SHIMADZU

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Novel Aspect

The Quantitation of short chain per- and polyfluoroalkyl substances (PFAS) in drinking water¹ by isotope dilution anion exchange solid phase extraction and liquid chromatography/tandem mass spectrometry (LC-MS/MS).

Introduction

Recently, EPA announced a new method for testing short chain per- and polyfluoroalkyl substances (PFAS) in drinking water. Structures for select short chain and long chain PFAS² are included in Figure 1. Method 533³ measures PFAS by isotope dilution anion exchange solid phase extraction and liquid chromatography/tandem mass spectrometry (LC-MS/MS). The lowest concentration minimum reporting levels (LCMRLs) for the method analytes range from 1.4 to 16 ng/L. Shimadzu Scientific Instruments was one of eight laboratories that participated in providing EPA with outside laboratory validation data along with a review of the method draft. This poster includes Shimadzu Scientific Instruments data from the validation study.

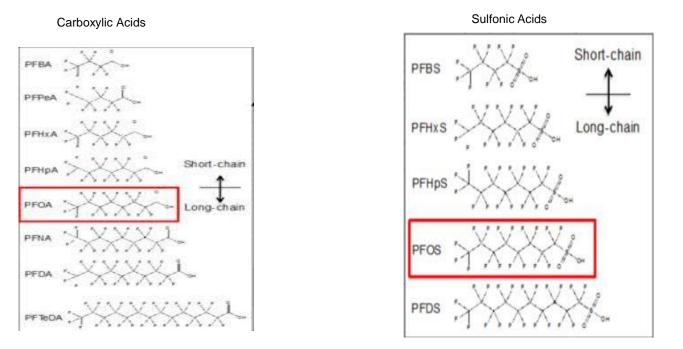


Figure 1. Structure of Long and Short Chain PFAS compounds.

SPE Method

Solid Phase Extraction (SPE) with a WAX sorbent (500 mg) was used for the extraction, as outlined in EPA method 533 (section 6.8.1). Each cartridge was cleaned and conditioned first, following EPA 533 (section 11.4.1). A vacuum manifold with a high-volume sampling kit outfitted with large bore PEEK tubing was used to reduce potential contamination.

All sample bottles were rinsed with the elution solvent prior to use. Each water sample (250 mL) is adjusted to pH 6-8 and fortified with PFAS analyte and Isotope dilution analytes, mixed, and loaded onto the conditioned cartridge. Compounds were eluted at a high pH from the solid phase with two 5 mL aliquots of methanol containing 2% ammonium hydroxide (v/v) and evaporated to dryness using nitrogen. Extracted samples were reconstituted to a final volume of 1 mL in 80:20 Methanol: H2O with internal standards added.

Extraction for P & A study was performed by fortifying five replicates of reagent water and tap water samples at 10 ng/L. For LCMRL calculations (results not shown in this poster) samples were extracted at eight concentration levels ranging from 0.2 ppt and 14 ppt. Four replicates were prepared at each concentration level and a minimum of four laboratory reagent blanks (LRB) were also included in the extraction batches



Figure 2. LCMS-8045 triple quadrupole mass spectrometer

Instrumental Method

The analysis of 25 PFAS compounds, with16 Isotope Dilution Analogues and 3 post extraction internal standards was performed using a UHPLC system coupled with a triple quadrupole mass spectrometer LC/MS/MS. MRM transitions were optimized using Flow Injection Analysis (FiA) for all compounds⁴. Source parameters were optimized to reduce fragmentation and increase sensitivity. Fluorotelomer acids, observed as [M-H]- and [M-HF-H]- can result in an ion with the same m/z as the unsaturated fluorotelomer acid. Even under optimized chromatography, these compounds have near identical retention times. The lower ESI heater temperature reduces HF loss and minimizes false identification of fluorotelomer acids. The chromatographic parameters are based on the chromatographic method used in EPA Method 533. A Shim-pack XR-ODS 50 x 3.0 mm column was used as a delay column, and a Phenomenex Gemini[™] C18, 2.0 mm ID × 50 mm, 3.0 µm particle size column was used as the analytical column. Quantitation was performed using MRM on tandem mass spectrometer (LC-MS/MS). LCMS system and instrumental conditions are included in Table 1 and MRM transitions are included in Table 2.
 Table 1. LCMS Method conditions

LCMS Instrume	nt		Shimadzu LO						
Analytical Colur	nn		Gemini 3µm C18 110A LC Column 50 x 2mm						
Solvent Delay C	olumn		Shim-pack XR-ODS 2.2-micron, 3.0 x 50mm						
Injection Volume			10 μL						
LC Flow Rate			0.25 mL/min						
Mobile Phase A			20 mM Amn	nonium Ac	cetate in LCMS-grade Water				
Mobile Phase B			Methanol						
Run / Acquisitio	on Cycle Time		35 minutes	(all 44 PFA	S comp	pounds are eluted in 20 minutes)			
		I							
		Time (min)	% MPA	%MPB	10	0			
		0	95	5	8				
		3	60	40	6	0			
Gradient C	Conditions	16	20	80	4	0		\	
		20	5	95	2	0			
		22	5	95		0		1	
		25	95	5		0	10	20	30
		Interface					ECI		ada
	Interface Temperature			ESI, Negative Mode 100 °C					
Desolvation Line Temperature				160 °C					
Heat Block Temperature				200 °C					
Heating Gas Flow					15 L/min				
Drying Gas Flow					5 L/min				
Nebulizing Gas Flow					3 L/min				
		Total MRMs				66			
		nimum Dwell				19 msec 124 msec			
	IVId		inne			124 IIISEC			

	CMS Instrument		Shimadzu LCMS-8045						
4	nalytical Column		Gemini 3µm C18 110A LC Column 50 x 2mm						
5	olvent Delay Column		Shim-pack XR-ODS 2.2-micron, 3.0 x 50mm						
r	ijection Volume		10 μL						
_	C Flow Rate		0.25 mL/min						
V	lobile Phase A		20 mM Ammonium Acetate in LCMS-grade Water						
V	lobile Phase B		Methanol						
2	un / Acquisition Cycle Time		35 minutes (all 44 PFAS compounds are eluted in 20 minutes)						
		Time (min)	% MPA	%MPB	3 100				
		0	95	5	80				
		3	60	40	60				
	Gradient Conditions	16	20	80	40				
		20	5	95	20				
		22	5	95	0				
		25	95	5	0 10 20 30				
		Interface			ESI, Negative Mode				
	Inte	erface Tempera	100 °C						
Desolvation Line Temperature					160 °C				
	Heat	Block Temper	200 °C						
		leating Gas Flo	15 L/min						
		Drying Gas Flo	5 L/min						
	Νε	ebulizing Gas F	3 L/min						
		Total MRMs			66				
		nimum Dwell 1 ximum Dwell 1			19 msec 124 msec				
_	IVIA		124 111580						

