SHIMADZU
Excellence in Science

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

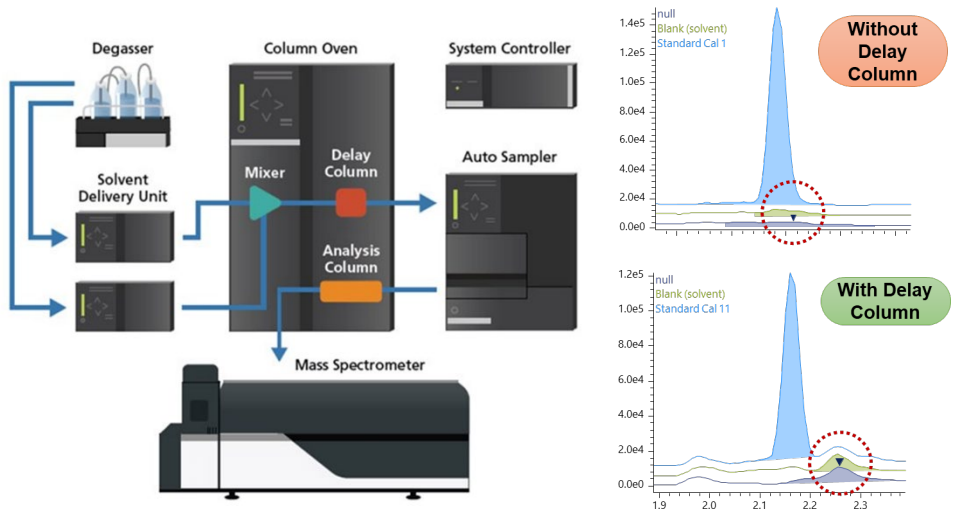

Figure 1: Placement of delay column in LC-MS/MS and effect on the delay of PFBA in the background.

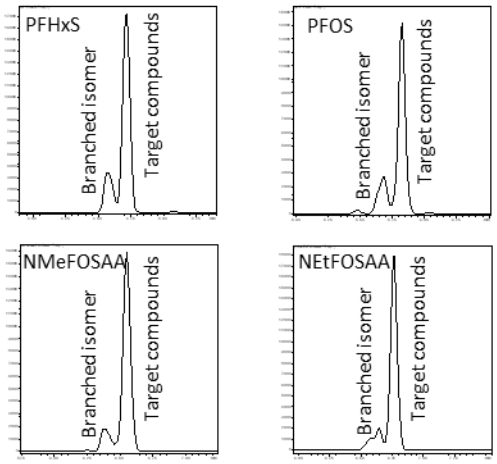

Figure 2: Separation of branched isomers: PFHxS, PFOS, NMeFOSAA and NEtFOSAA.

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.

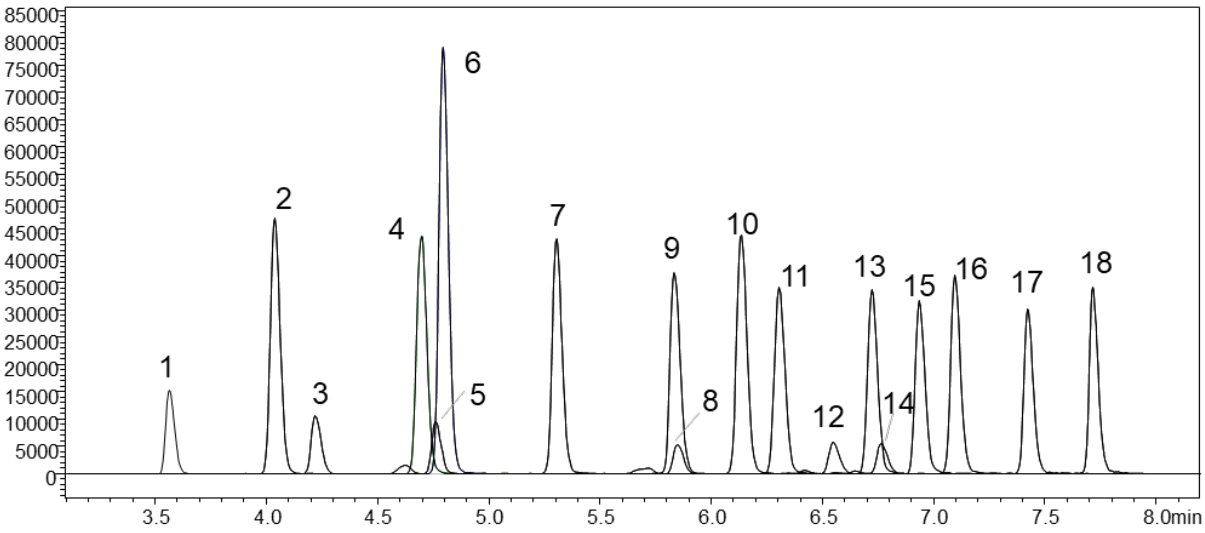

Figure 3: Example chromatogram of target compounds.

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.

