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Delay Columns: Additional Impacts to Delaying PFAS Present in the Background

Ruth Marfil-Vega, Om Shrestha, Kathleen Luo, Landon Wiest

Shimadzu Scientific Instruments

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Garden Grove, California

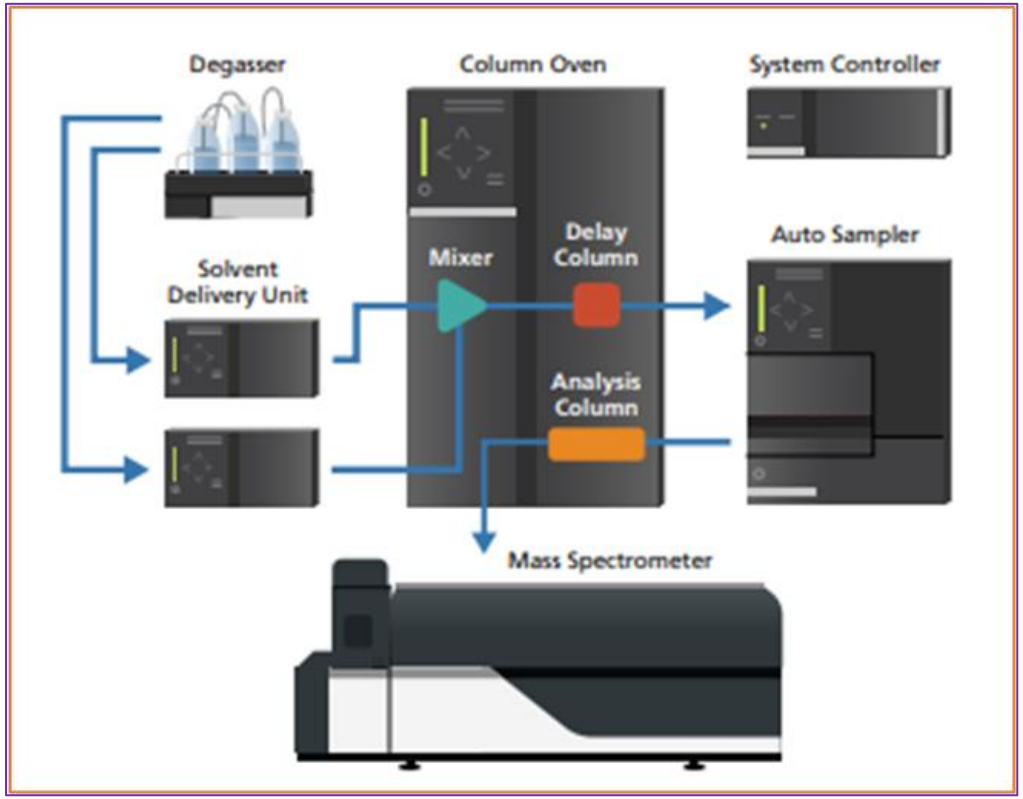
Sources of PFAS Contamination

Before the autosampler

- Solvents
- Buffers
- Tubing
- Degasser

After the autosampler

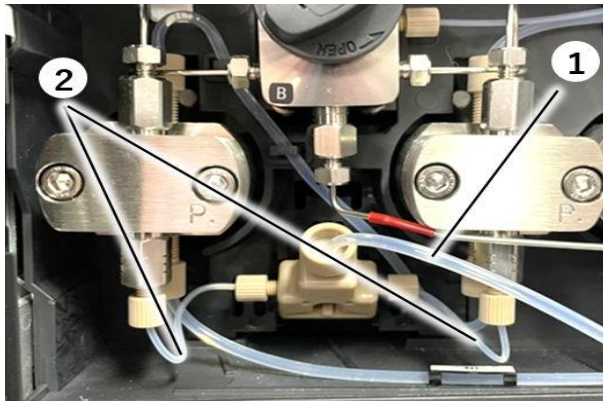
- Vials
- Vial Caps
- Materials in contact with samples (during collection, shipping and preparation)

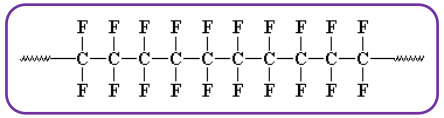


Approaches to mitigate PFAS in background

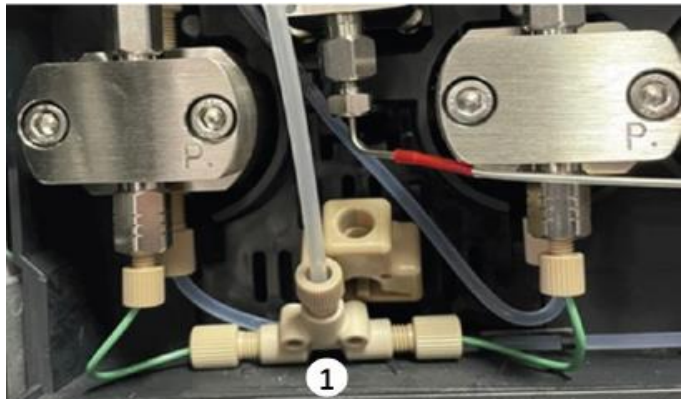
To replumb the LC with a “tubing kit” consisting of LC tubing made of alternatives to PTFE

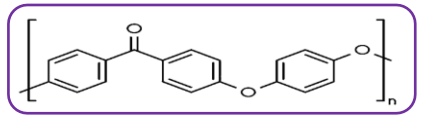
(PEEK or other materials)





PTFE



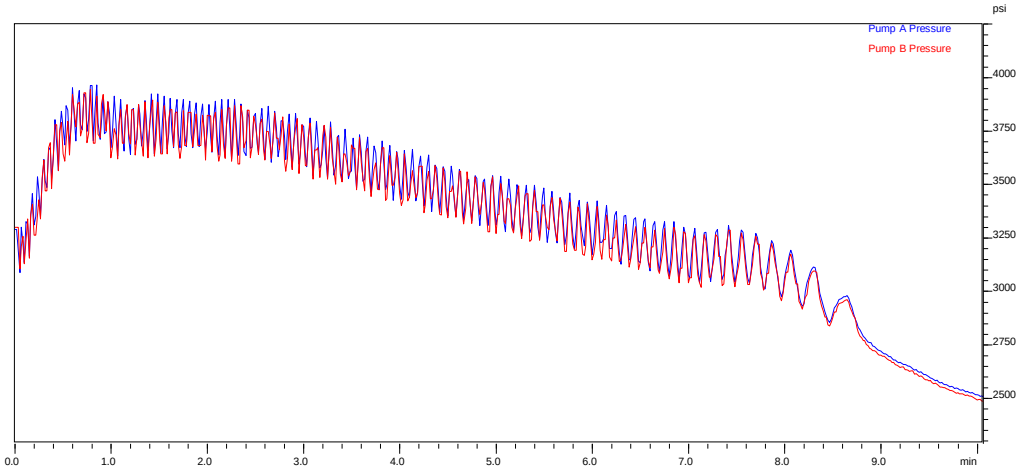


PEEK

Tubing Kit for PFAS Analysis

If installed:

- Mobile phase tubing bypass degasser:
  - ➢ increased frequency of air bubbles
  - ➢ required regular or continuous sparging/degassing of the mobile phase
- Autosampler rinsing options may be altered → increased chances of carryover
- It does not help with mitigating other sources of PFAS contamination



