Polyfluoroalkyl Substances (PFAS) in the Environment (Session 4)

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Meeting Mechanics

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Meeting Mechanics

- Phone lines and computer sound are muted when you join the call. Look for the Q&A feature in Webex and type in a Q&A question at any time during the presentation. **Choose to send the question to All Panelists.** Time permitting, there will be a Q&A session at the end of each presentation.
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Polyfluoroalkyl Substances (PFAS) in the Environment (session 4)

Session Chairs: Charles Neslund, Eurofins Lancaster Laboratories Environmental and Mike Chang, Restek Corporation

- 10:30 Challenges and Solutions: Approaches to Reducing Interferences and Adsorption in PFAS Environmental Analysis
  Matthew Giardina, Agilent Technologies, Inc.

11:00 BREAK

- 11:15 An Alternative Ionization Technique for LC-MS/MS Analysis of Perfluoroalkyl Substances (PFAS) in Environmental Samples
  Stuart Oehrle, Waters Corporation
- 11:45 Demonstrating Improvements in PFAS Sensitivity using a Microflow LC Approach for the EPA 537 Panel
  Katherine Hyland, SCIEX
- 12:15 Supercritical Fluid Extraction – A Solution for the Extraction of PFAS in Environmental Samples
  Ruth Marfil-Vega, Shimadzu Scientific Instruments

Agilent
Trusted Answers

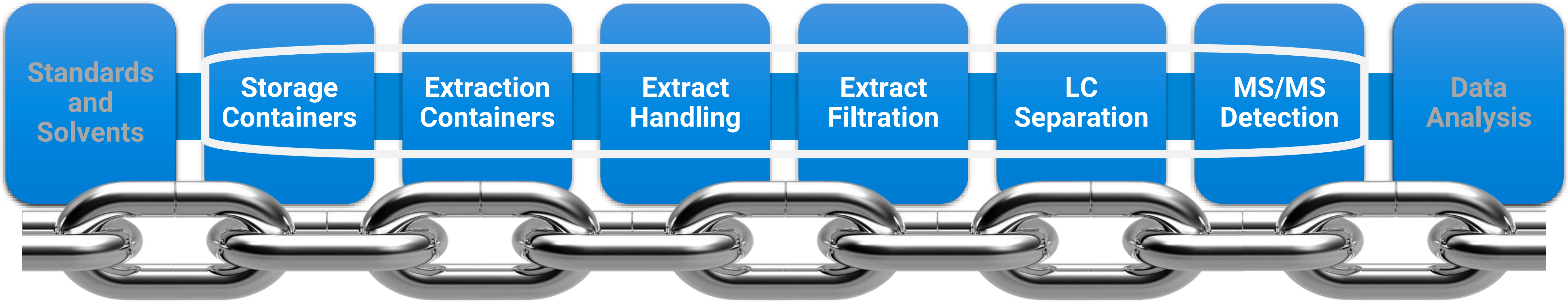
Challenges and Solutions:
Approaches to reducing interferences and adsorption in PFAS environmental analysis

Matthew Giardina, Ph.D.
Applications Chemist

August 21, 2020

Outline

- Discuss aspects of PFAS workflow optimization
- Focus on methods involving direct analysis (no solid phase extraction)



Workflow

Direct Methods

EPA 8327

- Analysis of 24 per- and polyfluoroalkyl substances in **waters and solids** by LC/MS/MS. Tested on **reagent water, surface water, groundwater, and wastewater**.

EPA Draft Method for Soils

- Analysis of 24 per- and polyfluoroalkyl substances in **soils** by LC/MS/MS. Tested on **sand, silt, fat clay, and lean clay.**

ASTM D7979-19

- Analysis of 21 per- and polyfluoroalkyl substances in a **water, sludge, influent, effluent, and wastewater** by LC/MS/MS.

ASTM D7968-17a

- Analysis of 21 per- and polyfluoroalkyl substances in a **soil** by LC/MS/MS. Tested on **sand, silt, fat clay, and lean clay.**

Similar sample workflow

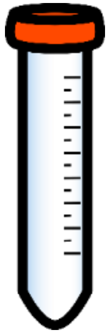
- Extraction/dilution with methanol/water
- Filtration
- LC/MS/MS

Compound List (8327 & EPA Draft Soil Method)

Targets	Surrogates
Perfluorobutyl sulfonic acid (PFBS)	Perfluoro-1-[1,2,3-^{13}C3]hexyl sulfonic acid (M3PFHxS)
Perfluorohexyl sulfonic acid (PFHxS)	Perfluoro-1-[^{13}C8]octyl sulfonic acid (M8PFOS)
Perfluorooctyl sulfonic acid (PFOS)	Perfluoro-n-[^{13}C4]butanoic acid (M4PFBA)
1H, 1H, 2H, 2H-Perfluorohexane sulfonic acid (4:2 FTS)	Perfluoro-n-[^{13}C5]pentanoic acid (M5PFPeA)
1H, 1H, 2H, 2H-Perfluorodecane sulfonic acid (8:2 FTS)	Perfluoro-n-[1,2,3,4,6-^{13}C5]hexanoic acid (M5PFHxA)
Perfluoro-1-pentanesulfonic acid (PFPeS)	Perfluoro-n-[1,2,3,4-^{13}C4]heptanoic acid (M4PFHpA)
Perfluoro-1-heptanesulfonic acid (PFHpS)	Perfluoro-n-[^{13}C8]octanoic acid (M8PFOA)
Perfluoro-1-nonanesulfonic acid (PFNS)	Perfluoro-n-[^{13}C9]nonanoic acid (M9PFNA)
Perfluoro-1-decanesulfonic acid (PFDS)	Perfluoro-n-[1,2,3,4,5,6-^{13}C6]decanoic acid (M6PFDA)
Perfluorobutanoic acid (PFBA)	Perfluoro-n-[1,2,3,4,5,6,7-^{13}C7]undecanoic acid (M7PFUnA)
Perfluoropentanoic acid (PFPeA)	Perfluoro-n-[1,2-^{13}C2]dodecanoic acid (MPFDoA)
Perfluorohexanoic acid (PFHxA)	Perfluoro-n-[1,2-^{13}C2]tetradecanoic acid (M2PFTeDA)
Perfluoroheptanoic acid (PFHpA)	1H, 1H, 2H, 2H-Perfluoro-(1,2-^{13}C2) hexyl sulfonic acid (M2-4:2 FTS)
Perfluorooctanoic acid (PFOA)	1H, 1H, 2H, 2H-Perfluoro-1(1,2-^{13}C2) decyl sulfonic acid (M2-8:2 FTS)
Perfluorononanoic acid (PFNA)	N-Methyl-d3-perfluoro-1-octanesulfonamidoacetic acid (d3-N-MeFOSAA)
Perfluorodecanoic acid (PFDA)	N-Ethyl-d5-perfluoro-1-octanesulfonamidoacetic acid (d5-N-EtFOSAA)
Perfluoroundecanoic acid (PFUdA)	Perfluoro-1-[^{13}C8]octanesulfonamide (M8FOSA)
Perfluorododecanoic acid (PFDoA)	~~Perfluoro-1-[2,3,4-13C3]butyl sulfonic acid (M3PFBS)~~
Perfluorotridecanoic acid (PFTrDA)	~~1H, 1H, 2H, 2H-perfluoro-1(1,2-^{13}C2) octyl sulfonic acid (M2-8:2 FTS)~~
Perfluorotetradecanoic acid (PFTeDA)	
N-Ethylperfluoro-1-octanesulfonamidoacetic acid (NEtFOSAA)	
N-Methylperfluoro-1-octanesulfonamidoacetic acid (NMeFOSAA)	
Perfluoro-1-octanesulfonamide (FOSA)	
~~1H, 1H, 2H, 2H-Perfluorooctane sulfonic acid (6:2 FTS)~~	

PFAS Direct Analysis – Consumables are Critical to the Workflow

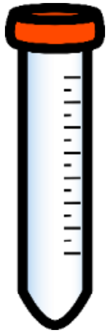
Sample workflow



15 mL Polypropylene Centrifuge Tube

Syringe Filter

Syringe



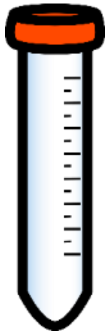
15 mL Polypropylene Centrifuge Tube

Autosampler Vial

LC Tubing, Gaskets, Delay and Analytical Columns

PFAS Direct Analysis – Consumables are Critical to the Workflow

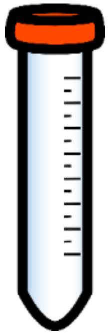
Optimization workflow



15 mL Polypropylene Centrifuge Tube

Syringe Filter

Syringe



15 mL Polypropylene Centrifuge Tube

LC Tubing, Gaskets, Delay and Analytical Columns

Autosampler Vial

Analysis – Special PFAS Considerations

- Thermal lability of some PFAS compounds
- Analyte sorption to containers
- Column selection consideration
- Analysis method

