

Analysis of Per- and Polyfluoroalkyl Substances (PFAS) using the LCMS-8050 Triple Quadrupole Mass Spectrometer According to EPA Draft Method 1633

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User Benefits

- ◆ Limits of Quantitation listed in EPA Draft 1633 were readily achieved with the Shimadzu LCMS-8050, a system that delivers high-quality data, throughput, and affordability.
- ◆ The robust LCMS-8050 performs well with complex environmental matrices, such as wastewater samples used in this study.
- ◆ This efficient instrument is field upgradeable to higher sensitivity instruments, which makes it a versatile and future-proof solution for PFAS analysis.

Keywords: Per- and Polyfluorinated Alkyl Substances, PFAS, Perfluorinated Compounds, PFCs, PFOA, PFOS, Triple Quad, EPA Draft 1633, EPAM1633, aqueous samples, water, wastewater

■ Abstract

This application note demonstrates that the Shimadzu LCMS-8050 meets and exceeds the Quality Assurance and Quality Control criteria and performance specified in Environmental Protection Agency (EPA) draft method 1633 for the analysis of Per- and Polyfluoroalkyl Substances (PFAS) in environmental samples. All analytes were reliably quantitated at or less than the Limit of Quantitation (LOQ) reported in the EPA draft method. This work proves the suitability of the Shimadzu LCMS-8050 for accurate and robust analysis of PFAS in routine laboratories in accordance with present demands in sensitivity and throughput. The method presented here can be optimized for other matrices or additional compounds.

This application note, part of the comprehensive suite of Shimadzu's vetted solutions for the analysis of PFAS², demonstrates the performance of the Shimadzu LCMS-8050 for the quantitation of PFAS in accordance with EPAM1633. The LCMS-8050 was selected for this work because of its known dependability to deliver the sensitivity and robustness expected by modern environmental laboratories for the routine analysis of PFAS using methods requiring sample preparation by Solid Phase Extraction (SPE), like EPAM1633, or with a large-volume injection (*e.g.*, EPA 8327, ASTM 8421-22).

■ Introduction

The EPA is continuing to standardize methods for the analysis of PFAS in environmental samples using Liquid Chromatography with Triple Quadrupole Spectrometry (LC-MS/MS). Shimadzu offers a full line of Ultra-Fast LC-MS/MS (UFMS™) systems for quantitating PFAS at environmentally relevant concentrations. EPA Draft Method 1633 (EPAM1633)¹ is the latest method proposed by the EPA in 2022 for the quantitation of targeted PFAS in aqueous, solid, biosolid, and tissue samples.

■ Experimental Approach and Instrumentation

This application note describes the analysis of the 40 target PFAS compounds, 23 Extracted Internal Standards (EIS), and 7 Non-Extracted Internal Standards (NIS) included in EPAM1633 in reagent water and wastewater using the Shimadzu LCMS-8050. PFAS types and acronyms used in EPAM1633 and This work are listed in Table 1. All standards used in the work presented here were purchased from Wellington Laboratories (PFAC-MXF, PFAC-MXG, PFAC-MXH, PFAC-MXI, PFAC-MXJ, MPFAC-HIF-ES, MPFAC-HIF-IS) as native mixes.

Table 1 Target analytes, EIS, and NIS included in this method

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

Standards and Calibration Curve Preparation

The native PFAS mixtures PFAC-MXF, PFAC-MXG, PFAC-MXH, PFAC-MXI, PFAC-MXJ, and methanol were mixed in a ratio of 20:10:10:10:12.5:37.5 for preparing Target Standard Solution I. Target standard solution II was prepared by diluting Target Standard Solution I 10 times with methanol. Native PFAS Mixtures MPFAC-HIF-ES, MPFAC-HIF, and methanol were mixed in a ratio of 15:15:270 for preparing the Internal standard solution. Calibration standards were prepared by mixing Target Standard Solution I, Target Standard Solution II, Internal Standard Solution, and methanol to achieve the desired concentrations listed in EPA draft method 1633 and Table 2 (targets: 0.2 – 250 ng/mL; EIS: 1.25 – 25 ng/mL; NIS: 1.25 – 5 ng/mL).

