EPA Method 537.1: Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

UCT Part Numbers

ECDVB156P
ENVIRO-CLEAN® DVB
500 mg, 6 mL cartridge, PE frits
Or
ECHLD156-P
ENVIRO-CLEAN® Highly Cross-Linked HLD
500 mg, 6 mL cartridge, PE frits

VMF016GL-PFAS
Complete 16 position glass block manifold for PFAS analysis

VMFSTFR06-PFC
Large Volume LLDPE Sample Transfer Tubes (6 ct) – For PFAS Analysis

CLTTP050
Polypropylene Clean-Thru Tips

VMF02116-PFAS
16 pack of Polyethylene (PE) Stopcocks

SLC-18100ID21-3UM
Selectra® C18 HPLC column
(50 × 2.1 mm, 3 µm)

SLC-1850ID46-5UM
Selectra® C18 Delay Column
(50 × 4.6 mm, 5 µm)

SLC-18GDC20-3UM
Selectra® C18 Guard Cartridges
(10 × 2.1 mm, 3 µm)

SLGRDHLDR

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Summary:

Per- and polyfluoroalkyl substances (PFAS) are a diverse group of synthetic organofluorine compounds that are widely used in industrial applications and consumer products. PFAS are persistent in the environment, are resistant to degradation, and are known to bioaccumulate in humans and wildlife. PFAS have historically been analyzed in drinking water according to EPA 537 (14 compounds) and 537.1 (18 compounds) [1,2]. An updated method, EPA 533, has been validated for the analysis of multiple short-chain PFAS, including telomers and precursor compounds, that cannot be measured by EPA 537.1 [3]. EPA 537.1 measures PFAS by styrenedivinylbenzene (SDVB) single polymer solid-phase extraction (SPE) and liquid chromatography/tandem mass spectrometry (LC-MS/MS).

This application note outlines the analysis of PFAS in drinking water according to EPA 537.1 utilizing UCT's Enviro-Clean® polymeric styrenedivinylbenzene (DVB and/or HLD) SPE cartridges (ECDVB156P/ECHLD156-P). LC-MS/MS analysis was carried out using a Selectra® C18 HPLC analytical column (SLC-18100ID21-3UM), while a short (5cm) C18 delay column (SLC-1850ID46-5UM) was used to reduce potential PFAS contamination from the HPLC system. For quantitation, a seven-point calibration (0.5-25 ng/mL) was performed, and all compounds were found to be linear with R2 values > 0.99. The extraction method was evaluated by spiking reagent water samples with PFAS at 2.5 and 20 ng/L. Recoveries of all analytes were within a range of 70-110% and RSD values <10%. Due to the prevalence of fluorochemicals used in lab equipment, excluding the use of any PTFE labware throughout the sampling and analytical processes (including HPLC solvent inlet tubing) is essential for accurate analysis of PFAS. The use of UCT's linear low-density polyethylene (LLDPE) large volume sample transfer tubes (VMFSTFR06-PFC) in conjunction with our complete Glass Block Manifold kit (VMF016GL-PFAS) geared towards PFAS analysis allows for simplified sample preparation and prevent any further introduction of contaminants to the samples.

Sample Pretreatment:

Samples must be collected in a 250-mL polypropylene bottle fitted with a polypropylene screw-cap. All Field and QC Samples, including the LRB, LFB and FRB, must contain the dechlorinating agent listed in Section 8.1.2 of Method 537.1 (Trizma 5g/L). Before extraction, verify that the sample pH is 7 ± 0.5. Check the pH of the water sample to ensure that it is in the range of pH 6-8. Spike sample with appropriate concentrations of surrogate standard and mix thoroughly (add target analytes for fortified samples).

SPE Procedure:

1. **SPE Conditioning**
   a) Rinse cartridge with 15mL MEOH.
   b) Rinse the cartridge with 18 mL of D.I. H_2O, being sure to not allow the water to drop below the top edge of the packing. Add 2–3 mL of D.I. H_2O to the cartridge reservoir.
2. **Sample Extraction/Drying**
   a) Attach a large volume sample transfer tube* (VMFSTFR06-PFC) to the top of each SPE cartridge and place the stainless-steel end of the transfer tube directly into the sample bottle.
   *Ensure transfer tubes are adequately rinsed prior to use.
   b) Adjust the vacuum so that the flow rate is approximately 10-15 mL/min. Do not allow the cartridge to go dry before all the sample has passed through.
   c) After the entire sample has passed through the cartridge, rinse the sample bottle with 2 x 7.5 mL of D.I. H_2O; Draw the rinsate through the sample transfer tubes and the cartridges.
   d) Add 1 mL of MEOH to the sample bottle and draw through the transfer tubes and SPE cartridges. This step is designed to remove most of the water from the transfer line and cartridge resulting in the reduction of the salt and water present in the eluate.
   e) Dry the cartridge under high vacuum (15-20 inHg) for 5 minutes to remove any residual water.
3. **Elution**
   a) Insert a collection rack containing 15 mL polypropylene collection tubes into the extraction manifold (VMF016GL-PFAS).
   b) Add 4 mL of MEOH to the sample container, cap and thoroughly rinse the sides with the elution solvent.
   *Note: Rinsing the sides of the container is important for obtaining good recovery of the long-chain hydrophobic PFAS.
   c) Elute the analytes from the cartridges by pulling the elution solvent through the sample transfer tubes and the cartridges. Use a low vacuum such that the solvent exits the cartridge in a dropwise fashion.
   d) Repeat sample bottle rinse and cartridge elution with a second 4 mL aliquot of MEOH.
4. **Concentration**
   a) Concentrate the extract to dryness under a gentle stream of nitrogen in a heated water bath (55–60°C).
   b) Reconstitute the extract with 1.0 mL of 96:4 MEOH: D.I. H_2O (v/v).
   c) Add the isotope performance standards to the extract and vortex.
   d) Transfer an aliquot of the final extract to a polypropylene autosampler vial (PTFE free).

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LC-MS/MS Parameters:

PFAS are ubiquitous in the laboratory environment, mainly through the widespread use of Teflon™ components in analytical equipment, including HPLC. To avoid high background in LC-MS/MS analysis, the Teflon™ solvent lines should be replaced with PEEK tubing. However, PFAS contamination is difficult to eliminate and depending on the analytical conditions used, any PFAS present in the mobile phase, solvent lines and online degasser can become concentrated in the analytical column and be detected at the same time as the injected sample analyte. To overcome this, a short C18 "delay column" is commonly installed after the solvent mixer and before the sample injector to separate the contaminant peak from any PFAS present in the sample. Alterations to existing HPLC systems can be readily performed, although it is recommended to check with your HPLC's vendor before proceeding. Additional information can also be found in EPA Method 537.1 (2).

HPLC Conditions	
HPLC system	Shimadzu Nexera LC-30AD
Delay column	UCT Selectra® C18, 50 × 4.6 mm, 5 µm (p/n: SLC-1850ID46-5UM)
HPLC column	UCT Selectra® C18, 50 × 2.1 mm, 3 µm (p/n: SLC-18100ID21-3UM)
Guard column	UCT Selectra® C18, 10 × 2.0 mm, 3 µm (p/n: SLC-18GDC20-3UM)
Guard column holder	p/n: SLGRDHLDR
Column temperature	45°C
Flow rate	300 µL/min
Injection volume	10 µL

Time (min)	Mobile Phase A (%): 20 mM Ammonium Acetate	Mobile Phase B (%): Methanol
0.0	95	5
0.5	50	50
7.5	5	95
8.5	5	95
8.6	95	5
11.0	95	5

MS Conditions	
MS/MS system	Shimadzu LCMS-8050
Ionization Mode	Electrospray Ionization in negative mode (ESI^-)
Interface Temperature	125°C
DL Temperature	200°C
Heat Block Temperature	250°C
Nebulizing Gas Flow	3 L/min
Heating Gas Flow	15 L/min
Drying Gas Flow	10 L/min

MRM Transitions:

