To Isolation and Beyond: A New Mixed Mode Column Approach for PFAS Chromatography

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Waters Corporation
NEMC 2023

What are some of the trends in PFAS Analysis?

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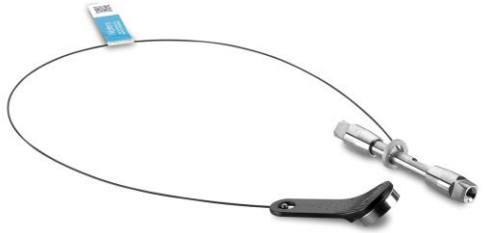
1. Sensitivity
   - How low can you (reliably) go? → **MS/MS Quan**
   - HALs, MCLs, daily intakes…
2. Short Chains and Ultra-Short Chains
   - How low can you (reliably) go? → **Chromatography**
3. Contamination
   - How low can you (reliably) go? → **Laboratory and Instrument**
   - Sample preparation
   - “PFAS free” Instruments
4. Total Fluorine/Total PFAS

OASIS®
SAMPLE EXTRACTION PRODUCTS

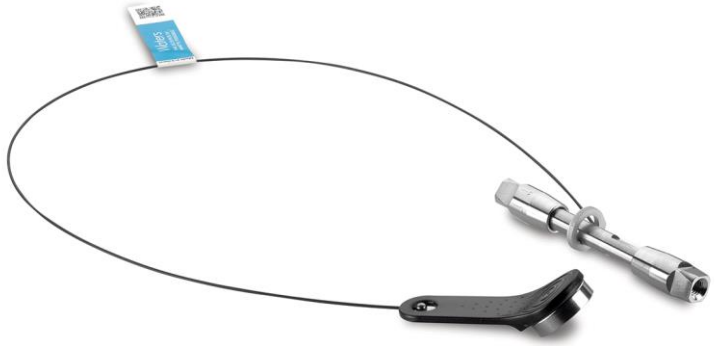
Weak Anion Exchange for Sample Preparation – contains reverse phase and ion exchange



So why not use both mechanisms for chromatography?

Atlantis™ Premier C18 AX Columns

Addressing System Contamination Using Mixed Mode Column Chemistry



Atlantis Premier C18 AX Columns

Starting with C18 AX as an isolator…

- Why?
  - The current isolator column is C18 phase:
    - Suitable for long chain PFAS like PFOA, PFOS, etc
    - Short chain compounds, such as PFBA, aren't retained enough
    - Can we lower the back pressure using the 5.0 µm C18 AX column?
- How?
  - Purposely contaminated mobile phase with PFAS standards to be able to ensure that we can see the contamination front and how it changes with column.
  - Mobile Phase Composition used standard PFAS solvents
    - A = 2 mM ammonium acetate in water, B = 2 mM ammonium acetate in methanol

C18 isolator – 0.025 ng/mL PFAS spiked in mobile phase

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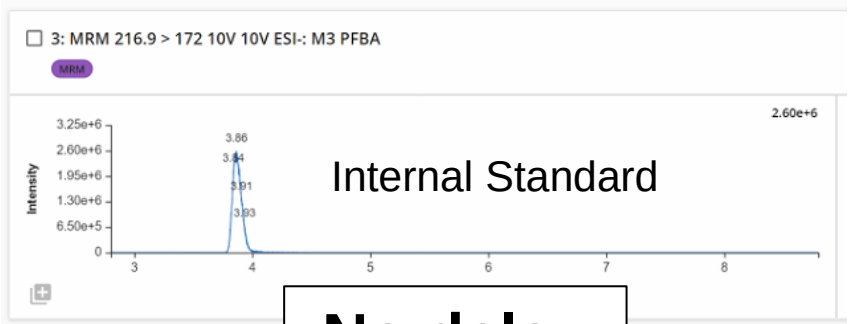
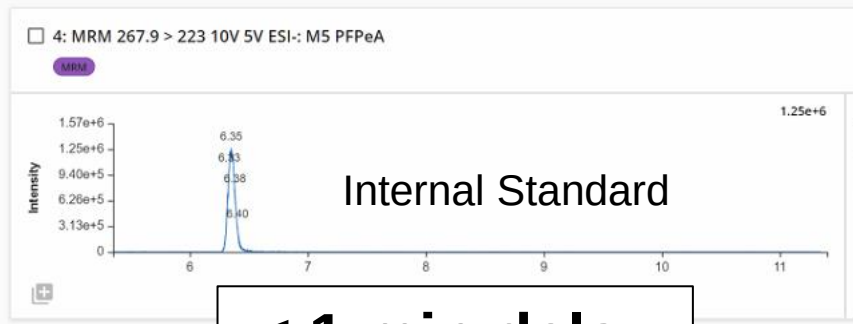
PFBA (C4 carboxylate)

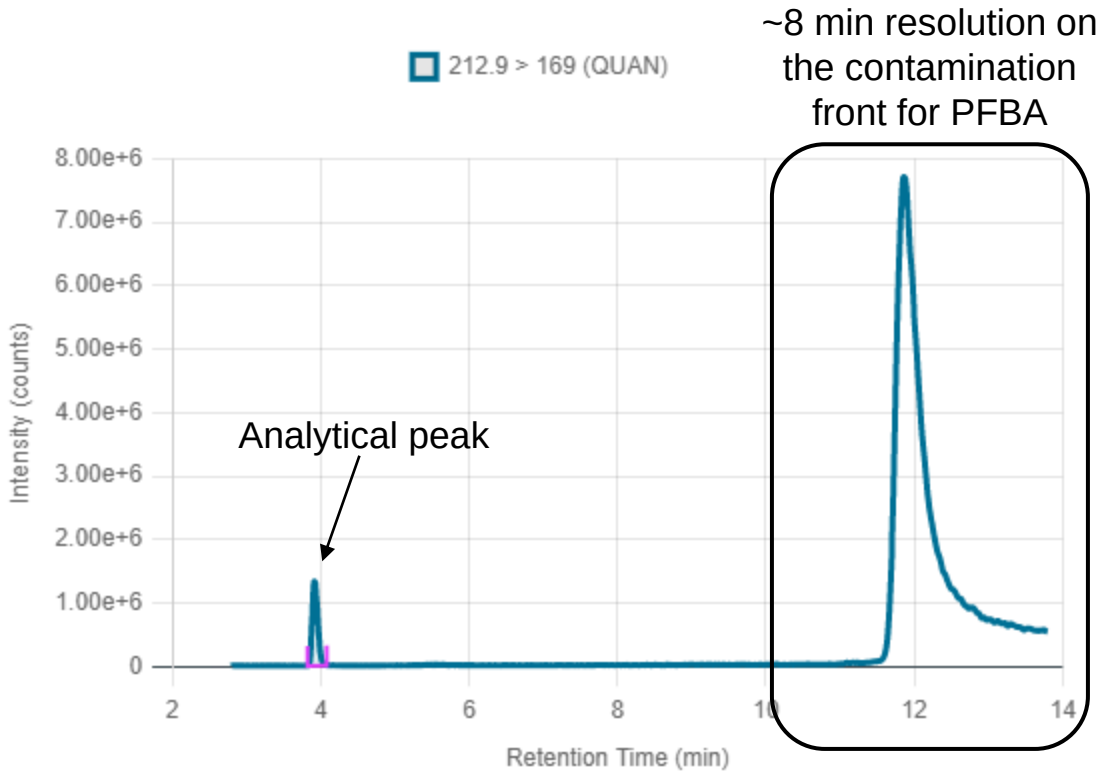


PFPeA – (C5 carboxylate)



PFPeA (C5)

C18 AX isolator - 0.025 ng/mL PFAS in mobile phase

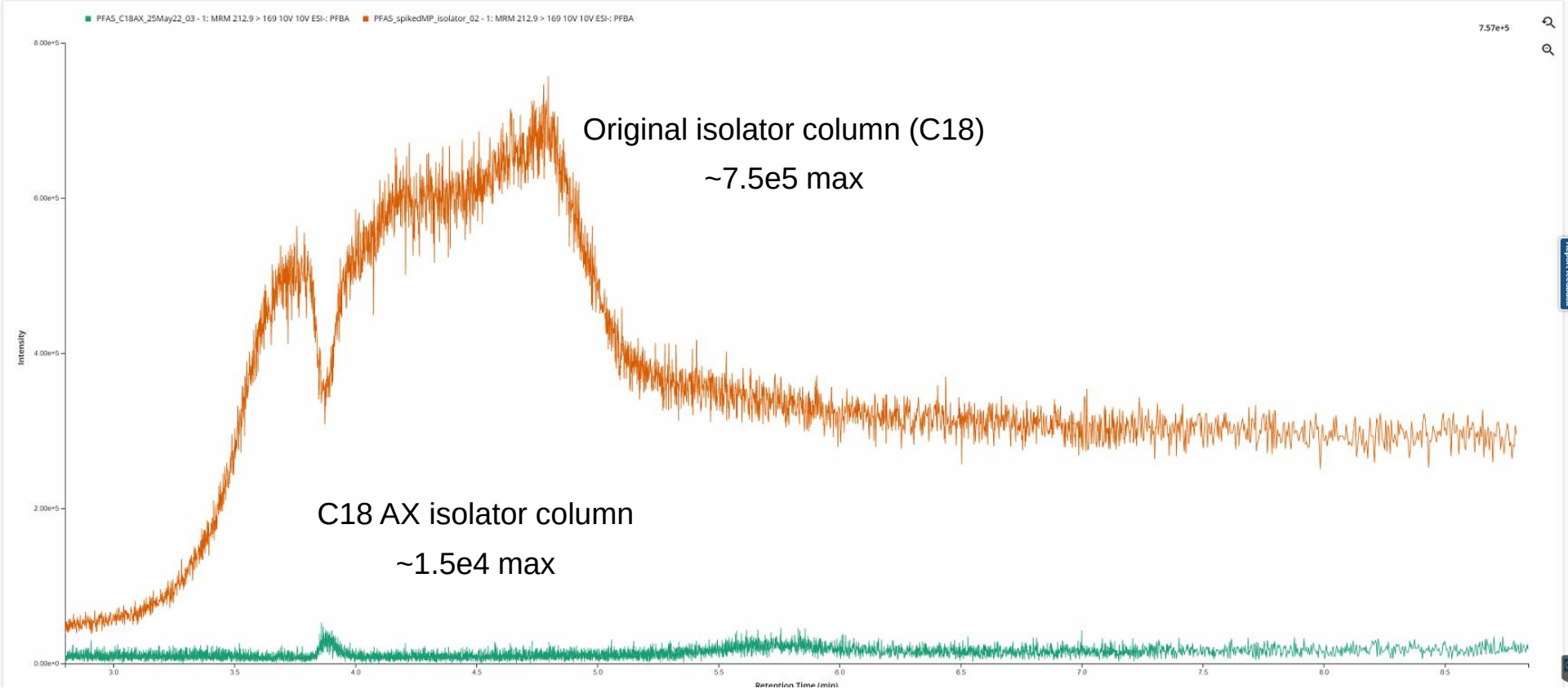


Backpressure Reduced:

~9500 for C18 isolator

~8500 for C18 AX isolator

Comparison of columns with spiked mobile phase



PFBA Comparison

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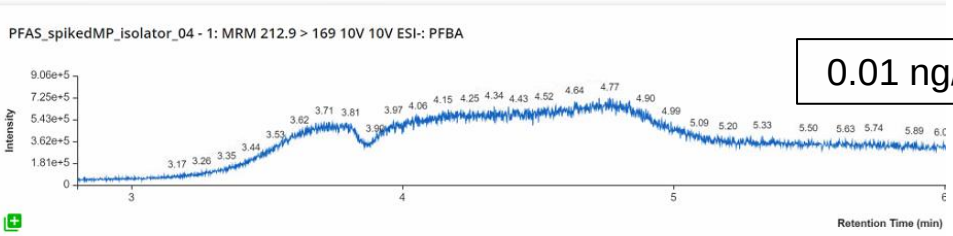
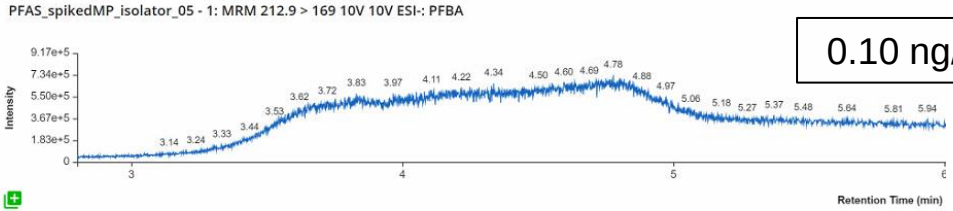
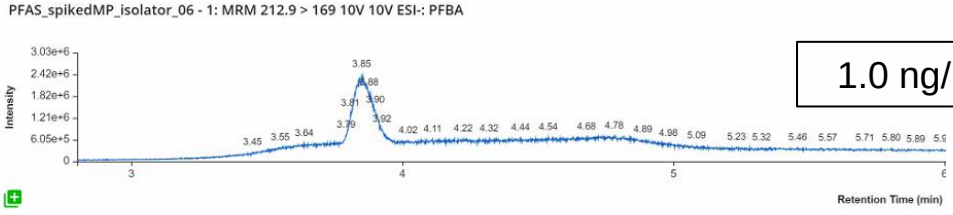
C18 Isolator Column



0.01 ng/mL

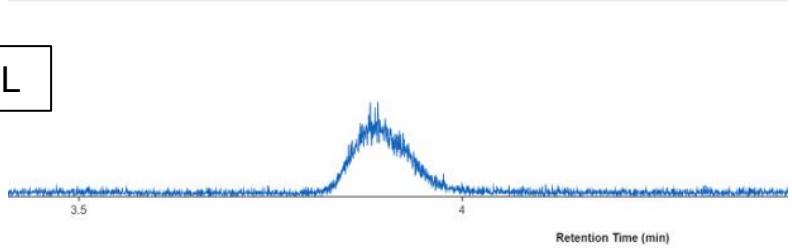


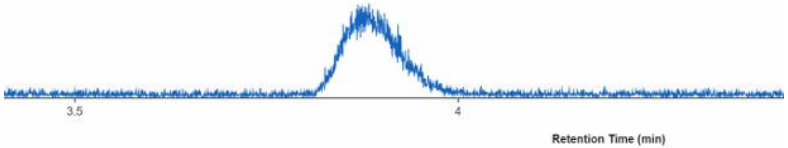
0.10 ng/mL

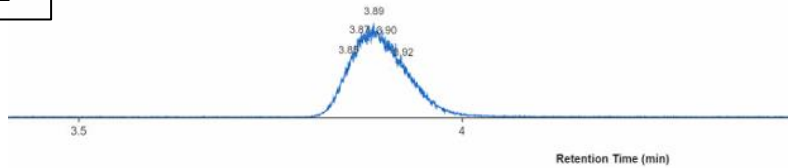


1.0 ng/mL

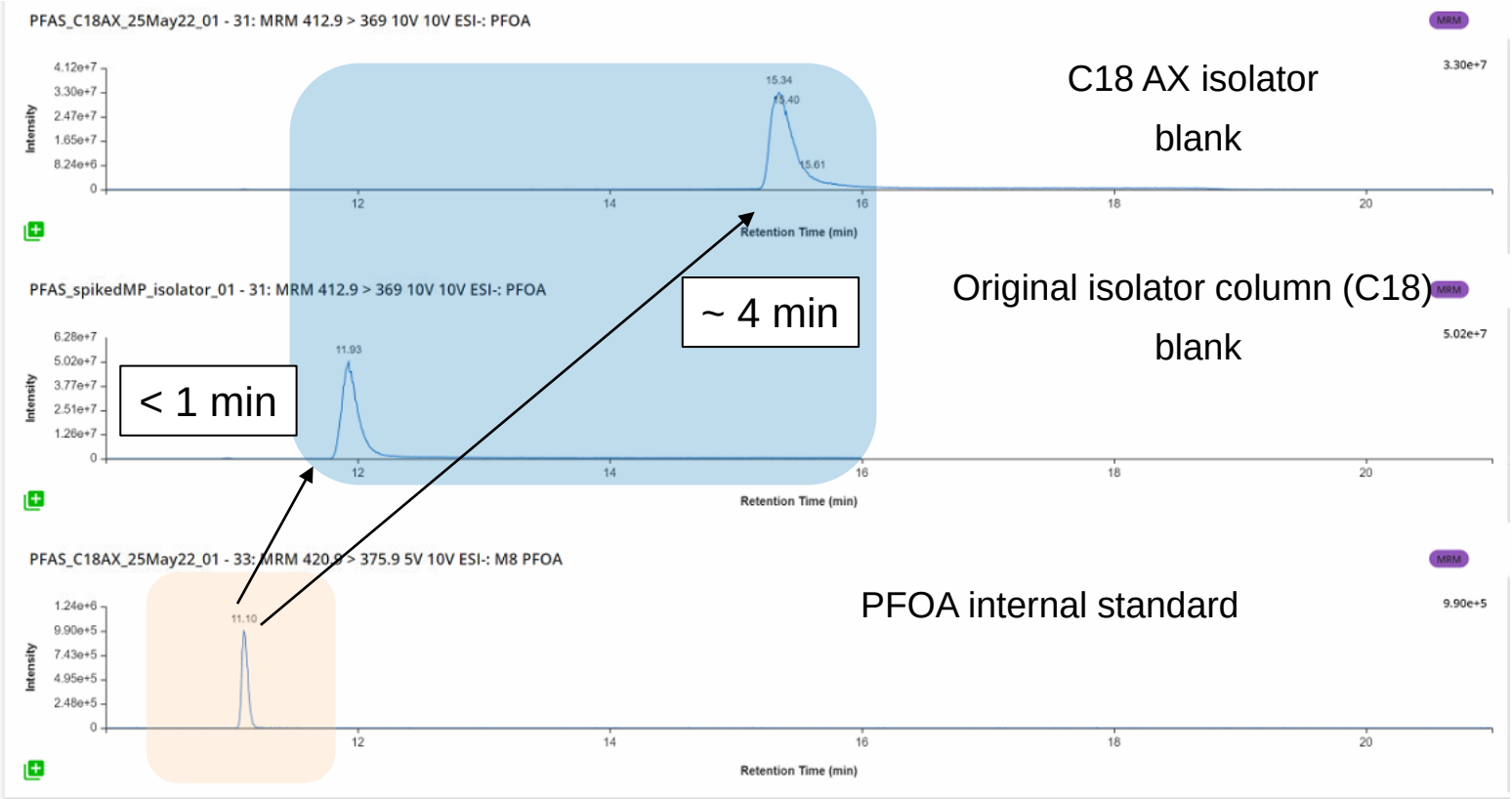
C18 AX Isolator Column







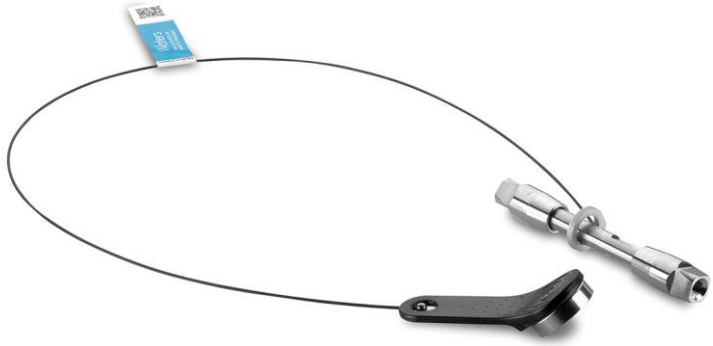
What about longer chain PFAS?