Sample Preparation

500 mL of wastewater effluent or ultra-pure water were collected into pre-cleaned polyethylene bottles and weighed. 25 µL of EIS stock solution was added to each sample. Pre-conditioning of the SPE cartridges with 0.1% methanolic ammonium, methanol, and reagent water was performed according to the product manual (Inert Sep mini MA-2: 5010-27235, 280 mg). Samples were loaded onto the SPE cartridges at 5 mL/min. Sample bottles were rinsed with 10 mL of reagent water and those rinses were loaded onto the SPE cartridges. The cartridge was dried with nitrogen gas and the analytes were eluted with 5 mL of 0.1% methanolic ammonium. 25 µL of NIS was added to each extract.

Instrument and operational conditions

The LC-MS/MS analysis was performed using a Shimadzu Nexera ultrahigh pressure liquid chromatography (UHPLC) system coupled with a triple quad LCMS-8050. A delay column was used in this work as the essential modification of hardware for minimizing possible PFAS background contamination from LC and solvents³.

A description of the LC-MS/MS parameters is included in Table 3. Sample-to-sample run time of 20 minutes includes the re-equilibration for both the delay and analytical columns after final wash out with concentrated acetonitrile to flush the column, remove background residual contaminants, and restore column performance before starting the next run.

All compound parameters, including precursor ion, product ions, and collision energies, were optimized using flow injection analysis (i.e., bypassing the analytical column) using LabSolutions™ software. Optimized retention times and precursor and product ions for multiple reaction monitoring (MRMs) are listed in Table 3.

Table 3 Chromatography and mass spectrometer conditions

Parameter	Value
LCMS	Shimadzu LCMS-8050
Analytical column	Shim-pack™ GIST-HP C18 2.1 × 50 mm, 3 μm
Delay column	Shim-pack GIST C18 3.0 × 50 mm, 5 μm
Column oven temp	40 °C
Injection volume	2 μL
Mobile phase	A: 2 mM Ammonium Acetate in 5 % (v/v) Acetonitrile in reagent water B: Acetonitrile
Gradient flow rate	0.4 mL/ Min
Run time	20 minutes
Nebulizing gas flow	3 L/ Min
Heating gas flow	15 L/Min
Interface temperature	190 °C
Desolvation line temperature	200 °C
Heat block temperature	300 °C
Drying gas flow	5 L/ min
Acquisition cycle time	16 min
Total MRMs	72

Table 2 Retention time, precursor ion, product ions, and calibration range used in this method.