_									
CMS Instrument			Shimadzu LCMS-8045						
1	nalytical Column		Gemini 3µm C18 110A LC Column 50 x 2mm						
5	olvent Delay Column		Shim-pack X	R-ODS 2.2-	.2-micron, 3.0 x 50mm				
r	jection Volume		10 µL						
.(C Flow Rate		0.25 mL/min						
/	lobile Phase A		20 mM Ammonium Acetate in LCMS-grade Water						
/	lobile Phase B		Methanol						
2	un / Acquisition Cycle Time		35 minutes (all 44 PFAS compounds are eluted in 20 minutes)						
		I							
		Time (min)	% MPA	%MPB	100				
		0	95	5	80				
		3	60	40	60				
	Gradient Conditions	16	20	80	40				
		20	5	95	20				
		22	5	95	0				
		25	95	5	0 10 20 30				
		Interface			ESI, Negative Mode				
	Inte		ature		100 °C				
Interface Temperature Desolvation Line Temperature					160 °C				
		Block Tempe	200 °C						
	H	leating Gas Flo	15 L/min						
		Drying Gas Flo	5 L/min						
	Ne	ebulizing Gas F	3 L/min						
		Total MRMs			66				
		nimum Dwell	19 msec						
_	Ма	ximum Dwell	124 msec						

Calibration

Standards available from Wellington Laboratories were used for these studies (EPA) method analyte stock 2 mL volume in methanol at 1 ug/L, Internal standard in methanol Wellington Catalog No. 533-IS and Isotope Dilution Analogue PDS in Methanol Wellington Catalog No. 533-ES). These standards were then diluted to working standards as outlined in Section 7.17.5 of EPA Method 533 using 20% water in methanol as diluent to match the extract solvent composition. The working standards were used to create a calibration curve ranging from 1 ng/L to 1000 ng/L for NFDHA, and from 0.1 ng/L to 100 ng/L for all other analytes. During this study Initial Calibration curve was ran 5 consecutive days. Figure 3 shows aggregate calibration curve for PFMPA and PFPeA and Figure 4 shows aggregate calibration curve for PFDA and example MRL 0.1 ng/L chromatogram. The chromatogram shown in Figure 5 is from a level 7, 6 ng/L calibrator. Figure 6 shows a clean instrument blank for 80% MeOH:20% H2O, indicating that the system is free from PFAS contamination as no PFAS was detected.

