SHIMADZU
Excellence in Science

PFAS Testing In Food And Environmental Analysis

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a diverse group of over 4000 organic fluorinated compounds, which have been widely used in industry since the 1950s. Their properties make these substances heat-resistant, oil-and-water-repellent and chemically and thermally stable.

For these reasons, PFAS have multiple uses and commonly function as surface treatment agents, water repellents, coatings and even fire extinguishers. Those very properties make them resistant to degradation and this has led to their persistence in the environment and bioaccumulation through food chains and other routes of exposure.

PFAS are now so ubiquitous that they have been labeled by the media as 'forever chemicals'. Scientists and engineers across the world are making progress to develop alternative materials and implement treatment technologies that can mitigate the presence of PFAS in industrial, consumer products, food and the environment. Concerns about their toxicity to humans through diet and drinking water has led to increasingly stringent regulations and directives from both the US Environmental

Contents

Protection Agency (EPA) and the European Union regarding permitted levels in food and drinking water. Recent advancements in analytical instruments and methodologies have significantly enhanced the accuracy, speed, and automation of PFAS detection, contributing to a deeper understanding of their occurrence, behavior, and impact across various sample types. These technological innovations are also paving the way for more efficient PFAS testing, ensuring compliance with current regulations and supporting future regulatory developments.

In this eBook, we explore Shimadzu Corporation's range of chromatography and mass spectrometry (MS) solutions for detecting very low levels of PFAS in matrices such as drinking water, wastewater, eggs, and fish fillets. Plus, learn through a series of case studies how Shimadzu's Triple Quadrupole LCMS instruments and other analytical methods meet and often exceed performance criteria included in EPA methods for PFAS analysis – generating accurate results with fast turnaround times designed to enhance lab productivity.

Optimized PFAS analysis

Accurate and fast analysis of PFAS in drinking water are crucial for protecting public health. In these two application notes (EPA 533 and EPA 537.1), explore the suitability and full method demonstrations of Shimadzu's Triple Quadrupole LCMS-8050 for PFAS analysis in drinking water. This system meets performance and quality control criteria from both EPA methods, achieving 50% faster run times and reducing injection volumes to just 2 µL.

Cost-effective PFAS analysis with LC/MS

The US EPA Method 1633 establishes a standardized method for the determination of 40 target PFAS in water, solid, biosolids, and tissue samples, to support analysis of PFAS in support of developing regulations. In this Application Note, discover how Shimadzu's Triple Quadrupole LCMS-8060NX, coupled with its Nexera™ 40 Series UHPLC, was used to quantify all 40 target PFAS. This was achieved at concentrations ten times lower than the limit of quantitation (LOQ) specified in the EPA method for water samples. Such gains in sensitivity allow a lab to minimize its operational costs by decreasing the volume of sample that needs to be collected and shipped. Plus, in this Poster, learn how Shimadzu's LCMS-8060NX combined with an automated solvent extraction system can deliver accurate and rapid PFAS analysis in soil, according to the EPA Method 1633.

Enhanced method for PFAS analysis in non-potable water by LC-MS/MS

The ASTM D8421 is a standard method that allows for the rapid and cost effective analysis of PFAS in wastewater. In this Application Note, find out how Shimadzu's LCMS-8060NX was employed to analyze 44 PFAS compounds and 24 labeled isotopes in non-potable water samples to meet and exceed the method performance criteria set down in ASTM D8421. This resource also highlights how optimized chromatographic conditions can achieve excellent peak shape, even for those compounds that elute early.

High-performance analysis of PFAS in food matrices

PFAS screening in foods are becoming increasingly important as concerns grow around bioaccumulation through food chain or within the risks they pose to human health. This Poster shows how Shimadzu's LCMS-8060NX coupled with its Nexera™ X3 UHPLC successfully analyzed 30 PFAS compounds in fish fillets within as little as 15 minutes.

Automation and streamlined workflows (with as fewer sample preparation steps as possible) are essential to support in timely manner the safety testing of food products.

In this Application Note, learn how the automated workflow using Shimadzu's LCMS-8060NX, paired with the Nexera™ X3 UHPLC system equipped for online solid phase extraction, provides a highly sensitive method for detecting 27 PFAS in egg matrices.

Detection of PFAS in fast food packaging

PFAS have been detected in food contact materials (FCM), such as paper wrappers and

beverage cups. This Application Note explores how Shimadzu's LCMS-8050 was used to quantify 15 target PFAS compounds in FCM. The results revealed 12 of those compounds in seven fast food packaging samples at concentrations far below the limits set by the Danish Government from 2020.

Non-Targeted Analysis in water using LC-Q-TOF

PFAS represent over 4000 different chemicals, many of which are not routinely monitored in current targeted methods. In this Application Note, discover how an Non-Targeted Analysis method for suspect and unknown PFAS in water samples was developed using Shimadzu's Q-TOF LCMS-9030, based on High-resolution accurate mass - data-independent acquisition. The method was verified using 14 PFAS standards before using it for the analysis of water samples, from which 16 PFAS were discovered and further characterized.

Exploring adsorbable organic fluorine (AOF) through CIC analysis

Adsorbable Organic Fluorine (AOF) compounds serve as a broad proxy for PFAS and other organic compounds containing fluorine. Analyzing these compounds using combustion ion chromatography (CIC) reveals the total PFAS content in a sample, including those not detected by more selective chromatography methods.

The US EPA's Method 1621 describes a screening method for determining AOF in water. Here the sample passes through a column of granular activated carbon, adsorbing the compounds on to the column for subsequent combustion and analysis. This application note details how Shimadzu's HIC-ESP Ion Chromatograph was used to analyze AOF in water, demonstrating excellent recovery and precision stipulated in the EPA Method 1621. It also explains how AOF detection in river water is possible down to parts per billion.

Application News

Liquid Chromatograph Mass Spectrometer LCMS-8050

EPA 533 for PFAS Analysis with the Triple Quad LCMS-8050: Demonstration of Instrument and Method Performance

Toshiya Matsubara, Landon Wiest, Ruth Marfil-Vega
Shimadzu Scientific Instruments

User Benefits

- Comprehensive suitability assessment and full method demonstration for Per- and Polyfluoroalkyl Substances (PFAS) analysis per EPA 533 performed on a Shimadzu LCMS-8050.
- Verified instrumental performance based on detection limits, precision, and accuracy to ensure effective and consistent achievement of the method's analytical requirements.
- Enhanced analytical process for greater efficiency, achieving over 50% faster run times and reduced injection volumes down to 2 µL. This leads to quicker analyses, less solvent use, and improved lab productivity.

■ Introduction

This application note demonstrates the performance of the Shimadzu LCMS-8050 as part of the complete workflow (including the sample preparation) for analyzing the target PFAS specified in EPA method 533[1]. After optimizing the LC-MS/MS method, two parallel studies were conducted: a demonstration of the individual performance of the LC-MS/MS and an Initial Demonstration of Capability (IDC) study, as required by EPA method 533, for laboratories to establish the laboratory's proficiency in running this method. This work provides a framework for laboratories to evaluate the performance of the individual steps performed in the laboratory (extraction and instrumental analysis) for successfully analyzing the targeted PFAS according to the quality control requirements outlined in EPA method 533.

■ Method Overview

This application details the analysis of 44 PFAS in drinking water, including 25 target compounds, 16 isotope dilution analogues and 3 isotope performance standards, as specified in EPA Method 533. The list of target compounds and their corresponding retention times in the optimized LC-MS/MS method are provided in Table 1. All standards were purchased from Wellington Laboratories.

PFAS may be present in sampling containers and other consumables employed during sample preparation and instrumental analysis. To minimize the contribution of PFAS background contamination, the Shimadzu LCMS-8050 was configured with the optional PFAS free kit (P/N: 225-46100-41).

The key element to mitigate the presence of PFAS in the background was using a Shim-pack GIST C18 50 mm x 5.0 mm, 5 µm column as a delay column (P/N: 227-30015-03). This column is situated before the autosampler and causes a delay in the elution of PFAS present in the background, allowing for their separation from the target analytes in the samples, as shown in Figure 1. Compounds were separated, including PFHxS and PFOS isomers, as shown in Figure 2, using a Shim-pack GIST C18, 3 µm, 2.1 x 50mm (P/N: 227-30008-03).

Table 1: Target compounds and retention time in the optimized LC-MS/MS method.

#	Compound	Retention time
1	PFBA	3.45
2	PFMPA	3.85
3	PFPeA	4.49
4	PFBS	4.66
5	PFMBA	4.80
6	PFEESA	5.06
7	NFDHA	5.28
8	4:2 FTS	5.33
9	PFHxA	5.40
10	PFPeS	5.50
11	HFPO-DA	5.67
12	PFHpA	6.16
13	PFHxS	6.20
14	ADONA	6.27
15	6:2 FTS	6.74
16	PFOA	6.78
17	PFHpS	6.80
18	PFOS	7.32
19	PFNA	7.32
20	9Cl-PF3ONS	7.57
21	8:2 FTS	7.77
22	PFDA	7.77
23	PFUNA	8.17
24	11Cl-PF3OUdS	8.34
25	PFDoA	8.53

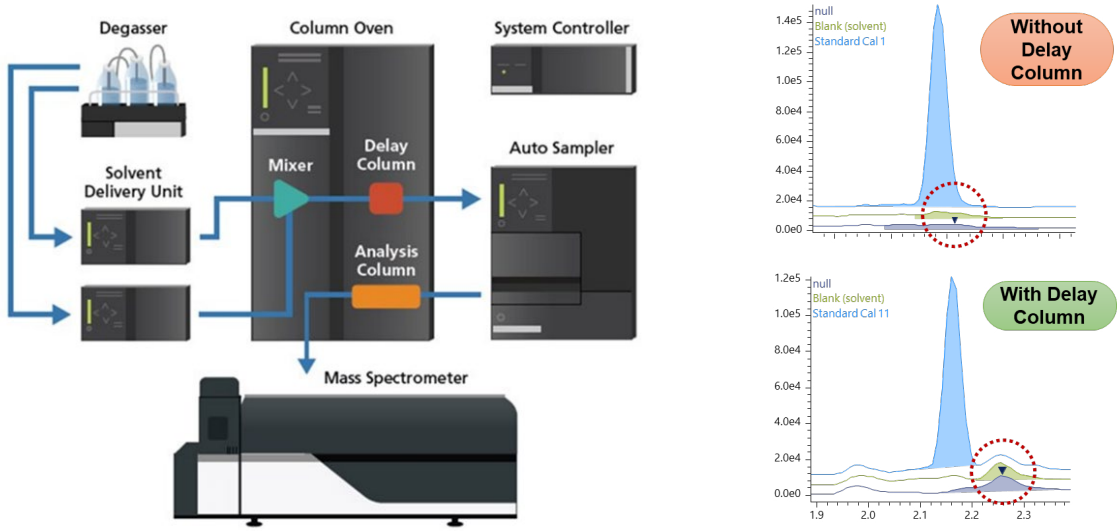


Figure 1: Placement of delay column in LC-MS/MS and effect on the delay of PFBA in the background.

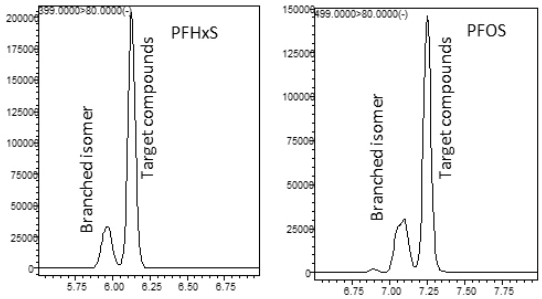


Figure 2: Separation of branched isomers: PFHxS and PFOS.

Chromatography was optimized to decrease the run time when compared to the original EPA method 533. The final run time of the method presented here is 15 minutes (50% shorter than the original method). All targets elute within 5.5 minutes.

Figure 3 shows an example chromatogram; the numbers in the figure correspond to the numbers listed in Table 1 to identify each target compound.

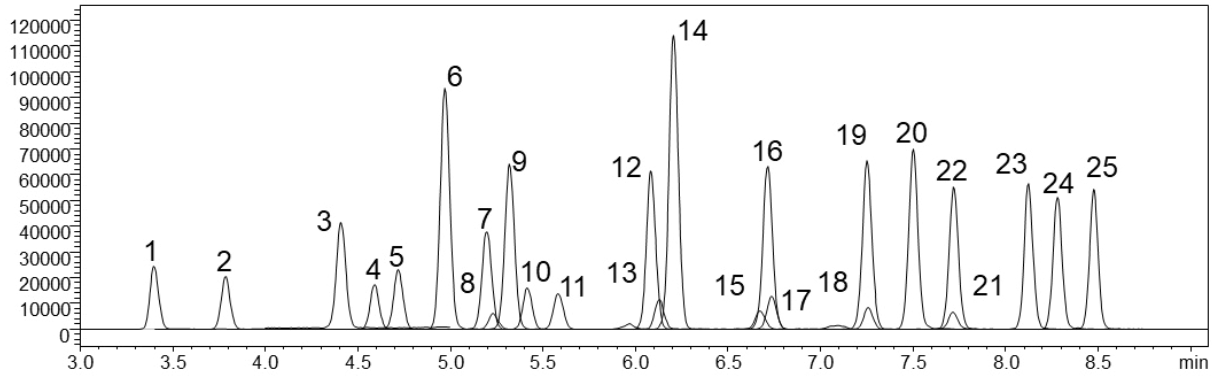


Figure 3: Example chromatogram of target compounds.

A series of samples, including Laboratory Reagent Blanks (LRB), Laboratory Fortified Blanks (LFB), spiked at different concentrations, Laboratory Fortified Matrix Sample (LFMS) and its duplicate (LFMD), and Field Reagent Blank (FRB), were prepared according to the extraction protocol described in EPA Method 533 to determine the QC parameters required for the IDC study and on-going QCs for each extraction batch.

Briefly, the isotope dilution analogues were added to 250 mL of preserved water (reagent or tap water) before they were extracted using SPE (Supelclean ENVI-WAX SPE, Millipore-Sigma, P/N: 54057) using a manual vacuum extraction manifold with stainless steel solvent guide needles (P/Ns: 57250-U and 57036) from Millipore Sigma.

Eluted extracts were concentrated down to dryness and reconstituted in 20% reagent water in methanol (v/v). The isotope performance standards were then added to the extracts for analysis by LC-MS/MS.

All consumables used for the sample preparation were tested prior to analysis to confirm the absence of detectable PFAS; a full list of consumables can be found in Shimadzu's webstore.

A detailed description of the LC-MS/MS parameters used for analysis in this work is included in Table 2. The optimized method (PFAS method package EPA 533, P/N: 225-45420-91) is commercially available to help laboratories accelerate their implementation of EPA Method 533.

Table 2: LC-MS/MS conditions from PFAS Method Package for EPA 533.

LC: Nexera HPLC	Parameters	MS/MS: LCMS-8050	Parameters
Analytical Column	Shim-pack GIST C18, 3 µm, 2.1 x 50mm	**Ion source**	ESI
Delay Column	Shim-pack GIST C18 50mm x 5.0 mm, 5 µm	**Polarity**	(-)
Flow rate	0.25 mL/min	**Interface temperature**	100 °C
Mobile phase A	5 mM ammonium acetate in water	**DL temperature**	150 °C
Mobile phase B	Methanol	**Heat block temperature**	250 °C
Gradient	5-95% B	**Injection Volume**	2 µL
Column Temp	45 °C	**Run time**	15 min

■ Results and Discussion

Initial Demonstration of Capabilities – Calibration

A series of 7 calibration standards with concentrations ranging from 0.5 to 50 ng/mL (concentration in vial) were analyzed in this study. These concentrations were used to reflect the 250-fold sample concentration required in EPA Method 533 (250 mL of sample are extracted and concentrated down to 1 mL for injection in the LC-MS/MS); the equivalent concentration in the sample ranged between 2 and 200 ng/L. The initial calibration curve for each target compound was calculated using the internal standard technique, based on the ratio of the peak areas of the target compounds to that of the isotope dilution analogue, with a linear fitting forced through zero and no weighting.

Table 3 lists the concentrations of the standards used to create the calibration curve and percent recovery for all targets in EPA Method 533. The %recovery for all targets were well within the acceptable ranges for this method (±50% for the lowest standard if lower than the MRL and ±30% for the other calibration levels).

Table 3. Concentration Calibration Standards, %accuracy of calibration standards, and demonstration of low system background.

#	Compound	r2	%Accuracy	LRB % of MRL
1	PFBA	0.9999	98.2-106.0	10.47
2	PFMPA	0.9999	90.2-100.3	4.29
3	PFPeA	0.9999	96.6-112.7	10.77
4	PFMBA	0.9999	92.7-107.2	4.01
5	PFBS	0.9999	99.5-132.2	2.89
6	PFEESA	0.9999	99.1-102.5	1.99
7	4:2FTS	0.9987	90.3-110.4	4.70
8	PFHxA	0.9999	94.5-103.7	2.19
9	NFDHA	0.9999	92.7-102.1	7.12
10	HFPO-DA	0.9998	95.7-106.0	2.61
11	PFHpA	0.9999	97.2-101.0	2.63
12	ADONA	0.9999	91.3-100.5	7.16
13	PFHxS	0.9996	98.1-105.2	1.72
14	PFPeS	0.9999	91.8-104.6	4.97
15	6:2FTS	0.9997	97.6-107.0	3.70
16	PFOA	0.9999	97.2-103.7	9.16
17	PFNA	0.9999	99.6-102.5	2.59
18	PFOS	0.9993	91.1-101.2	6.75
19	PFHpS	0.9974	84.4-101.8	2.12
20	9Cl-PF3ONS	0.9975	89.3-101.9	1.77
21	11Cl-PF3OUdS	0.9991	93.6-102.3	0.64
22	8:2FTS	0.9991	98.7-112.5	7.60
23	PFDA	0.9999	94.7-101.1	5.64
24	PFUNA	0.9998	97.0-103.4	1.93
25	PFDoA	0.9999	98.1-100.6	5.83

Initial Demonstration of Capabilities - Demonstration of Low System Background

The demonstration of low system background was performed after the LC-MS/MS method optimization was completed to evaluate the presence of PFAS in the background. Prior to analyzing any of the samples required for this study, a NULL injection was run to demonstrate the absence of detectable PFAS in the LC-MS/MS and mobile phases. With the NULL injection, a chromatographic run is performed without injecting a sample and without rotating the injection valve or high-pressure valve of the autosampler; this type of injection is also valuable for troubleshooting carryover issues during routine analysis.

Table 3 compares the area counts of each target PFAS in a standard with same concentration as the Minimum Reporting Limit (MRL) and in an LRB analyzed after a 50 ppb (in vial; 200 ng/L in sample equivalent concentration), prepared according to EPA method 533. All analytes were present in the LRB between 0.6% (8:2 FTS) and 10% (PFBA) of the MRL, exceeding the QC criteria from the method (<1/3 MRL or 33%) to demonstrate that any PFAS present in the background do not prevent the identification and quantification of the analytes of interest.

Initial Demonstration of Capabilities - Precision and Accuracy of LC-MS/MS and Method

Two precision and accuracy studies were conducted in this work. The first study assessed the long-term performance of the Shimadzu's LCMS-8050: seven replicates of a 4 ng/L standard (concentration in sample; equivalent concentration in the vial: 1 ng/mL) were quantified. The second study demonstrated the overall performance of the sample preparation protocol and LC-MS/MS as required in the IDC study outlined in EPA method 533. Seven replicates of a LFB spiked at 20 ng/L (concentration in sample; equivalent concentration in the vial: 5 ng/mL) were extracted and quantified.

The QC criteria for precision and accuracy listed in EPA method 533 apply to the overall analytical workflow. However, it is important to understand how the LC-MS/MS performs without the impact of the sample preparation. Table 4 summarizes the results for precision (assessed based on the %RSD) and accuracy (based on %recovery) from these two studies.

The %RSD for all targets was less than 10%, exceeding the precision criteria of <20%, in both studies. The percent recovery for all compounds ranged between 87% and 108% in both studies., well within the criteria accepted in the method (±30%). These results confirm that the individual precision and accuracy of the Shimadzu LC-MS/MS, as well as the overall precision and accuracy, are suitable for PFAS analysis according to EPA method 533.

EPA method 533 establishes for QC purposes that the percent recoveries of the isotope dilution analogues must be calculated using the integrated peak areas of isotope performance standards. If the %recoveries be within 50–200% of the true concentration. Figure 4 summarizes the %recovery of the isotope dilution analogues from the precision and accuracy of the method study.

Table 4. Precision and Accuracy of Shimadzu's LCMS-8050 and method.

		LC-MS/MS		Method	
#	Compound	Precision - %RSD (4 ng/L, n=7)	Accuracy – Mean %Recovery (4 ng/L, n=7)	Precision - %RSD (20 ng/L, n=7)	Accuracy – Mean %Recovery (20 ng/L, n=7)
1	PFBA	1.5%	97.7	3.1	100.2
2	PFMPA	3.3%	101.7	2.6	95.9
3	PFPeA	2.8%	95.8	3.1	98.7
4	PFBS	1.6%	99.9	5.0	97.9
5	PFMBA	2.6%	108.3	3.0	95.4
6	PFEESA	1.3%	101.8	4.9	99.6
7	NFDHA	7.4%	93.5	3.6	94.6
8	4:2 FTS	3.2%	87.3	4.3	102.7
9	PFHxA	3.4%	94.4	3.8	98.2
10	PFPeS	7.7%	104.1	4.6	97.4
11	HFPO-DA	2.3%	96.1	3.8	99.3
12	PFHpA	1.7%	98.1	4.0	98.0
13	PFHxS	4.0%	103.9	5.1	96.4
14	ADONA	3.1%	99.4	3.8	90.2
15	6:2 FTS	8.4%	98.1	4.8	101.8
16	PFOA	2.5%	97.2	2.6	98.2
17	PFHpS	2.4%	90.0	4.9	96.8
18	PFOS	3.2%	95.7	4.0	98.3
19	PFNA	9.7%	98.8	4.3	97.4
20	9Cl-PF3ONS	2.0%	98.7	4.5	94.0
21	8:2 FTS	5.4%	94.8	4.1	102.1
22	PFDA	8.3%	95.7	4.0	100.2
23	PFUNA	3.3%	97.2	4.8	96.2
24	11Cl-PF3OUdS	1.9%	99.2	7.2	91.4
25	PFDoA	1.4%	99.3	4.0	97.4

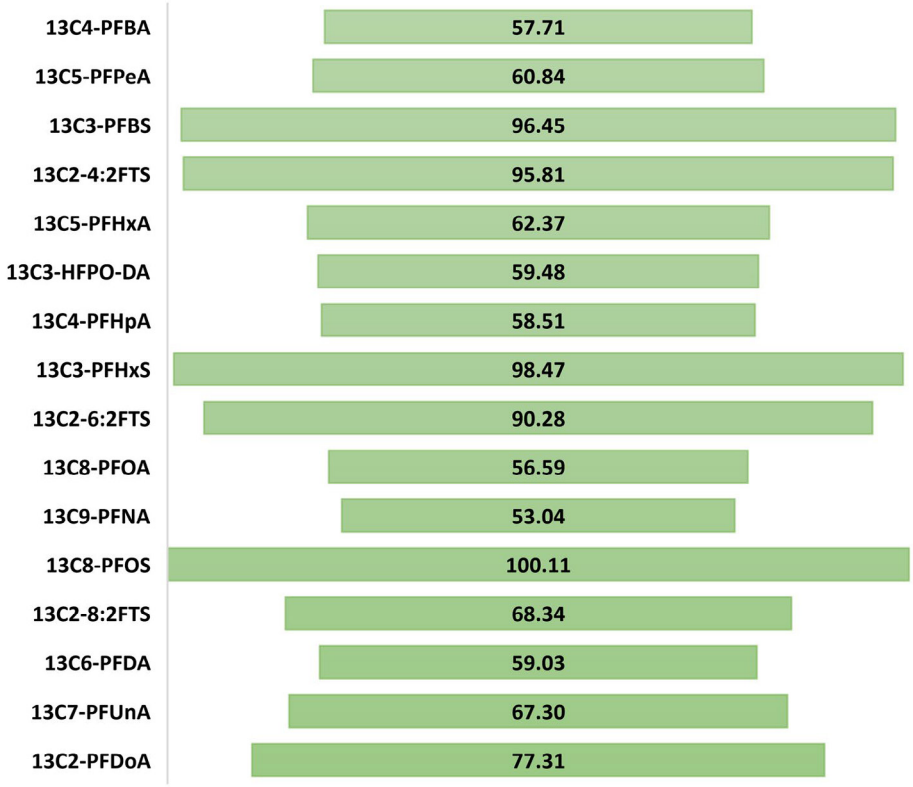


Figure 4: %recovery of the isotope dilution analogues from the precision and accuracy of the method.

Initial Demonstration of Capabilities – Instrument Detection Limit, Method Detection Limit and Minimum Reporting Limit

Two studies were also conducted to evaluate the sensitivity achieved with the Shimadzu's LCMS-8050 and the full analytical workflow.

In the first study, the instrument detection limit (IDL) was computed based on the analysis of a 0.5 ng/mL calibration standard (equivalent to 2 ng/L in sample). The IDL is derived from a statistical calculation like that used for the Method Detection Limit (MDL), per EPA guidelines. The main difference is that the IDL uses a standard, while the MDL uses a spiked sample that has undergone the full method. The IDL provides the analyte concentration (or on-column amount) that can be distinguished from baseline noise with 99% confidence. For methods requiring extensive sample prep, like EPA 533, the IDL better reflects the LC-MS/MS performance than MRL or MDL, which are affected by workflow variability and analyst proficiency. In the second study, the MRL and MDL were calculated per EPA 533, based on extracting seven 1 ng/mL (equivalent to 4 ng/L in sample) LFB replicates.

Table 5 summarizes IDLs, MDLs, MRLs and the upper and lower limits for the Prediction Interval of Results (Upper PIR and Lower PIR). IDLs ranged between 0.18 ng/L (PFEESA) and 1.38 ng/L (PFHpS) and MDLs ranged between 0.37 ng/L (PFHXA) and 1.33 ng/L (PFUnA). IDLs are not reported in the published EPA method 533. IDLs for all the carboxylic and sulfonic PFAS were <1 ng/L except for PFHpS; IDLs for the other classes of PFAS targeted in EPA 533 were <1.5 ng/L. These results were generated using an injection volume 5 times smaller than in EPA method 533 (2 µL instead of 10 µL), which helps with maintaining long-term performance of the instrument as less sample is introduced into the system. The MRLs reported in Table 5 were validated in the study as the Upper PIR for all analytes was <146% (PFUnA) and the Lower PIR was >63% (8:2 FTS), within the QC criteria from the method (Upper PIR <150%, Lower PIR >50%).

Table 5: Instrument detection limit, Method Detection Limit, Minimum Reporting Limit, Upper and Lower limits for the Prediction Interval of Results.

