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Lessons Learned from a PFAS: Stress Test of LCMS Instruments

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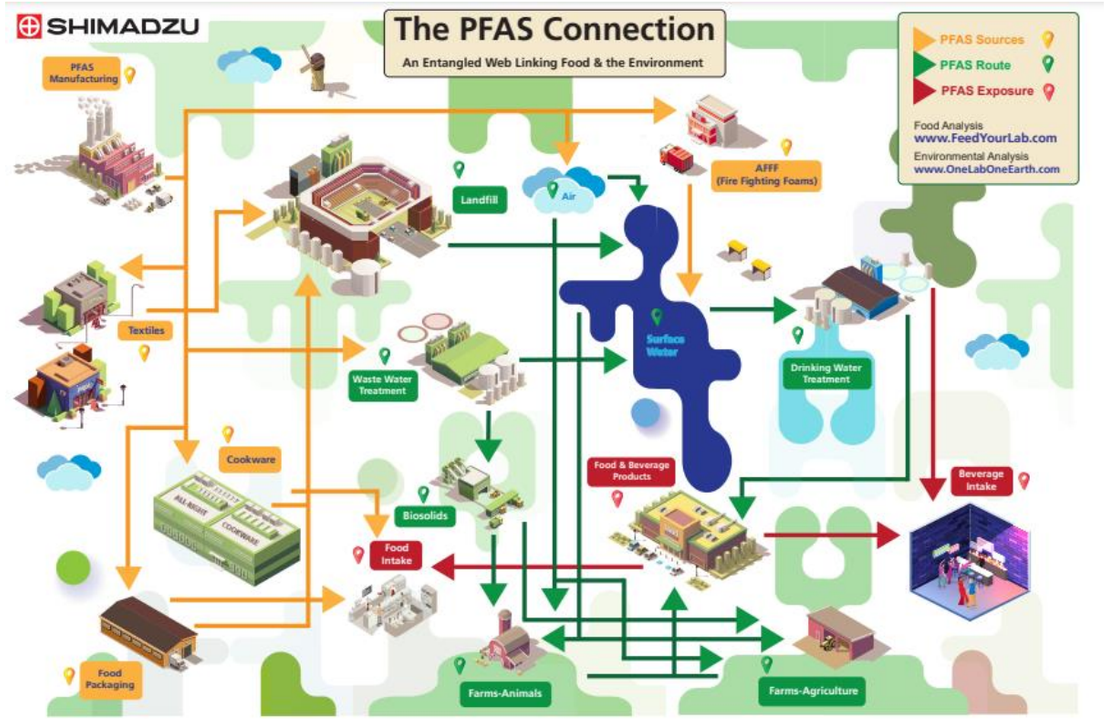
In today's presentation

1. Why PFAS?
2. Experimental plan
3. Results
4. Conclusions
5. Q&A

Why PFAS?

- ✓ PFAS are still the MOST RELEVANT Contaminants of Emerging Concern
- ✓ There is a large amount of funds from the Infrastructure Bill that will be allocated to PFAS remediation and treatment
- ✓ There are expected new regulations affecting all stakeholders in the environmental industry (and others)
- ✓ **There is a need for robust analytical workflows to address upcoming monitoring demands**



Why PFAS?

Recap from NEMC 2021…

A systematic evaluation of the sources of PFAS in the laboratory

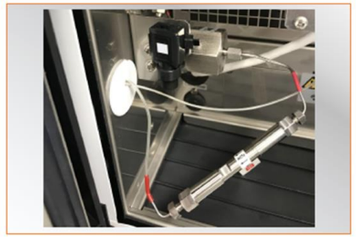
Targets – EPA 533

Find list of targets here

Tubing Type	With Degasser		Without Degasser	
	Without Delay Column	With Delay Column	Without Delay Column	With Delay Column
PEEK			✓	✓
FEP	✓	✓		
LLDPE	✓	✓	✓	

FEP: Fluorinated Ethylene Propylene

- ✓ All types of tested tubing displayed PFAS compounds
- ✓ Installation of delay column eliminates measurable PFAS background from all types of tubing tested - **Non-fluorinated tubing is not essential!**



Experimental Plan

Go back to basics, to re-evaluate instruments' performance (including sensitivity) for EPA 537.1 and EPA 533:

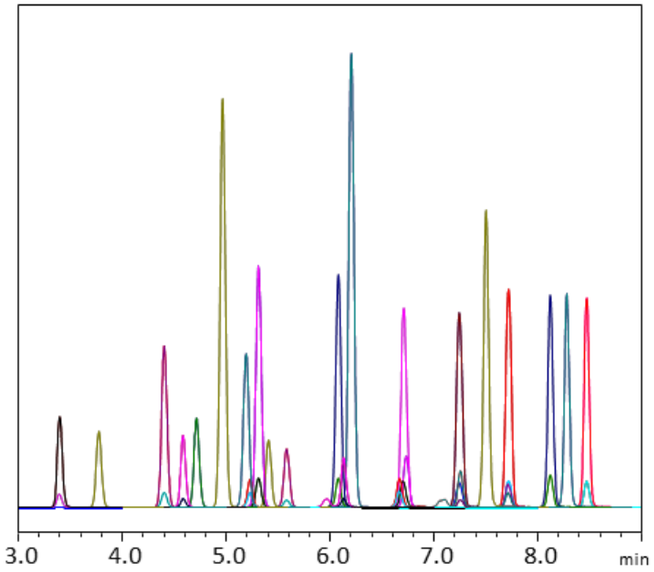
1. Instrumental analysis
2. Sample preparation

→

1. Crosstalk
2. Stress tests
3. Contamination sources

Experimental Plan – EPA 533

Nexera HPLC	Parameters	LCMS-8050	Parameters
Analytical Column	Shim-pack GIST C18, 3 um, 2.1 x 50mm	**Ion source**	ESI
Delay Column	Shim-pack GIST C18 50mmL. x 3.0mmID 5um, 227-30015-03	**Polarity**	(-)
Flow rate	0.25 mL/min	**Interface temperature**	100 °C
Mobile phase A	5 mM ammonium acetate in water	**DL temperature**	150 °C
Mobile phase B	Methanol	**Heat block temperature**	250 °C
Gradient	5-95% B	**Capillary position**	+1 mm
Column Temp	45 °C	**Run time**	15 min

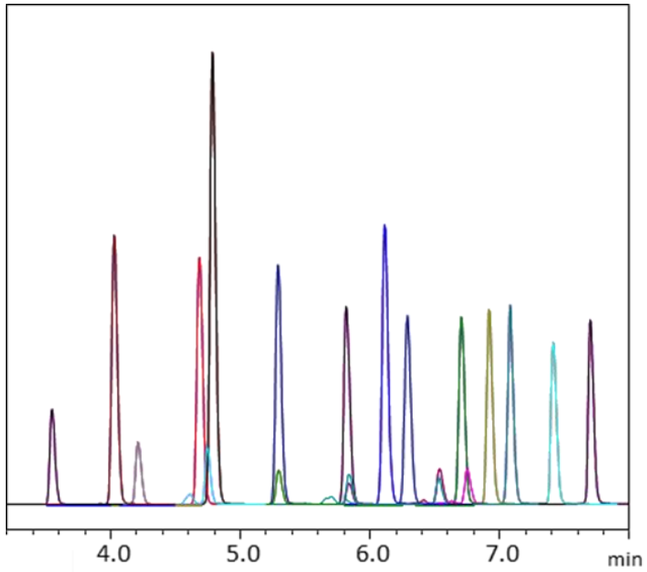


Cycle time: 15 min
Original cycle time in EPA method: 35 min

Additional method info

Experimental Plan – EPA 537.1

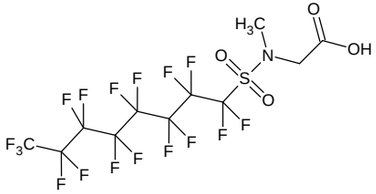
Nexera HPLC	Parameters	LCMS-8050	Parameters
Column	Shim-pack Velox SP-C18, 2.7 um, 2.1 x 50mm	**Ion source**	ESI
Delay Column	Shim-pack GIST C18 50mmL. x 3.0mmID 5um, 227-30015-03	**Polarity**	(-)
Flow rate	0.25 mL/min	**Interface temperature**	100 °C
Mobile phase A	5 mM ammonium acetate in water	**DL temperature**	150 °C
Mobile phase B	Methanol	**Heat block temperature**	250 °C
Gradient	5-100% B	**Capillary position**	+1 mm
Column Temp	45 °C	**Run time**	18 min



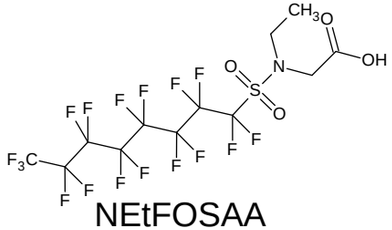
Cycle time: 18 min
Original cycle time in EPA method: 37 min

Additional method info

- ✓ Cycle time 3 min longer than EPA 533
  - 3 min additional MeOH rinse to minimize carry-over observed from NMeFOSAA and NEtFOSAA.

