

Analysis of PFAS Specified in EPA Method 537 and Beyond using Shimadzu UFMS™

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Abstract

This application news demonstrates the use, performance and compatibility of Shimadzu Ultra-fast Mass Spectrometry (UFMS™) for EPA Method 537 with an expanded compound panel of seven additional PFAS. A total of 27 PFAS compounds were extracted, separated and detected with triple quadrupole mass spectrometers, LCMS-8045 and LCMS-8050. Recoveries of 86–106% (LCMS-8050) and 77–104% (LCMS-8045) were well within the limits outlined in EPA 537. Method detection limits of 0.7–1.7 ng/L (ppt) (LCMS-8050) and 0.7–3.3 ng/L (LCMS-8045) were obtained and both systems fulfilled all of the EPA's requirements for PFAS analysis in drinking water.

Keywords: Per- and Polyfluorinated Alkyl Substances, PFAS, Perfluorinated Compounds, PFCs, Drinking Water, PFOA, PFOS, Persistent Organic Pollutants, POPs

Introduction

Per- and Polyfluorinated Alkyl Substances (PFAS) are a group of anthropogenic chemicals widely used as fire retardants, food packaging materials, and non-stick

coatings because of their heat-resistant, and oil- and water-repellent properties. These properties result in resistance to degradation, hence, PFAS accumulate in the environment. Moreover, PFAS are capable of long-range transport and can potentially affect human health (e.g. developmental and reproductive effects).

Over the past several years, the issue of PFAS contamination in drinking water has become a global concern. To safeguard public health and minimize human exposure to these chemicals, the US, EU and Australia have issued health advisory guidelines for perfluorooctanoic acid (PFOA) and perfluorooctane-sulfonic acid (PFOS) in drinking water (e.g. US: 70 ppt for combined PFOS and PFOA). Furthermore, some states in the US (e.g. California, Minnesota, Colorado, Michigan and New Jersey) have established similar or even stricter limits for PFAS and these can go as low as 13 ppt and 14 ppt for PFOS and PFOA respectively.

US Environmental Protection Agency (US EPA) has established Method 537 for PFAS in drinking water. It utilizes a solid phase extraction (SPE) followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) for the determination of 14 perfluorinated alkyl acids in drinking water. Besides the analysis of these