Type	Name	RT	Precursor Ion	Product Ion-1	Product Ion-2	Concentration CS1 (ng/mL)	Concentration CS7 (ng/mL)
Target	PFBA	2.024	213.0	169.0	N/A	0.8	250
Target	PFPeA	3.57	263.0	219.0	69.0	0.4	125
Target	PFHxA	4.906	313.0	269.0	119.0	0.2	62.5
Target	PFHpA	7.708	363.0	319.0	169.0	0.2	62.5
Target	PFOA	8.64	413.0	369.0	169.0	0.2	62.5
Target	PFNA	9.119	463.0	419.0	219.0	0.2	62.5
Target	PFDA	9.511	513.0	469.0	219.0	0.2	62.5
Target	PFUnA	9.901	563.0	519.0	269.0	0.2	62.5
Target	PFDoA	10.461	613.0	569.0	319.0	0.2	62.5
Target	PFTTrDA	11.345	663.0	619.0	168.9	0.2	62.5
Target	PFTeDA	12.237	713.0	669.0	168.9	0.2	62.5
Target	PFBS	4.841	299.0	80.0	99.0	0.2	62.5
Target	PFPeS	7.766	349.0	80.0	99.0	0.2	62.5
Target	PFHxS	8.776	399.0	80.0	99.0	0.2	62.5
Target	PFHpS	9.294	449.0	80.0	99.0	0.2	62.5
Target	PFOS	9.709	499.0	80.0	99.0	0.2	62.5
Target	PFNS	10.2	549.0	80.0	99.0	0.2	62.5
Target	PFDS	10.956	598.9	80.0	99.0	0.2	62.5
Target	PFDoS	12.524	698.9	80.0	99.0	0.2	62.5
Target	4:2FTS	4.404	327.0	307.0	80.9	0.8	250
Target	6:2FTS	8.398	427.0	407.0	80.9	0.8	250
Target	8:2FTS	9.323	527.0	507.0	80.9	0.8	250
Target	PFOSA	11.438	498.0	78.0	478.0	0.2	62.5
Target	NMeFOSA	13.178	512.0	219.0	169.0	0.2	62.5
Target	NEtFOSA	13.475	526.0	219.0	169.0	0.2	62.5
Target	NMeFOSAA	9.491	570.0	419.0	483.0	0.2	62.5
Target	NEtFOSAA	9.647	584.0	419.0	526.0	0.2	62.5
Target	NMeFOSE	13.016	616.0	59.0	N/A	2	625
Target	NEtFOSE	13.305	630.0	59.0	N/A	2	625
Target	HFPO-DA	5.693	285.0	169.0	185.0	0.8	250

Type	Name	RT	Precursor Ion	Product Ion-1	Product Ion-2	Concentration CS1 (ng/mL)	Concentration CS7 (ng/mL)
Target	ADONA	8.209	377.0	251.0	85.0	0.8	250
Target	9CI-PF3ONS	10.032	530.9	351.0	353.0	0.8	250
Target	11CI-PF3OUdS	11.728	630.9	451.0	453.0	0.8	250
Target	3:3 FTCA	2.937	241.0	177.0	117.0	1	312.5
Target	5:3 FTCA	6.076	341.0	237.0	217.0	5	1562.5
Target	7:3 FTCA	8.952	441.0	317.0	337.0	5	1562.5
Target	PFEESA	5.943	315.0	135.0	83.0	0.4	125
Target	PFMPA	2.681	229.0	85.0	N/A	0.4	125
Target	PFMBA	3.916	279.0	85.0	N/A	0.4	125
Target	NFDHA	4.715	295.0	201.0	85.0	0.4	125
EIS	13C4-PFBA	2.025	217.0	172.0	172.0	10	10
EIS	13C5-PFPeA	3.568	268.0	223.0	223.0	5	5
EIS	13C5-PFHxA	4.904	318.0	273.0	120.0	2.5	2.5
EIS	13C4-PFHpA	7.707	367.0	322.0	322.0	2.5	2.5
EIS	13C8-PFOA	8.64	421.0	376.0	376.0	2.5	2.5
EIS	13C9-PFNA	9.119	472.0	427.0	427.0	1.25	1.25
EIS	13C6-PFDA	9.51	519.0	474.0	474.0	1.25	1.25
EIS	13C7-PFUnA	9.899	570.0	525.0	525.0	1.25	1.25
EIS	13C2-PFDoA	10.458	615.0	570.0	570.0	1.25	1.25
EIS	13C2-PFTeDA	12.236	715.0	670.0	670.0	1.25	1.25
EIS	13C3-PFBS	4.835	302.0	80.0	99.0	2.5	2.5
EIS	13C3-PFHxS	8.775	402.0	80.0	99.0	2.5	2.5
EIS	13C8-PFOS	9.71	507.0	80.0	98.9	2.5	2.5
EIS	13C2-4:2FTS	4.405	329.0	309.0	80.9	5	5
EIS	13C2-6:2FTS	8.397	429.0	409.0	80.9	5	5
EIS	13C2-8:2FTS	9.323	529.0	509.0	80.9	5	5
EIS	13C8-PFOA	11.438	506.0	78.0	78.0	2.5	2.5
EIS	D3-NMeFOSA	13.174	515.0	219.0	168.9	2.5	2.5
EIS	D5-NEtFOSA	13.467	531.0	219.0	168.9	2.5	2.5
EIS	D3-NMeFOSAA	9.489	573.0	419.0	419.0	5	5
EIS	D5-NEtFOSAA	9.645	589.0	419.0	419.0	5	5
EIS	D7-NMeFOSE	12.997	623.1	59.0	59.0	25	25
EIS	D9-NEtFOSE	13.282	639.1	59.0	59.0	25	25
EIS	13C3-HFO-DA	5.688	287.0	169.0	185.0	10	10
NIS	13C3-PFBA	2.023	216.0	172.0	N/A	5	5
NIS	13C2-PFHxA	4.905	315.0	270.0	119.0	2.5	2.5
NIS	13C4-PFOA	8.637	417.0	172.0	N/A	2.5	2.5
NIS	13C5-PFNA	9.118	468.0	423.0	N/A	1.25	1.25
NIS	13C2-PFDA	9.51	515.0	470.0	N/A	1.25	1.25
NIS	18O2-PFHxS	8.775	403.0	84.0	N/A	2.5	2.5
NIS	13C4-PFOS	9.708	503.0	80.0	99.0	2.5	2.5
--	TDCA	8.401	498.3	124.0	80.0		