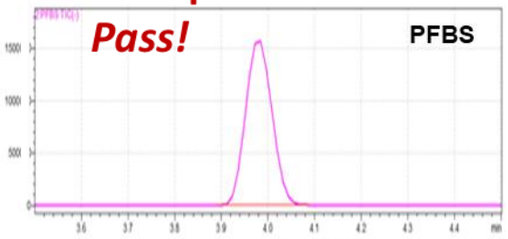


NMeFOSAA



NEtFOSAA

Peak shape?



Asymmetry: 1.16
EPA requirement: 0.8 - 1.5

Separation of isomers?
Pass!

Results – Crosstalk

Crosstalk occurs when two successive MRM transitions share a common product ion and the ions from the first MRM are still present in the final quadrupole when the second MRM is being acquired.

This phenomenon can cause FALSE POSITIVES or increase area counts of compound affected.

Results – Crosstalk

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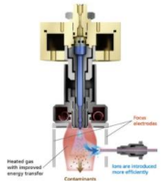
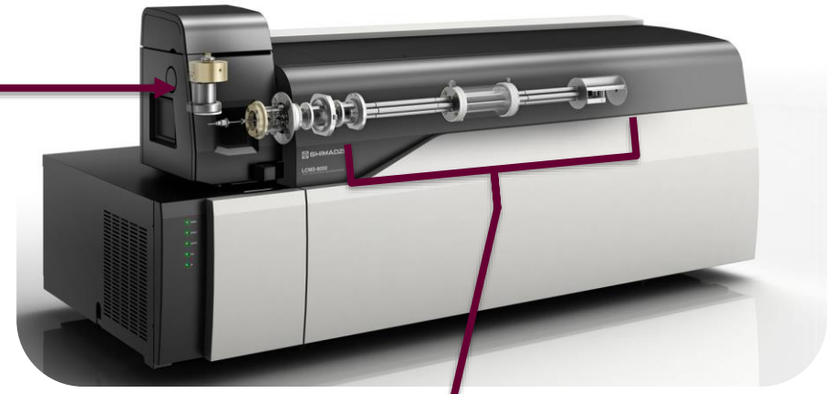
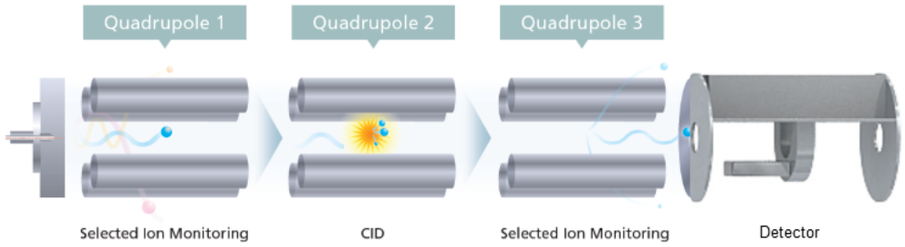
This phenomenon can cause FALSE POSITIVES or increase area counts of compound affected.



Electrospray Ionization Source



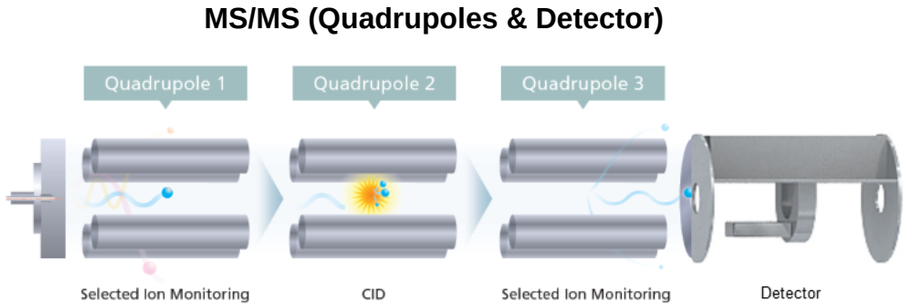
MS/MS (Quadrupoles & Detector)



