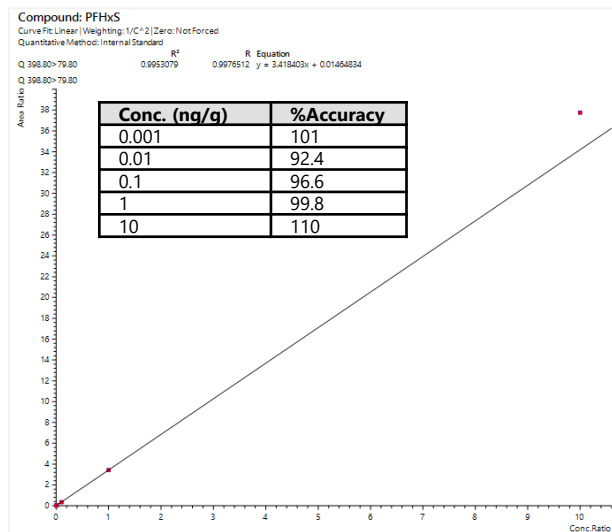


Figure 5: PFNA Calibration Curve

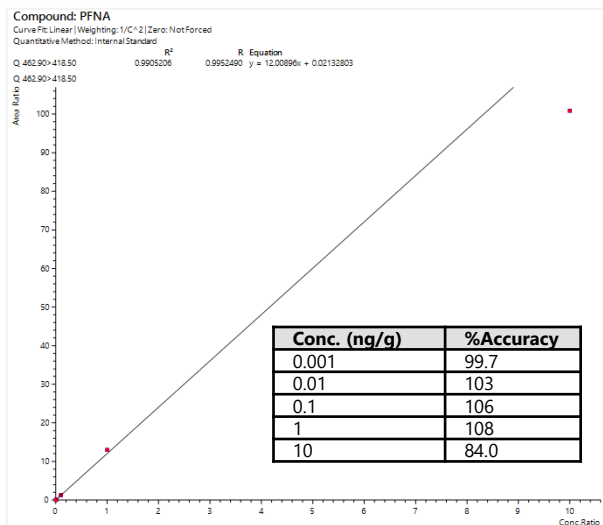
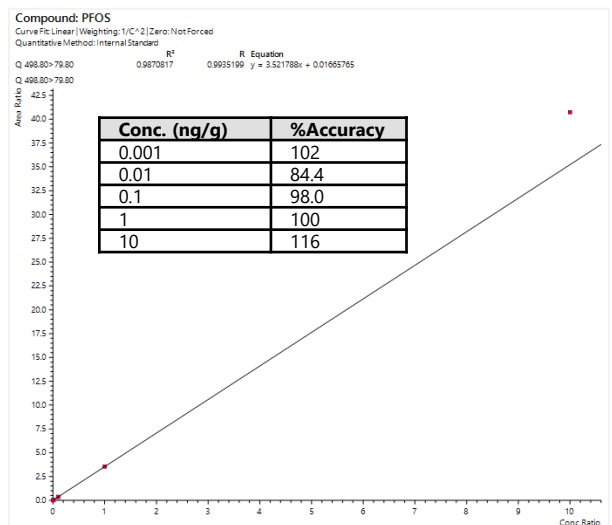


Figure 6: PFOS Calibration Curve



Blank matrixes and four different concentrations ranging from below the SMPR required LOQ to approximately 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).

Exact labeled analogs were used as isotope dilution standards, except in a few cases where we found that <sup>13</sup>C<sub>2</sub> 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.