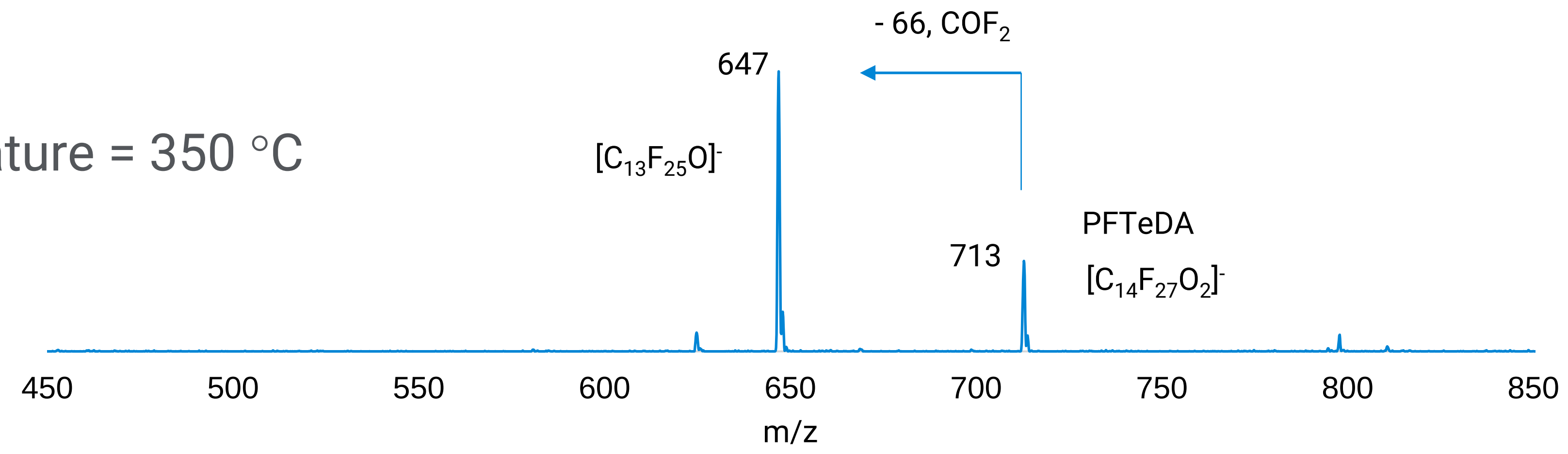
Today's focus

- Reduce LC/MS/MS system PFAS background
- Add delay column
- Use high purity solvents

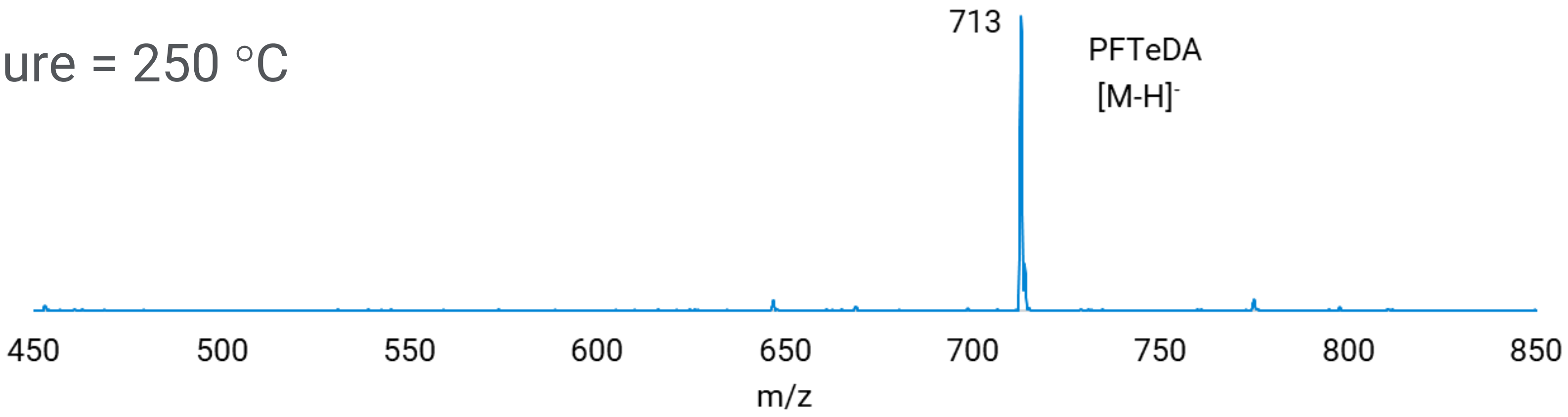
PFAS Thermal Lability of Perfluoroalkyl Acids