LC Pressure Trace with Air Bubbles

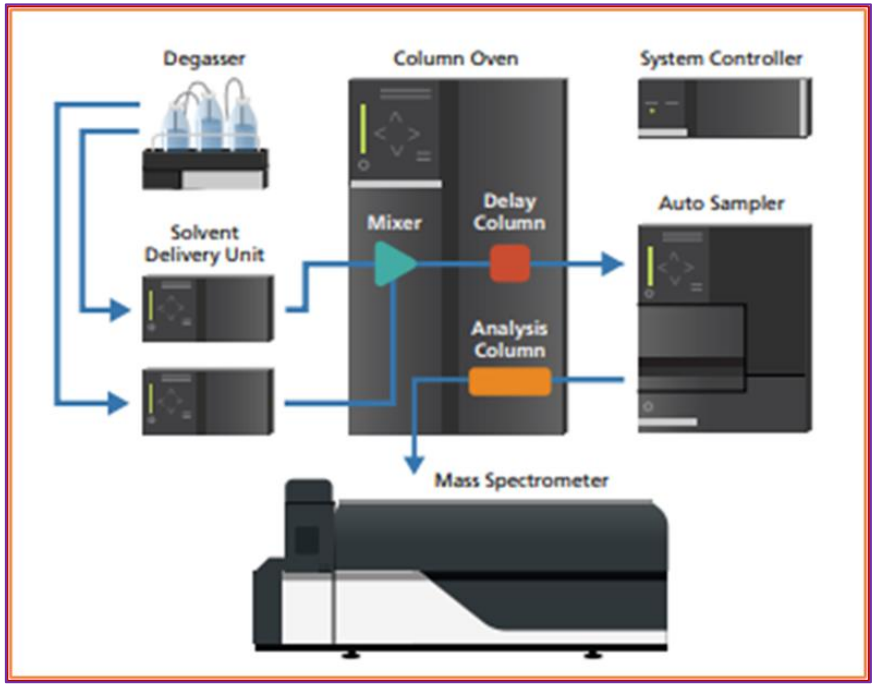
Before the autosampler

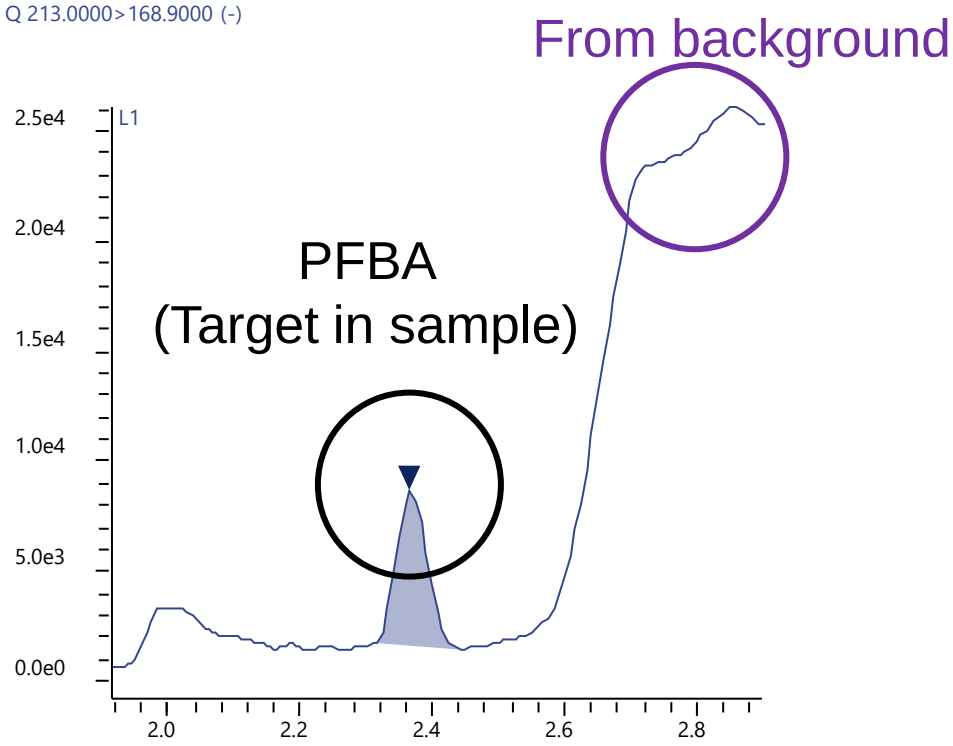
- Solvents
- Buffers
- ✓ Tubing
- ✓ Degasser

PFAS Tubing Kit is **Optional**

Approaches to mitigate PFAS in background

To use a delay column that separates PFAS present in the samples from those potentially present in consumables and sample flow path, minimizing false positives and interferences





Approaches to mitigate PFAS in background

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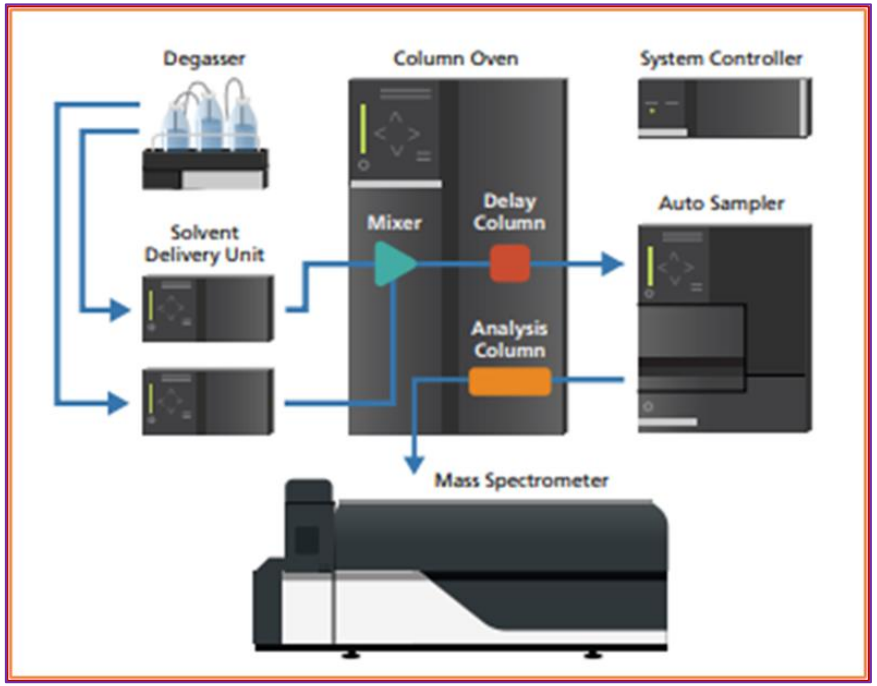


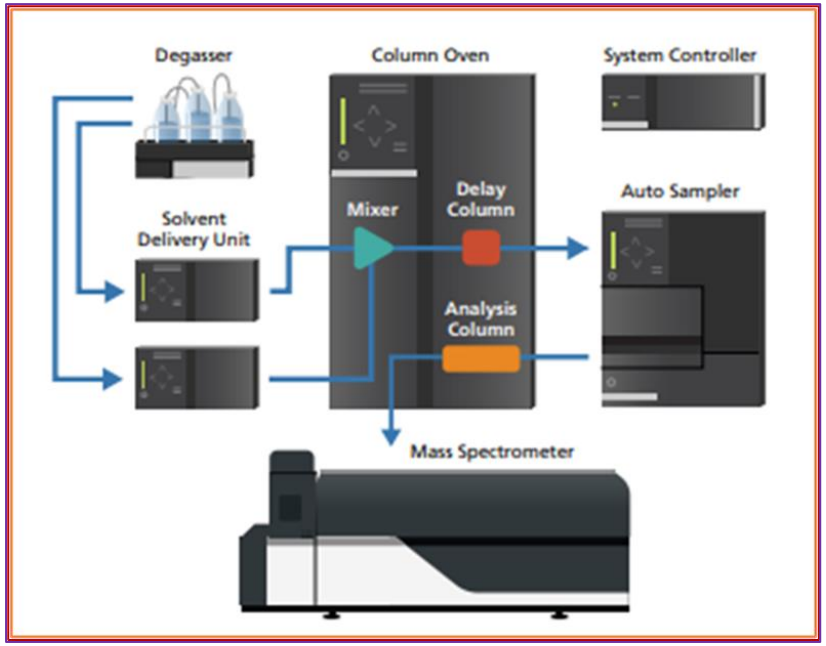
Before the autosampler

- ✓ Solvents
- ✓ Buffers
- ✓ Tubing
- ✓ Degasser

Systematic evaluation of PFAS in background

- Shimadzu LCMS-8060NX
- Standard configuration
  - Degasser in-line
  - Standard fluoropolymer containing tubing
- Data acquired according to EPA 1633
  - Using methods with standard injection and co-injection
- Replicated injections (n=7) of each condition tested
  1. No delay column: NULL, blank, blank co-injection
  2. Delay column: NULL, blank, blank co-injection
  3. Calibration curves
- Calibration curves range (compound dependent)
  - Standard injection: 0.8 - 250 ng/mL (PFBA)
  - Co-injection: 0.08 ng/mL – 25 ng/mL (PFBA)

Before the autosampler	**After the autosampler**
• Solvents • Buffers • Tubing • Degasser	• Vials • Vial Caps • Materials in contact with samples (during collection, shipping and preparation)