#	Compound	IDL, ng/L	MDL, ng/L	MRL, ng/L	Lower PIR	Upper PIR
1	PFBA	0.22	0.54	4.22	88.29	122.53
2	PFMPA	0.47	0.58	3.95	80.53	117.13
3	PFPeA	0.41	0.48	4.17	89.08	119.24
4	PFBS	0.22	0.41	3.91	84.76	110.84
5	PFMBA	0.37	0.59	3.91	79.06	116.26
6	PFEESA	0.18	0.43	3.94	85.11	112.03
7	NFDHA	1.08	0.76	3.90	73.72	121.37
8	4:2 FTS	0.46	0.75	4.19	81.18	128.25
9	PFHxA	0.48	0.37	4.01	88.50	112.07
10	PFPeS	1.11	0.55	3.86	79.06	113.77
11	HFPO-DA	0.33	0.41	4.06	88.64	114.48
12	PFHpA	0.24	0.45	4.03	86.50	114.99
13	PFHxS	0.56	0.39	3.98	87.18	111.64
14	ADONA	0.45	0.52	3.55	72.40	105.09
15	6:2 FTS	1.18	1.26	4.16	64.41	143.70
16	PFOA	0.36	0.57	4.11	84.70	120.56
17	PFHpS	0.34	0.43	4.10	88.86	116.08
18	PFOS	0.44	0.61	4.16	84.77	123.35
19	PFNA	1.38	0.65	4.18	84.18	124.90
20	9Cl-PF3ONS	0.29	0.67	4.02	79.37	121.57
21	8:2 FTS	0.75	1.09	3.78	60.22	129.01
22	PFDA	1.07	0.92	3.97	70.24	128.50
23	PFUNA	0.46	1.33	4.20	62.97	146.86
24	11Cl-PF3OUdS	0.27	0.78	3.87	72.22	121.32
25	PFDoA	0.20	1.15	4.16	67.59	140.24

Ongoing QC requirements – QC samples in each extraction batch

In addition to the samples mentioned in previous sections (LRB, LFB for MRL and precision and accuracy studies), LFSM, and LFSMD were also analyzed in this study, as they are required in each extraction batch per method EPA 533.

Table 6 summarizes the LFSM and LFSMD recovery (spike concentration: 5 ng/mL in vial, equivalent to 20 ng/L in sample) and variability. All parameters reported met the QC criteria listed in the method: % recoveries ranged between 91% and 108%, and %RPD was <7%.

Table 6: Analysis of LFSM and LFSMD.

#	Compound	LFSM %Recovery	LFSMD %Recovery	%RSD
1	PFBA	101.80	101.30	0.35
2	PFMPA	95.58	94.08	1.12
3	PFPeA	97.54	99.32	1.28
4	PFBS	100.74	93.38	5.36
5	PFMBA	96.24	94.98	0.93
6	PFEESA	99.26	99.56	0.21
7	NFDHA	97.90	98.28	0.27
8	4:2 FTS	103.00	108.38	3.60
9	PFHxA	99.30	99.18	0.09
10	PFPeS	97.82	103.04	3.68
11	HFPO-DA	100.60	98.44	1.53
12	PFHpA	98.54	98.68	0.10
13	PFHxS	98.10	99.04	0.67
14	ADONA	90.94	91.06	0.09
15	6:2 FTS	106.50	103.04	2.34
16	PFOA	105.22	99.34	4.07
17	PFHpS	101.76	99.74	1.42
18	PFOS	101.12	99.52	1.13
19	PFNA	99.98	96.24	2.70
20	9Cl-PF3ONS	94.96	96.28	0.98
21	8:2 FTS	104.80	94.82	7.07
22	PFDA	102.88	96.06	4.85
23	PFUNA	95.40	97.40	1.47
24	11Cl-PF3OUdS	92.34	94.20	1.41
25	PFDoA	99.08	97.74	0.96

■ Summary and Conclusions

Our study confirms that the Shimadzu LCMS-8050, coupled with Millipore Sigma's sample preparation consumables used in this research, meets or surpasses the performance standards outlined in EPA Method 533 for PFAS analysis. This comprehensive evaluation involved parallel studies to isolate the impact of each step within the entire workflow on overall method effectiveness. This information empowers laboratories to make informed decisions regarding optimization strategies.

Equipped with the PFAS Method Package for EPA 533, the Shimadzu LCMS-8050 delivers rapid (50% faster), reliable, and highly sensitive PFAS quantification in drinking water using a minimal injection volume of 2 µL. Beyond the immediate benefits of speed, reliability, and sensitivity, Shimadzu's solutions offer long-term advantages. The field-upgradable nature of the LCMS-8050 to the LCMS-8060NX ensures robust workflows that can adapt to evolving PFAS analysis demands, potentially reducing overall ownership costs.

■ Reference

[1] EPA method 533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution anion exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (U.S. Environmental Protection Agency, Washington, D.C., December 2019).

■ Acknowledgement

We would like to thank Millipore Sigma for their contributions.

Application News

Liquid Chromatograph Mass Spectrometer LCMS-8050

EPA 537.1 for PFAS Analysis with the Triple Quad LCMS-8050: Demonstration of Instrument and Method Performance

Toshiya Matsubara, Landon Wiest, Ruth Marfil-Vega
Shimadzu Scientific Instruments

User Benefits

- Comprehensive suitability assessment and full method demonstration for Per- and Polyfluoroalkyl Substances (PFAS) analysis per EPA 537.1 performed on a Shimadzu LMCS-8050.
- Verified instrumental performance based on detection limits, precision, and accuracy to ensure effective and consistent achievement of the method's analytical requirements for PFAS.
- Enhanced workflow that consistently achieves up to four times better detection limits for all targeted classes of PFAS with a small injection volume of 2 µL, in comparison to the detection limits specified by EPA Method 537.1.

■ Introduction

This application note demonstrates the performance of the Shimadzu LCMS-8050 as part of the complete workflow (including the sample preparation) for analyzing the target PFAS specified in EPA Method 537.1[1]. After optimizing the LC-MS/MS method, two parallel studies were conducted: a demonstration of the individual performance of the LC-MS/MS and an Initial Demonstration of Capability (IDC) study, as required by EPA Method 537.1, for laboratories to establish their proficiency in running this method. This work provides a framework for laboratories to evaluate the performance of each step performed in the laboratory (extraction and instrumental analysis), for successfully analyzing the targeted PFAS according to the quality control requirements outlined in EPA method 537.1.

■ Method Overview

This application details the analysis of 25 PFAS in drinking water, including 18 target compounds, 4 surrogates, and 3 internal standards, as specified in EPA Method 537.1.

The list of target compounds and their corresponding retention times in the optimized LC-MS/MS method are provided in Table 1. All standards were purchased from Wellington Laboratories.

PFAS may be present in sampling containers and other consumables employed during sample preparation and instrumental analysis. To minimize the contribution of PFAS background contamination, the Shimadzu LCMS-8050 was configured with the optional PFAS free kit (P/N: 225-46100-41). The key element used in this work to mitigate the presence of PFAS in the background was a Shim-pack GIST C18 50 mm x 3.0 mm, 5 µm column used as a delay column (P/N:227-30015-03). This column is situated before the autosampler and causes a delay in the elution of PFAS present in the background, allowing for their separation from the target analytes in the samples, as shown in Figure 1. Compounds were separated, including PFHxS and PFOS isomers, as shown in Figure 2, using a Shim-pack Velox SP-C18, 2.7 µm, 2.1 x 50 mm column (P/N: 227-32003-02).

Table 1: Target compounds and their respective retention time in the optimized LC-MS/MS method.

#	Compound	Retention time (min)	#	Compound	Retention time (min)
1	PFBS	3.57	10	9Cl-PF3ONS	6.12
2	PFHxA	4.02	11	PFDA	6.29
3	HFPO-DA	4.20	12	NMeFOSAA	6.52
4	PFHpA	4.67	13	PFUNA	6.70
5	PFHxS	4.73	14	NEtFOSAA	6.76
6	ADONA	4.77	15	11Cl-PF3OUdS	6.92
7	PFOA	5.27	16	PFDoA	7.08
8	PFNA	5.83	17	PFTrDA	7.41
9	PFOS	5.82	18	PFTA	7.70

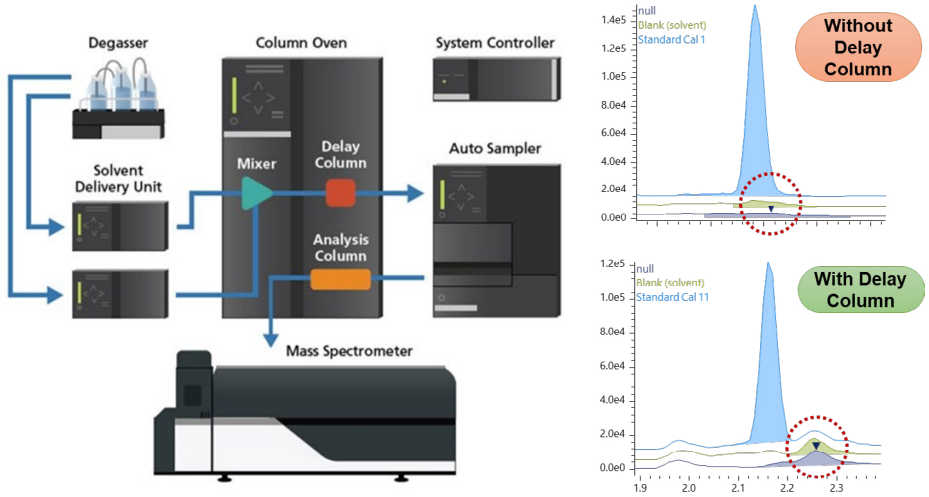


Figure 1: Placement of delay column in LC-MS/MS and effect on the delay of PFBA in the background.

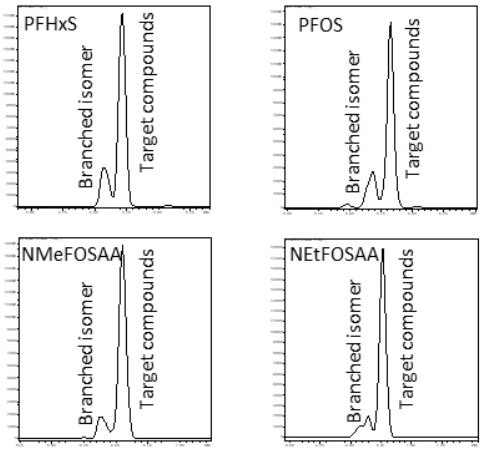


Figure 2: Separation of branched isomers: PFHxS, PFOS, NMeFOSAA and NEtFOSAA.

A series of samples, including Laboratory Reagent Blanks (LRB), Laboratory Fortified Blanks (LFB), spiked at different concentrations, Laboratory Fortified Matrix Sample (LFMS) and its duplicate (LFMD), and Field Reagent Blank (FRB), were prepared according to the extraction protocol described in EPA Method 537.1 to determine the QC parameters required for the IDC study and on-going QCs for each extraction batch.

Briefly, 250 mL of preserved water (reagent or tap water) were extracted using SPE (Supelclean ENVI-Chrome P SPE, Millipore-Sigma, P/N: 54226) using a manual vacuum extraction manifold with stainless steel solvent guide needles (P/Ns: 57250-Uand 57036) from Millipore Sigma. Eluted extracts were concentrated down to dryness and reconstituted in methanol:water (96:4% (v/v)) for LC-MS/MS analysis.

All consumables used for the sample preparation were tested prior to analysis to confirm the absence of detectable PFAS. A full list of consumables can be found in in Shimadzu's webstore.

A detailed description of the LC-MS/MS parameters used for analysis in this work is included in Table 2. The optimized method (PFAS method package EPA 537.1, P/N: 225-45420-91) is commercially available to help laboratories accelerate their implementation of EPA Method 537.1.

Table 2: LC-MS/MS conditions from PFAS Method Package for EPA 537.1.

LC: Nexera HPLC	**Parameters**	**MS/MS: LCMS-8050**	**Parameters**
Column	Shim-pack Velox SP-C18, 2.7 µm, 2.1 x 50mm	**Ion source**	ESI
Delay Column	Shim-pack GIST C18 50 mm x 5.0 mm 5 µm,	**Polarity**	(-)
Flow rate	0.25 mL/min	**Interface temperature**	100 °C
Mobile phase A	5 mM ammonium acetate in water	**DL temperature**	150 °C
Mobile phase B	Methanol	**Heat block temperature**	250 °C
Gradient	5-100% B	**Injection Volume**	2 µL
Column Temp	45 °C	**Run time**	18 min

■ Results and Discussion

Initial Demonstration of Capabilities - Calibration

A series of 7 calibration standards with concentrations ranging from 0.5 to 50 ng/mL (concentration in vial) were analyzed in this study. These concentrations were used to reflect the 250-fold sample concentration required in EPA Method 537.1 (250 mL of sample are extracted and concentrated down to 1 mL for injection in the LC-MS/MS). The equivalent concentration in the sample ranged between 2 and 200 ng/L. The initial calibration curve for each target compound was calculated using the internal standard technique with a linear fitting forced through zero. No weighting was used to quantitate the subsequent injections.

Table 3 lists the concentrations of the standards used to create the calibration curve and percent recovery for all targets in EPA Method 537.1. The %recovery for all targets were well within the acceptable ranges for this method (±50% for the lowest standard and ±30% for the other calibration levels)

.

Table 3: Concentration Calibration Standards and %recovery.

		Concentration Calibration Standards, ng/mL						
#	Compound	0.5	1	2	5	10	20	50
1	PFBS	90	100	99	97	98	99	100
2	PFHxA	98	104	104	103	102	99	100
3	HFPO-DA	97	107	101	100	102	99	100
4	PFHpA	101	112	104	102	102	98	100
5	PFHxS	96	102	99	99	99	98	100
6	ADONA	105	108	104	102	103	98	100
7	PFOA	107	111	108	102	102	98	100
8	PFOS	106	105	107	101	103	99	100
9	PFNA	107	111	106	102	104	98	100
10	9Cl-PF3ONS	94	98	103	96	99	97	101
11	PFDA	104	116	106	102	102	99	100
12	NMeFOSAA	99	109	104	105	101	99	100
13	PFUnA	117	120	107	106	103	99	100
14	NEtFOSAA	104	108	105	101	98	98	100
15	11Cl-PF3OUdS	107	114	110	104	100	98	100
16	PFDoA	88	116	104	104	103	98	100
17	PFTrDA	86	104	106	104	104	100	100
18	PFTA	95	111	110	103	104	99	100

Initial Demonstration of Capabilities – Chromatography and Asymmetry Factor

Chromatography was optimized to decrease the run time when compared to the original EPA Method 537.1, while ensuring that the QC requirements for asymmetry of PFBS and PFHxA were met (0.8-1.5) while also separating the PFHxS and PFOS branched isomers. The final run time of the method presented here is 18 minutes (50% shorter than the original method) and includes a 3-minute column rinse with methanol to eliminate the observed carry-over from NMeFOSAA and NEtFOSAA during method development.

All targets elute within 5 minutes, and the peak asymmetry factors were 1.2 and 1.1 for PFBS and PFHxA, respectively. Figure 3 shows an example chromatogram. The numbers in the figure correspond to the numbers listed in Table 1 to identify each target compound.

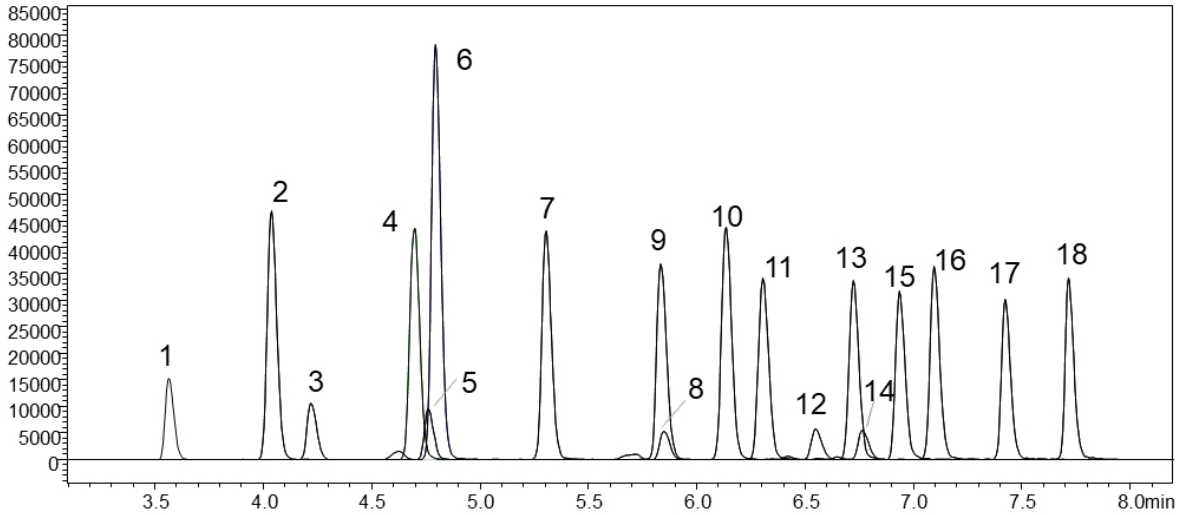


Figure 3: Example chromatogram of target compounds.

Initial Demonstration of Capabilities – Demonstration of Low System Background

The demonstration of low system background was performed after the LC-MS/MS method optimization was completed to evaluate the presence of PFAS in the background. Prior to analyzing any of the samples required for this study, a NULL injection was run to demonstrate the absence of detectable PFAS in the LC-MS/MS and mobile phases. With the NULL injection, a chromatographic run is performed without injecting a sample and without rotating the injection valve or high-pressure valve of the autosampler. This type of injection is also valuable for troubleshooting carry-over issues during routine analysis.

Table 4 compares the area counts of each target PFAS in a standard with the same concentration as the Minimum Reporting Limit (MRL) and in the LRB, prepared according to EPA method 537.1. All analytes were present in the LRB at <4% of the MRL, exceeding the QC criteria from the method (<1/3 MRL) to demonstrate that any PFAS present in the background do not prevent the identification and quantification of the analytes of interest.

Table 4: Demonstration of Low System Background.

#	Compound	Mean peak area at MRL	LRB peak area	LRB % of MRL
1	PFBS	20,209	212	1.0
2	PFHxA	58,179	1,424	2.4
3	HFPO-DA	19,005	199	1.0
4	PFHpA	50,975	1,201	2.4
5	PFHxS	16,475	0	0.0
6	ADONA	91,935	1,029	1.1
7	PFOA	43,464	1,516	3.5
8	PFOS	10,449	234	2.2
9	PFNA	34,881	752	2.2
10	9Cl-PF3ONS	41,001	613	1.5
11	PFDA	29,301	1,136	3.9
12	NMeFOSAA	8,651	136	1.6
13	PFUnA	23,106	379	1.6
14	NEtFOSAA	8,744	174	2.0
15	11Cl-PF3OUdS	24,918	473	1.9
16	PFDoA	24,549	695	2.8
17	PFTrDA	15,422	273	1.8
18	PFTA	20,443	342	1.7

Initial Demonstration of Capabilities – Precision and Accuracy of LC-MS/MS and Method

Two precision and accuracy studies were conducted in this work.

The first study assessed the long-term performance of Shimadzu's LCMS-8050. Seven replicates were quantified of a 4 ng/L standard, which represents the concentration in sample (equivalent concentration in the vial was 1 ng/mL). The second study demonstrated the overall performance of the sample preparation protocol and LC-MS/MS as required in the IDC study outlined in EPA Method 537.1. Five replicates of a LFB spiked at 20 ng/L (equivalent concentration in the vial was 5 ng/mL) were extracted and quantified.

The QC criteria for precision and accuracy listed in EPA Method 537.1 apply to the overall analytical workflow. However, it is important to understand how the LC-MS/MS performs without the impact of the sample preparation. Table 5 summarizes the results for precision (assessed based on the %RSD) and accuracy (based on %recovery) from these two studies. The %RSD for all targets was less than 7%, exceeding the precision criteria of <20%, in both studies. The percent recovery for all compounds was within ±10% also in both studies., up to three times better than the criteria accepted in the method (±30%). These results confirm that the individual precision and accuracy of the Shimadzu LC-MS/MS, as well as the overall precision and accuracy, are suitable for PFAS analysis according to EPA method 537.1.

Table 5: Precision and Accuracy of Shimadzu's LCMS-8050 and method.

		LC-MS/MS		Method	
#	Compound	Precision - %RSD (4 ng/L , n=7)	Accuracy – Mean %Recovery (4 ng/L, n=7)	Precision – %RSD (20 ng/L, n=5)	Accuracy – Mean %Recovery (20 ng/L, n=5)
1	PFBS	2.2	98.4	3.5	107.1
2	PFHxA	2.9	94.8	2.5	108.7
3	HFPO-DA	2.9	97.4	2.9	106.4
4	PFHpA	3.3	96.8	2.4	104.5
5	PFHxS	3.5	101.1	1.7	109.5
6	ADONA	1.7	94.8	3.1	108.1
7	PFOA	1.5	95.7	2.2	107.0
8	PFNA	6.7	96.8	4.9	100.8
9	PFOS	2.7	102.1	2.3	104.7
10	9Cl-PF3ONS	2.6	95.5	3.3	105.9
11	PFDA	4.7	100.8	2.6	103.4
12	NMeFOSAA	5.6	101.7	1.6	97.0
13	PFUNA	3.5	97.8	2.9	96.2
14	NEtFOSAA	8.3	105.9	3.0	97.9
15	11Cl-PF3OUdS	2.0	96.1	4.6	97.6
16	PFDoA	4.9	91.3	4.3	97.6
17	PFTrDA	3.3	91.8	3.4	100.6
18	PFTA	2.4	95.8	4.0	98.0

Initial Demonstration of Capabilities – Instrument Detection Limit, Method Detection Limit and Minimum Reporting Limit

Two studies were conducted to evaluate the sensitivity achieved with the Shimadzu's LCMS-8050 and the full analytical workflow.

In the first study, the instrument detection limit (IDL) was computed based on the analysis of a 0.5 ng/mL calibration standard (equivalent to 2 ng/L in sample). The IDL is derived from a statistical calculation like that used for the Method Detection Limit (MDL), per EPA guidelines.

The main difference is that the IDL uses a standard, while the MDL uses a spiked sample that has undergone the full method, including extraction. The IDL provides the analyte concentration (or on-column amount) that can be distinguished from baseline noise with 99% confidence. For methods requiring extensive sample prep, like EPA 537.1, the IDL better reflects the LC-MS/MS performance than MRL or MDL, which are affected by workflow variability and analyst proficiency.

In the second study, the MRL and MDL were calculated per EPA 537.1, based on extracting seven 1 ng/mL (equivalent to 4 ng/L in sample) LFB replicates, the same concentration used in the validation study published by EPA.

Table 6 summarizes IDLs, MDLs, MRLs and the upper and lower limits for the Prediction Interval of Results (Upper PIR and Lower PIR). IDLs ranged between 0.19 ng/L (PFOA) and 1.07 ng/L (NEtFOSAA) and MDLs ranged between 0.36 ng/L (PFUnA) and 0.76 ng/L (PFBS). IDLs are not reported in the published EPA method 537.1; however, the results obtained in this work demonstrate that with the LC-MS-8050 concentrations of <1 ng/L can be measured with 99% confidence. MDLs obtained in this work were up to 4 times better than those reported in EPA method 537.1. The optimized LC-MS/MS method from Shimadzu's PFAS Method Package for EPA 537.1, along with the consumables used in this study, demonstrated a more consistent performance across different classes of PFAS.

This is evidenced by the minimal variance between the highest and lowest method detection limits (MDLs) obtained, which was 0.4 ng/L, compared to the 2.27 ng/L difference reported in the standard method. These results suggest that when adding new PFAS to the method from the classes already included, similar sensitivity could be achieved for the new compounds. It is also important to highlight that these results were generated using an injection volume 5 times smaller than in EPA Method 537.1 (2 µL instead of 10 µL). This helps with maintaining long-term performance of the instrument as less sample is introduced into the system.

The MRLs were validated in the study as the Upper PIR for all analytes was <136% and the Lower PIR was >79%, well within the QC criteria from the method (Upper PIR <150%, Lower PIR >50%).

Table 6: Instrument detection limit, Method Detection Limit, Minimum Reporting Limit, Upper and Lower limits for the Prediction Interval of Results.

#	Compound	IDL, ng/L	MDL, ng/L	MRL, ng/L	Upper PIR	Lower PIR
1	PFBS	0.27	0.76	4.52	136.81	89.05
2	PFHxA	0.35	0.44	4.54	127.45	99.52
3	HFPO-DA	0.34	0.41	4.37	122.10	96.24
4	PFHpA	0.39	0.44	4.49	126.09	98.29
5	PFHxS	0.43	0.65	4.60	135.64	94.58
6	ADONA	0.21	0.46	4.46	126.09	97.02
7	PFOA	0.19	0.54	4.53	130.42	96.26
8	PFNA	0.78	0.48	4.22	120.80	90.26
9	PFOS	0.32	0.38	4.40	122.14	98.00
10	9Cl-PF3ONS	0.34	0.44	4.43	124.53	96.87
11	PFDA	0.57	0.38	4.43	122.83	98.74
12	NMeFOSAA	0.71	0.71	4.05	123.42	78.87
13	PFUNA	0.44	0.36	4.16	115.36	92.50
14	NEtFOSAA	1.07	0.56	4.21	123.03	87.68
15	11Cl-PF3OUdS	0.23	0.68	4.27	128.19	85.30
16	PFDoA	0.62	0.63	4.24	125.82	86.35
17	PFTrDA	0.42	0.70	4.21	127.40	83.31
18	PFTA	0.30	0.65	4.22	125.76	85.09

Ongoing QC requirements –
Internal Standards and Surrogates

EPA Method 537.1 establishes ongoing QC parameters for the internal standards and surrogates required in this method. For all injections, peak area counts for each internal standard must be within 50–150% of the average peak area in the initial calibration and within 70–140% of the most recent CCC. The recovery of each surrogate must be within 70–130% of its true concentration for all injections. If these criteria are not met for the internal standards, the corresponding target results are invalid, and for the surrogates, the results must be flagged as suspect.

Figures 4 and 5 show the results from all samples analyzed in this study, including LRB, LFB spiked at MRL concentration (n=7), LFB spiked at mid-level concentration (n=5), LFSM, LFSMD, and FRB. The %area of the 3 internal standards used in this method (13C2-PFOA, 13-C4-PFOS, d3-NMeFOSAA) based on the average peak area of the initial calibration, shown in Figure 4, met the required criteria during this study. The criteria for the surrogates' recovery (13C2-PFHxA, 13C3-HFPO-DA, 13C2-PFDA, d5-NEtFOSAA) were also met, as shown in Figure 5.

