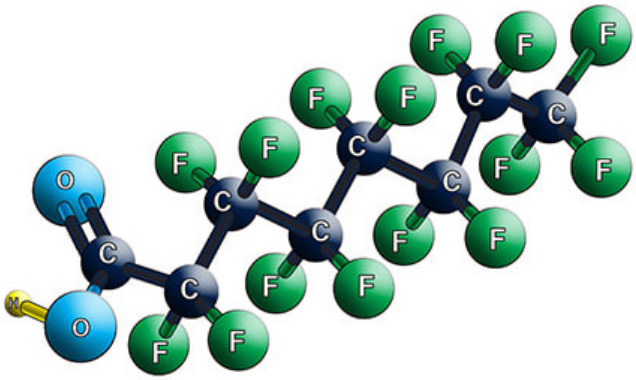
EPA Draft Method 1633: Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Samples by Solid-Phase Extraction and LC-MS/MS



UCT Part Numbers

ECWAX156-P*
ENVIRO-CLEAN® WAX
500 mg, 6 mL cartridge, PE frits

RFV00150P
Empty Polypropylene Reservoirs

AD0000AS
Cartridge Adaptor

EUCARB00X
Enviro-Clean® Graphitized Carbon (10g)

SCS27-C18GDC21
SelectraCore® C18 Guard Column
(5 × 2.1 mm, 2.7 µm)

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

VMF016GL
Complete 16 Position Glass Block Manifold

SCS27-C18521
SelectraCore® C18 HPLC Column
(50 × 2.1 mm, 2.7 µm)

SLGRDHLDR
Guard cartridge holder

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

Summary:

The significance of examining the levels of Per- and polyfluoroalkyl substances (PFAS) in various matrices cannot be overstated. In September 2021, the EPA published a draft method for analyzing 40 different PFASs in a wide range of matrices. At present, two validated and published USEPA methods are available for examining PFAS in drinking water. An updated version of USEPA 537, known as USEPA 537.1, is the first method [1,2]. The second method, USEPA 533, features a more comprehensive list of analytes and includes some shorter-chain PFASs [3]. Meanwhile, EPA 1633 utilizes isotope dilution, anion-exchange solid-phase extraction (SPE), and liquid chromatography/tandem mass spectrometry (LC-MS/MS) to measure PFAS levels. The EPA 1633 Draft Method encompasses 40 target PFASs and also includes 24 isotopically labelled extracted internal standards and 7 isotopically labelled non-extracted internal standards to ensure optimal method performance [4]. Additionally, many of the compounds listed in the fifth Unregulated Contaminant Monitoring Rule (UCMR5) released by the EPA are also incorporated into the USEPA 1633 draft method [5].

This application note outlines the analysis of 40 distinct PFASs in aqueous samples as per EPA 1633 3rd draft method by utilizing UCT's Enviro-Clean® polymeric weak-anion exchange (WAX) SPE cartridges. A SelectraCore® C18 HPLC analytical column was employed for LC-MS/MS analysis, while a short (5 cm) C18 delay column was used to reduce potential of PFAS contamination from the HPLC system. SelectraCore® column was able to achieve a baseline separation for many critical analytes, some of which are branched vs linear isomers like PFHxS and PFOS. A calibration curve specific to each analyte was performed according to the guidelines written in the method. All compounds were linear, with R^2 values >0.99. The extraction method was evaluated by spiking aqueous samples with PFAS's at high and low fortification levels, as indicated in the results table in this application note. Recoveries of all analytes were within a range of 80-110% and RSD values <10%.

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Sample Pretreatment:

For detailed information regarding standard preparation, sample collection, preservation, and mitigating PFAS background contamination refer EPA Method 1633 [4].

- 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 Native Standard Solution.
- Add an aliquot of the External Internal Standard (EIS) to each sample, including the laboratory reagent blank (LRB), then cap and invert to mix.

SPE Procedure:

1. System Set up

a) Pack clean silanized glass wool to half the height of the ECWAX SPE cartridge barrel.
b) Set up the vacuum manifold with one ECWAX SPE cartridge plus a reservoir and reservoir adaptor for each cartridge for each sample.

2. SPE Conditioning

a) Rinse the SPE cartridge (**ECWAX156-P**) with 15 mL of 1% methanolic ammonium hydroxide.
b) Rinse the cartridge with 10 mL of 0.3M formic acid, 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.

3. Sample Extraction/Drying

a) Pour the sample into the reservoir.
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 walls of the reservoir with 5 mL reagent water twice.
d) Rinse the walls of the reservoir with 5 mL of 1:1 0.1M formic acid/methanol.
e) Dry the cartridge under a high vacuum (15-20 in.Hg) for 5 minutes.

4. Elution

a) Insert a collection rack containing 15 mL polypropylene collection tubes into the extraction manifold. **DO NOT add (NIS)** Non-Extracted Internal Standards to these collection tubes.
b) Rinse the inside of the sample bottle with 5 mL of 1% methanolic ammonium hydroxide.