Analyte	R.T.	Precursor	Fragment Ion 1	Fragment Ion 2	R^2
PFBS	2.13	299.0	79.9	99.0	0.9989
PFHxA	2.67	313.0	269.1	118.9	0.9971
HFPO-DA	2.89	285.0	169.0	185.1	0.9986
PFHpA	3.42	362.8	319.1	169.1	0.9987
PFHxS	3.47	399.0	80.0	99.0	0.9979
ADONA	3.52	377.1	251.0	85.0	0.9982
PFOA	4.21	412.8	369.1	169.2	0.9976
PFOS	4.95	499.1	80.0	99.0	0.9979
PFNA	4.95	463.1	419.0	219.2	0.9978
9Cl-PF3ONS	5.33	530.9	351.0	-	0.9980
PFDA	5.61	513.1	468.9	219.1	0.9988
PFUnA	6.19	563.1	518.9	268.8	0.9984
11Cl-PF3OUdS	6.45	631.1	451.0	-	0.9977
PFDoA	6.70	612.9	569.0	319.1	0.9980
NEtFOSAA	6.20	584.1	419.1	526.1	0.9967
NMeFOSAA	5.90	569.7	418.9	482.9	0.9989
PFTrDA	7.14	662.9	618.9	162.2	0.9918
PFTA	7.53	713.0	668.9	169.1	0.9981
Isotope Performance Standards					
^{12}C2-PFOA	4.21	414.8	370.0	169.0	
^{13}C4-PFOS	4.95	502.5	80.0	99.0	
d_3-NMeFOSAA	5.90	572.8	419.0	483.0	
Isotope Dilution Standards					
$^{13}C_2$-PFHxA	2.67	314.9	270.0	120.1	
$^{13}C_2$-PFDA	5.61	514.8	470.1	269.1	
d_5-NEtFOSAA	6.19	588.9	419.1	531.1	
$^{13}C_3$-HFPO-DA	2.89	287.0	169.0	185.1	

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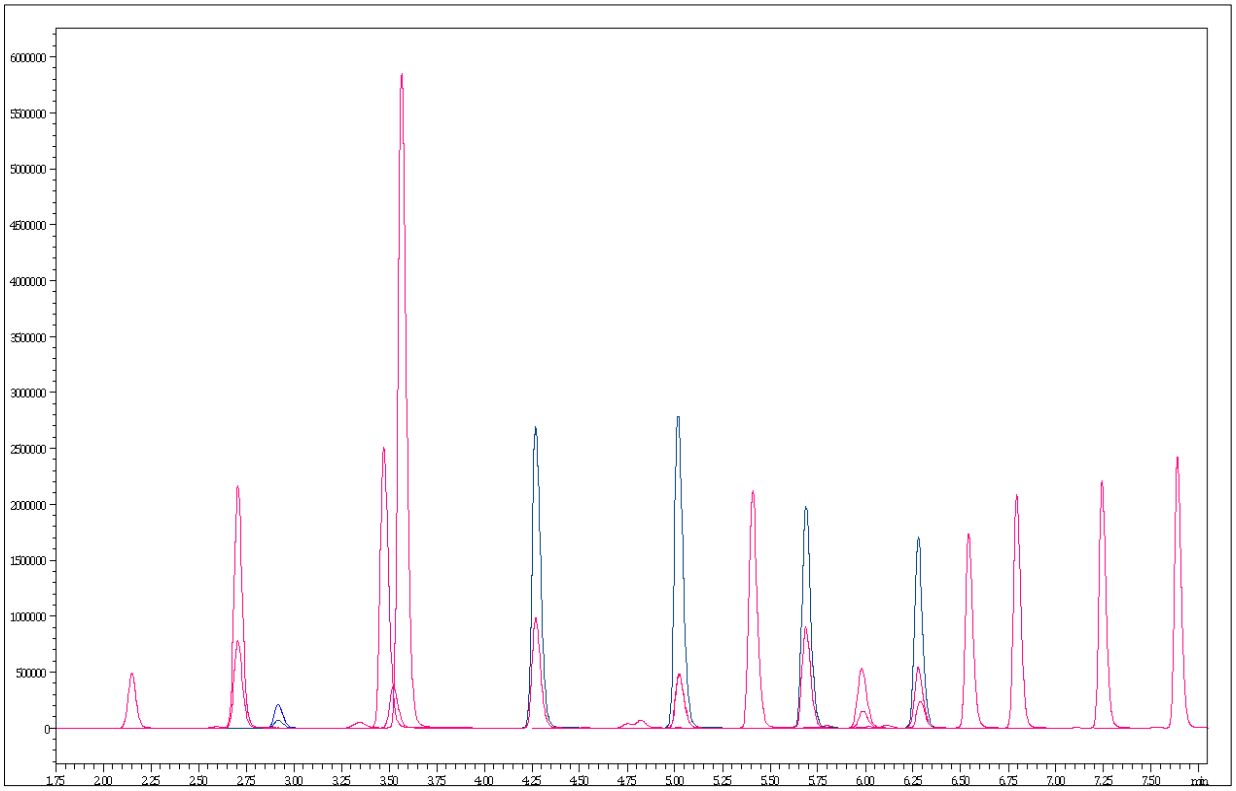

Figure 1: PFAS fortified at low fortification level 5 ng/L in reagent water (5 ng/mL in vial).