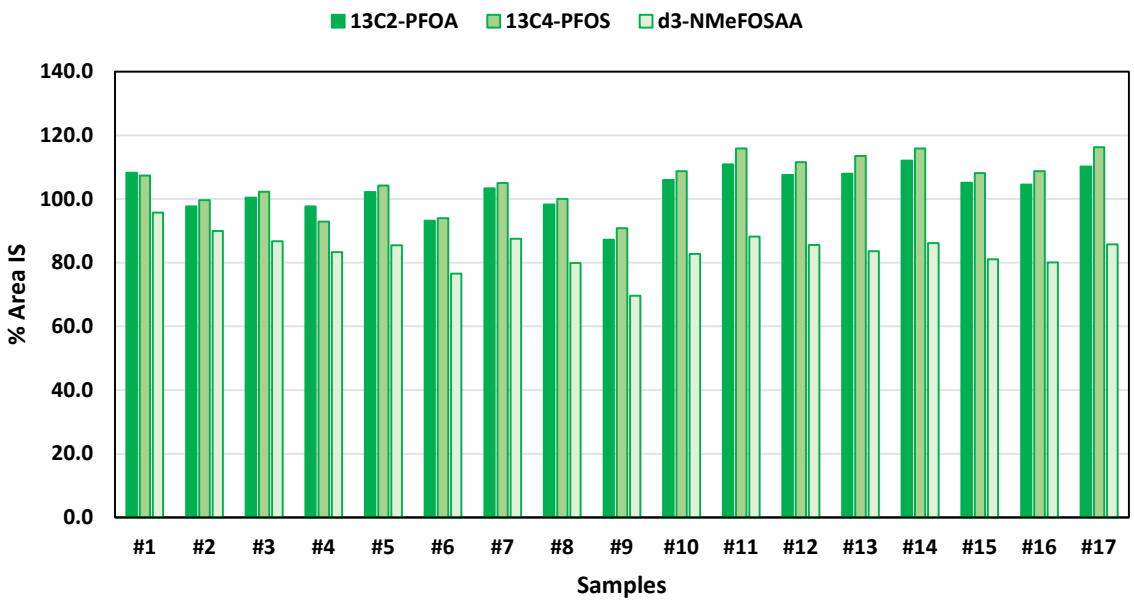


Figure 4: %Area of internal standards based on the average peak area of the initial calibration.

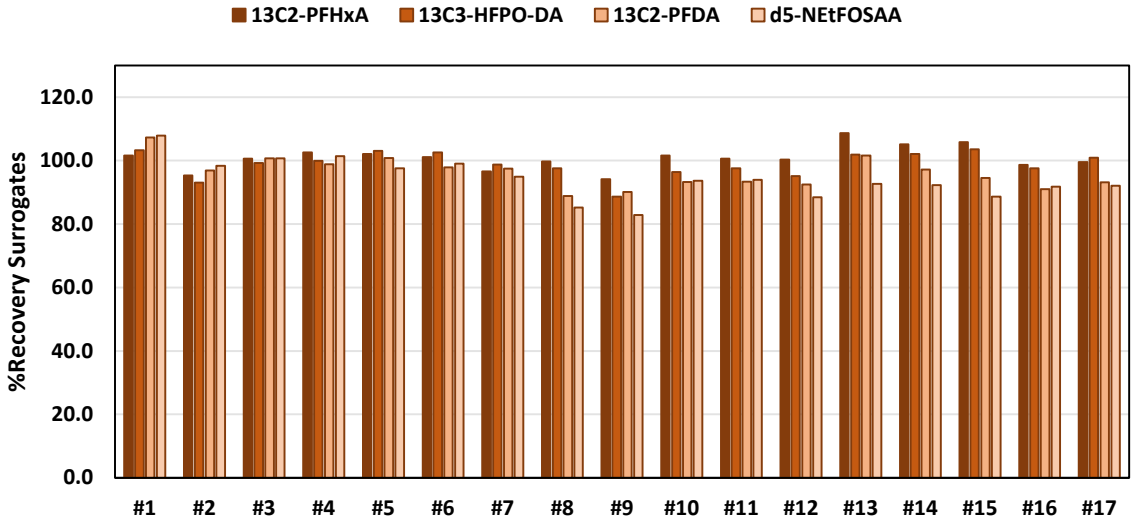


Figure 5: %recovery of surrogates.

Ongoing QC requirements – QC samples in each extraction batch

In addition to the samples mentioned in previous sections (LRB, LFB for MRL and precision and accuracy studies), LFSM, LFSMD, and FRB were also analyzed in this study, as they are required in each extraction batch per method EPA 537.1.

Table 7 summarizes the LFSM and LFSMD recovery (spike concentration: 5 ng/mL in vial, equivalent to 20 ng/L in sample) and variability, as well as the presence of PFAS in the FRB. All parameters reported met the QC criteria listed in the method: % recoveries ranged between 92% and 117%, and %RPD was <11.4%; the percent of MRL in the FRB was <5%.

Table 7: Analysis of LFSM, LFSMD and FRB.

#	Compound	LFSM - %Recovery	LFSMD - %Recovery	%RPD	FRB - % of MRL
1	PFBS	110.0	116.2	-5.5	1.2
2	PFHxA	107.5	115.7	-7.4	3.2
3	HFPO-DA	109.6	113.2	-3.3	1.0
4	PFHpA	103.8	112.1	-7.7	0.2
5	PFHxS	108.5	117.5	-8.0	0.5
6	ADONA	109.1	116.5	-6.5	1.0
7	PFOA	106.6	113.4	-6.2	4.4
8	PFOS	98.9	105.9	-6.9	3.1
9	PFNA	101.6	111.9	-9.6	2.6
10	9Cl-PF3ONS	104.7	112.7	-7.3	1.0
11	PFDA	99.5	109.8	-9.8	2.8
12	NMeFOSAA	93.9	101.0	-7.4	0.9
13	PFUnA	94.8	102.5	-7.9	2.4
14	NEtFOSAA	97.0	100.5	-3.6	1.5
15	11Cl-PF3OUdS	93.8	104.6	-10.9	1.3
16	PFDoA	98.5	101.3	-2.8	2.8
17	PFTrDA	92.8	104.0	-11.4	2.7
18	PFTA	91.9	102.4	-10.9	1.6

■ Summary and Conclusions

Our study demonstrates that the Shimadzu LCMS-8050 in combination with the sample preparation consumables from Millipore Sigma employed in this work meet or exceed the performance criteria specified in EPA Method 537.1 for PFAS analysis. The parallel studies conducted in this work aimed to provide laboratories with the information they need to demonstrate how individual steps of the full workflow impact the overall method performance.

Shimadzu LCMS-8050 equipped with the PFAS Method Package for EPA 537.1 achieves rapid (50% shorter), reliable, and highly sensitive quantitation of PFAS in drinking water using low injection volumes. The tested workflow provided improved (up to 4x better) and consistent MDLs for all classes of PFAS targeted in the method with lower injection volume (2 µL), compared to those reported in EPA Method 537.1.

Shimadzu's solutions presented in this work, along with the option for the LCMS-8050 to be upgraded in the field to the LCMS-8060NX, offer robust workflows that can also reduce long-term cost of ownership as requirements for PFAS analysis continue to evolve.

■ Reference

[1] Shoemaker, J. and Dan Tettenhorst. Method 537.1: Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Washington, DC, 2018.

■ Acknowledgement

We would like to thank Millipore Sigma for their contributions.

Application News

LCMS™-8060NX High Performance Liquid Chromatograph Mass Spectrometer

EPA Method 1633: Method Detection Limits of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Matrices using the Triple Quad LCMS-8060NX

Megan Davis, Om Shrestha, Kathleen Lou, Ruth Marfil-Vega, Landon Wiest, Evelyn Wang
Shimadzu Scientific Instruments, Inc.

User Benefits

- Reproducible results can be achieved with the Shimadzu LCMS 8060NX for the analysis of wastewater according to EPA Method 1633.
- Achieve quantification 10x lower than the EPA's Limit of Quantitation (LOQ).
- The excellent sensitivity achieved enables laboratories to reoptimize their sample preparation approach (i.e. reduce sample volume) while ensuring performance as required in EPA 1633

■ Introduction

This application note demonstrates that the LCMS-8060NX meets and exceeds the method detection limits, required by the Environmental Protection Agency (EPA) in Method 1633 for aqueous matrices.1 All 40 Per- and Polyfluoroalkyl Substances (PFAS) compounds were successfully quantified at concentrations 10x lower than the Limit of Quantitation (LOQ). This improved sensitivity allows laboratories to minimize operational cost by decreasing the volume of sample that needs to be collected, shipped, and extracted.

■ Method Overview

This application details the analysis of 40 native target PFAS compounds extracted from aqueous matrix along with 23 extracted internal standards (EIS), and 7 non-extracted internal standards (NIS). Stock standards were purchased from Wellington Laboratories as a series of native and mass-labelled PFAS mixtures in methanol (PFAC-MXF, PFAC-MXG, PFAC-MXH, PFAC-MXI, PFAC-MXJ, MPFAC-HIF-
ES, and MPFAC-HIF-IS). Three spiking standards was made containing the native targets, EIS, and NIS compounds by diluting the stock solutions in methanol. The calibration curve was made by preparing methanol with 4% water, 1% ammonium hydroxide, and 0.625% acetic acid. The stock standards were then diluted to make a curve that ranged from 0.025 to 10.0 µg/L for PFBA, 1.0 to 20.0 µg/L for EIS, and 1.0 to 4.0 µg/L for NIS. All standards were prepared for analysis in 200 µL silanized glass inserts in 1.5 mL amber silanized glass vials a with PE/Silicone blue screw caps.

Table 1: EPA Draft Method 1633 compound list

Type	Name	Type	Name
Target	PFBA	Target	NMeFOSE
Target	PFMPA	Target	NMeFOSA
Target	3:3 FTCA	Target	NEtFOSE
Target	PFPeA	Target	NEtFOSA
Target	PFMBA	EIS	13C4-PFBA
Target	4-2 FTS	EIS	13C5-PFPeA
Target	NFDHA	EIS	13C2-4:2 FTS
Target	PFHxA	EIS	13C5-PFHxA
Target	PFBS	EIS	13C3-PFBS
Target	HFPO-DA	EIS	13C3-HFPO-DA
Target	5:3 FTCA	EIS	13C4-PFHpA
Target	PFEESA	EIS	13C2-6:2FTS
Target	PFHpA	EIS	13C8-PFOA
Target	PFPeS	EIS	13C3-PFHxS
Target	ADONA	EIS	13C9-PFNA
Target	6-2 FTS	EIS	13C2-8:2FTS
Target	PFOA	EIS	D3-NMeFOSAA
Target	PFHxS	EIS	13C6-PFDA
Target	7:3 FTCA	EIS	D5-NEtFOSAA
Target	PFNA	EIS	13C8-PFOS
Target	PFHpS	EIS	13C7-PFUnA
Target	8-2 FTS	EIS	13C2-PFDoA
Target	NMeFOSAA	EIS	13C8-PFOSA
Target	PFDA	EIS	13C2-PFTeDA
Target	NEtFOSAA	EIS	D7-NMeFOSE
Target	PFOS	EIS	D3-NMeFOSA
Target	PFUnA	EIS	D9-NEtFOSE
Target	9Cl-PF3ONS	EIS	D5-NEtFOSA
Target	PFNS	NIS	13C3-PFBA
Target	PFDOA	NIS	13C2-PFHxA
Target	PFOSA	NIS	13C4-PFOA
Target	PFDS	NIS	18O2-PFHxS
Target	PFTrDA	NIS	13C5-PFNA
Target	11Cl-PF3OUdS	NIS	13C2-PFDA
Target	PFTeDA	NIS	13C4-PFOS
Target	PFDOS		

■ Sample Preparation and Extraction

500 mL of reagent water was spiked with 50 µL of EIS (800 µg/L 13C4-PFBA) and 200 µL of native compounds (2 µg/L PFBA). Method Blanks (MB) were also prepared and only spiked with EIS. Samples were extracted by solid-phase extraction (SPE) using Biotage EVOLUTE® EXPRESS WAX 150-mg/6-mL cartridges. Silanized glass wool was added to each cartridge before extraction, and each was pre-conditioned with 1% methanolic ammonium hydroxide and 0.3 M formic acid. Samples were loaded onto the WAX cartridges at a rate of 5 mL/min. The cartridges were then rinsed with LCMS grade water and 0.1 M formic acid/methanol and were left to dry for 15 seconds by vacuum. Elution was then carried out by rinsing the sample bottles with 1% methanolic ammonium hydroxide and eluted onto the WAX cartridge. Acetic acid and carbon were added to each extracted sample, then shaken by hand for a maximum of five minutes and centrifuged for ten minutes. The extracted samples were then filtered using a NYLON Choice 25, 0.22 µm filter into a new collection tube containing 50 µL of NIS (400µg/L 13C3-PFBA) spiking solution. A portion was transferred to a 1 mL silanized amber glass vial and vortexed for LCMS analysis.

■ Instrument and Operational Conditions

The LCMS analysis was performed by using a Shimadzu triple quadrupole mass spectrometer LCMS-8060NX, coupled with a Shimadzu Nexera -40 Series UHPLC. To minimize PFAS background contamination, a delay column was installed between the mixer and high-pressure valve shown in Figure 1. The LCMS parameters are included in Table 2. Samples run for calculating MDLs, according to EPA Method 1633, occurred over a minimum of three days. Day 1 analyses included, a calibration curve, instrument blank, a calibration verification (CV), three method blanks, and three spiked water samples were analyzed. Day 2 consisted of analyzing the instrument blank, CV, three method blanks, and three spiked water samples. This was repeated on Day 3 with the instrument blank, CV, two method blanks, and two spiked water samples. Before each LC-MS/MS batch, every vial was vortexed to resuspend PFAS compounds that may have adsorbed to the walls of their respective vials. This helps to improve relative standard error (RSE), as PFAS compounds are known to adsorb to the walls of sample vials.

Table 2: LCMS analysis method parameters

Parameter	Value
LCMS	Shimadzu LCMS-8060NX
Analytical Column	Shim-pack Scepter C18-120, 3.0 µm, 2.0 x 50mm
Delay Column	Shim-pack Scepter C18-120, 3.0 µm, 2.0 x 100mm
Injection Volume	10 µL
Pretreatment Mode	Co-Injection
Column Oven Temp.	40°C
Mobile Phase	A: 2 mM Ammonium Acetate in LCMS Grade Water
	B: Acetonitrile
Flow Rate	0.4 mL/min
Run Time	14 minutes

Figure 1: Installation/placement of a delay column for PFAS Analysis.

■ Calibration Curve Results

Relative standard error (RSE) of all native target PFAS and EIS compounds ranged between 1% and 19% and were below the maximum level of 20% required in the EPA method. Table 3 shows the concentration range from CS1 to CS9 for each compound along with its retention time and RSE. The calibration curve for NMeFOSA can be seen in Figure 2. Each curve contained a minimum of 7 calibration standards within the linear quantitative range.

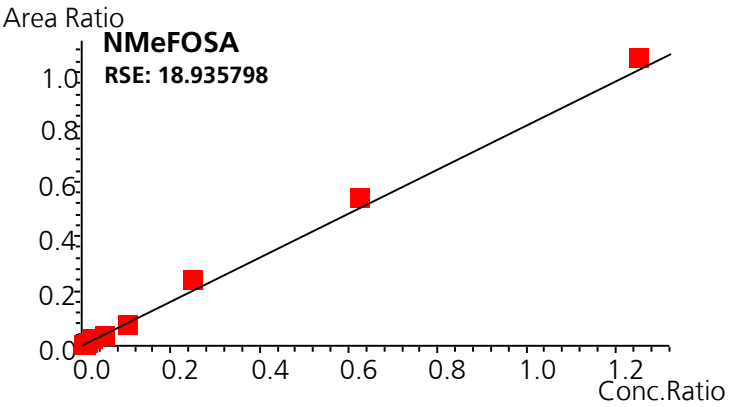


Figure 2. Calibration curve for NMeFOSA

Table 3: Retention time, calibration range, and resulted RSE for each target PFAS and EIS compound.

Type	Name	Ret. Time	CS1 (µg/L)	CS9 (µg/L)	RF RSE (curve)	Type	Name	Ret. Time	CS1 (µg/L)	CS9 (µg/L)	RF RSE (curve)
Target	PFBA	2.43	0.03	10.00	10.00	Target	PFTrDA	9.10	0.01	2.50	7.00
Target	PFMPA	2.73	0.01	5.00	9.00	Target	11Cl-PF3OUdS	9.23	0.03	10.00	10.00
Target	3:3 FTCA	2.82	0.03	12.50	11.00	Target	PFTeDA	9.40	0.01	2.50	15.00
Target	PFPeA	3.27	0.01	5.00	10.00	Target	PFDOS	9.59	0.01	2.50	10.00
Target	PFMBA	3.57	0.01	5.00	10.00	Target	NMeFOSE	9.42	0.06	25.00	9.00
Target	4-2 FTS	3.87	0.03	10.00	10.00	Target	NMeFOSA	9.50	0.01	2.50	19.00
Target	NFDHA	4.09	0.01	5.00	10.00	Target	NEtFOSE	9.60	0.06	25.00	9.00
Target	PFHxA	4.19	0.01	2.50	17.00	Target	NEtFOSA	9.67	0.01	2.50	10.00
Target	PFBS	4.33	0.01	2.50	9.00	EIS	13C4-PFBA	2.43	8.00	8.00	1.00
Target	HFPO-DA	4.57	0.03	10.00	17.00	EIS	13C5-PFPeA	3.27	4.00	4.00	4.00
Target	5:3 FTCA	4.56	0.16	62.50	9.00	EIS	13C2-4:2 FTS	3.87	4.00	4.00	4.00
Target	PFEESA	4.84	0.01	5.00	3.00	EIS	13C5-PFHxA	4.19	2.00	2.00	1.00
Target	PFHpA	5.13	0.01	2.50	9.00	EIS	13C3-PFBS	4.33	2.00	2.00	3.00
Target	PFPeS	5.39	0.01	2.50	9.00	EIS	13C3-HFPO-DA	4.56	8.00	8.00	11.00
Target	ADONA	5.45	0.03	10.00	9.00	EIS	13C4-PFHpA	5.13	2.00	2.00	3.00
Target	6-2 FTS	5.59	0.03	10.00	11.00	EIS	13C2-6:2FTS	5.60	4.00	4.00	5.00
Target	PFOA	5.97	0.01	2.50	14.00	EIS	13C8-PFOA	5.97	2.00	2.00	1.00
Target	PFHxS	6.32	0.01	2.50	11.00	EIS	13C3-PFHxS	6.33	2.00	2.00	1.00
Target	7:3 FTCA	6.23	0.16	62.50	11.00	EIS	13C9-PFNA	6.78	1.00	1.00	1.00
Target	PFNA	6.78	0.01	2.50	10.00	EIS	13C2-8:2FTS	7.16	4.00	4.00	2.00
Target	PFHpS	7.21	0.01	2.50	10.00	EIS	D3-NMeFOSAA	7.47	2.00	2.00	1.00
Target	8-2 FTS	7.16	0.03	10.00	11.00	EIS	13C6-PFDA	7.57	1.00	1.00	1.00
Target	NMeFOSAA	7.48	0.01	2.50	8.00	EIS	D5-NEtFOSAA	7.78	4.00	4.00	2.00
Target	PFDA	7.57	0.01	2.50	12.00	EIS	13C8-PFOS	8.03	2.00	2.00	2.00
Target	NEtFOSAA	7.81	0.01	2.50	15.00	EIS	13C7-PFUnA	8.30	1.00	1.00	2.00
Target	PFOS	8.03	0.01	2.50	9.00	EIS	13C2-PFDoA	8.77	1.00	1.00	3.00
Target	PFUnA	8.30	0.01	2.50	13.00	EIS	13C8-PFOSA	8.72	2.00	2.00	3.00
Target	9Cl-PF3ONS	8.51	0.03	10.00	8.00	EIS	13C2-PFTeDA	9.39	1.00	1.00	4.00
Target	PFNS	8.64	0.01	2.50	11.00	EIS	D7-NMeFOSE	9.41	20.00	20.00	4.00
Target	PFDOA	8.76	0.01	2.50	18.00	EIS	D3-NMeFOSA	9.50	2.00	2.00	6.00
Target	PFOSA	8.73	0.01	2.50	16.00	EIS	D9-NEtFOSE	9.59	20.00	20.00	3.00
Target	PFDS	9.00	0.01	2.50	9.00	EIS	D5-NEtFOSA	9.67	2.00	2.00	2.00

■ Method Detection Limit Calculations and Results

The method detection limits for spiked samples (MDLs) were computed by taking the standard deviation of each compound's concentration and multiplying it by the appropriate t-value (Equation 1). The method detection limit for the method blanks (MDLb) was computed if the compound was found to have a numerical result. If all seven samples did not give a numerical result, then it does not apply. If any of the method blanks gave a numerical result the MDLb is set to the highest recorded method blank. The MDLb was calculated using Equation 2. If the average concentration found was negative, then it was changed to zero) after multiplying the t-value and standard deviation of each compound.

Equation 1: $MDLs = t(n-1,1-\propto\equiv 0.99)Ss$

Equation 2: $MDLb = X(0) + t(n-1,1-\propto\equiv 0.99)Ss$

The greater value between the MDLs and MDLb for each compound becomes the initial MDL result.2 Out of the 40 compounds, 39 had higher MDLs values than their corresponding MDLb. PFHpA was the only compound for which the concentration quantified in the Method Blank (MB) was used to compute the max MDL value. This demonstrates that despite the high sensitivity achieved with this method, presence of PFAS in the MB was minimal and had negligible impact in the final MDLs. These results are all shown in Table 4 along with the values obtained by the EPA.

Overall, the MDLs ranged from 0.10 ng/L for PFEESA to 1.48 ng/L for 5:3 FTCA and were up to 13.4x better than those reported in EPA Method 1633. Figures 3 and 4 compare the MDLs reported in EPA Method 1633 compared with those from Shimadzu's LCMS-8060NX, based on the class of PFAS. For perfluoralkyl carboxylic and sulfonic acids (Figure 3), the highest MDL obtained with Shimadzu's LCMS-8060NX was 0.34 ng/L (PFOA), 1.6x better than MDL reported in the method. The results from the other classes of PFAS included in EPA 1633 are shown in Figure 4. MDLs reported in EPA Method 1633 ranged from 0.32 ng/L (PFOSA) and 9.59 ng/L (5:3 FTCA); those obtained with Shimadzu's LCMS-8060NX were between 0.1 ng/L (PFEESA) and 1.48 (5:3 FTCA). In addition to the improved sensitivity, which was up to 13.4x less as compared to results from the published EPA method, results presented less disparity in the concentrations determined from all PFAS classes targeted in the method. These results confirm concentrations of PFAS can be determined with 99% confidence at ppt levels and distinguishable from the method blank results.

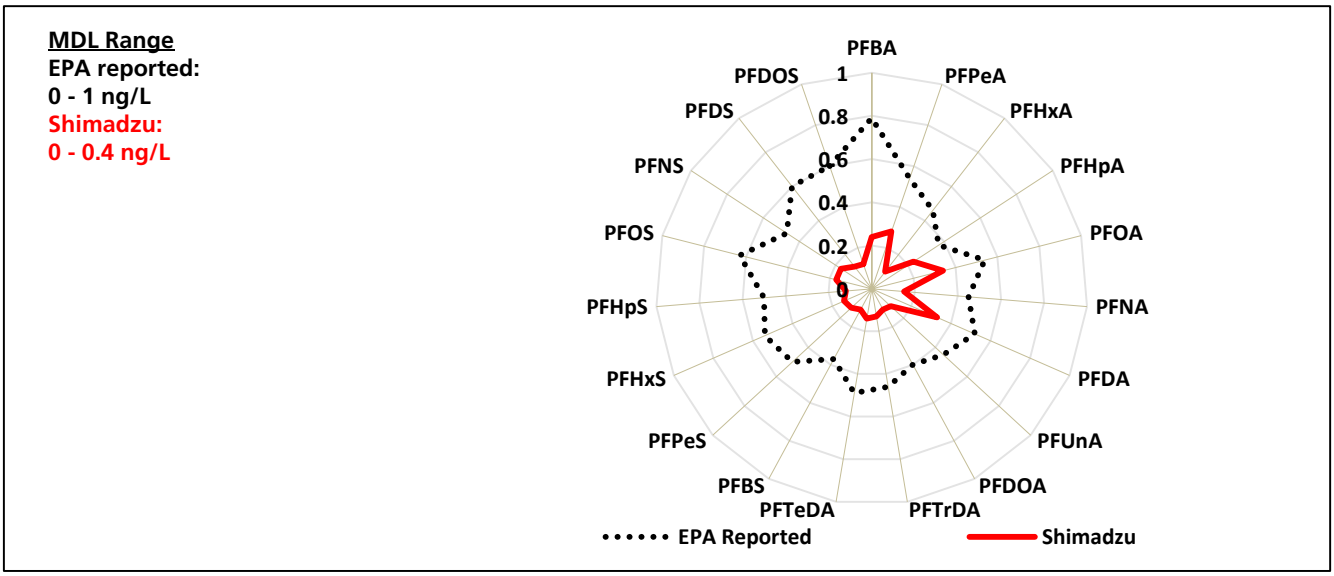


Figure 3: MDLs reported in EPA 1633 and obtained with Shimadzu's LCMS-8060NX of perfluoroalkyl carboxylic acids and sulfonic acids.

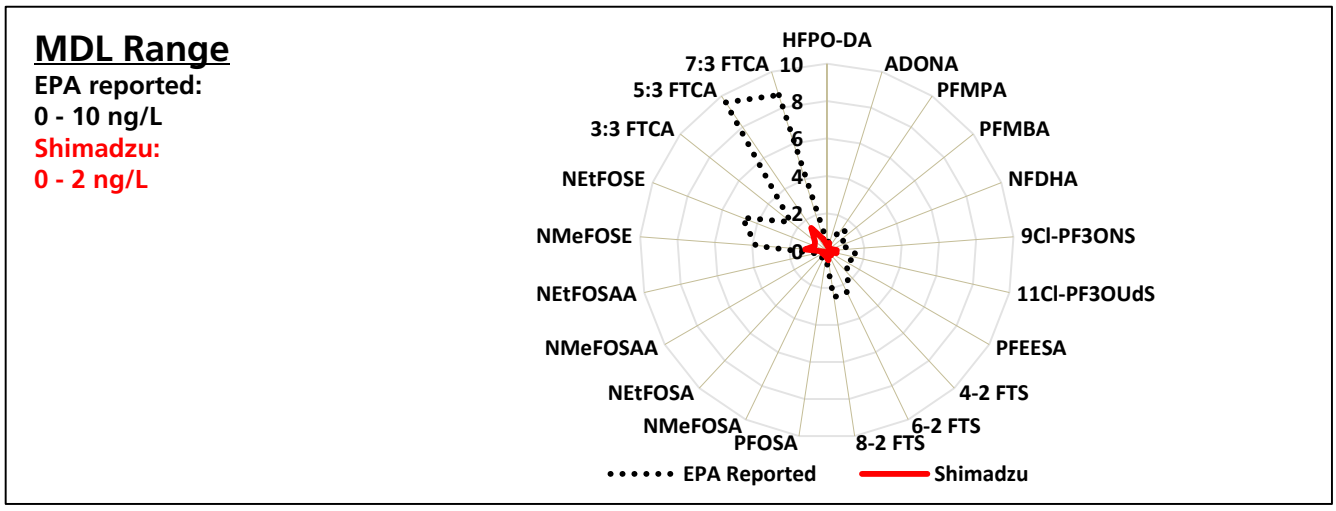


Figure 4: MDLs reported in EPA 1633 and obtained with Shimadzu's LCMS-8060NX of Per- and Polyfluoroether carboxylicacids, Ether sulfonic acids, Fluorotelomer sulfonic acids, Perfluorooctane sulfonamides, Perfluorooctane sulfonamidoacetic acids, Perfluorooctane sulfonamide ethanols, Fluorotelomer carboxylic acids.

