RESTEK Pure Chromatography

The Evolving Landscape of PFAS Detection, an Outline of Methods

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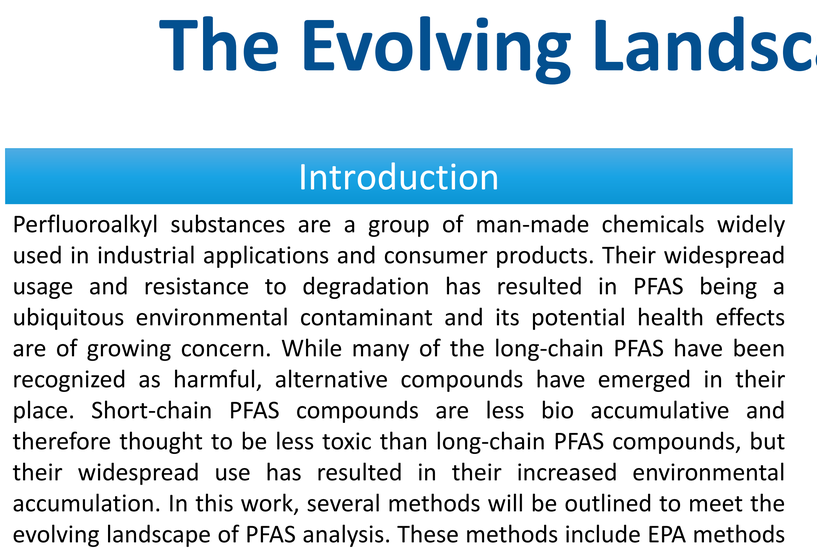
Introduction

Perfluoroalkyl substances are a group of man-made chemicals widely used in industrial applications and consumer products. Their widespread usage and resistance to degradation has resulted in PFAS being a ubiquitous environmental contaminant and its potential health effects are of growing concern. While many of the long-chain PFAS have been recognized as harmful, alternative compounds have emerged in their place. Short-chain PFAS compounds are less bio accumulative and therefore thought to be less toxic than long-chain PFAS compounds, but their widespread use has resulted in their increased environmental accumulation. In this work, several methods will be outlined to meet the evolving landscape of PFAS analysis. These methods include EPA methods 1633, 533, 8327, and 537.1 as well as a method for the analysis of ultra-short through short-chain PFAS(C1-C4).

EPA 1633 Aqueous, Solid, Biosolids, and Tissue Samples

In EPA 1633 the potential interference from bile acids to PFOS is outlined along with guidelines to follow for the analysis of 40 PFAS compounds. This potential interference from the bile acids to PFOS is affected by the organic modifier used in the analytical method. The document states that when using acetonitrile as the organic modifier, the bile acid taurodeoxycholic acid (TDCA) needs to be monitored and resolved with at least one minute or greater retention time difference between it and PFOS. If using any other organic modifier, such as methanol, it is necessary to also include taurochenodeoxycholic acid (TCDCA) and tauroursodeoxycholic acid (TUDCA) and achieve one minute or greater retention time difference between PFOS and all three bile acids. In addition to the retention time requirement, the bile acids may also not coelute with any of the PFAS analytes. A method was developed to meet these guidelines using acetonitrile.



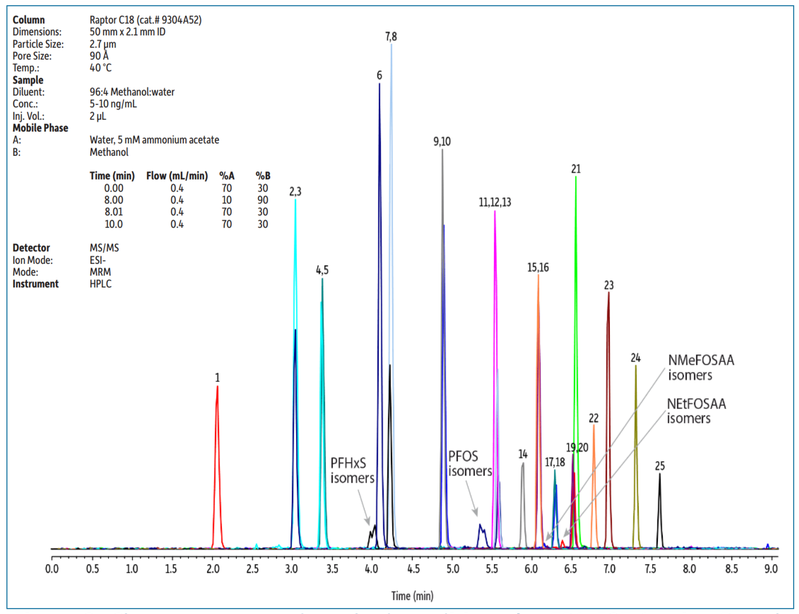
Column	Force C18 (cat.# 9634252)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	1.8 µm
Pore Size:	100 Å
Guard Column:	Force C18 EXP guard column cartridge 5 mm, 2.1 mm ID (cat.# 963450252)
Temp.:	40 °C
Standard/Sample	
Diluent:	Water, 5 mM ammonium acetate
Inj. Vol.:	1 µL
Mobile Phase	
A:	Water, 5 mM ammonium acetate
B:	Acetonitrile