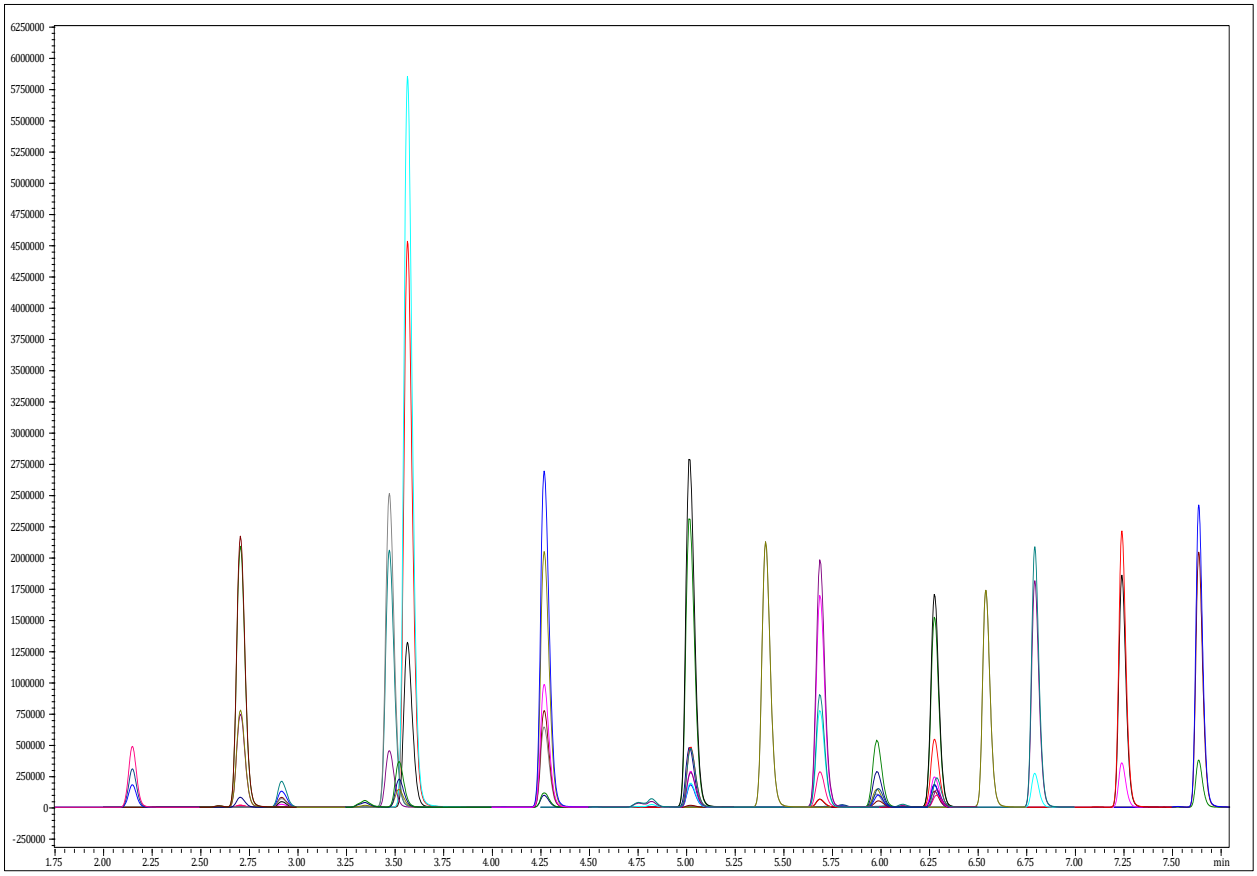

Figure 2: PFAS fortified at high fortification level 10 ng/L in reagent water (10 ng/mL in vial).