Table 4:Comparison of Method Detection Limits values obtained by this study and EPA Draft Method 1633

Compound	Pooled MDLs (ng/L)	Pooled MDLb (ng/L)	Maximum MDL Value (ng/L)	EPA 1633 Draft MDL (ng/L)	Ratio MDL EPA/8060NX
PFBA	0.24	0.2	0.24	0.79	3.3
PFPeA	0.28	0.11	0.28	0.54	1.9
PFHxA	0.1	0.08	0.1	0.46	4.6
PFHpA	0.16	0.23	0.23	0.37	1.6
PFOA	0.34	0.26	0.34	0.54	1.6
PFNA	0.15	0.01	0.15	0.45	3.0
PFDA	0.33	0	0.33	0.52	1.6
PFUnA	0.12	0	0.12	0.45	3.8
PFDOA	0.11	0	0.11	0.4	3.6
PFTrDA	0.13	0	0.13	0.46	3.5
PFTeDA	0.14	0.04	0.14	0.49	3.5
PFBS	0.11	0	0.11	0.37	3.4
PFPeS	0.13	0	0.13	0.5	3.8
PFHxS	0.14	0.08	0.14	0.54	3.9
PFHpS	0.12	0	0.12	0.5	4.2
PFOS	0.17	0.15	0.17	0.63	3.7
PFNS	0.17	0.02	0.17	0.47	2.8
PFDS	0.13	0	0.13	0.6	4.6
PFDOS	0.12	0	0.12	0.6	5.0
HFPO-DA	0.32	0	0.32	0.51	1.6
ADONA	0.45	0	0.45	0.5	1.1
PFMPA	0.16	0	0.16	1.46	9.1
PFMBA	0.13	0	0.13	1.41	10.8
NFDHA	0.28	0	0.28	0.75	2.7
9Cl-PF3ONS	0.54	0	0.54	1.38	2.6
11Cl-PF3OUdS	0.52	0	0.52	1.67	3.2
PFEESA	0.1	0	0.1	1.17	11.7
4-2 FTS	0.33	0.01	0.33	1.69	5.1
6-2 FTS	0.24	0.05	0.24	2.45	10.2
8-2 FTS	0.51	0.04	0.51	2.5	4.9
PFOSA	0.29	0.04	0.29	0.32	1.1
NMeFOSA	0.26	0.09	0.26	0.43	1.7
NEtFOSA	0.21	0	0.21	0.45	2.1
NMeFOSAA	0.28	0.01	0.28	0.68	2.4
NEtFOSAA	0.17	0.1	0.17	0.59	3.5
NMeFOSE	1.17	0.5	1.17	3.81	3.3
NEtFOSE	0.7	0	0.7	4.84	6.9
3:3 FTCA	0.8	0.11	0.8	2.47	3.1
5:3 FTCA	1.48	0.06	1.48	9.59	6.5
7:3 FTCA	0.65	0.36	0.65	8.71	13.4

■ Conclusions

- The Shimadzu LCMS-8060NX can detect 10x lower than EPA's LOQ in a neat standard matrix and extracted aqueous matrix.
- Low MDL values were determined using the Shimadzu LCMS-8060NX, confirming sufficient sensitivity and reproducibility to meet and exceed (up to 13.4x better) all EPA 1633 requirements.
- Passing calibration curve linearity was obtained using this analysis method and consumables that were tested to ensure they did not interact with or contain any detectable PFAS constituents.
  Users must test every new lot number of consumables used n this analysis to ensure absence of detectable PFAS.

■ References

(1) Method 1633* Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS
(2) Appendix B to Part 136, Title 40 -- Definition and Procedure for the Determination of the Method Detection Limit—Revision 2

SHIMADZU
Excellence in Science

Application News

Liquid Chromatography Mass Spectrometry

ASTM D8421-22 Standard Test Method for Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Matrices by Co-solvation Followed by Analysis Using the Shimadzu LCMS-8060NX

Nami Iwasa[1], Landon Wiest[2], William Lipps[2]
1 Shimadzu Corporation, 2 Shimadzu Scientific Instruments, Inc.

User Benefits

- The LCMS-8060NX easily meets and exceeds method performance criteria of ASTM D8421 for 44 PFAS and 24 surrogates.
- Optimized chromatography and MS conditions for excellent peak shape for improved precision and accuracy.
- ASTM D8421 is a simple extraction procedure validated by ASTM for the analysis of PFAS in wastewater samples.

■ Introduction and Background

ASTM International published ASTM D8421[1] for the analysis of 44 per- and polyfluorinated alkyl substances and 24 labeled isotopes in non-potable water samples. This method extracts the substances in a 1+1 ratio of sample and methanol, filters and then measures the targeted compounds using external standard calibration liquid chromatography-tandem mass spectrometry (LC/MS/MS). The minimum reporting limit is 10 ng/L with an analytical range of 10 - 400 ng/L for most compounds. The method requires standard solutions be prepared by the laboratory from neat compounds.

To save a laboratory's time and effort from preparing stock standards individually, we optimized the method using commercially available native and labeled calibration standard mixes. Additionally, we optimized chromatography, achieving better peak shape for early-eluting compounds, such as PFBA and PFPrA.

This application news summarizes the performance of the Shimadzu LCMS-8060NX Liquid Chromatography Mass Spectrometer (LC/MS/MS) (Fig. 1) for all analytes listed in ASTM D8421. Results meet or exceed the requirements outlined in the method.

The reporting range and the target analytes are listed in Table 1. The reporting limit (RL) for the test method is defined as an integer value that is equal to the concentration of the lowest calibration standard.

Fig. 1 Shimadzu LCMS-8060NX

Table 1 Analyte List with D8421 Reporting Range

Analyte Name	Acronym	CAS Number	Range (ng/L)
Perfluorotetradecanoic acid	PFTreA	376-06-7	10-400
Perfluorotridecanoic acid	PFTriA	72629-94-8	10-400
Perfluorododecanoic acid	PFDoA	307-55-1	10-400
Perfluoroundecanoic acid	PFUnA	2058-94-8	10-400
Perfluorodecanoic acid	PFDA	335-76-2	10-400
Perfluorononanoic acid	PFNA	375-95-1	10-400
Perfluorooctanoic acid	PFOA	335-67-1	10-400
Perfluoroheptanoic acid	PFHpA	375-85-9	10-400
Perfluorohexanoic acid	PFHxA	307-24-4	10-400
Perfluoropentanoic acid	PFPeA	2706-90-3	50-1000
Perfluorobutanoic acid	PFBA	375-22-4	50-1000
Perfluorodecanesulfonic acid	PFDS	335-77-3	10-400

Perfluorononanes`ulfonic acid	PFNS	68259-12-1	10-400
Perfluorooctanesulfonic acid	PFOS	1763-23-1	10-400
Perfluoroheptanesulfonic acid	PFHpS	375-92-8	10-400
Perfluorohexanesulfonic acid	PFHxS	355-46-4	10-400
Perfluoropentanesulfonic acid	PFPeS	2706-91-4	10-400
Perfluorobutanesulfonic acid	PFBS	375-73-5	10-400
Perfluorooctanesulfonamide	PFOSA	754-91-6	10-400
8:2 Fluorotelomer sulfonic acid	8:2 FTS	39108-34-4	10-400
6:2 Fluorotelomer sulfonic acid	6:2 FTS	27619-97-2	10-400
4:2 Fluorotelomer sulfonic acid	4:2 FTS	757124-72-4	10-400
N-Ethylperfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6	10-400
N-Methylperfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9	10-400
Perfluorododecanesulfonic acid	PFDoS	79780-39-5	10-400
N-Methylperfluorooctanesulfonamide	NMeFOSA	31506-32-8	10-400
N-Ethylperfluorooctanesulfonamide	NEtFOSA	4151-50-2	10-400
N-Methylperfluorooctanesulfonamidoethanol	NMeFOSE	24448-09-7	10-400
N-Ethylperfluorooctanesulfonamidoethanol	NEtFOSE	1691-99-2	10-400
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	10-400
4,8-dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4	10-400
9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1	10-400
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9	10-400
Pentafluorpropanoic acid	PFPrA	422-64-0	50-1000
Perfluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6	10-400
Perfluoro(2-ethoxyethane) sulfonic acid	PFEESA	113507-82-7	10-400
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1	10-400
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5	10-400
2H,2H,3H,3H-Perfluorohexanoic Acid	3:3 FTCA	356-02-05	10-400
2H,2H,3H,3H-Perfluorooctanoic Acid	5:3 FTCA	914637-49-3	10-400
2H,2H,3H,3H-Perfluorodecanoic acid	7:3 FTCA	812-70-4	10-400
2H-perfluoro-2-octenoic acid	FHUEA	70887-88-6	10-400
2H-perfluoro-2-decenoic acid	FOUEA	70887-84-2	10-400
Lithium Bis(trifluoromethane)sulfonimide *	HQ-115	90076-65-6	10-400
Surrogates			
Perfluoro-n-[^{13}C4] butanoic acid	MPFBA	NA	10-400
Perfluor0-n-[^{13}C5] pentanoic acid	M5PFPeA	NA	10-400
Perfluoro-n-[1,2,3,4,6-^{13}C5] hexanoic acid	M5PFHxA	NA	10-400
Perfluoro-n-[1,2,3,4-^{13}C4] heptanoic acid	M4PFHpA	NA	10-400
Perfluoro-n-[$^{13}C_8$] octanoic acid	M8PFOA	NA	10-400
Perfluoro-n-[$^{13}C_9$] nonanoic acid	M9PFNA	NA	10-400
Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$] decanoic acid	M6PFDA	NA	10-400
Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$] undecanoic acid	M7PFUnA	NA	10-400
Perfluoro-n-[1,2-$^{13}C_2$] dodecanoic acid	MPFDoA	NA	10-400
Perfluoro-n-[1,2-$^{13}C_2$] tetradecanoic acid	M2PFTreA	NA	10-400
Perfluoro-1-[$^{13}C_8$] octanesulfonamide	M8FOSA	NA	10-400
N-methyl-d_3-perfluoro-1-octanesulfonamidoacetic acid	D3-N-MeFOSAA	NA	10-400
N-ethyl-d_5-perfluoro-1-octanesulfonamidoacetic acid	D5-N-EtFOSAA	NA	10-400
N-methyl-d_3-perfluoro-1-octanesulfanamide	d-N-MeFOSA	NA	10-400
N-ethyl-d_5-perfluoro-1-octanesulfanamide	d-N-EtFOSA	NA	10-400
2-(N-methyl-d_3-perfluoro-1-octanesulfonamido) ethan-d_4-ol	d7-N-MeFOSE	NA	10-400
2-(N-ethyl-d_5-perfluoro-1-octanesulfonamido) ethan-d_4-ol	D9-N-EtFOSE	NA	10-400
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy-$^{13}C_3$-propanoic acid	MHFPO-DA	NA	10-400
1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$] hexane sulfonate	M4:2FTS	NA	10-400
1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]-octane sulfonate	M6:2FTS	NA	10-400
1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]-decane sulfonate	M8:2FTS	NA	10-400
Perfluoro-1-[$^{13}C_8$] octanesulfonate	M8PFOS	NA	10-400
Perfluoro-1-[2,3,4-$^{13}C_3$] butanesulfonate	MPFBS	NA	10-400
Perfluoro-1-[1,2,3-$^{13}C_3$] hexanesulfonate	M3PFHxS	NA	10-400

Compounds in red are not in the Method 1633 standards and need to be added separately

■ Materials and Methods

Stock standard solutions containing native analytes and labeled isotopes (surrogates) were diluted from commercially available mixed stock standards (Wellington Method 1633 standard mixes) to be within the calibration range per analyte as shown in Table 1.

The individual standard solution was prepared in 50:50 (vol: vol) methanol/water with 0.1% acetic acid to obtain final concentrations shown in Table 2.

Table 2 Concentrations of each Calibration Standard (CS) in ng/L

	Compounds	CS1	CS2	CS3	CS4	CS5	CS6	CS7	CS8	CS9	CS10	CS11	CS12	CS13	CS14	CS15
Analyte	All analytes unless otherwise noted	1	2.5	5	10	25	40	60	80	100	150	200	250	375	500	800
	PFPeA	2	5	10	20	50	80	120	160	200	300	400	500	750	1000	1600
	PFBA, 4:2-FTS, 6:2-FTS, 8:2-FTS	4	10	20	40	100	160	240	320	400	600	800	1000	1500	2000	3200
	PFPrA, 5:3 FTCA, 7:3 FTCA	5	12.5	25	50	125	200	300	400	500	750	1000	1250	1875	2500	4000
	NMeFOSE, NEtFOSE	10	25	50	100	250	400	600	800	1000	1500	2000	2500	3750	5000	8000
Surrogate	13C9-PFNA, 13C6-PFDA, 13C7-PFUnA, 13C2-PFDoA, 13C2-PFTreA	0.25	0.625	1.25	2.5	6.25	10	15	20	25	37.5	50	62.5	93.75	125	200
	13C5-PFHxA, 13C4-PFHpA, 13C8-PFOA, 13C8-PFOSA, D3-NMeFOSA, D5-NEtFOSA, 13C8-PFOS, 13C3-PFBS, 13C3-PFHxS	0.5	1.25	2.5	5	12.5	20	30	40	50	75	100	125	187.5	250	400
	13C5-PFPeA, 13C2-4:2FTS,13C2-6:2FTS, 13C2-8:2FTS, D3-NMeFOSAA, D5-NEtFOSAA	1	2.5	5	10	25	40	60	80	100	150	200	250	375	500	800
	13C4-PFBA, 13C3-HFPO-DA	2	5	10	20	50	80	120	160	200	300	400	500	750	1000	1600
	D7-NMeFOSE, D9-NEtFOSE	5	12.5	25	50	125	200	300	400	500	750	1000	1250	1875	2500	4000

These standards were not filtered. Calibration is performed using a 6 to 10-point curve, depending on the analyte. To obtain the calibration levels from the commercial stock solutions, 15 individual calibration standards were prepared and analyzed. Only the calibration points within the method-specified range were used.

The stock solutions were prepared and stored in PFAS-free polypropylene (PP) containers. Prior to the analysis, the solutions were shaken thoroughly, then transferred to a 2 mL PP LC vial and analyzed within 24 hours. If samples or standards are allowed to sit in the LC vials, some PFAS compounds may settle, rise, precipitate, or adsorb on the surface. To ensure a homogenous solution and optimum results, the solutions were vortexed prior to injection.

2.1 Sample Preparation

The surrogate spiking mix is added to 5 mL of sample contained in a 15 mL polypropylene vial. Add 5 mL of methanol and mix by vortex for ~2 minutes. After mixing, add acetic acid and adjust the pH ~4. Transfer an aliquot to a LC vial and cap with a Shimadzu GLC PP vial with septum confirmed to not contain PFAS. Analyze per the conditions shown in Table 3. Concentrations obtained from the curve are multiplied by two to obtain the final concentration in the samples.

2.2 Analytical Conditions

Table 3 Instrument Configuration and Analytical Conditions for ASTM D8421 PFAS using the Shimadzu LCMS-8060NX.

Mobile Phase	A: 2 mmol/L Ammonium Acetate in H_2O/ Acetonitrile = 95/5
	B: Acetonitrile
Delay Column	Shim-pack Scepter™ C18-120
	2.1 mm x 100 mm, 3 µm (P/N: 227-31014-05)
Analytical Column	Shim-pack™ GIST-HP C18
	3.0 mm x 100 mm, 3 um (P/N: 227-30040-04)
Gradient (%B)	10% (0 min) ⇒ 22% (2.3-3.0 min) ⇒ 45% (6.0 min) ⇒ 80% (13.0 min) ⇒ 95% (14.0-16 min) ⇒ 10% (16.01-20.0 min)
Interface	IonFocus ESI
Column Oven Temp.	40 °C
Flow rate	0.6 mL/min
Injection volume	25 µL
Multiple draw injection program	Co-injection 25 µL Sample →25 µL 0.1% Acetic acid in H2O
Interface Temp.	170 °C
Probe position	+3 mm
Neblizer gas flow	3 L/min
Heating gas flow	15 L/min
Interface Voltage	-0.5 kV (same value for all compounds)
DL Temp.	200 °C
Heatblock Temp.	300 °C
Drying gas flow	5 L/min
Focus bias	-2 kV (same value for all compounds)

■ Results and Discussion

A single laboratory validation of this method for specificity, linearity, recovery, and precision in nine wastewater matrices according to ASTM D8272[2] was previously described.[3] For this application news, a study was made to improve peak shape, particularly of early-eluting compounds, such as PFPrA and PFBA. This included evaluation of injection technique, columns, and flow rate. Co-injection of 25 µL sample with 25 µL 0.1% acetic acid in reagent water significantly improved the peak shapes of PFPrA, PFBA, and PFMPA (Fig. 2). A large diameter column with a long column length and large particle size, combined with a high flow rate, allowed greater axial diffusion, improving peak shape (Fig. 3).

Finally, to better separate impurities from the mobile phase, a new delay column was chosen, and the gradient program was modified (Fig. 4). Upon optimization of chromatography and mass spectrometer conditions, calibration mixtures (Table 2) were prepared and used for subsequent analysis. Compound parameters, including quantitation ion, confirmation ion and collision energies, were optimized using LabSolutions™ software. At least two MRM transitions, if available, were used.

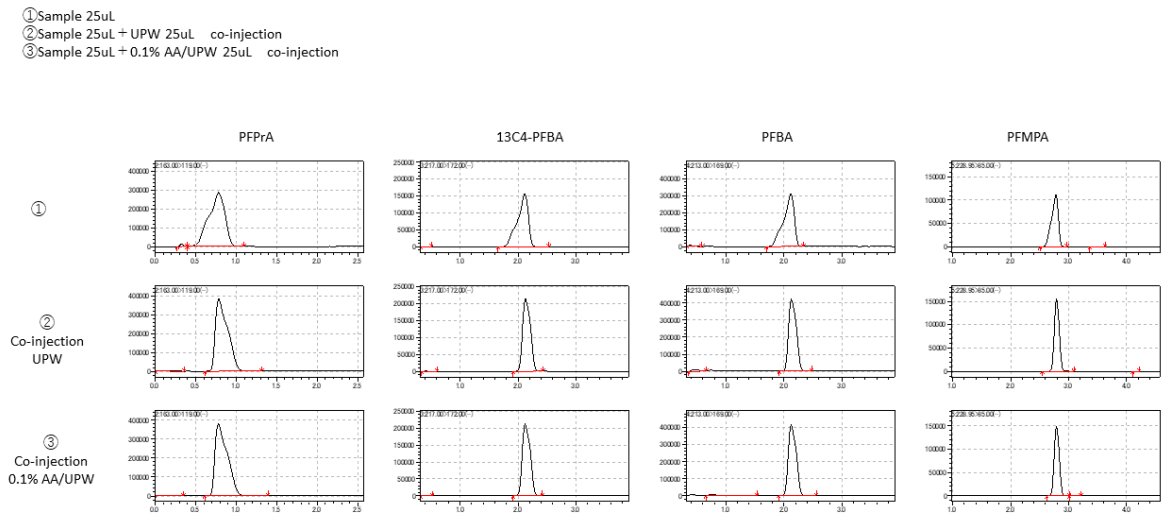


Fig. 2 Optimization of injection technique to improve peak shape

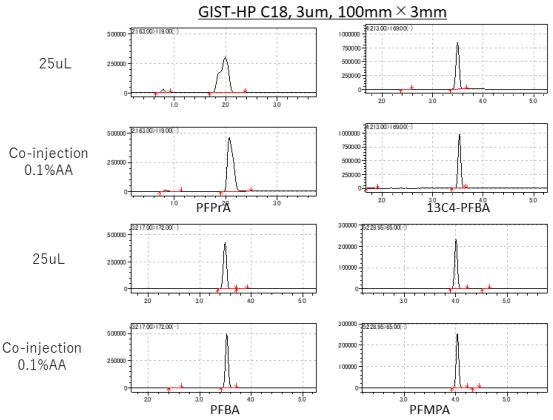


Fig. 3 Example chromatograms for final column and flow rate with co-injection applied

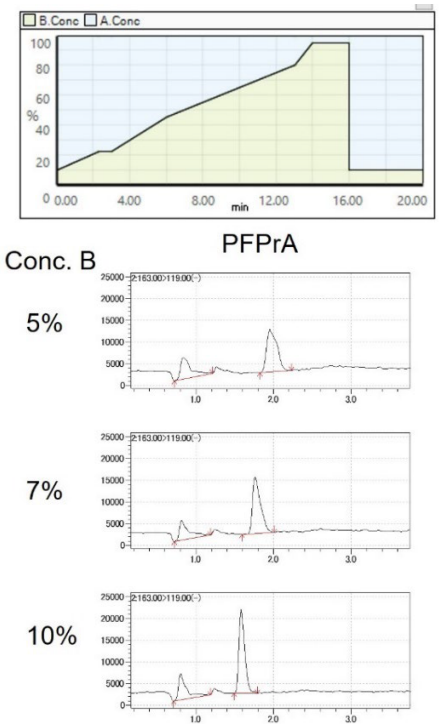


Fig. 4 Final gradient with chromatogram of PFPrA, the earliest eluting peak

Linearity Study

Calibration curves for each analyte were found by Shimadzu Lab Solutions Insight data processing software to have a % RSD of less than 30%, as required by ASTM D8421. Calibration data, MRM transitions for the quantitation and confirmation ions (when available), and retention times are shown in Table 4. Calibration curves along with a midpoint standard chromatogram of PFOA and PFOS are shown in Figures 5 and 6.

These compounds were chosen to illustrate because of their likelihood for regulation in wastewater. Additionally, calibration curves and midpoint chromatograms of PFPrA and NEtFOSE are shown in Figures 7 and 8. These compounds were chosen because they are the earliest and latest eluting compounds respectively.

Table 4 Summary of calibration data.

Compound	Quantitation Ion	Confirmation Ion	Retention Time (min)	r^2
PFTreA	712.95>668.95	712.95>169.00	11.756	0.9962
PFTriA	662.95>618.95	662.95>169.00	11.026	0.9978
PFDoA	612.95>568.95	612.95>319.00	10.286	0.9987
PFUnA	562.95>518.95	562.95>269.00	9.543	0.9991
PFDA	512.95>468.95	512.95>219.00	8.813	0.9967
PFNA	462.95>418.95	462.95>219.00	8.11	0.9921
PFOA	412.95>369.00	412.95>169.00	7.451	0.9929
PFHpA	362.95>319.00	362.95>169.00	6.807	0.9989
PFHxA	312.95>269.00	312.95>119.00	6.028	0.9976
PFPeA	263.00>219.00	263.00>69.00	4.728	0.9996
PFBA	213.00>169.00	----	3.026	0.9985
PFDS	598.90>79.95	598.90>98.95	10.785	0.9971
PFNS	548.95>79.95	548.95>98.95	10.033	0.9975
PFOS	498.95>79.95	498.95>98.95	9.275	0.9951
PFHpS	448.95>79.95	448.95>98.95	8.522	0.9951
PFHxS	398.95>79.95	398.95>98.95	7.783	0.9917
PFPeS	348.95>79.95	348.95>98.95	7.059	0.9917
PFBS	298.95>79.95	298.95>98.95	6.17	0.9990
PFOSA	497.95>77.95	497.95>477.95	11.075	0.9979
8:2FTS	526.95>506.95	526.95>80.90	8.426	0.9976
6:2FTS	426.95>406.95	426.95>80.90	7.148	0.9960
4:2FTS	326.95>306.95	326.95>80.90	5.678	0.9970

NEtFOSAA	584.00>418.95	584.00>526.00	9.01	0.9950
NMeFOSAA	569.95>418.95	569.95>482.95	8.703	0.9929
PFDoS	698.90>79.95	698.90>98.95	12.228	0.9959
NMeFOSA	511.95>219.00	511.95>169.00	13.556	0.9956
NEtFOSA	526.00>219.00	526.00>169.00	14.149	0.9988
NMeFOSE	616.00>59.00	----	13.246	0.9996
NEtFOSE	630.00>59.00	----	13.853	0.9998
HFPO-DA	285.00>169.00	285.00>185.00	6.365	0.9971
ADONA	376.95>251.00	376.95>85.00	7.064	0.9980
9Cl-PF3ONS	530.90>350.95	532.90>352.95	9.809	0.9994
11Cl-PF3OUdS	630.90>450.95	632.90>452.95	11.308	0.9994
PFPrA	163.00>119.00	----	1.589	0.9996
NFDHA	294.95>201.00	294.95>85.00	5.937	0.9953
PFEESA	314.95>135.00	314.95>82.95	6.628	0.9978
PFMPA	228.95>85.00	----	3.656	0.9981
PFMBA	278.95>85.00	----	5.279	0.9979
3:3 FTCA	241.00>177.00	241.00>117.00	3.804	0.9717
5:3 FTCA	341.00>237.00	341.00>217.00	6.375	0.9945
7:3 FTCA	441.00>317.00	441.00>337.00	7.752	0.9964
FHUEA	357.00>293.00	----	6.472	0.9962
FOUEA	456.95>393.00	----	7.704	0.9973
HQ-115	279.90>146.95	279.90>210.90	7.259	0.9988
13C4-PFBA_Surr	217.00>172.00	----	3.023	0.9982
13C5-PFPeA_Surr	268.00>223.00	----	4.726	0.9976
13C5-PFHxA_Surr	318.00>273.00	318.00>120.00	6.026	0.9972
13C4-PFHpA_Surr	367.00>322.00	----	6.806	0.9994
13C8-PFOA_Surr	421.00>376.00	----	7.45	0.9959
13C9-PFNA_Surr	472.00>427.00	----	8.108	0.9947
13C6-PFDA_Surr	519.00>474.00	----	8.81	0.9989
13C7-PFUnA_Surr	570.00>525.00	----	9.541	0.9979
13C2-PFDoA_Surr	614.95>569.95	----	10.285	0.9968
13C2-PFTreA_Surr	714.95>669.95	----	11.755	0.9952
13C8-PFOSA_Surr	505.95>77.95	----	11.077	0.9983
D3-NMeFOSAA_Surr	573.00>418.95	----	8.697	0.9933
D5-NEtFOSAA_Surr	589.00>418.95	----	9	0.9976
D3-NMeFOSA_Surr	515.00>219.00	515.00>168.90	13.548	0.9993
D5-NEtFOSA_Surr	531.00>219.00	531.00>168.90	14.131	0.9983
D7-NMeFOSE_Surr	623.05>59.00	----	13.206	0.9957
D9-NEtFOSE_Surr	639.10>59.00	----	13.807	0.9994
13C3-HFPO-DA_Surr	287.00>169.00	284.90>185.00	6.363	0.9921
13C2-4:2FTS_Surr	329.00>308.95	329.00>80.90	5.678	0.9943
13C2-6:2FTS_Surr	428.95>408.95	428.95>80.90	7.147	0.9903
13C2-8:2FTS_Surr	528.95>508.95	528.95>80.90	8.425	0.9956
13C8-PFOS_Surr	506.95>79.95	506.95>98.95	9.274	0.9966
13C3-PFBS_Surr	301.95>79.95	301.95>98.95	6.17	0.9953
13C3-PFHxS_Surr	401.95>79.95	401.95>98.95	7.782	0.9921