Delayed system contamination

Analytical peak retention time

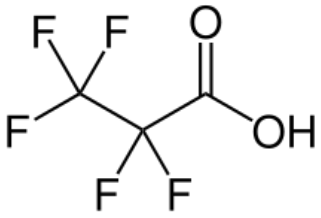
Improving Chromatography Using Mixed Mode Column Chemistry



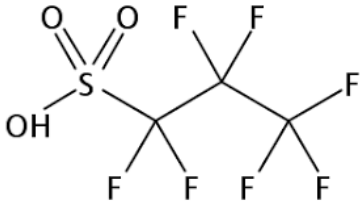
Atlantis Premier C18 AX Columns

Ultra Short Chains on Reverse Phase

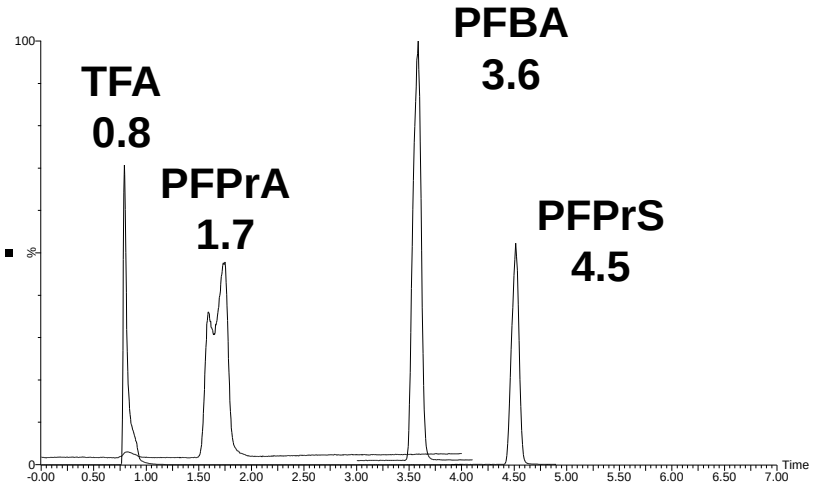
Time (min)	Flow (ml/min)	% A	% B	Curve
0	0.3	95	5	6
1	0.3	75	25	6
6	0.3	50	50	6
13	0.3	15	85	6
14	0.3	5	95	6
17	0.3	5	95	6
18	0.3	95	5	6
22	0.3	95	5	6



PFPrA (C3)



PFPrS (C3)



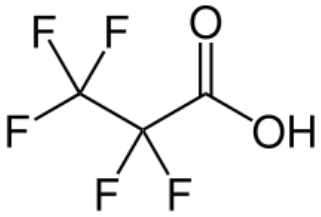
ACQUITY™ BEH™ **C18** 2.1 x 100 mm Column

Mobile Phase A: 2 mM ammonium acetate in water

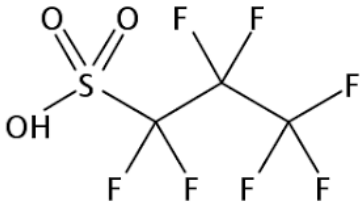
Mobile Phase B: 2 mM ammonium acetate in methanol

Ultra Short Chains on Mixed Mode

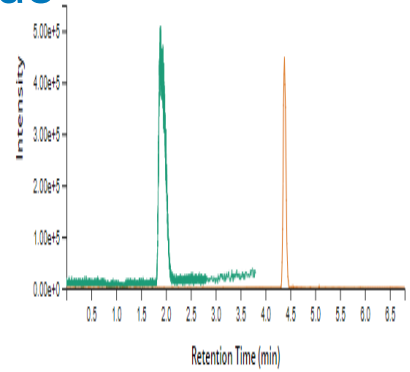
Time (min)	Flow (ml/min)	% A	% B	Curve
0	0.3	95	5	6
1	0.3	75	25	6
6	0.3	50	50	6
13	0.3	15	85	6
14	0.3	5	95	6
17	0.3	5	95	6
18	0.3	95	5	6
22	0.3	95	5	6



PFPrA (C3)

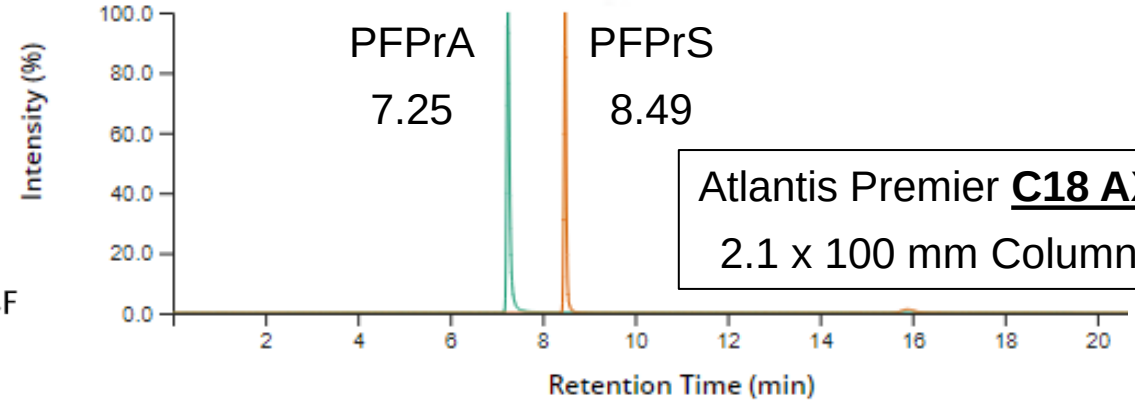


PFPrS (C3)



ACQUITY BEH **C18**

2.1 x 100 mm Column



Atlantis Premier **C18 AX**

2.1 x 100 mm Column

Mobile Phase A: 2 mM ammonium acetate in water

Mobile Phase B: 2 mM ammonium acetate in methanol

Ultra Short Chains on C18 AX

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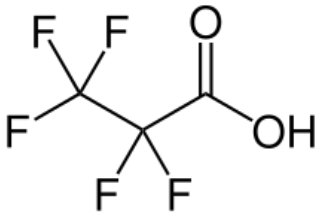
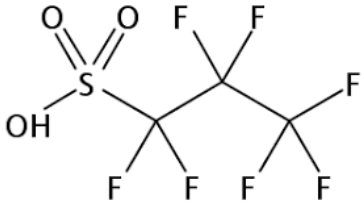
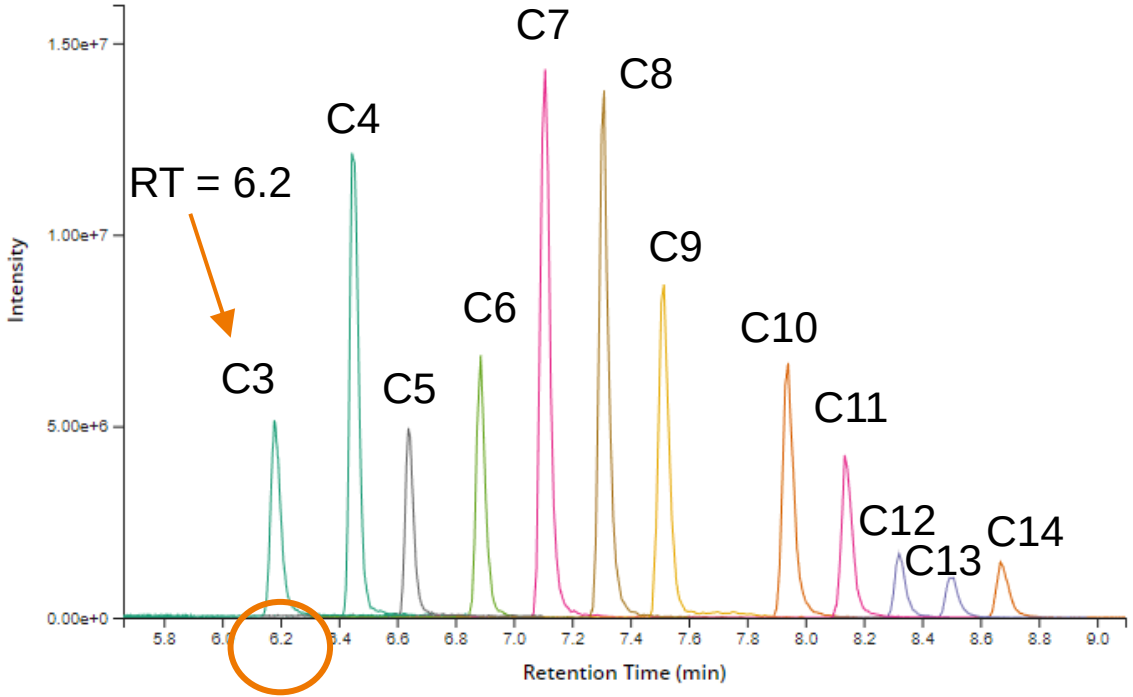
Time (min)	Flow (ml/min)	% A	% B	Curve
0	0.3	80	20	6
1	0.3	80	20	6
4	0.3	50	50	6
9	0.3	1	99	3
14	0.3	1	99	6
14.01	0.3	80	20	6
18	0.3	80	20	6



PFPrA (C3)



PFPrS (C3)

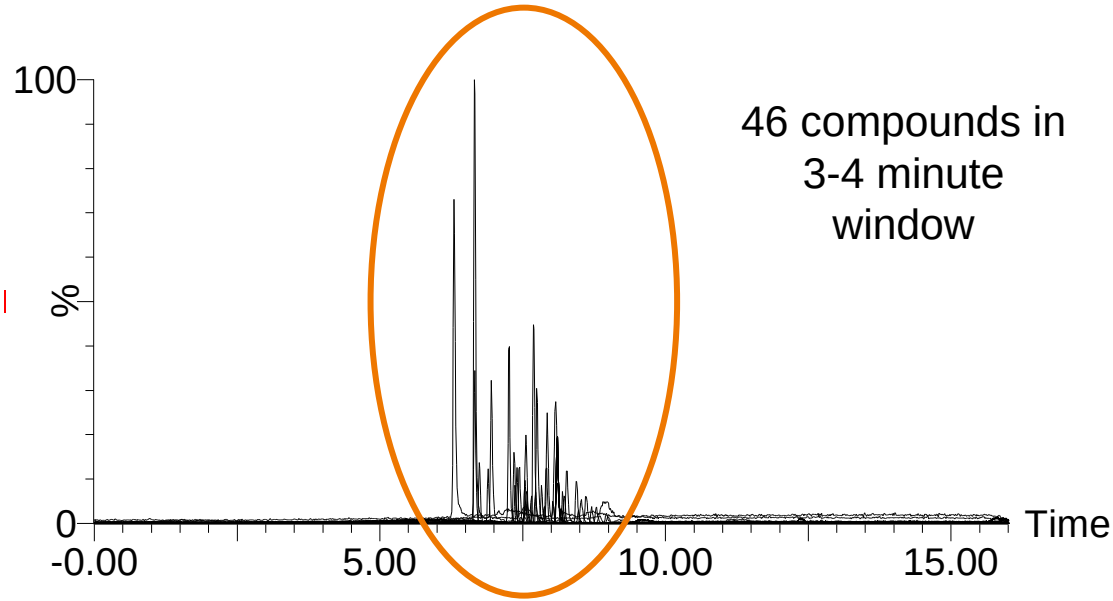


Mobile Phase A: 2 mM ammonium acetate in water

Mobile Phase B: 2 mM ammonium acetate in methanol

The Full Picture

Time (min)	Flow (ml/min)	% A	% B	Curve
0	0.3	95	5	6
1	0.3	75	25	6
6	0.3	50	50	6
13	0.3	15	85	6
14	0.3	1	99	6
17	0.3	1	99	6
18	0.3	95	5	6
22	0.3	95	5	6



Atlantis Premier C18 AX
2.1 x 100 mm Column

Mobile Phase A: 2 mM **ammonium acetate** in water

Mobile Phase B: 2 mM **ammonium acetate** in methanol

Learning from SPE Sample Preparation

Waters™

Prep Samples (250 mL)

pH adjust to < 3 – **activates ion exchange sites in WAX sorbent when loaded**

Condition Cartridge

Load Sample

Rinse Cartridge

Elute

Eluent with pH > 8 – **“turns off” ion exchange sites in WAX sorbent to release PFAS**

