



PFAS Analysis: From Fundamentals to Advanced Techniques

Part 2: Analytical Challenges and Developing LC-MS Methods for PFAS

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Analysis PFAS: Challenges

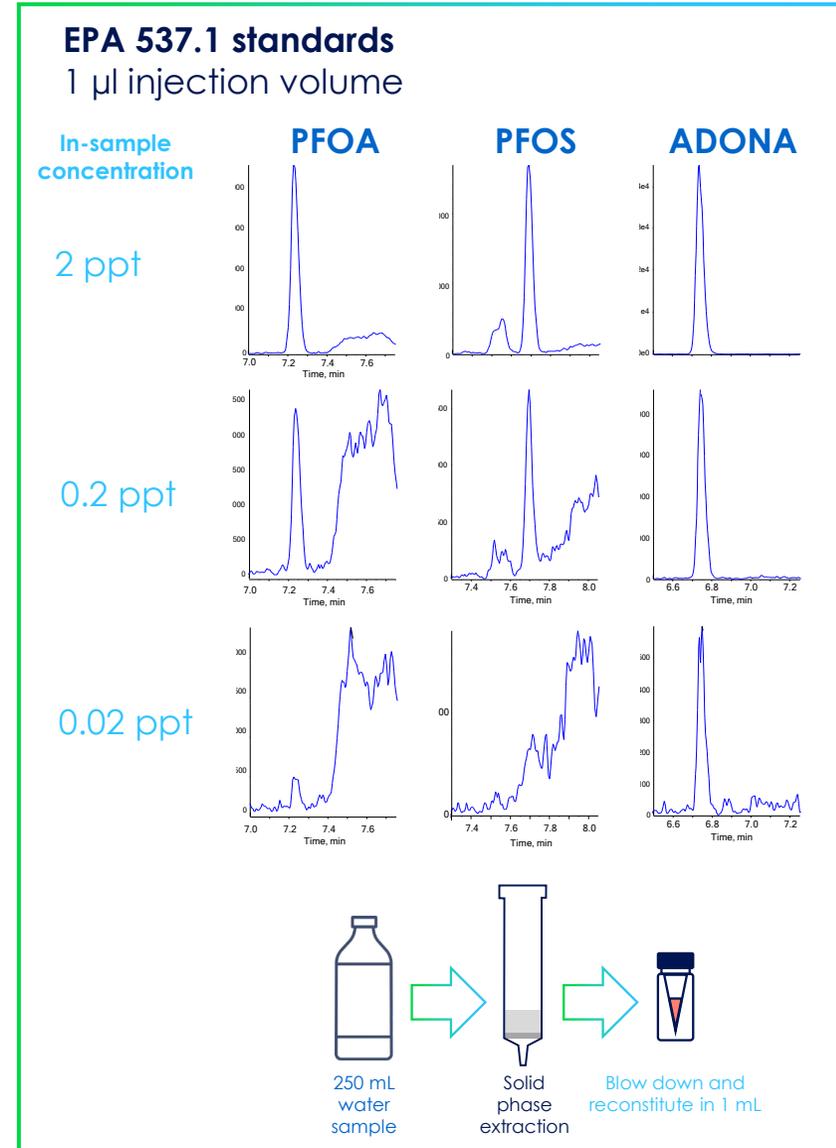
- High sensitivity required – guided by Regulatory limits (parts per trillion (ppt) = ng/L)
- E.g. drinking water guidelines:
- Background PFAS
 - Use of PFAS containing materials ubiquitous in the lab e.g. PTFE
 - PFAS may be used in production of equipment and consumables
 - PFAS may be present in solvents and the analytical instrument itself
- Wide range of target analyte structures
- Wide range of target matrices

US: Final PFAS National Primary Drinking Water Regulation

	Maximum contaminant level goal (MCLG)	Maximum contaminant level (MCL)
PFOA	0	4.0 ppt
PFOS	0	4.0 ppt
PFHxS	10 ppt	10 ppt
HFPO-DA (GenX)	10 ppt	10 ppt
PFNA	10 ppt	10 ppt

Why Mass Spectrometry?

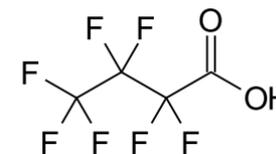
- Mass Spectrometers are:
 - Very sensitive, detection limits typically 10 – 100 than UV
 - ppt – ppq achievable, especially with SPE
 - Very specific, the ability to tune into a unique mass / compound
 - Very fast analysis, potentially co-elution is not a problem due to specificity
 - Data collection rates can be limiting
- Mass Spectrometers are expensive:
 - Initial purchase, higher than a UV detector
 - Operational costs higher
 - Time to develop detector conditions



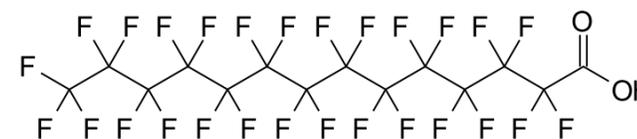
LC-MS methods for PFAS

LC-MS/MS analysis of PFAS

- LC-MS typically the most widely used approach for PFAS analysis
 - Quadrupole, ion trap and time of flight MS used
- LC-MS/MS with triple quadrupole instruments
 - Quantitative targeted analysis
 - High specificity
 - High sensitivity (ppt – ppq), especially with SPE
- Negative ion mode
- Electrospray ionisation (ESI)
- Wide range of hydrophobicity: gradient separation



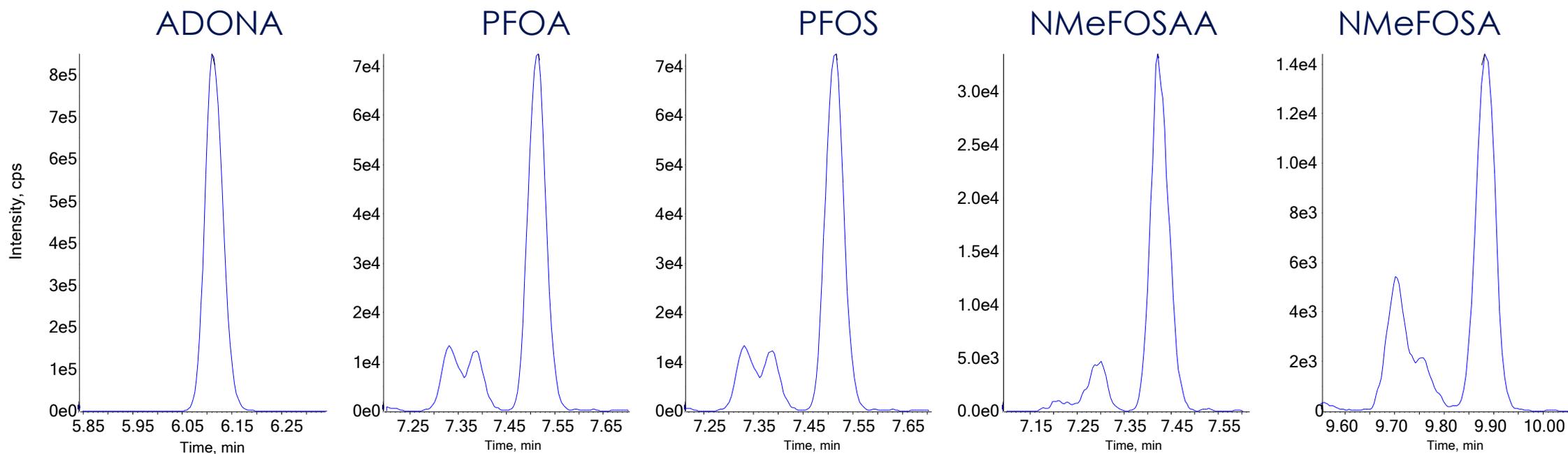
$$\text{PFBA } \text{LogD}_{\text{pH}7.0} = -1.22$$