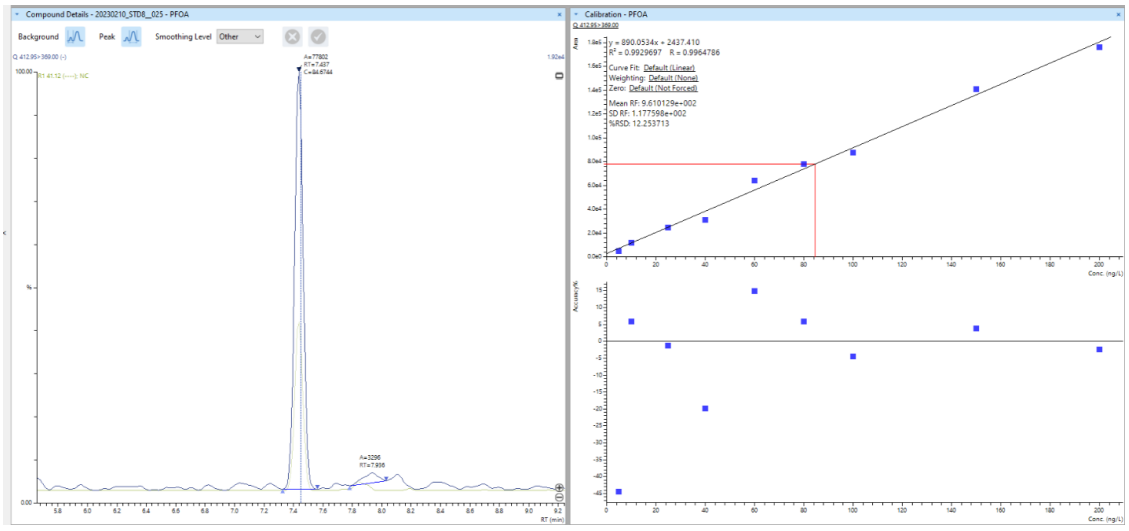


Fig. 5 Calibration curve and midpoint chromatogram for PFOA

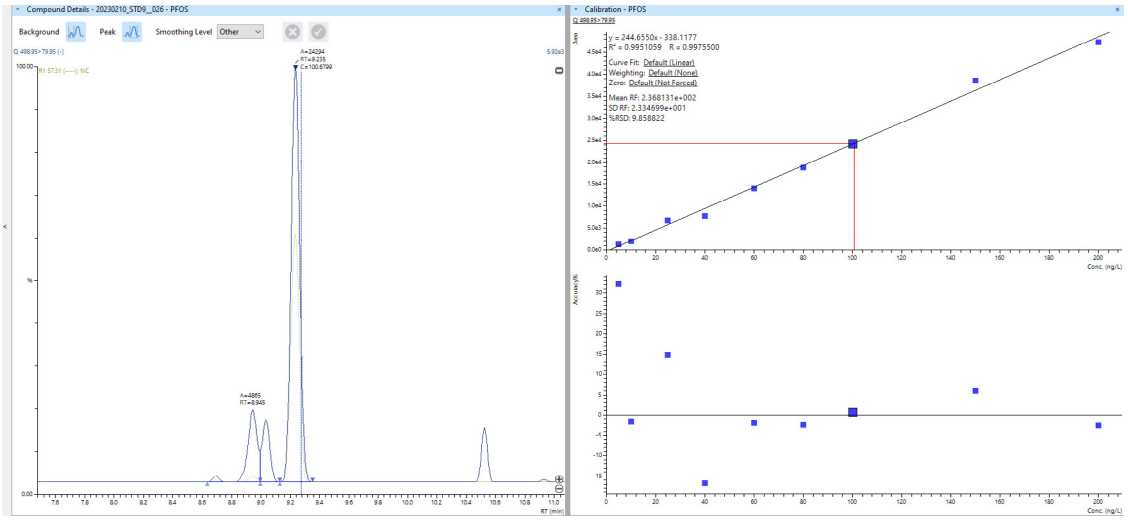


Fig. 6 Calibration curve and midpoint chromatogram for PFOS

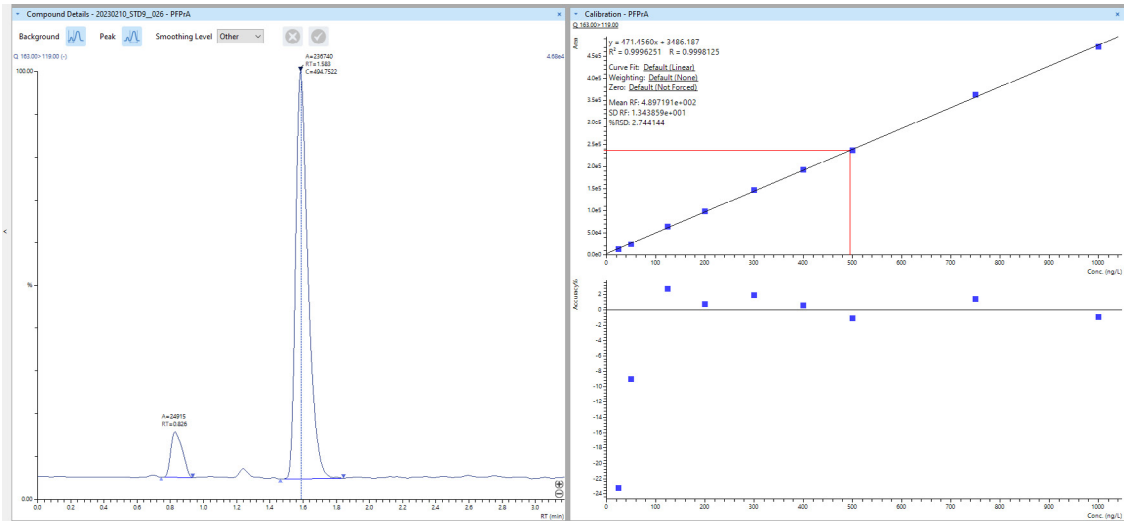


Fig. 7 Calibration curve and midpoint chromatogram for PFPrA

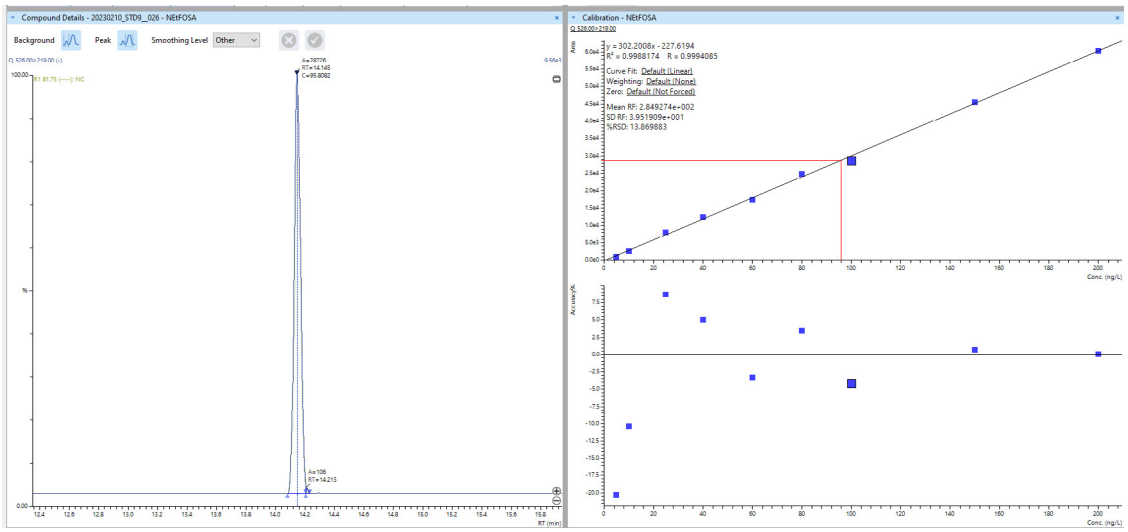


Fig. 8 Calibration curve and midpoint chromatogram for NEtFOSE

Recovery and Repeatability Study

Recovery and repeatability (Table 5) were evaluated in reagent water and wastewater, each spiked four times at the concentration indicated. Recovery was calculated after subtracting the native PFAS found in the unspiked sample matrices.

These data are well within the 70 -130 % recovery and ≤ 30 %RSD limits of the method.

Table 5 Recovery and Repeatability in Reagent Water and Wastewater

Compound	Spike Concentration (ppt)	Reagent Water % Recovery	Reagent Water %RSD (n=4)	Wastewater % Recovery	Wastewater %RSD (n=4)
PFTreA	160	110	3.76	119	2.71
PFTriA	160	109	2.08	79.9	4.82
PFDoA	160	104	4.33	107	4.6
PFUnA	160	113	5.53	105	2.49
PFDA	160	113	2.67	102	4.5
PFNA	160	113	6.71	107	2.34
PFOA	160	111	7.52	112	5.7
PFHpA	160	116	4.13	108	4.46
PFHxA	160	114	6.83	115	3.41
PFPeA	320	106	4.37	108	2.47
PFBA	640	107	0.55	108	2.06
PFDS	160	112	8.89	112	3.73
PFNS	160	113	3.72	116	5.58
PFOS	160	110	3.83	122	4.02
PFHpS	160	115	6.09	102	6.91
PFHxS	160	113	5.93	113	13.15
PFPeS	160	124	4.94	119	9.49
PFBS	160	109	4.59	114	5.12
PFOSA	160	101	2.44	100	4.59
8:2FTS	640	113	6.24	103	4.97
6:2FTS	640	119	3.2	107	3.3
4:2FTS	640	121	0.7	100	2.47
NEtFOSAA	160	113	7.5	89.4	10.15
NMeFOSAA	160	111	13.35	88.0	7.42
PFDoS	160	106	5.23	108	9.31
NMeFOSA	160	102	3.68	91.5	5.59
NEtFOSA	160	100	0.73	90.5	3.33
NMeFOSE	1600	97.2	0.34	93.6	0.94
NEtFOSE	1600	96.8	0.9	93.5	1.39
HFPO-DA	160	109	2.35	112	9.66
ADONA	160	110	1.01	104	4.22
9Cl-PF3ONS	160	111	2.01	111	1.92
11Cl-PF3OUdS	160	112	3.59	111	2.78
PFPrA	800	108	1.8	105	0.77
NFDHA	160	107	9.13	110	4.16
PFEESA	160	112	4.71	115	4.45
PFMPA	160	106	2.37	102	5.83
PFMBA	160	113	7.07	115	2.55
3:3 FTCA	160	87.1	20.57	94.8	14.52
5:3 FTCA	800	95.7	6.91	90.4	4.22
7:3 FTCA	800	92.5	2.56	88.8	2.73
FHUEA	160	99.1	3.91	95.3	2.16
FOUEA	160	102	4.2	97.2	3.5
HQ-115	160	112	2.56	111	0.78
Surrogates					
13C4-PFBA_Surr	320	102	1.7	96.4	2.88
13C5-PFPeA_Surr	160	108	6.03	96.4	3.36
13C5-PFHxA_Surr	80	110	3.7	101	5.35
13C4-PFHpA_Surr	80	104	6.43	105	5.99
13C8-PFOA_Surr	80	107	11.32	100	10.19
13C9-PFNA_Surr	40	98.2	13.76	92.2	17.29

13C6-PFDA_Surr	40	106	5.74	96.5	8.59
13C7-PFUnA_Surr	40	98.6	6.01	90.8	6.68
13C2-PFDoA_Surr	40	96.1	4.31	94.9	5.43
13C2-PFTreA_Surr	40	98.9	10.44	119	9.46
13C8-PFOSA_Surr	80	92.2	2.22	91.4	6.54
D3-NMeFOSAA_Surr	160	98.4	3.95	90.6	6.56
D5-NEtFOSAA_Surr	160	95.0	3.55	81.3	3.26
D3-NMeFOSA_Surr	80	89.3	12.1	82.6	8.57
D5-NEtFOSA_Surr	80	90.5	8.83	84.2	8.94
D7-NMeFOSE_Surr	800	88.8	0.49	85.4	1.68
D9-NEtFOSE_Surr	800	89.6	0.97	85.9	1.49
13C3-HFPO-DA_Surr	320	103	3.19	101	8.25
13C2-4:2FTS_Surr	160	116	3.15	96.2	6.88
13C2-6:2FTS_Surr	160	119	2.25	97.3	5.65
13C2-8:2FTS_Surr	160	104	0.73	97.4	7.53
13C8-PFOS_Surr	80	103	9.45	105	9.61
13C3-PFBS_Surr	80	121	5.06	94.0	6.33
13C3-PFHxS_Surr	80	116	9.99	108	6.65

■ Conclusion

This application news demonstrates the analysis of 44 PFAS and 24 surrogate compounds in non-potable water by ASTM D8421 using the Shimadzu LCMS-8060NX Liquid Chromatography Mass Spectrometer (LC/MS/MS). Chromatographic conditions were optimized to achieve excellent peak shape, even for the earliest eluting compounds, such as PFPrA and PFBA.

The highly sensitive Shimadzu LCMS-8060NX easily exceeds method performance criteria of the ASTM method and provides testing laboratories with highly accurate and reliable, repeatable results for PFAS in wastewater samples.

■ References

1. ASTM Test Method D8241 Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Matrices by Co-solvation followed by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)
2. ASTM Standard D8272-19 -Standard Guide for Development and Optimization of D19 Chemical Analysis Methods Intended for EPA Compliance Reporting / ASTM International / West Conshohocken / PA / 2020 / 10.1520/D8272-19/ https://www.astm.org/
3. Lipps, W., ASTM D8421-22 Standard Test Method for Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Matrices by Co-solvation followed by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS), Shimadzu Whitepaper, August 2023.

WP 280

Utilization of Automated Solvent Extraction with a Triple Quadrupole Mass Spectrometer following EPA Method 1633 for PFAS Analysis in Soil

Landon A Wiest[1]; Om K Shrestha[1]; Benedict Liu[2]; Kathleen K Luo[1]; Megan Davis[1]; Evelyn H Wang[1]; Alicia Stell[2]; Ruth Marfil-Vega[1]

[1]Shimadzu Scientific Instruments, Columbia, MD; [2]CEM Corporation, Matthews, NC

1. Introduction

◆ Manual solid sample extraction is error prone and resource consuming, making automation desirable. With the increasing interest in meeting regulatory requirements and understanding PFAS levels in various sample types, automated workflows are essential for improving lab productivity. This work will demonstrate the combined performance of an automated solvent extraction system for soil extraction coupled with a robust LC-MS/MS for PFAS analysis according to EPA Method 1633 to help laboratories with providing accurate results and fast turn-around-times.

2. Methods

◆ Soil samples comprised of 5 g Ottawa sand were extracted, with the extract being filtered using the CEM EDGE PFAS™ Automated Extraction system with the method detailed below. Samples were weighed into pre-assembled 2-piece Q-Cup® sample cells with Q-Disc® PFAS filter disc and spiked with native PFAS compounds and extracted internal standard. Each sample was then extracted in sequence via the automated addition of solvent via pressurized fluid extraction. Each of the 12 samples was extracted in under 10 minutes, including automated extraction, and automated cleaning of the system (**Figure 1**). Extracts were cleaned-up according to EPA Method 1633 (Millipore-Sigma Carbopack Adsorbent and SupelClean ENVI-WAX SPE Tube) before LCMS analysis.

◆ The 40 PFAS (targets, non-extracted and extracted internal standards) were chromatographically separated with a C18 column (50x2.1 mm, 3µm) by gradient elution. A C18 delay column was used to remove the interference system PFAS contaminants. The LC and MS parameters used are outlined in **Table 1**.

Table 1. Shimadzu LCMS-8060NX parameters

LC Time Program		Mobile Phase	
Time	**B.Conc**	**A**	2mM ammonium acetate in water
0	2	**B**	Acetonitrile
0.21	20	**Flow Rate**	0.4 mL/min
7	55	**Gas Flow**	
9	98	**Nebulizing**	2 L/min
10.25	98	**Heating**	15 L/min
10.26	2	**Drying**	5 L/min
Injection Volume	15 µL	**Interface Temp.**	250 °C

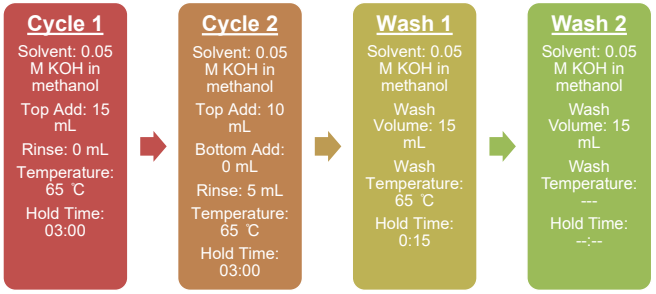


Figure 1. Sample extraction process following EPA 1633 Method with the CEM EDGE PFAS Automated Extraction system

3. Results

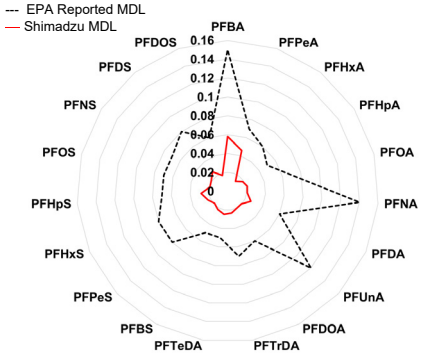
◆ A calibration curve ranging from 0.02 – 1.25 ng/mL with appropriate Non-Extracted Internal Standard concentrations was prepared. Calibrants were set at concentration starting at 10 times lower than EPA Method 1633 Cal 1 (PFBA: 0.08 ng/mL; variable concentration of targets as listed in EPA 1633[1]) to demonstrate that accurate quantitation of spiked soils can be achieved at limits below the method requirements. The EPA Method 1633 requires an RSE equal to or lower than 20%. The RSE values calculated from the calibration curve were all below 18%.

◆ Calibration verification was performed after every 10 sample injections as specified by the EPA method; the average %accuracy ranged from 90 – 128% for all targeted PFAS analytes throughout the analysis.

◆ Sample recovery ranged from 63% (PFDOS) to 115% (PFHxA) and were within the acceptable range listed in EPA Method 1633.

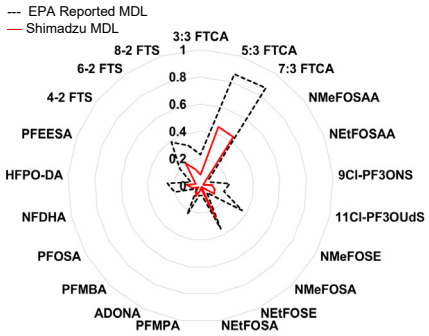
◆ The method detection limits for spiked samples (MDL_s) were calculated by taking the standard deviation from the concentration of each compound and multiplying it by the appropriate t-value[2]. **Figures 2** and **3** compare the MDLs reported in EPA Method 1633 compared with those from the workflow used in this study combining the EDGE and the LCMS-8060NX, based on the class of PFAS.

References
(1) Method 1633* Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS
(2) Appendix B to Part 136, Title 40 -- Definition and Procedure for the Determination of the Method Detection Limit—Revision 2



EPA Range: 0.05 – 0.15 ng/g; Shimadzu Range: 0.01 – 0.06 ng/g

Figure 2. MDLs comparison between the levels reported in EPA Method1633 and obtained from this work of perfluoroalkyl carboxylic acids and sulfonic acids.



EPA Range: 0.04 – 0.87 ng/g; Shimadzu Range: 0.01 – 0.45 ng/g

Figure 3. MDLs comparison between the levels reported in EPA Method 1633 and obtained from this work of the listed PFAS compounds.

3. Results (Cont.)

◆ For perfluoralkyl carboxylic and sulfonic acids (**Figure 2**), MDLs obtained in this work ranged from 0.01 ng/g (PFHxA) to 0.06 ng/g (PFBA). The results from the other classes of PFAS included in EPA Method 1633 are shown in **Figure 3**; results obtained in this work ranged from 0.01 ng/g (NMeFOSA) to 0.45 ng/g (5:3 FTCA).

4. Conclusions

◆ Overall, the calculated MDLs from this workflow using the CEM EDGE PFAS combined with the Shimadzu LCMS-8060NX were 2 times better than those reported in EPA Method 1633 in soils.

◆ The combination of the automated solvent extraction system with optimized extraction parameters and the robust sensitive LC-MS/MS demonstrated performance that met the requirements in the final EPA Method 1633.

1The authors are affiliated and funded by Shimadzu Corporation.
2The named authors declare no competing financial interest.

SHIMADZU

ThP 263

Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Fish Fillet with LC-MS/MS

Nozomi Maeshima[1], Kota Ishioka[1], Toshiya Matsubara[2], Manami Kobayashi[1]
1) SHIMADZU Corporation, Kawasaki, Japan; 2) Shimadzu Scientific Instruments, Columbia, MD

1. Introduction

Per- and Polyfluoroalkyl Substances (PFAS) is the collective name for a chemical group of organic fluorinated compounds, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are representative compounds of PFAS. They have been used water repellents, surface treatment agents, fire extinguishers, and coatings. PFAS are persistent and bioaccumulative in the environment because of their stable structure, it is known that they are present in a wide range of environmental water and wildlife. Due to concerns about human exposure through diet, studies on the status of food contamination by PFAS are being conducted in various countries. We have examined a quantitative analysis method for thirty PFAS compounds in fish fillet samples.

2. Methods

2-1. Sample and equipment

Standard compounds were purchased from Wellington Laboratories. Fish fillet for sample was purchased from a local grocery store and homogenized using a freeze grinder FST-4000 (AiSTI SCIENCE). Quantification was performed with a triple quadrupole mass spectrometer LCMS-8060NX equipped with Nexera™ X3 UHPLC (Shimadzu Corporation, figure 1). The system configuration is shown below. To prevent contamination from an equipment, a delay column was added between a mixer and an autosampler.

Nexera X3 system
Column : Shim-pack Scepter™ C18-120 (100 mm x 2.1 mm I.D., 3 µm)
Delay column : Shim-pack Scepter C18-120 (50 mm x 2.1 mm I.D., 3 µm)
Mobile phase A : Acetonitrile/water = 5:95(v/v) with 2 mmol/L Ammonium acetate
Mobile phase B : Acetonitrile
Rinse : Methanol/water = 50:50(v/v)
Flow rate : 0.3 mL/min (0.6 mL/min only between 10.01-12 min)
Time program : B conc. 20% (0 min) → 100% (10-12 min) → 20% (12.01-15 min) The flow was introduced into the mass spectrometer between 1 to 9.6 min using a flow switching valve.
Column temp. : 40 ºC Injection vol. : 5 µL

LCMS-8060NX
Ionization : ESI, Negative mode
DL temp. : 200 ºC
Interface temp. : 250 ºC
Heat block temp. : 400 ºC
Nebulizer gas : 2 L/min
Drying gas : 10 L/min
Heating gas : 10 L/min
Probe position : +2 mm

Figure 1. Nexera X3 and LCMS-8060NX

2-2. Extraction

The extraction procedure was performed with reference to the QuEChERS method. The flow is shown in Figure 2. A frozen and ground sample of 10 g was weighed and added with 10 mL of acetonitrile, then vigorously shaken for 1 minute. One packet of Q-sep QuEChERS extraction salt (Restek, P/N: 25849) was added and immediately shaken vigorously by hand for 1 minute. The mixture was centrifuged at 4,000 rpm at room temperature for 5 minutes, and the acetonitrile layer was collected. This acetonitrile layer was diluted 5 times with water to obtain the extraction solution.

2-3. Purification

EVOLUTE PFAS (Biotage, 150 mg/6 mL) and PRESSURE+ pressurized manifold (Biotage) was used for solid-phase purification. The flow is shown in figure 3. After conditioning with 5 mL of 28% ammonium hydroxide/methanol (1:100, v/v) and 5 mL of formic acid/methanol (1:1000, v/v), 40 mL of extraction solution (equivalent to 8 g of fish sample) was loaded. After washing with 5 mL of water and 5 mL of formic acid/methanol/water (1:400:600, v/v/v), the elution was performed with 5 mL of 28% ammonium hydroxide/methanol/water (1:90:10, v/v/v). The eluted solution was taken 500 µL and mixed with 2 µL of formic acid, then analyzed by LC-MS/MS.

3. Results

3-1. MS chromatogram and calibration curve

Figure 4 shows the MS chromatograms for simultaneous analysis of thirty PFAS compounds, and Figure 5 shows the calibration curves for representative compounds.

All compounds eluted within 8 minutes, indicating good separation. Additionally, although not shown in the figures, it has been confirmed that taurodeoxycholic acid (TDCA), taurolithocholic acid (TCDCA), and tauroursodeoxycholic acid (TUDCA) are sufficiently separated from PFOS using these conditions.

Good calibration curves can be obtained for all compounds in the range of 0.05 to 5 µg/kg, and the coefficient of determination R^2 was 0.98 for 10:2 FTS, and 0.99 or more for all other compounds, indicating good linearity.

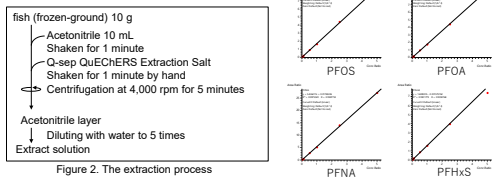


Figure 2. The extraction process

Figure 5. Calibration curves ranging from 0.05 - 5 µg/kg (spiked conc.)

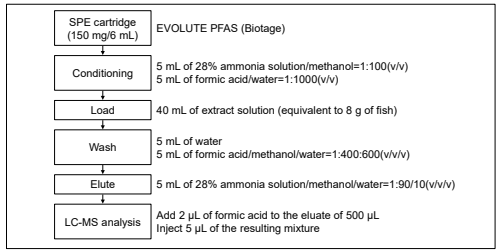


Figure 3. The purification process

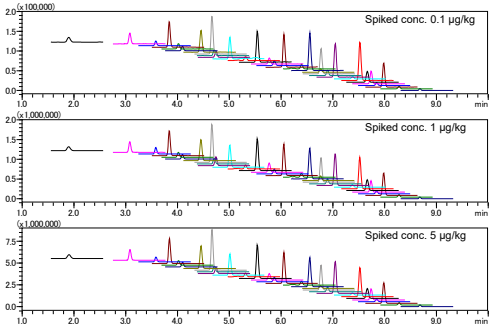


Figure 4. MS chromatograms of samples spiked with thirty PFAS compounds

3-2. Recovery rate test

Recovery tests were conducted at concentrations of 0.1, 1, and 5 µg/kg to verify the recovery rates and repeatability. Preprocessing was performed in triplicate, and matrix-matched calibration curves were used for quantification. According to the requirements of the AOAC SMPR, PFOS, PFOA, PFNA, and PFHxS have a LOQ of 0.1 µg/kg, a recovery rate of 80-120%, and a repeatability of less than 20%. Other PFAS compounds have a LOQ of 1.0 µg/kg, a recovery rate of 65-135%, and a repeatability of 25%. For all compounds, the recovery rates were within 80-120% and the repeatability was below 20% at the spiked concentrations of 0.1, 1, and 5 µg/kg.

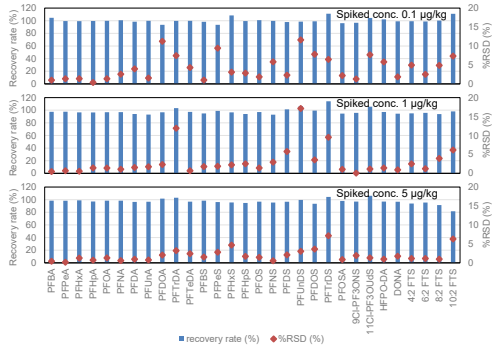


Figure 6. Recovery rate and repeatability (n=3)

3. Conclusions

- An LC-MS method for thirty PFAS within fifteen minutes analysis were created.
- The development of the pre-processing step, and a recovery test were conducted at 0.1, 1, and 5 µg/kg, resulting in favorable results. Recovery rates within 80-120% and repeatability below 20% were achieved for all compounds.