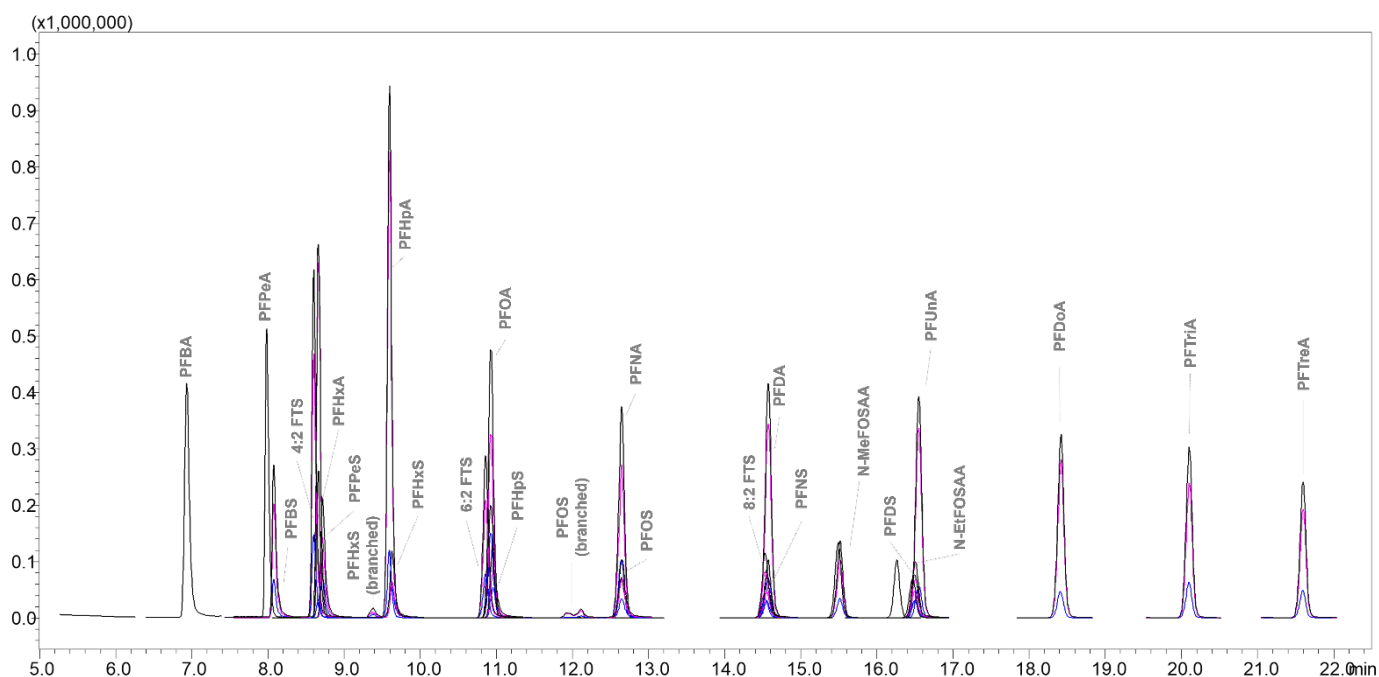


Figure 1. MRM (pink and blue) and TIC (black) chromatograms of all PFAS in a mixed standard solution with each PFAS at 20 ng/mL (LCMS-8050)

PFAS (e.g. PFOA and PFOS), there are other classes gaining attention due to their increasing use, occurrence and persistence in the environment. In this study, apart from the compounds listed in EPA Method 537, the scope of PFAS has been expanded to include seven additional compounds such as fluorotelomeric alcohols (precursor of PFOA).

This application news describes and demonstrates the use and performance of Shimadzu UFMS™ for the analysis of 21 PFAS (including all 14 stated in EPA Method 537) in drinking water. Shimadzu triple quadrupole mass spectrometers, LCMS-8045 and LCMS-8050, were used in this study. Possessing an ultra-fast acquisition rate of 555 MRM/sec and a high polarity switching speed (5 msec), these UFMS™ instruments achieve rapid, reliable and highly-sensitive quantitation of PFAS in drinking water.

□ Experimental

PFAS and Preparation of Calibration Standards

Table 1 lists the 27 PFAS compounds (21 target compounds, 3 internal standards (IS) and 3 surrogates) used in this study. All PFAS standards were purchased from Wellington Laboratories (Guelph, Ontario). A series of 10 calibration standards at concentrations of 1.25, 2.50, 5.00, 10.0, 15.0, 20.0, 25.0, 37.5, 50.0 and 100 ng/mL were prepared by dilution with 96:4% (vol/vol) methanol:water. These concentrations were 250 times higher than the target concentration range in consideration of analyte enrichment over the course of sample preparation, such that a 1.25 ng/mL calibration standard was equivalent to 5 ng/L of field sample.

Table 1. List of PFAS (target compounds, internal standards and surrogates)