$$\text{PFTA } \text{LogD}_{\text{pH}7.0} = 5.79$$

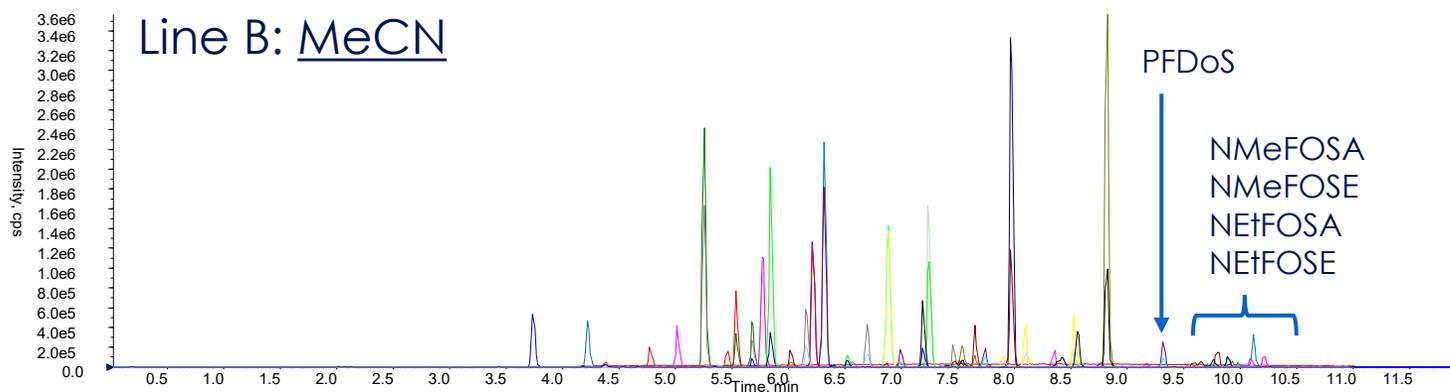
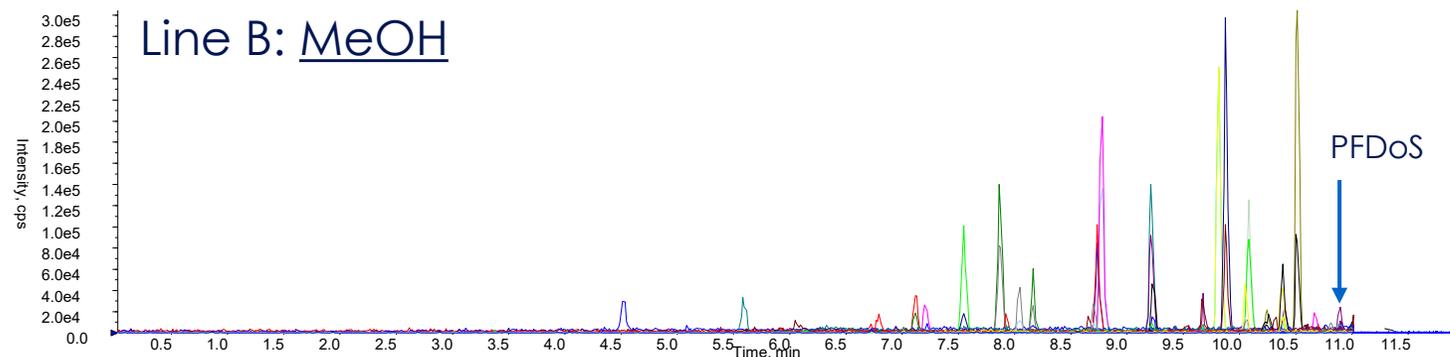
Linear and branched PFAS isomers

- Many PFAS are manufactured using electrochemical fluorination (ECF) and telomerisation
 - Telomerisation produces primarily straight chain PFAS
 - ECF produces mixtures of linear and branched PFAS
 - E.g. PFOA: 78% n-PFOA, 22% br-PFOA*
 - Branched isomers typically elute first
 - Typically integrate isomers as one peak
 - Published methods often require chromatographic separation



Mobile phase and column selection

- 5-20 mM Ammonium acetate commonly used
- Methanol is often utilised as the organic modifier
- For more hydrophobic PFAS, acetonitrile may be required



Columns:

Avantor® ACE® Excel 3 C18, 100 x 2.1 mm

Mobile Phase:

A: 5 mM ammonium acetate (aq)

B: MeOH or MeCN

Gradient:

t/min	%B
0	5
0.5	5
10.5	95
11.5	95
11.6	5
14.6	5

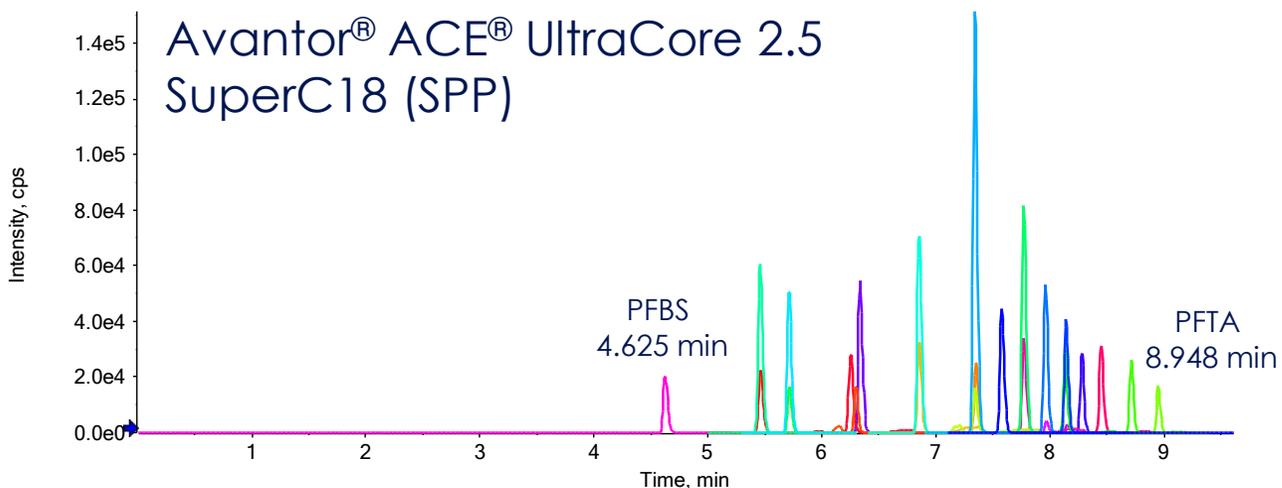
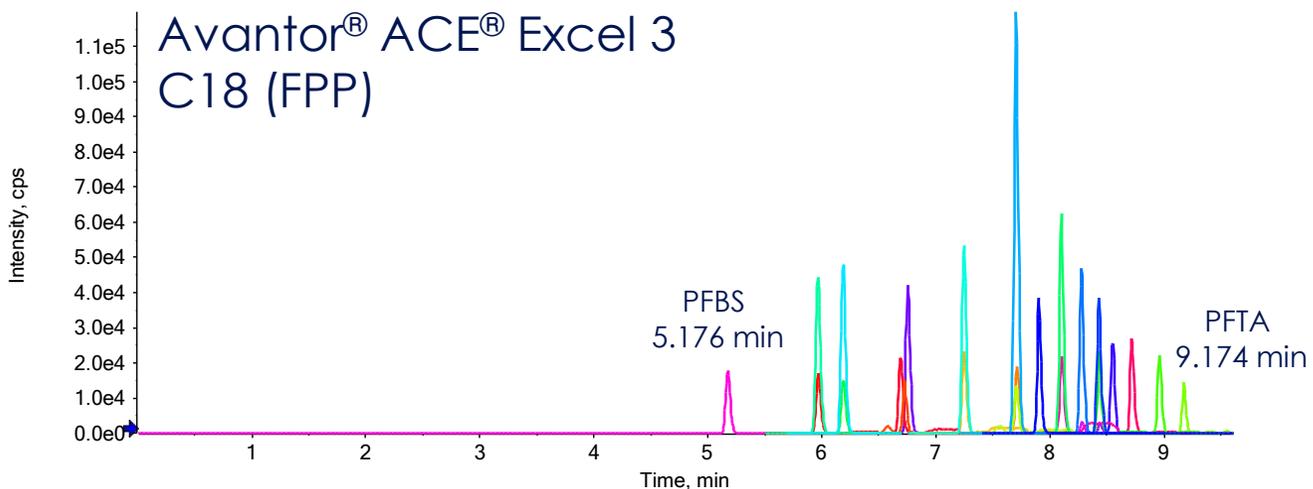
Flow Rate: 0.4 ml/min

Temperature: 40 °C

Injection volume: 1 µl

Sample: EPA 1633 Native PFAS mix

Fully porous particles (FPP) vs Solid core particles (SPP)



Columns:

100 x 2.1 mm

Mobile Phase:

A: 10 mM ammonium acetate (aq)