■ Results

Calibration was performed for all targeted PFAS using a seven-point calibration curve and following the recommendations included in EPA draft method 1633; concentrations ranged from 0.2 – 250 ng/mL for targets, 1.25 – 25 ng/mL for EISs, and 1.25 – 5 ng/mL as shown in Table 3. The LOQs for each target compound are listed in Table 3 and equivalent to CS1; all LOQs meet the concentration listed in EPAM1633.

Fig. 1 shows the MRM transitions from CS1; this figure demonstrates the separation and peak shape of targets at the lowest concentration standard included in the calibration curve.

Table 4 summarizes signal-to-noise (S/N), accuracy, and instrument linearity for the initial calibration, and the instrument detection limit (IDL) for all target compounds. The S/N of CS1 ranged from 8 to >30,000; this demonstrates that lower concentrations of the targeted PFAS could be easily measured with the LCMS-8050. Accuracy for all target compounds in CS1 ranged between 80% and 116%, exceeding results reported and accepted in EPAM1633. The instrument linearity for the calibration curve was evaluated by calculating the relative standard error (RSE); RSE for all target compounds was ≤20% in accordance with EPAM1633. The IDLs and %RSDs at the IDLs for each target are shown in Table 4. The IDLs ranged between 1.9 ng/mL (7:3 FTCA) and 0.01 ng/mL (multiple analytes), approximately one order of

magnitude lower than the LOQs.