PFAS Compounds	Abbreviation	CAS	Molecular Weight	Molecular Formula	IS, Surrogates and its Abbreviation	PFAS Listed in EPA Method 537
<u>PERFLUOROALKYLCARBOXYLIC ACIDS</u>						
Perfluorohexanoic acid	PFHxA	307-24-4	314.06	C ₆ F ₁₁ O ₂ H	M2PFHxA (Surr.) (¹³ C ₂ ¹² C ₄ F ₁₁ O ₂ H)	✓
Perfluoroheptanoic acid	PFHpA	375-85-9	364.06	C ₇ F ₁₃ O ₂ H	-	✓
Perfluorooctanoic acid	PFOA	335-67-1	414.07	C ₈ F ₁₅ O ₂ H	M2PFOA (IS) (¹³ C ₂ ¹² C ₆ F ₁₅ O ₂ H)	✓
Perfluorononanoic acid	PFNA	375-95-1	464.08	C ₉ F ₁₇ O ₂ H	-	✓
Perfluorodecanoic acid	PFDA	335-76-2	514.09	C ₁₀ F ₁₉ O ₂ H	M2PFDA (Surr.) (¹³ C ₂ ¹² C ₈ F ₁₉ O ₂ H)	✓
Perfluoroundecanoic acid	PFUnA	2058-94-8	564.09	C ₁₁ F ₂₁ O ₂ H	-	✓
Perfluorododecanoic acid	PFDoA	307-55-1	614.10	C ₁₂ F ₂₃ O ₂ H	-	✓
Perfluorotridecanoic acid	PFTriA	72629-94-8	664.11	C ₁₃ F ₂₅ O ₂ H	-	✓
Perfluorotetradecanoic acid	PFTreA	376-06-7	714.12	C ₁₄ F ₂₇ O ₂ H	-	✓
<u>PERFLUOROALKYLSULFONATES</u>						
Perfluorobutyl sulfonate	PFBS	375-73-5	300.10	C ₄ F ₉ SO ₃ H	-	✓
Perfluoropentane sulfonate	PFPeS	2706-91-4	350.11	C ₅ F ₁₁ SO ₃ H	-	Additional
Perfluorohexyl sulfonate	PFHxS	355-46-4	400.11	C ₆ F ₁₃ SO ₃ H	-	✓
Perfluorohexyl sulfonate	PFHpS	375-92-8	450.12	C ₇ F ₁₅ SO ₃ H	-	Additional
Perfluoroheptane sulfonate	PFOS	1763-23-1	500.13	C ₈ F ₁₇ SO ₃ H	M4PFOS (IS) (¹³ C ₄ ¹² C ₄ F ₁₇ SO ₃ Na)	✓
Perfluorooctyl sulfonate	PFNS	68259-12-1	550.14	C ₉ F ₁₉ SO ₃ H	-	Additional
Perfluorononane sulfonate	PFDS	335-77-3	600.14	C ₁₀ F ₂₁ SO ₃ H	-	Additional
<u>FLUORINATED TELOMER SULFONATES</u>						
1H, 1H, 2H, 2H-perfluorohexane sulfonic acid	4:2 FTS	757124-72-4	328.15	C ₆ H ₅ F ₉ SO ₃	-	Additional
1H, 1H, 2H, 2H-perfluorooctane sulfonic acid	6:2 FTS	27619-97-2	428.17	C ₈ H ₅ F ₁₃ SO ₃	-	Additional
1H, 1H, 2H, 2H-perfluorodecane sulfonic acid	8:2 FTS	39108-34-4	528.18	C ₁₀ H ₅ F ₁₇ SO ₃	-	Additional
<u>PERFLUOROOCETANESULFONAMIDE AND PERFLUOROOCETANESULFONAMIDOACETIC ACIDS</u>						
2-(N-Methylperfluorooctanesulfonamido) acetic acid	N-MeFOSAA	2355-31-9	571.21	C ₁₁ H ₆ F ₁₇ NSO ₄	d3-NMeFOSAA (IS) (C ₁₁ ² H ₃ F ₁₇ NSO ₄)	✓
2-(N-Ethylperfluorooctanesulfonamido) acetic acid	N-EtFOSAA	2991-50-6	585.24	C ₁₂ H ₈ F ₁₇ NSO ₄	d5-NEtFOSAA (Surr.) (C ₁₂ ² H ₅ H ₃ F ₁₇ NSO ₄)	✓

Preparation of Samples

Sample preparation and SPE were carried out according to EPA Method 537. A vacuum manifold with a high-volume sampling kit fitted with PEEK tubing was used to reduce potential PFAS contamination. Extractions were performed using Biotage-ISOLUTE® 101 polystyrenedivinylbenzene (SDVB) cartridges (500 mg / 6 mL, Part No. 101-0050-C). Each cartridge was first conditioned with methanol, followed by LCMS-grade water. The water sample was first fortified with surrogates and passed through the SPE cartridge. Compounds were eluted from the solid phase with 8 mL of methanol and evaporated to dryness using nitrogen. Extracted samples were reconstituted to a

final volume of 1 mL in 96:4% methanol:water after adding IS. It is recommended to vortex the LC vials prior to injection and analysis by LC-MS/MS to ensure that all solutions are homogenized and consistent results are obtained.

LCMS Analytical and Instrument Conditions

PFAS analyses were carried out by injecting 1 µL of the extracted aliquot into the Shimadzu LCMS-8045 and LCMS-8050 with the conditions shown in Table 2 and Table 3. PFAS compounds, including branched and linear isomers of PFHxS and PFOS, were separated using a Shim-pack™ Velox SP-C18 column.