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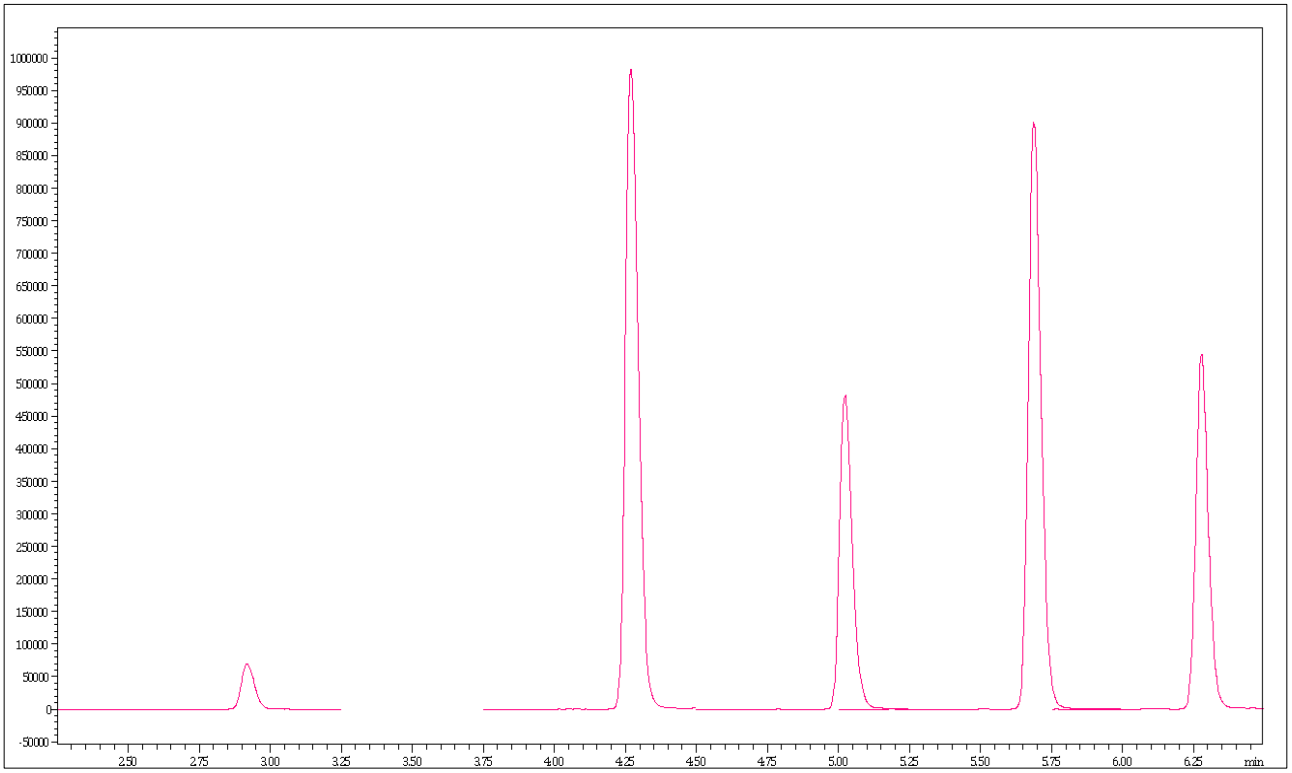

Figure 3: Chromatogram of a LRB sample containing Isotope Dilution and Isotope Performance standards.