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	80	20
7.00	0.4	55	45
12.00	0.4	0	100
12.01	0.4	80	20
14.00	0.4	80	20

Detector	Shimadzu 8060 MS/MS
Ion Source:	Electrospray
Ion Mode:	ESI-
Mode:	MRM
Instrument	Shimadzu Nexera X2
Sample Preparation	Samples were aliquoted into a 2.0 mL, 9 mm screw-thread polypropylene vials (cat.# 23242) and capped with 9 mm solid-top polyethylene caps (cat.# 23244).
Notes	A PFAS delay column (cat.# 27854) was installed before the injector.

Figure 1: Chromatogram and method conditions for EPA 1633 using acetonitrile to resolve the matrix interference TDCA (peak 16) and PFOS (peak 26) with >1 minute retention using a Force C18 column.

EPA 537.1 Drinking Water



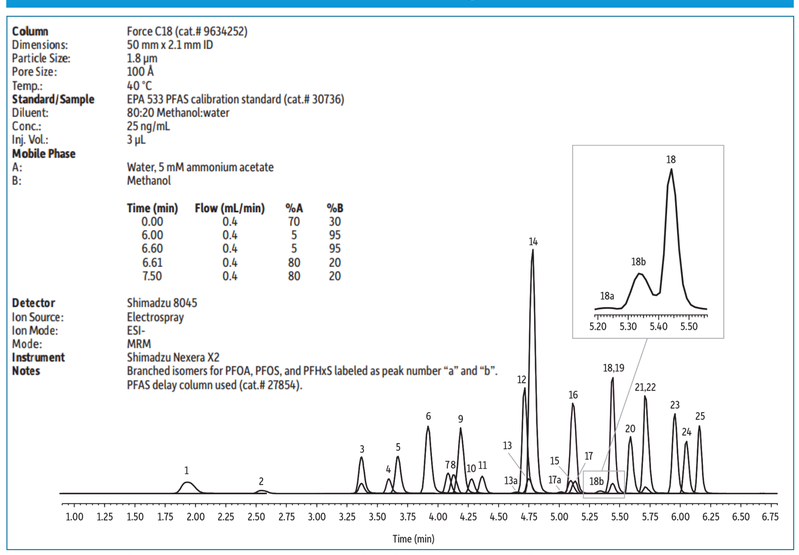
Column	Raptor C18 (cat.# 9304A52)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	2.7 µm
Pore Size:	90 Å
Temp.:	40 °C
Sample	
Diluent:	96:4 Methanol:water
Conc.:	5-10 ng/mL
Inj. Vol.:	2 µL
Mobile Phase	
A:	Water, 5 mM ammonium acetate
B:	Methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	70	30
8.00	0.4	10	90
8.01	0.4	70	30
10.0	0.4	70	30

Detector	MS/MS
Ion Mode:	ESI-
Mode:	MRM
Instrument	HPLC

Figure 2: Chromatogram and method conditions for EPA 537.1 using Restek standard Cat# 30735 on a Raptor C18 column.

EPA 533 Drinking Water



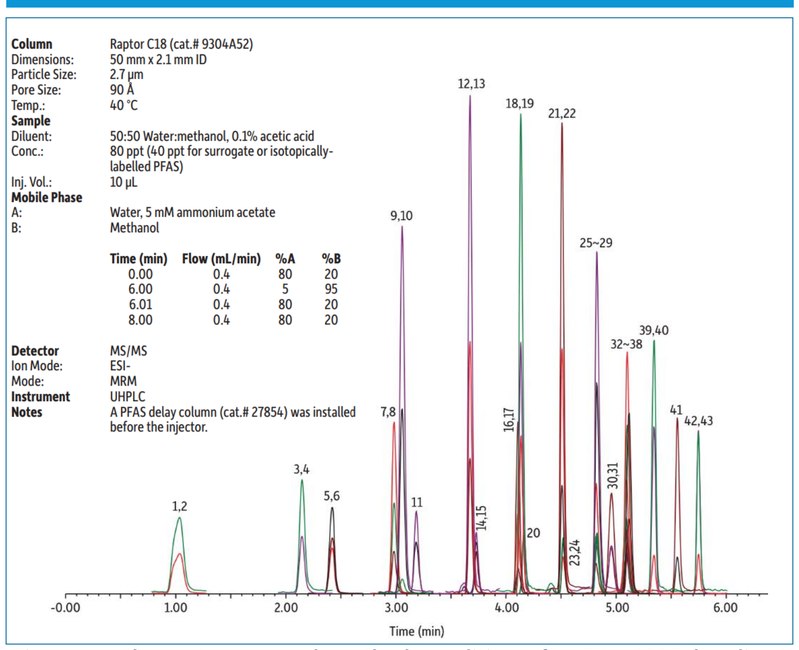
Column	Force C18 (cat.# 9634252)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	1.8 µm
Pore Size:	100 Å
Temp.:	40 °C
Standard/Sample	EPA 533 PFAS calibration standard (cat.# 30736)
Diluent:	80:20 Methanol:water
Conc.:	25 ng/mL
Inj. Vol.:	3 µL
Mobile Phase	
A:	Water, 5 mM ammonium acetate
B:	Methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	70	30
6.00	0.4	5	95
6.60	0.4	5	95
6.61	0.4	80	20
7.50	0.4	80	20