Dry and dilute

Oasis WAX Cartridge
6 cc
150 mg

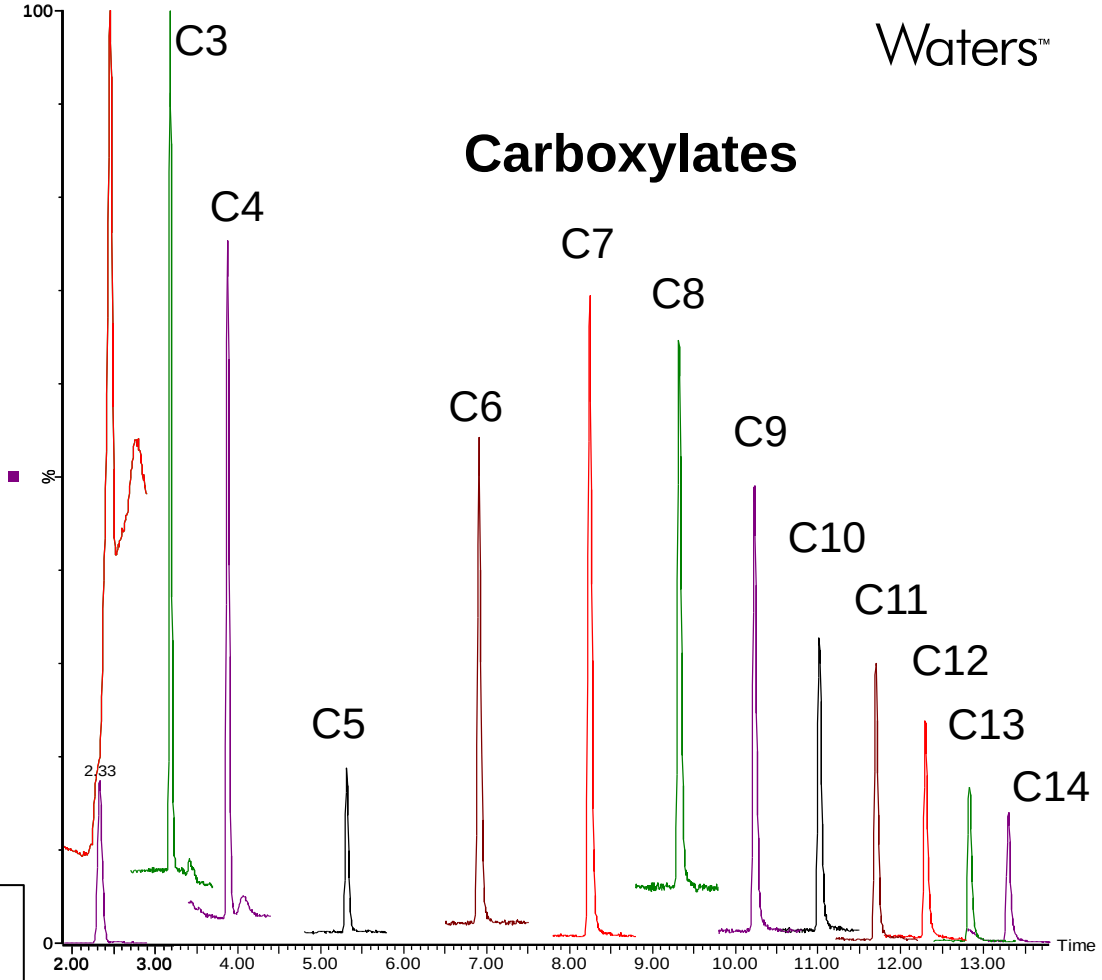
Playing with Mobile Phase pH

Time (min)	Flow (ml/min)	% A	% B	Curve
0	0.3	99	1	6
2	0.3	99	1	6
3	0.3	75	25	6
8	0.3	50	50	6
15	0.3	15	85	6
16	0.3	0	100	6
20	0.3	0	100	6
20.1	0.3	99	1	6
24	0.3	99	1	6

Mobile Phase A: 2 mM ammonium acetate in water

Mobile Phase B: **0.1% ammonium hydroxide** in methanol

Atlantis Premier C18 AX 2.1 x 100 mm Column



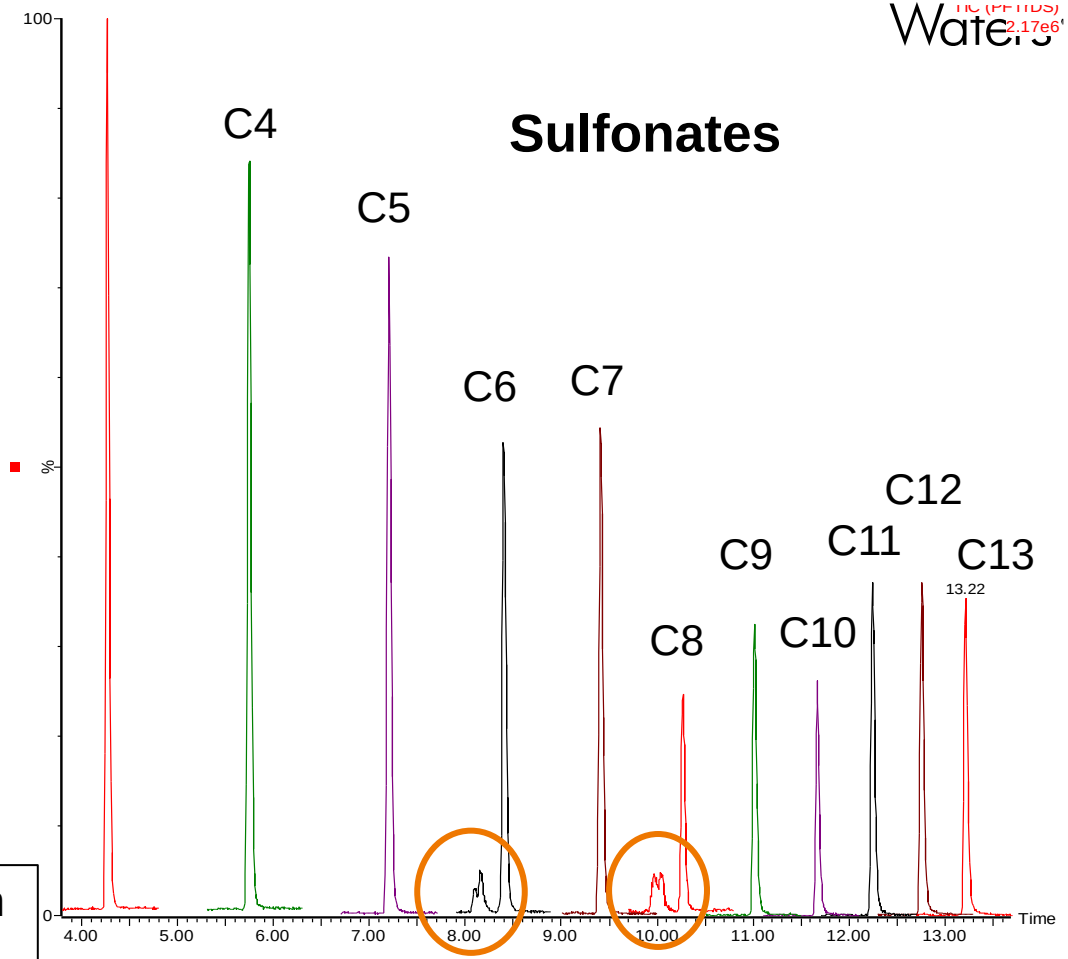
Playing with Mobile Phase pH

Time (min)	Flow (ml/min)	% A	% B	Curve
0	0.3	99	1	6
2	0.3	99	1	6
3	0.3	75	25	6
8	0.3	50	50	6
15	0.3	15	85	6
16	0.3	0	100	6
20	0.3	0	100	6
20.1	0.3	99	1	6
24	0.3	99	1	6

Mobile Phase A: 2 mM ammonium acetate in water

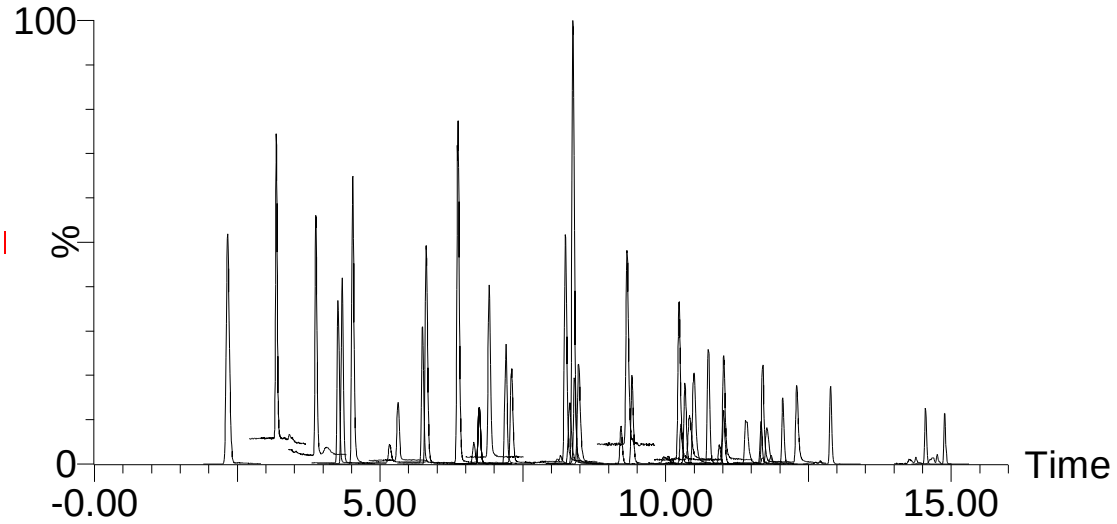
Mobile Phase B: **0.1% ammonium hydroxide** in methanol

Atlantis Premier C18 AX 2.1 x 100 mm Column



The Full Picture (Again)

Time (min)	Flow (ml/min)	% A	% B	Curve
0	0.3	99	1	6
2	0.3	99	1	6
3	0.3	75	25	6
8	0.3	50	50	6
15	0.3	15	85	6
16	0.3	0	100	6
20	0.3	0	100	6
20.1	0.3	99	1	6
24	0.3	99	1	6