4 PFMPA 0.025 0.1 Target 1 229.00>85.00 6.2 5 PFPeA 0.05 0.2 Target 1 263.00>219.00 7.95 6 MSPFPeA Surrogate 1 268.00>213.00 7.44 7 M3PFBS Surrogate 1 302.00>80.00 8.54 8 PFBS 0.11 4 Target 1 302.00>80.00 8.72 10 PFESA 0.025 0.1 Target 1 298.00>30.00 10.22 12 M2-42 FTS Surrogate 2 327.00>30.00 10.22 13 4-2 FTS 1 4 Target 1 312.90>269.00 10.42 14 PFHxA 0.05 0.2 Target 1 285.00>10.00 11.22 13 4-2 FTS 1 4.025 0.1 Target 1 285.00>10.01 132 14 PFHxA 0.025 </th <th>ID#</th> <th>Compound</th> <th>MRL in vial (ng/mL)</th> <th>MRL in sample (ng/L)</th> <th>Туре</th> <th>ISTD Group#</th> <th>m/z</th> <th>RT</th>	ID#	Compound	MRL in vial (ng/mL)	MRL in sample (ng/L)	Туре	ISTD Group#	m/z	RT
3 PFBA 0.05 0.2 Target 1 212.90>16.80 5.18 4 PFMPA 0.025 0.1 Target 1 229.00>85.00 6.2 5 PFPA 0.05 0.2 Target 1 263.00>219.00 7.94 6 MSPFPA Surrogate 1 302.00>80.00 8.55 7 M3PFBS 0.1 4 Target 1 279.00>85.00 8.72 10 PFEBS 0.025 0.1 Target 1 279.00>85.00 8.72 11 NFDHA 5 20 Target 1 279.00>85.00 8.72 12 M2-42 FTS 1 4 Target 2 329.00>30.00 10.22 13 4-2 FTS 1 4 Target 1 285.00>10.0 10.42 15 PFPES 0.1 0.4 Target 1 280.00>10.0 11.22 16 HFPO-DA	1	M3PFBA			ISTD	3	216.00>172.00	5
4 PFMPA 0.025 0.1 Target 1 229.00>85.00 6.2 5 PFPeA 0.05 0.2 Target 1 263.00>219.00 7.95 6 MSPFPeA Surrogate 1 268.00>213.00 7.44 7 M3PFBS Surrogate 1 302.00>80.00 8.54 8 PFBS 0.11 4 Target 1 302.00>80.00 8.72 10 PFESA 0.025 0.1 Target 1 298.00>30.00 10.22 12 M2-42 FTS Surrogate 2 327.00>30.00 10.22 13 4-2 FTS 1 4 Target 1 312.90>269.00 10.42 14 PFHxA 0.05 0.2 Target 1 285.00>10.00 11.22 13 4-2 FTS 1 4.025 0.1 Target 1 285.00>10.01 132 14 PFHxA 0.025 </td <td>2</td> <td>MPFBA</td> <td></td> <td></td> <td>Surrogate</td> <td>1</td> <td>217.00>172.00</td> <td>5</td>	2	MPFBA			Surrogate	1	217.00>172.00	5
PFPeA 0.05 0.2 Target 1 26.3.00>219.00 7.95 6 MSPFPeA Surrogate 1 268.00>223.00 7.94 7 M3PFBS Surrogate 1 302.00>88.00 8.55 8 PFBS 0.1 4 Target 1 279.00>85.00 8.55 9 PFMBA 0.025 0.1 Target 1 279.00>85.00 8.55 10 PFEESA 0.025 0.1 Target 1 25.00>201.15 10.02 12 M2-4-2 FTS Surrogate 2 27.00>37.00 10.42 14 PFHxA 0.05 0.2 Target 1 28.00>16.00 11.22 15 PFPeS 0.1 0.4 Target 1 28.00>16.00 11.22 16 HFPO-DA 0.025 0.1 Target 1 28.00>16.00 12.20 13 3C4-FFD <da< td=""> Surrogate</da<>	3	PFBA	0.05	0.2	Target	1	212.90>168.90	5.18
6 MSPFPeA Surogate 1 268.00>223.00 7.94 7 M3PFBS Surogate 1 302.00>80.00 8.54 8 PFBS 0.1 4 Target 2 288.90>80.10 8.55 9 PFMBA 0.025 0.1 Target 1 279.00>80.0 8.72 10 PFEESA 0.025 0.1 Target 1 279.00>301.0 10.23 11 NFDHA 5 20 Target 1 295.00>201.5 10.0 12 M2-4-2 FTS 1 4 Target 2 327.00>307.00 10.2 13 4-2 FTS 1 4 Target 1 312.90>307.00 10.2 14 PFHA 0.025 0.1 Target 1 327.00>80.00 11.2 15 PFPPA 0.025 0.1 Target 1 367.00>32.00 12.5 18 PFHpA 0.025 <t< td=""><td>4</td><td>PFMPA</td><td>0.025</td><td>0.1</td><td>Target</td><td>1</td><td>229.00>85.00</td><td>6.2</td></t<>	4	PFMPA	0.025	0.1	Target	1	229.00>85.00	6.2
M3PFBS Surrogate 1 302.00>80.00 8.54 8 PFBS 0.1 4 Target 2 298.90>80.10 8.55 9 PFMBA 0.025 0.1 Target 1 279.00>80.10 8.55 9 PFESA 0.025 0.1 Target 1 279.00>80.00 8.72 10 PFESA 0.025 0.1 Target 1 314.90>13.43 9.54 11 NFDHA 5 20 Target 1 314.90>13.43 9.54 12 M2-4-2 FTS 1 4 Target 1 312.90>26.00 10.42 15 PFPA 0.025 0.1 Target 1 285.00>16.00 11.22 16 HFPO-DA 0.025 0.1 Target 1 367.00>320.00 12.02 17 13C-HFPO-DA Surrogate 1 367.00>320.00 12.02 18 PFHpA Sur	5	PFPeA	0.05	0.2	Target	1	263.00>219.00	7.95
8 PFBS 0.1 4 Target 2 288.0980.10 8.55 9 PFMBA 0.025 0.1 Target 1 279.00>85.00 8.72 10 PFEESA 0.025 0.1 Target 1 314.90>134.85 9.54 11 NFDHA 5 20 Target 1 295.00>200 10.2 12 M2-42 FTS 1 4 Target 2 329.00>30.00 10.2 13 4-2 FTS 1 4 Target 2 349.0>80.00 10.2 14 PFHxA 0.05 0.2 Target 1 287.00>10.0 11.2 15 PFPeS 0.1 0.4 Target 1 362.90>10.1 12.5 16 HFPO-DA 0.025 0.1 Target 1 367.00>32.00 12.5 17 13C-HFPO-DA Surrogate 2 402.00>80.01 12.6 21 PFHpA 0.025<	6	M5PFPeA			Surrogate	1	268.00>223.00	7.94
PFMBA 0.025 0.1 Target 1 279.00>85.00 8.72 10 PFEESA 0.025 0.1 Target 1 314.90>134.85 9.54 11 NFDHA 5 20 Target 1 295.00>201.15 10.02 12 M2.4-2 FTS 1 4 Target 2 329.00>30.00 10.22 13 4-2 FTS 1 4 Target 2 329.00>30.00 10.22 14 PFHxA 0.05 0.2 Target 1 312.90>269.00 10.22 15 PFPeS 0.1 0.4 Target 1 285.00>469.00 11.22 16 HFPO-DA Surrogate 1 362.90>31.00 12.52 18 PFHpA 0.025 0.1 Target 1 367.00>28.90 12.55 19 M4PFHpA Surrogate 1 367.00>28.90 12.05 11 PFHpS 0.1 0	7	M3PFBS			Surrogate	1	302.00>80.00	8.54
PFEESA 0.025 0.1 Target 1 314.90>134.8 9.54 11 NFDHA 5 20 Target 1 295.00>201.15 10.02 12 M2-4-2 FTS Surrogate 2 329.00>307.00 10.2 13 4-2 FTS 1 4 Target 2 329.00>307.00 10.2 14 PFHxA 0.05 0.2 Target 1 312.00>269.00 10.2 15 PFPeS 0.1 0.4 Target 1 362.00>1.00 11.2 16 HFPO-DA 0.025 0.1 Target 1 362.00>1.00 12.2 18 PFHpA 0.025 0.1 Target 1 362.00>8.00 12.2 19 M4PFHpA Surrogate 1 367.00>32.00 12.5 10 MAPFHpA Surrogate 1 367.00>32.00 12.5 11 MAPFHpA 0.01 0.4 <td< td=""><td>8</td><td>PFBS</td><td>0.1</td><td>4</td><td>Target</td><td>2</td><td>298.90>80.10</td><td>8.55</td></td<>	8	PFBS	0.1	4	Target	2	298.90>80.10	8.55