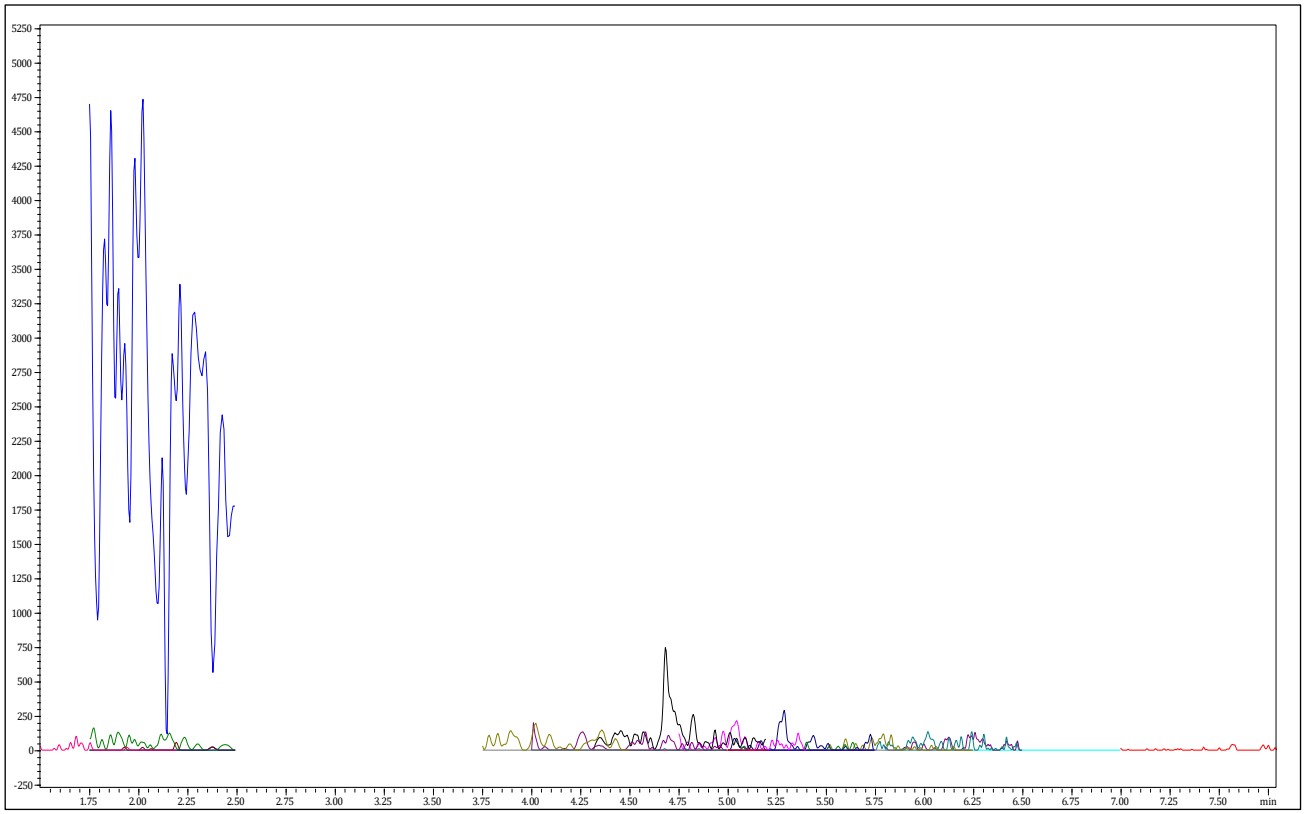

Figure 4: Chromatogram of a blank solvent injection demonstrating low system background levels.