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	0.002			
	0.055	0.051	1.38	1.48	93.3
	0.55	0.528	1.30	1.36	95.9
	5.5	5.664	0.26	0.26	103.0
PFPeA	Blank	0.004			
	0.0055	0.006	10.48	9.67	108.4
	0.055	0.050	4.96	5.42	91.5
	0.55	0.486	0.50	0.57	88.3
PFHxA	5.5	5.364	0.87	0.90	97.5
	Blank	0.004			
	0.0055	0.005	4.52	4.82	93.8
	0.055	0.055	0.62	0.62	100.3
PFHpA	0.55	0.550	1.37	1.36	100.0
	5.5	6.107	1.10	0.99	111.1
	Blank	0.000			
	0.0055	0.005	4.39	4.62	94.9
PFOA	0.055	0.053	1.04	1.09	95.6
	0.55	0.542	0.49	0.50	98.5
	5.5	6.271	0.92	0.80	114.0
	Blank	0.000			
PFNA	0.0055	0.005	5.50	6.19	88.9
	0.055	0.054	2.41	2.46	97.9
	0.55	0.557	1.11	1.10	101.3
	5.5	6.338	2.33	2.02	115.2
PFDA	Blank	0.000			
	0.0055	0.005	1.66	1.67	99.1
	0.055	0.056	2.05	2.01	102.0
	0.55	0.592	0.59	0.54	107.6
PFUnA	5.5	6.122	8.80	7.91	111.3
	Blank	ND			
	0.0055	0.005	3.66	3.91	93.7
	0.055	0.055	0.98	0.99	99.7
PFDoA	0.55	0.569	2.10	2.03	103.5
	5.5	6.085	7.05	6.37	110.7
	Blank	0.000			
	0.0055	0.005	3.20	3.35	95.5
PFTrDA	0.055	0.057	2.15	2.10	102.8
	0.55	0.587	0.85	0.80	106.8
	5.5	6.281	8.37	7.33	114.2
	Blank	ND			
PFTeDA	0.0055	0.004	12.06	16.81	71.7
	0.055	0.047	6.41	7.53	85.1
	0.55	0.504	1.88	2.05	91.6
	5.5	5.892	1.60	1.49	107.1
PFBS	Blank	0.000			
	0.0055	0.005	9.21	10.21	90.2
	0.055	0.047	3.12	3.63	85.8
	0.55	0.515	0.81	0.86	93.6
PFPeS	5.5	6.927	3.26	2.59	125.9
	Blank	0.002			
	0.0055	0.006	24.12	21.48	112.3
	0.055	0.040	0.61	0.83	73.5
PFBS	0.55	0.508	0.91	0.98	92.4
	5.5	7.220	3.10	2.36	131.3
	Blank	ND			
	0.0055	0.004	7.72	10.25	75.4
PFPeS	0.055	0.051	0.61	0.66	93.1
	0.55	0.534	2.17	2.23	97.1
	5.5	5.763	1.50	1.43	104.8
	Blank	0.001			
PFPeS	0.0055	0.005	17.67	18.11	97.6
	0.055	0.051	4.60	4.99	92.2
	0.55	0.516	0.67	0.71	93.9
	5.5	5.494	1.49	1.49	99.9

PFHxS	Blank	ND			
	0.0055	0.006	21.39	19.16	111.7
	0.055	0.051	1.16	1.24	93.7
	0.55	0.533	1.27	1.32	96.9
	5.5	5.779	1.15	1.09	105.1
PFHpS	Blank	0.001			
	0.0055	0.005	2.93	2.98	98.3
	0.055	0.057	3.84	3.73	103.0
	0.55	0.515	1.29	1.38	93.6
	5.5	5.058	3.91	4.25	92.0
PFOS	Blank	0.002			
	0.0055	0.006	5.17	5.28	98.1
	0.055	0.051	2.30	2.49	92.4
	0.55	0.520	0.29	0.31	94.6
	5.5	5.563	1.78	1.76	101.2
PFNS	Blank	0.000			
	0.0055	0.005	10.26	11.84	86.7
	0.055	0.048	1.71	1.94	88.1
	0.55	0.523	2.52	2.65	95.1
	5.5	6.736	3.30	2.69	122.5
PFDS	Blank	ND			
	0.0055	0.005	2.25	2.47	91.3
	0.055	0.050	2.42	2.67	90.5
	0.55	0.503	1.82	1.99	91.5
	5.5	5.541	3.26	3.23	100.8
PFUnDS	Blank	0.001			
	0.0055	0.005	4.47	5.03	88.9
	0.055	0.049	3.86	4.36	88.7
	0.55	0.536	2.55	2.62	97.4
	5.5	6.074	2.62	2.37	110.5
PFDoS	Blank	ND			
	0.0055	0.004	7.73	11.46	67.5
	0.055	0.048	1.70	1.94	87.7
	0.55	0.548	1.64	1.65	99.7
	5.5	5.444	3.69	3.72	99.0
PFTrDS	Blank	ND			
	0.055	0.045	1.86	2.25	82.5
	0.55	0.514	1.37	1.46	93.4
	5.5	6.457	3.92	3.34	117.4
	PFOSA	Blank	ND		
0.0055		0.005	10.12	12.42	81.5
0.055		0.054	2.96	3.00	98.7
0.55		0.539	1.73	1.77	98.1
5.5		5.676	2.21	2.14	103.2
9CI-PF3ONS	Blank	0.000			
	0.0055	0.006	6.82	6.82	100.0
	0.055	0.048	1.26	1.45	86.6
	0.55	0.507	1.59	1.73	92.2
	5.5	7.191	2.75	2.10	130.7
11CI-PF3OUdS	Blank	0.002			
	0.0055	0.006	4.92	4.23	116.2
	0.055	0.045	0.96	1.18	81.4
	0.55	0.494	2.01	2.23	89.9
	5.5	7.332	3.60	2.70	133.3
HFPO-DA	Blank	0.000			
	0.0055	0.005	10.01	10.88	92.0
	0.055	0.052	0.50	0.53	94.7
	0.55	0.533	1.25	1.29	96.9
	5.5	5.695	1.62	1.56	103.5
DONA	Blank	0.000			
	0.0055	0.005	3.26	3.24	100.6
	0.055	0.057	0.53	0.51	104.2
	0.55	0.583	0.31	0.29	106.1
	5.5	6.148	9.36	8.38	111.8
4:2 FTS	Blank	0.017			
	0.0055	0.005	3.67	4.43	82.7
	0.055	0.052	3.28	3.44	95.5
	0.55	0.548	2.03	2.04	99.6
	5.5	5.733	2.18	2.09	104.2