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13 4-2 FTS 1 4 Target 2 327.0>307.00 10.2 14 PFHxA 0.05 0.2 Target 1 312.9>269.00 10.43 15 PFPeS 0.1 0.4 Target 1 23.00>169.00 10.43 16 HFPO-DA 0.025 0.1 Target 1 285.00>169.00 1.23 17 13C-HFPO-DA Surrogate 1 362.90>319.00 12.53 19 M4PFHpA 0.025 0.1 Target 1 367.00>322.00 12.53 20 M3PFHxS 0.1 0.4 Target 2 398.90×80.00 12.73 21 PFHxS 0.1 0.4 Target 2 398.90×80.00 12.73 22 ADONA 0.025 0.1 Target 2 398.90×80.00 12.73 23 6-2 FTS 0.5 2 Target 1 421.00>36.00 14.12 24 M2	11	NFDHA	5	20	Target	1	295.00>201.15	10.08
14 PFHXA 0.05 0.2 Target Target 1 312.90>269.00 10.44 15 PFPeS 0.1 0.4 Target 2 349.00>80.00 10.83 16 HFPO-DA 0.025 0.1 Target 1 285.00>169.00 11.23 17 13C-HFPO-DA Surrogate 1 362.90>319.00 12.53 19 M4PFHpA 0.025 0.1 Target 1 362.90>319.00 12.53 20 M3PFHXS Surrogate 2 398.90>80.10 12.02 21 PFHXS 0.1 0.4 Target 1 377.00>250.09 12.83 22 ADONA 0.025 0.1 Target 1 377.00>250.09 14.12 23 G-2 FTS 0.5 2 Target 1 472.00>470.00 14.12 24 M26-2 FTS 0.5 2 Target 1 421.00>360.01 14.22 <td< td=""><td>12</td><td>M2-4-2 FTS</td><td></td><td></td><td>Surrogate</td><td>2</td><td>329.00>309.00</td><td>10.22</td></td<>	12	M2-4-2 FTS			Surrogate	2	329.00>309.00	10.22
Image: Prese biological biologic	13	4-2 FTS	1	4	Target	2	327.00>307.00	10.2
Image: https://without.com/section	14	PFHxA	0.05	0.2	Target	1	312.90>269.00	10.48
13C-HFPO-DA Surrogate 1 287.00>169.20 1.1.2 18 PFHpA 0.025 0.1 Target 1 362.90>319.00 12.57 19 M4PFHpA Surrogate 1 367.00>32.00 12.57 20 M3PFHxS Surrogate 2 402.00>80.00 12.75 21 PFHxS 0.1 0.4 Target 2 398.90>80.10 12.02 22 ADONA 0.025 0.1 Target 1 377.00>250.90 12.8 23 6-2 FTS 0.5 2 Target 2 429.00>40.00 14.12 24 M2-6-2 FTS 0.5 2 Target 1 421.00>376.00 14.22 25 M8PFOA Surrogate 1 421.00>376.00 14.22 26 PFOA 0.1 0.4 Target 1 412.00>36.00 14.22 27 M2PFOA Surrogate	15	PFPeS	0.1	0.4	Target	2	349.00>80.00	10.82
18 PFHpA 0.025 0.1 Target 1 362.99319.00 12.57 19 MAPFHpA Surrogate 1 367.09322.00 12.57 20 M3PFHxS Surrogate 2 402.0>80.00 12.75 21 PFHxS 0.1 0.4 Target 2 398.9>80.10 12.02 22 ADONA 0.025 0.1 Target 1 377.09250.90 12.8 23 6-2 FTS 0.5 2 Target 2 427.09407.00 14.12 24 M2-6-2 FTS 0.5 2 Target 1 421.09376.00 14.12 25 M8PFOA Surrogate 1 421.09376.00 14.22 26 PFOA 0.1 0.4 Target 1 412.90369.00 14.22 27 M2PFOA Surrogate 1 412.0937.00 14.22 28 PFHpS 0.1 0.4	16	HFPO-DA	0.025	0.1	Target	1	285.00>169.00	11.21
M4PFHpA Surrogate 1 367.00>322.00 12.57 20 M3PFHxS Surrogate 2 402.00>80.00 12.72 21 PFHxS 0.1 0.4 Target 2 398.90>80.10 12.02 22 ADONA 0.025 0.1 Target 1 377.00>250.90 12.82 23 6-2 FTS 0.5 2 Target 2 427.00>40.00 14.12 24 M2-6-2 FTS 0.5 2 Target 2 429.00>409.00 14.12 25 M8PFOA Surrogate 1 421.00>376.00 14.22 26 PFOA 0.1 0.4 Target 1 412.90>369.00 14.22 27 M2PFOA ISTD 1 412.00>370.00 14.22 28 PFHpS 0.1 0.4 Target 1 462.90>418.90 15.73 30 M8PFOS Surrogate 3	17	13C-HFPO-DA			Surrogate	1	287.00>169.20	11.21
Name Surrogate 2 402.00>80.00 12.75 21 PFHxS 0.1 0.4 Target 2 398.90>80.10 12.05 22 ADONA 0.025 0.1 Target 1 377.00>250.90 12.8 23 6-2 FTS 0.5 2 Target 2 427.00>40.00 14.12 24 M2-6-2 FTS 0.5 2 Target 2 429.00>409.00 14.12 25 M8PF0A Surrogate 1 412.00>376.00 14.22 26 PF0A 0.1 0.4 Target 1 412.90>369.00 14.23 27 M2PF0A ISTD 1 415.00>37.00 14.23 28 PFHpS 0.1 0.4 Target 2 449.00\$80.00 15.73 30 M8PF0S Surrogate 3 507.00>80.00 15.73 31 M9PFNA Surrogate 1 472.0>42.00	18	PFHpA	0.025	0.1	Target	1	362.90>319.00	12.57
PFHxS 0.1 0.4 Target 2 398.90-80.10 12.02 22 ADONA 0.025 0.1 Target 1 377.00>250.90 12.8 23 G-2 FTS 0.5 2 Target 2 427.00>407.00 14.12 24 M2-6-2 FTS Surrogate 2 429.00>409.00 14.12 25 M8PFOA Surrogate 1 421.00>376.00 14.22 26 PFOA 0.1 0.4 Target 1 412.90>369.00 14.22 27 M2PFOA ISTD 1 412.90>369.00 14.23 28 PFHpS 0.1 0.4 Target 1 462.90>418.90 15.76 30 M8PFOS Surrogate 3 507.00>80.00 15.73 31 M9PFNA Surrogate 3 507.00>80.00 15.73 32 PFOS 0.05 0.2 Target 1	19	M4PFHpA			Surrogate	1	367.00>322.00	12.57
22 ADONA 0.025 0.1 Target 1 377.00>250.90 14.12 23 6-2 FTS 0.5 2 Target 2 427.00>407.00 14.12 24 M2-6-2 FTS Surrogate 2 429.00>409.00 14.12 25 M8PFOA Surrogate 1 421.00>376.00 14.22 26 PFOA 0.1 0.4 Target 1 412.00>376.00 14.22 27 M2PFOA ISTD 1 412.00>376.00 14.22 28 PFHpS 0.1 0.4 Target 1 462.90>41.80 15.76 29 PFNA 0.05 0.2 Target 1 462.90>41.80 15.76 30 M8PFOS Surrogate 3 507.00>80.00 15.76 31 M9PFNA Surrogate 1 472.00>42.70 15.76 32 PFOS 0.05 0.2 Target	20	M3PFHxS			Surrogate	2	402.00>80.00	12.75