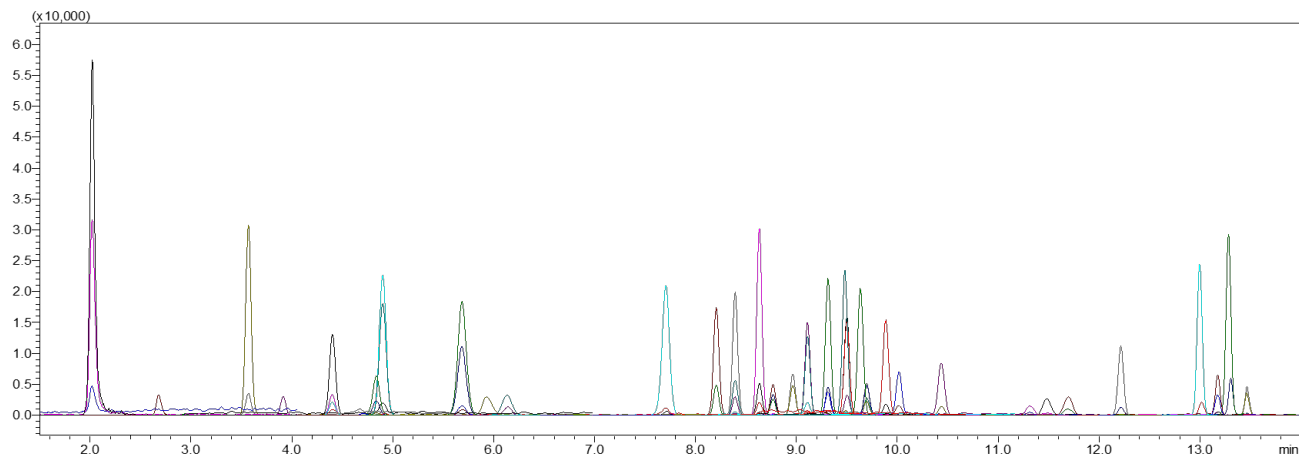


Fig. 1 MRM transitions of all PFAS (target, EIS, NIS) at the lowest calibration standard (CS1) concentration.

Table 4 Method performance summary of neat standards using the Shimadzu LCMS-8050.

Name	CS1 Average S/N (n=7)	%Accuracy CS1 (n=7)	RSE	IDL ng/mL (n=7)	%RSD IDL (n=7)
PFBA	12.36	102.68	3.64	0.16	5.77
PFPeA	18.73	105.25	2.98	0.18	12.52
PFHxA	25.93	106.18	4.75	0.13	21.55
PFHpA	7.85	102.14	4.77	0.11	15.03
PFOA	16.56	103.79	8.42	0.08	11.19
PFNA	26.59	105.89	7.06	0.10	15.65
PFDA	32.95	105.47	9.36	0.07	11.79
PFUnA	44.23	104.42	7.51	0.10	15.78
PFDoA	56.80	99.14	6.67	0.15	20.17
PFTTrDA	47.26	109.92	6.73	0.11	16.01
PFTeDA	145.08	86.49	7.07	0.08	10.89
PFBS	106.61	104.01	8.32	0.12	23.82
PFPeS	57.58	116.00	8.97	0.12	16.54
PFHxS	287.07	103.13	9.56	0.18	31.19
PFHpS	96.74	100.94	10.68	0.07	10.80
PFOS	12.97	104.09	11.42	0.21	50.62
PFNS	32.95	113.14	10.88	0.12	16.77
PFDS	101.25	94.83	11.37	0.18	34.16
PFDoS	62.67	109.76	6.70	0.17	19.79
4:2FTS	28989.63	83.92	15.20	0.24	9.70
6:2FTS	24990.49	80.44	15.82	0.50	26.45
8:2FTS	34754.78	108.43	10.40	0.32	12.78
PFOSA	304.11	113.56	11.92	0.22	35.66
NMeFOSA	107.66	110.25	10.66	0.14	19.22
NEtFOSA	537.58	107.01	9.04	0.12	15.94
NMeFOSAA	111.13	102.36	5.69	0.09	15.71
NEtFOSAA	18.72	112.22	10.59	0.18	34.30
NMeFOSE	150.32	92.74	6.52	0.85	11.82
NEtFOSE	158.18	101.23	6.01	0.38	5.63
HFPO-DA	626.89	100.05	4.20	0.22	9.04
ADONA	2149.52	101.71	3.84	0.13	5.14
9Cl-PF3ONS	486.71	94.21	4.70	0.16	6.11
11Cl-PF3OUdS	671.33	111.72	9.08	0.35	14.03
3:3 FTCA	3924.23	104.45	8.49	0.31	12.02
5:3 FTCA	196.34	112.09	6.11	0.84	5.14
7:3 FTCA	1179.93	111.16	5.90	1.89	11.20
PFEEESA	87.20	105.06	3.19	0.11	7.91
PFMPA	476.40	104.14	2.67	0.09	6.87
PFMBA	38489.86	113.02	7.73	0.14	10.19
NFDHA	2035.17	85.56	8.37	0.24	17.57

Repeatability was evaluated at CS1 (n=7), CS4 (n=6) and CS7 (n=6). In CS1, the lowest calibration standard, 75% of the targeted compounds showed a %RSD of less than 20%; the %RSD of 12.5% of the targets ranged between 20% and 30%, and %RSD was >30% for the remaining 12.5% of the targets (5 compounds).

