EPA Method 533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water via Anion-Exchange SPE and LC-MS/MS

UCT Part Numbers

ECWAX156-P*
ENVIRO-CLEAN® WAX
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

SLC-18100ID21-3UM
Selectra® C18 HPLC column
(100 × 2.1 mm, 3 µm)

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

SLGRDHLDR
Guard cartridge holder

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

* **ECWAX126-P (**200 mg, 6 mL cartridge)
Available for extraction of 100mL samples

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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 533 measures PFAS by isotope dilution anion-exchange solid-phase extraction (SPE) and liquid chromatography/tandem mass spectrometry (LC-MS/MS). In total, EPA 533 covers 25 PFAS. Furthermore, it includes the use of 16 isotopically labeled Isotope Dilution Standards and 3 Isotope Performance Standards to ensure optimum method performance. In light of the EPA's recent announcement on the fifth Unregulated Contaminant Monitoring Rule (UCMR5), which includes 29 PFAS, the use of EPA 533 is set to become increasingly important to water testing labs [4].

This application note outlines the analysis of PFAS in drinking water according to EPA 533 utilizing UCT's Enviro-Clean®polymeric weak-anion exchange (**WAX)**SPE cartridges (**ECWAX156-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. The chromatography was optimized to obtain maximum resolution with minimum co-elution of isomers, including the critical linear and branched isomers of PFHxS and PFOS. For quantitation, a seven-point calibration (0.5-25 ng/mL) was performed, and all compounds were found to be linear with R^2 values > 0.99. The extraction method was evaluated by spiking reagent water samples with PFAS at 10 and 80 ng/L. Recoveries of all analytes were within a range of 70-130% and RSD values <20%. 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:

For detailed information regarding standard preparation, sample collection, preservation, and mitigating PFAS background contamination consult EPA Method 533 [3].

Verify that the sample containing 1 g/L ammonium acetate has a pH between 6.0 and 8.0. Acetic acid may be added as needed to reduce the sample pH.
Fortify the laboratory fortified blanks (LFB), laboratory fortified sample matrix (LFSM), and laboratory fortified sample matrix duplicate (LFSMD) samples with an appropriate volume of analyte primary dilution standard.
Add an aliquot of the isotope dilution analogue primary dilution standard to each sample, including the laboratory reagent blank (LRB), then cap and invert to mix.

SPE Procedure:

1. SPE Conditioning

a) Rinse SPE cartridge (**ECWAX156-P**) with 10mL methanol.
b) Rinse the cartridge with 10 mL of 0.1M pH 7 phosphate buffer, being sure to not allow the water to drop below the top edge of the packing.
c) Close the valve and add 2–3 mL of phosphate buffer to the cartridge reservoir and fill the remaining volume with reagent water.

Note: Do not allow cartridge packing material to go dry during any of the conditioning steps. If the cartridge goes dry during the conditioning phase, the conditioning must be repeated.

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.
b) Adjust the vacuum so that the flow rate is approximately 5 mL/min. Flow rates above 5 mL/min during loading may cause low analyte recovery.
c) After the entire sample has passed through the cartridge, rinse the sample bottle with 10 mL of 1 g/L ammonium acetate in reagent water. Draw the rinsate through the sample transfer tubes and the cartridges.
d) Add 1 mL of methanol 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 in.Hg) for 5 minutes.

3. Elution

a) Insert a collection rack containing 15 mL polypropylene collection tubes into the extraction manifold (**VMF016GL-PFAS**).
b) Add 5 mL of methanol containing 2% NH_4OH (v/v) to the sample container, cap and thoroughly rinse the sides with the elution solvent.

Note: Due to the volatility of NH_4OH, it is highly recommended to use fresh 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 5 mL aliquot of elution solvent.

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 20% reagent water in methanol (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. In order 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 completely 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 533 [3].

HPLC Conditions	
HPLC system	Shimadzu Nexara LC-30AD
Delay column	UCT Selectra® C18, 50 × 4.6 mm, 5 µm (p/n: SLC-1850ID46-5UM)
HPLC column	UCT Selectra® C18, 100 × 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
2.0	60	40
18.0	5	95
20.0	5	95
20.1	95	5
25.0	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

***Note:** If the source temperature is too high this may result in poor signal of HFPA-DA.

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MRM Transitions:

Analyte	R.T.	Precursor	Fragment Ion 1	Fragment Ion 2	R^2
PFBA	4.11	213.0	169.1	-	0.9984
PFMPA	4.79	229.0	85.0	-	0.9977
PFPeA	6.09	263.0	219.0	141.1	0.9987
PFBS	6.47	299.0	79.9	99.0	0.9989
PFMBA	6.72	279.1	85.0	-	0.9985
PFEESA	7.29	314.9	135.0	69.0	0.9987
NFDHA	7.80	295.0	201.0	85.0	0.9970
4:2FTS	7.96	327.0	307.1	81.0	0.9987
PFHxA	8.14	313.0	269.1	118.9	0.9971
PFPeS	8.37	349.0	80.0	99.1	0.9986
HFPO-DA	8.67	285.0	169.0	185.1	0.9986
PFHpA	9.90	362.8	319.1	169.1	0.9987
PFHxS	10.01	399.0	80.0	99.0	0.9979
ADONA	10.09	377.1	251.0	85.0	0.9982
6:2FTS	11.31	427.1	407.0	81.0	0.9978
PFOA	11.37	412.8	369.1	169.2	0.9976
PFHpS	11.42	449.1	80.0	99.1	0.9967
PFOS	12.61	499.1	80.0	99.0	0.9979
PFNA	12.62	463.1	419.0	219.2	0.9978
9Cl-PF3ONS	13.19	530.9	351.0	-	0.9980
PFDA	13.68	513.1	468.9	219.1	0.9988
8:2FTS	13.68	527.1	506.8	81.0	0.9979
PFUnA	14.61	563.1	518.9	268.8	0.9984
11Cl-PF3OUdS	14.96	631.1	451.0	-	0.9977
PFDoA	15.40	612.9	569.0	319.1	0.9980
Isotope Performance Standards					
$^{13}C_3$-PFBA	4.11	216.1	172.1	-	
$^{13}C_2$-PFOA	11.36	415	370	169.1	
$^{13}C_4$-PFOS	12.61	502.9	80	99.0	
Isotope Dilution Standards					
$^{13}C_4$-PFBA	4.11	217.1	172.1	-	
$^{13}C_5$-PFPeA	6.09	268	223.2	70.1	
$^{13}C_3$-PFBS	6.47	302.1	79.9	99.0	
$^{13}C_2$-4:2FTS	7.96	328.9	309	80.9	
$^{13}C_5$-PFHxA	8.13	318.1	273	121.1	
$^{13}C_3$-HFPO-DA	8.67	287	169	185.1	
$^{13}C_4$-PFHPA	9.90	367.1	322.1	-	
$^{13}C_3$-PFHxS	10.01	401.9	80	99.0	
$^{13}C_2$-6:2FTS	11.31	429.1	409	81.0	
$^{13}C_8$-PFOA	11.36	421.1	376.1	172.2	
$^{13}C_8$-PFOS	12.61	507.1	80	98.9	
$^{13}C_9$-PFNA	12.62	471.5	427	-	
$^{13}C_6$-PFDA	13.68	519	474	-	
$^{13}C_2$-8:2FTS	13.68	529	508.9	81.0	
$^{13}C_7$-PFUnA	14.61	570	525	-	
$^{13}C_2$-PFDoA	15.40	612.9	569	319.1	