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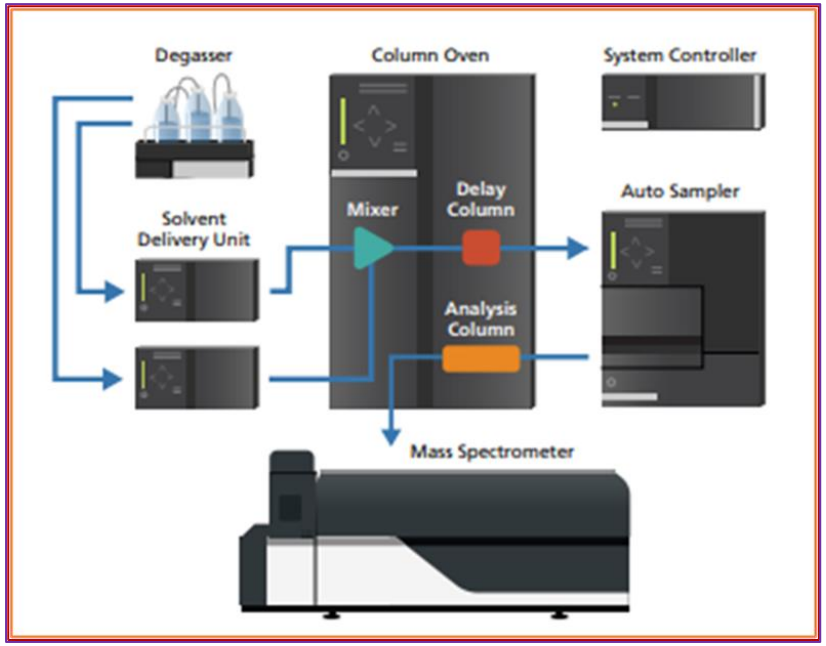
- Solvents
- Buffers
- Tubing
- Degasser

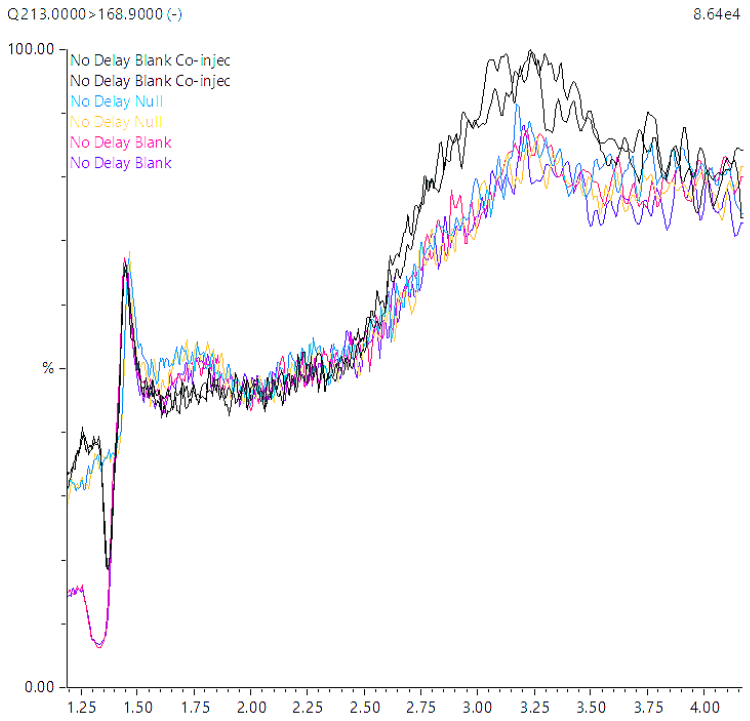
After the autosampler
- Vials
- Vial Caps
- Materials in contact with samples (during collection, shipping and preparation)



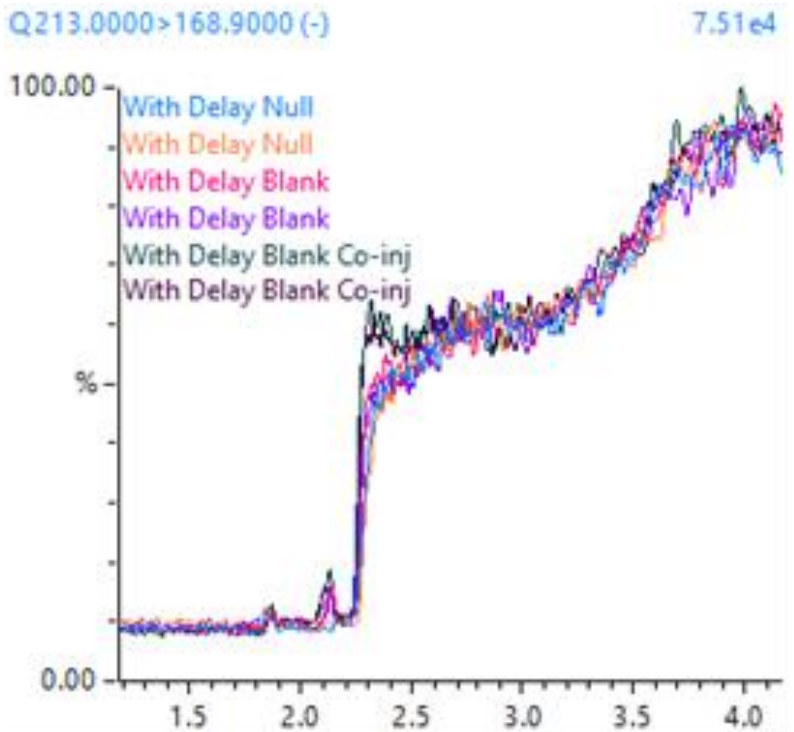
Systematic evaluation of PFAS in background