Mobile Phase A: 2 mM ammonium acetate in water

Mobile Phase B: **0.1% ammonium hydroxide** in methanol

Atlantis Premier C18 AX 2.1 x 100 mm Column

46 native PFAS covering both ASTM 8421 and EPA 1633 Lists

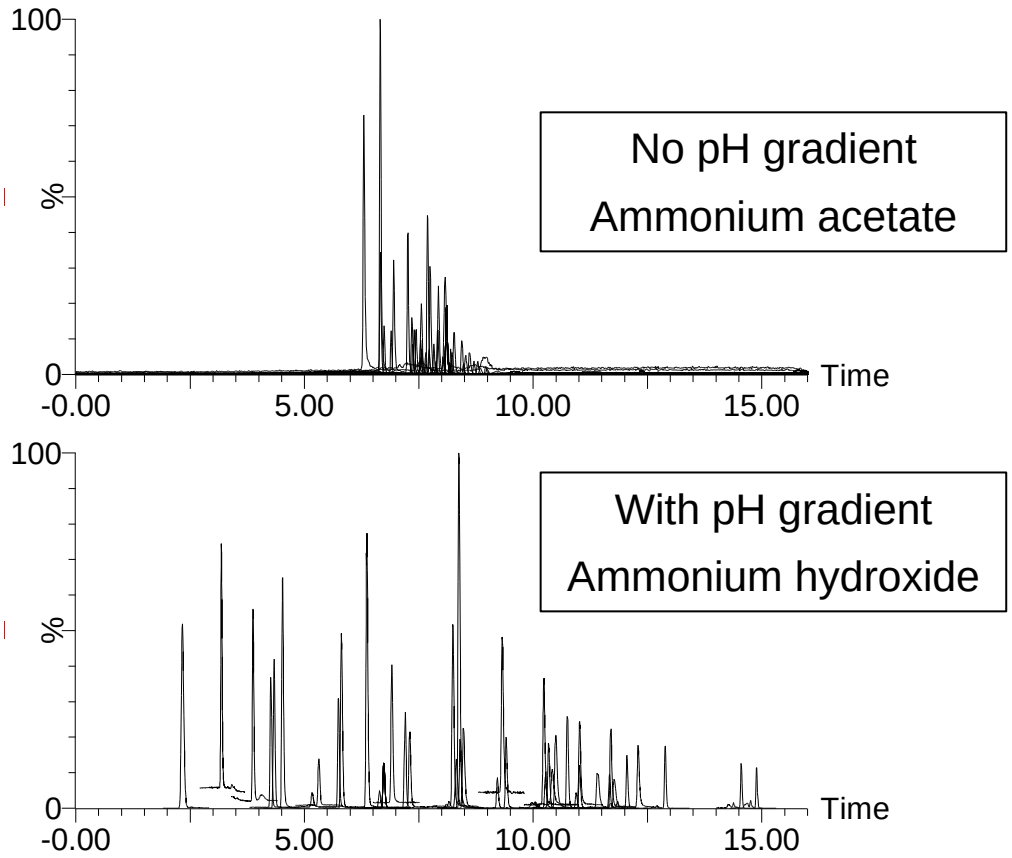
The Full Picture (Again)

Time (min)	Flow (ml/min)	% A	% B	Curve
0	0.3	99	1	6
2	0.3	99	1	6
3	0.3	75	25	6
8	0.3	50	50	6
15	0.3	15	85	6
16	0.3	0	100	6
20	0.3	0	100	6
20.1	0.3	99	1	6
24	0.3	99	1	6

Mobile Phase A: 2 mM ammonium acetate in water

Mobile Phase B: **0.1% ammonium hydroxide** in methanol

Atlantis Premier C18 AX 2.1 x 100 mm Column



Final Instrument Methods

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Source Parameters

- Instrument: Xevo™ TQ Absolute MS
- Ion Mode: ESI-
- Capillary Voltage: 0.5 kV
- Desolvation Temperature: 350° C
- Desolvation Flow: 900 L/hr
- Cone Flow: 150 L/hr

UHPLC Configuration

- Instrument: ACQUITY Premier BSM FTN System with PFAS Kit
- Isolator Column: Atlantis Premier BEH C18 AX Column 2.1x50 mm, 5.0 µm
- Analytical Column: Atlantis Premier BEH C18 AX Column 2.1x100 mm, 1.7 µm

LC Method

- Mobile Phase A: Water + 2 mM ammonium acetate
- Mobile Phase B: Methanol + 0.1% ammonium hydroxide
- Flow Rate: 0.3 mL/min
- Injection Volume: 10 uL
- Gradient:

Time (min)	%A	%B	Curve
0	99	1	initial
2	99	1	6
3	75	25	6
8	50	50	6
15	15	85	6
16	0	100	6
20	0	100	6
20.1	100	0	6
23.5	100	0	6
24	99	1	6

Samples and Sample Preparation

Designation: D8421 – 22

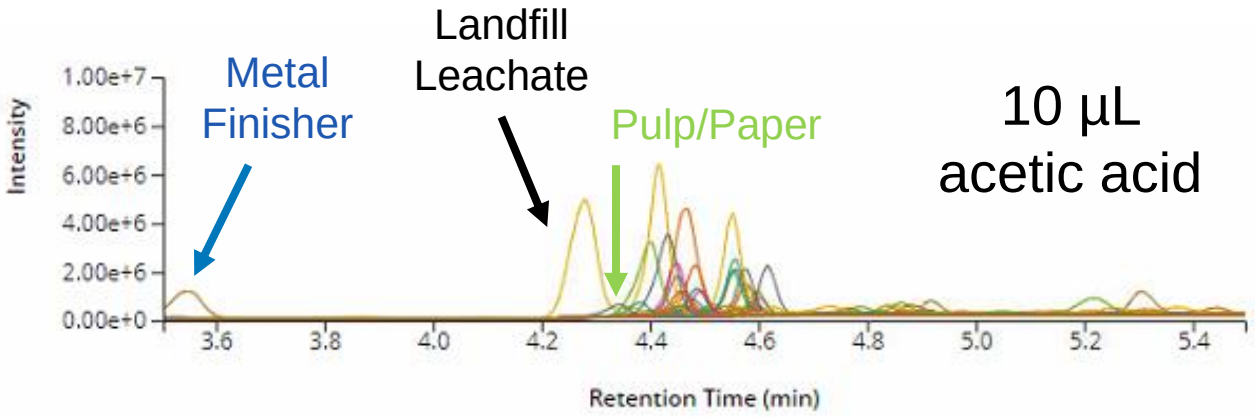
Standard Test Method for
Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Matrices by Co-solvation followed by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)[1]

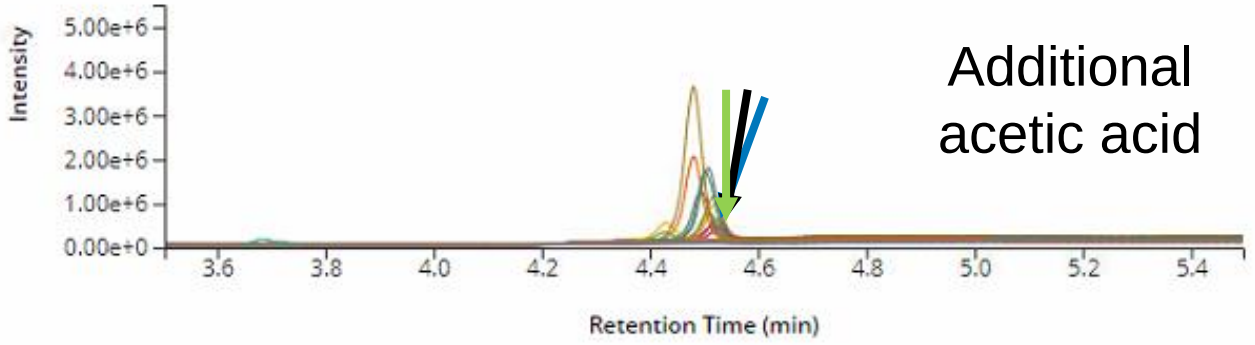
- 5 mL water sample
- Add 5 mL methanol
- Add 10 µL acetic acid
- Syringe filter
- Inject

Sample Types

- Landfill leachate
- Metal finisher
- Wastewater effluent
- Wastewater influent
- Hospital discharge
- Bus washing station
- Powerplan
- Pulp and paper factory
- Ground water
- Surface water

Sample pH Considerations





- Metal Finisher pH 5.5
- Landfill Leachate pH 5.0
- Pulp/paper pH 4.5
- All other samples pH 4