Q1 **Q3**

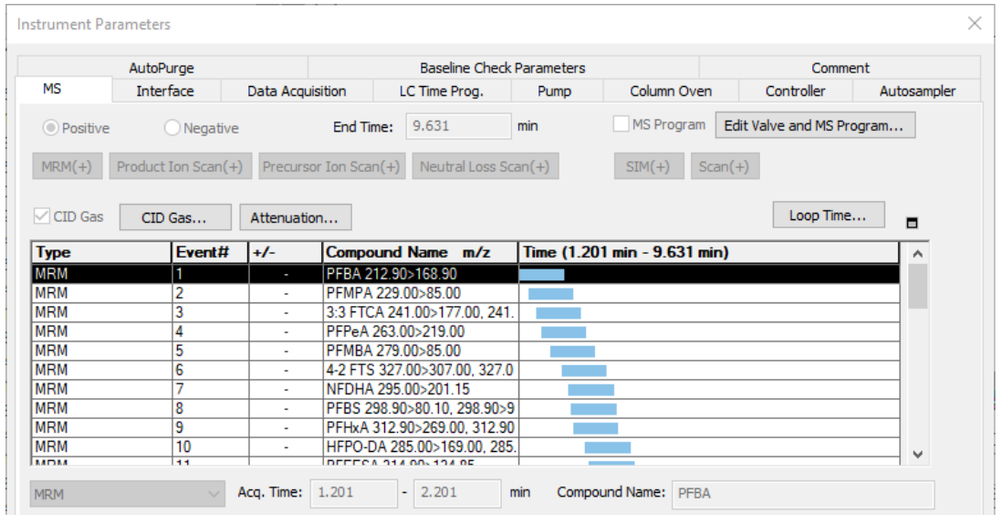
MRM: m/z parent ion → m/z product ion

Results – Crosstalk



Q1 **Q3**

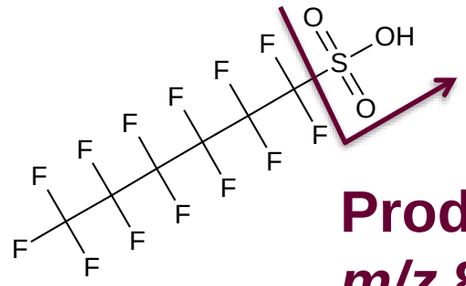
MRM: m/z parent ion → m/z product ion



PFHpS

RT $_{533}$= 6.80

m/z = 440

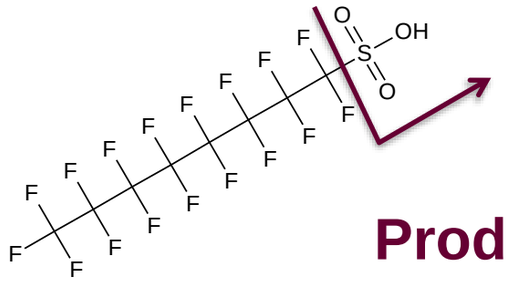


Product ion *m/z* 80

PFOS

RT $_{533}$= 7.32

m/z = 499



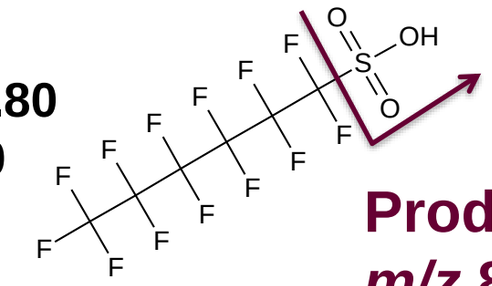
Product ion *m/z* 80

Creating Conditions for Crosstalk

PFHpS

RT $_{533}$= 6.80

m/z = 440



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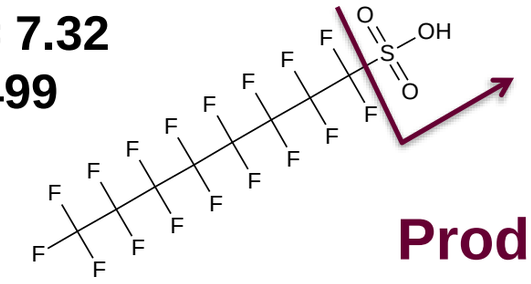
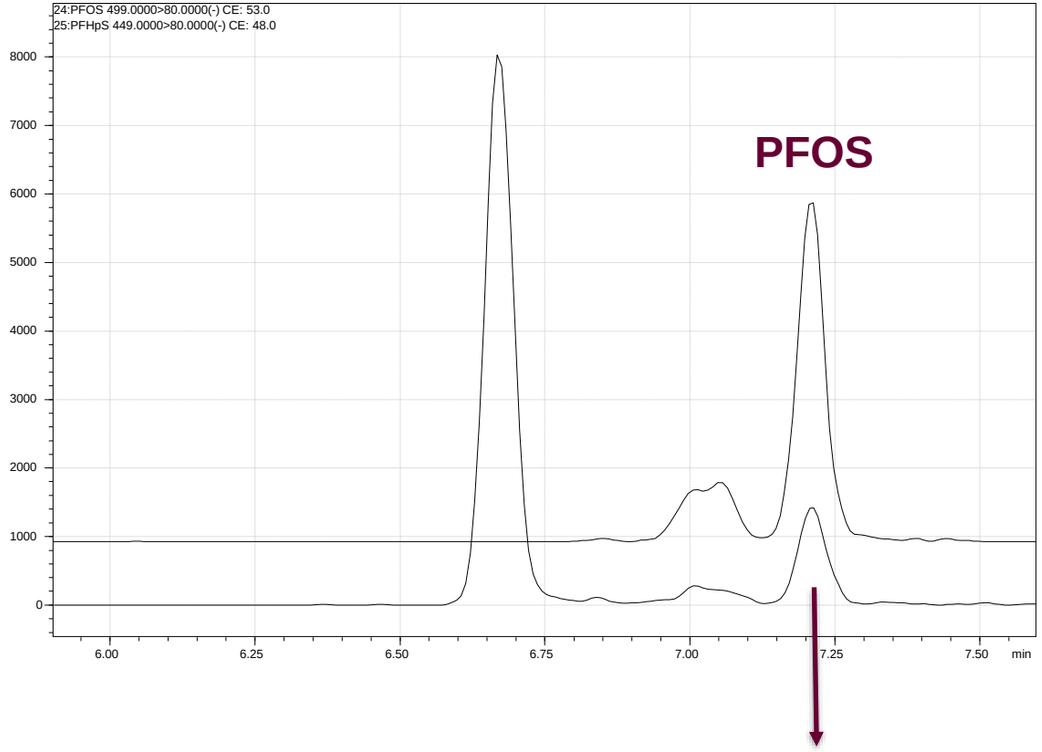


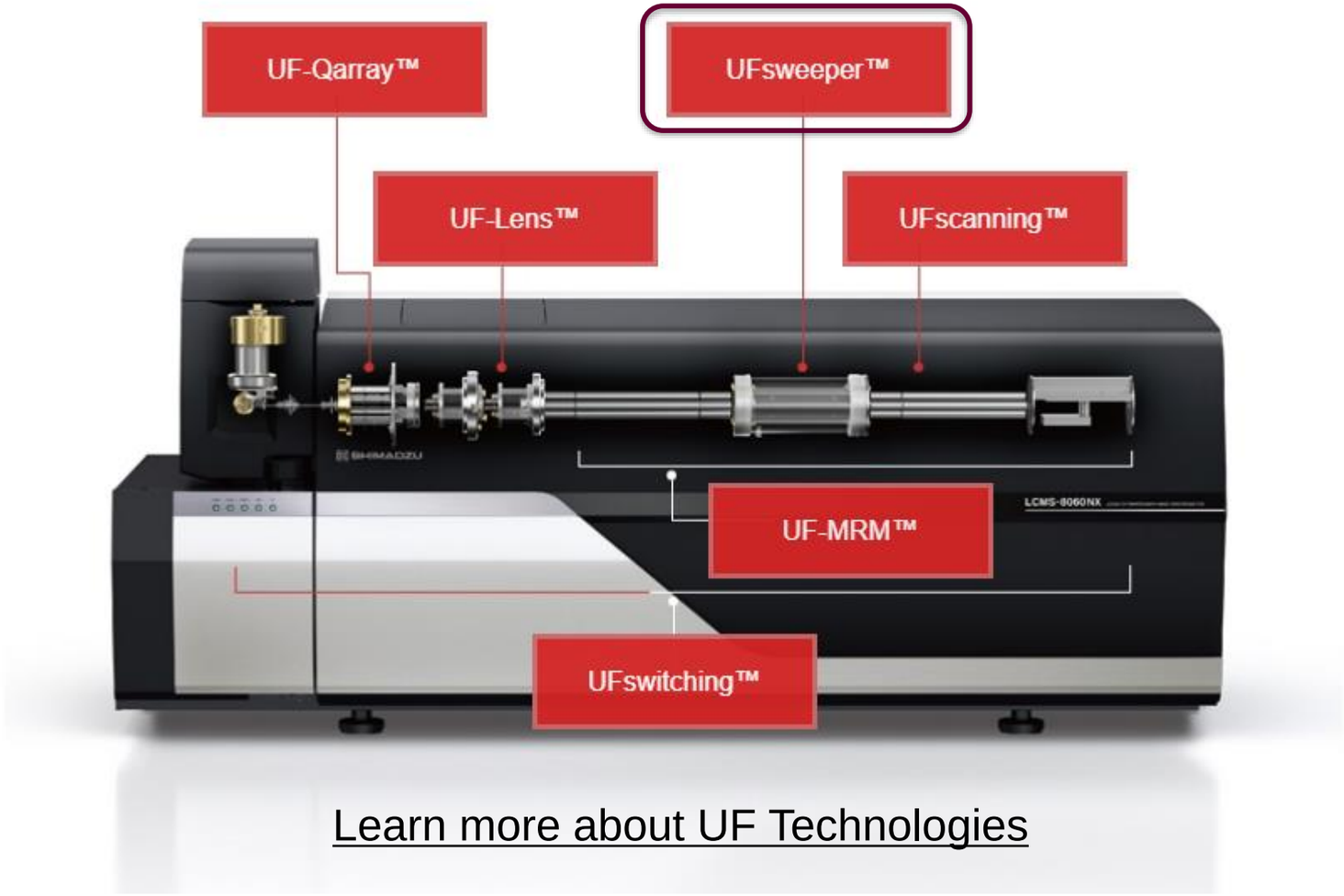
Product ion *m/z* 80

PFHpS



Crosstalk invoked

Results - Crosstalk



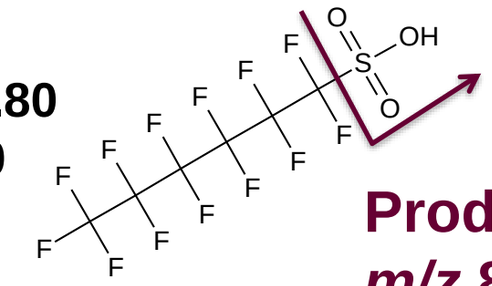
Learn more about UF Technologies

Crosstalk Demonstrated

PFHpS

RT $_{533}$= 6.80

m/z = 440



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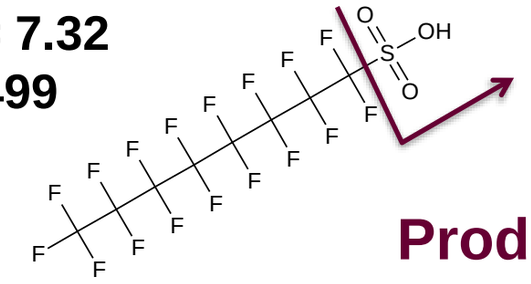