Note: It is highly recommended to use a fresh elution solvent, due to the volatility of NH_4OH. Rinsing the sides of the container is important for obtaining good recovery of the long-chain hydrophobic PFASs.

c) Transfer the rinse to the SPE reservoir, washing the walls of the reservoir. Use a low vacuum such that the solvent exits the cartridge in a dropwise fashion.
d) Add 25 µL of concentrated acetic acid to each sample eluted in the collection tubes and vortex.
e) Add 10 mg of carbon (**EUCARB00X**) to each sample and hand-shake occasionally for no more than 5 minutes.
f) Vortex and centrifuge at 2800 rpm for 10 minutes.
g) Add NIS solution to a clean collection tub.
h) Place a syringe filter (25-mm filter, 0.2-µm nylon membrane) on a 5-mL polypropylene syringe. Take the plunger out and carefully decant the sample supernatant into the syringe barrel. Replace the plunger and filter the entire extract into new collection tube containing NIS.
i) Vortex and transfer a portion of the extract into a 1-mL polypropylene autosampler vial for LC-MS/MS analysis.

LC-MS/MS Parameters:

PFASs 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 completely 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 the HPLC's vendor before proceeding. Additional information can also be found in EPA Draft Method 1633 [4].

HPLC Conditions	
HPLC system	SCIEX Exion LC
Delay column	UCT Selectra® C18, 50 × 4.6 mm, 5 µm (p/n: SLC-1850ID46-5UM)
HPLC column	UCT SelectraCore® C18, 50 × 2.1 mm, 2.7 µm (p/n: SCS27-C18521)
Guard column	UCT SelectraCore® C18, 5 × 2.1 mm, 2.7 µm (p/n: SCS27-C18GDC21)
Guard column holder	p/n: SLGRDHLDR
Column temperature	40°C
Flow rate	0.400 µL/min
Injection volume	2 µL

Time (min)	Mobile Phase A (%): 20 mM Ammonium Acetate	Mobile Phase B (%): Acetonitrile
0.0	98	2
7.5	0	100
8.50	0	100
8.51	98	2
11.50	98	2

MS Conditions	
MS/MS system	ABSCIEX QTrap 6500+
Ionization Mode	Electrospray Ionization in negative mode (-ESI-)
Ion Spray Voltage (IS)	-4500.00
Temperature (TEM)	300°C
Curtain Gas (CUR)	40
Ion Source Gas 1 (GS1)	50
Ion Source Gas 2 (GS2)	50

***Note:** Too high of a source temperature may result in a poor signal of HFPA-DA

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MRM Transitions:

Analyte	R.T.	Precursor	Fragment Ion 1	Fragment Ion 2	R^2
Target Analytes					
PFBA	1.82	212.8	168.9	NA	0.9948
PFPeA	2.64	262.7	218.9	68.9	0.9939
PFHxA	3.13	312.8	268.9	118.9	0.9891
PFHpA	3.50	362.7	318.9	168.9	0.9875
PFOA	3.83	413.0	369.0	169.0	0.9905
PFNA	4.15	463.0	419.0	219.0	0.9915
PFDA	4.46	512.9	469.0	219.0	0.9960
PFUnA	4.76	563.1	519.0	269.1	0.9894
PFDoA	5.06	613.1	569.0	319.0	0.9929
PFTrDA[2]	5.35	663.0	619.0	168.9	0.9986
PFTeDA	5.64	713.1	669.0	168.9	0.9964
PFBS	3.15	298.7	79.9	98.8	0.9965
PFPeS	3.58	349.1	79.9	98.9	0.9941
PFHxS	3.95	398.7	79.9	98.9	0.9988
PFHpS	4.29	449.0	79.9	98.8	0.9948
PFOS	4.61	498.9	79.9	98.8	0.9959
PFNS	4.92	548.8	79.9	98.8	0.9955
PFDS	5.22	599.0	79.9	98.8	0.9909
PFDoS	5.80	699.1	79.9	98.8	0.9931
4:2 FTS	2.98	327.1	307.0	80.9	0.9820
6:2 FTS	3.69	427.1	407.0	80.9	0.9837
8:2 FTS	4.31	527.1	507.0	80.8	0.9977
PFOSA	5.26	498.1	77.9	478.0	0.9872
NMeFOSA	6.23	511.9	219.0	169.0	0.9923
NEtFOSA	6.49	526.0	219.0	169.0	0.9950
NMeFOSAA	4.48	570.1	419.0	483.0	0.9846
NEtFOSAA	4.59	584.2	419.1	526.0	0.9957
NMeFOSE	6.13	616.1	58.9	NA	0.9930
NEtFOSE	6.38	630.0	58.9	NA	0.9936
HFPO-DA	3.26	284.9	168.9	184.9	0.9874
ADONA	3.62	376.9	250.9	84.8	0.9885
9Cl-PF3ONS	4.84	530.8	350.8	98.8	0.9898
11Cl-PF3OUdS	5.44	630.9	450.8	98.8	0.9888
3:3 FTCA	2.93	241.0	177.0	117.0	0.9934
5:3 FTCA	3.33	341.0	237.1	217.0	0.9920
7:3 FTCA	4.04	441.0	316.9	336.9	0.9953
PFEESA	3.35	314.8	134.9	82.9	0.9937
PFMPA	2.22	229.0	84.9	NA	0.9939
PFMBA	2.81	279.0	85.1	NA	0.9908
NFDHA	3.07	295.0	201.0	84.9	0.9899