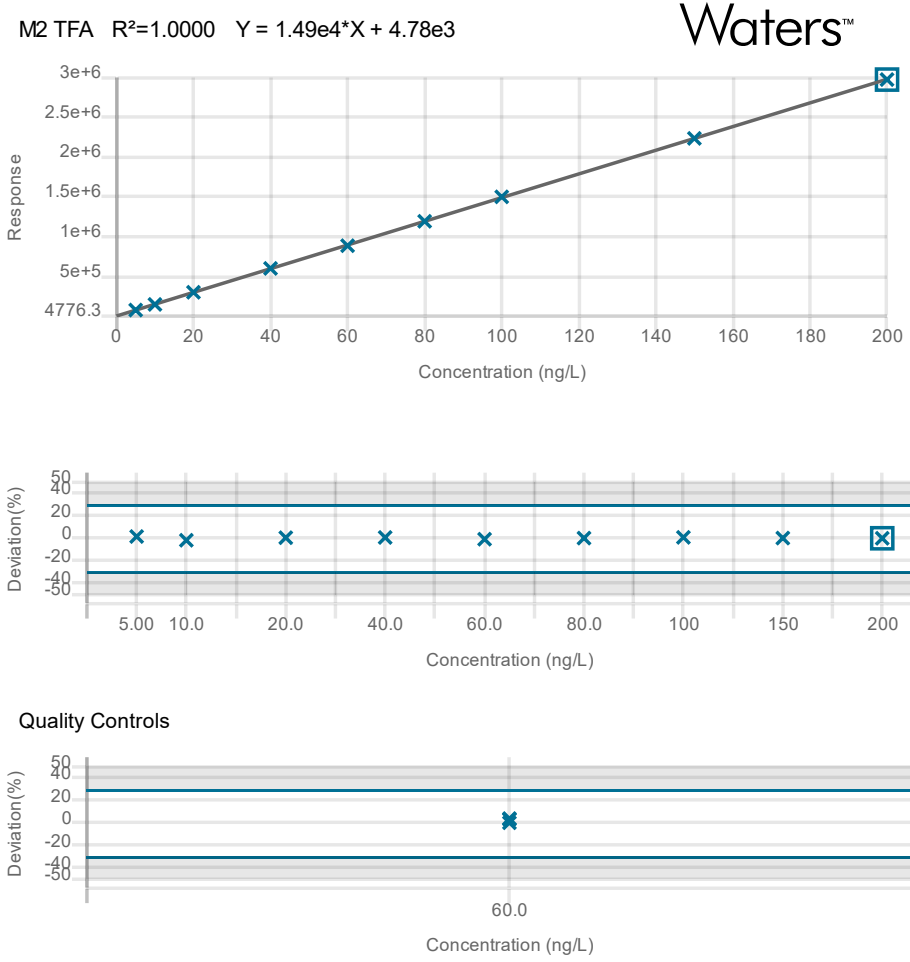
ASTM 8421 Data Quality Guidelines

Waters™

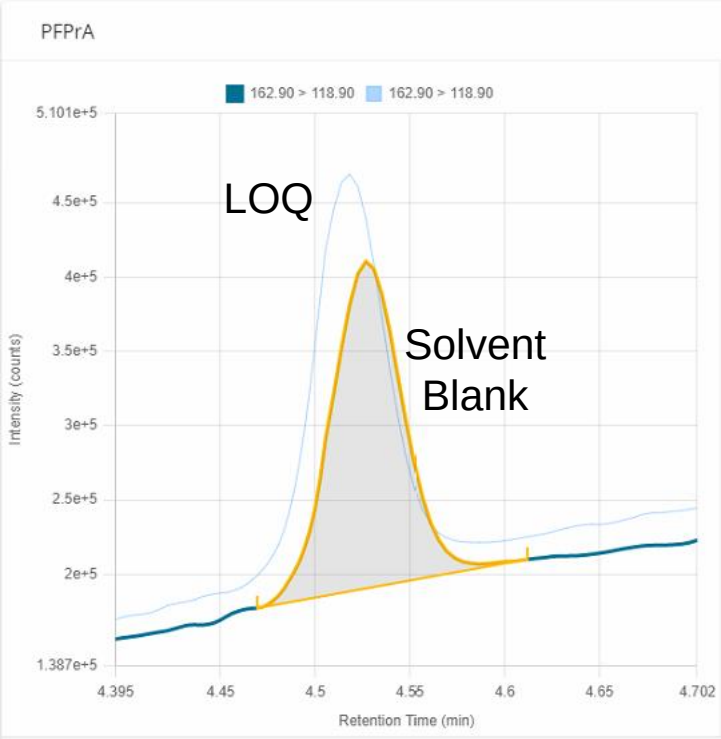
Calibration Curve	Minimum 5-point linear curve	✓
	Deviation ± 30%	✓
QC	Deviation ± 30%	✓
Blank Response	< 50% of LLOQ	✓ TFA PFPrA
Internal Standards	± 30% of median batch response	✓
Retention Times	5% of expected	✓
Ion Ratios	± 30% of reference peaks	✓

ASTM 8421 Data Quality Guidelines

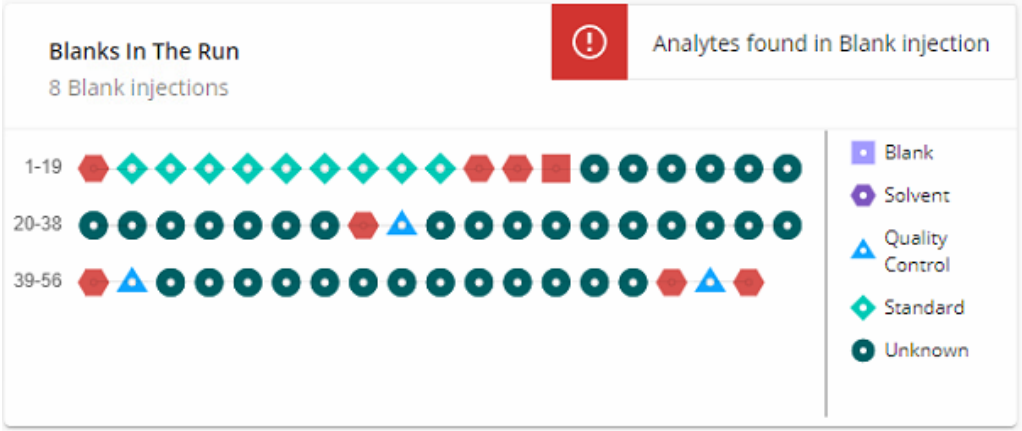
Calibration Curve	Minimum 5-point linear curve	✓
	Deviation ± 30%	✓
QC	Deviation ± 30%	✓
Internal Standards	± 30% of median batch response	✓