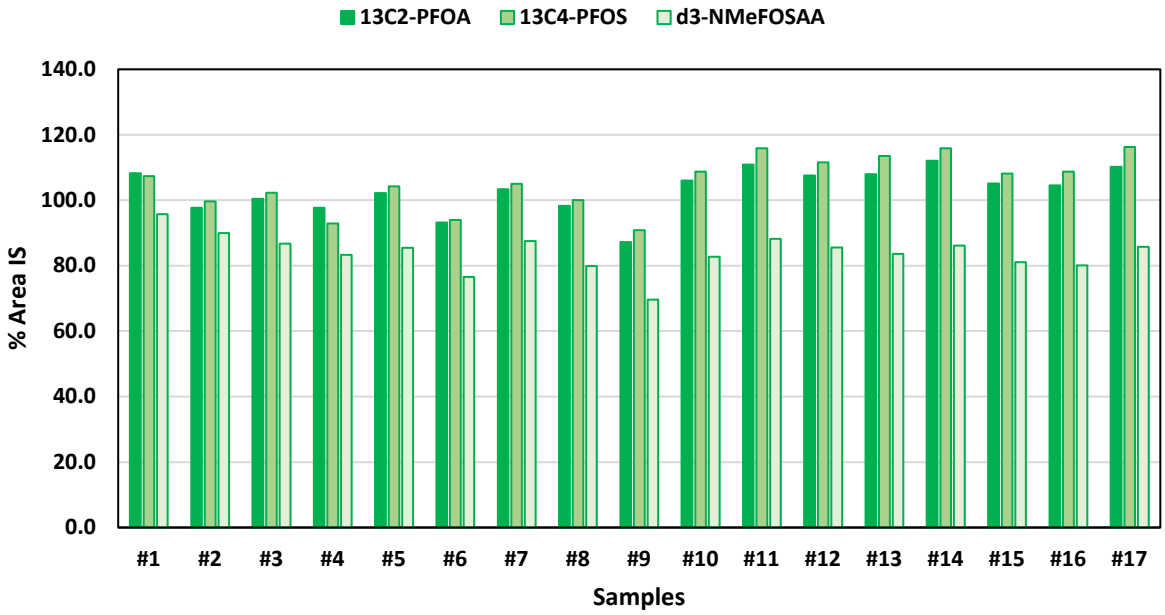

Figure 4: %Area of internal standards based on the average peak area of the initial calibration.

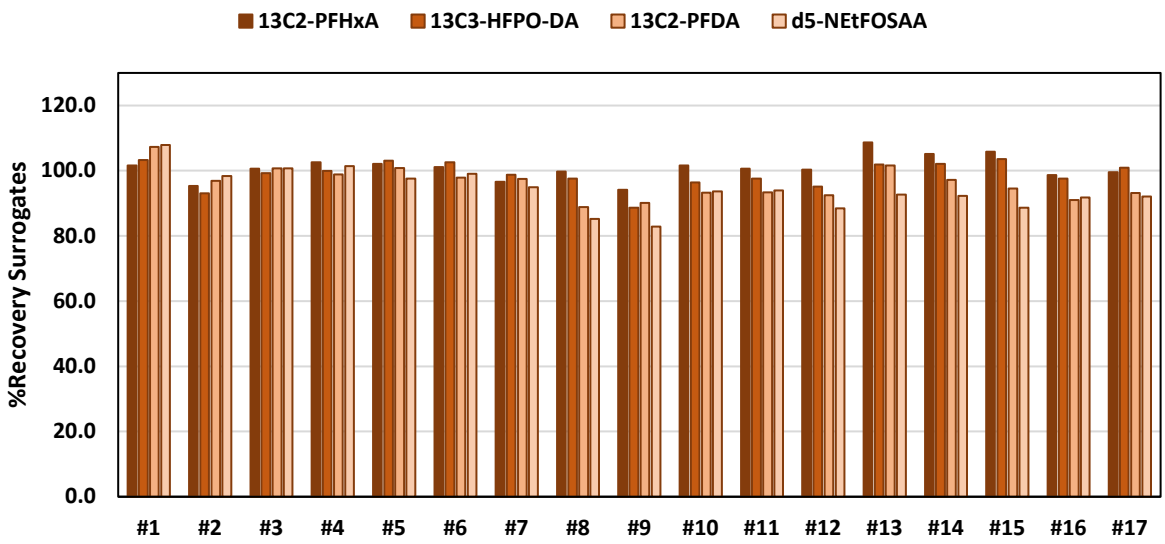

Figure 5: %recovery of surrogates.

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.

ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8045


LCMS-8050


LCMS-8060NX


LCMS-2020


LCMS-2050


Q-TOF LCMS-9030/9050

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

For information about Shimadzu Scientific Instruments and to contact your local office, please visit our Web site at www.ssi.shimadzu.com

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/

SHIMADZU SCIENTIFIC INSTRUMENTS, INC.
Applications Laboratory
7102 Riverwood Drive, Columbia, MD 21045
Phone: 800-477-1227 Fax: 410-381-1222
www.ssi.shimadzu.com

Reference: SSI-LCMS-159

First Edition: June 2024