23 6-2 FTS 0.5 2 Target 2 427.00>407.00 14.12 24 M2-6-2 FTS Surrogate 2 429.00>409.00 14.12 25 M8PFOA Surrogate 2 429.00>409.00 14.12 26 PFOA 0.1 0.4 Target 1 412.90>369.00 14.22 27 M2PFOA Surrogate 1 412.90>369.00 14.22 28 PFHpS 0.1 0.4 Target 1 412.90>369.00 14.22 29 PFNA 0.05 0.2 Target 1 462.90>418.90 15.79 30 M8PFOS Surrogate 3 507.00>80.00 15.79 31 M9PFNA Surrogate 1 472.00>427.00 15.79 32 PFOS 0.05 0.2 Target 1 50.30.980.00 15.79 33 M4PFOS Surrogate	21	PFHxS	0.1	0.4	Target	2	398.90>80.10	12.08
24 M2-6-2 FTS Surrogate 2 429.00>409.00 14.14 25 M8PFOA Surrogate 1 421.00>376.00 14.27 26 PFOA 0.1 0.4 Target 1 412.90>369.00 14.27 27 M2PFOA ISTD 1 415.00>370.00 14.28 28 PFHpS 0.1 0.4 Target 2 449.00>80.00 14.32 29 PFNA 0.05 0.2 Target 1 462.90>418.90 15.76 30 M8PFOS Surrogate 3 507.00>80.00 15.76 31 M9PFNA Surrogate 1 472.00>427.00 15.76 32 PFOS 0.05 0.2 Target 1 472.00>427.00 15.76 33 M4PFOS Surrogate 1 530.90>30.00 15.76 34 9CI-PF30NS 0.025 0.1 Target	22	ADONA	0.025	0.1	Target	1	377.00>250.90	12.8
25 M8PFOA Surrogate 1 421.00>376.00 14.23 26 PFOA 0.1 0.4 Target 1 412.90>369.00 14.23 27 M2PFOA ISTD 1 415.00>370.00 14.23 28 PFHpS 0.1 0.4 Target 2 449.00>80.00 14.33 29 PFNA 0.05 0.2 Target 1 462.90>41.89 15.76 30 M8PFOS Surrogate 3 507.00>80.00 15.75 31 M9PFNA Surrogate 1 472.00>427.00 15.75 32 PFOS 0.05 0.2 Target 1 472.00>427.00 15.75 33 M4PFOS Surrogate 1 530.0>80.00 15.76 34 9CI-PF3ONS 0.025 0.1 Target 1 530.90>351.00 16.97 35 8-2 FTS 1 4 Target	23	6-2 FTS	0.5	2	Target	2	427.00>407.00	14.12
26 PFOA 0.1 0.4 Target 1 412.90>369.00 14.23 27 M2PFOA ISTD 1 415.00>370.00 14.23 28 PFHpS 0.1 0.4 Target 2 449.00>80.00 14.33 29 PFNA 0.05 0.2 Target 1 462.90>41.89 15.76 30 M8PFOS Surrogate 3 507.00>80.00 15.75 31 M9PFNA Surrogate 1 472.00>427.00 15.75 32 PFOS 0.05 0.2 Target 1 472.00>427.00 15.75 33 M4PFOS Surrogate 1 472.00>427.00 15.75 34 9CI-PF3ONS 0.025 0.1 Target 1 530.90>351.00 16.57 35 8-2 FTS 1 4 Target 1 512.90>50.00 16.97 36 M2-8-2 FTS 1 4	24	M2-6-2 FTS			Surrogate	2	429.00>409.00	14.14
27 M2PFOA ISTD 1 415.00>370.00 14.28 28 PFHpS 0.1 0.4 Target 2 449.00>80.00 14.33 29 PFNA 0.05 0.2 Target 1 462.90>418.90 15.76 30 M8PFOS Surrogate 3 507.00>80.00 15.75 31 M9PFNA Surrogate 1 472.00>427.00 15.75 32 PFOS 0.05 0.2 Target 2 498.90>80.10 15.75 33 M4PFOS Surrogate 1 472.00>427.00 15.76 34 9CI-PF3ONS 0.05 0.2 Target 2 498.90>80.10 15.23 35 8-2 FTS 1 4 Target 1 53.00>80.00 15.76 36 M2-8-2 FTS 0.025 0.1 Target 1 53.00>80.00 16.97 37 PFDA 0.025 0.1	25	M8PFOA			Surrogate	1	421.00>376.00	14.27
28PFHpS0.10.4Target2449.0>80.0014.3329PFNA0.050.2Target1462.90>418.9015.7630M8PFOSSurrogate3507.00>80.0015.7531M9PFNASurrogate1472.00>427.0015.7532PFOS0.050.2Target2498.90>80.1015.7633M4PFOSISTD2503.00>80.0015.76349CI-PF3ONS0.0250.1Target1530.90>351.0016.57358-2 FTS14Target2527.00>507.0016.9736M2-8-2 FTS14Target1512.90>509.0016.9737PFDA0.0250.1Target1318.00>273.0010.4238MPFHxASurrogate1318.00>273.0010.4239PFUnA0.0250.1Target1562.90>51.0018.1240M7PFUnA0.0250.1Target1562.90>51.0018.124111CI-PF3OUdS0.0250.1Target1630.70>451.0018.6242PFDoA0.0250.1Target1612.90>568.9019.0643M2PFDA0.0250.1Target1612.90>568.9019.0644M2M2PFDA0.0250.1Target1612.00>57.0018.6744 </td <td>26</td> <td>PFOA</td> <td>0.1</td> <td>0.4</td> <td>Target</td> <td>1</td> <td>412.90>369.00</td> <td>14.25</td>	26	PFOA	0.1	0.4	Target	1	412.90>369.00	14.25
29 PFNA 0.05 0.2 Target 1 462.90>418.90 15.76 30 M8PFOS Surrogate 3 507.00>80.00 15.75 31 M9PFNA Surrogate 1 472.00>427.00 15.75 32 PFOS 0.05 0.2 Target 2 498.90>80.10 15.75 33 M4PFOS Surrogate 1 472.00>427.00 15.75 34 9CI-PF3ONS 0.05 0.2 Target 2 498.90>80.10 15.76 35 8-2 FTS 0.025 0.1 Target 1 530.90>351.00 16.57 36 M2-8-2 FTS 1 4 Target 1 530.90>351.00 16.57 37 PFDA 0.025 0.1 Target 1 529.00>50.00 16.97 38 MPFHxA Surrogate 1 318.00>273.00 10.42 39 PFUnA 0.025 <td< td=""><td>27</td><td>M2PFOA</td><td></td><td></td><td>ISTD</td><td>1</td><td>415.00>370.00</td><td>14.28</td></td<>	27	M2PFOA			ISTD	1	415.00>370.00	14.28
M8PFOS Surrogate 3 507.00>80.00 15.7 31 M9PFNA Surrogate 1 472.00>427.00 15.7 32 PFOS 0.05 0.2 Target 2 498.90>80.10 15.2 33 M4PFOS ISTD 2 503.00>80.00 15.7 34 9CI-PF3ONS 0.025 0.1 Target 1 530.90>80.00 15.7 35 8-2 FTS 1 4 Target 2 503.00>80.00 16.9 36 M2-8-2 FTS 1 4 Target 2 529.00>509.00 16.9 37 PFDA 0.025 0.1 Target 1 512.90>468.90 17.0 38 MPFHxA Surrogate 1 562.90>51.00 18.12 39 PFUnA 0.025 0.1 Target 1 562.90>51.00 18.12 40 M7PFUnA Surrogate 3	28	PFHpS	0.1	0.4	Target	2	449.00>80.00	14.33
31 M9PFNA Surrogate 1 472.00>427.00 15.73 32 PFOS 0.05 0.2 Target 2 498.90>80.10 15.23 33 M4PFOS ISTD 2 503.00>80.00 15.76 34 9CI-PF3ONS 0.025 0.1 Target 1 530.90>351.00 16.57 35 8-2 FTS 1 4 Target 2 527.00>507.00 16.97 36 M2-8-2 FTS 1 4 Target 2 529.00>509.00 16.97 37 PFDA 0.025 0.1 Target 1 512.90>468.90 17.04 38 MPFHXA Surrogate 1 318.00>273.00 10.48 39 PFUnA 0.025 0.1 Target 1 562.90>519.00 18.14 40 M7PFUnA Surrogate 3 570.00>525.00 18.14 41 11CI-PF30UdS 0.025 <	29	PFNA	0.05	0.2	Target	1	462.90>418.90	15.76