The %RSD for all targets in CS4 and CS7 (i.e., the mid-point and high point of the calibration curve, respectively) was <20%, with most compounds showing a %RSD of less than 10%.

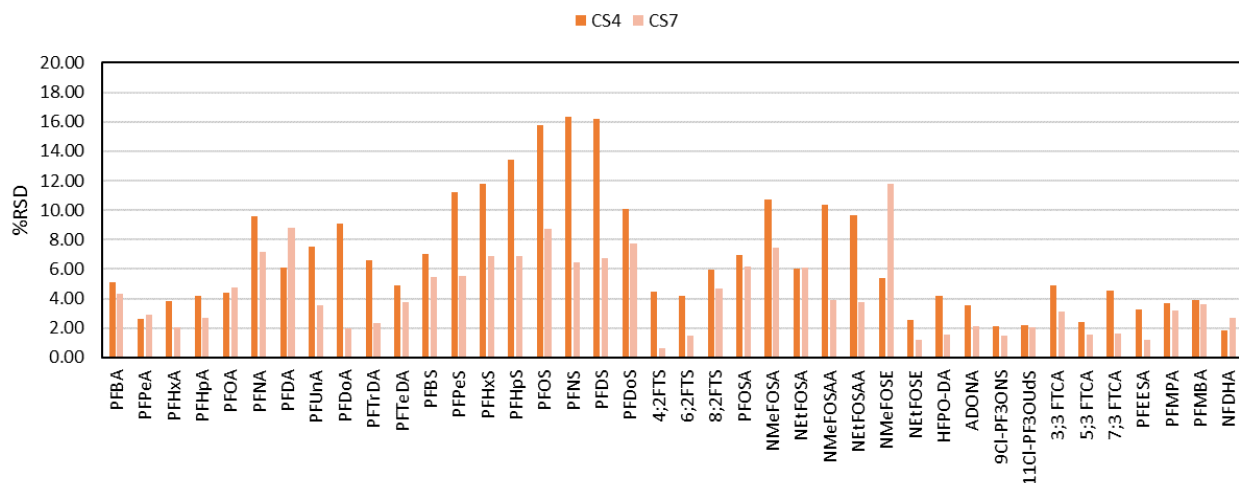


Fig. 2 The %RSDs of target compounds from CS4 (n=6) and CS7 (n=6).

Two types of aqueous samples were analyzed following the extraction procedure outlined in EPAM1633: ultrapure reagent water (n=5) and wastewater (n=7). Both sample types were spiked at concentrations equal to those in CS1. Precision and accuracy are normally evaluated by spiking the samples with a mid-level concentration. CS1 was used instead to better assess the method performance at a more challenging concentration.

Table 5 shows the %recoveries of the EISs in the ultrapure reagent water and wastewater samples. In ultrapure reagent water, the recoveries from all EISs were within 50% and 150%, except D5-NEtFOA(48%). The recoveries of the EIS in wastewater were slightly lower for all compounds than in ultrapure reagent water. The %recoveries of the EIS in both types of samples exceed the reported ranges in EPAM1633. Fig. 3 shows the recovery of the target compounds spiked at CS1 concentrations in ultrapure reagent water (n=5) and wastewater (n=3), with error bars displaying the %RSD. The %recovery and %RSD for all the targeted PFAS in ultra-pure water and wastewater, except for 3:3 FTCA, are within acceptable ranges for the analysis of PFAS in environmental samples when spiked at the lowest standard of the calibration curve.