B: MeOH

Gradient:

t/min	%B
0	5
0.1	20
8.5	95
10.5	95
10.6	5
13.2	5

Flow Rate: 0.4 ml/min

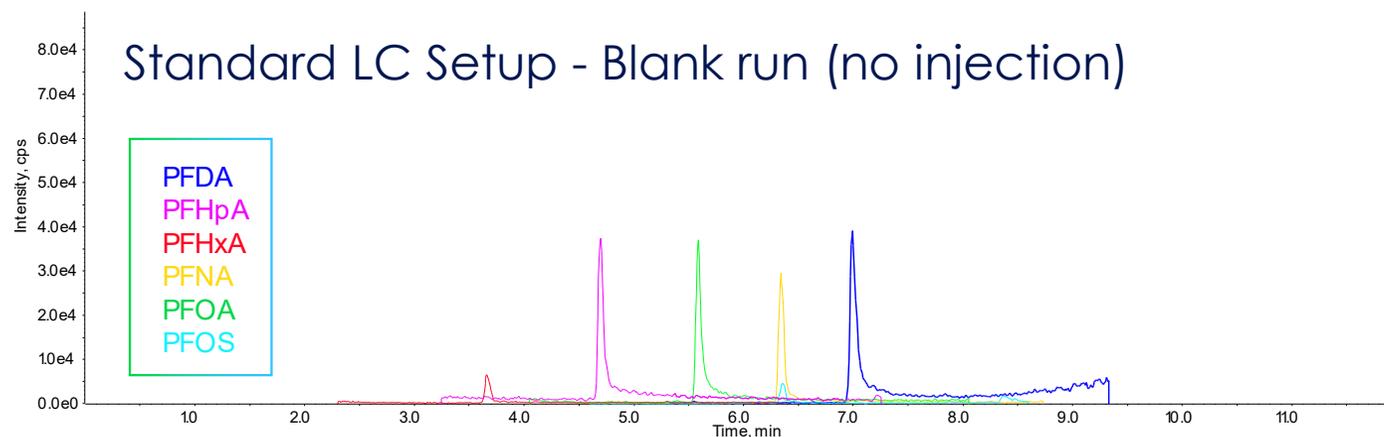
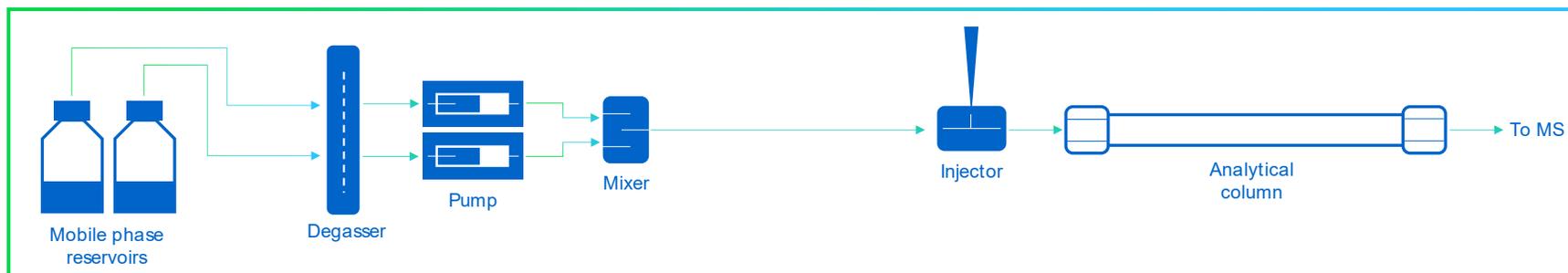
Temperature: 40 °C

Injection volume: 1 µl

Sample: EPA 1633 Native PFAS mix

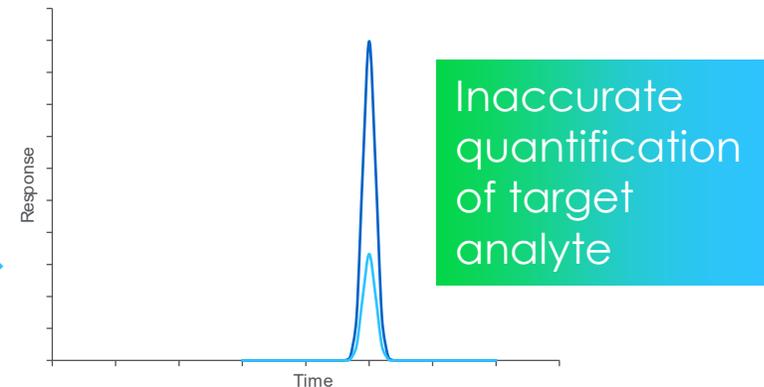
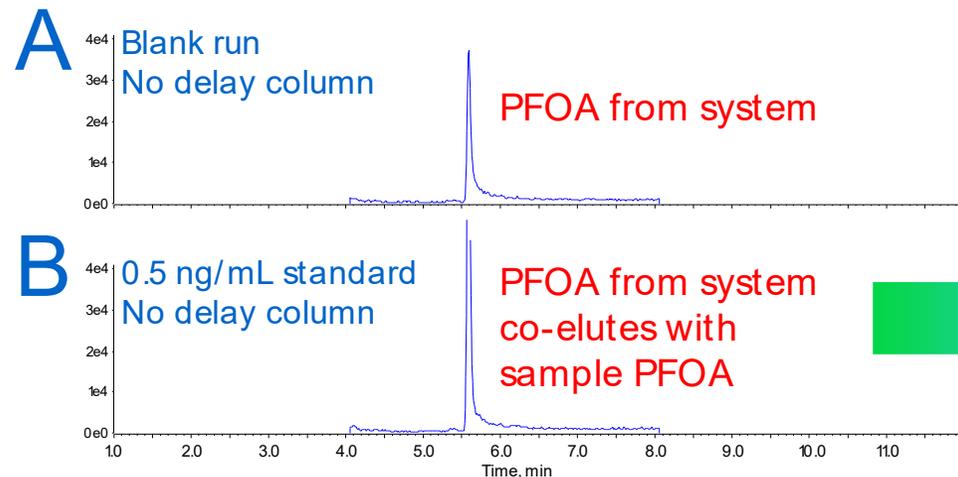
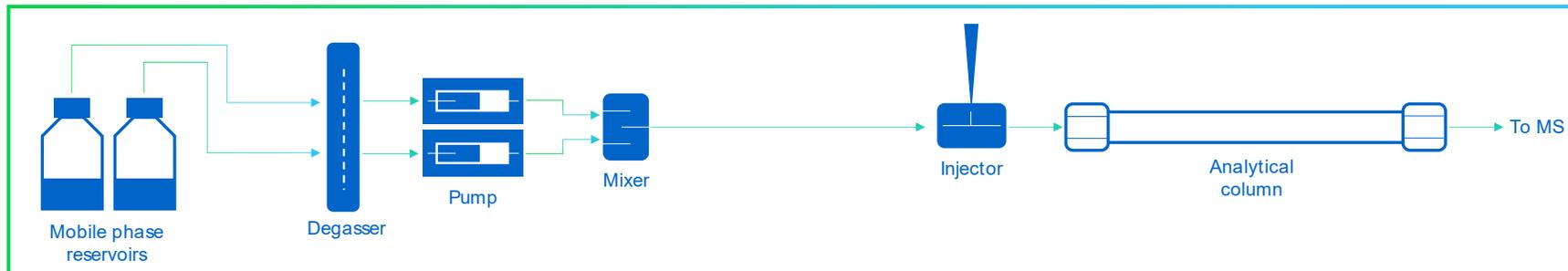
PFAS delay column

- Background PFAS compounds may originate from:
 - Solvents & buffers
 - LC system components (e.g. solvent lines, solvent bottles)
- Using a standard LC setup, these will accumulate on the analytical column and elute as discrete peaks