***Note:** Calibration curve concentrations are specific to each analyte in the method [4].

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MRM Transitions:

Analyte	R.T.	Precursor	Fragment Ion 1	Fragment Ion 2	R^2
Extracted Internal Standards					
$^{13}C_4$-PFBA	1.81	216.8	171.9	NA	NA
$^{13}C_5$-PFPeA	2.65	268.3	223.0	NA	NA
$^{13}C_5$-PFHxA	3.12	318.0	273.0	120.3	NA
$^{13}C_4$-PFHpA	3.49	367.1	322.0	NA	NA
$^{13}C_8$-PFOA	3.80	421.1	376.0	NA	NA
$^{13}C_9$-PFNA	4.15	472.1	427.0	NA	NA
$^{13}C_6$-PFDA	4.45	519.1	474.1	NA	NA
$^{13}C_7$-PFUnA	4.76	570.0	525.1	NA	NA
$^{13}C_2$-PFDoA	5.05	615.1	570.0	NA	NA
$^{13}C_2$-PFTeDA	5.63	715.2	670.0	NA	NA
$^{13}C_3$-PFBS	3.14	302.1	79.9	98.9	NA
$^{13}C_3$--PFHxS	3.95	402.1	79.9	98.9	NA
$^{13}C_8$-PFOS	4.61	507.1	79.9	98.9	NA
$^{13}C_2$-4:2FTS	2.98	329.1	80.9	309.0	NA
$^{13}C_2$-6:2FTS	3.69	429.1	80.9	409.0	NA
$^{13}C_2$-8:2FTS	4.31	529.1	80.9	509.0	NA
$^{13}C_8$-PFOSA	5.27	506.1	77.8	NA	NA
D_3-NMeFOSA	6.23	515.0	219.0	NA	NA
D_5-NEtFOSA	6.48	531.1	219.0	NA	NA
D_3-NMeFOSAA	4.46	573.2	419.0	NA	NA
D_5-NEtFOSAA	4.59	589.2	419.0	NA	NA
D_7-NMeFOSE	6.11	623.2	58.9	NA	NA
D_9-NEtFOSE	6.37	639.2	58.9	NA	NA
$^{13}C_3$-HFPO-DA	3.26	286.9	168.9	184.9	NA
Non-Extracted Internal Standards					
$^{13}C_3$--PFBA	1.84	216.0	172.0	NA	NA
$^{13}C_2$-PFHxA	3.12	315.1	270.0	119.4	NA
$^{13}C_4$-PFOA	3.83	417.1	172.0	NA	NA
$^{13}C_5$-PFNA	4.15	468.0	423.0	NA	NA
$^{13}C_2$-PFDA	4.45	515.1	470.1	NA	NA
$^{18}O_2$-PFHxS	3.95	403.0	83.9	NA	NA
$^{13}C_4$-PFOS	4.61	502.8	79.9	98.9	NA

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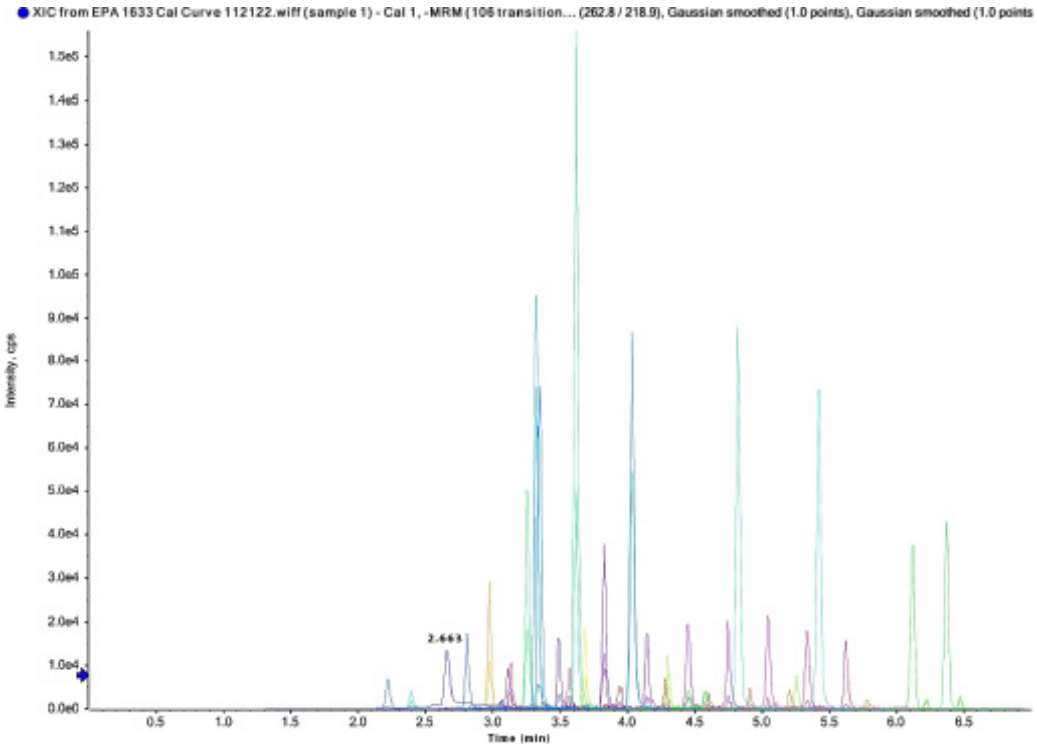