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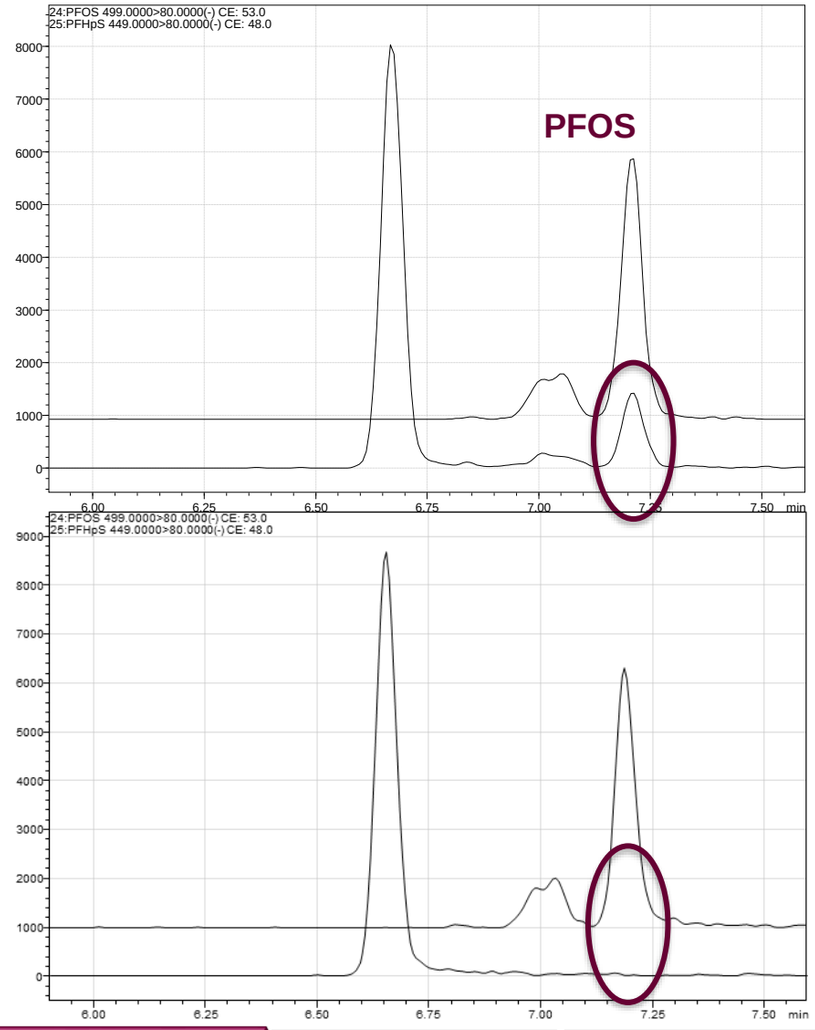
PFHpS



Crosstalk Shown (UF Sweeper Off)

Normal Conditions (UF Sweeper On)

Results – Contamination – STRESS TEST!

GOAL: Demonstrate how to manage contamination from HPLC, by spiking a known amount of PFAS in instrument

Experimental approach

- Method 533 conditions
  - Delay column – Shim-pack GIST C18, 5um, 3.0mm x 50mm
  - Analytical column - Shim-pack GIST C18, 3 um, 2.1 x 50mm
- Flow: 0.25 mL/min
- Both mobile phases spiked
- Concentration spike: 20 ppb analytical standard mix

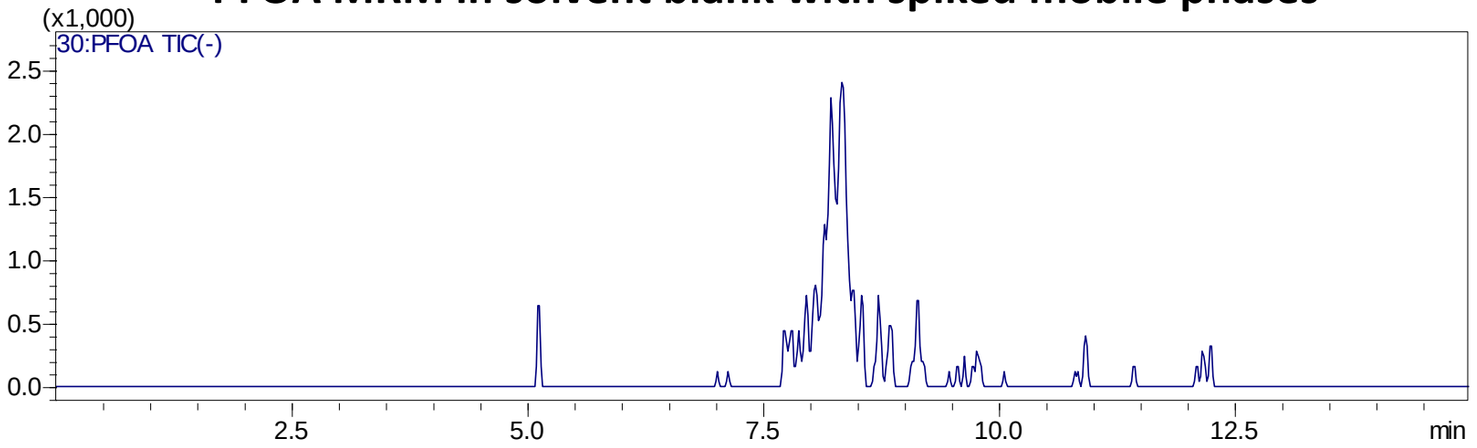
Results – Contamination – STRESS TEST!

- Spiked MP flowing through system before injections
- Mass of individual PFAS flowing through system: 5 ng/min
- HPLC configuration
- System recovery after test: standard methanol flush

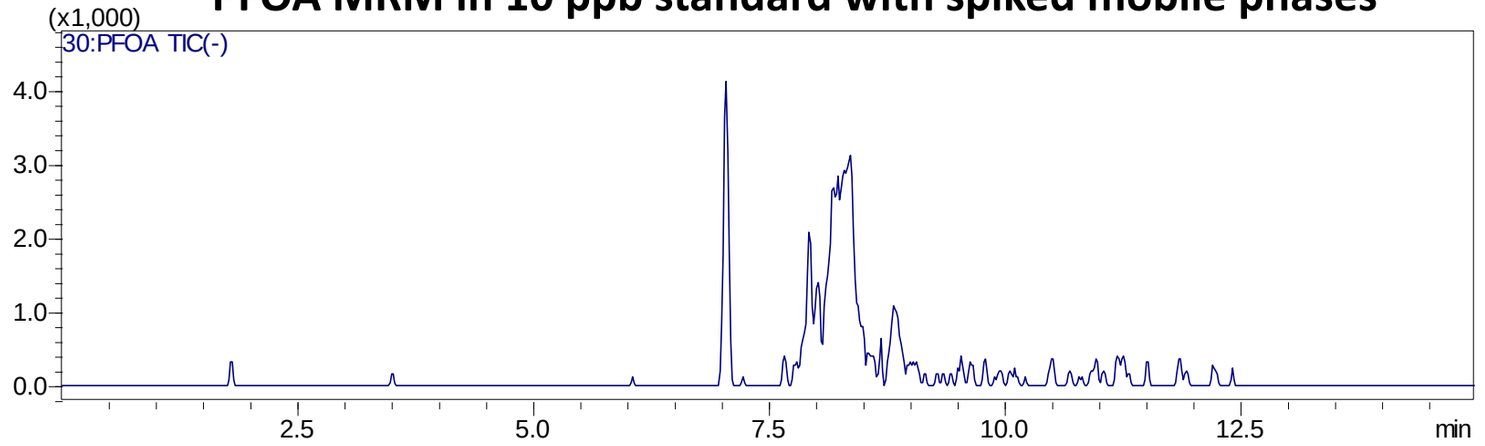
Optional kit for PFAS analysis

Results – Contamination – STRESS TEST!

PFOA MRM in solvent blank with spiked mobile phases



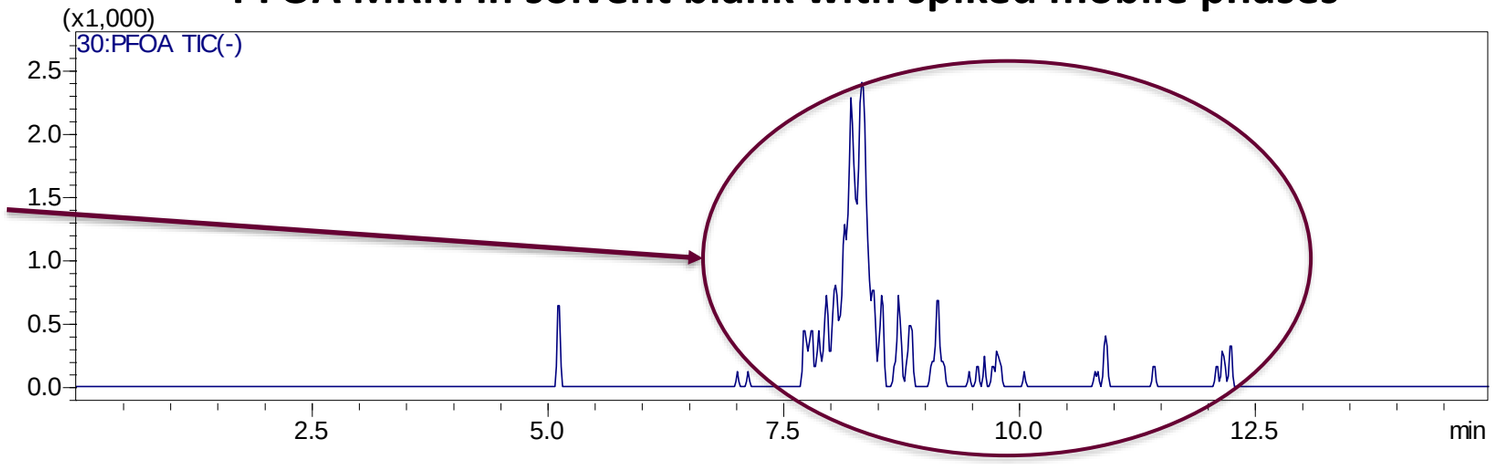
PFOA MRM in 10 ppb standard with spiked mobile phases