***Note:** Calibration curve concentrations = 0.5, 1, 2.5, 5, 10, 20 and 25 ng/mL.

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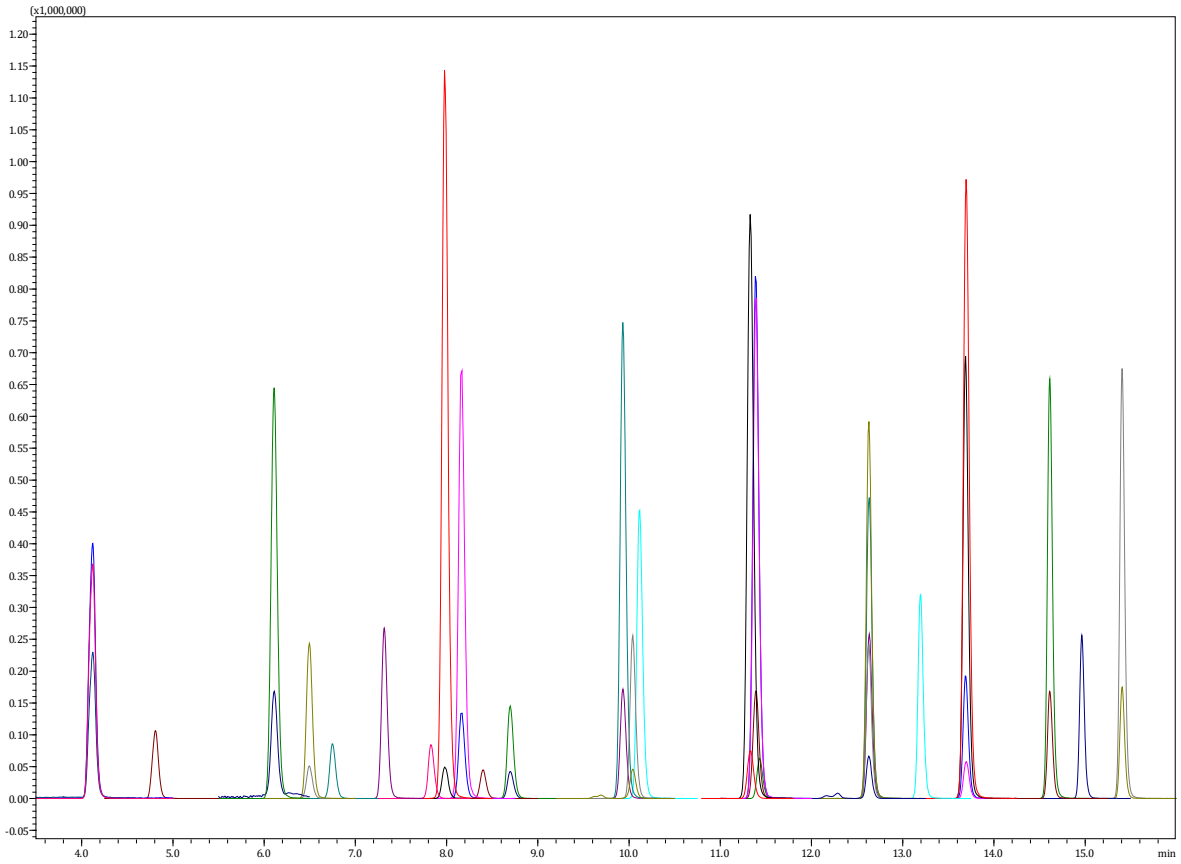

Figure 1: PFAS fortified at low fortification level 10 ng/L in reagent water (2.5 ng/mL in vial).

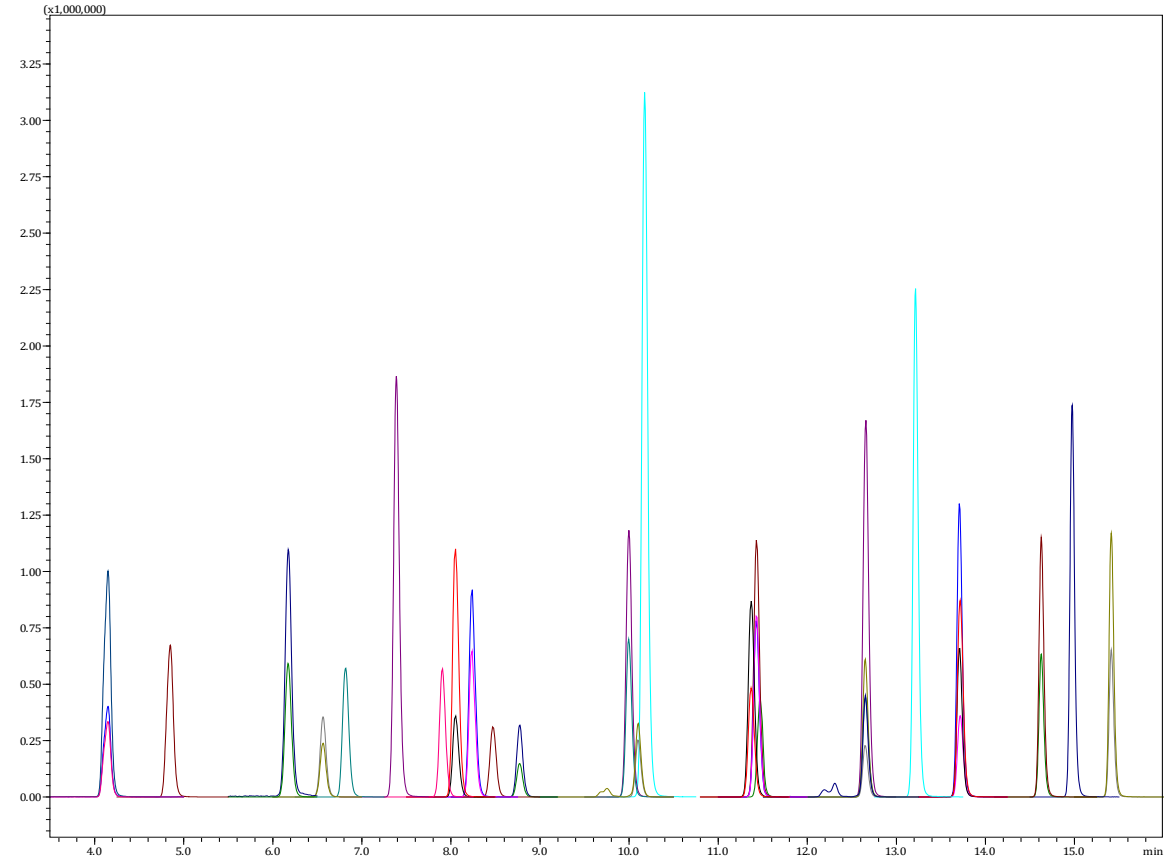

Figure 2: PFAS fortified at high fortification level 80 ng/L in reagent water (20 ng/mL in vial).