Figure 1: Target Analytes spiked at low fortification level

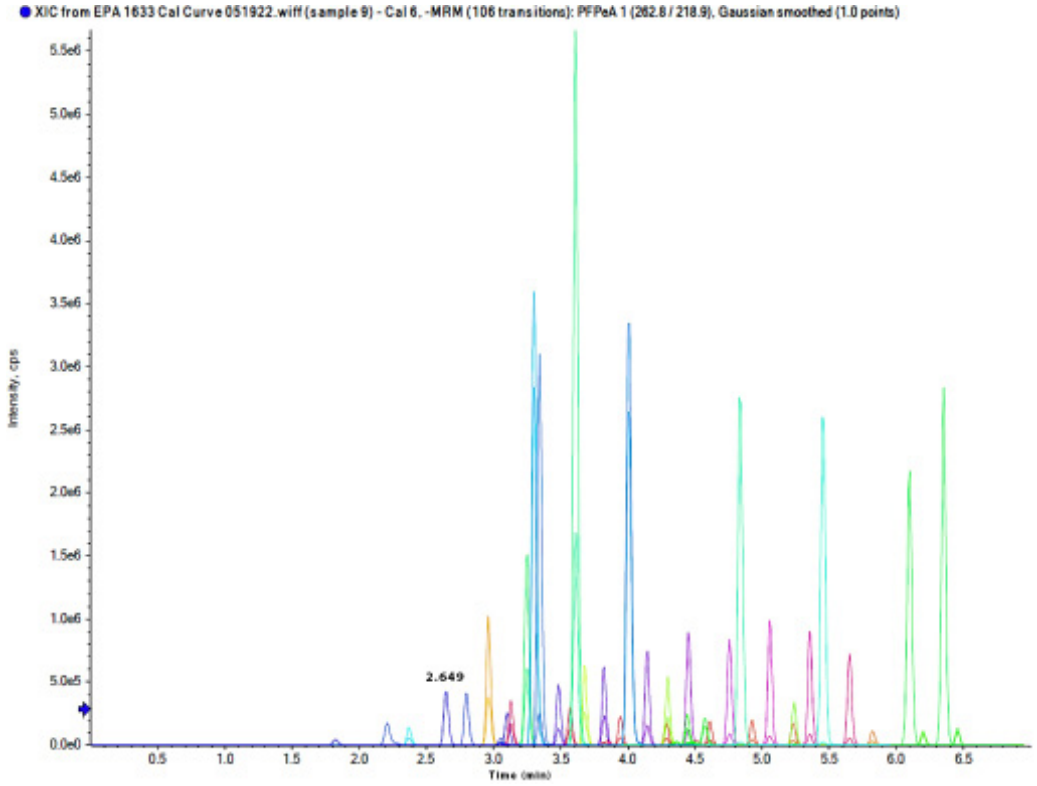


Figure 2: Target Analytes spiked at a high fortification level

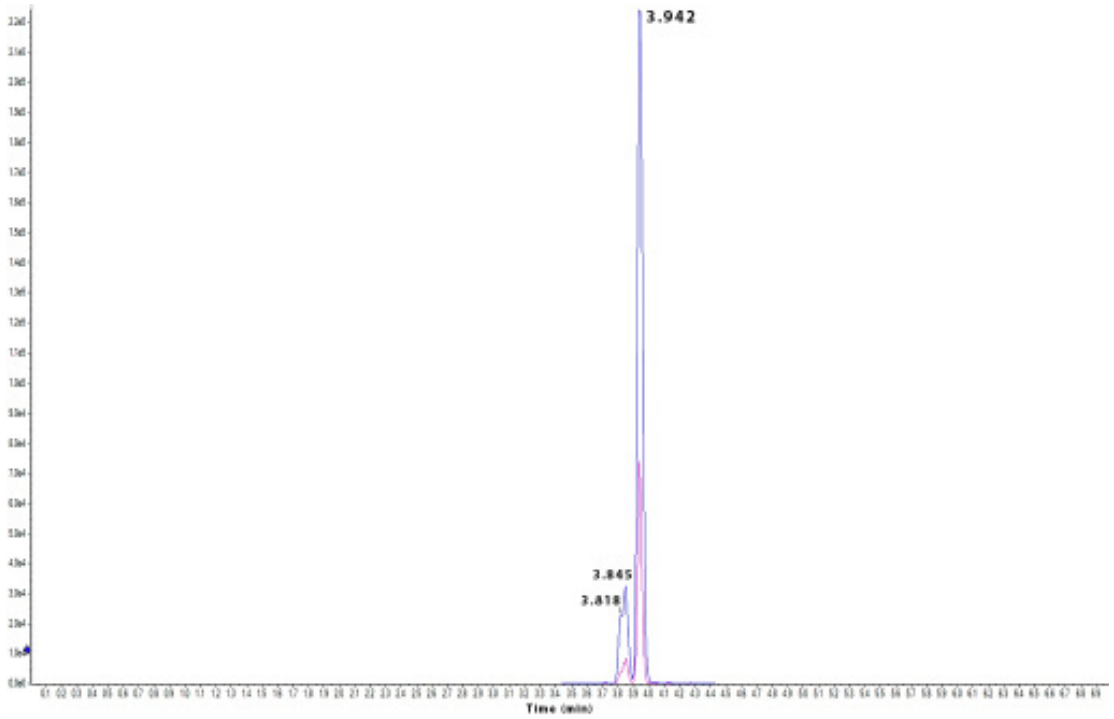


Figure 3. Chromatogram showing baseline