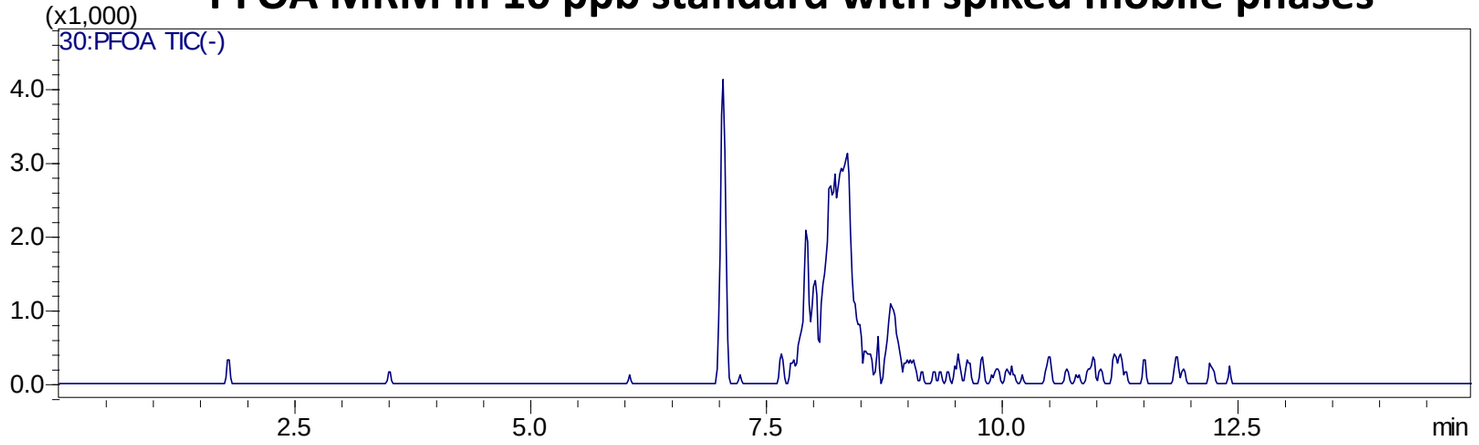
Results – Contamination – STRESS TEST!

Contamination from spike in mobile phase

PFOA MRM in solvent blank with spiked mobile phases

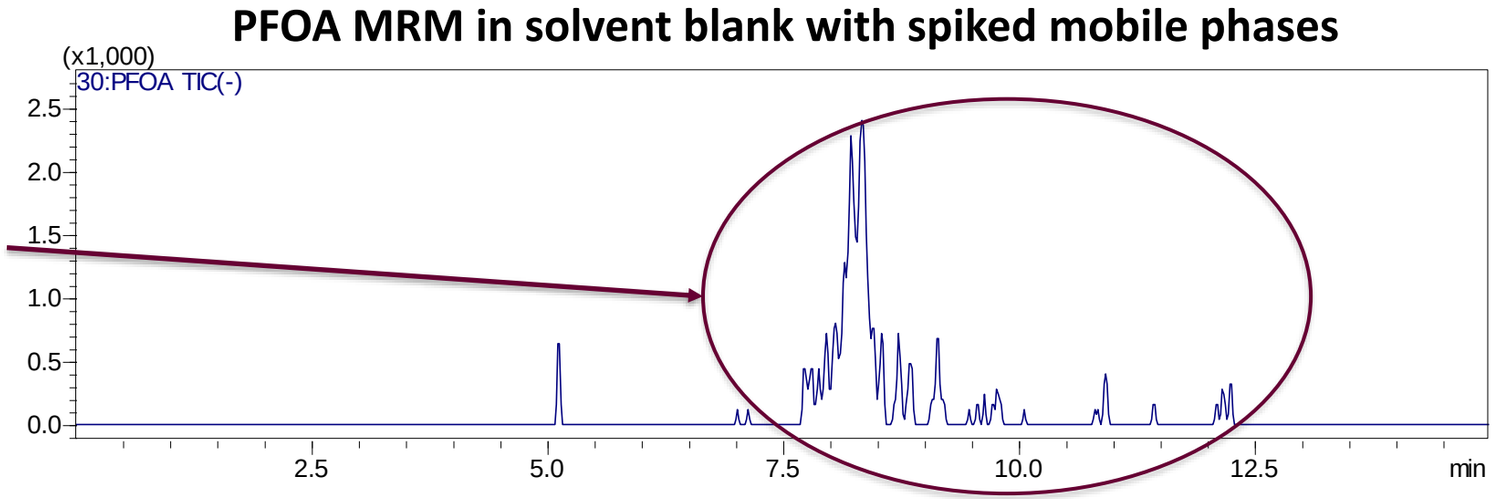


PFOA MRM in 10 ppb standard with spiked mobile phases

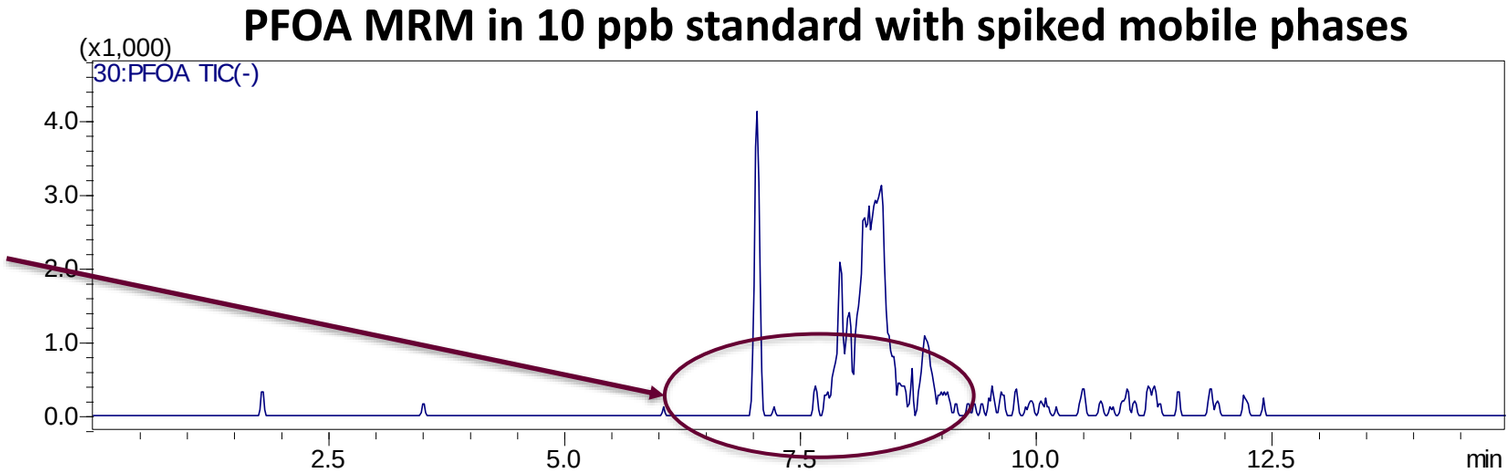


Results – Contamination – STRESS TEST!