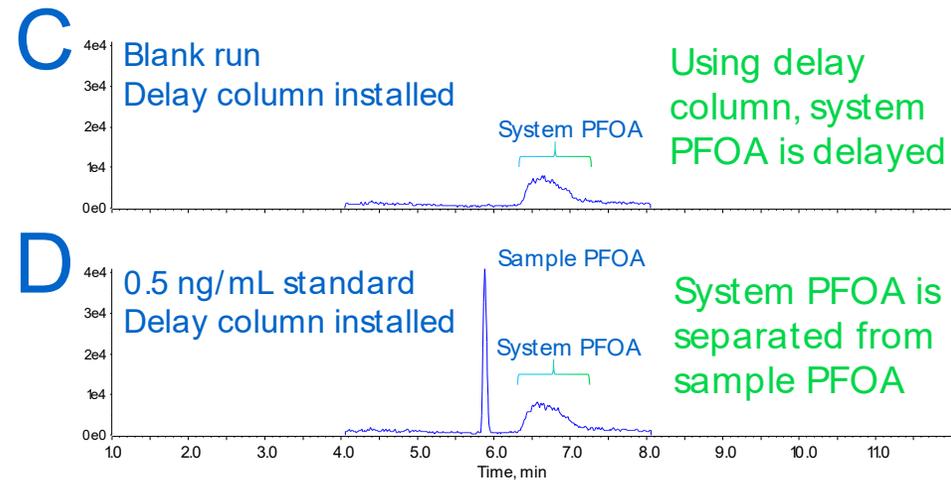
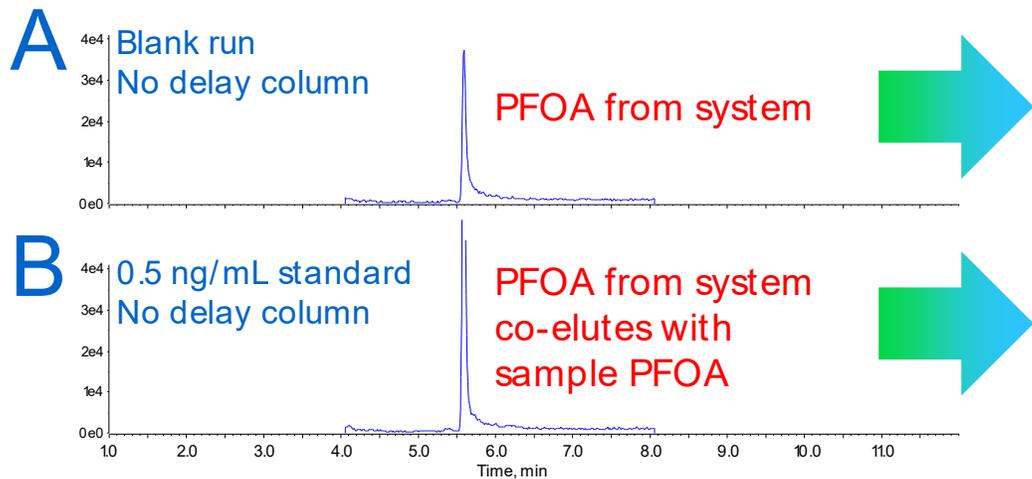
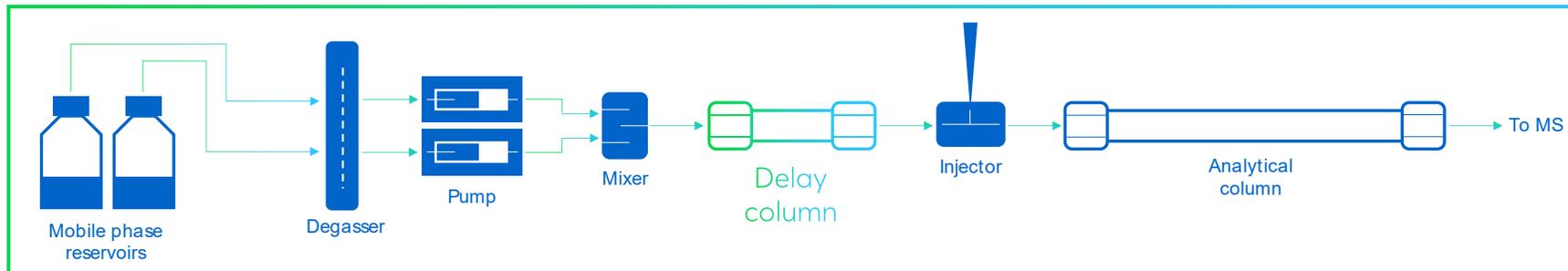
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 - Solvents & buffers
 - LC system components (e.g. solvent lines, solvent bottles)
- Using a standard LC setup, these will accumulate on the analytical column and elute as discrete peaks



PFAS delay column

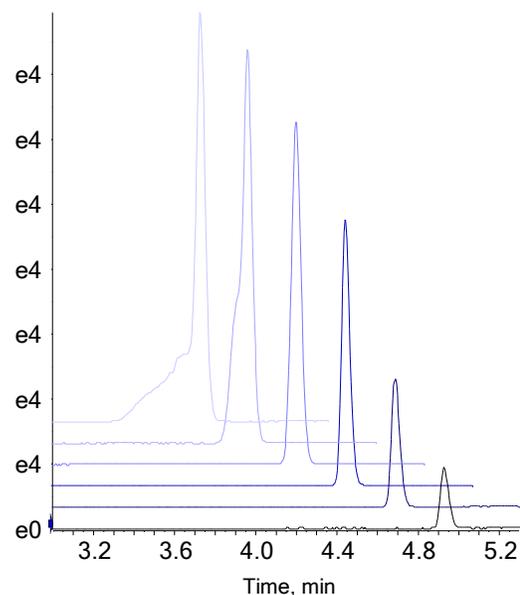
- Use of a delay column is essential for accurate determination of PFAS by LC-MS
- Installed immediately before the LC injector
- Traps background PFAS and allows chromatographic separation of background components from target analytes



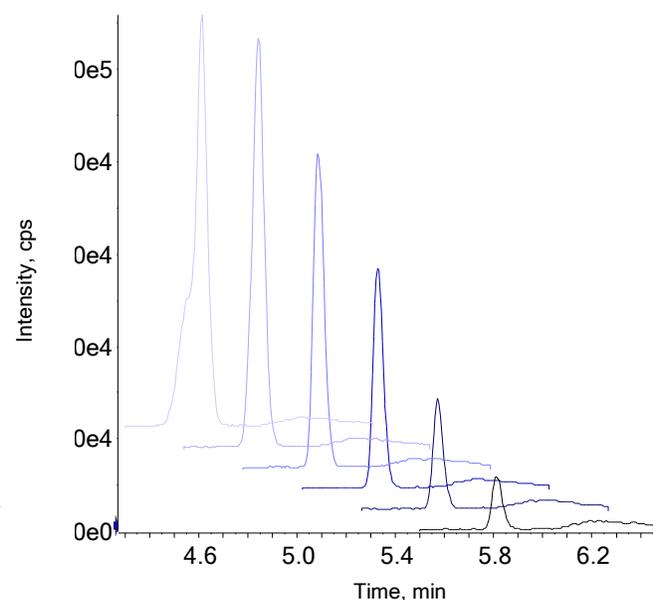
Sample injection volume

- Many PFAS LC-MS methods require sample diluents containing high % organic
- This places limitations on the sample volume that can be injected onto the LC system
- Peak shape for early eluting peaks become distorted at high injection volume