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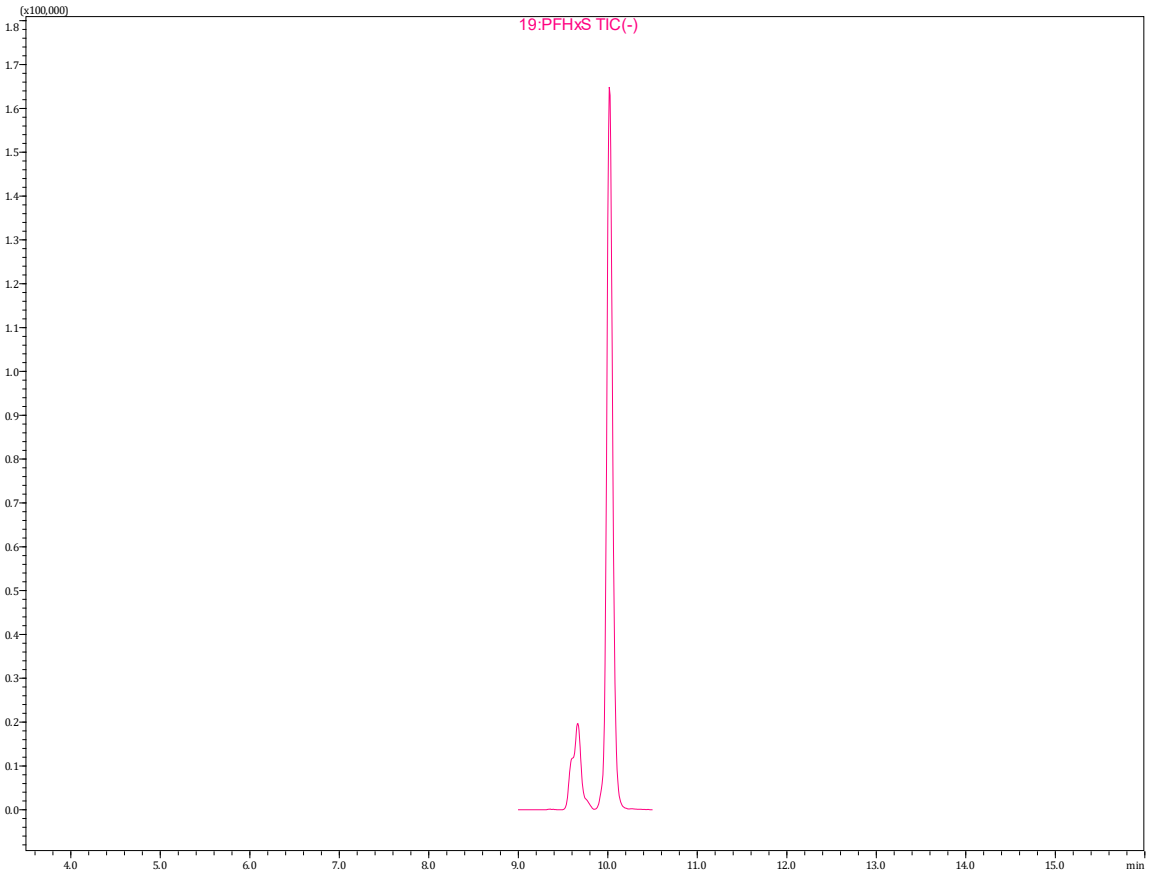

Figure 3. Chromatogram showing baseline separation of PFHxS isomers (branched vs linear).

