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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

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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

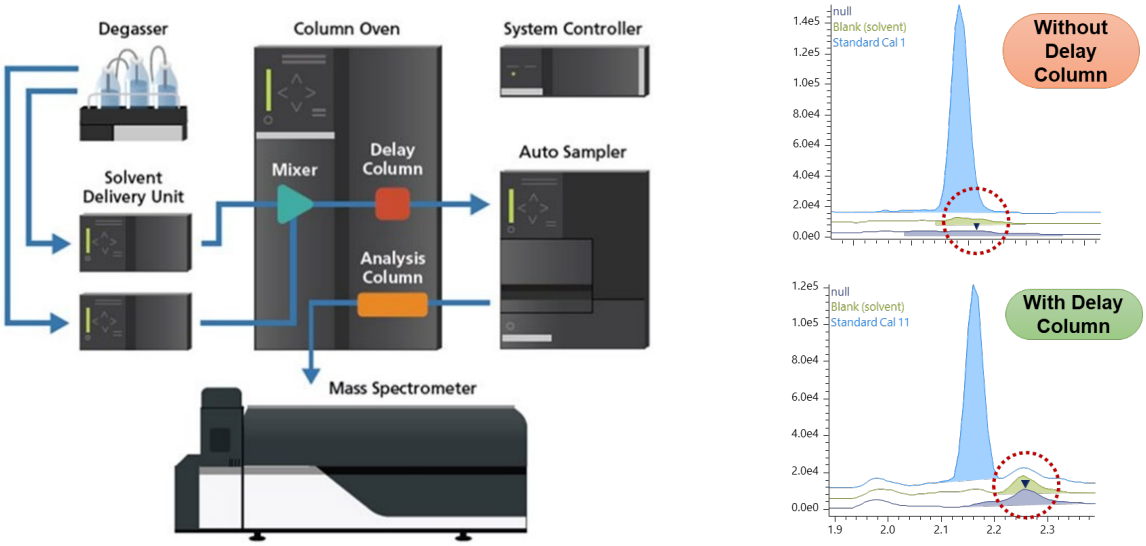

Figure 1: Placement of delay column in LC-MS/MS and effect on the delay of PFBA in the background.

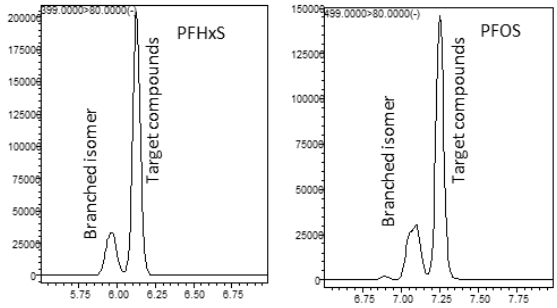

Figure 2: Separation of branched isomers: PFHxS and PFOS.

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.

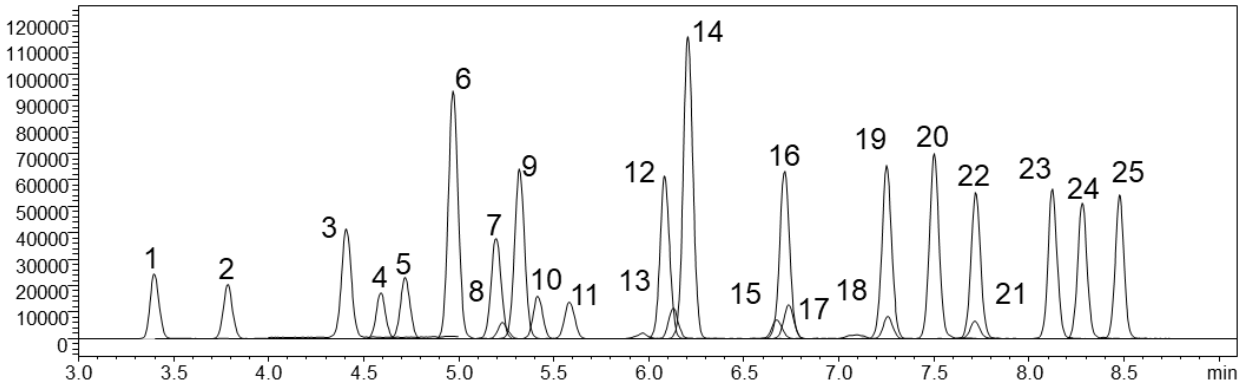

Figure 3: Example chromatogram of target compounds.

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

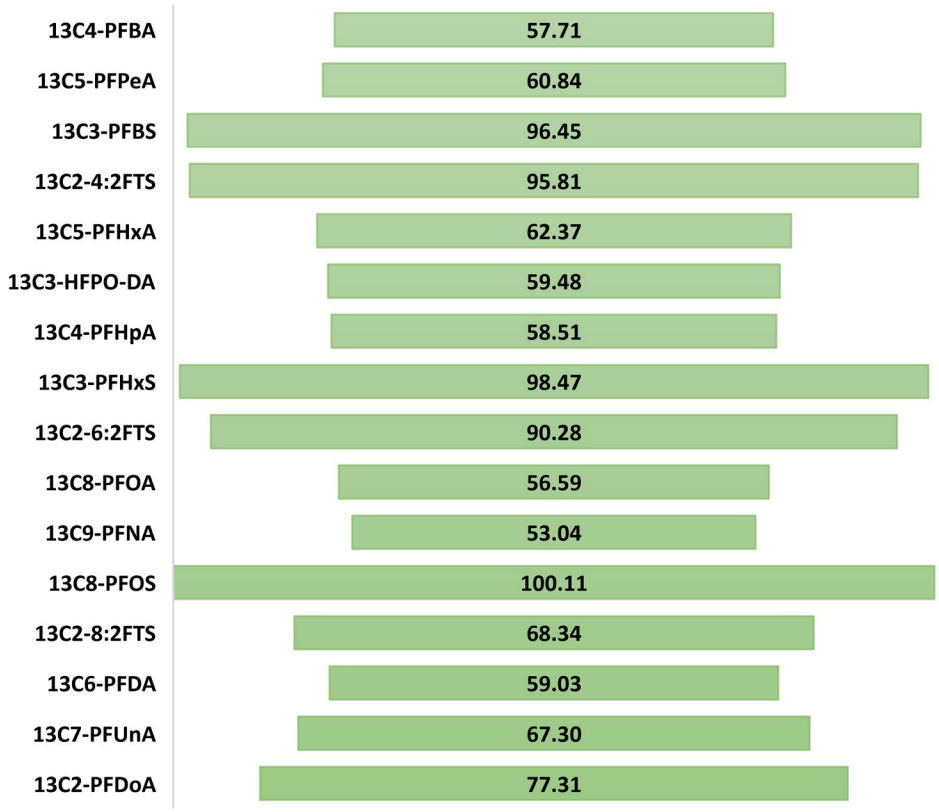

Figure 4: %recovery of the isotope dilution analogues from the precision and accuracy of the method.

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.

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