Ion source temperatures that are too high can cause thermal degradation of acids

Source N_2 temperature = 350 °C

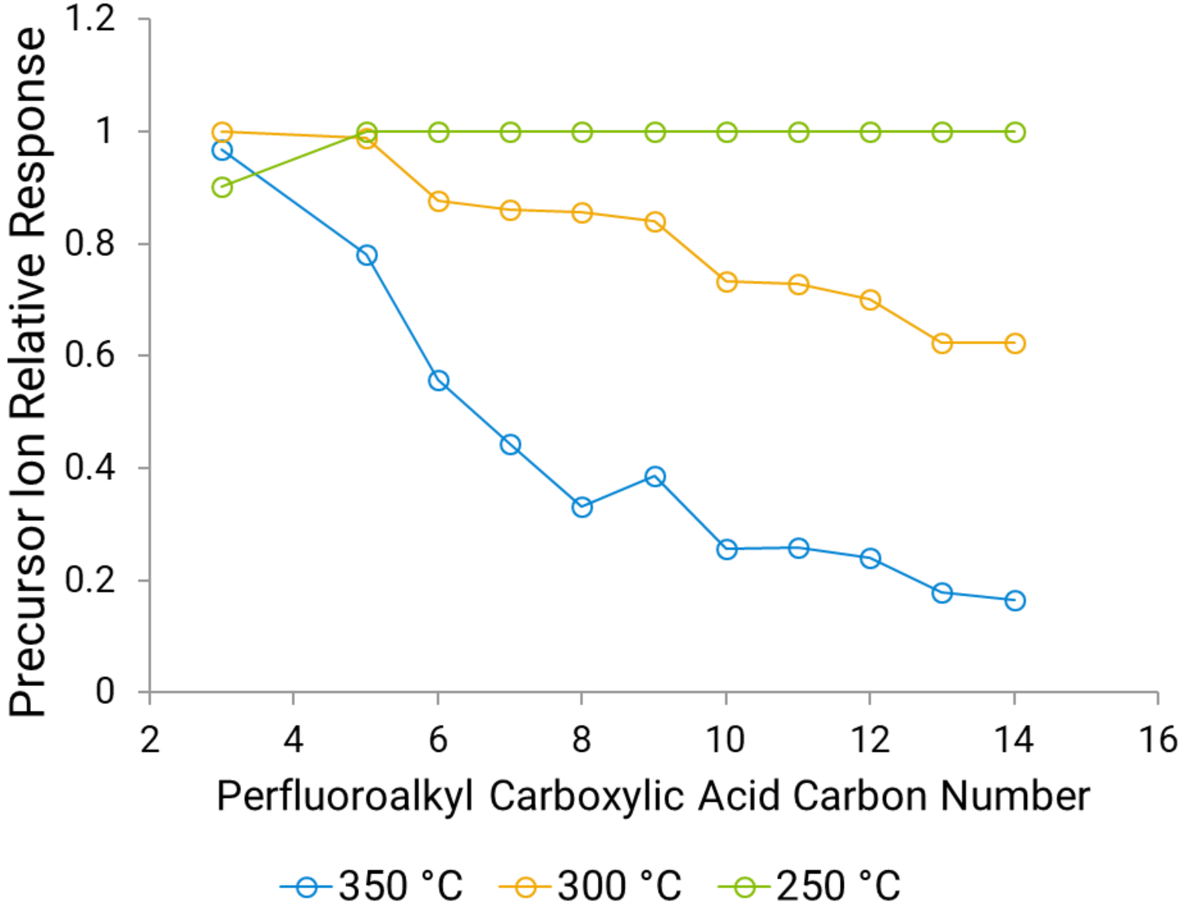


Source N_2 temperature = 250 °C

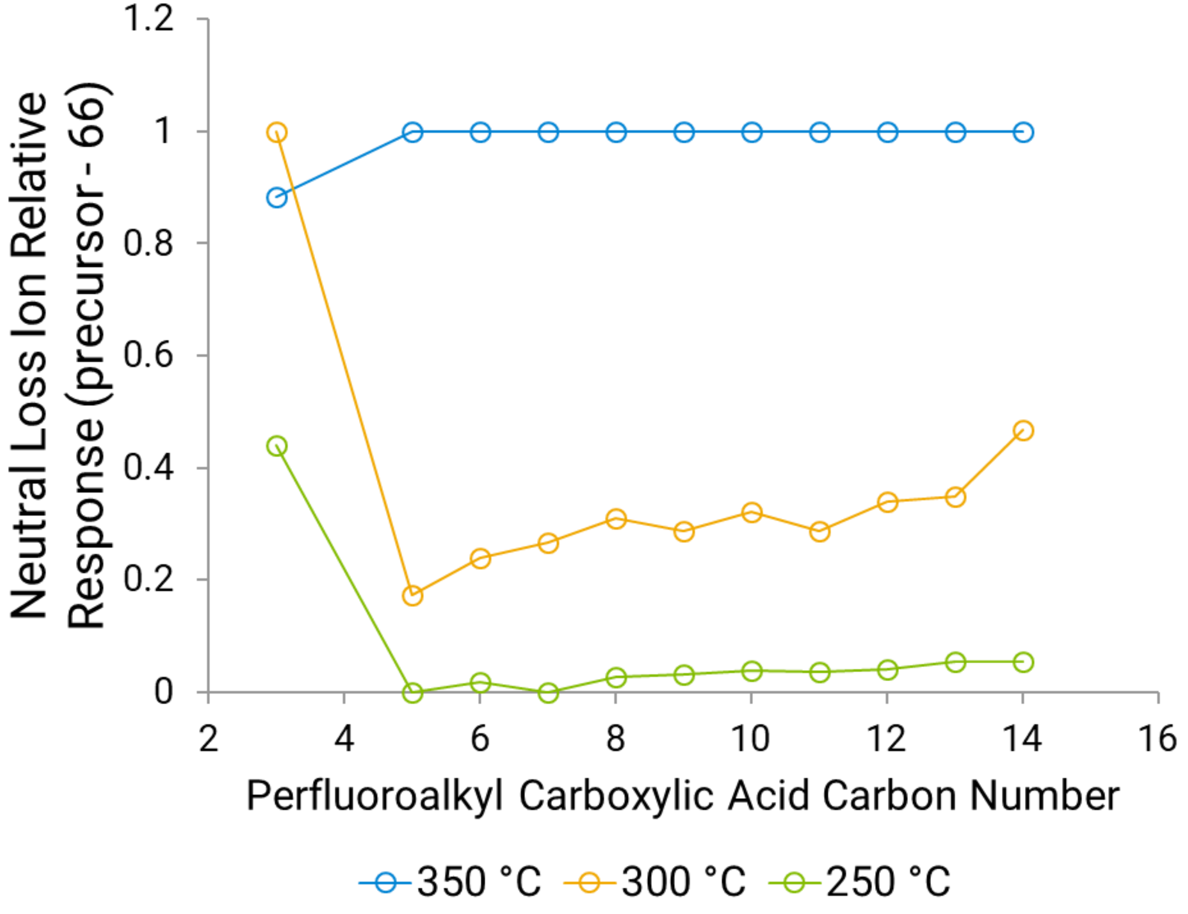


PFAS Thermal Lability of Perfluoroalkyl Acids

Lability increases with alkyl chain length



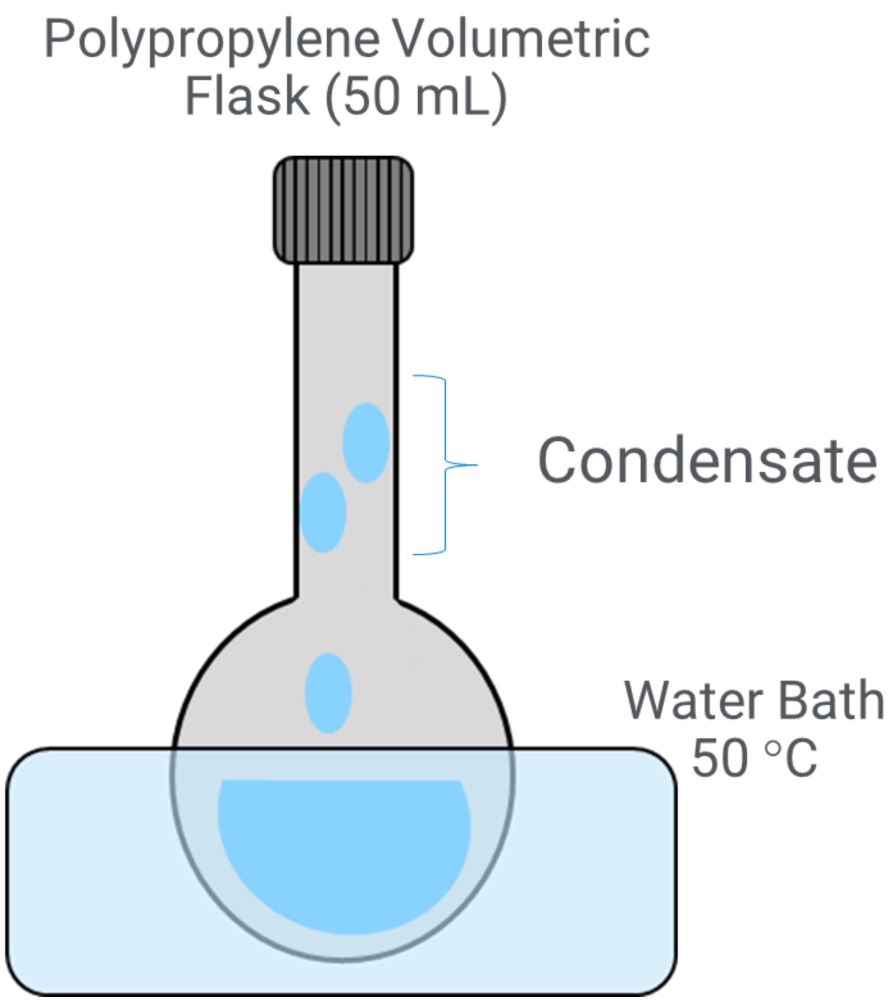
(Sheath N_2 temperature)



(Sheath N_2 temperature)

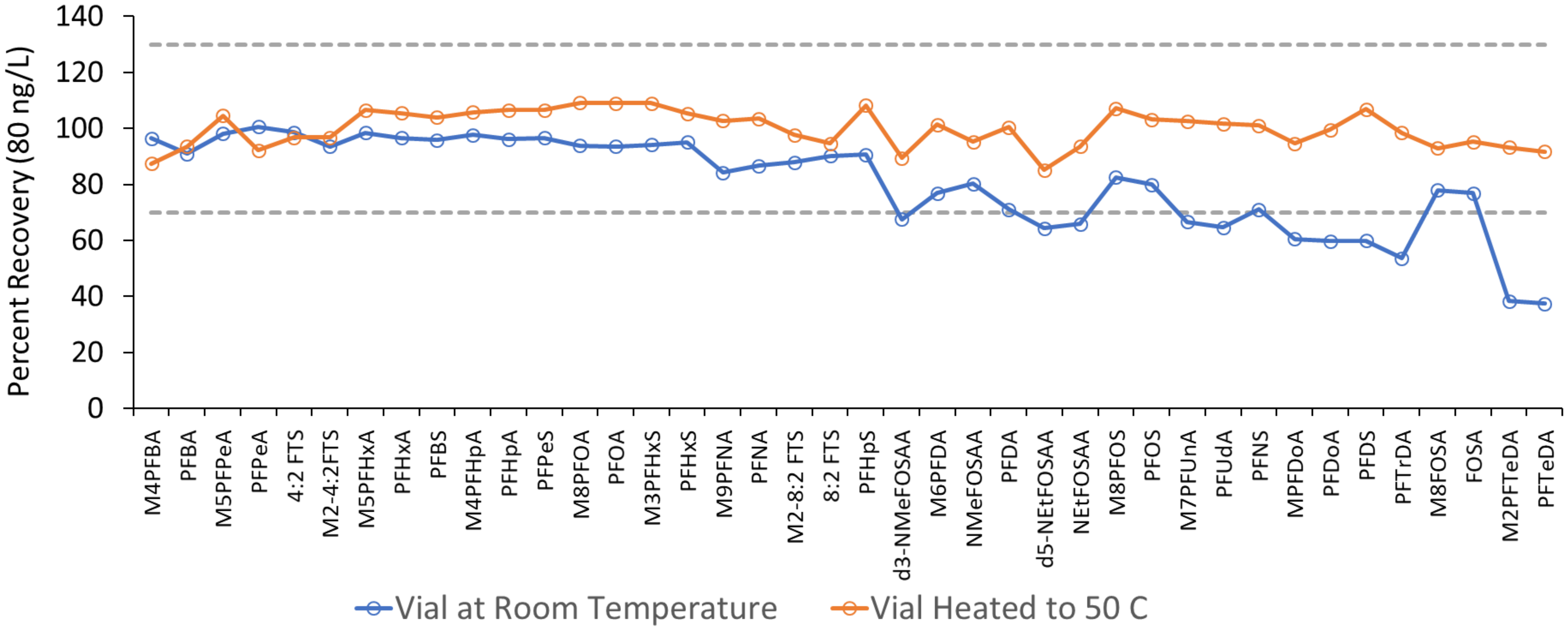
Loss of Analytes in Storage Vials and Containers

- "Reduced solubility or sorptive losses can occur particularly for stock solutions stored at ≤ 6 °C."
- Bringing solutions to room temperature is not sufficient to reduce these losses.
- Heating stock solutions in a water bath at 50 °C for up to 30 minutes followed by vortexing was sufficient at reducing losses.
- Reused stock solution (200 ng/L 50/50 methanol/water) for over a month stored in a 50 mL volumetric flask at 4 °C and no evaporative losses was evident after repeated heating.