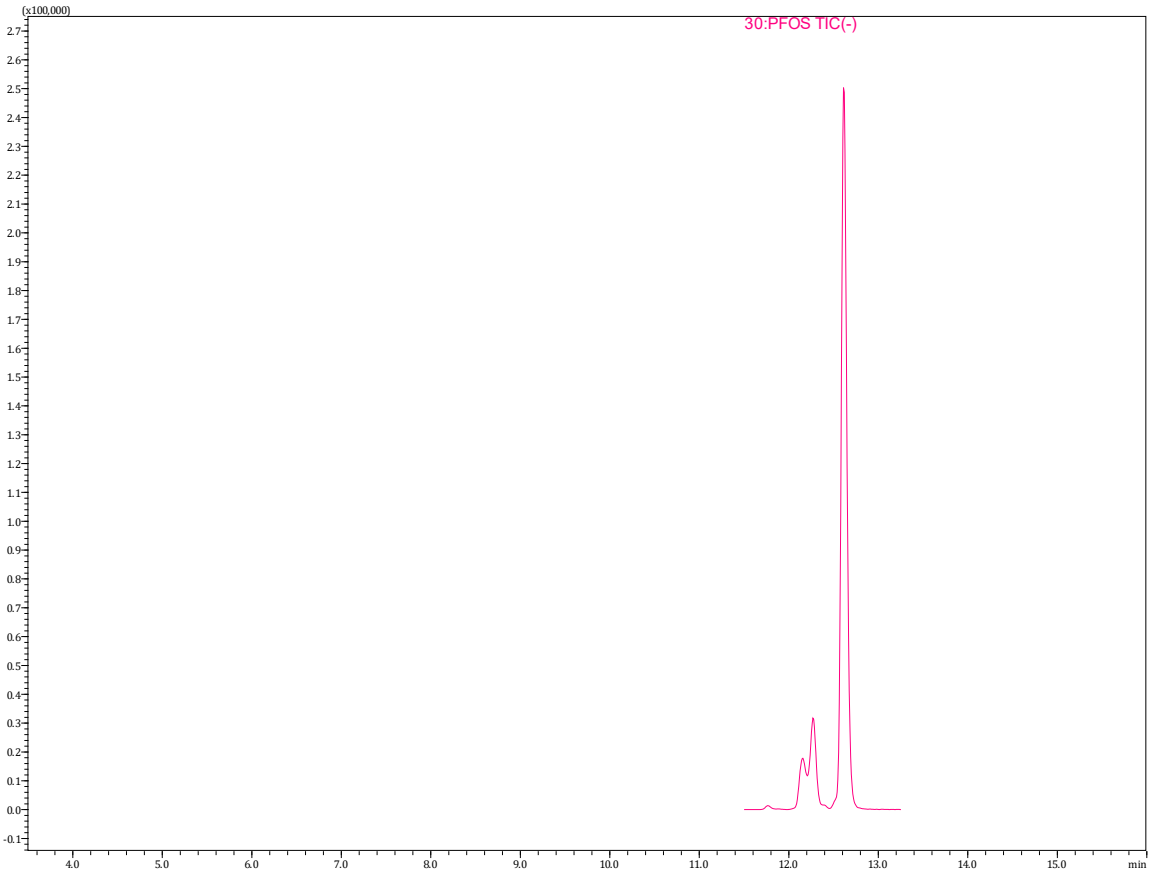

Figure 4. Chromatogram showing baseline separation of PFOS isomers (branched vs linear).

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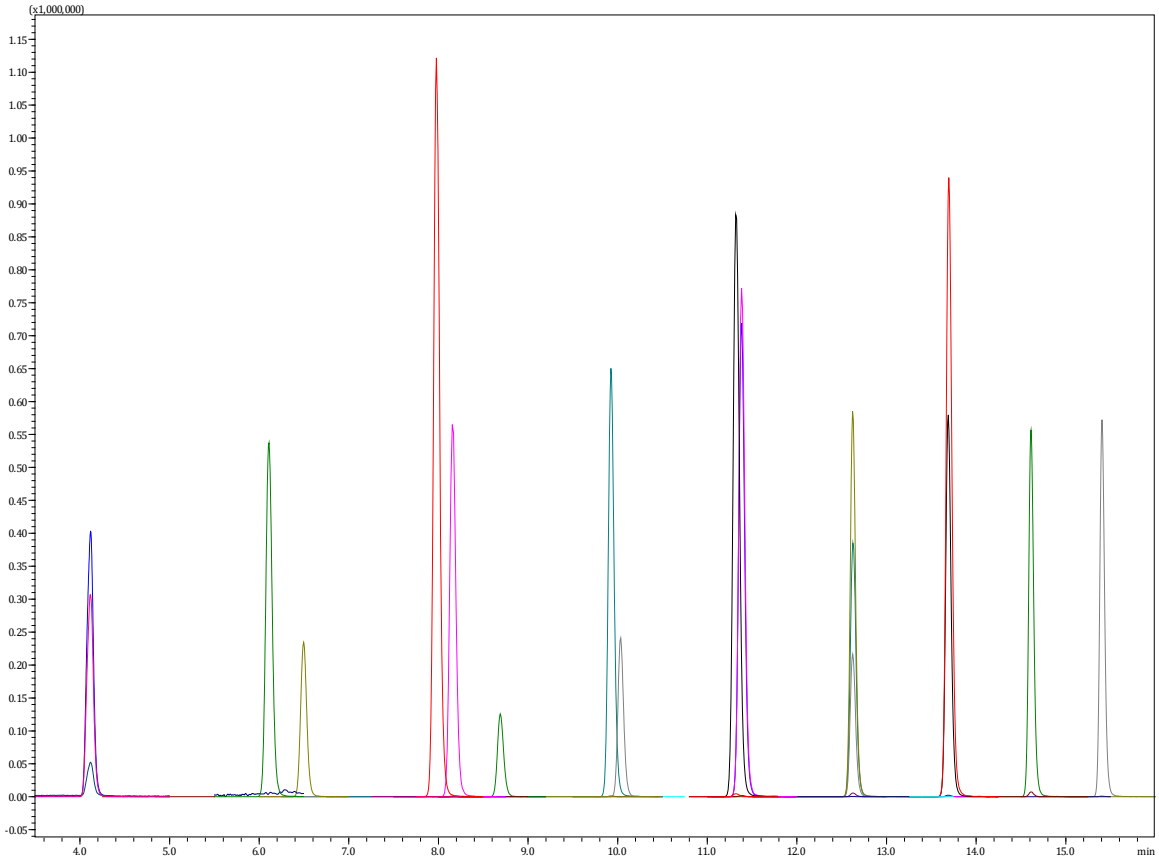