Table 2. LC System and Parameters

LC System	: Nexera™-X2 UHPLC System
Analytical Column	: Shim-pack™ Velox SP-C18, 150mm x 2.1mm x 2.7µm, Part No. 227-32003-04
Solvent Delay Column	: Shim-pack™ XR-ODS II, 75mm x 2mm x 2.2µm, Part No. 228-41605-93
Column Temp.	: 40 °C
Injection Volume	: 1 µL [#]
Mobile Phase	: A: 20 mM Ammonium Acetate B: Methanol
Flow Rate	: 0.25 mL/min
Run Time	: 35 minutes

[#]A much lower injection volume was used as compared to the injection volume of 10 µL in EPA Method 537.

Table 3. LCMS Acquisition Parameters

MS Instrument	: LCMS-8045 and LCMS-8050
Interface	: Electrospray Ionization (ESI)
Interface Temp.	: 300 °C
Desolvation Line Temp.	: 100 °C
Heat Block Temp.	: 200 °C
Heating Gas Flow	: 15 L/min
Drying Gas Flow	: 5 L/min
Nebulizing Gas Flow	: 3 L/min
Total MRMs	: 48

Table 4. Retention times (RT) and MRM transitions

PFAS Compound	RT (mins)	Precursor Ion	Product Ion
PFBS	8.046	298.90	80.10*
			99.10
4:2 FTS	8.558	327.00	80.90*
			307.00
PFHxA	8.614	312.90	269.00*
			119.10
M2PFHxA (Surr.)	8.650	315.00	270.00*
PFPeS	8.666	348.90	79.90*
			98.90
PFHpA	9.512	362.90	319.00*
			169.00
PFHxS	9.558	398.90	80.10*
			99.10
6:2 FTS	10.770	427.00	406.90*
			80.00
PFOA	10.840	412.90	369.00*
			169.00
PFHpS	10.859	448.90	79.90*
			98.90
M2PFOA (IS)	10.877	415.00	370.00*
PFNA	12.545	462.90	418.90*
			219.00
PFOS	12.550	498.90	80.10*
			99.10
M4PFOS (IS)	12.575	503.00	80.00*
8:2 FTS	14.436	526.90	506.90*
			80.90
PFNS	14.469	548.90	79.90*
			98.90
M2PFDA (Surr.)	14.484	515.00	469.95*
PFDA	14.486	512.90	468.90*
			219.00
M-N-MeFOSAA (IS)	15.403	572.90	419.00*
N-MeFOSAA	15.423	569.90	419.00*
			482.90
M-N-EtFOSAA (Surr.)	16.357	588.90	419.00*
PFDS	16.397	598.90	79.90*
			98.90
N-EtFOSAA	16.411	583.90	419.00*
			482.90
PFUnA	16.449	562.90	519.00*
			269.00
PFDoA	18.339	612.90	568.90*
			169.00
PFTriA	20.035	662.00	618.90*
			169.00
PFTreA	21.549	712.90	668.90*
			169.00

* Quantifying ions

Since PFAS is ubiquitously present on laboratory equipment such as tubing and HPLC systems, it is impossible to completely eliminate PFAS from LC mobile phases even if LCMS-grade reagent solvents have been used. This necessitates the use of a solvent delay column for high-sensitivity analysis. A small C18 column that have higher retention of PFAS than the analytical column is placed directly upstream of autosampler to trap all PFAS contained in the mobile phase. During chromatographic elution, the analytical column gives sample-derived PFAS peaks first, separated from secondary peaks derived from mobile phase contamination trapped on the delay column.

□ Results and Discussion

Chromatographic Separation

Two MRM transitions (one for quantifying and the other for confirmation) were selected for each target PFAS (Table 4). Figure 1 shows the overlaid MRM and total ion current (TIC) chromatograms of all PFAS in a mixed standard solution (20 ng/mL), obtained using LCMS-8050 to demonstrate the representative separation profile.

The branched and linear isomers of PFHxS (Figure 2) and PFOS (Figure 3 and Figure 4) were chromatographically separated using the Shim-pack™ Velox SP-C18 column.