ASTM 8421 Data Quality Guidelines



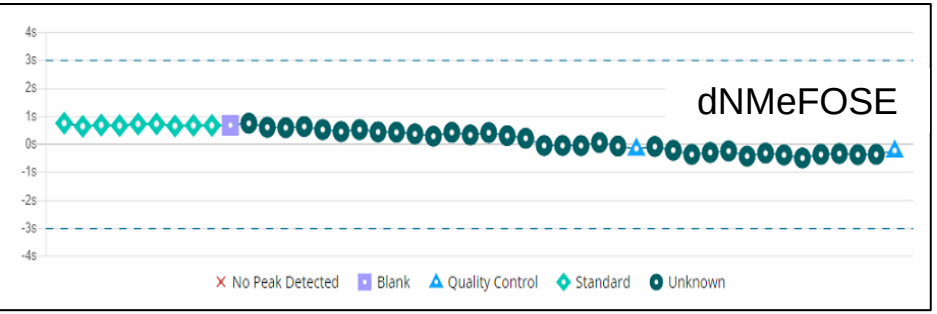
Blank Response	< 50% of LLOQ	TFA PFPrA

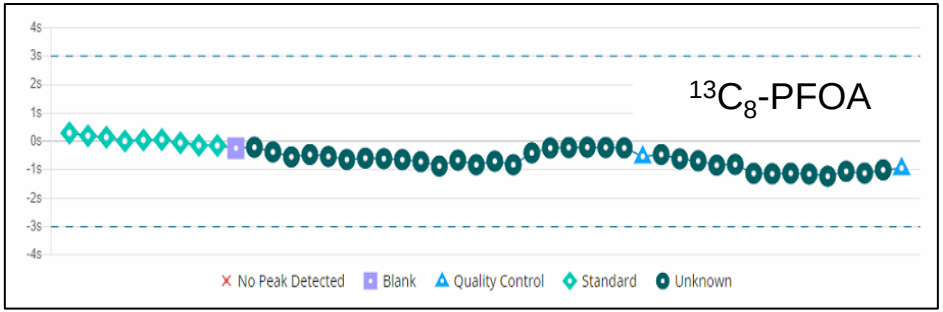


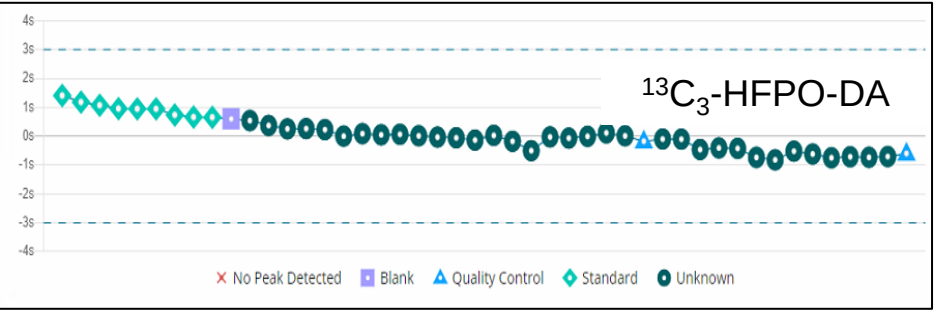
ASTM 8421 Data Quality Guidelines

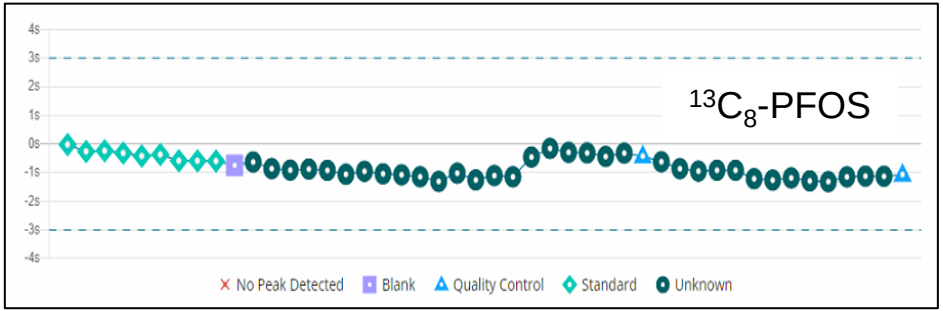
Waters™

Retention Times	5% of expected	✓

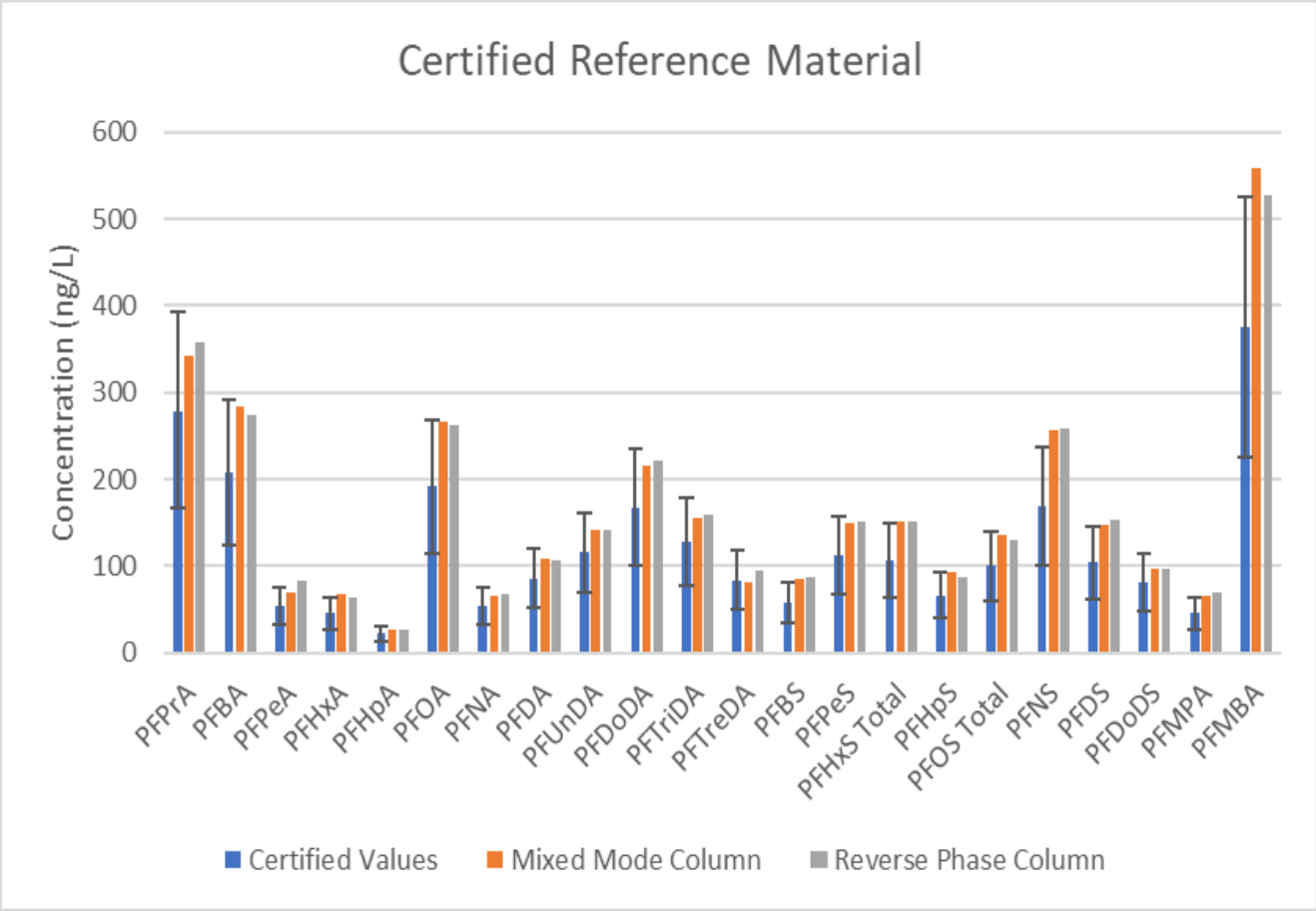




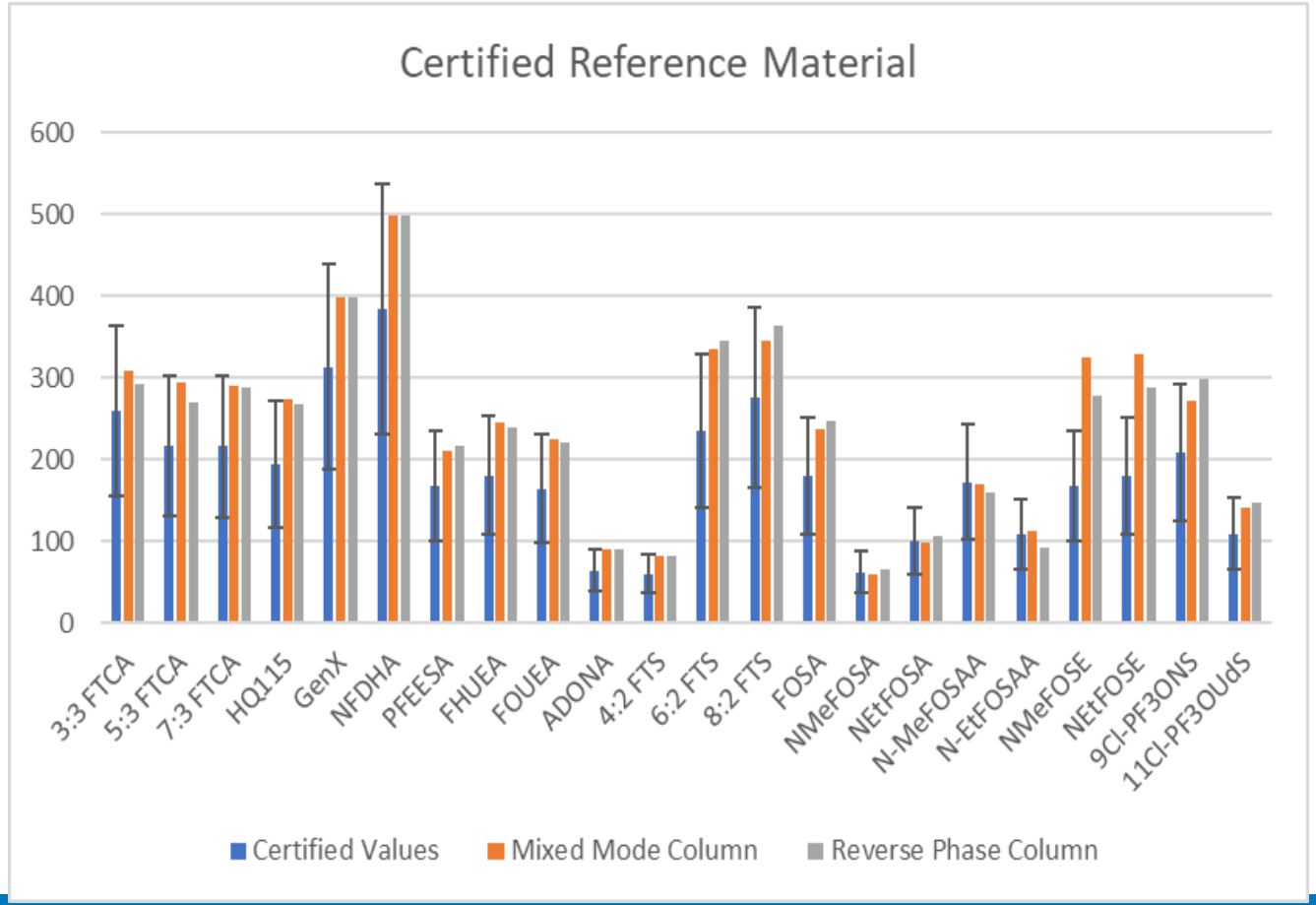




ERA Certified Reference Material - Wastewater

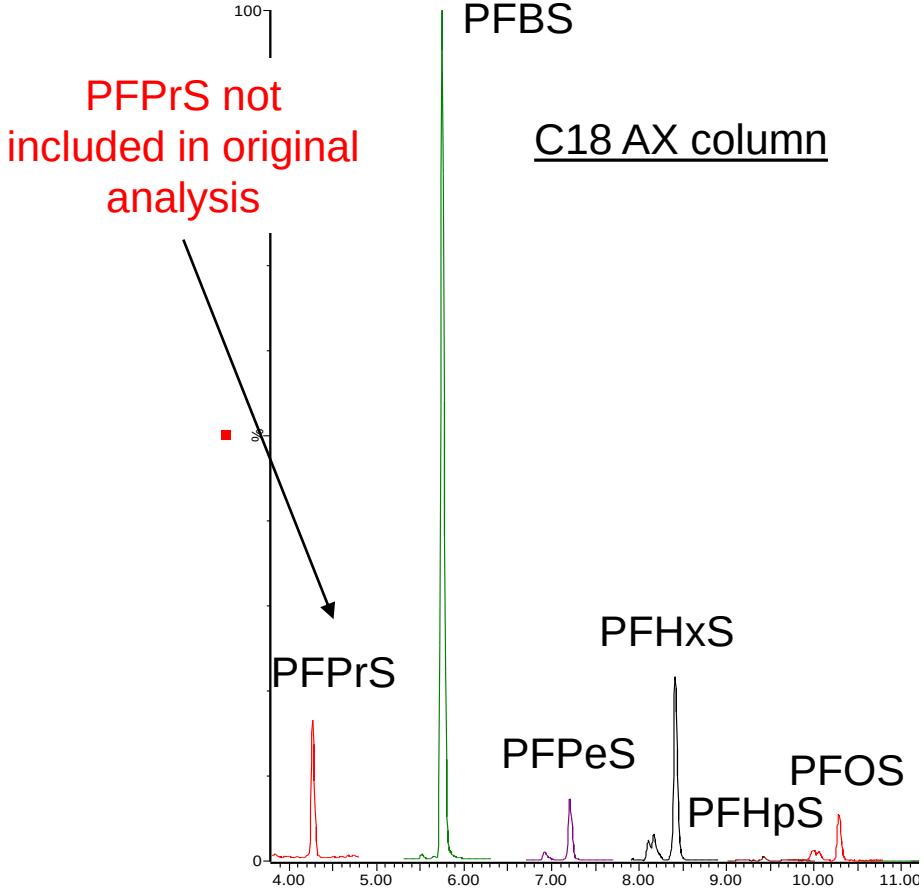


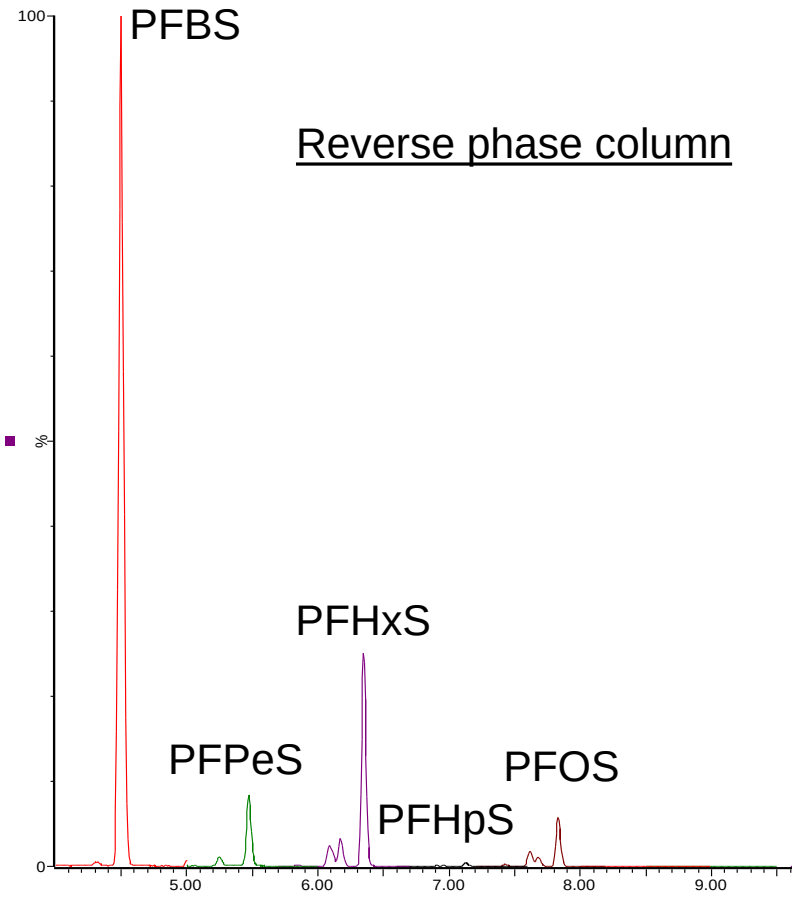
ERA Certified Reference Material - Wastewater