Example - PFBA

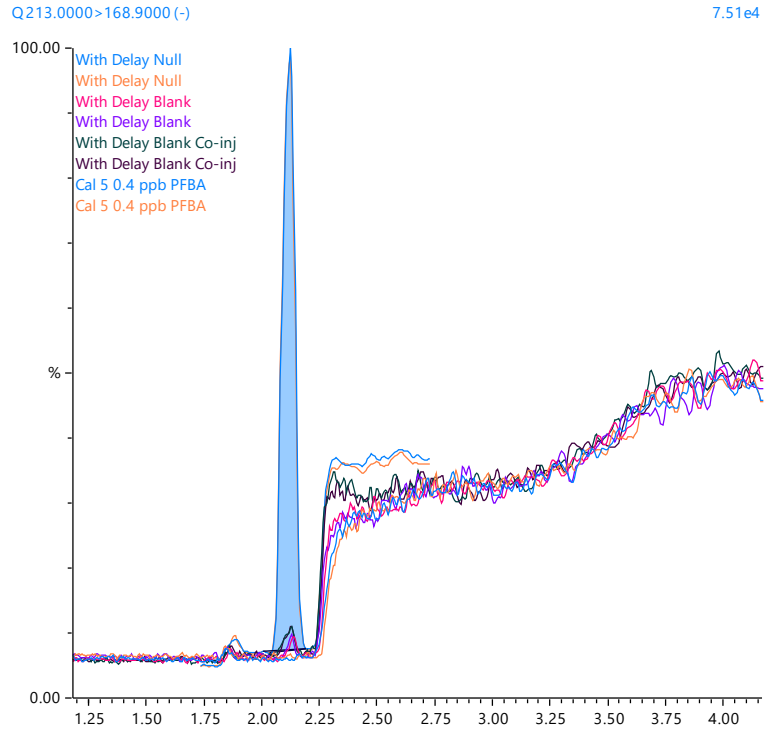
No delay column



With delay column



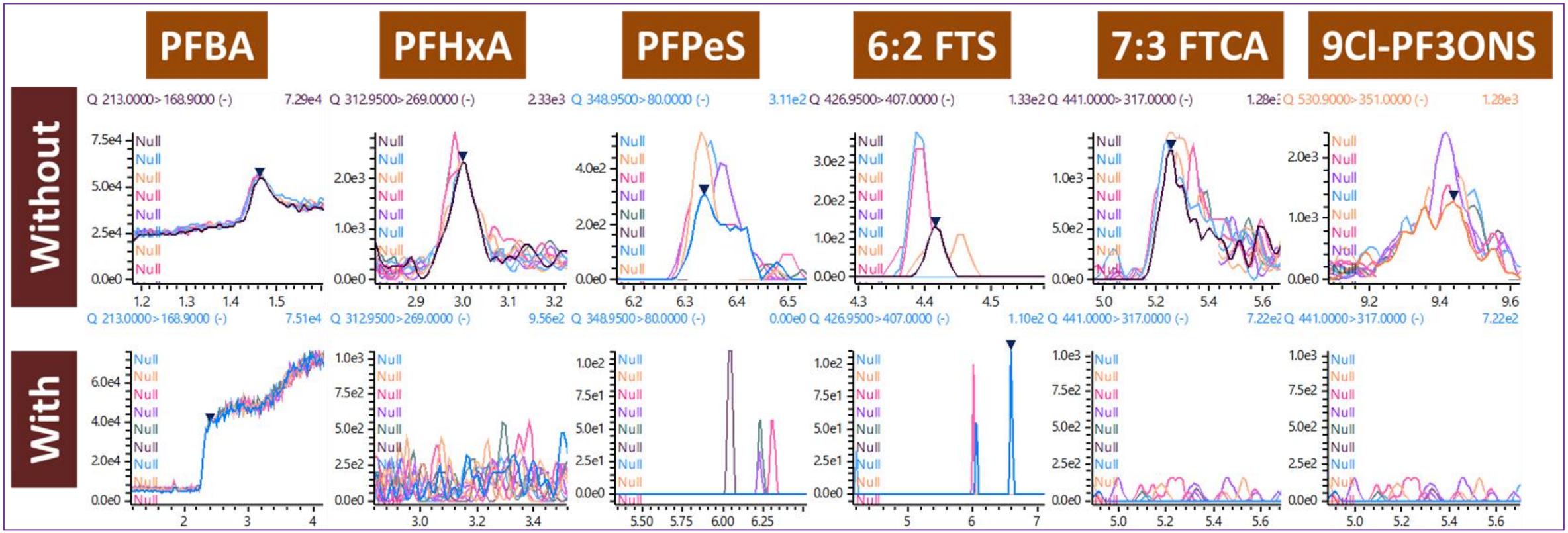
Standard, with delay column



Data in plots includes only n=2 per condition

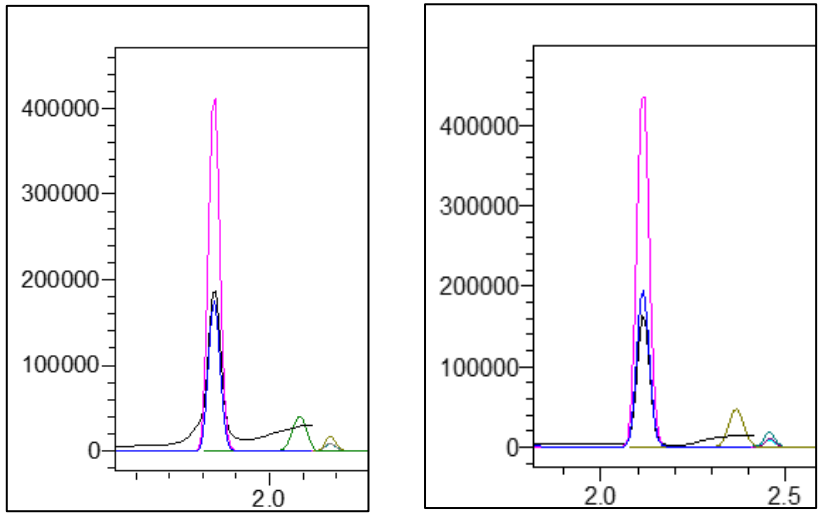
Let’s take a dive into…

PFAS detected in the background



What matters in a delay column?

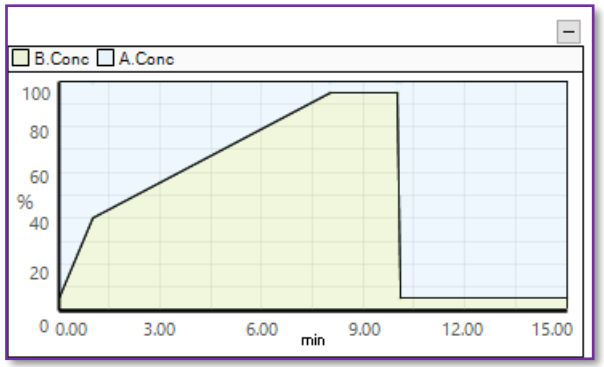
- Sufficient volume to retain/delay contaminants beyond the integrated peak or MRM window (phase, surface area, internal volume)
- High-pressure capability (column hardware, mechanical stability of particle)
- Minimal pressure contribution (particle size, column diameter, column length)

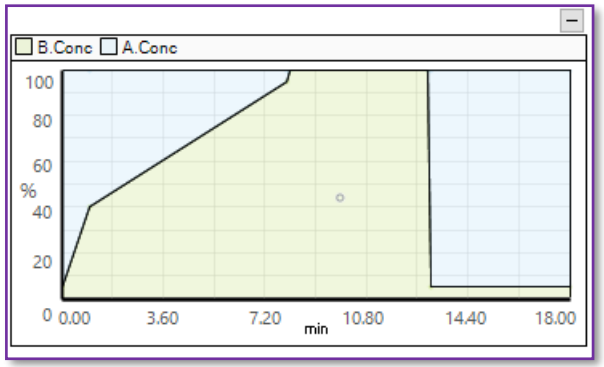


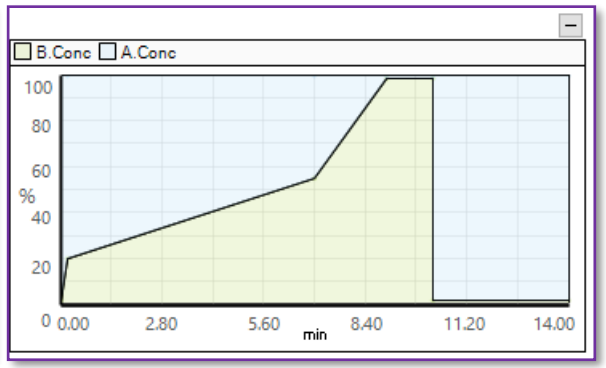
Different delay columns
Both results pass peak asymmetry requirements

Methods for PFAS Analysis

	EPA 533	EPA 537.1	EPA1633
Mobile phases	A: 5 mM Ammonium Acetate in Water B: Methanol	A: 5 mM Ammonium Acetate in Water B: Methanol	A: 2 mM Ammonium Acetate in Water B: Acetonitrile
Delay Column	**Continue listening to this presentation!**		
Analytical Column	Shim-pack GIST C18 3um, 2.1 x 50mm	Shim-pack Velox SP-C18 2.7um, 2.1 x 50mm	Shim-pack Scepter C18-120, 3um, 2.1 x 50mm





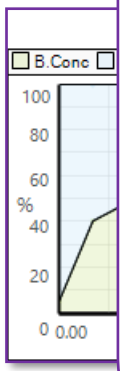


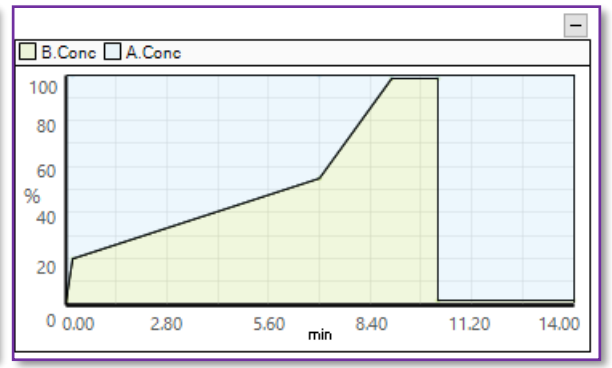
Methods for PFAS Analysis

	EPA1633
Mobile phases	A: 2 mM Ammonium Acetate in Water B: Acetonitrile
Delay Column	tation!
Analytical Column	Shim-pack Scepter C18-120, 3um, 2.1 x 50mm

EPA 1633

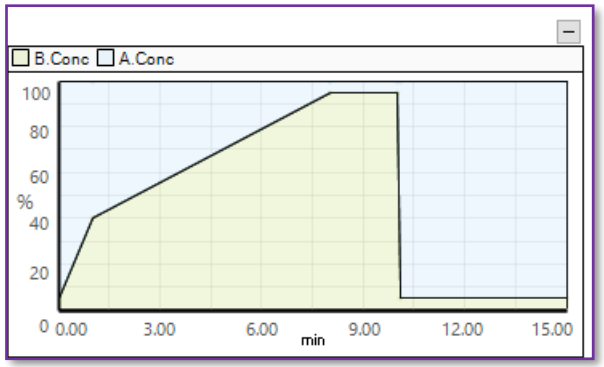
- Done in-house
- Shimadzu's LCMS-8060NX
- 5 delay columns tested
- Standard tubing
- Standards EPA cal 1
- Injection volume: 2 µL
- Replicates: n=7

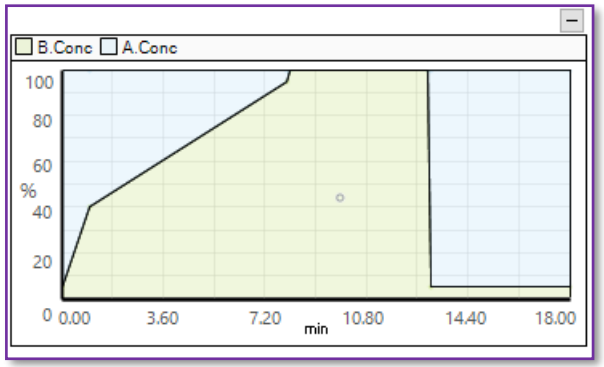




Methods for PFAS Analysis

	EPA 533	EPA 537.1
Mobile phases	A: 5 mM Ammonium Acetate in Water B: Methanol	A: 5 mM Ammonium Acetate in Water B: Methanol
Delay Column	**Continue listening to this present**	
Analytical Column	Shim-pack GIST C18 3um, 2.1 x 50mm	Shim-pack Velox SP-C18 2.7um, 2.1 x 50mm