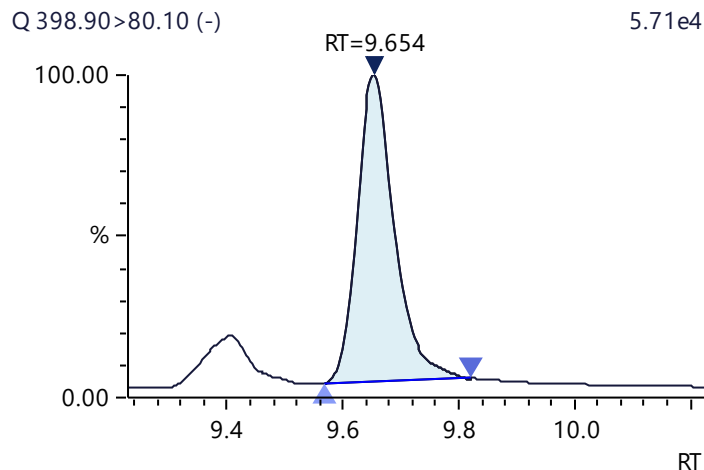


Figure 2. Separation of PFHxS isomers (20 ng/mL)

Calibration Curve Linearity and Continuing Calibration Check

The calibration solutions for target PFAS were prepared and analyzed at one injection each to generate ten-point calibration curves. All calibration curves (Table 5) had the regression coefficient (R^2) higher than 0.99 and quantitation of PFAS was performed using these calibration curves. Continuing Calibration Checks (CCC) was conducted at low (20 ng/mL), mid (50 ng/mL) and high (100 ng/mL) concentrations by 4 repeat injections over the course of

Table 5. Calibration curve linearity (1.25-100 ng/mL and %RSD of CCC (n = 4) using LCMS-8045

Compound	Linearity (R^2)	Low Concentration (20 ng/mL)		Mid Concentration (50 ng/mL)		High Concentration (100 ng/mL)	
		Conc.	%RSD	Conc.	%RSD	Conc.	%RSD
PFBS	0.9977	21	2	46	3	103	2
4:2FTS [^]	0.9928	22	2	45	7	94	1
PFHxA	0.9968	21	4	48	6	102	3
PFPeS [^]	0.9985	21	2	46	2	100	1
PFHpA	0.9974	21	5	46	5	101	2
PFHxS	0.9968	21	3	46	5	104	3
6:2 FTS [^]	0.9968	21	4	44	4	95	2
PFOA	0.9967	21	5	47	7	103	3
PFHpS [^]	0.9982	21	4	45	8	104	6
PFOS	0.9986	20	6	44	7	103	12
PFNA	0.9975	21	10	47	2	100	3
8:2 FTS [^]	0.9940	23	14	46	13	94	13
PFNS [^]	0.9978	21	2	46	6	100	5
PFDA	0.9969	21	3	47	3	98	2
N-MeFOSAA	0.9979	21	3	47	1	100	3
N-EtFOSAA	0.9980	22	4	48	2	102	5
PFDS [^]	0.9970	21	4	45	11	103	5
PFUnA	0.9973	21	4	48	4	100	6
PFDoA	0.9975	21	4	48	3	103	6
PFTriA	0.9967	20	5	45	5	101	5
PFTreA	0.9966	21	5	47	4	103	3

[^]Additional PFAS compounds not listed in EPA Method 537.

two weeks' investigation. The recovery of all PFAS compounds at the three concentration levels were well within the EPA's CCC criteria (i.e. 70–130% of the true value). The data demonstrated that both the LCMS-8045 and LCMS-8050 were capable of reliably

covering the concentration range required by EPA 537, though the LCMS-8050 has a superior signal-to-noise ratio as illustrated in Figure 3 (LCMS-8045) and Figure 4 (LCMS-8050).