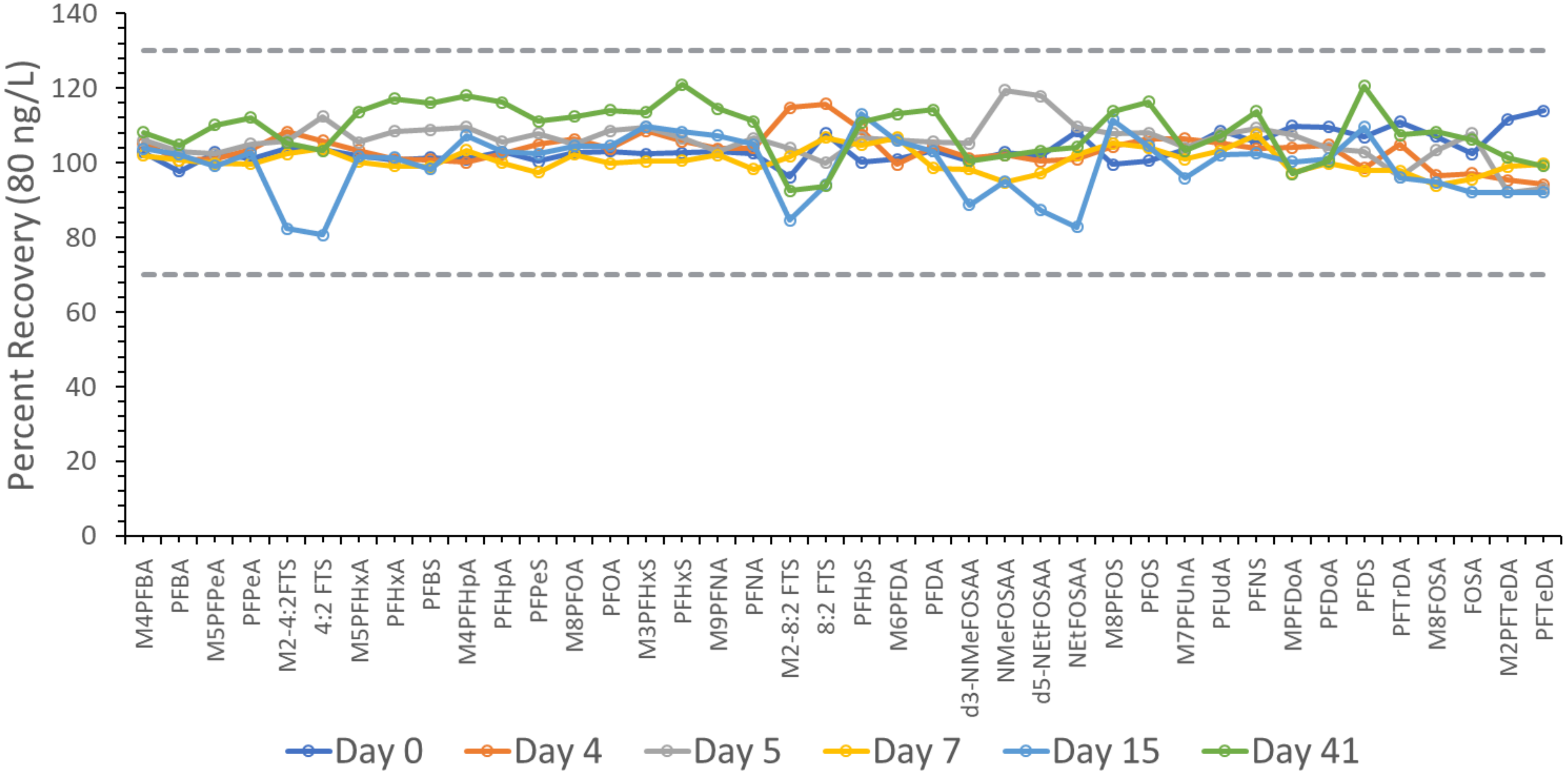
Loss of Analytes in Storage Vials and Containers

Heating stock compared to not heating stock



Loss of Analytes in Storage Vials and Containers

Heating/cooling cycles over 41 days

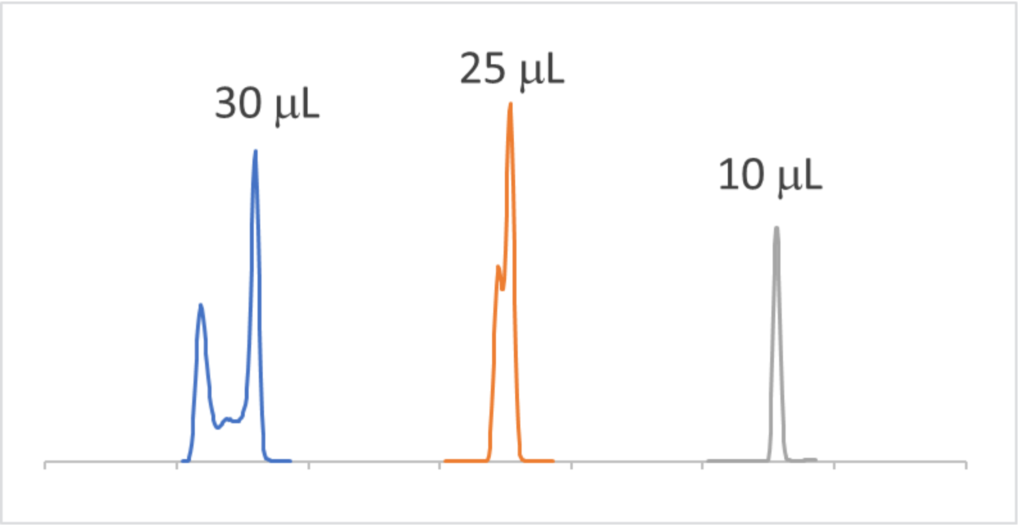


Day	Average Recovery
0	104 %
4	103 %
5	106 %
7	101 %
15	99 %
41	109 %

Column Loading

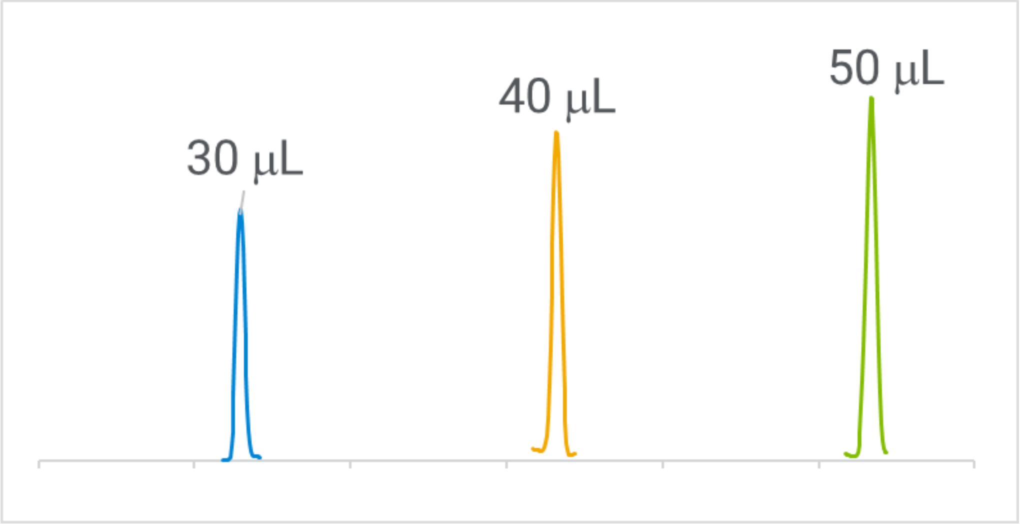
- A large injection volume is required to improve detection (10 to 30 μL).
- This problem is amplified by the injection in a relatively strong diluent (50/50 methanol/water).
- Column selection is important to achieving good peak shape with large injection volume.