Landfill Leachate Sample

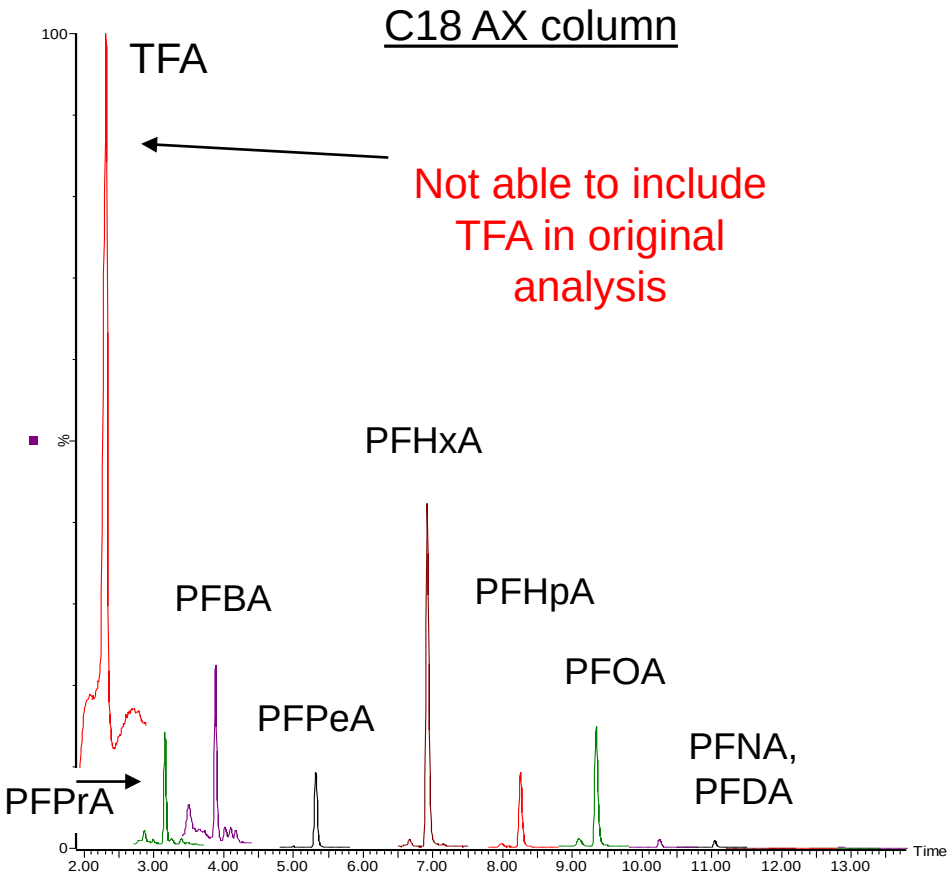
Waters™

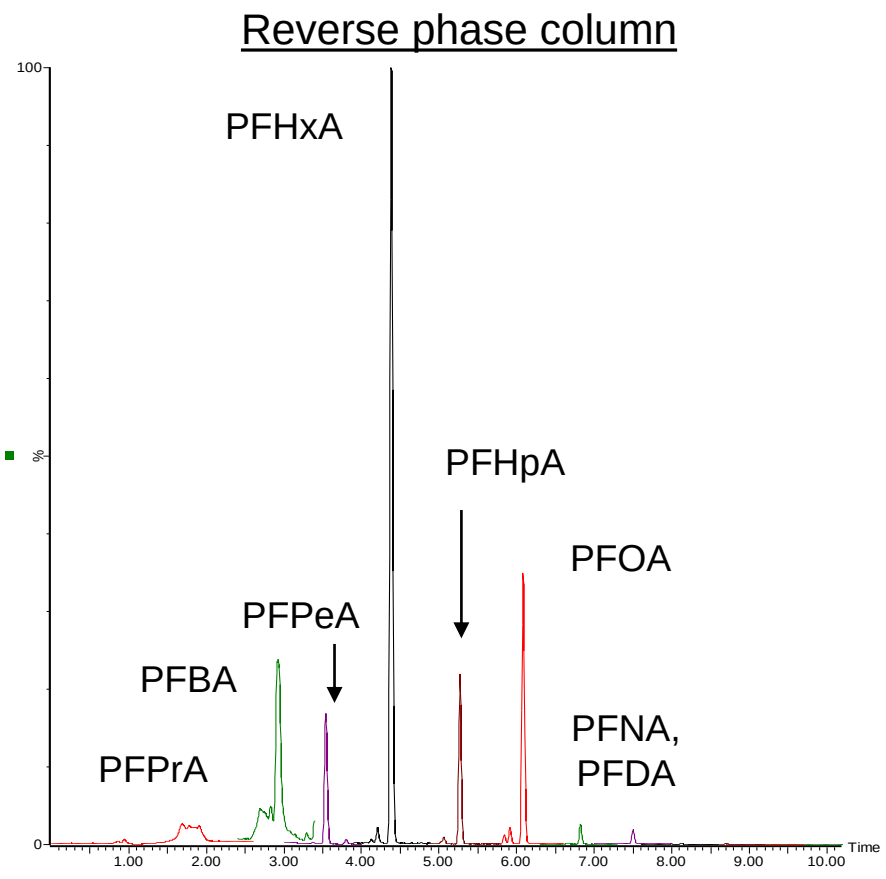




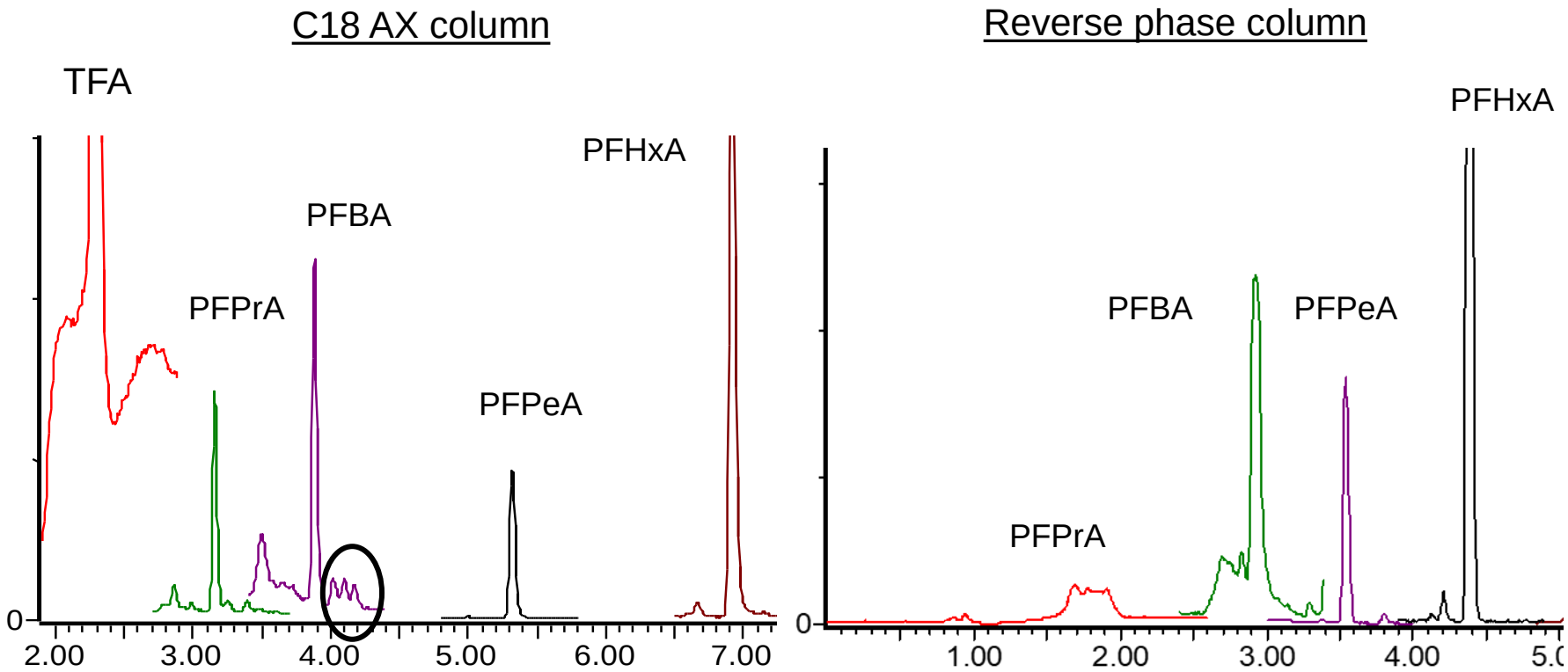
Landfill Leachate Sample

Waters™





Landfill Leachate Sample (zoom)



Landfill Leachate Sample

Waters™

Compound	C18 AX (ng/L)	Reverse Phase (ng/L)	% Difference
TFA	7790	-	NA
PFPrA	1063.6	1204	11.7
PFBA	1904.8	2853.2	33.2
PFPeA	3150.8	3351.4	6.0
PFHxA	5004.4	5002.4	0.0
PFHpA	743.2	682	9.0
PFOA	1431	1379	3.8
PFNA	133	129.2	2.9
PFDA	153.4	147.4	4.1
PFUnDA	ND	ND	ND
PFDoDA	ND	ND	ND
PFTriDA	ND	ND	ND
PFTreDA	ND	ND	ND
PFPrS	552	-	NA
PFBS	4055.8	4293.4	5.5
PFPeS	348	361	3.6
PFHxS	1133.8	1158.4	2.1
PFHpS	29.4	32.2	8.7
PFOS	452.8	454	0.3
PFNS	ND	ND	ND
PFDS	ND	ND	ND
PFDoDS	ND	ND	ND
HQ115	689.6	639	7.9

Compound	C18 AX (ng/L)	Reverse Phase (ng/L)	% Difference
PFMPA	10.6	9.8	8.2
PFMBA	3	2.8	7.1
3:3 FTCA	183.8	210	12.5
5:3 FTCA	6343.8	6176.2	2.7
7:3 FTCA	151.4	147.4	2.7
GenX	4.2	4	5.0
NFDHA	ND	ND	ND
PFEESA	ND	ND	ND
FHUEA	48.8	48.4	0.8
FOUEA	ND	ND	ND
ADONA	ND	ND	ND
4:2 FTS	42.2	40.4	4.5
6:2 FTS	6829.2	7012.2	2.6
8:2 FTS	69.2	69.4	0.3
FOSA	11	10	10.0
NMeFOSA	ND	ND	ND
NEtFOSA	ND	ND	ND
N-MeFOSAA	254.6	222.4	14.5
N-EtFOSAA	76.4	71.4	7.0
NMeFOSE	ND	ND	ND
NEtFOSE	ND	ND	ND
9Cl-PF3ONS	ND	ND	ND
11Cl-PF3OUdS	ND	ND	ND

Acknowledgements

Waters™

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