EPA 533 and 537.1

- Sent selected delay columns to >4 customers
- Shimadzu's LCMS-8050, LCMS-8060, LCMS-8060NX
- Varied configurations (PFAS tubing kit, with and without degasser, standard tubing)
- Standards and samples analyzed
- EPA 537.1, 533, and 1633

Parameters evaluated

1) Max Pressure
2) Back Pressure
3) Peak Asymmetry (early eluter)
4) Resilience

The PFAS Delay Column Relay

GIST 5um 50x3.0

Nexcol 5um 50x3.0

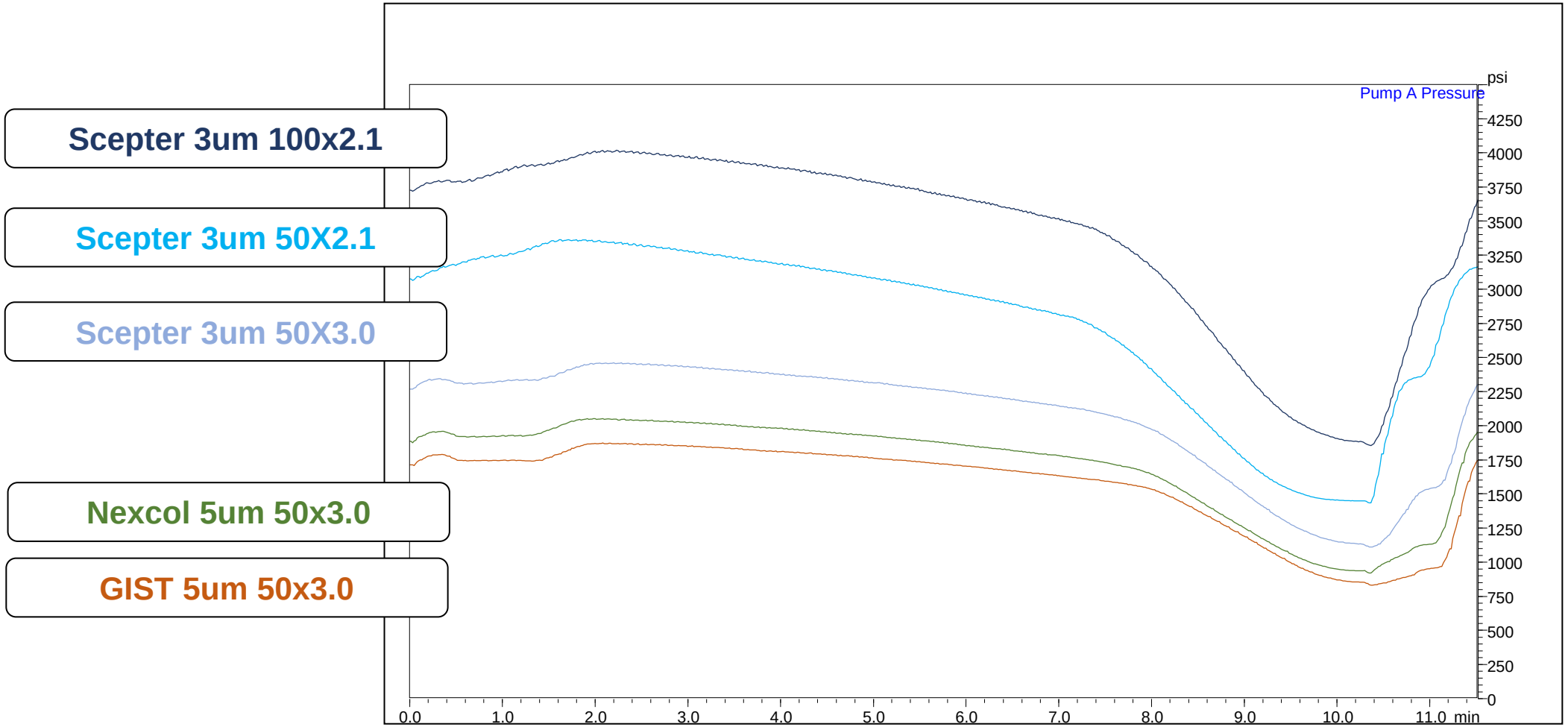
Scepter 3um 50X3.0

Scepter 3um 50X2.1

Scepter 3um 100x2.1

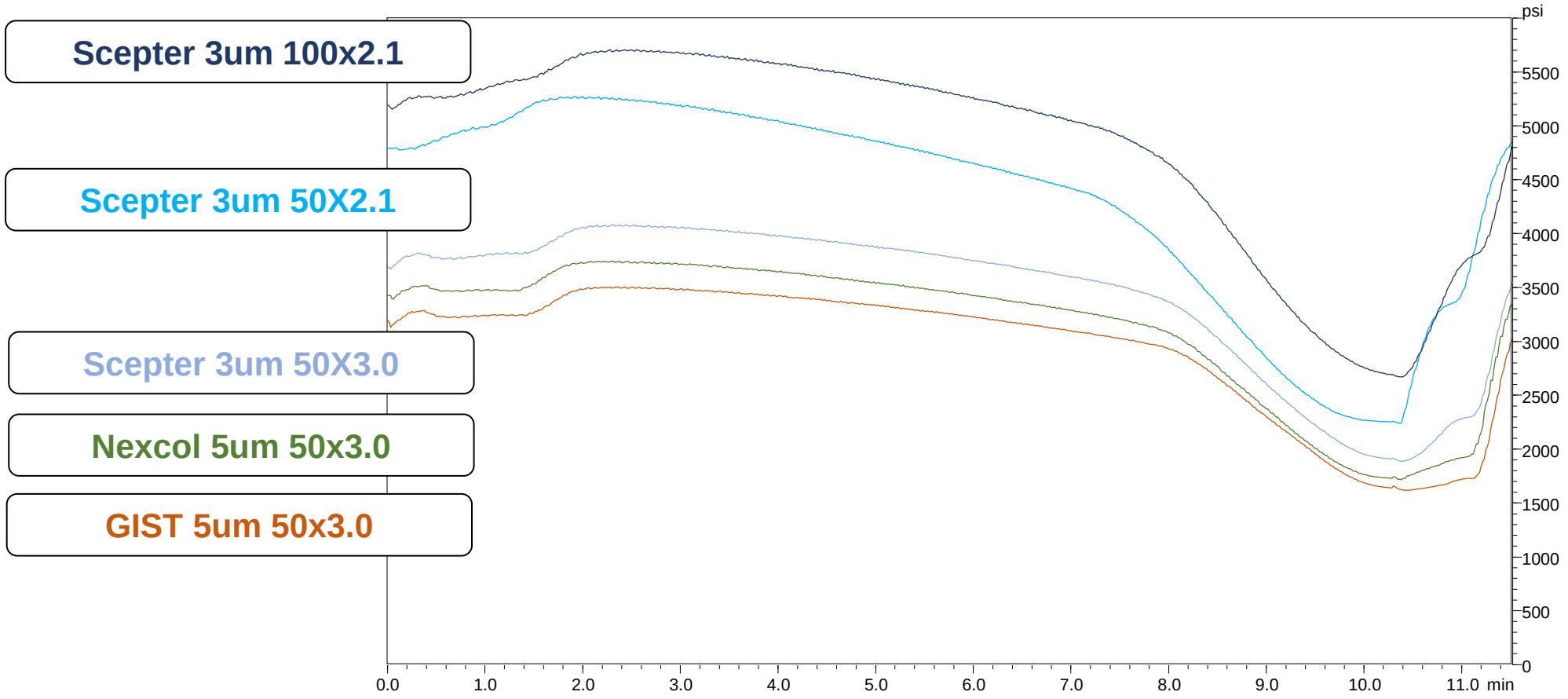
Pressure traces with various delay columns

Scepter analytical column C18-120 3µm 2.1X50mm

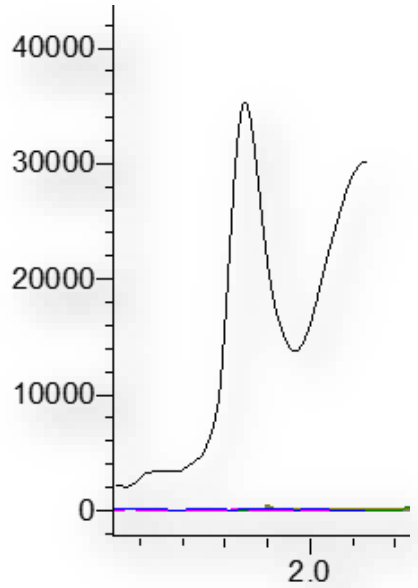


Pressure traces with various delay columns

Scepter analytical column C18-120 1.9µm 2.0X50mm

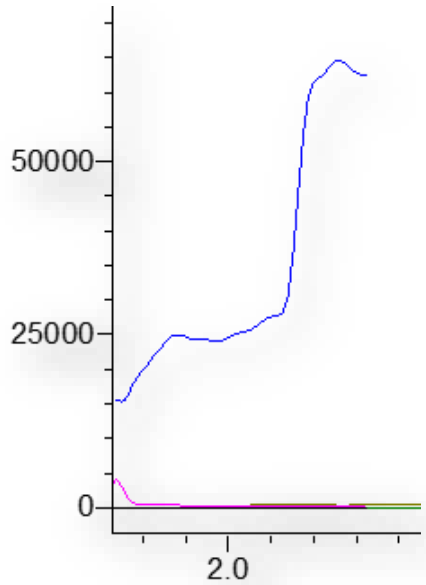


Influence of Delay Columns on PFBA (Solvent Blank)