Detector	Shimadzu 8045
Ion Source:	Electrospray
Ion Mode:	ESI-
Mode:	MRM
Instrument	Shimadzu Nexera X2
Notes	Branched isomers for PFOA, PFOS, and PFHxS labeled as peak number "a" and "b". PFAS delay column used (cat.# 27854).

Figure 3: Chromatogram and method conditions for EPA 533 using Restek standard Cat#30736 on a Force C18 column.

EPA 8327 Surface Water, Groundwater, & Wastewater



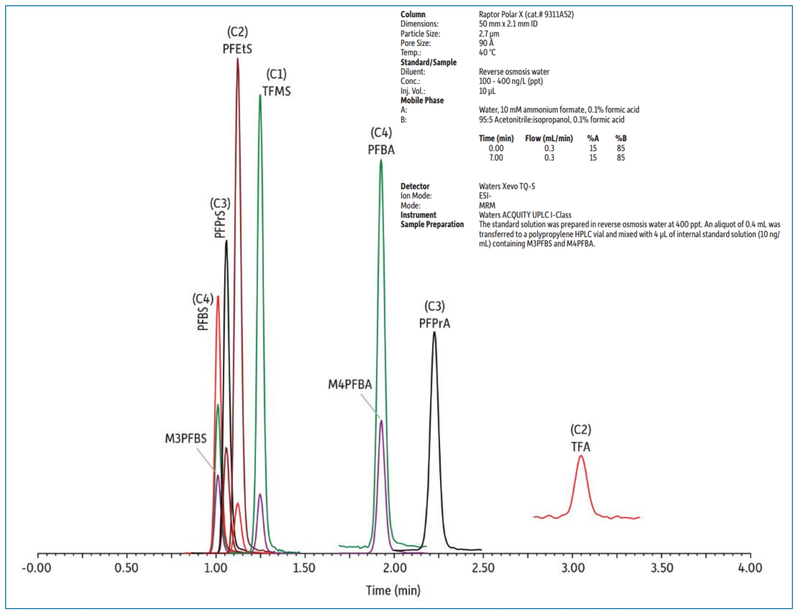
Column	Raptor C18 (cat.# 9304A52)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	2.7 µm
Pore Size:	90 Å
Temp.:	40 °C
Sample	
Diluent:	50:50 Water:methanol, 0.1% acetic acid
Conc.:	80 ppt (40 ppt for surrogate or isotopically-labelled PFAS)
Inj. Vol.:	10 µL
Mobile Phase	
A:	Water, 5 mM ammonium acetate
B:	Methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	80	20
6.00	0.4	5	95
6.01	0.4	80	20
8.00	0.4	80	20

Detector	MS/MS
Ion Mode:	ESI-
Mode:	MRM
Instrument	UHPLC
Notes	A PFAS delay column (cat.# 27854) was installed before the injector.

Figure 4: Chromatogram and method conditions for EPA 8327 by direct injection. Restek standard Cat# 30733 for 24 analytes and Cat#30734 for 28 analytes can be used and analyzed on a Raptor C18 column.

Ultrashort Through Short-chain (C1-C4)



Column	Raptor Polar X (cat.# 9311A52)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	2.7 µm
Pore Size:	90 Å
Temp.:	40 °C
Standard/Sample	
Diluent:	Reverse osmosis water
Conc.:	100 - 400 ng/L (ppt)
Inj. Vol.:	10 µL
Mobile Phase	
A:	Water, 10 mM ammonium formate, 0.1% formic acid
B:	95:5 Acetonitrile:isopropanol, 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.3	15	85
7.00	0.3	15	85

Detector	Waters Xevo TQ-S
Ion Mode:	ESI-
Mode:	MRM
Instrument	Waters ACQUITY UPLC I-Class
Sample Preparation	The standard solution was prepared in reverse osmosis water at 400 ppt. An aliquot of 0.4 mL was transferred to a polypropylene HPLC vial and mixed with 4 µL of internal standard solution (10 ng/mL) containing M3PFBS and M4PFBA.