32 PFOS 0.05 0.2 Target 2 498.90>80.10 15.2 33 M4PFOS ISTD 2 503.00>80.00 15.76 34 9CI-PF3ONS 0.025 0.1 Target 1 530.90>351.00 16.57 35 8-2 FTS 1 4 Target 2 527.00>507.00 16.97 36 M2-8-2 FTS 1 4 Target 2 529.00>509.00 16.97 37 PFDA 0.025 0.1 Target 1 512.90>509.00 16.97 38 MPFHxA Surrogate 1 512.90>509.00 16.97 39 PFUA 0.025 0.1 Target 1 562.90>519.00 18.12 40 M7PFUnA Surrogate 3 570.00>525.00 18.12 41 11Cl-PF3OUdS 0.025 0.1 Target 1 630.70>451.00 18.63 42 PFDoA 0.025	30	M8PFOS			Surrogate	3	507.00>80.00	15.75
33 M4PFOS ISTD 2 503.00>80.00 15.76 34 9CI-PF3ONS 0.025 0.1 Target 1 530.90>351.00 16.5 35 8-2 FTS 1 4 Target 2 527.00>507.00 16.97 36 M2-8-2 FTS 1 4 Target 2 529.00>509.00 16.97 37 PFDA 0.025 0.1 Target 1 512.90>509.00 16.97 38 MPFHXA Surrogate 2 529.00>509.00 16.97 39 PFUA 0.025 0.1 Target 1 512.90>509.00 10.48 39 PFUAA Surrogate 1 562.90>519.00 18.12 40 M7PFUAA Surrogate 3 570.00>525.00 18.12 41 11CI-PF3OUdS 0.025 0.1 Target 1 630.70>451.00 18.63 42 PFDoA 0.025 <t< td=""><td>31</td><td>M9PFNA</td><td></td><td></td><td>Surrogate</td><td>1</td><td>472.00>427.00</td><td>15.73</td></t<>	31	M9PFNA			Surrogate	1	472.00>427.00	15.73
34 9CI-PF3ONS 0.025 0.1 Target 1 530.90>35.00 16.5 35 8-2 FTS 1 4 Target 2 527.00>507.00 16.9 36 M2-8-2 FTS Surrogate 2 529.00>509.00 16.9 37 PFDA 0.025 0.1 Target 1 512.90>468.90 17.0 38 MPFHxA Surrogate 1 318.00>273.00 10.48 39 PFUnA 0.025 0.1 Target 1 562.90>519.00 18.42 40 M7PFUnA Surrogate 3 570.00>525.00 18.42 41 11Cl-PF30UdS 0.025 0.1 Target 1 630.70>525.00 18.42 42 PFDoA 0.025 0.1 Target 1 630.70>505.00 18.42 43 M2PFDoA 0.025 0.1 Target 1 612.90>568.90 19.02 43 M2PFDoA 0.025 0.1 Target 1 615.00>570.00 10.02 <td>32</td> <td>PFOS</td> <td>0.05</td> <td>0.2</td> <td>Target</td> <td>2</td> <td>498.90>80.10</td> <td>15.23</td>	32	PFOS	0.05	0.2	Target	2	498.90>80.10	15.23
35 8-2 FTS 1 4 Target 2 527.00>507.00 16.97 36 M2-8-2 FTS Surrogate 2 529.00>509.00 16.97 37 PFDA 0.025 0.1 Target 1 512.90>468.90 17.04 38 MPFHxA Surrogate 1 318.00>273.00 10.48 39 PFUnA 0.025 0.1 Target 1 562.90>519.00 18.14 40 M7PFUnA Surrogate 3 570.00>525.00 18.14 41 11Cl-PF3OUdS 0.025 0.1 Target 1 630.70>451.00 18.63 42 PFDoA 0.025 0.1 Target 1 612.90>568.90 19.06 43 M2PFDoA 0.025 0.1 Target 1 615.00>570.00 19.06	33	M4PFOS			ISTD	2	503.00>80.00	15.76
36 M2-8-2 FTS Surrogate 2 529.00>509.00 16.98 37 PFDA 0.025 0.1 Target 1 512.90>468.90 17.04 38 MPFHxA Surrogate 1 318.00>273.00 10.48 39 PFUnA 0.025 0.1 Target 1 562.90>519.00 18.14 40 M7PFUnA Surrogate 3 570.00>525.00 18.14 41 11Cl-PF3OUdS 0.025 0.1 Target 1 630.70>451.00 18.63 42 PFDoA 0.025 0.1 Target 1 612.90>568.90 19.04 43 M2PFDoA 0.025 0.1 Target 1 615.00>570.00 19.04	34	9CI-PF3ONS	0.025	0.1	Target	1	530.90>351.00	16.5
37 PFDA 0.025 0.1 Target 1 512.90>468.90 17.44 38 MPFHxA Surrogate 1 318.00>273.00 10.48 39 PFUnA 0.025 0.1 Target 1 562.90>519.00 18.14 40 M7PFUnA Surrogate 3 570.00>525.00 18.14 41 11Cl-PF3OUdS 0.025 0.1 Target 1 630.70>451.00 18.14 42 PFDoA 0.025 0.1 Target 1 612.90>568.90 19.06 43 M2PFDoA Surrogate 3 615.00>570.00 19.06	35	8-2 FTS	1	4	Target	2	527.00>507.00	16.97
38 MPFHxA Surrogate 1 318.00>273.00 10.48 39 PFUnA 0.025 0.1 Target 1 562.90>519.00 18.14 40 M7PFUnA Surrogate 3 570.00>525.00 18.14 41 11Cl-PF3OUdS 0.025 0.1 Target 1 630.70>451.00 18.63 42 PFDoA 0.025 0.1 Target 1 612.90>568.90 19.06 43 M2PFDoA Surrogate 3 615.00>570.00 19.06	36	M2-8-2 FTS			Surrogate	2	529.00>509.00	16.98
39 PFUnA 0.025 0.1 Target 1 562.90>519.00 18.14 40 M7PFUnA Surrogate 3 570.00>525.00 18.14 41 11Cl-PF3OUdS 0.025 0.1 Target 1 630.70>451.00 18.63 42 PFDoA 0.025 0.1 Target 1 612.90>568.90 19.06 43 M2PFDoA Surrogate 3 615.00>570.00 19.06	37	PFDA	0.025	0.1	Target	1	512.90>468.90	17.04
40 M7PFUnA Surrogate 3 570.00>525.00 18.12 41 11Cl-PF3OUdS 0.025 0.1 Target 1 630.70>451.00 18.62 42 PFDoA 0.025 0.1 Target 1 612.90>568.90 19.02 43 M2PFDoA Surrogate 3 615.00>570.00 19.02	38	MPFHxA			Surrogate	1	318.00>273.00	10.48
41 11Cl-PF3OUdS 0.025 0.1 Target 1 630.70>451.00 18.65 42 PFDoA 0.025 0.1 Target 1 612.90>568.90 19.06 43 M2PFDoA Surrogate 3 615.00>570.00 19.06	39	PFUnA	0.025	0.1	Target	1	562.90>519.00	18.14
42 PFDoA 0.025 0.1 Target 1 612.90>568.90 19.06 43 M2PFDoA Surrogate 3 615.00>570.00 19.06	40	M7PFUnA			Surrogate	3	570.00>525.00	18.11
43 M2PFDoA Surrogate 3 615.00>570.00 19.06	41	11Cl-PF3OUdS	0.025	0.1	Target	1	630.70>451.00	18.63
	42	PFDoA	0.025	0.1	Target	1	612.90>568.90	19.06
44 MPFDA Surrogate 1 519.00>474.10 17.04	43	M2PFDoA			Surrogate	3	615.00>570.00	19.06
	44	MPFDA			Surrogate	1	519.00>474.10	17.04