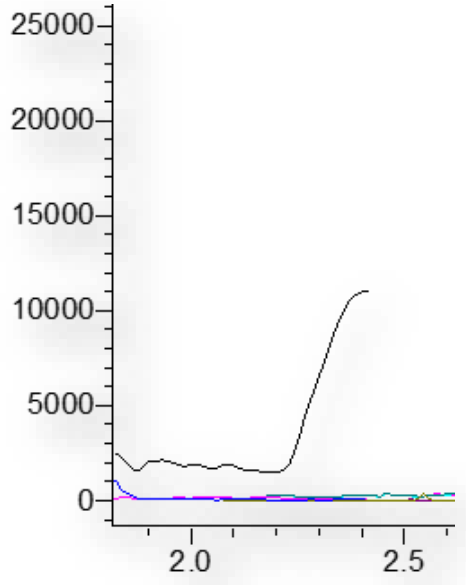
Shim-Pack
Scepter 3um 50X2.1

Insufficient delay



Shim-Pack
GIST 5um 50x3.0

Good delay
High baseline



Nexcol 5um 50x3.0

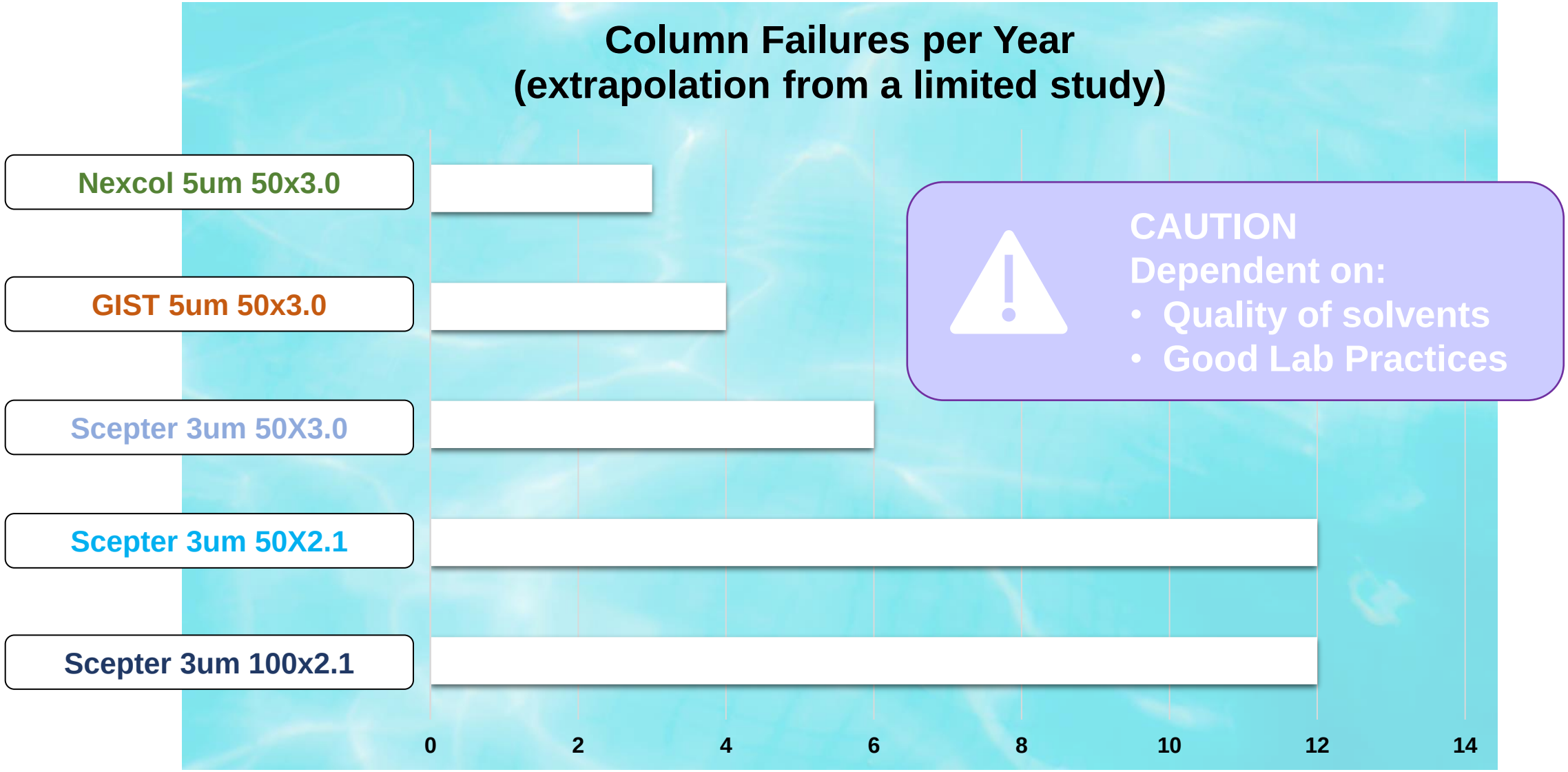
Good delay
Good baseline

Resilience

- Determined by failure frequency in house and in the field
  - High pressure originating at delay column
  - Delay column leaking
  - Adverse affects to chromatography

- What causes failures?
  - Mobile phase contaminants
  - Smaller particles foul more quickly
  - High pressures cause column stress

Resilience

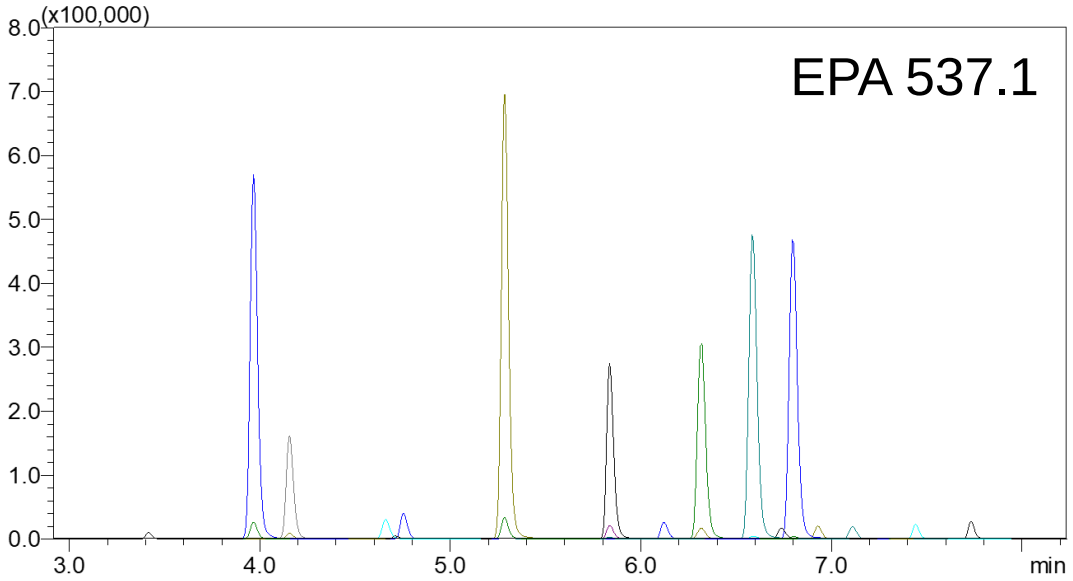

Column Failures per Year
(extrapolation from a limited study)
Nexcol 5um 50x3.0
GIST 5um 50x3.0
Scepter 3um 50X3.0
Scepter 3um 50X2.1
Scepter 3um 100x2.1
0
2
4
6
8
10
12
14
CAUTION
Dependent on:
• Quality of solvents
• Good Lab Practices