References
1) AOAC SMPR®2023.003

SHIMADZU
Excellence in Science

Application News

Liquid Chromatograph Mass Spectrometer LCMS-8060NX

Determination of various PFAS in egg matrix using stacked injection on-line SPE coupled to LC-MS/MS

Anja Grüning
Shimadzu Europa GmbH

User Benefits

- Single vendor solution for UHPLC and MS system
- Quantification of 27 PFAS in ng/mL range using an on-line SPE approach
- Increased sensitivity due to the stacked injection combined with on-line SPE

■ Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) refer to a class of more than 4000 individual chemicals that have been widely used since the 1950s, e.g. as fire retardants, food packaging materials or non-stick coatings. These compounds offer heat-resistant, and oil- and water-repellant properties as well as chemical and thermal stability, resistance to UV light and weathering. Due to their anthropogenic origin, PFAS cannot be degraded, and hence they accumulate and can now be detected ubiquitously in the environment. Due to this PFAS also found their way into the food chain and accordingly into our food. Concerns about human exposure through diet, studies on the status of food contamination are being conducted in various countries.

Here we describe the determination of various PFAS in egg matrix in a relevant concentration range. The analysis is based on a simple QuEChERS extraction coupled to an on-line SPE approach. This omits additional sample preparation steps like dSPE.

■ Materials and Methods

Fast, sensitive and robust LC-MS/MS systems provide the basis for routine analysis in food testing laboratories. For the described application, a Shimadzu LCMS-8060NX triple-quadrupole mass spectrometer coupled with a Nexera X3 UHPLC system was used (Figure 3).

27 PFAS standards and one IS-mixture (ISO 21675-LSS) were purchased (Wellington Laboratories / neochema). Stock solutions of these PFAS were diluted with methanol and combined to a single standard mixture with a final a concentration of 1ng/µL for each compound. Further dilutions of this mixture were produced to spike either the egg matrix before extraction or in case of calibrators, extracted egg matrix. Calibration samples in egg matrix were determined in the concentration range from 0.001 -0.025 ng/mL to 1 ng/mL. All samples (except blanks) were spiked with IS to a final concentration of 0.04 ng/mL.

Samples were extracted on the basis of QuEChERS AOAC method (Figure 1, RESTEK Q-Sep QuEChERS Extraction Packets AOAC Method). 50µL of sample was injected directly on a SPE-trap column using the stacked injection function offered by the Nexera SIL-40 autosampler. This results in 5x10 µL injections, where each injection is followed by aqueous sample loading phase removing the organic solvent from the sample extraction. This leads to improved trapping capability. With this approach higher volumes of the pure QuEChERS extract can be injected.

Analysis was performed within 15 minutes using MRM acquisition with at least two transitions for each compound (except PFBA, where only one transition is available).

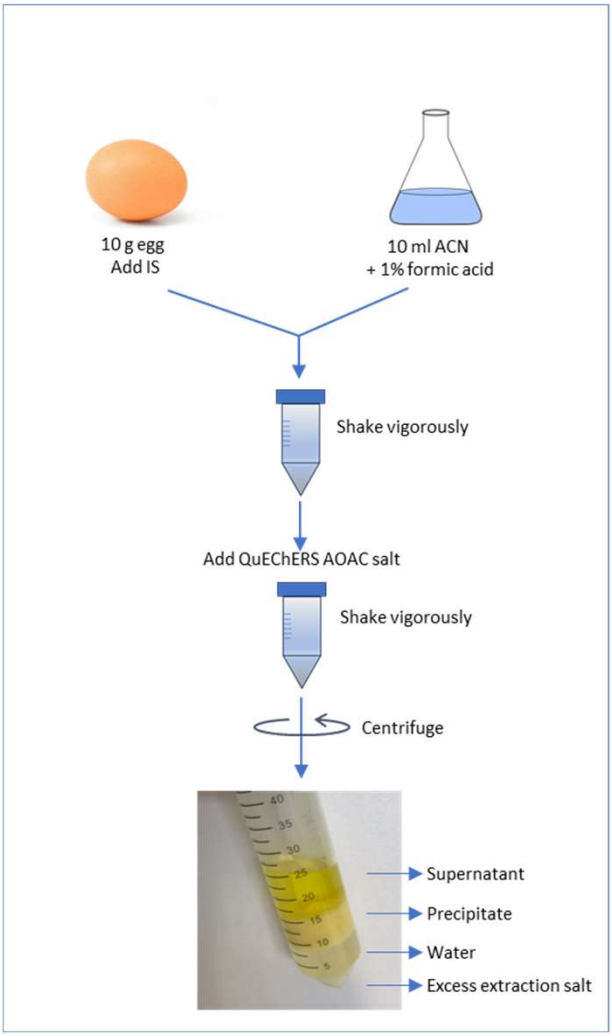


Figure 1 Extraction process

Analytical conditions are listed in Table 1. The optimized MRM transitions are summarized in Table 2.

Since PFAS can be present in reagents, glassware, pipettes, tubing, degassers and other parts from the LC-MS/MS instrument, the use of a solvent delay column is necessary. Small C18 columns are placed between mixer and autosampler respectively between mixer and valve to delay possible PFAS contaminations and separate them from sample-derived PFAS.

Table 1 Analytical conditions

Mass Spectrometer	: LCMS-8060NX
Ionization	: Electrospray Ionization (ESI), negative
Interface Voltage	: -1 kV
Focus Voltage	: -2.5 kV
Heating Gas	: 15 L/min
DL Temp.	: 150 ˚C
Interface Temp.	: 300 ˚C
Nebulizing Gas	: 3 L/min
Drying Gas	: 3 L/min
Heat Block	: 400 ˚C
Dwell-/Pause-time	: 4 (3 for IS) / 1 msec
CID	: 270 kPa

UHPLC	: Nexera X3
Pump A (Analytical)	: 2 mM ammonium acetate in H_2O
Pump B (Analytical)	: 2 mM ammonium acetate in Methanol
Pump C (Trap)	: H_2O + modifier (sample loading)
Pump D (Trap)	: Methanol (washing of SPE and delay column)
Analytical column	: Shim-pack™ Scepter 1.9 µm, C18-120, 2.1 x 50 mm
Delay column	: Shim-pack™ GIST HP 3 µm, C18-AQ, 3 x 30 mm
Trap column	: EVOLUTE® Express ABN on-line SPE cartridge
Injection Volume	: 5 x 10 µL
Cooler temperature	: 8 ˚C
Column Oven	: 50 ˚C
UHPLC	: Nexera X3

■ Results

Matrix matched calibration curves were calculated using weighted (1/conc) linear regression with an R^2 of >0.98 for all PFAS. Exemplary calibration curves and respective MRM-chromatograms at 0.1 ng/mL are shown in Figure 2.

All tested eggs already contained certain PFAS. These PFAS were marked with an asterisk. Lowest calibration point was adapted accordingly. Depending on availability of an appropriate ISTD either internal or external standard method was used for quantification.

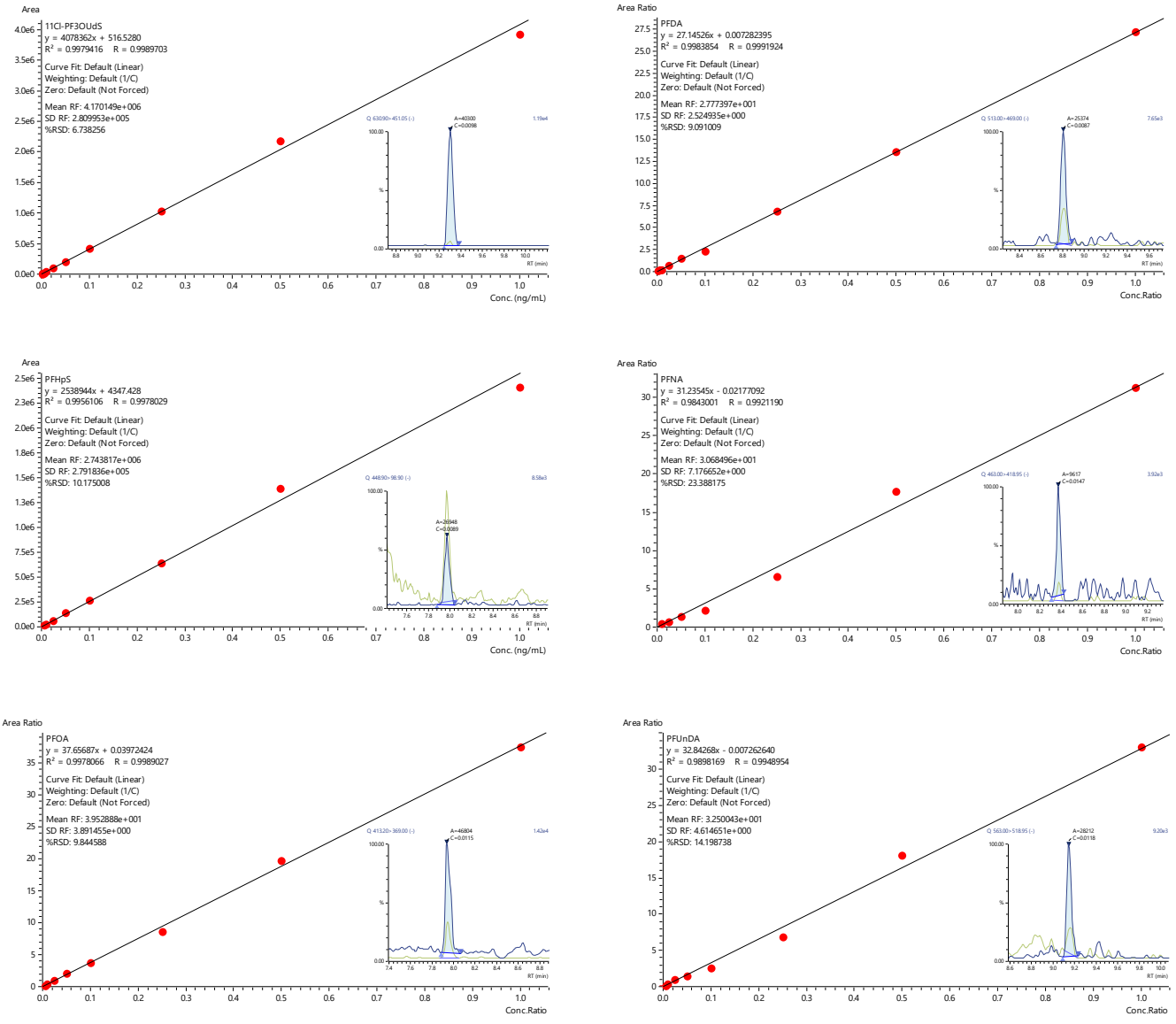


Figure 2 Exemplary calibration curves and a typical chromatogram at 0.01 ng/mL level

Five eggs from different origins were purchased locally and analysed together with the calibration samples. Results are shown in Table 4. In addition, these eggs were spiked with PFAS before extraction at concentrations of 0.01 ng/mL and 0.1 ng/ml.

The percentage relative standard deviation was typically lower than 20% (for 95% of the determined compounds resp. QCs) from these spiked samples (Table 3). Eggs where some PFAS could be detected at a relatively high level were not taken into account for the respective calculations.

Table 2 MRM transitions and calibration information

Acronym	RT	Type	ISTD used	Quantifier	Qualifier	Calibration range	Unit	R^2
11Cl-PF3OUdS	9.309	Target	----	630.90>451.05	630.90>82.95	0.001-1	ng_mL	0.9979
9Cl-PF3ONS	8.648	Target	PFOS-IS	530.90>351.10	530.90>82.90	0.001-1	ng_mL	0.9989
DONA	7.479	Target	PFHpA-IS	377.10>251.00	377.10>84.95	0.001-1	ng_mL	0.9957
FOSA	9.313	Target	FOSA-IS	497.90>77.90	497.90>478.15	0.01-1	ng_mL	0.9959
FOSA-IS	9.312	ISTD	----	505.90>78.00	505.90>172.00	----	ng_mL	----
HFPO-DA*	6.946	Target	HFPO-DA-IS	284.95>169.05	284.95>185.05	0.01-1	ng_mL	0.9945
HFPO-DA-IS	6.946	ISTD	----	286.85>168.90	286.85>118.85	----	ng_mL	----
PFDoS	9.674	Target	PFDoDA-IS	699.00>79.90	699.00>98.90	0.0025-1	ng_mL	0.9912
PFTrDS	9.878	Target	PFDoDA-IS	749.00>99.10	749.00>79.90	0.0025-1	ng_mL	0.9867
PEESA	6.538	Target	----	315.00>135.00	315.00>82.90	0.001-1	ng_mL	0.9989
PFBA**	4.547	Target	PFBA-IS	213.00>169.00	----	0.01 -1	ng_mL	0.9846
PFBA-IS	4.541	ISTD	----	216.90>172.00	----	----	ng_mL	----
PFBS**	5.982	Target	PFBS-IS	299.00>79.90	299.00>98.90	0.01 -1	ng_mL	0.9997
PFBS-IS	6.139	ISTD	----	301.90>98.80	301.90>79.80	----	ng_mL	----
PFDA	8.802	Target	PFDA-IS	513.00>469.00	513.00>219.05	0.0025-1	ng_mL	0.9984
PFDA-IS	8.814	ISTD	----	519.00>473.90	519.00>219.00	----	ng_mL	----
PFDoDA	9.454	Target	PFDoDA-IS	613.00>568.95	613.00>169.10	0.01-1	ng_mL	0.9979
PFDoDA-IS	9.451	ISTD	----	614.90>570.10	614.90>269.10	----	ng_mL	----
PFDS	9.155	Target	PFOS-IS	598.80>79.95	598.80>98.85	0.0001-1	ng_mL	0.9971
PFHpA	7.389	Target	PFHpA-IS	363.10>319.00	363.10>169.00	0.0025-1	ng_mL	0.9905
PFHpA-IS	7.381	ISTD	----	367.00>322.10	367.00>169.00	----	ng_mL	----
PFHpS	7.974	Target	----	448.90>98.90	448.90>79.90	0.005-1	ng_mL	0.9956
PFHxA	6.693	Target	PFHxA-IS	313.10>269.00	313.10>119.00	0.01-1	ng_mL	0.9994
PFHxA-IS	6.692	ISTD	----	317.90>273.00	317.90>120.10	----	ng_mL	----
PFHxDA-IS	10.208	ISTD	----	814.90>769.90	814.90>369.00	----	ng_mL	----
PFHxS**	7.468	Target	PFHxS-IS	398.90>79.95	398.90>98.90	0.005-1	ng_mL	0.9988
PFHxS-IS	7.636	ISTD	----	402.00>79.90	402.00>98.80	----	ng_mL	----
PFNA	8.392	Target	PFNA-IS	463.00>418.95	463.00>219.00	0.01-1	ng_mL	0.9843
PFNA-IS	8.375	ISTD	----	471.90>427.00	471.90>223.00	----	ng_mL	----
PFNS	8.809	Target	----	549.10>79.90	549.10>98.90	0.005-1	ng_mL	0.9965
PFOA**	7.943	Target	PFOA-IS	413.20>369.00	413.20>169.05	0.005-1	ng_mL	0.9978
PFOA-IS	7.951	ISTD	----	421.00>376.10	421.00>172.00	----	ng_mL	----
PFOS	8.387	Target	PFOS-IS	498.90>98.90	498.90>169.05	0.025-1	ng_mL	0.9858
PFOS-IS	8.368	ISTD	----	506.90>79.90	506.90>98.80	----	ng_mL	----
PFPeA	5.771	Target	PFPeA-IS	263.10>219.00	263.10>69.10	0.01-1	ng_mL	0.9989
PFPeA-IS	5.861	ISTD	----	267.90>223.00	267.90>69.10	----	ng_mL	----
PFPeS / PFPS	6.992	Target	----	349.20>79.95	349.20>98.95	0.005-1	ng_mL	0.9972
PFTeDA	9.896	Target	PFTeDA-IS	713.00>669.05	713.00>169.05	0.005-1	ng_mL	0.9804
PFTeDA-IS	9.892	ISTD	----	714.90>670.00	714.90>368.90	----	ng_mL	----
PFTrDA	9.698	Target	PFDoDA-IS	663.00>619.00	663.00>169.00	0.005-1	ng_mL	0.9877
PFUnDA	9.143	Target	PFUnDA-IS	563.00>518.95	563.00>269.05	0.005-1	ng_mL	0.9898
PFUnDA-IS	9.15	ISTD	----	570.00>524.90	570.00>268.90	----	ng_mL	----
PFUnDS	9.601	Target	----	649.00>79.95	649.00>98.95	0.0025-1	ng_mL	0.9917

* Contamination from ISTD
** Contamination from egg matrix

Table 3 Reproducibility of spiked samples

	11Cl-PF3OUdS		9Cl-PF3ONS		DONA		FOSA		HFPO-DA		L-PFDoS		L-PFTrDS		PEESA		PFBA	
	0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL	
	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy
Egg A QC 0.01	0.0101	100.64	0.0097	96.95	0.0102	102.08	0.0117	116.99	0.0088	88.39	0.0104	104.38	0.0067	67.41	0.0102	101.8	below LOQ	
Egg B QC 0.01	0.0107	106.84	0.0093	93.10	0.0096	95.63	0.0103	102.72	0.0062	61.63	0.0149	149.13	0.0117	117.46	0.0101	101.04	below LOQ	
Egg C QC 0.01	0.0092	91.70	0.0104	104.12	0.0100	100.08	0.0115	114.69	0.0112	111.54	0.0102	101.98	0.0108	108.27	0.0104	104.31	below LOQ	
Egg D QC 0.01	0.0113	113.08	0.0093	93.36	0.0087	87.41	0.0116	115.99	0.0099	98.96	0.0100	100.01	0.0061	61.48	0.0105	104.70	below LOQ	
Egg E QC 0.01	0.0109	108.64	0.0096	96.32	0.0098	98.12	0.0101	101.33	0.0073	72.54	0.0113	112.64	0.0117	117.26	0.0102	102.18	below LOQ	
Mean		104.18		96.77		96.66		110.34		86.61		113.63		94.38		102.81		
SD		8.28		4.45		5.70		7.65		19.99		20.42		27.65		1.61		
%RSD		7.95		4.60		5.89		6.94		23.09		17.97		29.30		1.57		
	0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL	
	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy
Egg A QC 0.1	0.0992	99.25	0.0922	92.24	0.1027	102.72	0.0965	96.47	0.1102	110.25	0.1105	110.53	0.0984	98.35	0.1062	106.16	0.1551	*155.15
Egg B QC 0.1	0.0816	81.65	0.0877	87.75	0.0971	97.10	0.1115	111.48	0.1055	105.46	0.1281	128.08	0.1112	111.16	0.1052	105.17	0.3562	*356.16
Egg C QC 0.1	0.0894	89.42	0.0916	91.58	0.0945	94.55	0.0985	98.47	0.1010	100.99	0.1028	102.79	0.0956	95.60	0.1018	101.79	0.1057	*105.71
Egg D QC 0.1	0.0923	92.31	0.0831	83.09	0.0963	96.33	0.1136	113.57	0.0993	99.32	0.1037	103.71	0.1143	114.29	0.1020	101.96	0.1197	*119.69
Egg E QC 0.1	0.1060	105.99	0.0919	91.92	0.0934	93.35	0.0906	90.64	0.1064	106.37	0.1022	102.22	0.0992	99.21	0.1061	106.13	0.2795	*279.55
Mean		93.72		89.32		96.81		102.13		104.48		109.47		103.72		104.24		
SD		9.32		3.93		3.62		9.95		4.38		10.93		8.40		2.20		
%RSD		9.94		4.40		3.74		9.74		4.19		9.98		8.10		2.11		

	PFBS		PFDA		PFDoDA		PFDS		PFHpA		PFHpS		PFHxA		PFHxS		PFNA	
	0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL	
	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy
Egg A QC 0.01	0.0100	99.89	0.0124	123.67	0.0081	80.88	0.0097	97.40	0.0097	97.33	0.0099	99.04	0.0107	106.80	0.0101	101.16	0.0093	92.70
Egg B QC 0.01	0.0117	116.9	0.0347	*346.61	0.0314	*314.12	0.0093	92.53	0.0125	*124.63	0.0108	107.58	0.0111	111.20	0.0205	*204.82	0.0253	*252.65
Egg C QC 0.01	0.0119	119.42	0.0107	106.55	0.0106	105.54	0.0088	87.75	0.0096	96.29	0.0103	102.74	0.0103	103.00	0.0089	88.72	0.0153	153.41
Egg D QC 0.01	0.0095	95.05	0.0105	104.55	0.0090	90.09	0.0072	71.81	0.0091	90.63	0.0098	97.96	0.0115	114.63	0.0111	111.11	0.0115	115.49
Egg E QC 0.01	0.0092	92.33	0.0297	*297.08	0.0315	*314.53	0.0085	85.27	0.0148	*147.64	0.0116	116.28	0.0121	121.23	0.0370	*370.26	0.0348	*347.85
Mean		104.72		111.59		92.17		86.95		94.75		104.72		111.37		100.33		120.53
SD		12.60		10.51		12.46		9.66		3.61		7.48		7.05		11.22		30.67
%RSD		12.03		9.42		13.52		11.11		3.81		7.14		6.33		11.18		25.44
	0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL	
	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy
Egg A QC 0.1	0.0975	97.52	0.1148	114.81	0.0974	97.43	0.0901	90.11	0.0973	97.26	0.1074	107.45	0.1039	103.89	0.1038	103.78	0.0906	90.59
Egg B QC 0.1	0.0945	94.50	0.1168	116.80	0.1076	107.56	0.0926	92.62	0.1013	101.27	0.1107	110.70	0.0986	98.58	0.1082	108.25	0.0925	92.55
Egg C QC 0.1	0.1001	100.07	0.0832	83.20	0.0863	86.28	0.0979	97.90	0.1051	105.10	0.1127	112.74	0.0986	98.62	0.0816	81.56	0.1043	104.30
Egg D QC 0.1	0.0965	96.50	0.1006	100.57	0.0921	92.13	0.0991	99.13	0.0980	98.05	0.1087	108.71	0.0964	96.41	0.0956	95.64	0.1056	105.57
Egg E QC 0.1	0.0937	93.66	0.1312	131.21	0.1040	104.03	0.0854	85.37	0.0938	93.79	0.1153	115.34	0.1040	103.99	0.1111	111.13	0.1194	119.44
Mean		96.45		109.32		97.49		93.03		99.09		110.99		100.30		100.07		102.49
SD		2.54		18.19		8.64		5.66		4.28		3.15		3.44		11.89		11.62
%RSD		2.64		16.64		8.86		6.09		4.32		2.84		3.43		11.88		11.34

	PFNS		PFOA		PFOS		PFPeA		PFPS		PFTeDA		PFTrDA		PFUnDA		PFUnDS	
	0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL	
	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy
Egg A QC 0.01	0.0109	109.26	0.0098	97.74	below LOQ		0.0070	69.92	0.0102	102.39	0.0110	109.83	0.0085	85.08	0.0110	109.54	0.0086	86.25
Egg B QC 0.01	0.0108	108.41	0.0510	*509.67	below LOQ		0.0084	84.20	0.0104	103.83	0.0183	*183.07	0.0265	*265.47	0.0248	*247.73	0.0123	122.98
Egg C QC 0.01	0.0100	99.86	0.0115	114.81	below LOQ		0.0118	117.66	0.0104	103.71	0.0091	90.60	0.0074	73.54	0.0111	111.06	0.0090	90.32
Egg D QC 0.01	0.0095	95.28	0.0105	104.76	below LOQ		0.0104	103.82	0.0097	97.22	0.0115	115.26	0.0085	85.21	0.0111	111.27	0.0082	82.14
Egg E QC 0.01	0.0106	106.16	0.0923	*922.92	below LOQ		0.0091	90.57	0.0113	113.23	0.0196	*196.07	0.0330	*329.81	0.0230	*229.98	0.0111	111.09
Mean		103.79		105.77				93.23		104.08		105.23		81.28		110.62		98.56
SD		6.02		8.58				18.31		5.78		12.96		6.70		0.94		17.62
%RSD		5.80		8.11				19.64		5.56		12.31		8.24		0.85		17.88
	0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL	
	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy
Egg A QC 0.1	0.0971	97.10	0.1009	100.88	0.0856	85.60	0.0986	98.56	0.1131	113.11	0.1189	118.90	0.1067	106.69	0.1024	102.41	0.0900	89.99
Egg B QC 0.1	0.0855	85.53	0.1410	*140.97	0.3793	*379.3	0.1044	104.40	0.1036	103.57	0.1480	*148.04	0.1265	126.50	0.1198	119.76	0.0796	79.64
Egg C QC 0.1	0.0881	88.14	0.1042	104.22	0.0953	95.29	0.0976	97.58	0.1015	101.55	0.1143	114.29	0.0943	94.32	0.0992	99.23	0.0894	89.45
Egg D QC 0.1	0.1008	100.85	0.0908	90.83	0.0999	99.93	0.0949	94.93	0.1006	100.64	0.1053	105.34	0.1060	105.96	0.0976	97.57	0.0794	79.39
Egg E QC 0.1	0.1035	103.45	0.1707	*170.69	0.43	*429.96	0.0955	95.49	0.1087	108.69	0.1514	*151.42	0.1179	117.94	0.1181	118.07	0.0956	95.59
Mean		95.01		98.64		93.61		98.19		105.51		112.84		110.28		107.41		86.81
SD		7.85		6.97		7.31		3.77		5.27		6.89		12.33		10.66		7.08
%RSD		8.27		7.07		7.81		3.84		4.99		6.11		11.18		9.93		8.16

*compound already found in sample

Table 4 Sample results (positive results only)

	PFBA	PFBS	PFDA	PFDoDA	PFHpA	PFHpS	PFHxS	PFNA	PFOA	PFOS	PFPeA	PFTeDA	PFTrDA	PFUnDA	PFUnDS
	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.
Egg A	<LOQ	<LOQ	----	<LOQ	----	----	----	----	----	----	----	----	----	----	----
Egg B	0.2662	<LOQ	0.0221	0.0167	0.0032	----	0.0125	0.0273	0.0411	0.3121		0.0125	0.0182	0.0144	----
Egg C	<LOQ	<LOQ	----	----	----	----	----	----	<LOQ	----	0.0114	----	----	----	----
Egg D	<LOQ	<LOQ	----	----	----	----	<LOQ	----	<LOQ	----	----	----	----	----	----
Egg E	0.1912	<LOQ	0.0207	0.0153	0.0043	<LOQ	0.0257	0.0198	0.1009	0.2567	----	0.0151	0.0162	0.013	0.0026

Figure 3 Scheme of the Nexera on-line SPE LCMS-8060NX system

■ The Package

❑ *Main Unit*

LCMS-8060NX:	TQ Mass spectrometer
Nexera X3:	Liquid chromatograph CBM-40 DGU-405 2x LC-40D X3 LC-40B X3 SIL-40C X3 CTO-40S 2x Reservoir Tray

❑ *Accessory*

Valve:	FCV-0206H3
Mixer:	2x Mir20 µL
Loop:	50 µL

❑ *Main Consumables*:

Shim-pack Scepter C18
(50 mm x 2.1 mm I.D., 1.9 µm; P/N 227-31012-03)

Shim-pack GIST HP C18-AQ (2x)
(30 mm x 3.0 mm I.D., 3 µm; P/N 227-30766-01)

EVOLUTE® Express ABN on-line SPE cartridge (Biotage)
(30 mm x 2.1 mm I.D; P/N OSPE-620-32150)

Shimadzu LabTotal Vial for LC/LCMS
(P/N 227-34001-01)

RESTEK® Q-Sep QuEChERS Extraction Packets / AOAC Method
(P/N 25851)

❑ *Software and Libraries*

LabSolutions LCMS

LabSolutions Insight

■ Conclusions

This application note describes an on-line SPE LC-MS/MS method to monitor 27 PFAS and internal standards in egg matrix. This proof of concept study using the LCMS-8060NX coupled with a Nexera UHPLC system equipped for on-line SPE demonstrates a sensitive method for PFAS analysis in egg matrix with minimal sample preparation steps.