separation of PFHxS isomers (branched vs linear) using SelectraCore® C18 HPLC Column

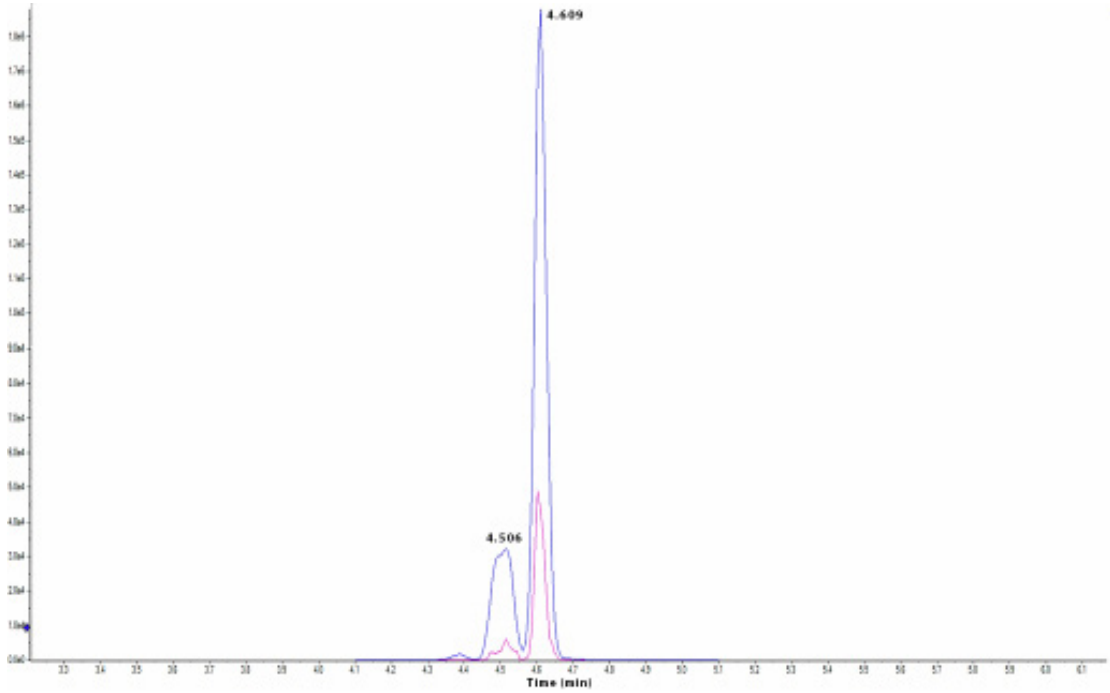


Figure 4. Chromatogram showing baseline separation of PFOS isomers (branched vs linear) using SelectraCore® C18 HPLC Column