Figure 3: Chromatogram of a LRB sample containing Isotope Dilution and Isotope Performance standards.

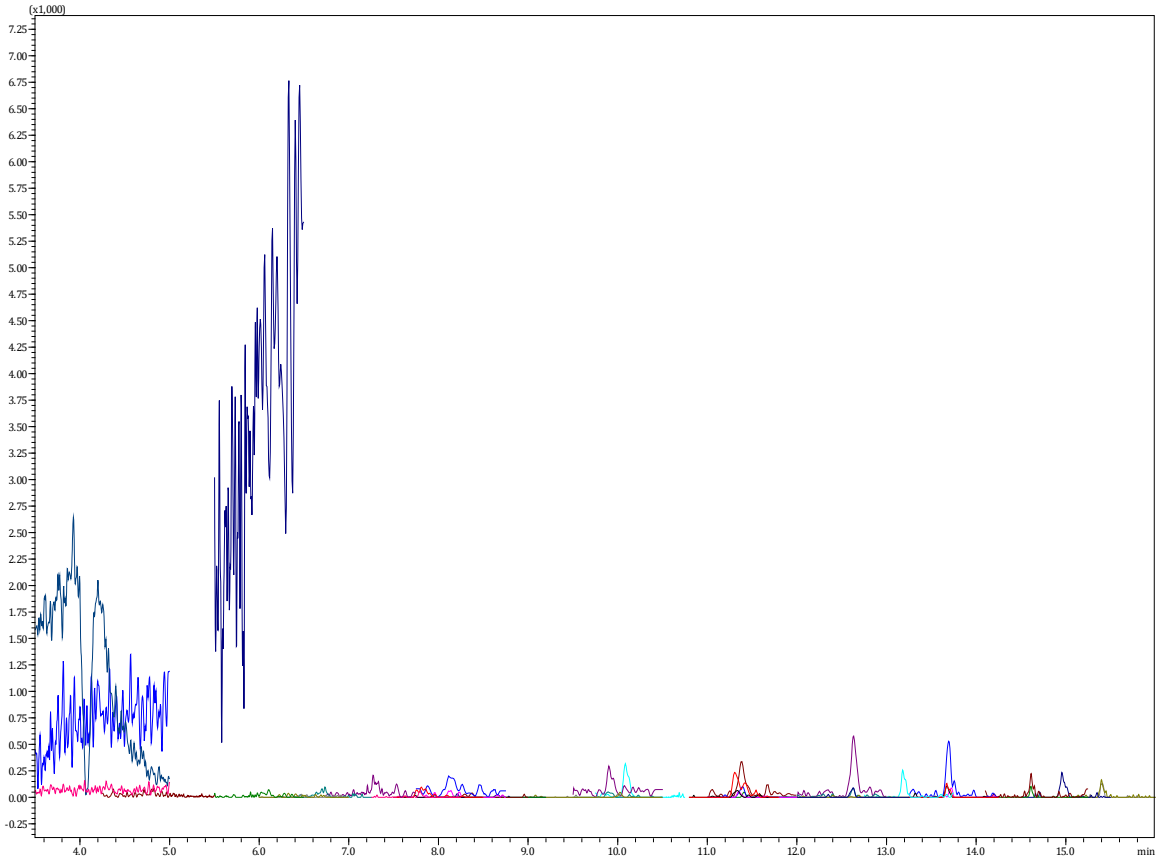

Figure 4: Chromatogram of a blank solvent injection demonstrating low system background levels.