Contamination from spike in mobile phase



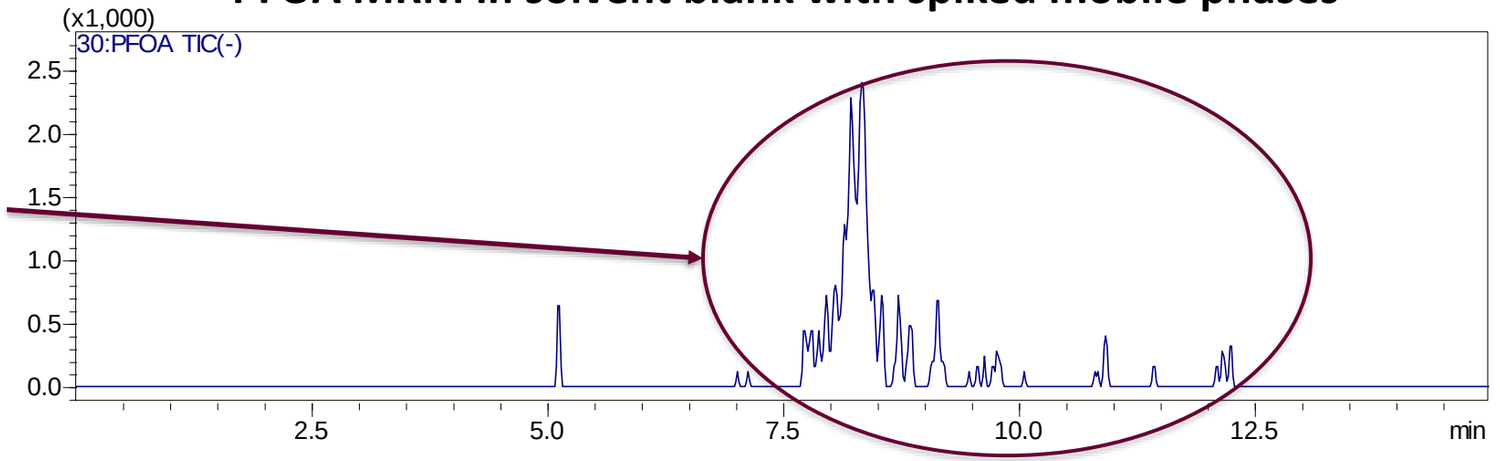
Separation between target and contamination



Results – Contamination – STRESS TEST!

Contamination from spike in mobile phase

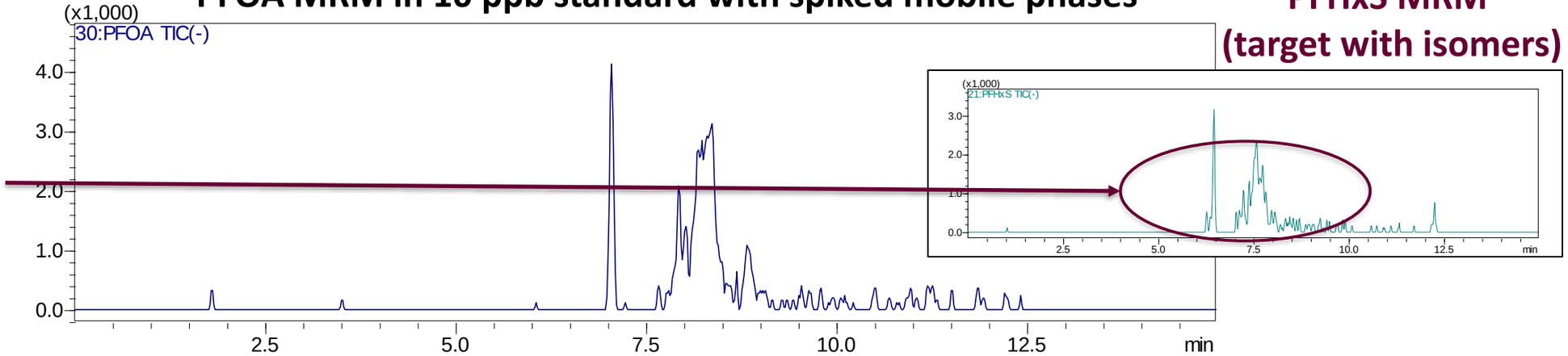
PFOA MRM in solvent blank with spiked mobile phases



Separation between target and contamination

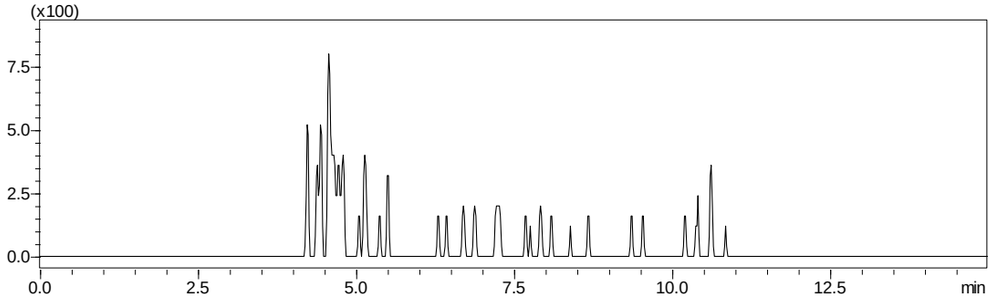
PFOA MRM in 10 ppb standard with spiked mobile phases

PFHxS MRM (target with isomers)

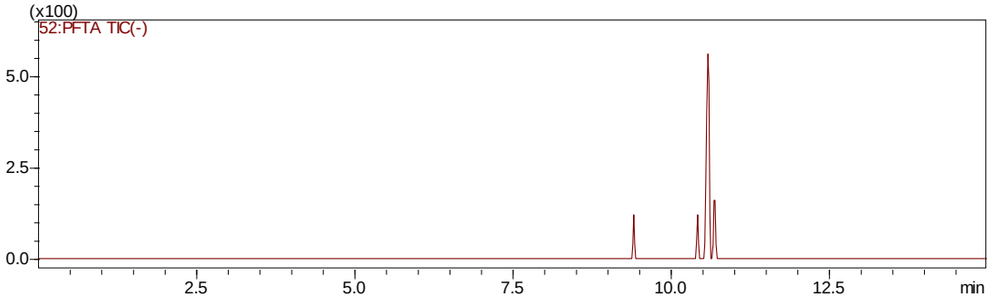


Results – Contamination – STRESS TEST!

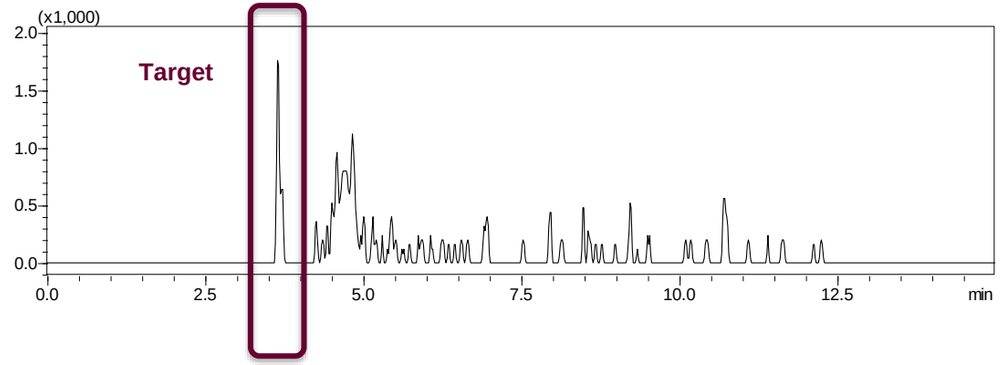
PFBA MRM in solvent blank with spiked mobile phases



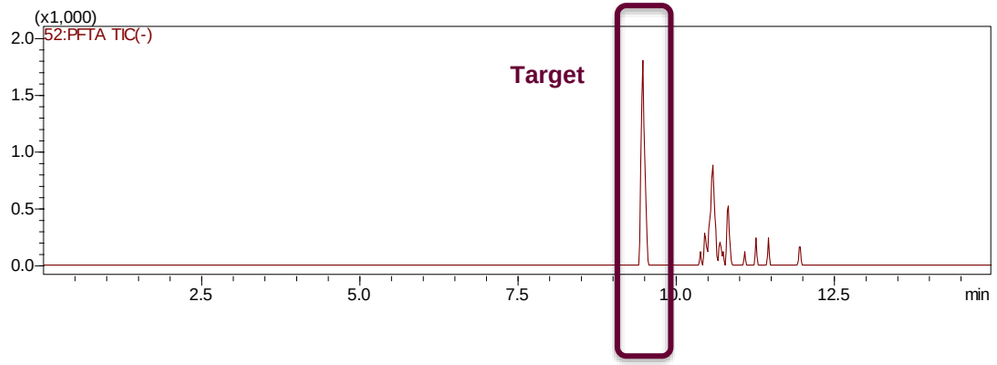
PFTA MRM in solvent blank with spiked mobile phases