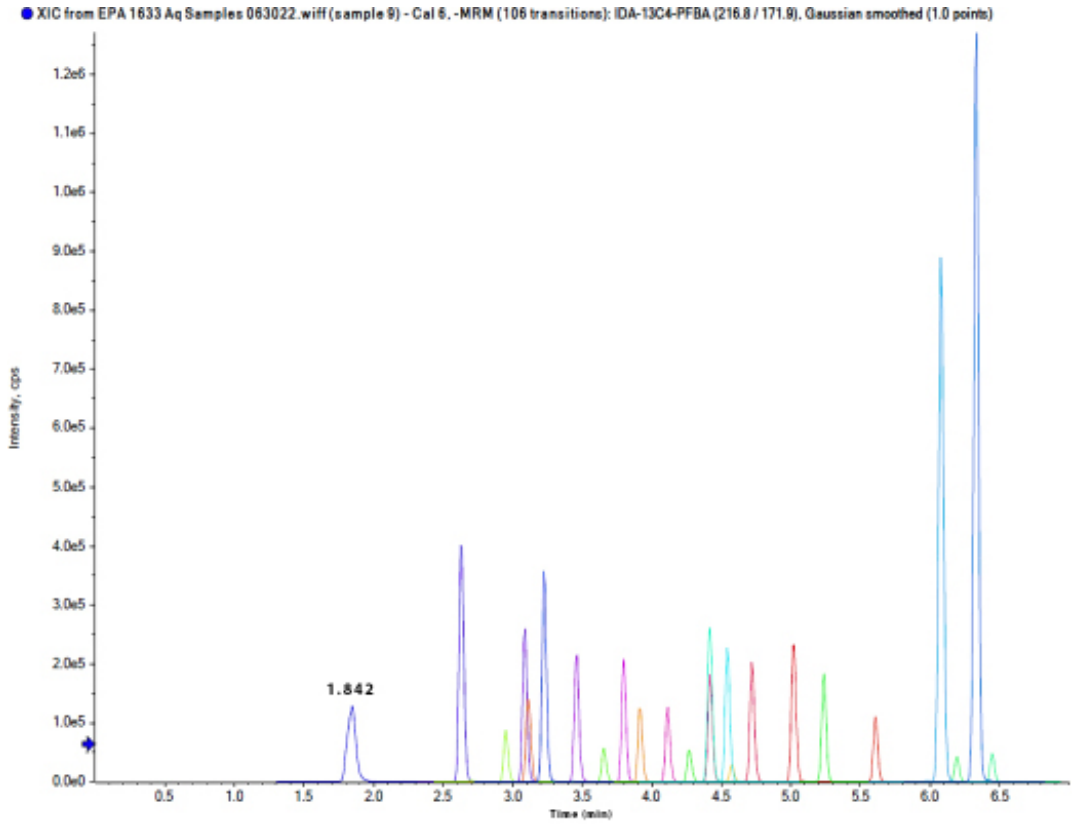


Figure 5. Extracted Internal Standards

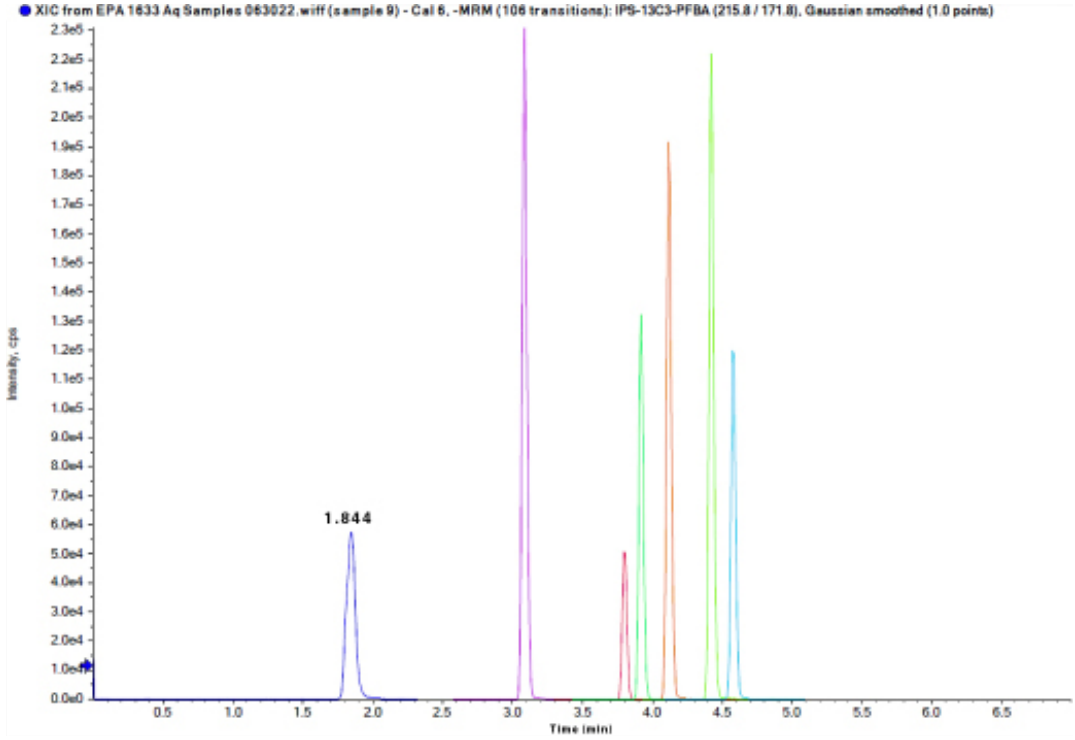