Peak splitting for early eluters



PFPeA (EIC m/z 263)

Zorbax Eclipse Plus C18, 2.1 x 150 mm, 1.8 μm



PFPeA (EIC m/z 263)

Instrumental Analysis Method

Parameter	Value
MS	Agilent 6470 Triple Quadrupole with Agilent Jet Stream ESI Source
Source Parameters	
Polarity	Negative
Drying Gas	230 °C, 4 L/min
Sheath Gas	250 °C, 12 L/min
Nebulizer Gas	15 psi
Capillary Voltage	2500 V
Nozzle Voltage	0 V
Acquisition	
Cycle Time	500 ms
Total MRMs	61
Max Concurrent MRM	30
Min/Max Dwell	14.18 ms/247.76 ms

Parameter	Value			
LC	Agilent 1290 Infinity II LC System			
Analytical Column	Zorbax RR Eclipse Plus C18, 2.1 x 100 mm, 1.8 μm			
Delay Column	InfinityLab PFC Delay Column			
Column Temperature	30 °C			
Injection Volume	30 μL			
Mobile Phase	A. 20 mM Ammonium acetate in 95% water/5 % acetonitrile B. 10 mM ammonium acetate in 95% acetonitrile/5% water			
Gradient	Time (min)	A%	B%	Flow (mL/mim)
	0	100	0	0.3
	1	70	30	0.3
	6	50	50	0.3
	13	15	85	0.3
	14	0	100	0.4
	17	0	100	0.4
	18	100	0	0.4
	21	100	0	0.4

Calibration

Target calibration accuracy and correlation coefficient of determination (5 to 200 ng/L)

Concen (ng/L)	PFBA	PFPeA	4:2 FTS	PFHxA	PFBS	PFHpA	PFPeS	PFOA	PFHxS	PFNA	8:2 FTS	PFHpS	PFDA	Nme-FOSAA	Net-FOSAA	PFOS	PFUdA	PFNS	PFDoA	PFDS	PFTrDA	FOSA	PFTeDA
5	99	95	101	99	100	92	89	99	96	94	101	73	87	95	113	94	88	91	102	90	105	107	103
10	103	103	114	101	96	106	98	94	101	106	95	107	110	105	87	105	102	98	97	105	90	92	94
20	99	99	83	95	100	99	109	102	101	92	98	114	88	96	85	94	95	98	85	93	87	95	82
40	98	99	98	102	100	99	103	101	99	103	100	103	107	100	103	104	108	106	110	107	113	103	118
60	99	103	99	101	104	102	103	102	100	102	101	104	109	102	108	100	104	106	101	101	98	102	97
80	98	101	104	101	101	103	100	103	103	103	108	100	103	103	108	101	109	105	110	107	111	102	114
100	106	101	103	103	101	101	100	101	104	103	101	104	102	101	105	107	101	101	99	103	100	101	98
150	99	100	100	101	102	102	101	101	101	99	100	97	100	103	97	100	99	99	100	100	102	101	99
200	100	98	99	97	97	97	97	97	97	98	96	97	95	96	96	96	95	96	96	95	94	97	95
R^2	0.999	1.000	0.998	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.998	0.997	0.996	0.999	0.995	0.998	0.997	0.998	0.996	0.997	0.994	0.999	0.991

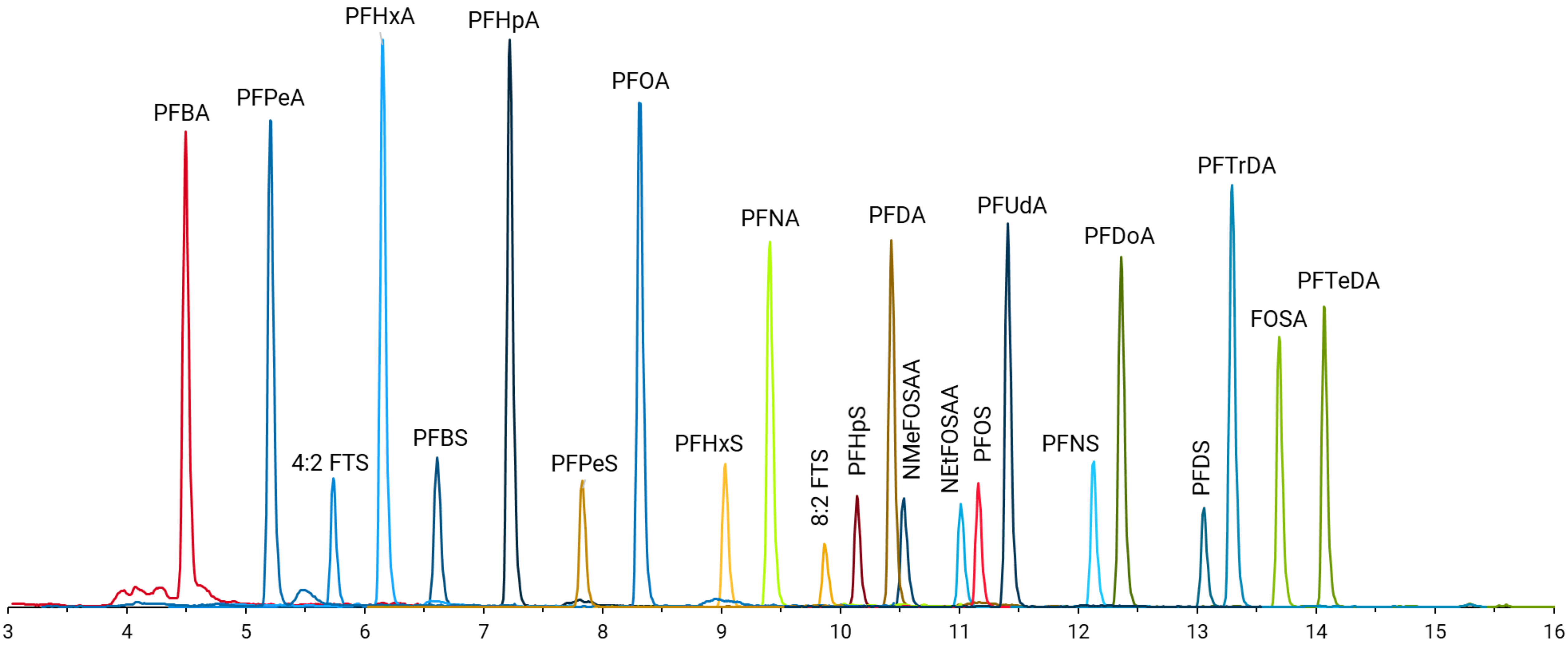
Calibration

Surrogate calibration accuracy and correlation coefficient of determination (5 to 200 ng/L)

Concen (ng/L)	M4PFBA	M5PFPeA	M2-4:2FTS	M5PFHxA	M4PFHpA	M8PFOA	M3PFHxS	M9PFNA	M2-8:2 FTS	M6PFDA	d3-NMeFOSAA	d5-NEtFOSAA	M8PFOS	M7PFUnA	MPFDoA	M8FOSA	M2PFTeDA
5	96	98	103	99	97	95	104	109	90	91	102	101	93	96	94	111	105
10	103	101	97	101	102	104	88	94	113	101	89	99	102	96	103	87	91
20	96	95	93	97	99	98	104	88	93	99	102	94	99	96	91	94	85
40	101	104	102	100	99	101	100	105	113	108	104	107	103	108	107	103	114
60	103	100	102	102	100	101	104	100	93	102	108	102	104	104	100	102	100
80	103	103	104	102	101	103	102	103	96	101	100	102	100	104	110	107	112
100	103	102	101	101	104	103	100	103	104	101	97	97	101	101	98	99	99
150	100	101	99	101	101	99	103	102	95	102	99	99	102	99	104	100	101
200	97	97	99	98	97	98	96	97	103	96	99	100	97	97	94	98	94
R^2	0.999	0.999	0.999	1.000	0.999	0.999	0.998	0.998	0.996	0.998	0.999	0.999	0.999	0.998	0.996	0.998	0.994

Chromatogram

Midpoint calibration (80 ng/L)



Evaluation of Consumables

15 mL Polypropylene
Centrifuge Tube

Disposable Syringes

Syringe Filters

Study Objectives

1. Demonstrate that 15 mL centrifuge tubes, syringes, and syringe filters are free from PFAS contamination (i.e. less than ½ LLOQ) and do not interfere with low-level quantitation.

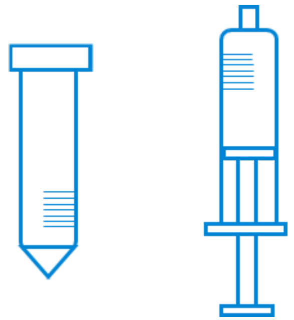
2. Demonstrate that the 15 mL centrifuge tubes, syringes, and syringe filters do not adsorb PFAS compounds and do not interfere with low-level quantitation.

3. Determine appropriate syringe filter material.

Measuring Interferences/Contamination/Background

Test procedure for tubes and syringes

Centrifuge Tubes and Syringes



1. Fill with 10 mL of methanol and water solution (1:1)
2. Adjust pH to 8.5 - 9.0 with ammonium hydroxide

Tumble on rotator for 1 hour

Acidify to pH 3.5 – 4.0 with acetic acid.

Transfer to polypropylene autosampler vial for LC-MS/MS analysis.