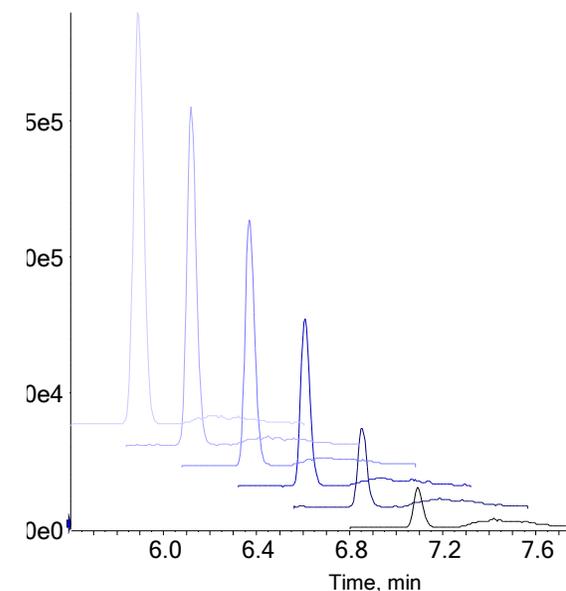
PFBS



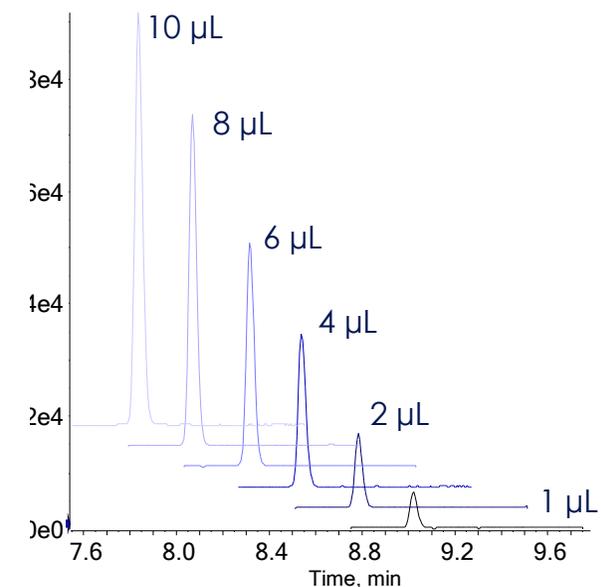
PFHxA



PFOA



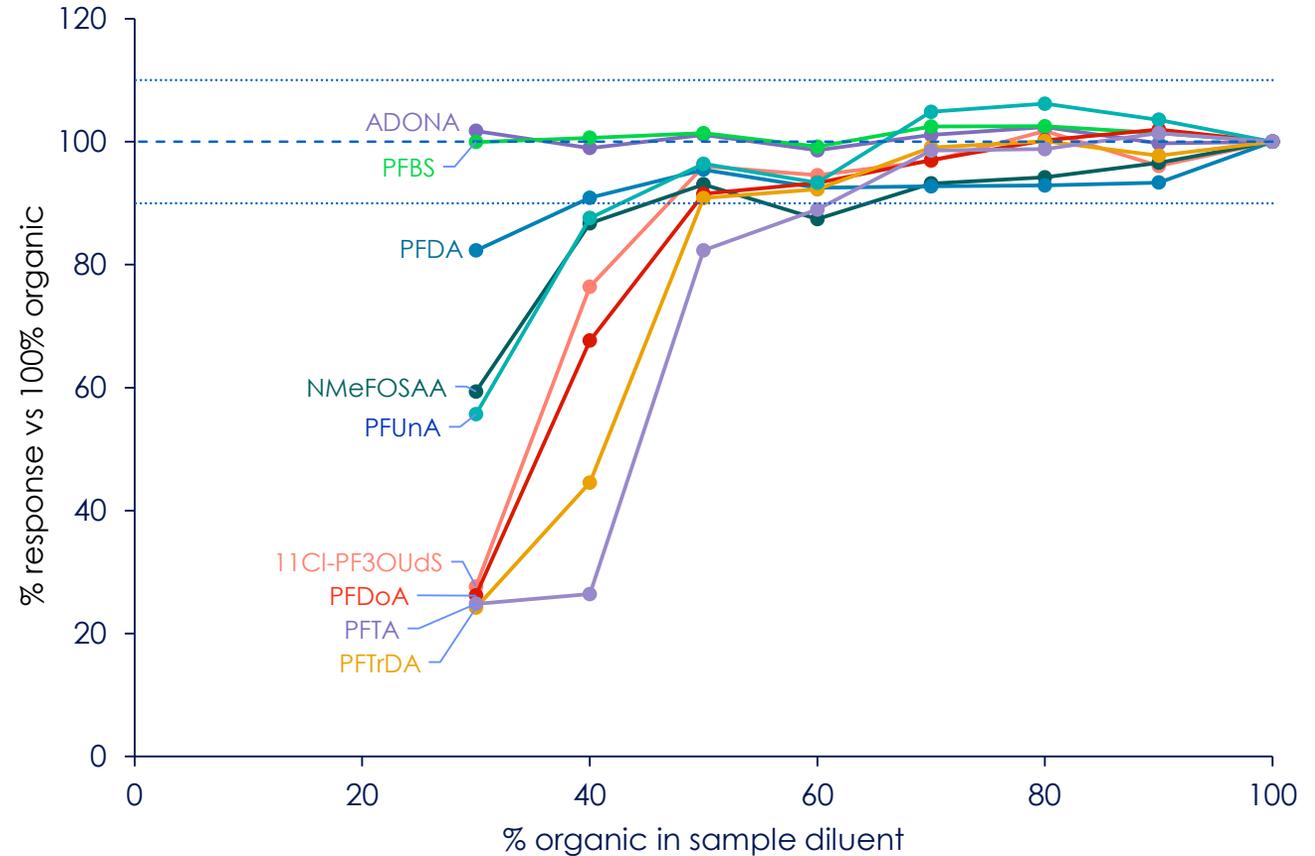
PFTA



Sample: EPA 537.1 PFAS standards (200 ng/l = 0.8 ppt in sample) injected in MeOH:H₂O (96:4 v/v) on an Avantor[®] ACE[®] Excel 3 C18, 100 x 2.1 mm column

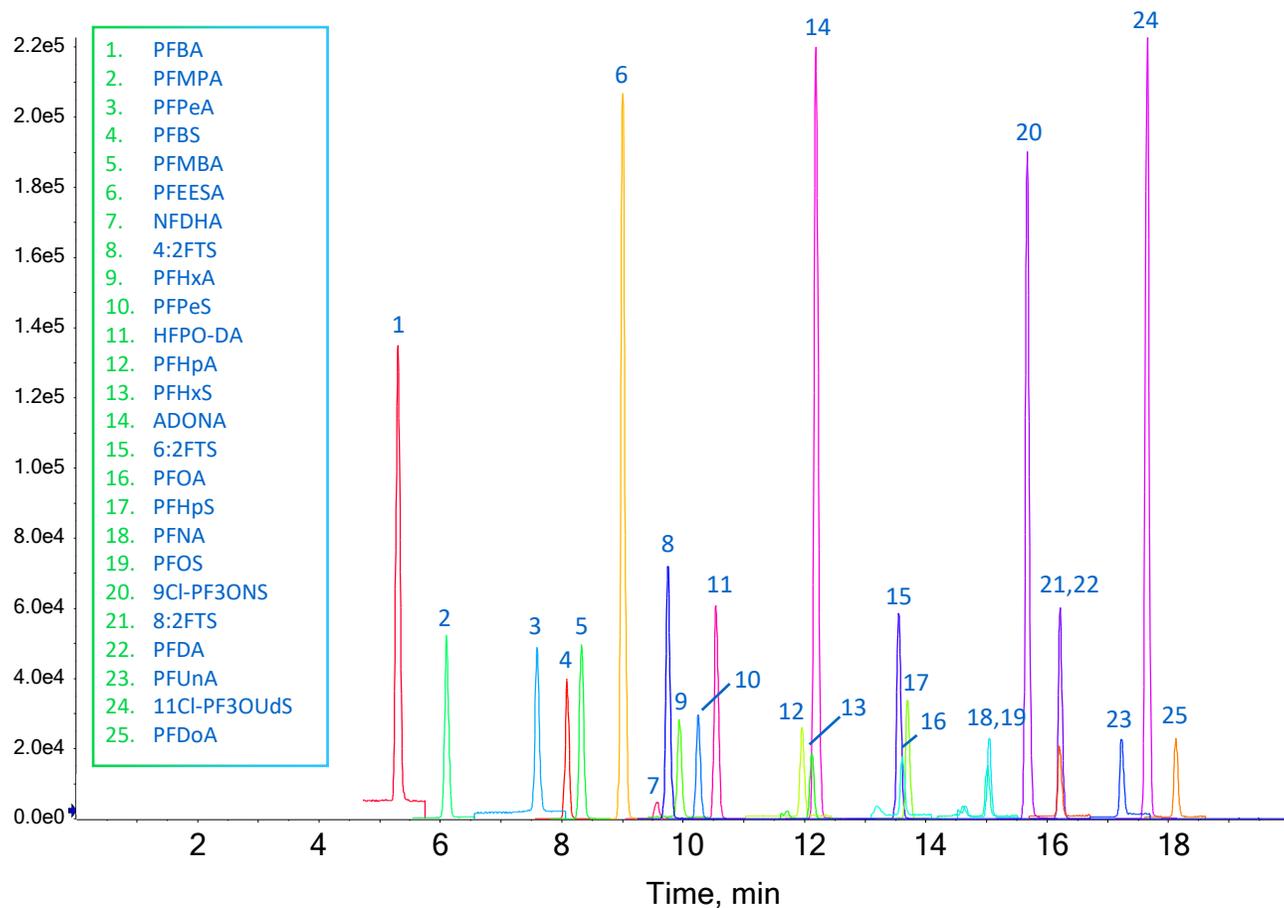
Direct injection

- For some methods, direct injection/dilute & shoot may be an option:
 - Filter or centrifuge sample
 - Dilute with organic
 - Inject
- Not as sensitive as SPE approach
- Faster process than SPE
- Less potential for sample contamination during preparation
- Percentage organic in the final injected sample is critical



Applications

Application 1: EPA 533



Sample: PFAS standards at 100 - 2500 ng/L (corresponding to an in-sample concentration of 0.4 – 10.0 ng/L, taking into account 250x sample pre-concentration during sample preparation specified in EPA method 533).