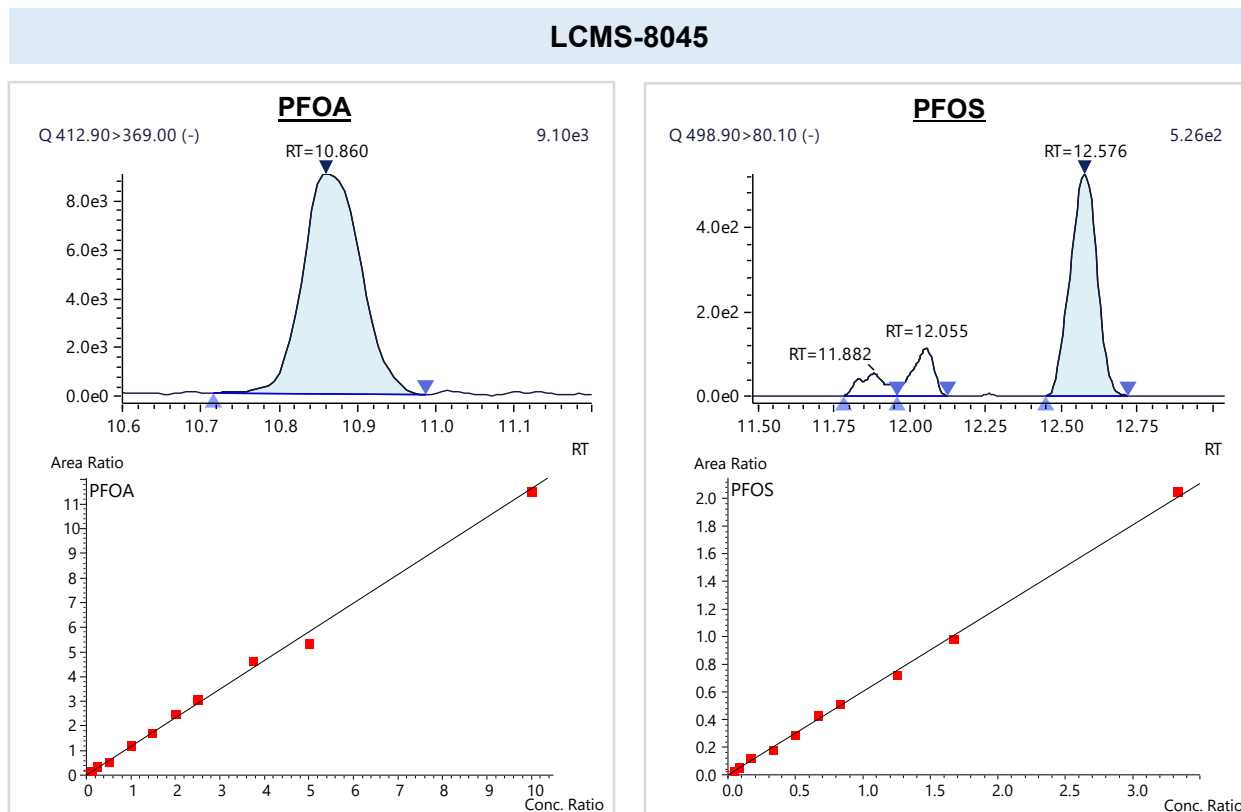


Figure 3. MRM chromatogram (for 1.25 ng/mL) and calibration curve for PFOA and PFOS using LCMS-8045