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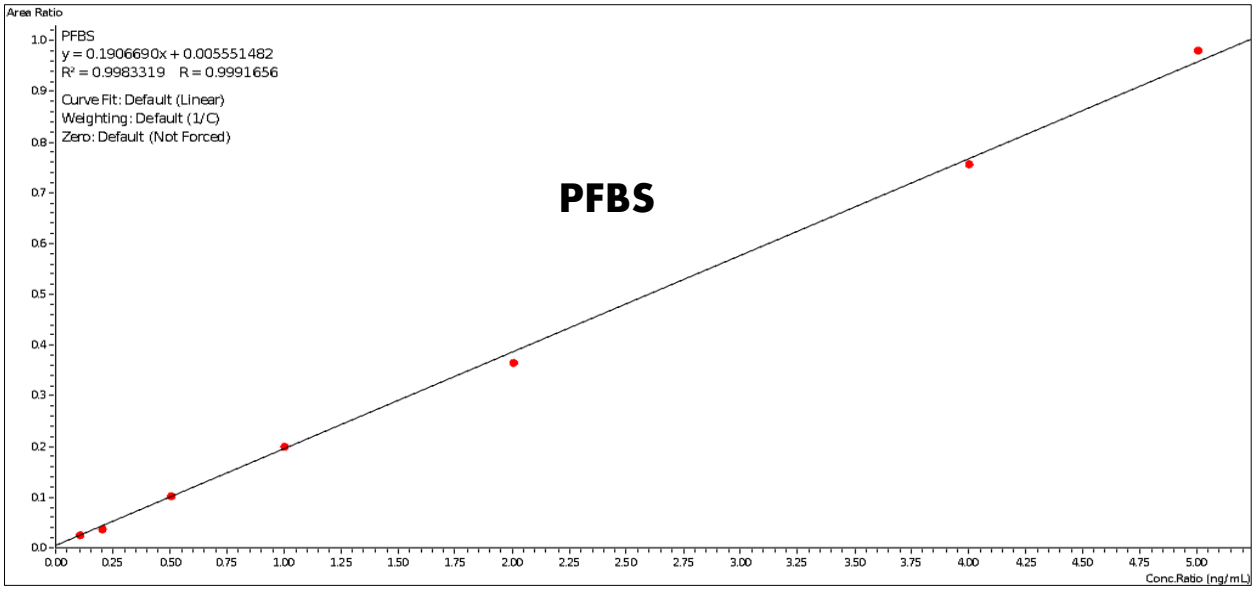
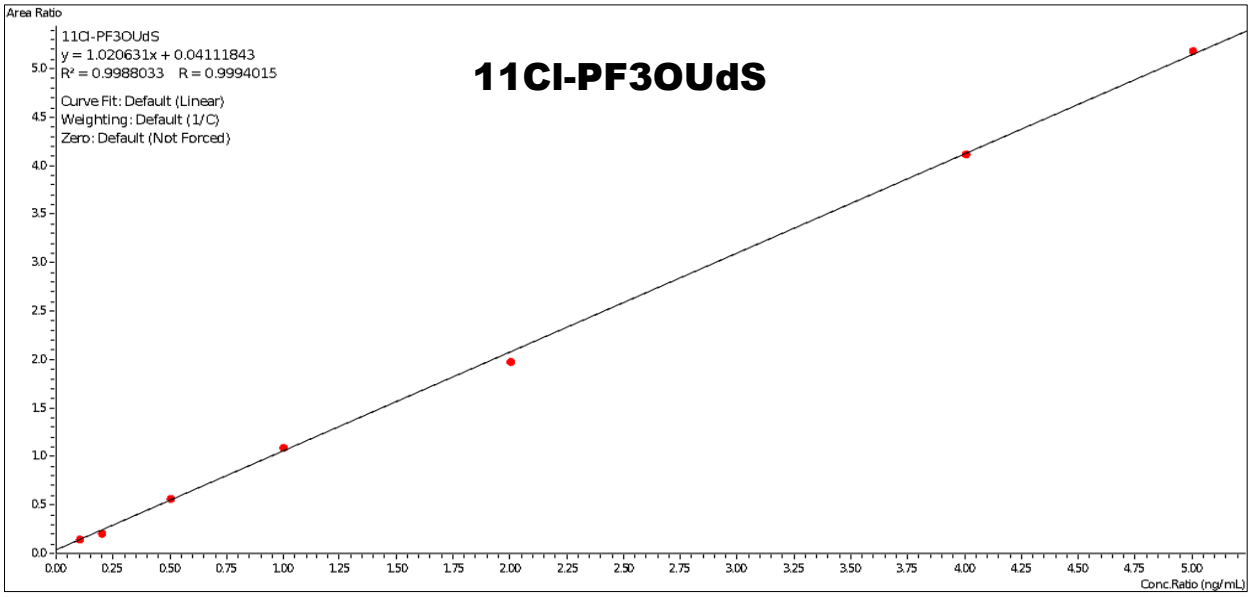
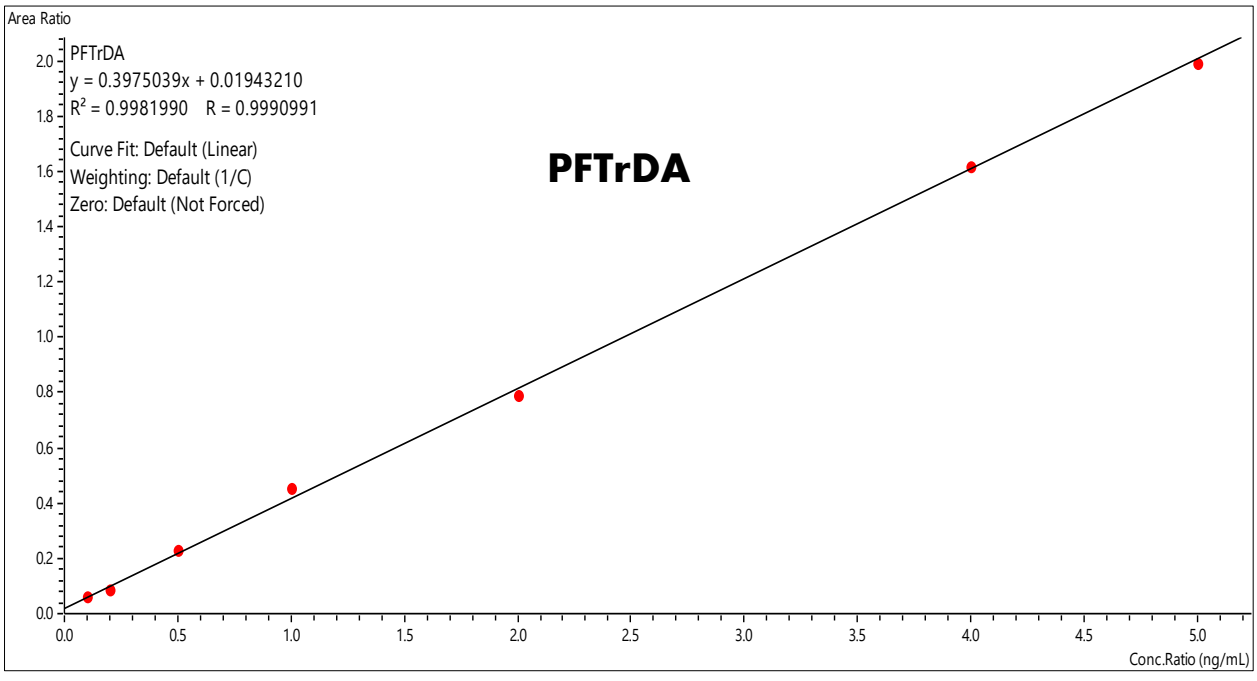
Calibration Curves:







