

Application News

LCMSTM-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

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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-PFOA
Target	PFDA	EIS	13C2-PFTeDA
Target	NEtFOSAA	EIS	D7-NMeFOSE
Target	PFOS	EIS	D3-NMeFOA
Target	PFUnA	EIS	D9-NEtFOSE
Target	9CI-PF3ONS	EIS	D5-NEtFOA
Target	PFNS	NIS	13C3-PFBA
Target	PFDOA	NIS	13C2-PFHxA
Target	PFOSA	NIS	13C4-PFOA
Target	PFDS	NIS	18O2-PFHxS
Target	PFTTrDA	NIS	13C5-PFNA
Target	11CI-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.

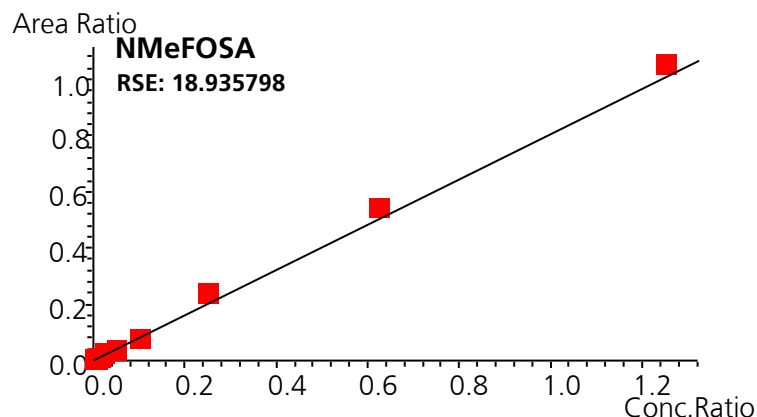


Figure 2. Calibration curve for NMeFOSA

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-PFOA	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.

$$\text{Equation 1: } MDLs = t(n-1, 1-\alpha \cong 0.99) Ss$$

$$\text{Equation 2: } MDLb = X(\bar{0}) + t(n-1, 1-\alpha \cong 0.99) Ss$$

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 PFEEESA 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 perfluoroalkyl 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 (PFEEESA) 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.

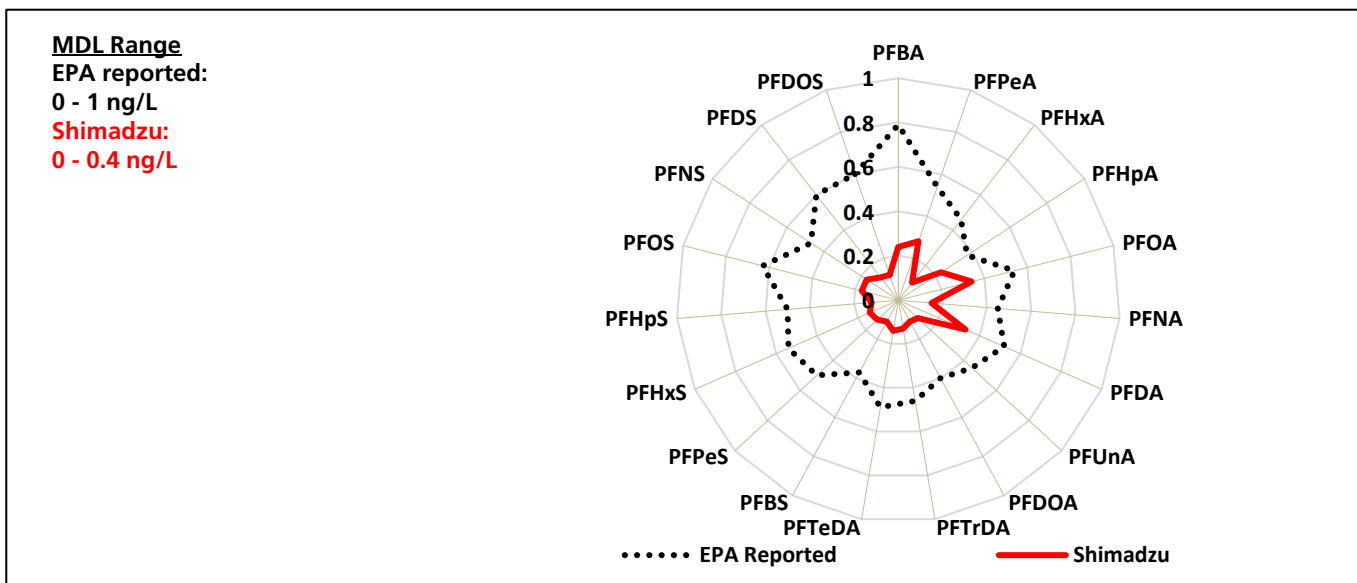


Figure 3: MDLs reported in EPA 1633 and obtained with Shimadzu's LCMS-8060NX of perfluoroalkyl carboxylic acids and sulfonic acids.

MDL Range

EPA reported:

0 - 10 ng/L

Shimadzu:

0 - 2 ng/L

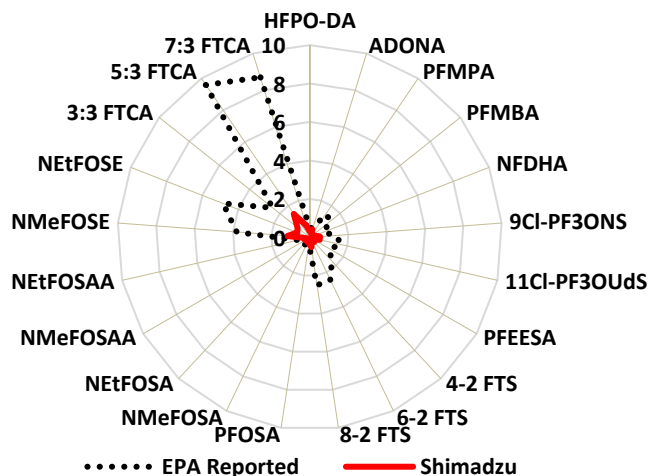


Figure 4: MDLs reported in EPA 1633 and obtained with Shimadzu's LCM5-8060NX of Per- and Polyfluoroether carboxylic acids, Ether sulfonic acids, Fluorotelomer sulfonic acids, Perfluorooctane sulfonamides, Perfluorooctane sulfonamidoacetic acids, Perfluorooctane sulfonamide ethanols, Fluorotelomer carboxylic 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
PFTeDA	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 in 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

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