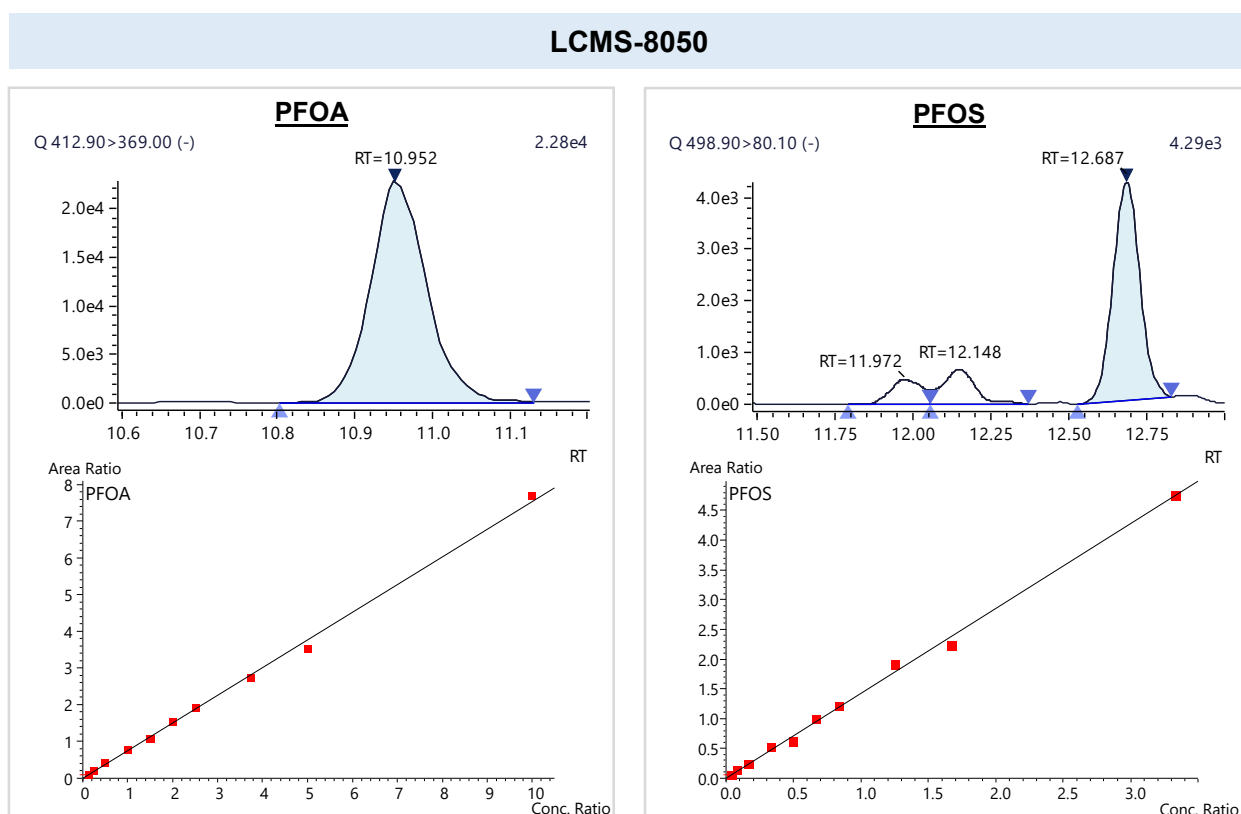


Figure 4. MRM chromatogram (1.25 ng/L) and calibration curve for PFOA and PFOS using LCMS-8050

Method Detection Limit

A Method Detection Limit (MDL) study was conducted by spiking the 250 mL water samples to obtain a spiked concentration of 5 ng/L (5 ppt) for each PFAS. These samples were then pretreated and concentrated

to a final volume of 1 mL in 96:4 % methanol:water. Nine such samples were pretreated over the course of three days. The results of the MDL study using the LCMS-8045 and LCMS-8050 are tabulated (Table 6), MDLs ranging from 0.7–3.3 ng/L and 0.7–1.6 ng/L, respectively, were achieved.

Table 6. Results for Method Detection Limits (MDL) study using LCMS-8045 and LCMS-8050

Compound	Spiked Conc. (ng/L)	LCMS-8045				LCMS-8050			
		Calculated Conc. (ng/L), n = 9	% Recovery	% RSD	MDL (ng/L)	Calculated Conc. (ng/L), n = 9	% Recovery	%RSD	MDL
PFBS	5	4.2	83	12	1.5	5.1	102	8	1.2
4:2FTS^	5	5.2	104	14	2.1	4.9	98	9	1.3
PFHxA	5	4.1	81	10	1.2	4.7	94	7	1.0
PFPeS^	5	4.1	81	13	1.5	4.8	96	9	1.4
PFHpA	5	4.2	84	8	1.1	4.7	94	7	1.0
PFHxS	5	4.3	85	6	0.7	4.8	96	8	1.2
6:2 FTS^	5	4.6	92	17	2.3	4.8	96	7	1.1
PFOA	5	4.6	92	12	1.6	4.7	94	7	1.0
PFHpS^	5	4.0	80	9	1.0	4.7	95	11	1.6
PFOS	5	4.0	81	15	1.7	4.6	92	6	0.8
PFNA	5	4.0	80	7	0.8	4.8	97	5	0.7
8:2 FTS^	5	5.0	100	22	3.3	5.3	106	11	1.7
PFNS^	5	4.0	81	9	2.1	4.4	91	8	1.1
PFDA	5	4.1	83	8	1.0	4.8	95	10	1.4
N-MeFOSAA	5	3.9	78	15	1.7	4.6	91	9	1.2
N-EtFOSAA	5	3.8	77	11	1.2	4.4	88	10	1.3
PFDS^	5	4.1	82	18	2.2	4.6	92	10	1.4
PFUnA	5	4.1	82	12	1.5	4.4	88	11	1.4
PFDoA	5	4.0	79	14	1.6	4.3	86	9	1.2
PFTriA	5	3.9	78	13	1.4	4.4	87	10	1.3
PFTreA	5	4.0	79	15	1.8	4.3	86	11	1.3

^Additional PFAS compounds not listed in EPA Method 537.