Table 5 The %recoveries of EIS in ultra-pure water and wastewater samples.

EIS	% Recovery CS1 Spike in UPW (n=5)	% Recovery CS1 Spike in WW (n=7)
13C4-PBFA	94.09	32.78
13C5-PFPeA	96.48	85.57
13C5-PFHxA	99.69	93.64
13C4-PFHpA	100.51	91.56
13C8-PFOA	104.16	93.28
13C9-PFNA	107.61	98.75
13C6-PFDA	104.58	89.06
13C7-PFUnA	97.69	72.58
13C2-PFDoA	82.12	58.55
13C2-PFTeDA	65.92	42.09
13C3-PFBS	96.04	92.31
13C3-PFHxS	105.44	100.97
13C8-PFOs	109.04	99.40
13C2-4:2FTS	86.72	82.76
13C2-6:2FTS	93.63	83.95
13C2-8:2FTS	93.09	84.67
13C8-PFOA	72.53	85.68
D3-NMeFOA	52.33	54.40
D5-NEtFOA	47.86	44.46
D3-NMeFOSAA	102.61	79.32
D5-NEtFOSAA	91.70	61.64
D7-NMeFOSE	56.78	49.43
D9-NEtFOSE	53.77	45.35

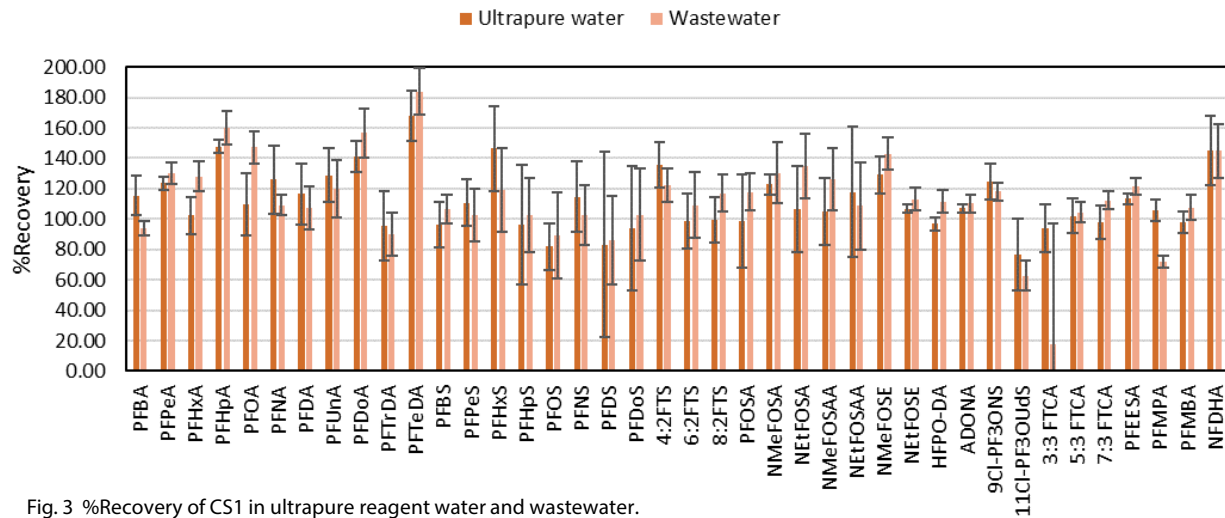


Fig. 3 %Recovery of CS1 in ultrapure reagent water and wastewater.

■ Summary and Conclusions

The Shimadzu LCMS-8050 was evaluated for its ability to analyze PFAS in wastewater samples in accordance with EPAM1633. The results demonstrated the excellent performance of the LCMS-8050 for key quality control and performance parameters defined in the draft method and indicate that lower detection limits are easily achievable.

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UFMS

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LCMS-8045



LCMS-8050



LCMS-8060NX



LCMS-2020



LCMS-2050



Q-TOF LCMS-9030/9050

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