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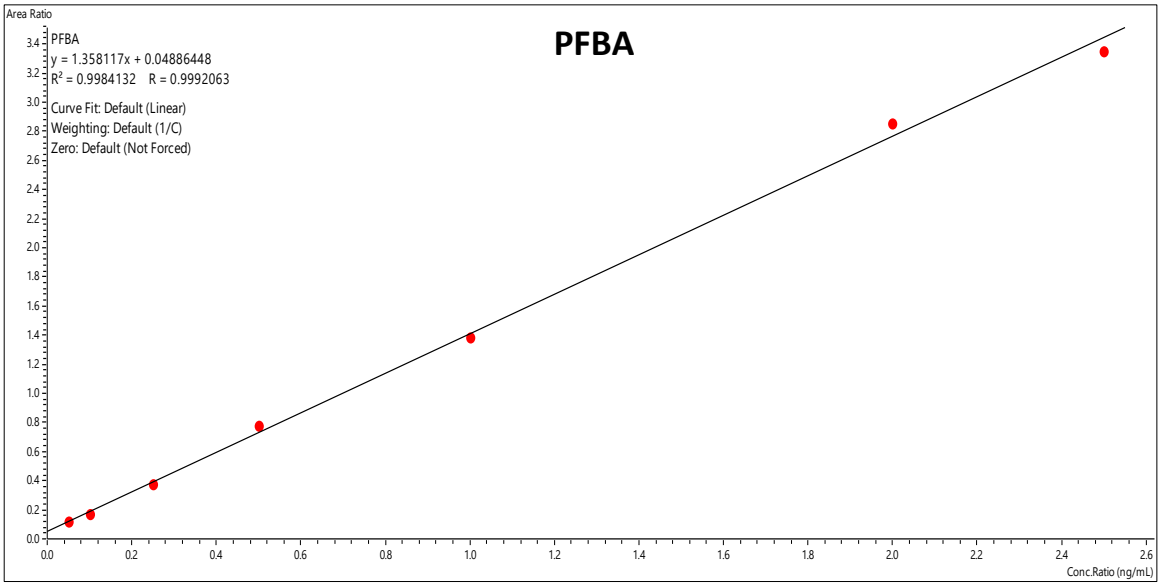
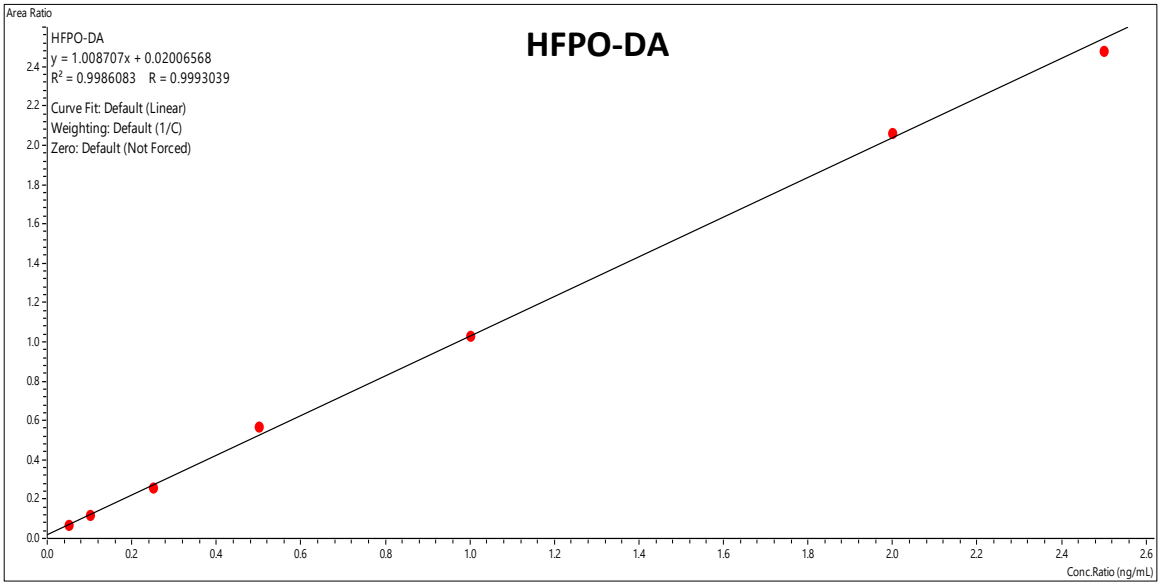
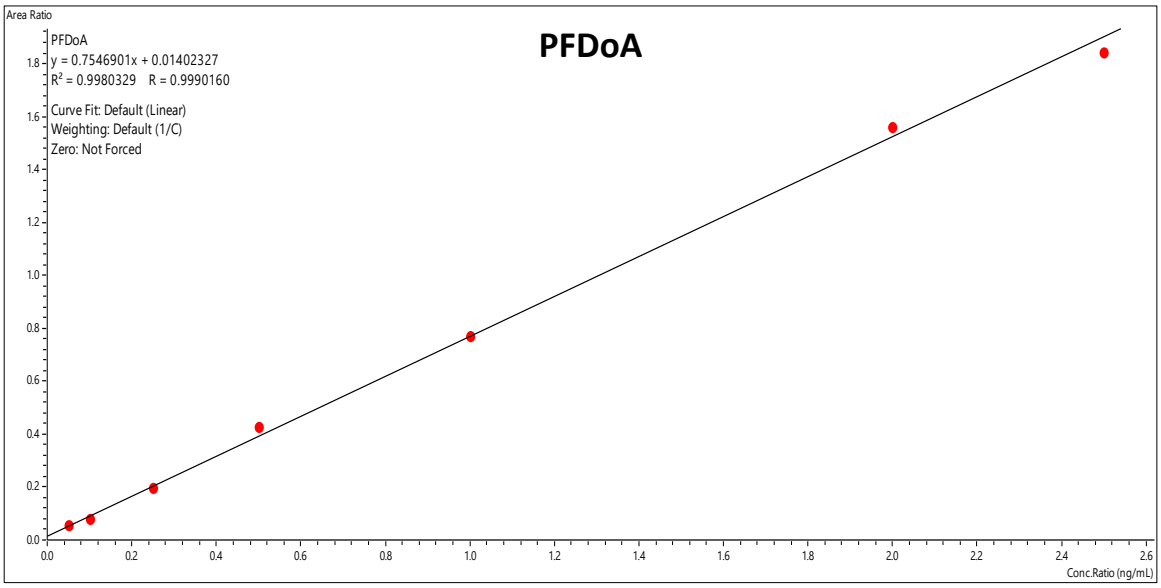
Calibration Curve Examples:







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SPE Results:

Results in Reagent Water				
Analyte	Low Fortification (10 ng/L; n=6)		High Fortification (80 ng/L; n=6)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
PFBA	115.89	9.22	108.04	10.71
PFMPA	102.20	4.71	100.47	1.24
PFPeA	100.93	5.10	100.70	2.33
PFBS	107.67	5.92	108.84	2.09
PFMBA	103.33	5.45	103.18	0.45
PFEESA	101.27	5.28	103.78	1.90
NFDHA	98.07	5.46	95.73	2.29
4:2FTS	107.27	5.89	105.98	1.90
PFHxA	100.47	5.73	102.22	2.34
PFPeS	107.40	5.97	112.27	2.69
HFPO-DA	106.07	7.09	105.08	2.50
PFHPA	106.60	5.01	107.54	1.96
PFHxS	104.13	5.32	106.32	1.35
ADONA	99.87	5.16	99.94	1.76
6:2FTS	127.00	13.24	102.90	1.54
PFOA	106.47	5.74	106.16	1.91
PFHpS	101.07	5.84	99.50	3.68
PFOS	101.73	4.96	100.48	2.05
PFNA	99.93	5.61	99.12	2.60
9Cl-PF3ONS	105.07	5.10	103.32	5.22
PFDA	106.47	4.84	106.89	2.38
8:2FTS	108.93	4.42	108.40	1.94
PFUnA	110.00	5.21	108.53	2.46
11Cl-PF3OUdS	101.07	5.28	97.37	10.74
PFDoA	109.13	4.85	107.22	1.99

References:

1. EPA Method 537: Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). https://cfpub.epa.gov/si/si_public_file_download.cfm?p_download_id=525468&Lab=NERL.

2. 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). https://cfpub.epa.gov/si/si_public_file_download.cfm?p_download_id=537290&Lab=NERL.

3. 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. https://www.epa.gov/sites/production/files/2019-12/documents/method-533-815b19020.pdf

4. Unregulated Contaminant Monitoring Rule 5 (**UCMR5**), accessed online March 2021, https://www.epa.gov/dwucmr/fifth-unregulated-contaminant-monitoring-rule; https://www.govinfo.gov/content/pkg/FR-2021-03-11/pdf/2021-03920.pdf.

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 • 215.781.9255
www.unitedchem.com Email: methods@unitedchem.com