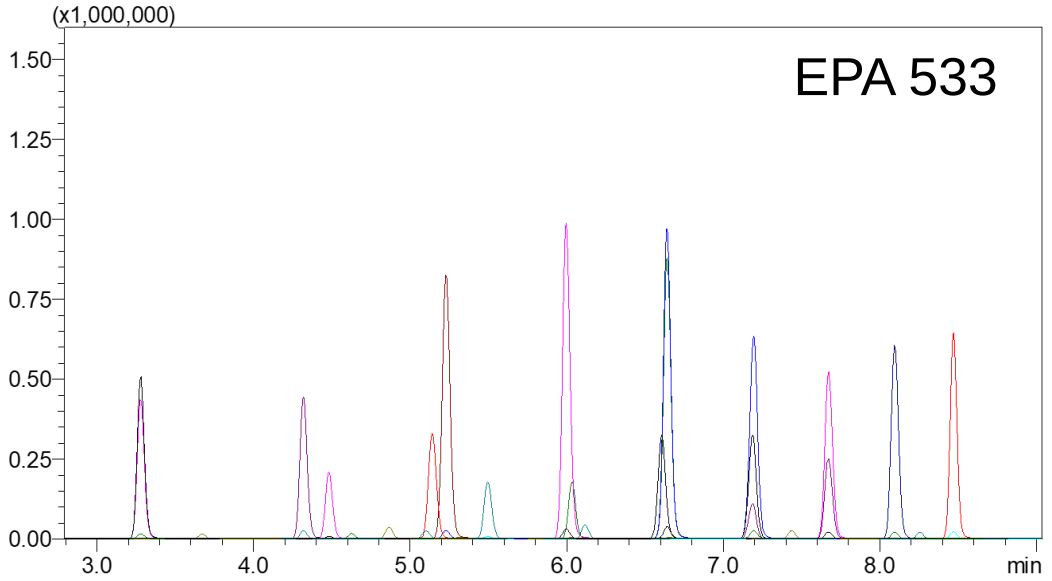
Summary Outcomes

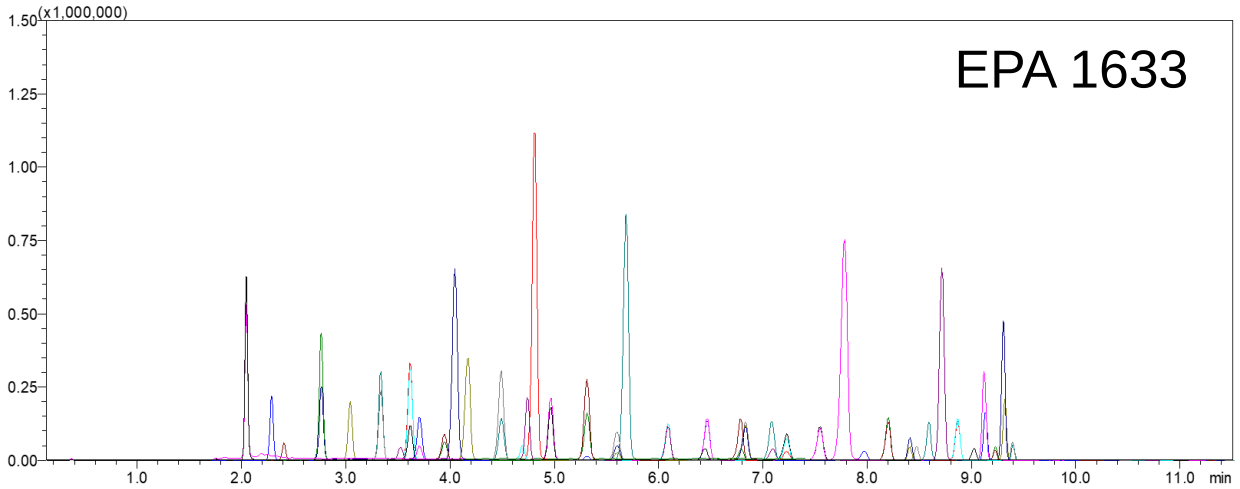
Delay Column	Dimensions (L x D; particle size)	Frit Housing	Max Press	Back Press	Asymmetry	Resilience
Scepter	100 x 2.1 mm; 3 µm	PEEK	+++	+	++	+
Scepter	50 x 2.1 mm; 3 µm	PEEK	+++	+	+	+
Scepter	50 x 3.0 mm; 3 µm	PEEK	++	++	++	++
GIST	50 x 3.0 mm; 5 µm	PEEK	+	++++	++	+++
Nexcol	50 x 3.0 mm; 5 µm	Stainless	++++	+++	+++	++++

The PFAS Delay Column Relay - Results

Lowest Calibration Level with Nexcol Delay Column







Common PFAS at the Lowest Calibration Level

With Nexcol Delay Column

Sources of PFAS Contamination

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After the autosampler

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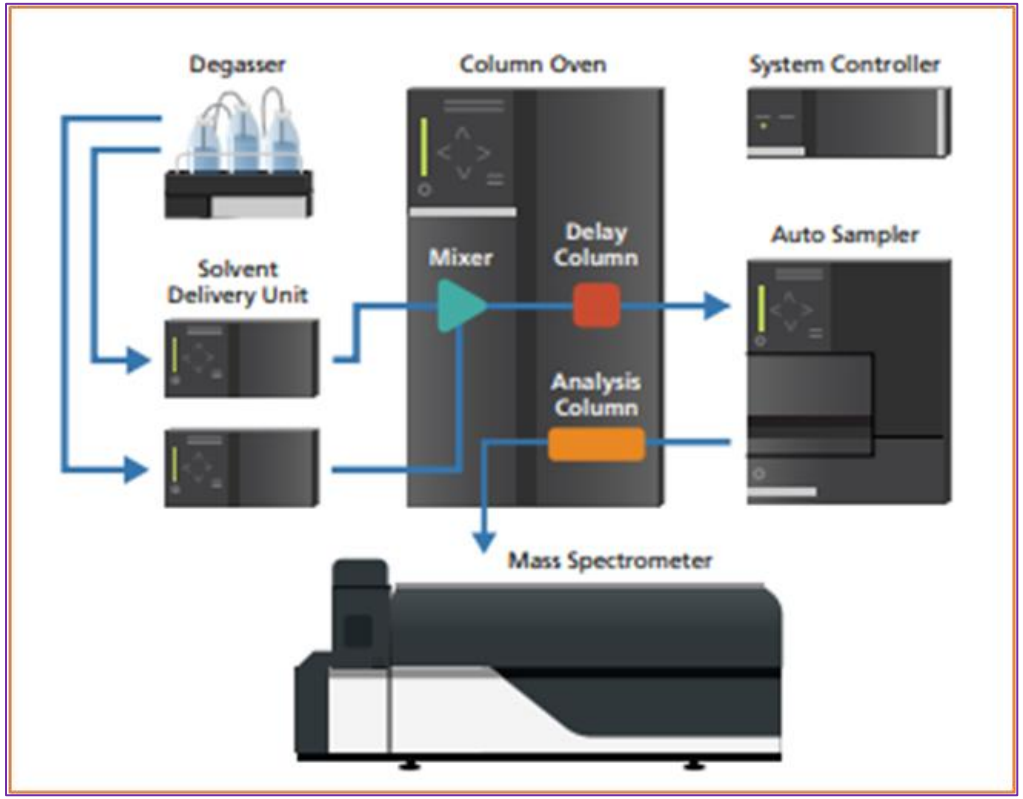


Mitigated with the help of a delay column

TEST YOUR CONSUMABLES!