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/

SHIMADZU Europa GmbH,
www.shimadzu.eu

05-SCA-210-070-EN First Edition: Aug. 2023

PFAS in Food Contact Materials (FCM) / LCMS-8050

Application News

Analysis of Per- and Polyfluoroalkyl Substances in Fast Food Packaging by LC-MS/MS Method

Elyson Keith P. Encarnacion[1], Anne C. Alcantara[1], Harold E. Armario[1], Winnie P. Alejandro[1], Zhaoqi Zhan[2], Zhe Sun[2], Lin Ng[3]
1 Industrial Technology Development Institute, Philippines, 2 Shimadzu (Asia Pacific), Singapore
3 Internship Student from School of Chemical & Life Sciences, Singapore Polytechnic

User Benefits

- A direct LC-MS/MS method was established for quantitative determination of 15 targeted PFAS compounds in food contact materials (FCM) using LCMS-8050.
- The results show that 12 out of 15 targeted PFAS were present in seven fast food packaging samples. The concentrations of the PFAS were far below the limit set by the Danish Ministry of Environmental and Food Guideline in 2015.

■ Introduction

Per- and polyfluoroalkyl substances (PFAS) have been detected in waters, soils, sediments, fish, foods and human blood among others. Recently, PFAS were reported in disposable fast food packaging such as paper wrappers, paperboard clamshells and beverage cups [1,2]. In 2015, the Danish Ministry of Environmental and Food set a guideline to limit the maximum total organic fluorine to 0.35 ug/dm^2 for food contact materials (FCM) [3]. This value corresponds to 0.5 ug of PFOA/dm^2. However, the same organization has banned the use of all fluorinated substances in FCM since 2020 [4].

Targeted screening and quantitation of PFAS in drinking water by LC-MS/MS are well established with reference to the US EPA Method 537 [5]. Publications on PFAS analysis in FCM have become available in recent years. Laurel A. Schaider et al. [6] reported their results of PFAS analysis using PIGE and LC-HRMS methods on over 400 FCM samples. In this Application News, an LC-MS/MS method for the detection and quantitation of 15 PFAS, including PFOA and PFOS, in fast food packaging samples is presented. The same sample pre-treatment used by Schaider et al. [6] was adopted to extract PFAS from the samples.

■ Experimental

Reagents and PFAS Standards

Acetonitrile (LC-MS grade) and methanol (LC-MS grade) were obtained from commercial suppliers. Ammonium acetate (>99%) of LC-MS grade was used as additive in the mobile phase prepared with Milli-Q water. Fifteen PFAS standards (Table 2) were purchased from Wellington Laboratories and Apollo Scientific. M-PFOS (with $^{13}C_4$) and M-PFOA (with $^{13}C_4$) were used as internal standards during method development.

Sample preparation

Seven FCM samples including paper wrappers, paperboard clamshells and beverage cups were cut into 10 cm x 10 cm (100 cm^2) and weighed. Each sample was further cut into smaller pieces for extraction and immersed in 20 mL of MeOH in a polypropylene (PP) centrifuge tube. Approximately 4 mL of the extract was cleaned using Supelclean™ ENVI-Carb™ SPE (6 mL/500 mg). The collected extract was evaporated to dryness with N_2 on a TurboVap LV evaporator (Biotag). The dried sample was reconstituted with 0.8 mL of 5 mM ammonium acetate solution and transferred into a 1.5 mL glass vial for LC-MS analysis (Fig. 1)

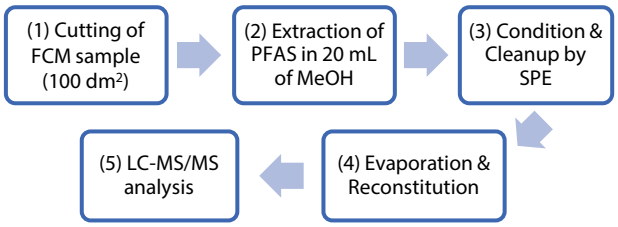


Figure 1 Flowchart of sample pre-treatment for PFAS in food contact materials (FCM).

LC-MS/MS analytical conditions

Details of the analytical conditions for PFAS using LCMS-8050 (Shimadzu Corporation, Japan) with a Shim-pack Velox C18 column are shown in Table 1.

Table 1 Analytical conditions of PFAS on LCMS-8050

LC Conditions	
Column	Shim-pack Velox™, C18 (2.1 X 100 mm, 2.7 µm)
Flow Rate	0.4 mL/min
Mobile Phase	A: 5 mM Ammonium acetate in water B: Acetonitrile
Elution mode	Gradient elution, 12 mins
Oven Temp.	40°C
Injection Vol.	10 µL
Interface Conditions	
Interface	Heated ESI
Interface Temp.	300°C
DL Temp.	250°C
Heat Block Temp.	400°C
Nebulizing Gas	2 L/min
Heating Gas Flow	10 L/min
Drying Gas Flow	10 L/min
MS mode	MRM, negative mode

■ Results and Discussion

MRM Method Setup

An LC-MS/MS method was developed for the detection and quantitation of 15 PFAS compounds in negative MRM mode (Table 2). A mixed standard was prepared from individual stock solutions to generate calibration curves. Approximately 40 µL of each PFAS stock solution (50 ppm) was transferred into a 1.5 mL LC sample vial and diluted with 400 µL of pure water, resulting in a 2 ppm mixed standard. Calibration series, with or without internal standards (M-PFOS and M-PFOA), were prepared from the mixed standard using pure water as the diluent.

Two MRM transitions were optimized for each compound except for PFBA and PFPeA with only one (Table 2).

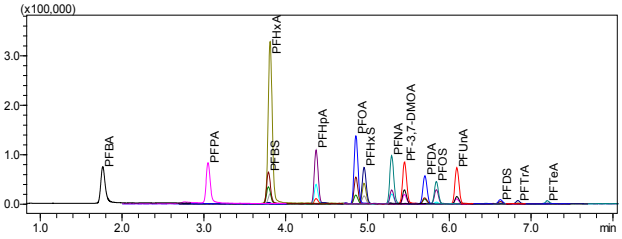


Figure 2 MRM peaks of 15 PFAS standards (1 ppb each).

With an optimized LC gradient elution, all 15 PFAS were eluted within 8 mins (Figure 2).

Table 2 MRM parameters, retention times and calibration curve ranges of 15 PFAS using LCMS-8050

Event #	PFAS (Abbr.)	Formula	CAS No.	Exact Mass	MRM (quantifier)	CE (V)	RT (min)	Range (ng/mL)	R^2
1	PFBA	C4HF7O2	375-22-4	214.0	212.9>169.1	10	1.76	0.1 ~ 10	0.999
2	PFPeA	C5HF9O2	2706-90-3	264.0	262.9>219.1	8	3.05	0.1 ~ 10	0.999
3	PFBS	C4F9SO3H	29420-49-3	300.0	298.9>79.9	31	3.79	0.1 ~ 10	0.993
4	PFHxA	C6HO2F11	307-24-4	314.0	313.0>269.1	9	3.81	0.1 ~ 10	0.998
5	PFHpA	C7HF13O2	375-85-9	364.0	362.9>319.1	10	4.37	0.1 ~ 10	0.999
7	PFOA	C8HF15O2	335-67-1	414.0	413.0>369.1	10	4.86	0.1 ~ 10	0.999
8	PFHxS	C6F13HO3S	82382-12-5	399.9	398.9>79.9	45	4.96	0.1 ~ 10	0.996
9	PFNA	C9HF17O2	375-95-1	464.0	463.0>419.0	10	5.30	0.1 ~ 10	0.998
10	PF-3,7-DMOA	C10HF19O2	172155-07-6	514.0	469.0>269.1	22	5.45	0.1 ~ 10	0.996
11	PFDA	C10HF19O2	335-76-2	514.0	513.0>469.1	11	5.70	0.1 ~ 10	0.987
13	PFOS	C8F17O3HS	4021-47-0	499.9	499.0>79.9	54	5.84	0.1 ~ 10	0.999
14	PFUnA	C11HF21O2	2058-94-8	564.0	563.0>519.1	11	6.09	0.1 ~ 10	0.952
15	PFDS	C10HF21SO3	2806-15-7	599.9	599.0>79.9	55	6.62	0.2 ~ 10	0.988
16	PFTrA	C13HO2F25	72629-94-8	664.0	663.0>619.0	13	6.84	0.5 ~ 10	0.996
17	PFTeA	C14HO2F27	376-06-7	714.0	712.9>668.9	13	7.20	1 ~ 10	0.988

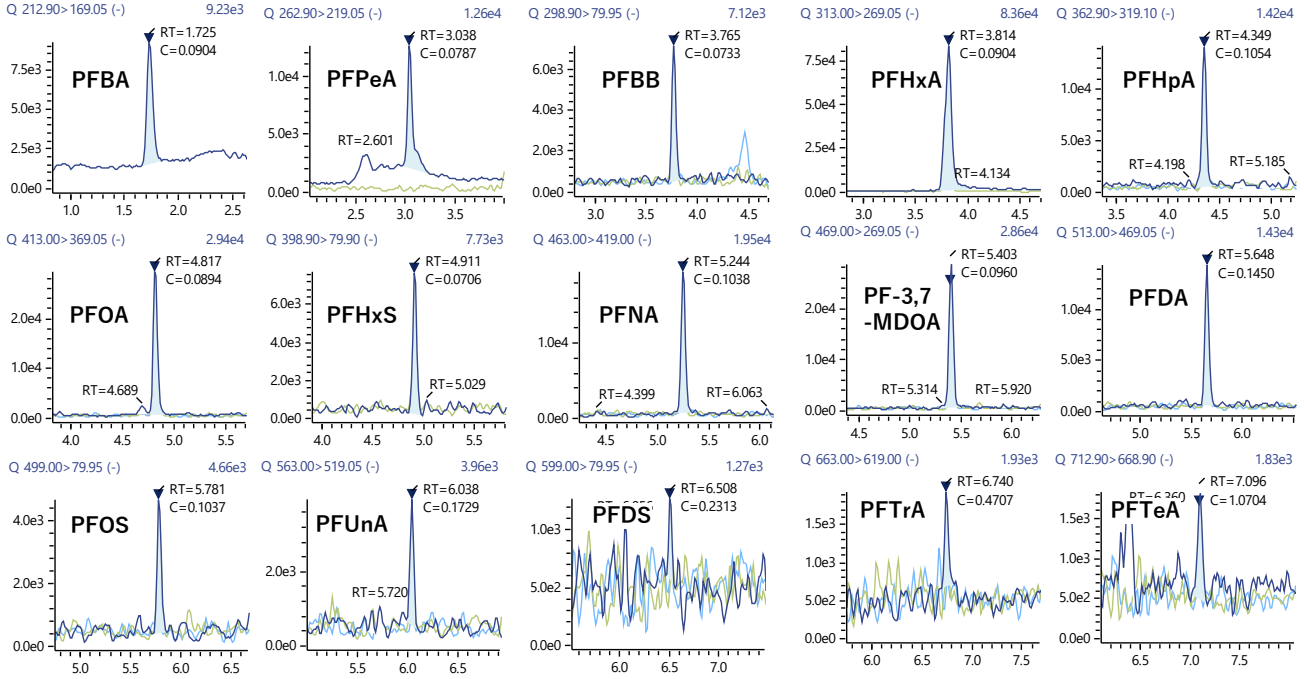


Figure 3 Individual MRM peaks of 15 PFAS compounds at the lowest calibration levels (refer to Table 2) using LCMS-8050

Calibration curves of 15 PFAS

Linear calibration curves were established with or without internal standards. Table 2 shows the R^2 values and ranges without the use of internal standards. The LODs of the method are lower than 0.1 ppb for most PFAS except for PFDS, PFTrA and PFTeA. The MRM peaks of the lowest calibration levels are shown in Figure 3.

Results of PFAS in fast food packaging

Seven fast food packaging samples (Table 3) were analysed using the validated LC-MS/MS method with external calibration. Sample pre-treatment described previously (Figure 1) was employed using MeOH for extraction and SPE for removal of pigments, dyes etc. The same pure water (Milli-Q) for preparing the standards was used as blank in the batch run of LC-MS/MS analysis.

Table 3 Fast food packaging samples for PFAS screening by LC-MS/MS method

S. No.	FCM Type	Description	Weight (mg /100 cm^2)
P1	paper	wrapping paper	302.4
P2	paper	pouch	551.3
P3	paper	wrapping paper	306.5
P4	paperboard	clamshell	2686.9
P5	paperboard	clamshell	2831.0
P6	paperboard	clamshell	2765.6
P7	paperboard	beverage cup	2406.2

Each sample was analysed in triplicate using LC-MS/MS to ensure repeatability of results (RSD < 10%). The average results of the seven fast food packaging samples are shown in Table 4, while PFAS profiles of P1, P3 and P6 are shown in Figure 4. Multiple PFAS were found in every sample. PFOA, a PFAS banned under the POPs regulation in 2020, was detected in every sample ranging from 0.19 ng/dm^2 to 1.69 ng/dm^2. In contrast, PFOS was not detected in all the samples. The highest amount PFAS found is PF-3,7-DMOA (up to 53.03 ng/dm^2). It is worth to note that the total amounts of the 15 PFAS measured range from 6.07 ng/dm^2 (P3) to 95.9 ng/dm^2 (P1), which levels are far below the limit set by the Danish Ministry of Environment and Food in 2015 for total organic fluorine (0.35 ug/dm^2) in food contact materials (FCM). Laurel A. Schaider et al. [6] reported various fluorinated compounds, including known PFAS, in 20 FCM samples by LC-HRMS, with 70% having a total fluorine level greater than 200 nmol/cm^2.

■ Conclusion

An LC-MS/MS method was established for quantitative determination of 15 PFAS compounds in food contact materials using LCMS-8050. Twelve (12) out of the 15 PFAS compounds studied were present in seven fast food packaging samples. The total amounts of PFAS (6.07-95.9 ng/dm^2) were far below the limit set by Danish Ministry of Environment and Food in 2015.

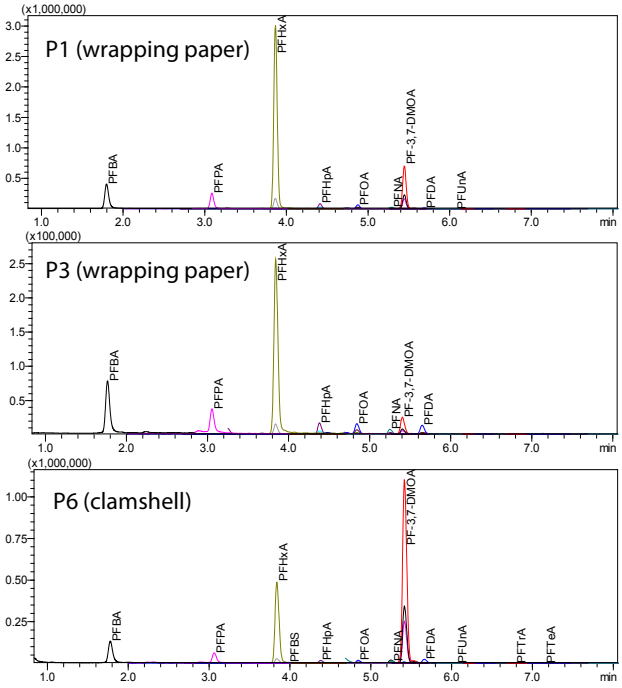


Figure 4 PFAS detected in P1, P3 and P 6 samples.

Table 4 Types and amounts of PFAS in seven fast food packaging samples

PFAS (Abbr.)	PFAS Content (ng/dm^2)						
	P1	P2	P3	P4	P5	P6	P7
PFBA	19.89	2.59	2.59	4.43	6.32	7.02	3.93
PFPeA	10.93	1.46	1.47	1.87	2.48	3.20	2.1
PFBS	-	-	-	-	-	0.32	-
PFHxA	30.47	1.68	1.15	2.91	3.66	5.33	2.42
PFHpA	2.58	0.44	0.21	0.62	0.46	0.59	0.53
PFOA	1.69	0.23	0.17	0.73	0.19	0.33	0.24
PFHxS	-	-	-	-	-	-	-
PFNA	0.32	0.11	0.09	0.44	0.17	0.41	0.17
PF-3,7-DMOA	28.98	0.36	0.2	2.14	1.41	53.03	0.56
PFDA	0.92	0.36	0.2	2.1	0.59	1.73	0.51
PFOS	-	-	-	-	-	-	-
PFUnA	0.12	-	-	-	-	0.07	-
PFDS	-	-	-	-	-	-	-
PFTrA	-	-	-	0.92	1.23	0.91	-
PFTeA	-	-	-	1.73	1.96	2.24	-
Total	**95.9**	**7.23**	**6.07**	**17.88**	**18.47**	**75.17**	**10.45**

■ References

1. Strakova, J., Cingotti, N., et al., "Throwaway packing, Forever Chemicals: European wide survey of PFAS in disposable food packaging and tableware", May 2021.
2. Trier, X et al., "Polyfluorinated surfactants (PFS) in paper and board coatings for food packaging", Env Sci Pollut Res 2011; 18(7):1108-20.
3. Ministry of Environment and Food of Denmark, "Fluorinated substances in paper and board food contact materials (FCM)", 2015.
4. Ministry of Environment and Food of Denmark, "Ban on fluorinated substances in paper and board food contact materials (FCM)", 2020.
5. Brahm P. et al., "Analysis of PFAS Specified in EPA Method 537 using Shimadzu UFMS", Shimadzu Application News C184A (2019).
6. Laurel A. Schaider et al., "Fluorinated Compounds in U.S. Fast Food Packaging", Environ Sci Technol Lett. 2017; 4(3): 105–111.

04-AD-0269-EN First Edition: May 2024

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/

SHIMADZU (Asia Pacific) Pte. Ltd,
www.shimadzu.com.sg

SHIMADZU
Excellence in Science

PFAS untargeted screening / LCMS-9030

Application News

Untargeted Screening of Per- and Polyfluoroalkyl Substances by HRAM-DIA Method on LCMS-9030

Zhaoqi Zhan[1], Zhe Sun[1], Jade Ting Shuen Chan[2]
[1] Shimadzu (Asia Pacific) Pte. Ltd, Singapore

User Benefits

- A sensitive untargeted screening method was established based on HRAM-DIA data acquisition on LCMS-9030. The method was successfully verified with 14 PFAS standards at 1 ng/mL in water.
- Data analysis was performed using LabSolutions Insight Explore™ – Analyze. PFAS-like species could be extracted by specific elemental settings which functions as mass defect filtering for PFAS. This procedure was used for an unknown sample, and 16 PFAS-like species were discovered and characterized.

■ Introduction

Contamination of per- and polyfluoroalkyl substances (PFAS) are found everywhere in water, soils, sediments, fish, foods, textiles and human blood etc. Targeted screening and quantitation of PFAS in drinking water by LC-MS/MS are established in reference to the US EPA Method 537 and ISO 21675: 2019, ASTM D7979 etc. Such MRM-based methods are widely adopted in the analysis of up to 29 PFAS or more [1]. However, PFAS represents a large collective of compounds [2] and many of them are not determined by the existing methods. This study aims to establish a method of untargeted screening for both known and undiscovered PFAS in water samples. The method was established based on LC-Q-TOF data, i.e., HRAM spectrum (MS) and DIA deconvolution spectrum (MS/MS), relying on the specific mass defect feature of PFAS [3, 4] using LabSolutions Insight Explore – Analyze program. Fourteen PFAS including PFOA and PFOS were used as standards for verifying the performance of this HRAM-DIA method in terms of detection sensitivity and identification. The established approach was applied to real water sample analysis and the found PFAS-like species were further characterized via database and library searches, and structural elucidation using LabSolutions Insight Explore – Assign program.

■ Experimental

Reagents, PFAS standards and samples

Acetonitrile (LCMS grade) and methanol (LCMS grade) were obtained from commercial suppliers. Ammonium acetate (>99%) of LCMS grade was used as additives in the mobile phase prepared from Milli-Q water. Sixteen PFAS standards (Table 2) were purchased from Wellington Laboratories and Apollo scientific. M-PFOS (with $^{13}C_4$) and M-PFOA (with $^{13}C_4$) were used as internal standards in method development. Water samples were collected and subjected to analysis for the discovery of unknown PFAS in the study.

LC-Q-TOF analytical conditions

Details of the analytical conditions on LCMS-9030, Q-TOF system (Shimadzu Corporation, Japan) are shown in Table 1. LabSolutions v5.114 and LabSolutions Insight Explore v3.8 SP4 were used for data acquisition in MS and DIA mode and data processing of HRAM spectra and DIA MS/MS spectra for efficient detection and identification of targeted and untargeted PFAS.

Table 1 Analytical conditions of PFAS on LCMS-9030

LC Conditions	
Column	Shim-pack™ Velox, C18 (2.1x100 mm, 2.7 µm)
Flow Rate	0.4 mL/min
Mobile Phase	A: 5 mM Ammonium acetate in water B: Acetonitrile
LC gradient	B: 10% (0-0.5 min) → 85% (8.5 min-9 min) → 10% (9.1 min-12 min) → stop
Delay column	Shim-pack Velox, C18 (2.1x50 mm, 2.7 µm)
Oven Temp.	40°C
Injection Vol.	50 µL
Interface Conditions and MS mode	
Interface	ESI Heated
Interface Temp.	300°C
DL Temp.	250°C
Heat Block Temp.	400°C
Nebulizing Gas	3 L/min (N2)
Heating Gas Flow	10 L/min (Air)
Drying Gas Flow	10 L/min (N2)
MS mode	MS (-), m/z 100~1000 DIA (-), m/z 50~1000; with CE 25V and Spread (+/-) 20V Loop time: 1.01 sec

Untargeted screening method

As shown in Table 1, MS and DIA events were set up for data acquisition. Data analysis and processing were performed with the LabSolutions Insight Explore suite, which include Analyze and Assign etc. The Analyze is for the deconvolution of DIA data to generate a precursor list and provide various functions of in-depth data analysis such as deconvoluted MS/MS spectrum, formula prediction and library search etc. The Assign is for identification and structural elucidation, which links to database searches such as ChemSpider and PubChem. Both Analyze and Assign were highly efficient and flexible in data processing and result display. Two PFAS libraries were installed and used in this study: (1) an in-house PFAS HRMS MS/MS library including spectra of 34 PFAS standards [4] and (2) MS-DIAL PFAS_Neg library [5].

[2] *Internship Student from Faculty of Science, National University of Singapore*

■ Results and Discussion

1. Detection of PFAS by HRAM

Table 2 shows the results of detection of 14 PFAS and 2 ISTD in pure water from HRAM data (event 1) acquired on LCMS-9030 using the method as described above. The XIC chromatograms of 1 ng/mL sample is displayed in Figure 1a and the spectrum of the first XIC peak (PFPA) is shown in Figure 1b. The lowest detectable concentrations by HRAM are 0.01 ng/mL for 11 PFAS, 0.02 ng/mL for PFDA, 0.05 ng/mL for PFPA and 0.1 ng/mL for PFTrA and PFTeA. The mass accuracy is better than (+/-) 3.3 ppm, with most compounds less than (+/-) 2 ppm. Linear calibration curves were established from the lowest concentrations to 5 ng/mL for all the PFAS with R^2 between 0.94 and 0.99. PFOA, PFOS and their isotope labelled standards (M-PFOA, M-PFOS) could also be detected at 0.01 ng/mL level. These results indicate that a highly-sensitive screening and quantitation method could be established based on HRAM on LCMS-9030.

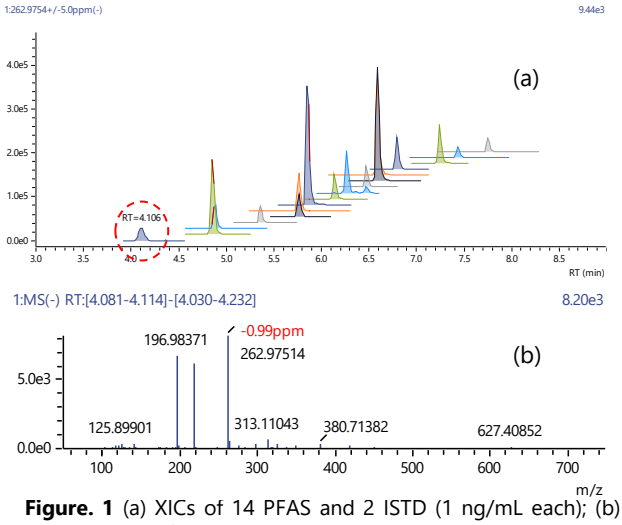


Figure. 1 (a) XICs of 14 PFAS and 2 ISTD (1 ng/mL each); (b) MS spectrum of the 1st peak (PFPA).