Five replicates

Measuring Interferences/Contamination/Background

Test procedure for syringe filters

Syringe Filters

1. Fill capped syringe with 10 mL of methanol and water solution (1:1)
2. Adjust pH to 8.5 - 9.0 with ammonium hydroxide

→ Attach filter and filter all 10 mL into 15 mL centrifuge tube

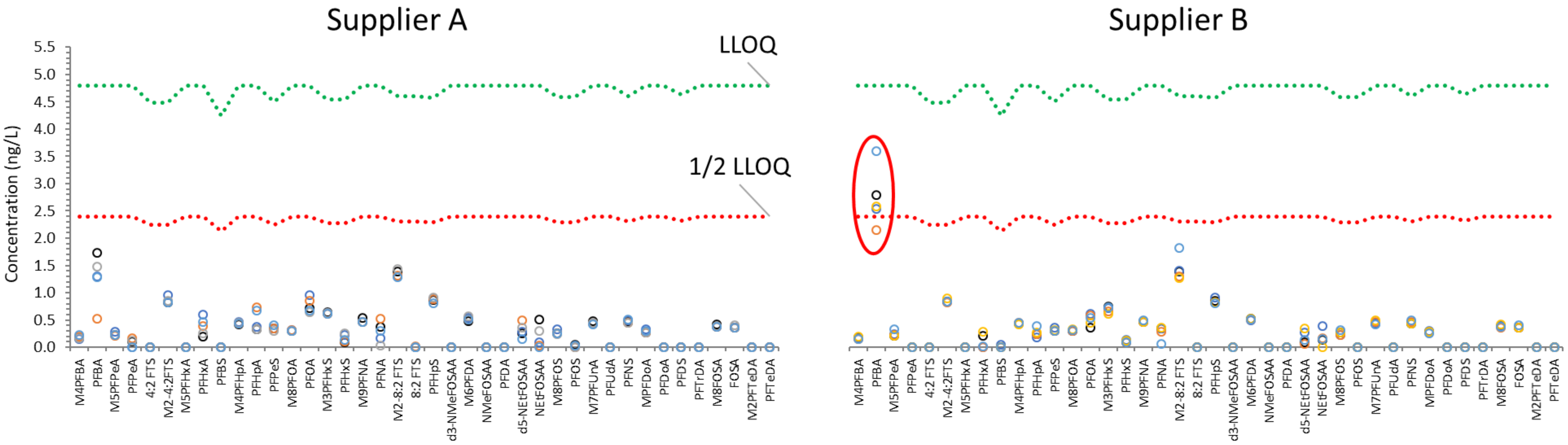
→ Acidify to pH 3.5 – 4.0 with acetic acid

→ Transfer from centrifuge tube to polypropylene autosampler vial for LC-MS/MS analysis.

→
- Five replicates for disposable syringes
- Two replicates for glass syringes

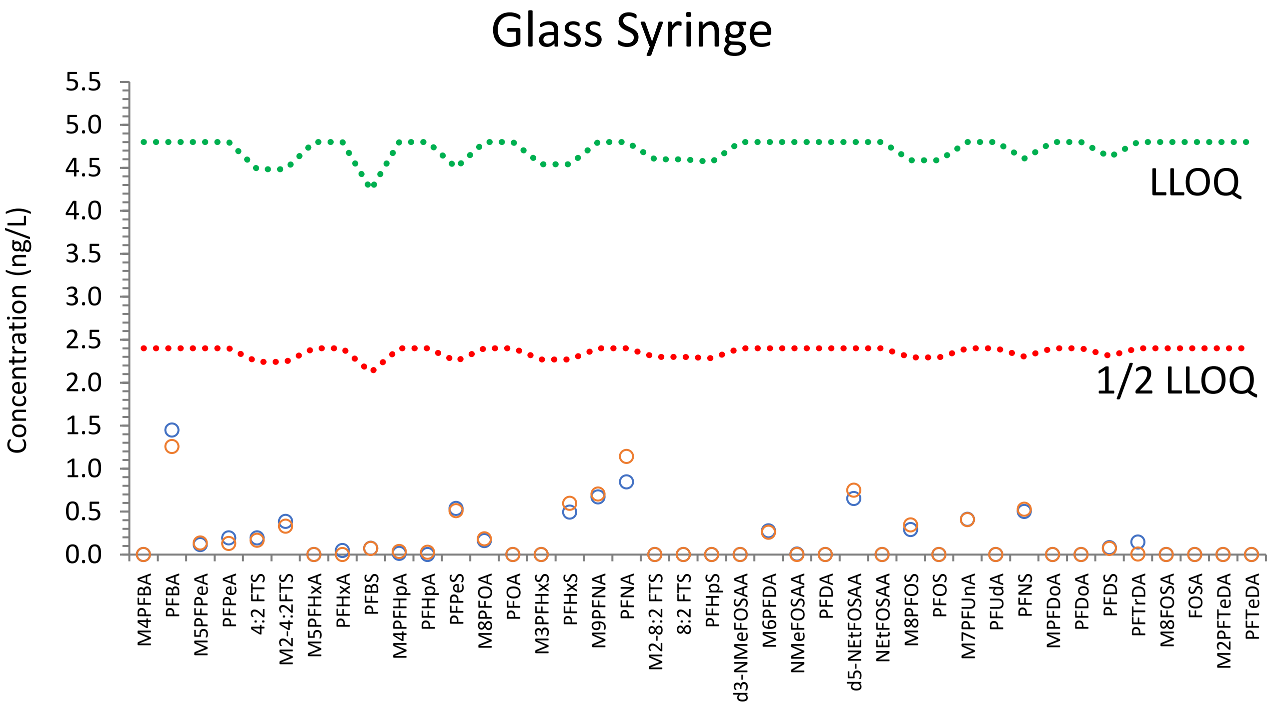
Measuring Interferences/Contamination/Background

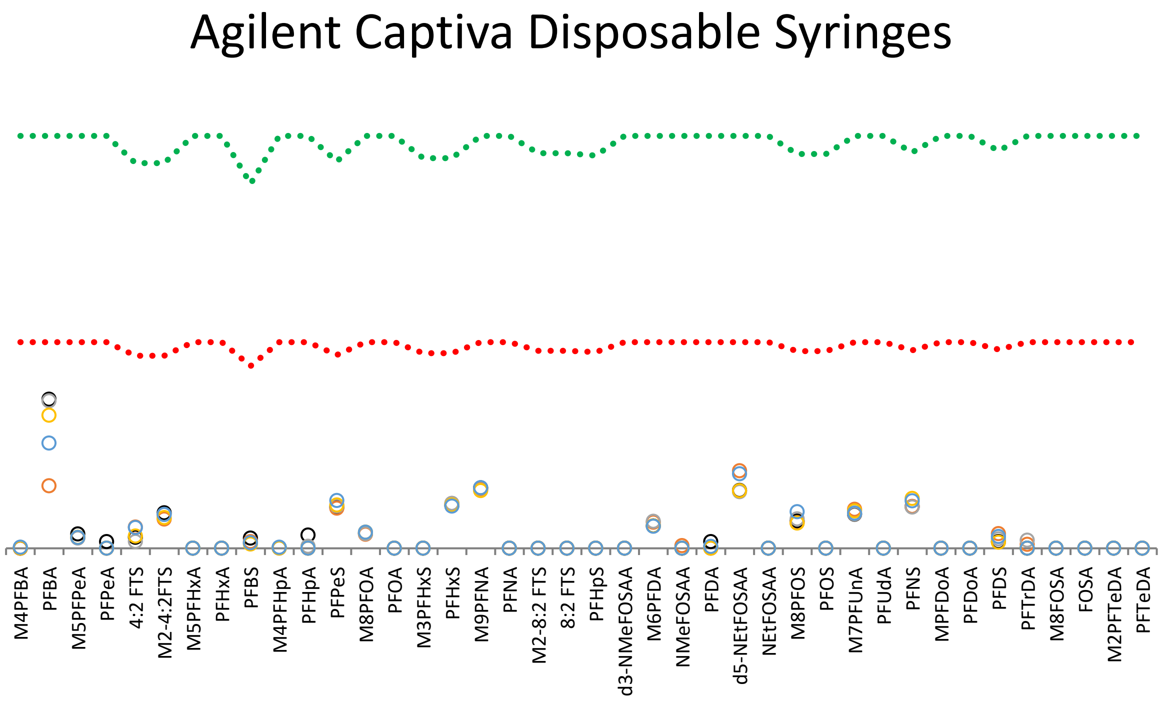
Results for 15 mL polypropylene centrifuge tubes (tested 4 suppliers, 2 shown)