Column: Avantor® ACE® Excel® C18
 Particle Size: 3 µm
 Dimensions: 100 x 2.1 mm
 Delay Column: Avantor® ACE® PFAS Delay Column
 Dimensions: 50 x 2.1 mm
 Mobile Phases: A: 5 mM ammonium acetate in H₂O
 B: MeOH

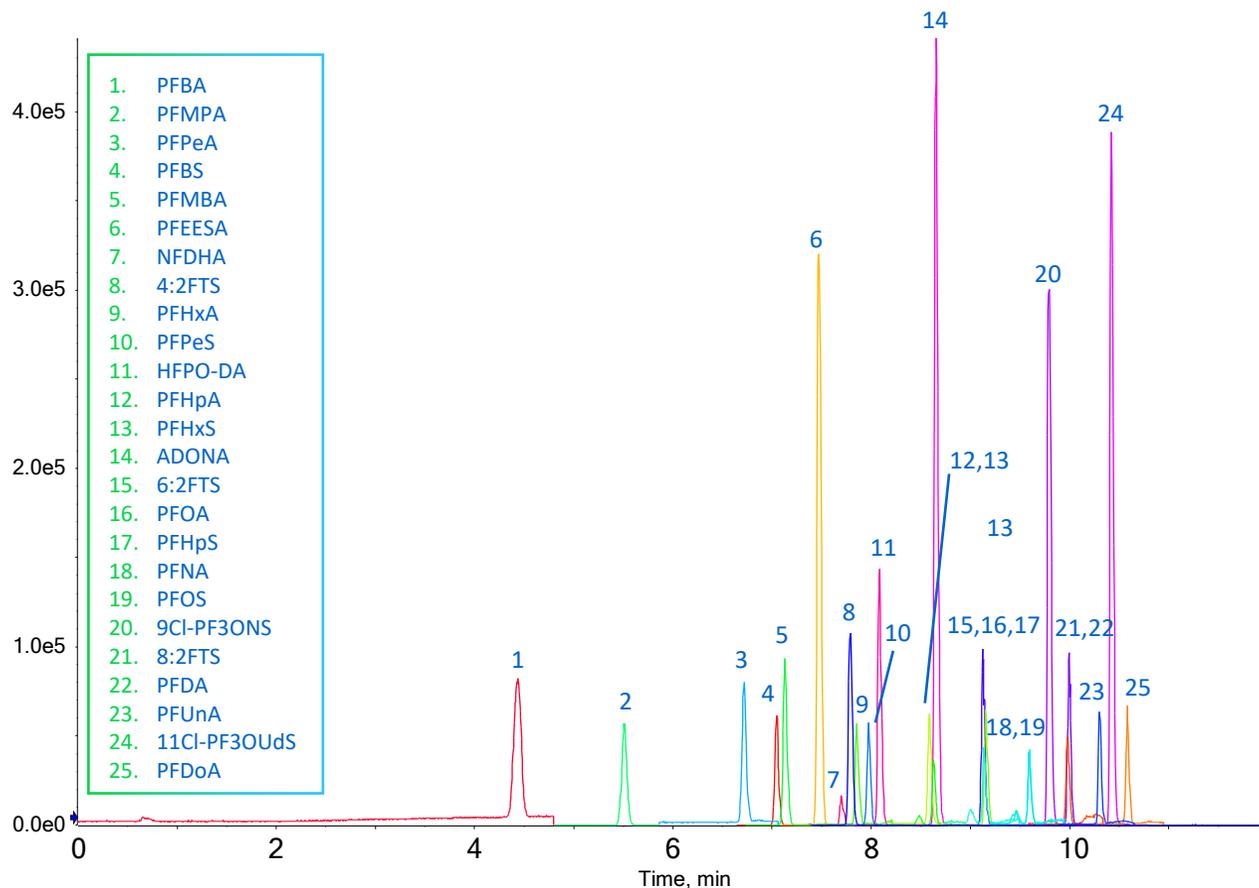
Gradient:

Time (mins)	% B
0	5
0.5	5
3.0	40
16.0	80
18.0	80
20.0	95
22.0	95
25.0	5

Flow Rate: 0.25 mL/min
 Temperature: 40 °C
 Injection volume: 5 µL

Detection: Sciex QTRAP® 6500+ LC-MS/MS system
 Ionisation mode: ESI, negative mode;
 Temp: 550 °C; Curtain gas: 35 psig;
 Source voltage: -3500 V;
 Ion source gas: 50 psig

Application 2: EPA 533 (rapid)



Sample: PFAS standards at 100 - 2500 ng/L (corresponding to an in-sample concentration of 0.4 – 10.0 ng/L, taking into account 250x sample pre-concentration during sample preparation specified in EPA method 533).

Column: Avantor® ACE® Excel® C18
 Particle Size: 3 µm
 Dimensions: 100 x 2.1 mm
 Delay Column: Avantor® ACE® PFAS Delay Column
 Dimensions: 50 x 2.1 mm
 Mobile Phases: A: 5 mM ammonium acetate in H₂O
 B: MeOH

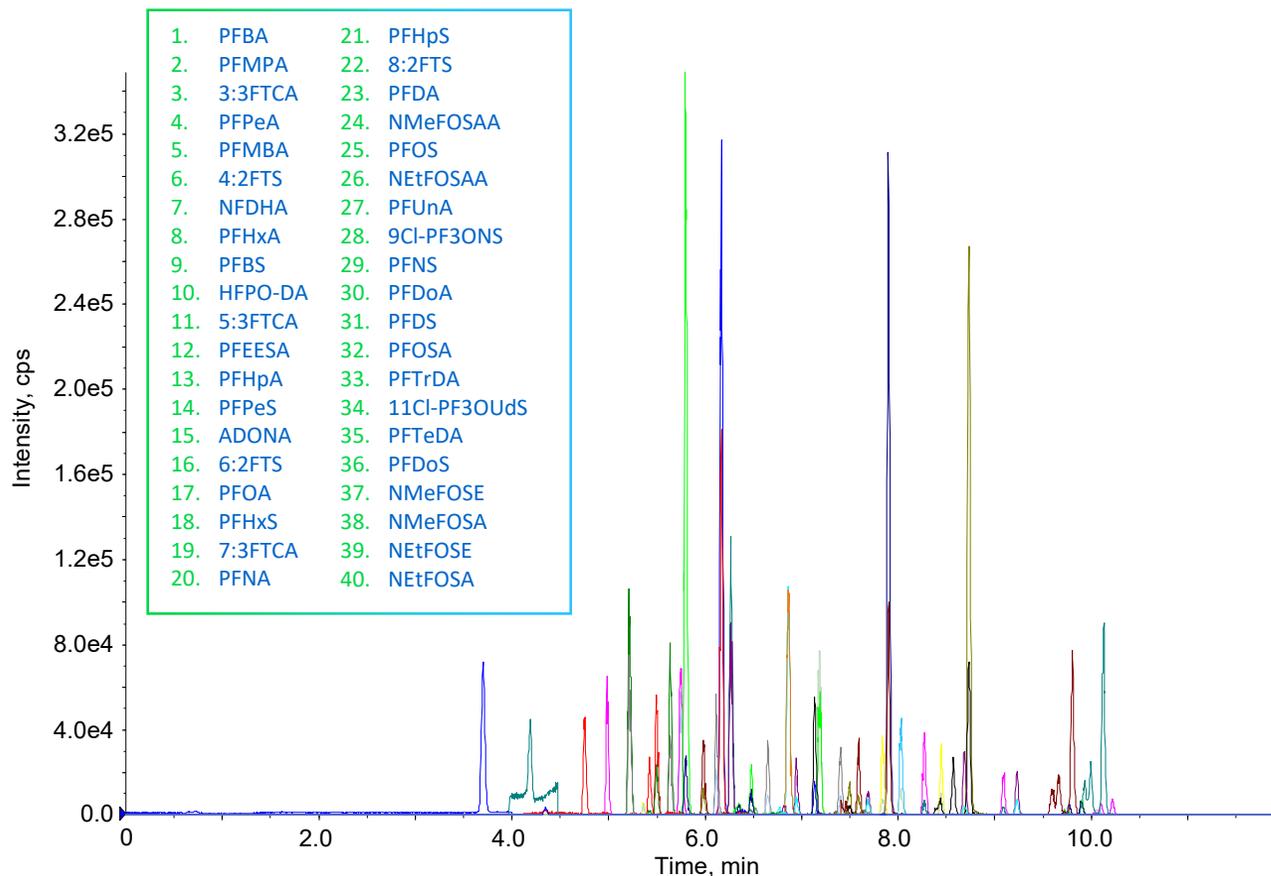
Gradient:

Time (mins)	% B
0	5
0.5	5
10.5	95
12.0	95
12.5	5

Flow Rate: 0.4 mL/min
 Temperature: 40 °C
 Injection volume: 5 µL

Detection: Sciex QTRAP® 6500+ LC-MS/MS system
 Ionisation mode: ESI, negative mode;
 Temp: 550 °C; Curtain gas: 35 psig;
 Source voltage: -3500 V;
 Ion source gas: 50 psig

Application 3: EPA 1633 (40 PFAS)



Sample: PFAS standards at 0.25 – 6.25 ng/mL in MeOH:H₂O (96:4 v/v)
+ 1% ammonium hydroxide +0.6% acetic acid.