Table 2. Target and labelled PFAS m/z, retention times, and correlation coefficients from the aggregate curve (Days 1-5)

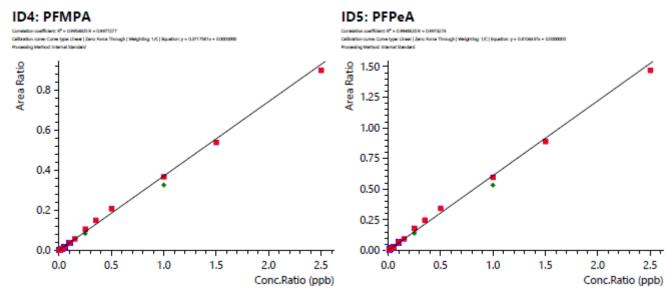
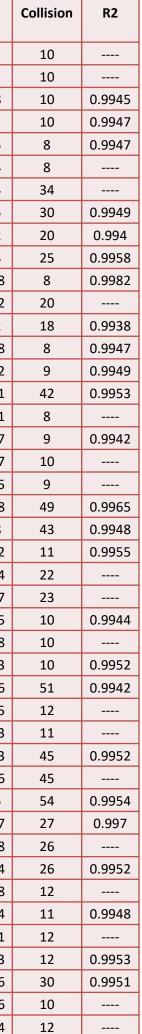
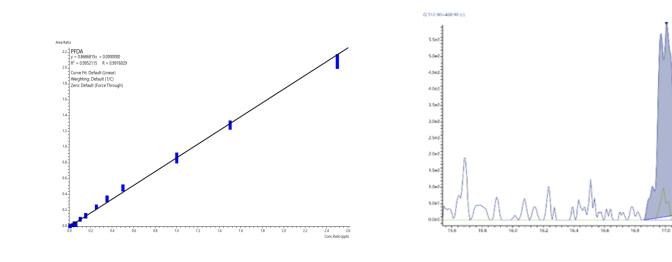
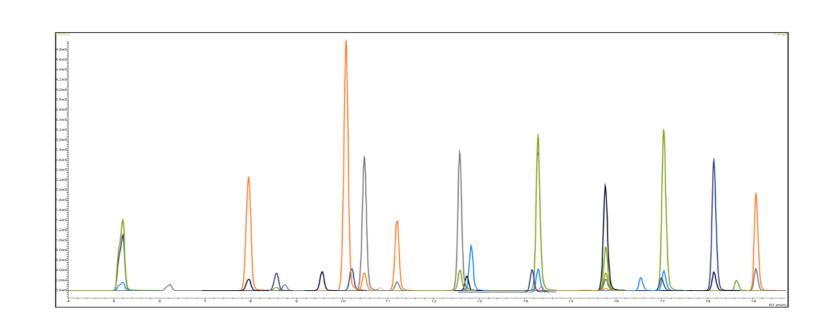