PFBA MRM in 10 ppb standard with spiked mobile phases



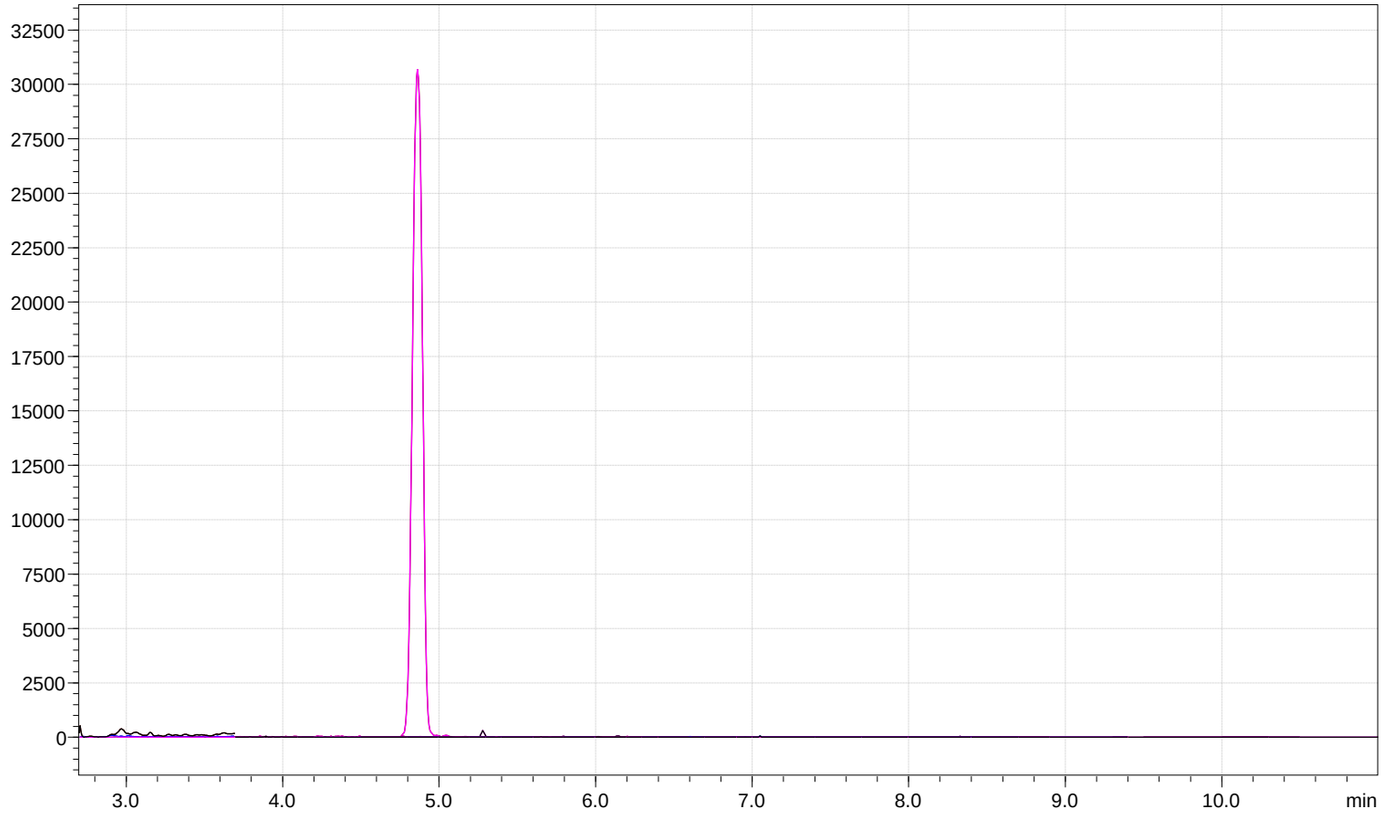
PFTA MRM in 10 ppb standard with spiked mobile phases



1st eluter → **Last eluter**

Slight increase in separation between targets and contamination during run

Results – Contamination – SEPTA



MRM from PFBS in blank (glass vial with silicone septa)

Type of vials[1] tested:

- Amber glass
- Clear glass
- Silanized clear vials
- PP vials

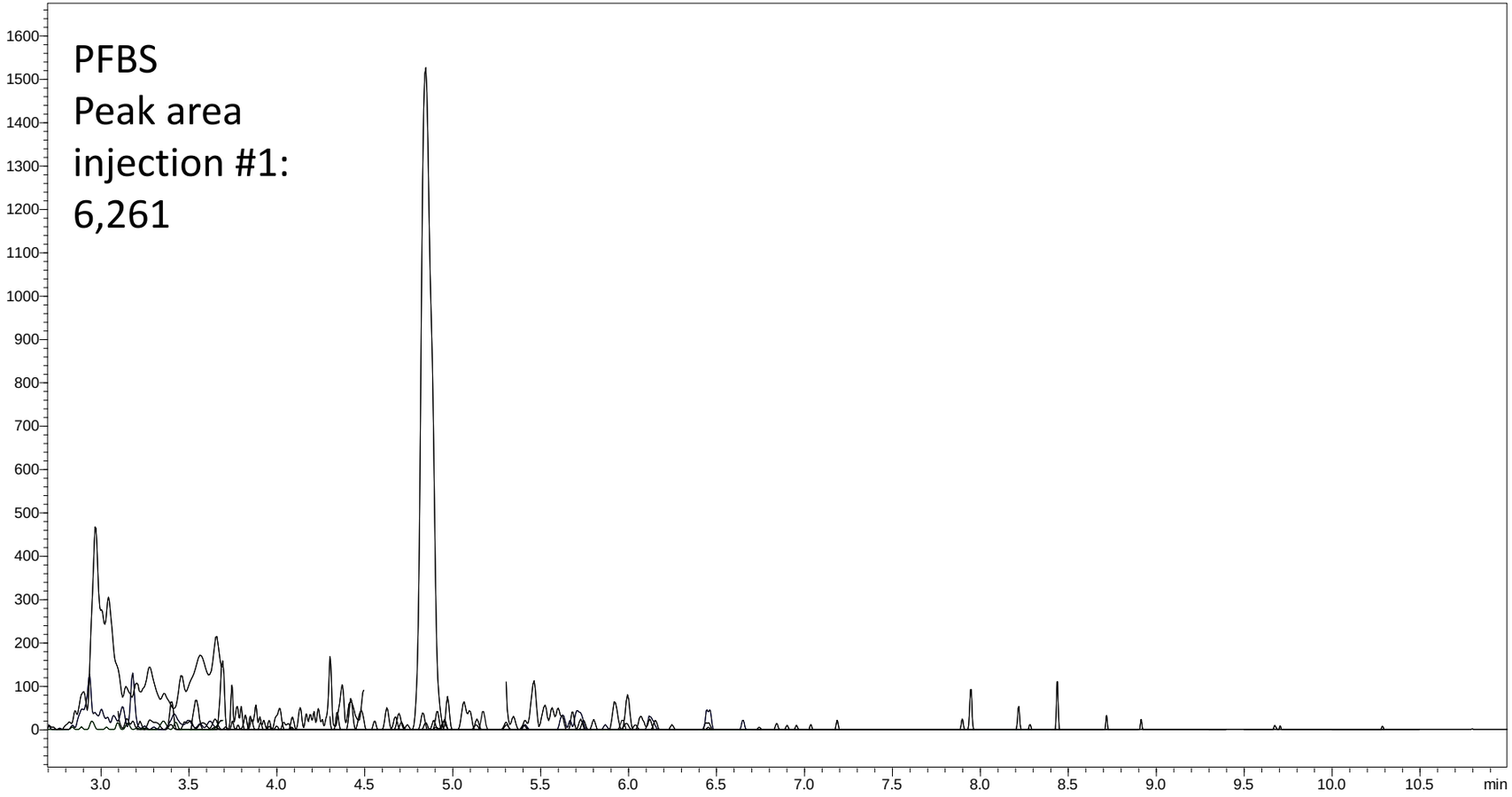
Type of caps[2] tested:

- Silicone lined
- PP

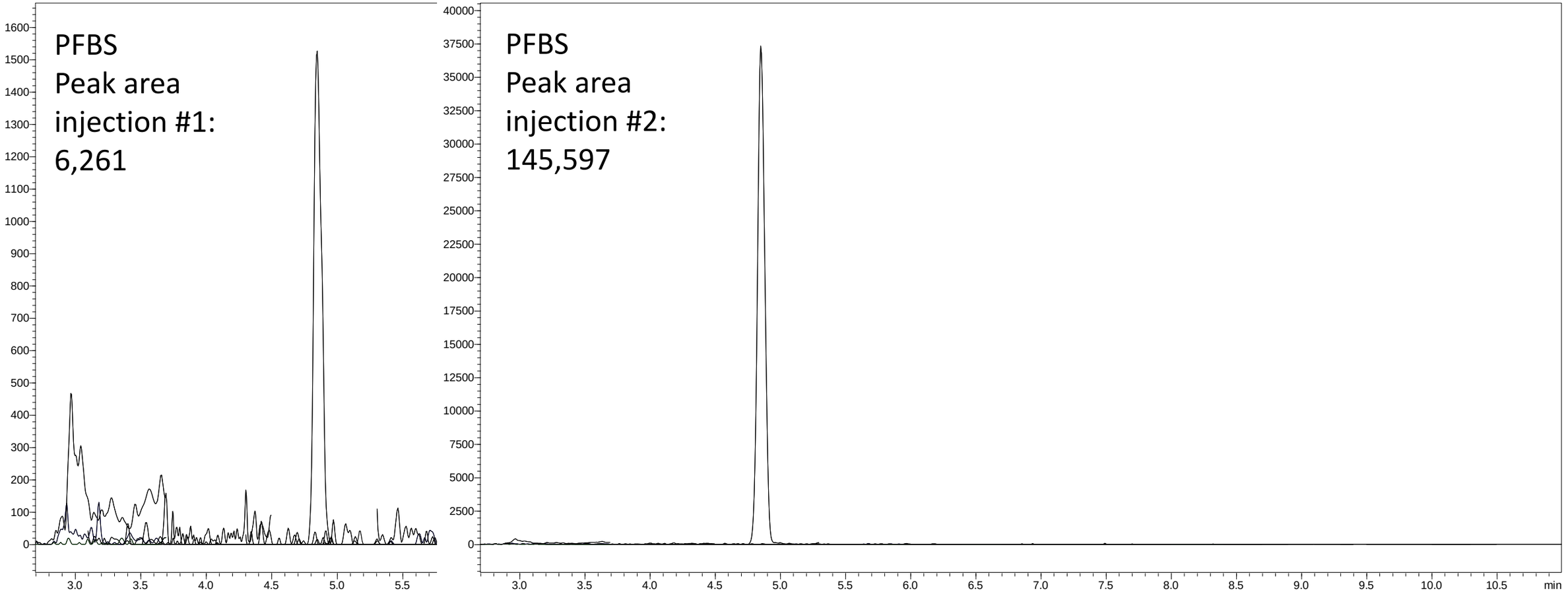
Silicone lined septa:
PFBS contamination found; same results with several septa (n>5) from same lot.

[1,2] From different manufacturers

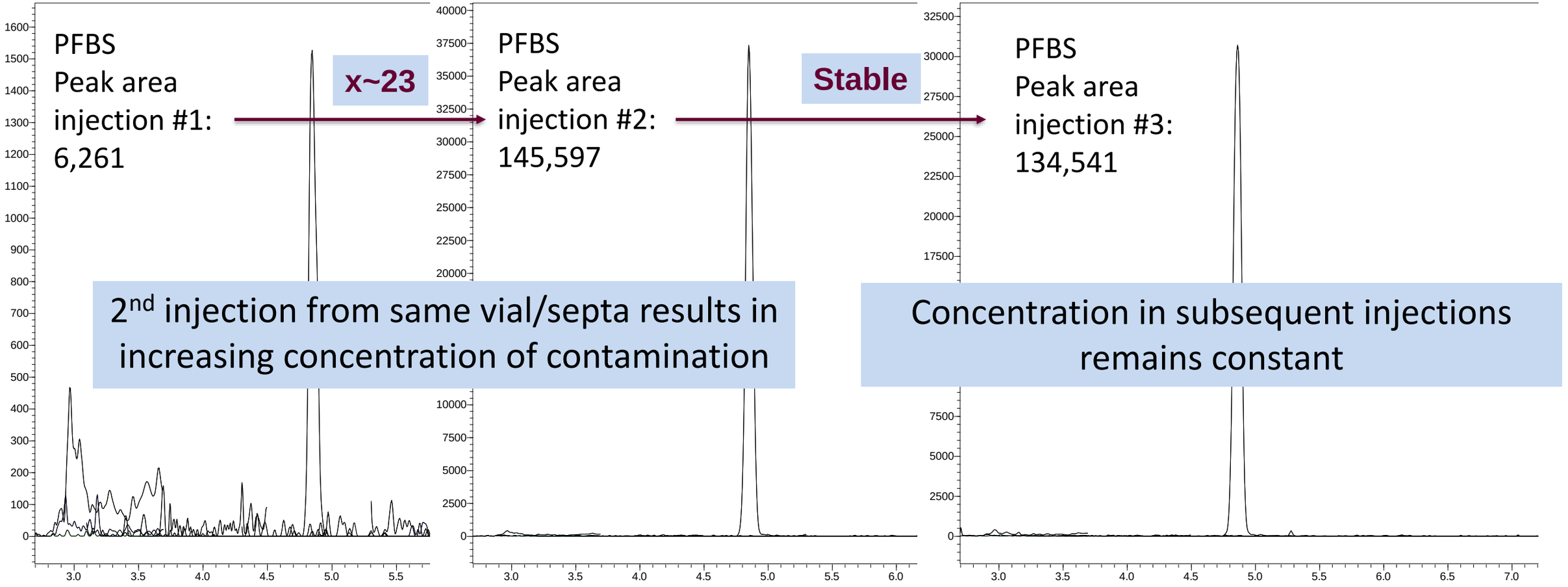
Results – Contamination – SEPTA



Results – Contamination – SEPTA



Results – Contamination – SEPTA



2nd injection from same vial/septa results in increasing concentration of contamination

Concentration in subsequent injections remains constant

Conclusions

- ✓ Method 537.1 and 533 re-optimized to ensure robust workflows that can easily be implemented in routine operations for addressing upcoming monitoring needs
  (Data not shown today)
- ✓ Absence of crosstalk is essential to avoid biased (high) results during PFAS analysis
  Modern mass spectrometers use built-in technology (i.e., UFsweeper™) to minimize it
- ✓ Delay column is sufficient HPLC modification to eliminate measurable PFAS background
- ✓ Frequent testing of consumables used during analysis is unavoidable

Acknowledgments

William Hedgepeth
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Tairo Ogura
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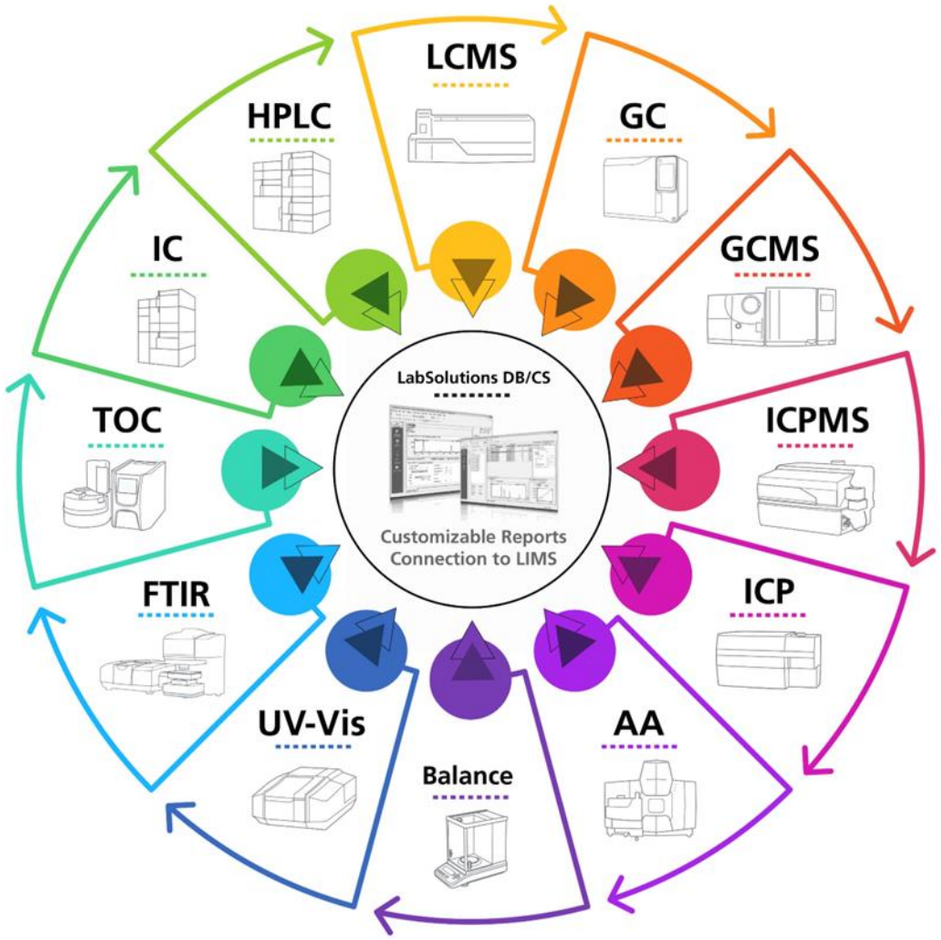
Christopher Gilles
Sr Product Manager - LCMS



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PFAS microsite