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SPE Results:

Results in Reagent Water – ECHLD156-P				
Analyte	Low Fortification (2.5 ng/L; n=4)		High Fortification (10 ng/L; n=4)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
PFBS	93%	5%	97%	2%
PFHxA	82%	3%	89%	2%
HFPO-DA	75%	4%	72%	4%
PFHpA	81%	4%	91%	1%
PFHxS	108%	4%	96%	1%
ADONA	77%	3%	88%	1%
PFOA	85%	4%	93%	1%
PFOS	99%	4%	90%	2%
PFNA	82%	3%	101%	1%
9Cl-PF3ONS	91%	3%	98%	2%
PFDA	84%	2%	105%	1%
PFUnA	85%	1%	106%	1%
11Cl-PF3OUdS	88%	4%	95%	2%
PFDoA	81%	3%	93%	1%
N-EtFOSAA	77%	4%	81%	2%
N-MeFOSAA	108%	2%	87%	3%
PFTA	80%	2%	93%	2%
PFTrDA	79%	5%	89%	1%

ENVIRO-CLEAN® Highly Cross-Linked HLD
500 mg, 6 mL cartridge, PE frits
Selectra® C18 HPLC

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Results in Reagent Water – ECDVB156P		
Analyte	One Point Fortification (5 ng/L; n=4)	
	Recovery (%)	RSD (%)
PFBS	96%	2%
PFHxA	96%	2%
HFPO-DA	86%	4%
PFHpA	95%	3%
PFHxS	101%	2%
ADONA	93%	3%
PFOA	95%	3%
PFOS	96%	3%
PFNA	101%	3%
9Cl-PF3ONS	92%	3%
PFDA	99%	5%
PFUnA	98%	5%
11Cl-PF3OUdS	95%	3%
PFDoA	92%	3%
N-EtFOSAA	98%	2%
N-MeFOSAA	106%	4%
PFTA	87%	4%
PFTrDA	85%	3%

References:

1.Unregulated Contaminant Monitoring Rule 3 (UCMR3), accessed online November 2017, http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/ucmr3/.

2.EPA Method 537.1: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), November 2018, EPA/600/R-18/352.

3.Shimadzu's Parts Compatibility Guide for LCMS Analysis of PFC's; accessed from Shimadzu website on November 2017; http://www.ssi.shimadzu.com/products/literature/lcms/085_Shimadzu%E2%80%99s%20Guide%20to%20US%20DOD_DOE%20Analysis%20of%20PFCs%20using%20the%20LCMS-8060.pdf.

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