Figure 3. Aggregate calibration curves for PFMPA, and PFPeA

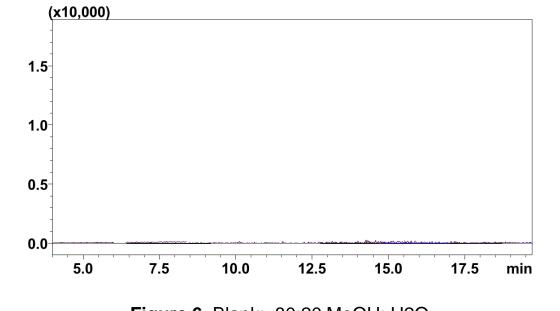










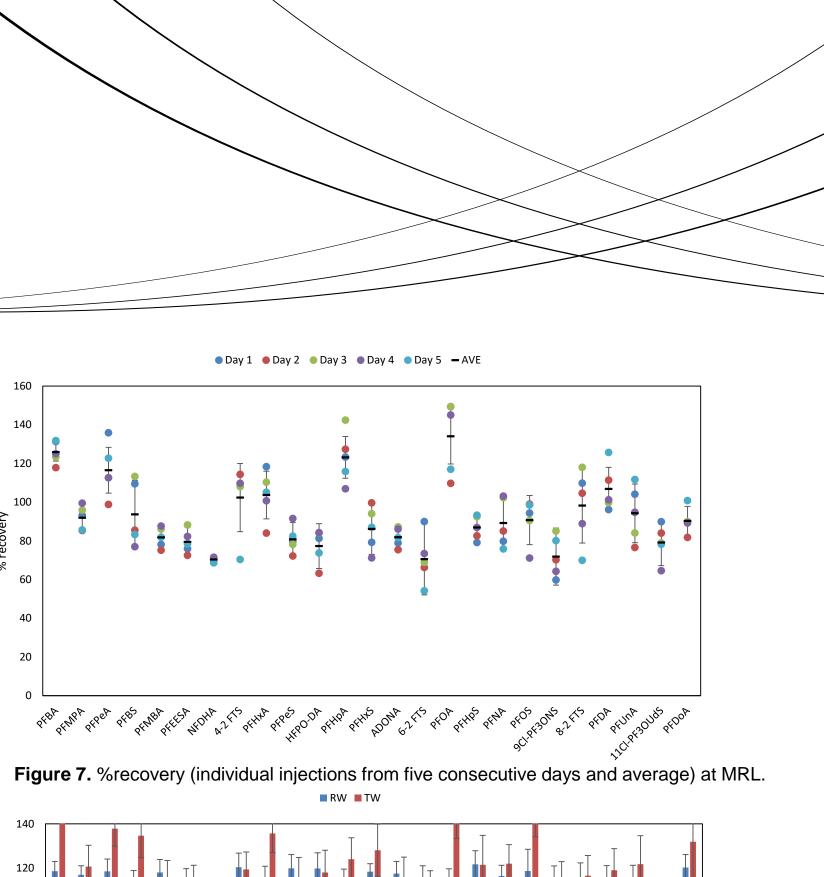


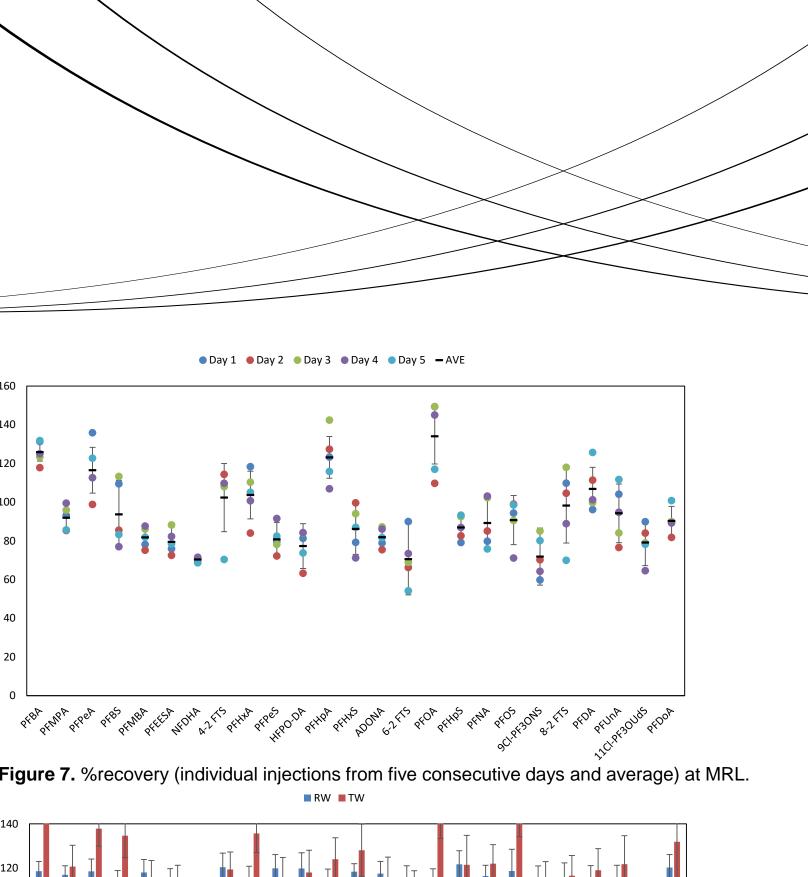


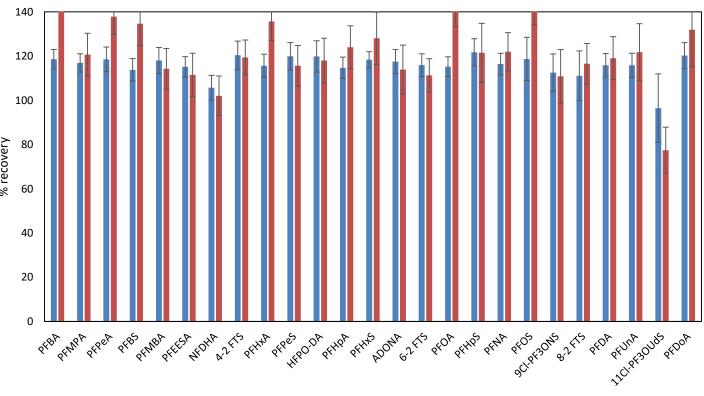
Results

Method development for PFAS

The use of a Phenomenex Gemini[™] C18, 2.0 mm ID × 50 mm, 3.0 µm particle size analytical column and a Shim-Pack XR-ODS 50 x 3.0 mm column as a delay column provided a good chromatographic separation for all compounds including branched and linear isomers. Calibration curves for PFAS analytes were prepared in the range of 0.025 – 25 ng/mL, representing pre-SPE sample concentrations of 0.1 – 100 ng/L (except for NFDHA which was analyzed from 0.25 – 250 ng/L). All calibration curves (aggregate curve and Day 1-5 individual curves) demonstrated r2 values greater than 0.99. All RSD results for the aggregate curve were less than 20%. All MRL level accuracies were between 50 – 150%. Accuracies at the MRL for each day (against the aggregate curve), and %RSDs are shown in Figure 7. Precision and accuracy studies in reagent water (RW) and tap water (TW) were performed at 10 ng/L and recoveries of majority of analytes were within 70-130% with %RSDs below 20% for all method analytes. The P & A study results were within EPA method 533 requirements; the data is included in Figure 8.









Conclusions

This study showed good chromatographic separation for all compounds listed in the method using the delay and analytical columns recommended by EPA. Recoveries for most target compounds and precision and accuracy data for all target analytes in reagent water and tap water were within EPA requirements of 70 -130%, with %RSD below 20% for all method analytes. This data was generated as part of the EPA method 533 second laboratory validation organized by EPA. Shimadzu participated in this validation, as acknowledged in the final method.

References

Figure 8. Precision and accuracy results/

(1) EPA Method 537 rev1.1, Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) (U.S. Environmental Protection Agency, Washington, D.C., Sept. 2009).

(2) Evoqua Water Technologies, Webinar, March 6, 2018

(3) 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).

(4) Shimadzu Application News No. C184, "Analysis of PFAS Specified in EPA Method 537 and Beyond Using Shimadzu UFMS", 2019