A few shots before closing the presentation

Solvents - LC Grade vs LCMS Grade Acetonitrile

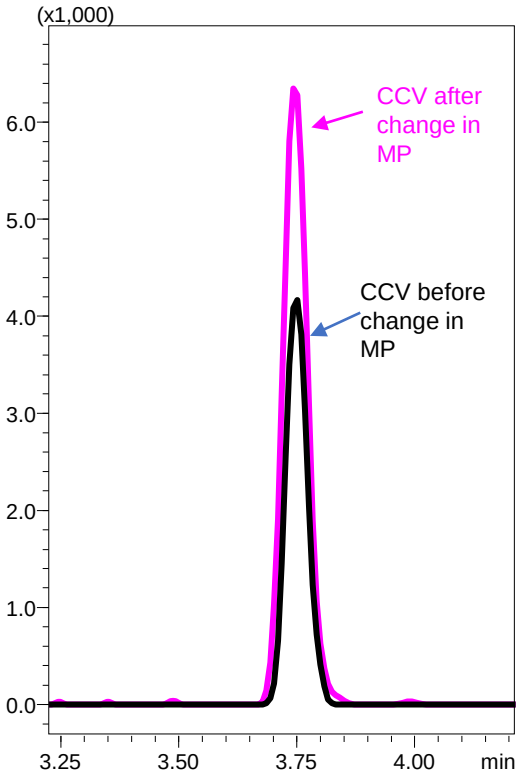
%RSD >20
%RSD 15-20
%RSD <15%

Name	LC grade ACN %RSD (Area)	LCMS grade ACN %RSD (Area)	Name	LC grade ACN %RSD (Area)	LCMS grade ACN %RSD (Area)
PFBA	31.8	4.7	NMeFOSAA	6.4	2.7
PFMPA	29.6	5.1	PFDA	7.2	3.4
3:3 FTCA	34.0	2.2	NEtFOSAA	5.0	5.8
PFPeA	22.0	3.6	PFOS	11.8	3.0
PFMBA	22.0	4.8	PFUnA	11.4	2.9
4-2 FTS	21.0	6.6	9Cl-PF3ONS	10.8	2.5
NFDHA	7.1	4.6	PFNS	16.1	4.0
PFHxA	19.1	6.5	PFDOA	13.3	2.0
PFBS	20.9	7.2	PFOSA	16.1	7.4
HFPO-DA	14.2	5.0	PFDS	15.5	4.5
5:3 FTCA	19.2	2.8	PFTrDA	11.2	1.2
PFEESA	21.0	4.1	11Cl-PF3OUdS	16.6	1.6
PFHpA	22.7	5.4	PFTeDA	13.3	2.3
PFPeS	21.8	5.6	PFDOS	10.9	7.2
ADONA	20.4	6.6	NMeFOSE	7.7	8.1
6-2 FTS	17.3	2.1	NMeFOSA	16.8	10.5
PFOA	16.3	5.0	NEtFOSE	3.9	8.3
PFHxS	11.8	5.7	NEtFOSA	17.2	6.1
7:3 FTCA	9.8	3.6	10 FTS	10.6	6.2
PFNA	9.2	4.5	PFHxDA	11.7	5.3
PFHpS	7.9	5.6	PFODA	7.7	3.1
8-2 FTS	4.0	1.6			

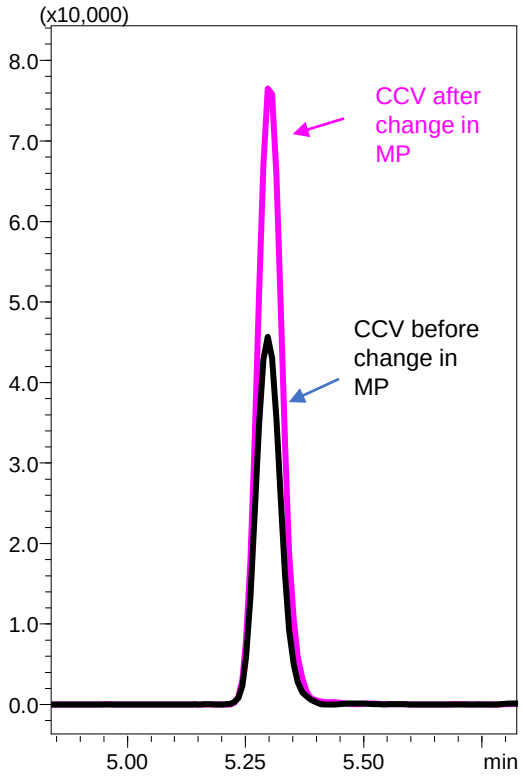
Robust and reproducible results with LCMS grade solvent

Solvents - Mobile Phase Lot Variability

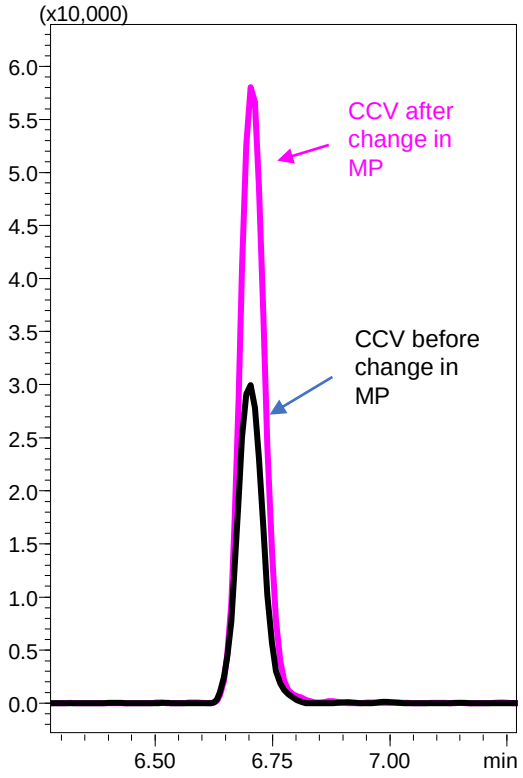
4-2 FTS



6-2 FTS



8-2 FTS



Different lot of solvents may affect the signal intensity

Final thoughts on Delay Columns – Don’t forget!

- A delay column is an analytical column
- Delay columns fail
  - Guard column could help extending lifetime
- Delay columns are not interchangeable unless the inner volume is similar
- Troubleshooting the performance of a delay column is more difficult than an analytical column

Acknowledgements

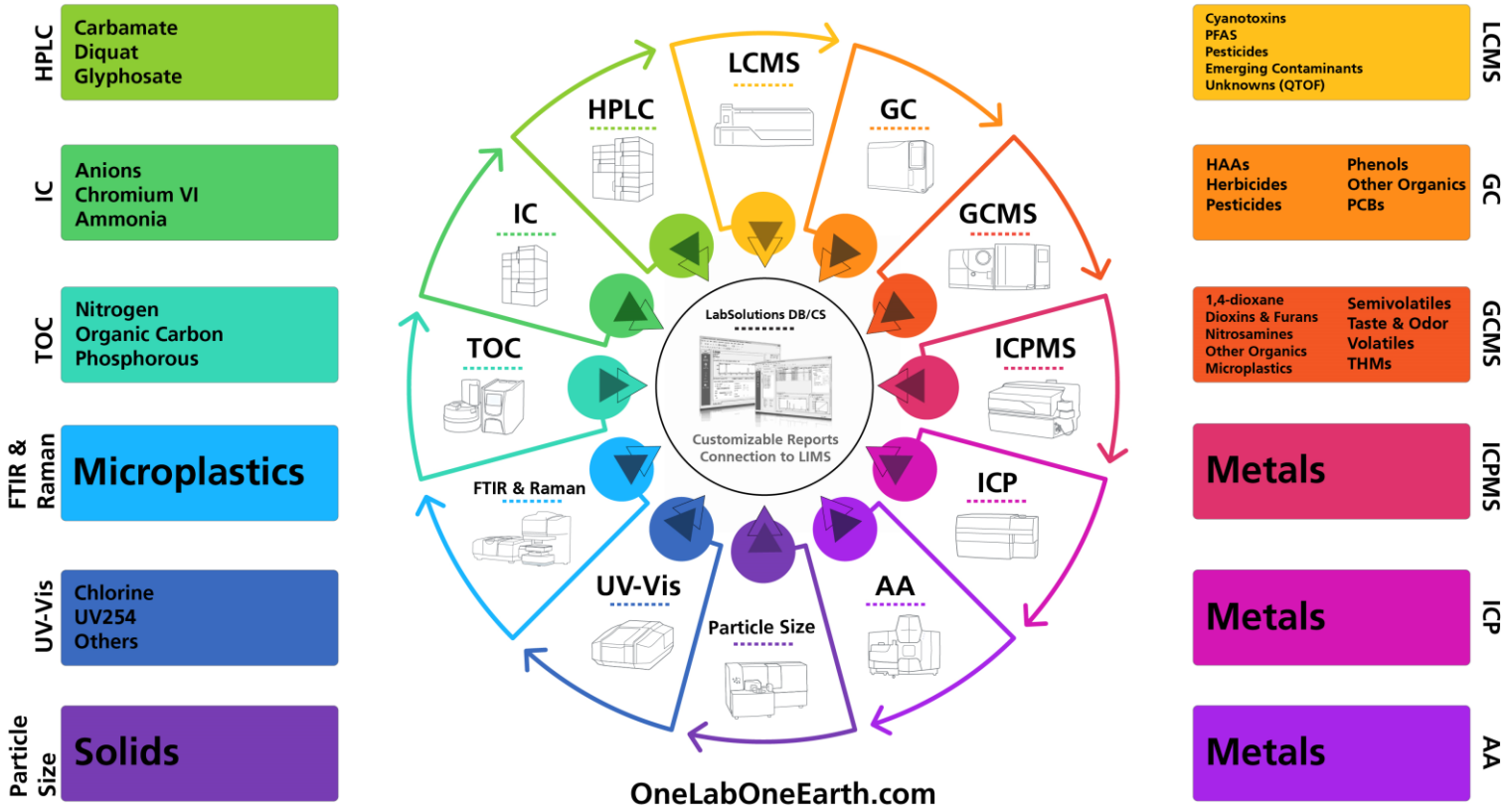
Om Shrestha

Kathleen Luo

Landon Wiest

Megan Davis

… and the customers
and teams in the field



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