Column: Avantor® ACE® Excel® C18
 Particle Size: 3 µm
 Dimensions: 100 x 2.1 mm
 Delay Column: Avantor® ACE® PFAS Delay Column
 Dimensions: 50 x 2.1 mm
 Mobile Phases: A: 5 mM ammonium acetate in H₂O
 B: MeCN

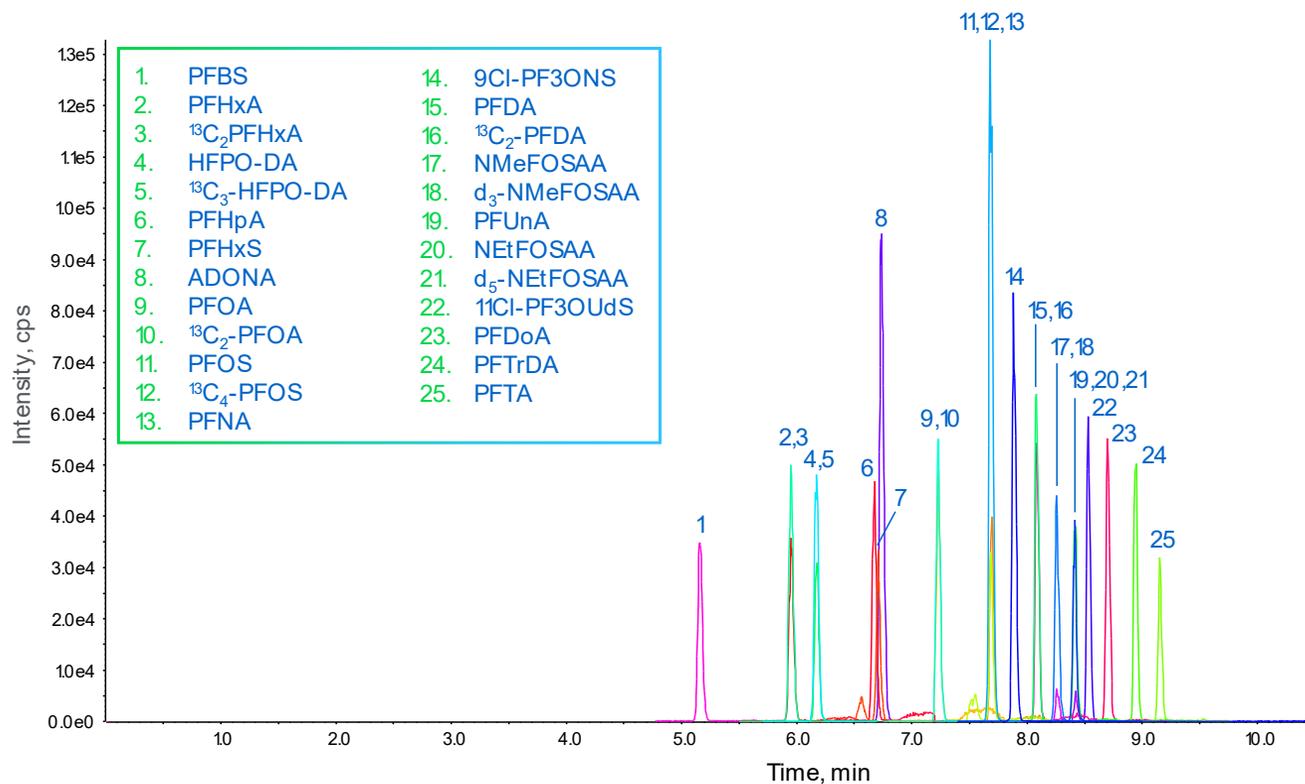
Gradient:

Time (mins)	% B
0	5
0.5	5
10.5	95
11.5	95
11.6	5
14.6	5

Flow Rate: 0.4 mL/min
 Temperature: 40 °C
 Injection volume: 4 µL

Detection: Sciex QTRAP® 6500+ LC-MS/MS system
 Ionisation mode: ESI, negative mode;
 Temp: 600 °C; Curtain gas: 30 psig;
 Source voltage: -4000 V;
 Ion source gas: 30 psig

Application 4: EPA 537.1



Sample: Calibration standard with PFAS standards, internal standards and surrogate standards at 1000 ng/L (corresponding to an in-sample concentration of 4 ng/L, taking into account 250x sample pre-concentration during sample preparation specified in EPA method 537.1).

Column: Avantor® ACE® Excel® C18
 Particle Size: 3 µm
 Dimensions: 100 x 2.1 mm
 Delay Column: Avantor® ACE® PFAS Delay Column
 Dimensions: 50 x 2.1 mm
 Mobile Phases: A: 10 mM ammonium acetate in H₂O
 B: MeOH

Gradient:

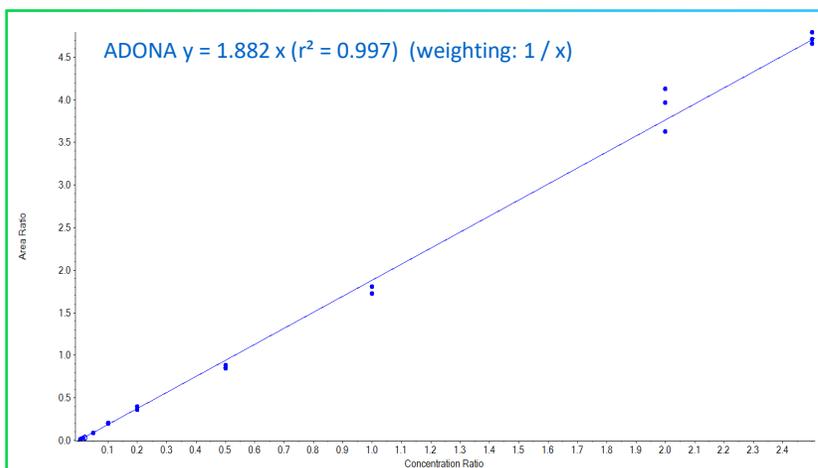
Time (mins)	% B
0	5
0.1	20
8.5	95
10.5	95
10.6	5

Flow Rate: 0.4 mL/min
 Temperature: 40 °C
 Injection volume: 1 µL

Detection: Sciex QTRAP® 6500+ LC-MS/MS system
 Ionisation mode: ESI, negative mode;
 Temp: 450 °C; Curtain gas: 30 psig;
 Source voltage: -4500 V;
 Ion source gas: 60 psig

Application 4: EPA 537.1

- Excellent accuracy and linearity for calibration curves
- Excellent accuracy and precision for QC samples