Measuring Interferences/Contamination/Background

Comparison of 10 mL polypropylene disposable and glass syringe

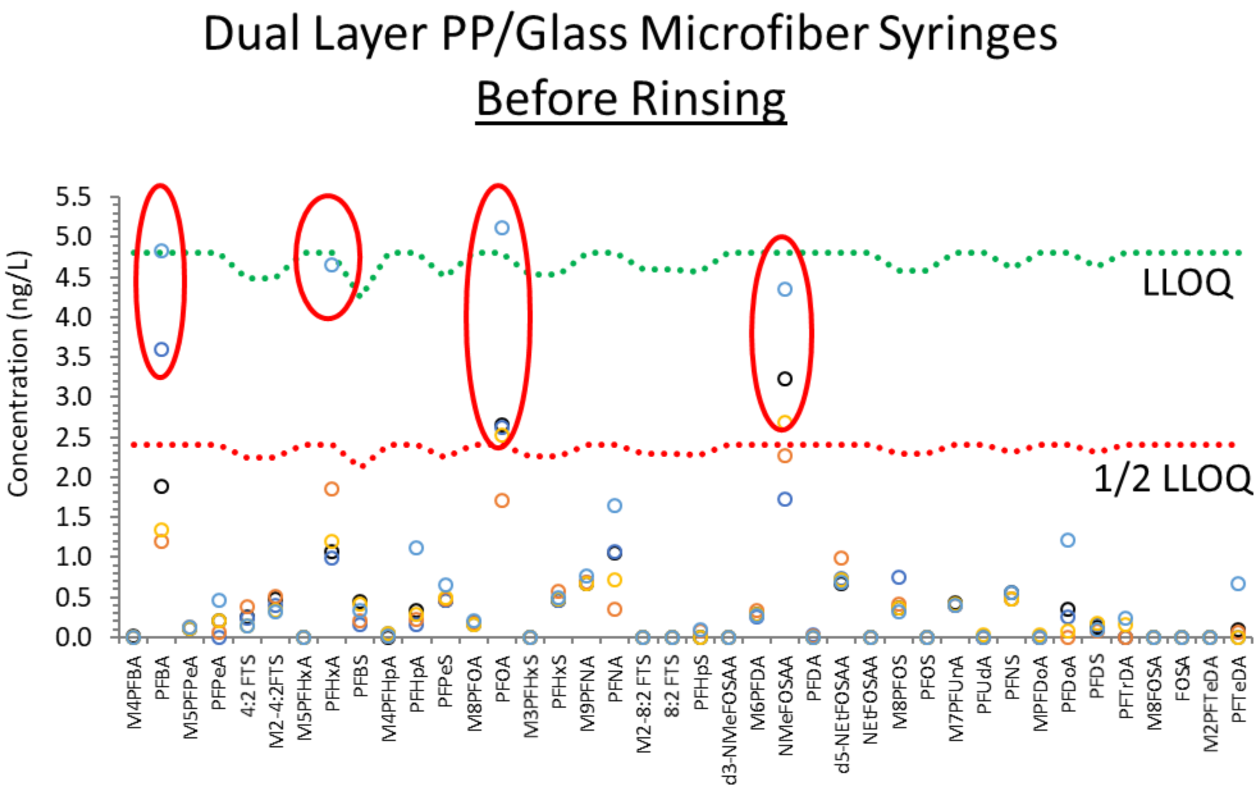


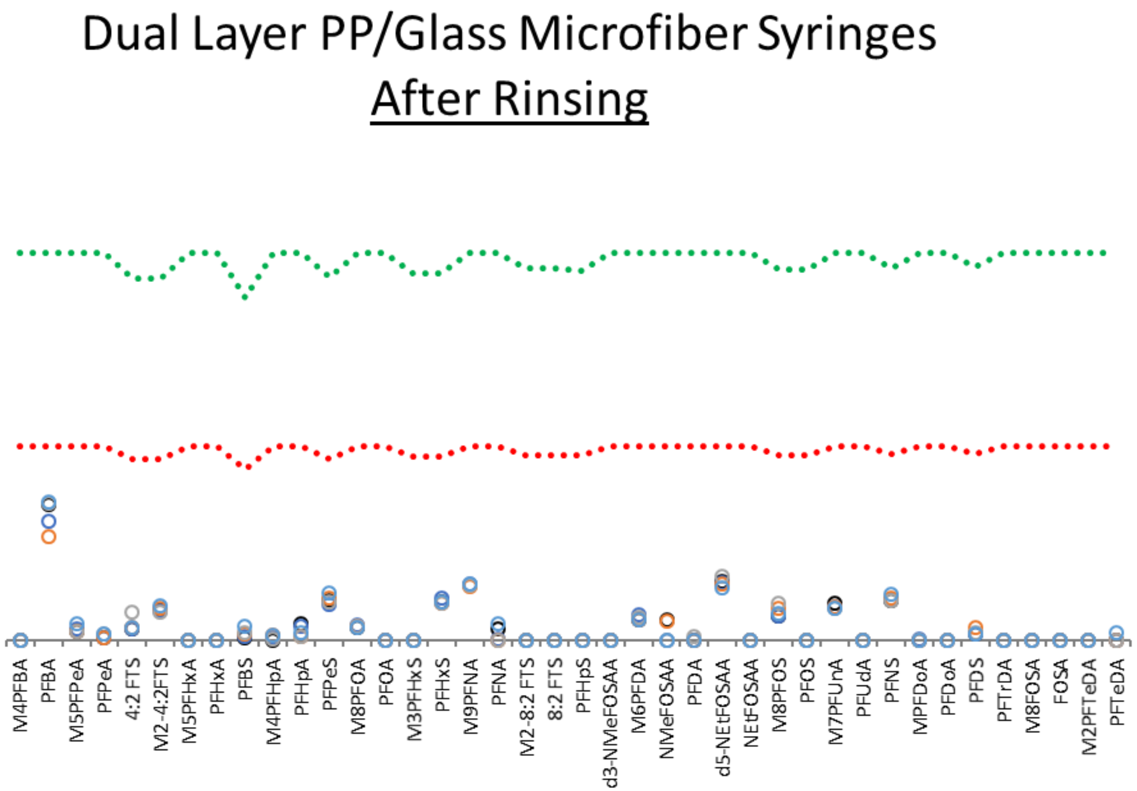


Disposable syringes provide a significant savings in time and solvent usage

Measuring Interferences/Contamination/Background

- The soil methods were validated with polypropylene/glass microfiber syringe filters
- These filters required rinsing to reduce background before use





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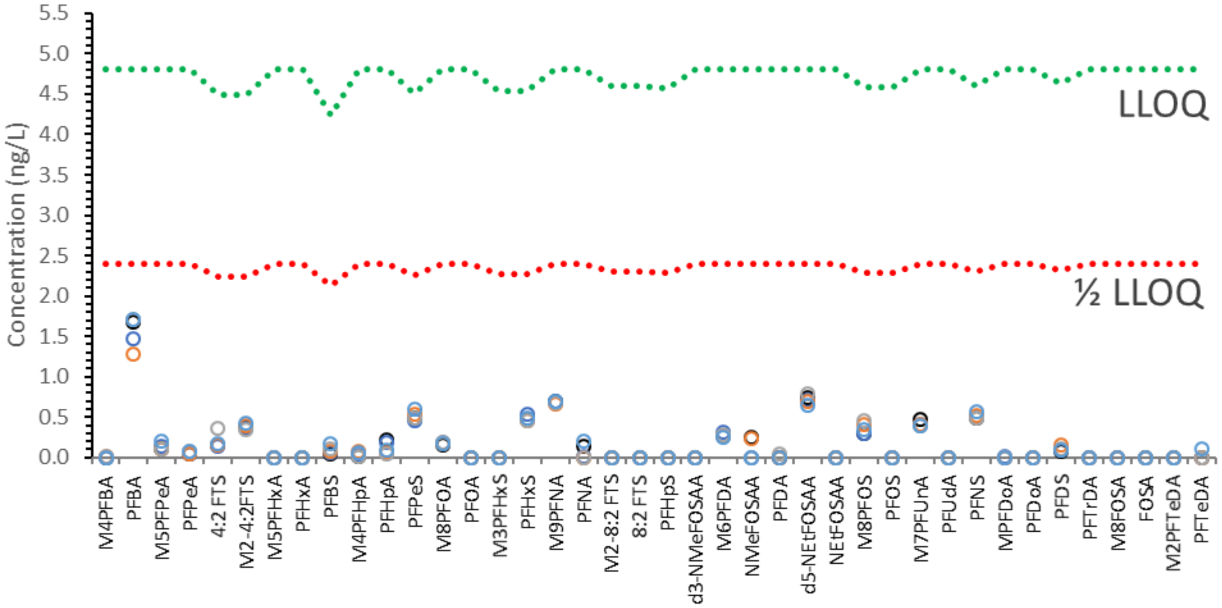
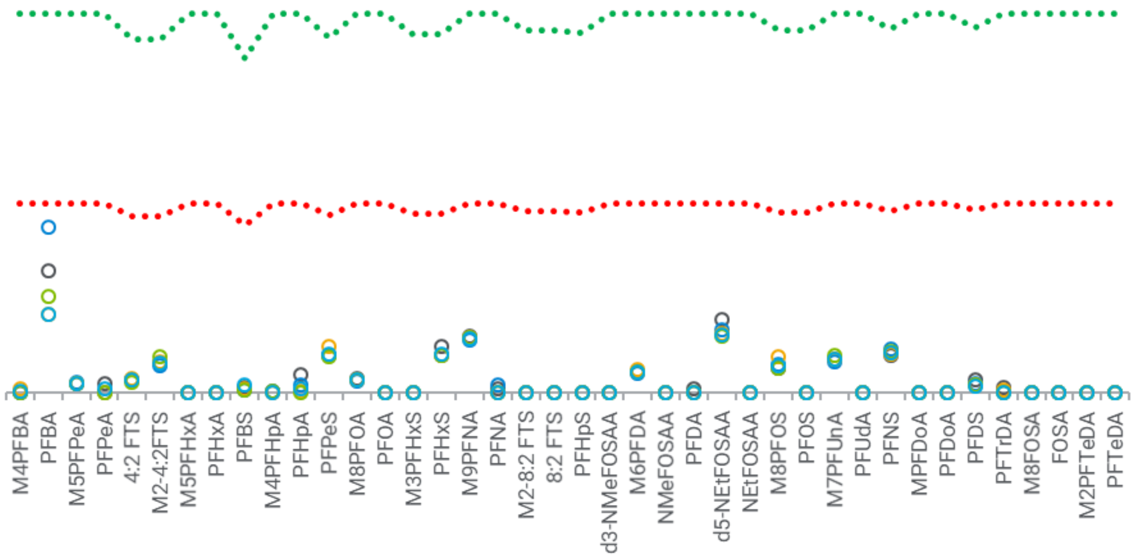
Measuring Interferences/Contamination/Background