6:2 FTS	Blank	0.000			
	0.0055	0.005	14.57	15.09	96.6
	0.055	0.053	2.80	2.88	97.2
	0.55	0.538	1.99	2.03	97.9
	5.5	5.480	1.60	1.61	99.7
8:2 FTS	Blank	0.000			
	0.0055	0.004	2.76	3.47	79.5
	0.055	0.056	3.32	3.29	101.2
	0.55	0.613	2.81	2.52	111.6
	5.5	4.938	3.12	3.47	89.8
10:2 FTS	Blank	0.000			
	0.0055	0.005	10.90	13.79	79.1
	0.055	0.052	1.12	1.17	95.5
	0.55	0.596	2.71	2.50	108.3
	5.5	4.553	4.08	4.93	82.8

ND = average results less than zero

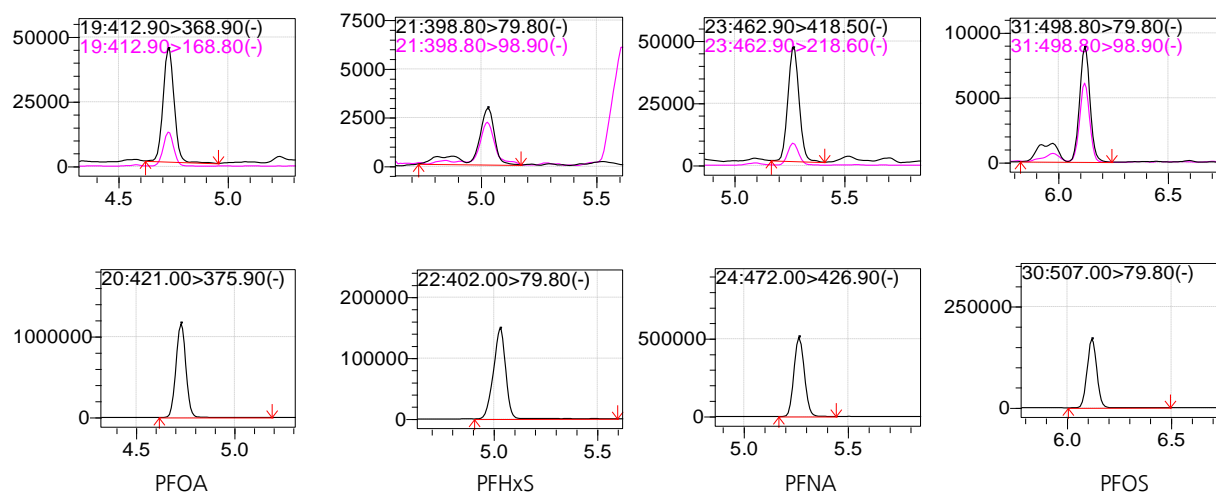


Figure 7: LOQ Peaks with Internal Standards

## 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 an egg 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

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.

For information about Shimadzu Scientific Instruments and to contact your local office, please visit our Web site at [www.ssi.shimadzu.com](http://www.ssi.shimadzu.com)

02-SSI-LCMS-165 First Edition: September 2024



Shimadzu Corporation  
[www.shimadzu.com/an/](http://www.shimadzu.com/an/)

SHIMADZU Scientific Instruments Inc.  
[www.ssi.shimadzu.com](http://www.ssi.shimadzu.com)

**For Research Use Only. Not for use in diagnostic procedures.**

This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. See <http://www.shimadzu.com/about/trademarks/index.html> for details.

Third party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not they are used with trademark symbol "TM" or "®".


Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own.

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

[› Please fill out the survey](#)

## Related Products

Some products may be updated to newer models.



Ultrafast Track to Your Success  
**LCMS-TQ RX Series**

› **LCMS-TQ RX Series**  
Triple Quadrupole LC-MS/MS  
Triple Quadrupole LC-MS/MS

## Related Solutions

› [Food and Beverages](#)

› [Price Inquiry](#)

› [Product Inquiry](#)

› [Technical Service / Support Inquiry](#)

› [Other Inquiry](#)