Accuracy and Precision

The initial demonstration of accuracy and precision was carried out using seven replicate LCMS-grade water blanks fortified with each PFAS at 60 ng/L (ppt). Table 7 shows the average measured concentrations

of the seven replicates and the results of accuracy and precision evaluation expressed as percentage recovery and relative standard deviation (RSD). The recoveries obtained using the LCMS-8045 and LCMS-8050 were all within $\pm 20\%$ of the true value, meeting the criteria listed by EPA.

Table 7. Accuracy (% Recovery) and precision (% RSD) of target PFAS at 60 ng/L for LCMS-8045 and LCMS-8050

Compound	LCMS-8045			LCMS-8050		
	Average Conc. (ng/L), n = 7	% Recovery	% RSD	Average Conc. (ng/L), n = 7	% Recovery	% RSD
PFBS	52	87	13	54	90	6
4:2FTS^	54	90	13	56	94	8
PFHxA	52	87	12	52	87	9
PFPeS^	54	90	14	54	90	9
PFHpA	53	88	16	52	87	10
PFHxS	54	89	13	54	90	8
6:2 FTS^	55	92	15	55	92	9
PFOA	52	86	14	53	88	11
PFHpS^	54	90	13	53	89	9
PFOS	53	89	17	51	85	12
PFNA	51	86	16	64	107	21
8:2 FTS^	51	86	19	56	93	9
PFNS^	54	89	15	55	92	11
PFDA	52	87	13	52	87	10
N-MeFOSAA	53	88	15	53	88	9
N-EtFOSAA	54	90	15	56	93	10
PFDS^	52	86	17	53	89	9
PFUnA	51	85	11	53	88	10
PFDoA	51	86	14	51	85	9
PFTriA	49	82	14	51	85	9
PFTreA	49	82	14	49	81	8

^Additional PFAS compounds not listed in EPA Method 537.

Table 8. Average conc. (n = 7), % recovery and % RSD of surrogates in spiked samples using LCMS-8045 and LCMS-8050

Compound	Fortified Conc. (ng/L)	LCMS-8045			LCMS-8050		
		Average Conc (ng/L), n=7	% Recovery	% RSD	Average Conc (ng/L), n=7	% Recovery	% RSD
M2PFHxA	40	43	107	14	40	101	10
M2PFDA	40	44	109	12	42	106	13
M-N-EtFOSAA	160	175	109	14	160	100	12

Surrogate Recovery

Surrogate recovery was similarly investigated by first spiking the water samples with surrogates, prior to sample extraction. The spiked water samples undergo the sample preparation procedures and LCMS analysis. The calculated recovery of the surrogates must be in the range of 70–130% (EPA, Section 9.3.5) to demonstrate good method performance.

Seven water samples were spiked with 10 ng of M2PFHxA, 10 ng of M2PFDA and 40 ng of M-N-EtFOSAA giving a concentration of 40 ng/L for M2PFHxA and M2PFDA and 160 ng/L for M-N-EtFOSAA in the 250 mL water sample. The calculated recoveries obtained using LCMS-8045 and LCMS-8050 are shown in Table 8 using a Mean Response Factor. All recoveries were within $\pm 10\%$, well achieving EPA requirements.

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Summary and Conclusions

This application note described and demonstrated the use, performance and compatibility of Shimadzu UFMS™ for EPA Method 537 with seven additional PFAS targets. In this study, all samples and blanks were extracted by the same SPE procedure and analyzed using the same LC-MS/MS method on different instrument models. Method detection limits of 0.7–1.7 ng/L (LCMS-8050) and 0.7–3.3 ng/L (LCMS-8045) were obtained and both systems fulfilled all of the EPA's requirements for PFAS analysis in drinking water. This was achieved with a 1 μ L injection volume, in contrast to 10 μ L as described in the original EPA M537. The smaller injection volume (less burden on the LCMS) would make the method more robust and reduce the long-term cost of ownership. Moreover, Shimadzu's high-speed and high-sensitivity UFMS™ instruments allow users to further increase productivity by running multiple EPA methods on the same system, in which case using the LCMS-8050 is recommended.

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