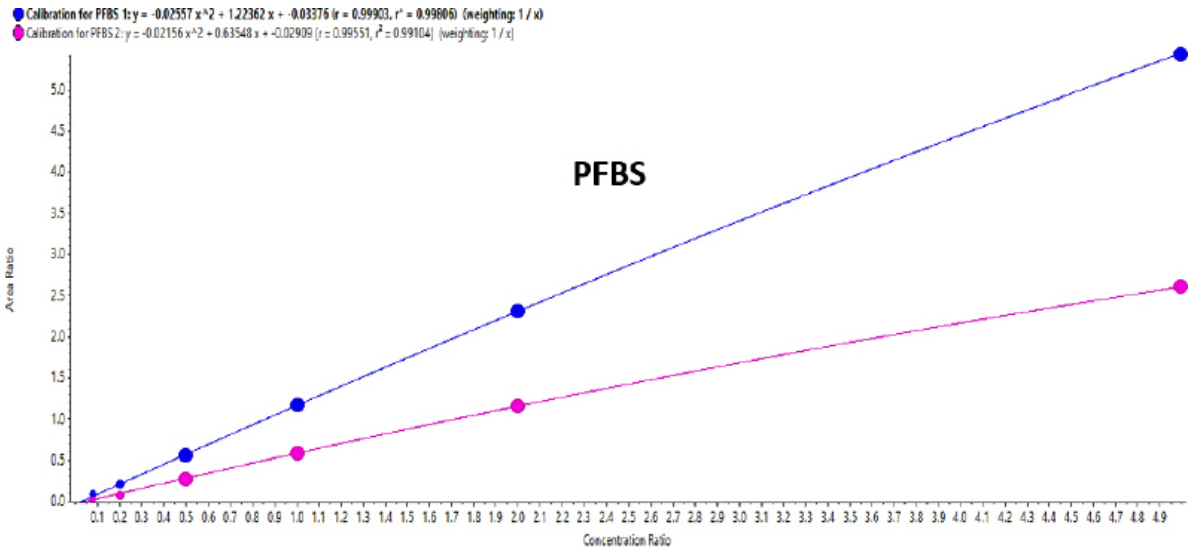
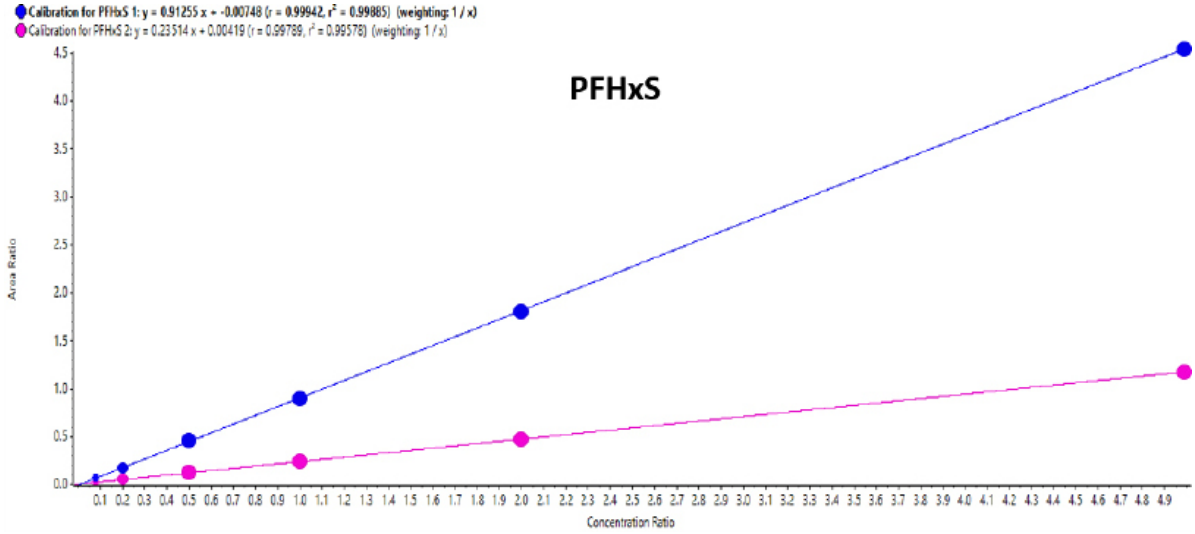
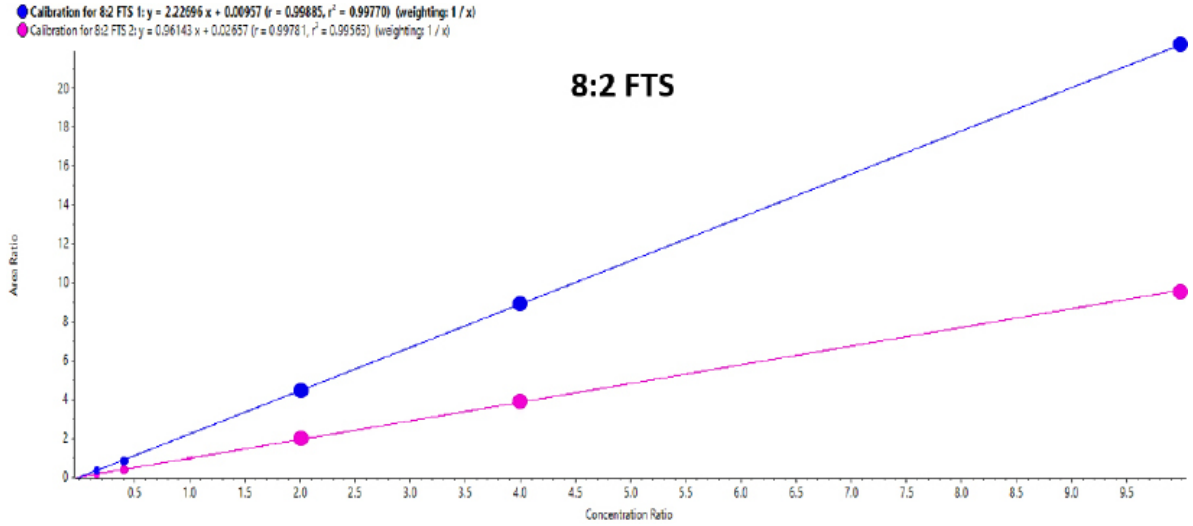