Ultrashort Through Long-chain (C2-C8)

Column	Raptor Polar X (cat.# 9311A52)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	2.7 µm
Temp.:	40 °C
Sample	
Diluent:	50:50 Water:methanol
Conc.:	400 ng/L
Inj. Vol.:	10 µL
Mobile Phase	
A:	Water, 10 mM ammonium formate, 0.05% formic acid
B:	60:40 Acetonitrile:methanol, 0.05% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.5	15	85
8.00	0.5	15	85

Detector	MS/MS
Ion Mode:	ESI-
Mode:	MRM
Instrument	UHPLC

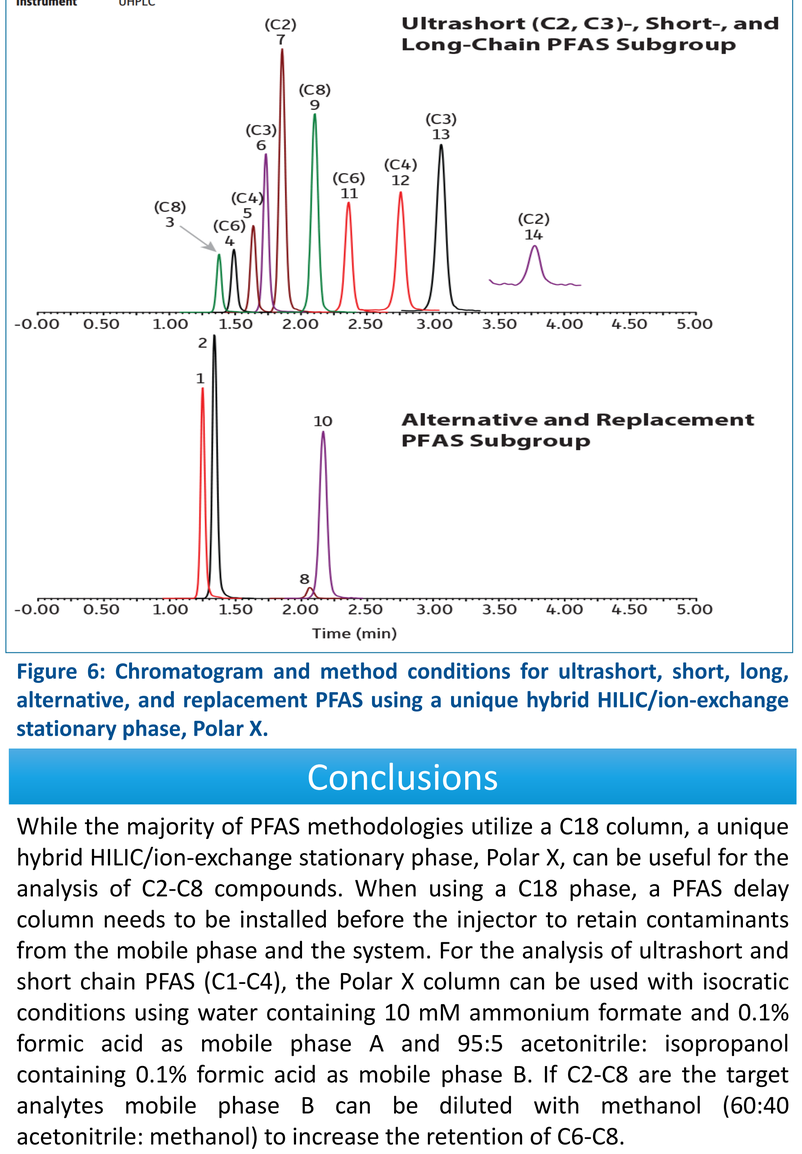


Figure 6: Chromatogram and method conditions for ultrashort, short, long, alternative, and replacement PFAS using a unique hybrid HILIC/ion-exchange stationary phase, Polar X.

Conclusions

While the majority of PFAS methodologies utilize a C18 column, a unique hybrid HILIC/ion-exchange stationary phase, Polar X, can be useful for the analysis of C2-C8 compounds. When using a C18 phase, a PFAS delay column needs to be installed before the injector to retain contaminants from the mobile phase and the system. For the analysis of ultrashort and short chain PFAS (C1-C4), the Polar X column can be used with isocratic conditions using water containing 10 mM ammonium formate and 0.1% formic acid as mobile phase A and 95:5 acetonitrile: isopropanol containing 0.1% formic acid as mobile phase B. If C2-C8 are the target analytes mobile phase B can be diluted with methanol (60:40 acetonitrile: methanol) to increase the retention of C6-C8.