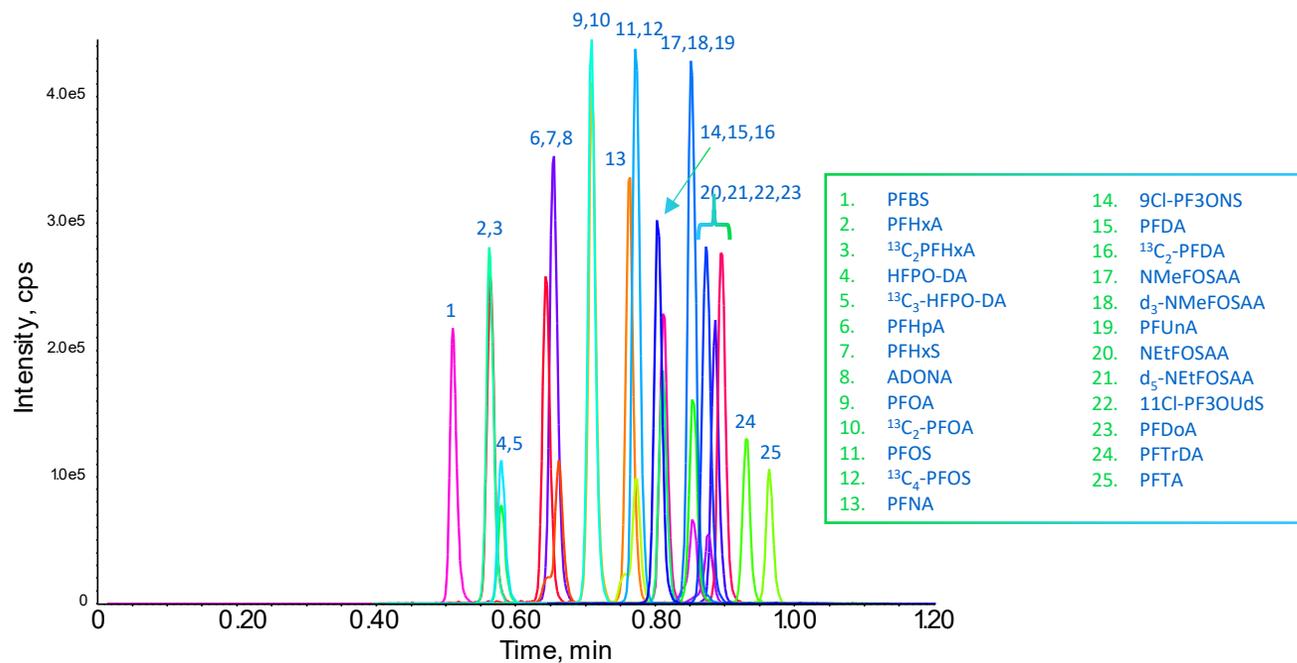
Concentration (in vial) ng/L	Concentration (in sample) ng/L*	% Accuracy
5	0.02	110.7
10	0.04	101.7
15	0.06	110.1
50	0.2	93.0
100	0.4	106.8
200	0.8	102.4
500	2.0	91.7
1000	4.0	94.6
2000	8.0	103.8
2500	10.0	100.4

Accuracy and Precision data for QC sample (6 replicates)

Analyte	Concentration (in vial) ng/L	Mean calculated ng/L	% Precision	% Accuracy
9CI-PF3ONS	100	106.0	5.3	106.0
11CI-PF3OUdS	100	104.9	1.9	104.9
ADONA	100	104.8	3.3	104.8
NEtFOSAA	100	104.9	6.2	104.9
NMeFOSAA	100	99.7	8.5	99.71
PFBS	100	105.1	3.8	105.1
PFDA	100	107.2	8.2	107.2
PFDaA	100	109.4	5.0	109.4
PFHpA	100	109.3	4.4	109.3
PFHxA	100	108.5	5.0	108.5
PFHxS	100	104.7	4.5	104.7
PFNA	100	109.9	5.3	109.9
PFOA	100	111.3	4.1	111.3
PFOS	100	113.6	3.8	113.6
PFTA	100	110.5	3.5	110.5
PFTrDA	100	109.0	3.7	109.0
PFUnA	100	104.4	2.7	104.4
HFPO-DA	100	103.6	5.2	103.6

*The corresponding in sample concentration takes into account the 250x concentration step during sample preparation specified in EPA method 537.1

Application 5: Rapid analysis with Avantor® ACE® HTP-MS columns



Sample: Calibration standard with PFAS standards at 1000-4000 ng/L, internal standards and surrogate standards at 1000-4000 ng/L in MeOH:H₂O (1:1, v/v).

Column: Avantor® ACE® HTP-MS
 Particle Size: 2 µm
 Dimensions: 10 x 2.1 mm
 Delay Column: Avantor® ACE® PFAS Delay Column
 Dimensions: 50 x 2.1 mm
 Mobile Phases: A: 5 mM ammonium acetate in H₂O
 B: MeOH

Gradient:

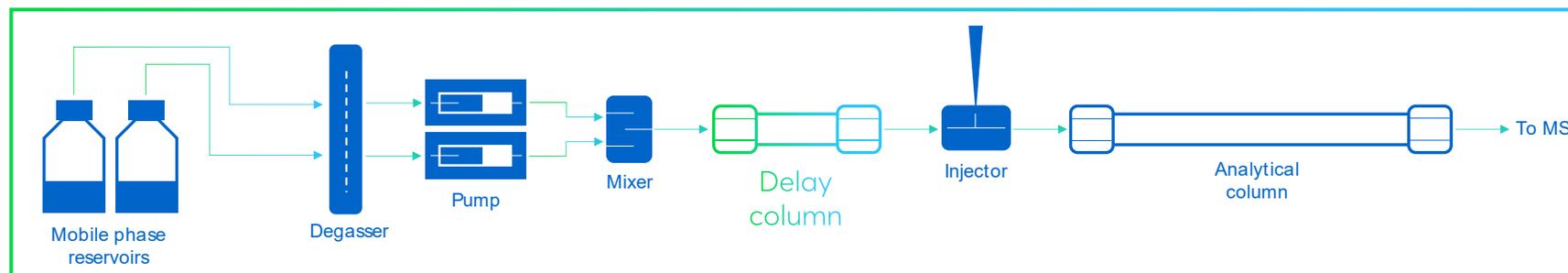
Time (mins)	% B
0	5
0.05	40
1.0	100
1.2	100
1.3	5
1.6	5

Flow Rate: 1.0 mL/min
 Temperature: 22 °C
 Injection volume: 4 µL

Detection: Sciex QTRAP® 6500+ LC-MS/MS system
 Ionisation mode: ESI, negative mode;
 Temp: 450 °C; Curtain gas: 40 psig;
 Source voltage: -4500 V;
 Ion source gas: 70 psig

Conclusions

- LC-MS is a powerful tool for the determination of PFAS.
- Key considerations when developing methods:
 - Consider analyte physicochemical properties
 - Mobile phase and column selection
- Use of a PFAS delay column is essential



- Pay attention to injection volume
- Careful consideration of percentage organic in diluent is required for direct inject approaches

Literature and resources

Workflow solutions for PFAS analysis:

Avantor® ACE® Application Notes (<https://www.mac-mod.com/applications/#app-brand-avantor-ace-excel>)

Avantor® ACE® Technical Notes (<https://www.mac-mod.com/resources/#resource-brand-avantor-ace>)

Chromatography Solutions

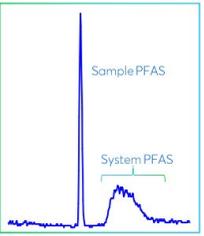
Technical note #035

Use of the Avantor® ACE® PFAS Delay Column to resolve background system interference in the LC-MS/MS determination of PFAS

INTRODUCTION

The ubiquitous use of poly and perfluoroalkyl substances (PFAS) in a wide range of industrial, commercial and consumer products, combined with their chemical inertness and longevity, means that widespread environmental PFAS contamination now exists on a global scale. Concerns over their impact to human health and the environment means that monitoring PFAS levels within natural and human environments is increasingly important.

One of the most powerful analytical techniques used for PFAS determination is LC-MS/MS. However, due to the presence of background PFAS within the laboratory environment, strict laboratory practices must be implemented to reduce the potential for inadvertent sample contamination or contamination of the chromatographic system which leads to inaccurate quantification. This technical note outlines one of the mandatory precautions that must be taken when using LC-MS/MS; the use of a PFAS Delay column to chromatographically separate native sample PFAS from



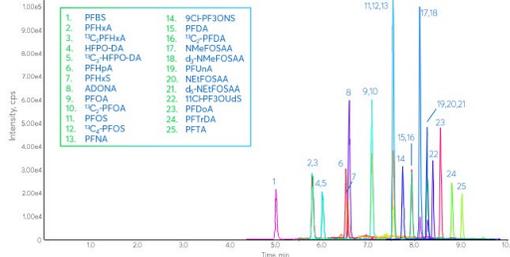
Avantor® ACE®

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Chromatography Solutions

Application note #7820

PFAS Analysis by EPA method 537.1 using VWR® HiPerSolv CHROMANORM® PFAS grade solvents

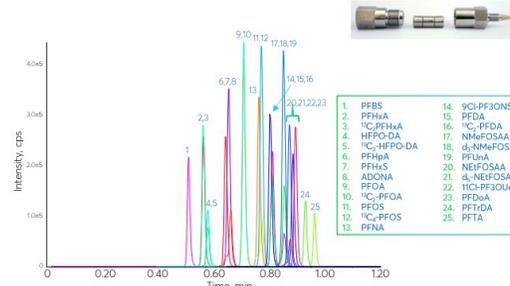


Avantor® ACE®

Chromatography Solutions

Application note #7830

Rapid LC-MS/MS screening of 18 PFAS compounds using an Avantor® ACE® HTP-MS column



Avantor® ACE®



**Thank you for your
attention**