Table 2 Sixteen PFAS information, accurate mass, measured error, RT and calibration curve range on LCMS-9030

PFAS (Abbr.)	Formula	CAS No.	[M-H]- (Meas.)	[M-H]- (Calc.)	Error (ppm)	RT (min)	Range (ng/mL)	R^2
PFPA	$C_5HF_9O_2$	2706-90-3	262.9751	262.9760	-3.27	4.12	0.05 ~ 5	0.989
PFBS	$C_4F_9SO_3H$*	29420-49-3	298.9421	298.9430	-3.01	4.86	0.01 ~ 5	0.941
PFHxA	$C_6HO_2F_{11}$*	307-24-4	312.9718	312.9728	-2.91	4.88	0.01 ~ 5	0.981
PFHpA	$C_7HF_{13}O_2$*	375-85-9	362.9687	362.9696	-2.20	5.36	0.01 ~ 5	0.982
PFOA	$C_8HF_{15}O_2$*	335-67-1	412.9655	412.9664	-1.99	5.77	0.01 ~ 5	0.982
M-PFOA	$^{13}C_4C_4HF_{15}O_2$	N.A.	416.9791	398.9366	-1.73	5.77	0.01 ~ 5	0.982
PFHxS	$C_6F_{13}HO_3S$*	82382-12-5	398.9358	462.9632	-1.75	5.86	0.01 ~ 5	0.950
PFNA	$C_9HF_{17}O_2$*	375-95-1	462.9624	512.9600	-1.60	6.13	0.01 ~ 5	0.983
PF-3,7-DMOA	$C_{10}HF_{19}O_2$	172155-07-6	468.9692	512.9600	-1.83	6.27	0.01 ~ 5	0.985
PFDA	$C_{10}HF_{19}O_2$*	335-76-2	512.9592	498.9302	-1.58	6.47	0.02 ~ 5	0.988
PFOS	$C_8F_{17}O_3HS$*	4021-47-0	498.9296	502.9436	-1.22	6.59	0.01 ~ 5	0.955
M-PFOS	$^{13}C_4C_4F_{17}O_3HS$	N.A.	502.9429	562.9568	-1.31	6.59	0.01 ~ 5	0.969
PFUnA	$C_{11}HF_{21}O_2$*	2058-94-8	562.9562	598.9238	-1.12	6.80	0.02 ~ 5	0.998
PFDS	$C_{10}HF_{21}SO_3$	2806-15-7	598.9232	662.9505	-1.09	7.24	0.01 ~ 5	0.987
PFTrA	$C_{13}HO_2F_{25}$*	72629-94-8	662.9497	712.9473	-1.27	7.43	0.1 ~ 5	0.991
PFTeA	$C_{14}HO_2F_{27}$*	376-06-7	712.9463	262.9760	-1.42	7.75	0.1 ~ 5	0.983

** Targeted PFAS by EPA 537 method*

2. Detection of PFAS by DIA

The DIA data of the same data set was processed with the LabSolutions Insight Explore - Analyze to generate a long list of precursors via deconvolution, followed by applying predicting formula. To look for PFAS-like compounds and species, the key settings of elements include F: 6~50, H: 1~5, O: 1~5, C: 1~50, S: 0~1 and N: 0~1. These settings restrict the elemental composition of candidate with a negative mass defect and could be used to find PFAS-like species [4]. Figure 2 shows an example of this approach. A precursor peak (*m/z*262.9751) generated from DIA data (Figure 2a) appeared at the same RT as PFPA in MS XIC (Figure 1a). The mass defect measured is -24.9 mDa, which is very close to the calculated mass defect of PFPA (-24.0 mDa). The corresponding deconvoluted MS/MS spectrum as shown in Figure 2b matches perfectly to the PFPA MS/MS spectrum in the PFAS library (Figure 2c). These results confirm that PFPA of 1 ng/mL spiked in water can be detected firmly via the DIA analysis approach.

The detection and identification results of the 14 spiked PFAS in water are compiled into Table 3. As can be seen from the Table, all the 14 PFAS were detected and identified via the above approach using the Analyze.

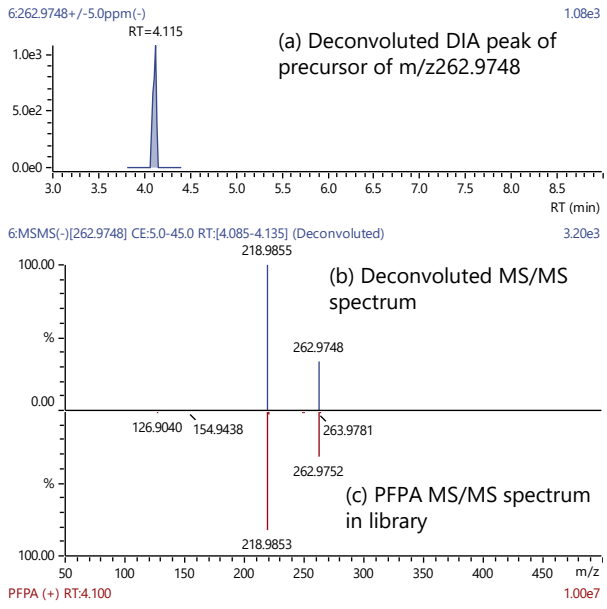


Figure 2 Detection of PFPA (1 ng/mL in water) by DIA peak (a) and deconvoluted spectrum (b), which matches to the library spectrum of PFPA (c)

Table 3 Detection and identification of 14 PFAS (1 ng/mL) from DIA data using Analyze program

PFAS (Abbr.)	PFAS Formula	Precursor generated from DIA data	Precursor RT (min)	Precursor ion formula obtained	Deconvoluted spectrum from DIA data	PFAS Lib Search
PFPA	$C_5HF_9O_2$	262.9748	4.12	$[C_5HO_2F_9\text{-}H]^-$	262.9748, 218.9855	Confirmed
PFBS	$C_4F_9SO_3H$	298.9421	4.86	$[C_4HO_3F_9S\text{-}H]^-$	298.9421, 98.9552, 79.9565	Confirmed
PFHxA	$C_6HO_2F_{11}$	312.9721	4.88	$[C_6HO_2F_{11}\text{-}H]^-$	312.9721, 268.9819, 118.9918	Confirmed
PFHpA	$C_7HF_{13}O_2$	362.9695	5.35	$[C_7HO_2F_{13}\text{-}H]^-$	362.9695, 318.9791, 168.9888	Confirmed
PFOA	$C_8HF_{15}O_2$	412.9656	5.77	$[C_8HO_2F_{15}\text{-}H]^-$	412.9656, 368.9757, 218.9857, 168.9884	Confirmed
PFHxS	$C_6F_{13}HO_3S$	398.9357	5.85	$[C_6HO_3F_{13}S\text{-}H]^-$	398.9357, 118.9918, 98.9548, 79.9567	Confirmed
PFNA	$C_9HF_{17}O_2$	462.9625	6.14	$[C_9HO_2F_{17}\text{-}H]^-$	462.9625, 418.9725, 218.9856, 168.9885	Confirmed
PF-3,7-DMOA	$C_{10}HF_{19}O_2$	468.9693	6.26	$[C_9HF_{19}\text{-}H]^{-*}$	468.9693, 446.9687, 268.9822	Confirmed
PFDA	$C_{10}HF_{19}O_2$	512.9592	6.46	$[C_{10}HO_2F_{19}\text{-}H]^-$	512.9592, 468.9694, 268.9823, 218.9855, 168.9884	Confirmed
PFOS	$C_8F_{17}O_3HS$	498.9294	6.58	$[C_8HO_3F_{17}S\text{-}H]^-$	498.9294, 168.9883, 118.9921, 98.9551, 79.9565	Confirmed
PFUnA	$C_{11}HF_{21}O_2$	562.9565	6.80	$[C_{11}HO_2F_{21}\text{-}H]^-$	562.9565, 518.9660, 318.9789, 268.9818, 218.9848, 168.9882	Confirmed
PFDS	$C_{10}HF_{21}SO_3$	598.9232	7.24	$[C_{10}HO_3F_{21}S\text{-}H]^-$	598.9232	Confirmed
PFTrA	$C_{13}HO_2F_{25}$	662.9486	7.43	$[C_{13}HO_2F_{25}\text{-}H]^-$	662.9486, 618.9597	Confirmed
PFTeA	$C_{14}HO_2F_{27}$	712.9465	7.75	$[C_{14}HO_2F_{27}\text{-}H]^-$	712.9465, 668.9566, 168.9880	Confirmed

** PF-3,7-DMOA is ionized to form $[M\text{-}HCOO]^- = [C_9HF_{19}\text{-}H]^-$*

The results indicate that PFAS can be detected from DIA data, which is the basis to use the HRMS-DIA method for untargeted screening of PFAS in water samples.

3. Untargeted screening by HRAM-DIA method

The above data processing using Analyze was adopted for untargeted screening of PFAS in unknown samples. The obtained DIA data of a water sample was processed with Analyze and a long list of precursors (>800) was produced. By applying formula prediction with specific elemental settings as described above to all precursors, 16 PFAS-like precursors were generated (Table 4), which feature with the characteristic negative mass defects. Upon applying library search, five candidates were found in the PFAS libraries: PFBA (SI=94%), PFCA-unsaturated (SI=65%), 6:2 fluorotelomer sulfonic acid (SI=55%), PFOA (SI=61%) and PF-3,7-DMOA (SI=76%). In addition, PFCA-diether H_substituted was found to match to *m/z*626.9530 spectrum with a very low SI (23%). For such poor library matched and the remaining totally un-matched species, their identities could rely only on the characterization through structural elucidation analysis using the Assign program.

The 16 PFAS-like precursor peaks extracted from DIA data via Analyze are displayed in Figure 3a. Taking the peak at 1.824 min (*m/z*212.9787) as an example, the deconvoluted MS/MS spectrum and library search are shown in Figure 3b and 3c. The results confirm the presence of PFBA in the sample. If the deconvoluted MS/MS spectrum was sent to Assign program which links to database search (ChemSpider), heptafluorobutyric acid (CAS No.: 375-22-4) was found as a matched structure and the precursor as well as a fragment were annotated (Figure 3d). It is actually same as perfluorobutanoic acid (PFBA). Another representative example is candidate #15, which was detected at 6.26 min with a precursor ion of

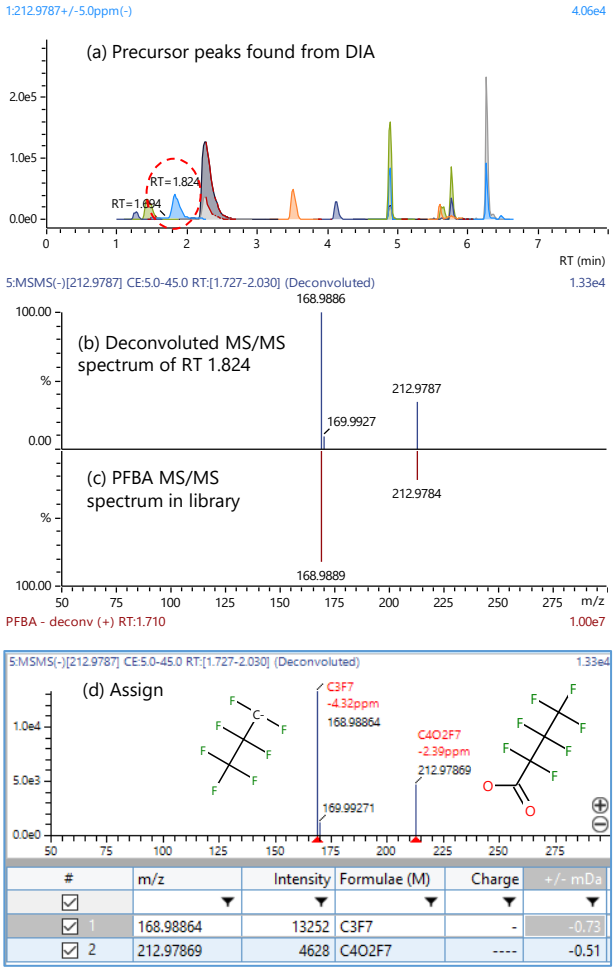
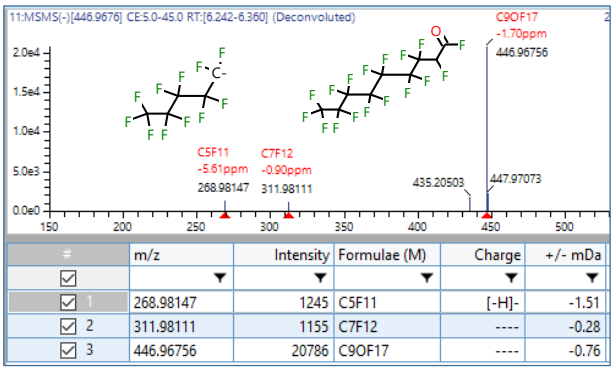


Figure 3 (a) Detection of unknown PFAS-like compounds from DIA, (b) deconvoluted spectrum of peak at RT 1.824, (c) matches to library spectrum of PFBA; (d) Assign to Heptafluorobutyric acid structure and fragment (same as PFBA).

Table 4 Untargeted screening for detection and identification of PFAS from a water sample by HRAM-DIA on LCMS-9030

Candi-date #	Precursor from DIA	RT (min)	Precursor ion Formula	Error (ppm)	Deconvoluted DIA MS/MS spectrum	PFAS Library search	ID by Assign (ChemSpider)
1	402.9979	1.26	$[C_{13}H_4OF_{12}\text{-}H]^-$	-4.6	402.9979	Not found	(2E)-2,3,4,4,5,5,6,6,7,7,7-Undecafluoro-1-(4-fluorophenyl)-2-hepten-1-one
2	404.0146	1.43	$[C_{15}H_5NOF_{10}\text{-}H]^-$	1.9	404. 0146, 376.9950	Not found	N-[2,3,5,6-Tetrafluoro-4-(trifluoromethyl) phenyl]-2-(trifluoromethyl) benzamide
3	212.9787	1.82	$[C_4HO_2F_7\text{-}H]^-$	-2.4	212.9787, 168.9886	Perfluorobutanoic acid (PFBA)	Heptafluorobutyric acid
4	220.9864	2.23	$[C_8H_2O_3F_4\text{-}H]^-$	-1.6	220.9864, 138.9956, 79.9567	Not found	2,3,5,6-Tetrafluoro-4-formylbenzoic acid
5	219.9835	2.27	$[C_5HNO_2F_6\text{-}H]^-$	-1.5	219.9835, 81.9525	Not found	4-Cyano-2,2,3,3,4,4-hexafluorobutanoic acid
6	247.9786	3.51	$[C_6HNO_3F_6\text{-}H]^-$	-0.8	247.9786, 219.9838, 79.9566	Not found	bis(trifluoromethyl)-1,2-oxazole-4-carboxylic acid
7	196.9836	4.12	$[C_4HOF_7\text{-}H]^-$	-3.6	196.9836, 130.9923, 80.9951, 68.9951	Not found	butanal, heptafluoro-
8	246.9805	4.88	$[C_5HOF_9\text{-}H]^-$	-2.4	246.9805, 180.9887, 130.9918, 118.9919	Not found	2,2,3,3,4,4,5,5,5-Nonafluoropentanal
9	626.9530	4.88	$[C_{12}H_2O_4F_{22}\text{-}H]^-$	0.1	626. 9530, 354.9605, 312.9722, 268.9822	PFCA-diether H_substituted	No result
10	224.9786	4.89	$[C_5HO_2F_7\text{-}H]^-$	-2.8	224.9786, 174.9816	PFCA-unsaturated	1,1,1,3,5,5,5-Heptafluoro-2,4-pentanedione
11	374.9738	4.89	$[C_{10}H_5O_3F_9S\text{-}H]^-$	-1.4	374.9738, 312.9720, 268.9821	Not found	Phenyl 1,1,2,2,3,3,4,4,4-nonafluoro-1-butanesulfonate
12	426.9681	5.60	$[C_8H_5O_3F_{13}S\text{-}H]^-$	0.4	426.9677	6:2 Fluorotelomer sulfonic acid	1H,1H,2H,2H-PERFLUOROOCTANESULFONIC ACID
13	346.9742	5.77	$[C_7HOF_{13}\text{-}H]^-$	-1.6	346.9742, 280.9816, 96.9595	Not found	2,2,3,3,4,4,5,5,6,6,7,7-Dodecafluoroheptanoyl fluoride
14	412.9654	5.77	$[C_8HO_2F_{15}\text{-}H]^-$	-2.5	412.9654, 368.9753, 218.9856, 168.9887	Perfluorooctanoic acid (PFOA)	Perfluorooctanoic Acid (PFOA)
15	446.9676	6.26	$[C_9HOF_{17}\text{-}H]^-$	-1.7	446. 9676, 311.9811, 268.9815	Not found	2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-Hexadecafluorononanoyl fluoride
16	468.9697	6.26	$[C_9HF_{19}\text{-}H]^-$	-3.8	468.9697, 446.9678, 268.9822, 218.9854, 168.9886	PF-3,7-DMOA	2,2,3,4,4,5,5,6,6,7,8,8,8-tridecafluoro-3,7-bis(trifluoromethyl)octanoic acid, C10HF19O2

*m/z*446.9676. PFAS Library search did not generate any result. The deconvoluted MS/MS spectrum was sent to Assign with inputting a formula of C_9HOF_{17} for searching in ChemSpider database. The Assign program generated a list of candidates for the formula and spectrum. One of most-likely candidate is 2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-Hexadecafluorononanoyl fluoride. As can be seen in Figure 4, the precursor ion and two fragments match the structure essentially. However, it is worth to note that, although the identification results using Assign program and ChemSpider database may provide reference structures and information, the results are not considered as conclusions and further structural analysis is required.



#	m/z	Intensity	Formulae (M)	Charge	+/- mDa
1	268.98147	1245	C5F11	[-H]-	-1.51
2	311.98111	1155	C7F12	----	-0.28
3	446.96756	20786	C9OF17	----	-0.76

Figure 4 Deconvoluted MS/MS spectrum of precursor peak at 6.26 min (*m/z*446.9676); Assign: 2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-Hexadecafluorononanoyl fluoride, C_9HOF_{17}, from ChemSpider.

■ Conclusion

In this study, an untargeted screening method based on HRAM-DIA data acquisition was established on LCMS-9030. The DIA data obtained was deconvoluted using the LabSolutions Insight Explore – Analyze to generate a list of precursors. Then, PFAS-like species were extracted by applying formula prediction with specific elemental settings. This approach was successfully verified with 14 PFAS standards at 1 ng/mL in water and applied to analyze an unknown water sample. Sixteen PFAS-like species were found. PFAS library search and structural elucidation using the Assign program were conducted for these PFAS-like species.

■ References

1. Brahm Prakash, et. al., "Ultra-fast LC-MS/MS analysis of PFAS in environmental samples", Shimadzu White Paper (2019).
2. Wang Z. et. al., "A Never-Ending Story of PFASs?", Env. Sci. & Tech., Vol. 51(5), 2017, 2508–2518.
3. Y. Liu et al., "High-resolution mass spectrometry methods for non target discovery and characterization of PFASs in environmental and human samples", Trends in Ana. Chem., Vol 121 Dec 2019, 115420
4. Lee J. X. et. al., "Development of Screening Analysis Method for PFAS in Surface Water on LC-Q-TOF", Shimadzu Application News, AD-0200A (2020),
5. Jeremy P. Koelmel et. al., "Toward Comprehensive PFAS Annotation Using FluoroMatch Software and Intelligent HR Tandem Mass Spectrometry Acquisition", *Anal. Chem.* 2020, 92, 16, 11186–11194.

04-AD-0280-EN First Edition: Oct. 2022

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/

SHIMADZU (Asia Pacific) Pte. Ltd,
www.shimadzu.com.sg

Suppressor Ion Chromatograph HIC-ESP

Application News

Analysis of Adsorbable Organic Fluorine (AOF) by Combustion Ion Chromatography(CIC)

Tomoka Kaseda

User Benefits

- The combination of the combustion unit and IC can perform AOF analysis according EPA Draft Method 1621.
- AOF analysis is a simplified and useful technique for screening PFAS.
- The CIC system enables automation of the entire process from sample combustion to ion chromatography analysis.

■ Introduction

The US Environmental Protection Agency (USEPA) has published Draft Method 1621, a screening method for the determination of AOF in aqueous matrices by CIC[1]. This method detects organic fluorine compounds that are dissolved in water and adsorbed by passing the sample through a column of granular activated carbon (GAC). The common sources of organic fluorine compounds are PFAS and non-PFAS fluorinated compounds such as pesticides and pharmaceuticals.

CIC system, AOF compounds adsorbed on the GAC from the sample are decomposed by combustion. The generated combustion gas containing fluorine is collected in an absorbing solution and analyzed by ion chromatography. An advantage of this technique is that it provides information on the total amount of PFAS that may not be targeted by other selective chromatography methods.

In this article, we introduce the analysis of AOF with CIC. Perfluorohexane sulfonic acid (PFHxS), the prescribed spiking compound in EPA Draft Method 1621, was evaluated for determining the initial precision and recovery (IPR) and a river water sample analyzed.

■ Experimental

The Shimadzu HIC-ESP ion chromatograph was equipped with the Nittoseiko Analytech Co., Ltd. AQF-2100H combustion unit (Fig.1). The sample preparation and analysis process are summarized below.

1. The sample is passed through the TXA-04 absorption unit.(Nittoseiko Analytech Co., Ltd.)
2. GAC is transferred to the ceramic boat and combusted
3. Combustion products are captured in the absorption solution
4. Absorption solution is analyzed by Ion chromatography

The Draft Method 1621 states that laboratory water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents should be analyzed as method blank. Also, at least two method blanks must be analyzed at the beginning and end of each batch to ensure the absence of contamination.

Table 1 shows the analytical conditions for combustion and chromatography.

Table 1 Analysis Conditions for AQF-2100H and HIC-ESP

System	: AQF-2100H
Sample boat	: Ceramic
Pyrolysis tube	: Ceramic inner + quartz outer tube
Furnace inlet temperature	: 1000 ˚C
Furnace outlet temperature	: 1100 ˚C
Oxygen flow	: 400 mL/min
Argon flow	: 200 mL/min
Humidified argon flow	: 100 mL/min
Absorption solution	: Reagent Water
Final absorption solution volume	: 10.3 mL

System	: HIC-ESP
Column	: Shim-pack™ IC-SA2*1 (4.0 mm × 250 mm I.D., 9 µm)
Mobile phase	: 1.8 mmol/L Na_2CO_3 1.7 mmol/L $NaHCO_3$
Flow rate	: 1.0 mL/min
Column temperature	: 30 ˚C
Injection volume	: 50 µL
Suppresoe unit	: ICDS™-40A
Detection	: Conductivity

*1 P/N：228-38983-91

Fig.1 Combustion Ion Chromatograph
Nittoseiko Analytech Co., Ltd. AQF-2100H Combustion unit(right) with Shimadzu HIC-ESP Ion Chromatograph(left)

■ Calibration

A 5-point calibration curve was prepared using the analysis results of five anion-mixed standard solutions with concentrations ranging from 0.01 mg/L to 0.5 mg/L. The correlation coefficient was 0.999 or higher for all components. Fig.2 shows a chromatogram of the mixed anion standard.

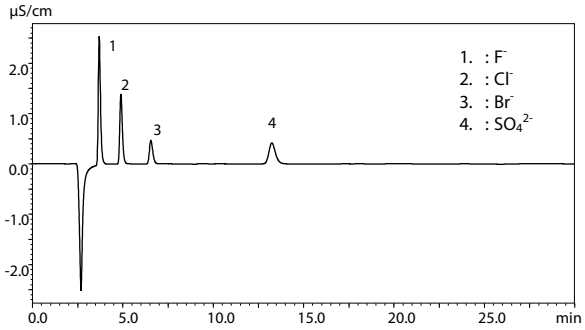


Fig.2 Chromatograms of each 0.5 mg/L mixed anionic standard solution

■ Initial Precision and Recovery (IPR)

Six 100 mL reagent water replicates spiked with PFHxSNa solution to 19.5 µg/L as fluoride ion and two method blanks were extracted and analyzed by CIC. The average concentration of the two method blanks concentration (1.6 µg/L) was subtracted from each of the six spiked samples to calculate the IPR. IPR is evaluated by calculating the average perecent recovery and the relative standard deviation (RSD) of concentration. Fig.3 shows the chromatogram of a PFHxS standard solution and Table 3 provides the results of the IPR along with the EPA Draft Method 1621 acceptance criteria.

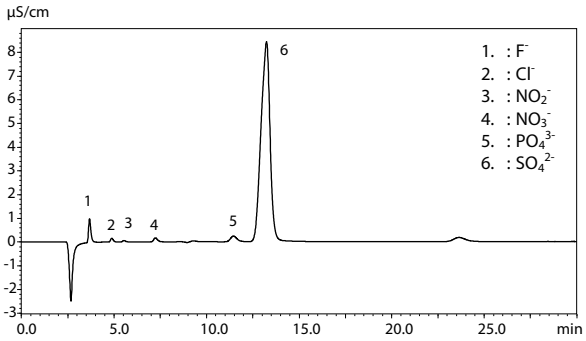


Fig.3 Chromatogram of PFHxS Standard Solution

Table 3 IPR results and acceptance criteria

	Result	Criteria
Average Recovery (%)	93.0	70-130
RSD	8.30	< 20
Method Blank (µg/L)	1.6	< 3.0

■ Analysis of river water

100 mL of river water was extracted and analyzed. The results are shown in Table 4. The average of the two method blanks concentration (1.4 µg/L) was subtracted from a river water sample. Fig.4 and 5 show an example chromatogram of the river water sample with trace anions detected, including fluoride.

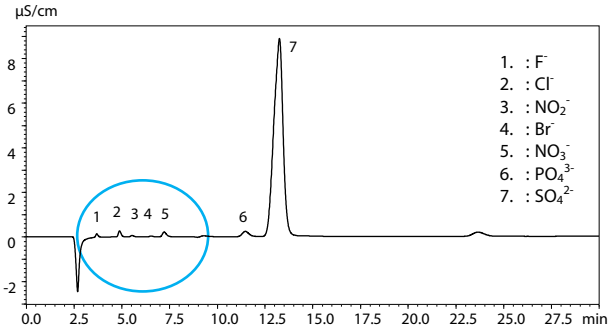


Fig.4 An example chromatogram of the river water sample

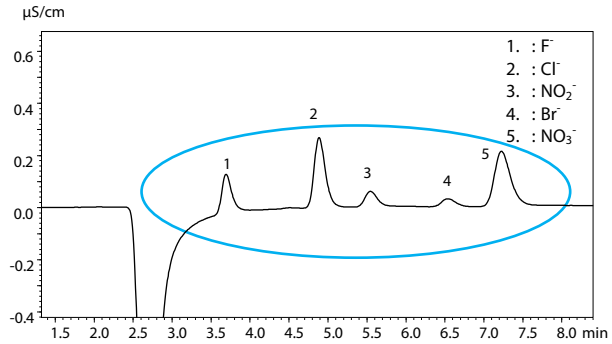


Fig.5 Enlarged example chromatogram of Fig.4

Table 4 Results of River Water Sample

	Result (µg/L)
River Water Sample	1.6

■ Conclusion

This Application News demonstrates the analysis of AOF using the Shimadzu HIC-ESP Ion Chromatograph equipped with the Nittoseiko Analytech Co., Ltd. AQF-2100H Combustion unit. Excellent recovery and precision were acheived in the IPR test using PFHxS as described in the EPA Draft Method 1621. Analysis of a river water sample demonstrates detection at the part per billion level is possible.

<References>

1) EPA 1621 Screening Method for the Determination of AOF in Aqueous Matrices by CIC

01-00657-EN First Edition: Feb. 2024

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/

LCMS-9050
by Shimadzu Corporation

The LCMS-9050 is a cutting-edge Q-TOF mass spectrometer designed to reliably and accurately acquire mass data in real laboratory environments. The design allows for rapid polarity switching, enabling confident identification of complex compounds and efficient screening of numerous unknowns. The instrument's versatility is enhanced by a wide range of optional accessories and front-end configurations, maximizing its analytical capabilities across various applications.

Prominence Ion Chromatograph
by Shimadzu Corporation

SCIENTISTS' CHOICE AWARDS 21
NOMINEE

The Prominence Ion Chromatograph with built-in electrodialytic suppressor boasts the same low carryover and excellent injection precision characteristics of Shimadzu HPLCs to bring you highly reliable results.

LCMS-TQ RX Series
by Shimadzu Corporation

The LCMS-TQ RX series (LCMS-8060RX, LCMS-8050RX, and LCMS-8045RX) triple quadrupole high-performance liquid chromatograph mass spectrometers provide higher sensitivity, stability, and convenient operability.

SHIMADZU
Excellence in Science

Shimadzu (Asia Pacific) Pte Ltd.
www.shimadzu.com.sg