Figure 6. Non-Extracted Internal Standards

Calibration Curve Examples:







SPE Results:

Results in Aqueous Samples						
	Low Fortification (n=5)			High Fortification (n=5)		
Analyte	Conc (ng/mL)	Recovery (%)	RSD (%)	Conc (ng/mL)	Recovery (%)	RSD (%)
PFBA	5.0	104.6	0.88	25	97.5	1.06
PFPeA	2.5	98.2	0.98	12.5	95.4	3.68
PFHxA	1.25	99.3	0.67	6.25	102.5	1.05
PFHpA	1.25	99.6	0.58	6.25	98.3	1.99
PFOA	1.25	106.3	2.06	6.25	97.1	2.59
PFNA	1.25	103.5	1.31	6.25	98.9	1.17
PFDA	1.25	95.4	0.87	6.25	102.4	1.4
PFUnA	1.25	96.4	0.64	6.25	103.6	1.69
PFDoA	1.25	99.4	0.74	6.25	105.6	1.03
PFTrDA2	1.25	95.7	2.58	6.25	99.3	4.27
PFTeDA	1.25	101.6	0.59	6.25	98.1	0.87
PFBS	1.25	92.8	0.8	6.25	103.4	1.81
PFPeS	1.25	94.6	0.74	6.25	99.6	1.86
PFHxS	1.25	100.3	1.31	6.25	105.3	5.52
PFHpS	1.25	94.2	1.2	6.25	99.3	5.85
PFOS	1.25	98.4	0.97	6.25	99.6	4.65
PFNS	1.25	101.07	0.85	6.25	99.5	1.42
PFDS	1.25	93.5	0.81	6.25	97.2	1.42
PFDoS	1.25	99.6	0.95	6.25	97.5	0.92
4:2 FTS	5.0	97.2	0.85	25	99.5	0.84
6:2 FTS	5.0	99.7	0.87	25	102.4	4.13
8:2 FTS	5.0	98.5	1.27	25	99.3	3.03
PFOSA	1.25	100.2	0.97	6.25	99.7	1.78
NMeFOSA	1.25	96.8	1.3	6.25	103.5	4.51
NEtFOSA	1.25	101.3	1.94	6.25	99.7	2.65
NMeFOSAA	1.25	94.7	1.49	6.25	94.3	2.85
NEtFOSAA	1.25	96.7	1.06	6.25	94.3	1.71
NMeFOSE	12.5	96.4	1.67	6.25	94.9	1.71
NEtFOSE	12.5	98.6	2.8	6.25	97.4	0.7
HFPO-DA	5.0	97.5	4.16	25	95.6	3.89
ADONA	5.0	96.2	0.75	25	96.3	2.68
9Cl-PF3ONS	5.0	97.6	2.88	25	96.4	1.23
11Cl-PF3OUdS	5.0	99.1	1.49	25	96.8	0.30
3:3 FTCA	6.25	99.3	2.60	31.2	97.6	4.16
5:3 FTCA	31.3	101.6	0.83	156	98.4	0.60
7:7 FTCA	31.3	105.3	0.95	156	100.9	0.27
PFEESA	2.5	89.6	0.82	12.5	95.2	0.94
PFMPA	2.5	97.9	0.84	12.5	99.4	1.42
PFMBA	2.5	103.6	0.79	12.5	106.4	1.42
NFDHA	2.5	96.4	0.59	12.5	98.6	0.92

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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] 3rd Draft Method 1633: Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS.

[5] 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

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