- Regenerated cellulose filters were tested as a replacement for the polypropylene/glass microfiber filters
- Regenerated cellulose filters did not require rinsing before use

Dual Layer PP/Glass Microfiber Syringes
After Rinsing



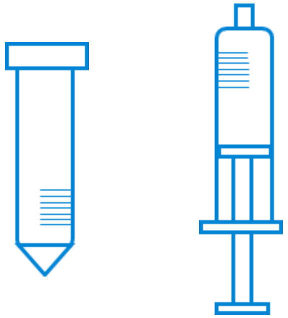
Agilent Captiva RC Syringe Filters
Not Rinsed



Measuring Low Level Spike Recoveries

Test procedure for tubes and syringes

Centrifuge Tubes and Syringes



1. Fill with 10 mL of methanol and water solution (1:1)
2. Spike with targets and surrogates at 5 ng/L and 80 ng/L, respectively
3. Adjust pH to 8.5 - 9.0 with ammonium hydroxide

Tumble on rotator for 1 hour

Acidify to pH 3.5 – 4.0 with acetic acid.

Transfer to polypropylene autosampler vial for LC-MS/MS analysis.

Five replicates

Measuring Low Level Spike Recoveries

Test procedure for syringe filters

Syringe Filters

1. Fill capped syringe with 10 mL of methanol and water solution (1:1)
2. Spike with targets and surrogates at 5 ng/L and 80 ng/L, respectively
3. Adjust pH to 8.5 - 9.0 with ammonium hydroxide

Rotate for 1 hour

Attach filter and filter all 10 mL into 15 mL centrifuge tube

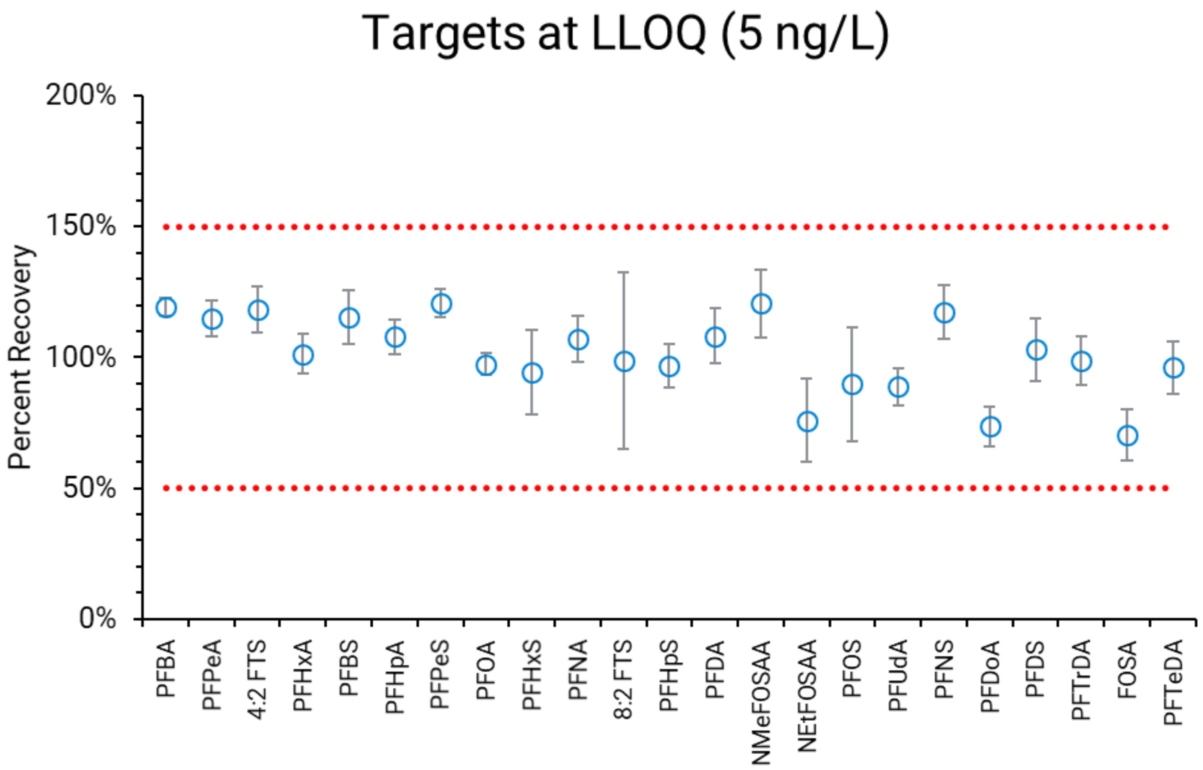
Acidify to pH 3.5 – 4.0 with acetic acid

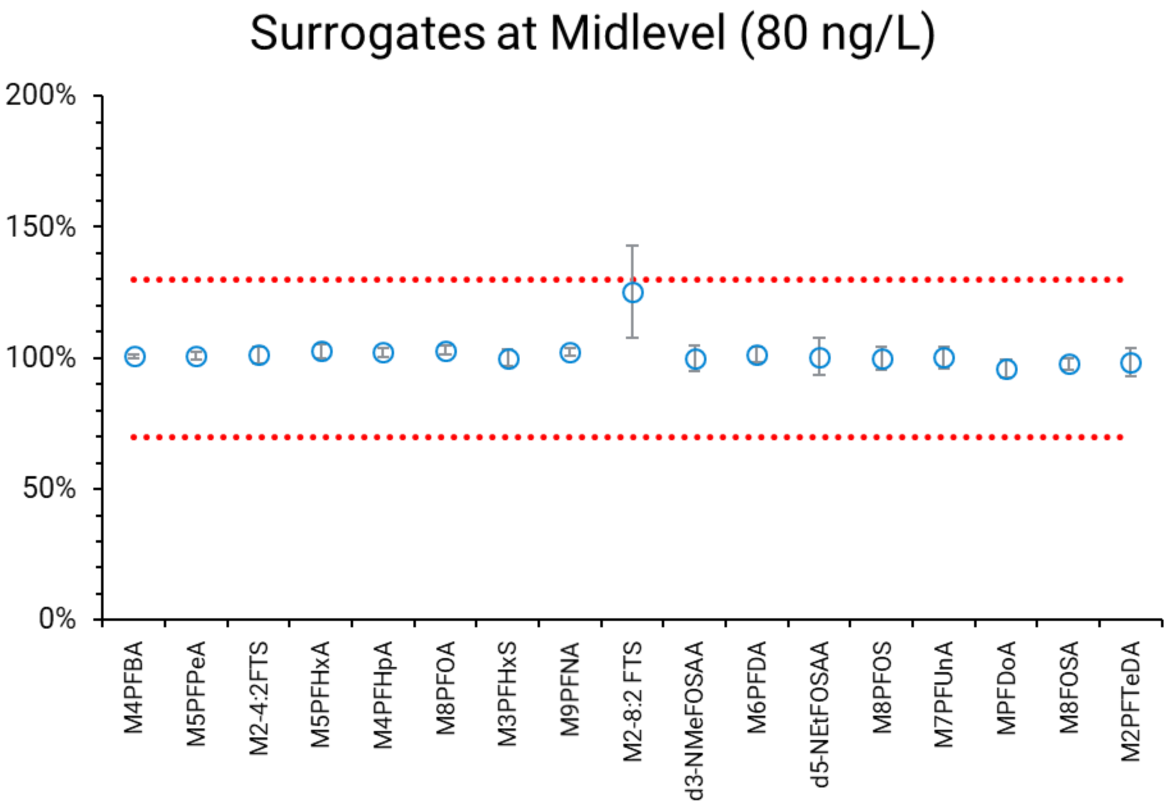
Transfer from centrifuge tube to polypropylene autosampler vial for LC-MS/MS analysis.

5 replicates

Measuring Low Level Spike Recoveries

Results for 15 mL polypropylene centrifuge tubes





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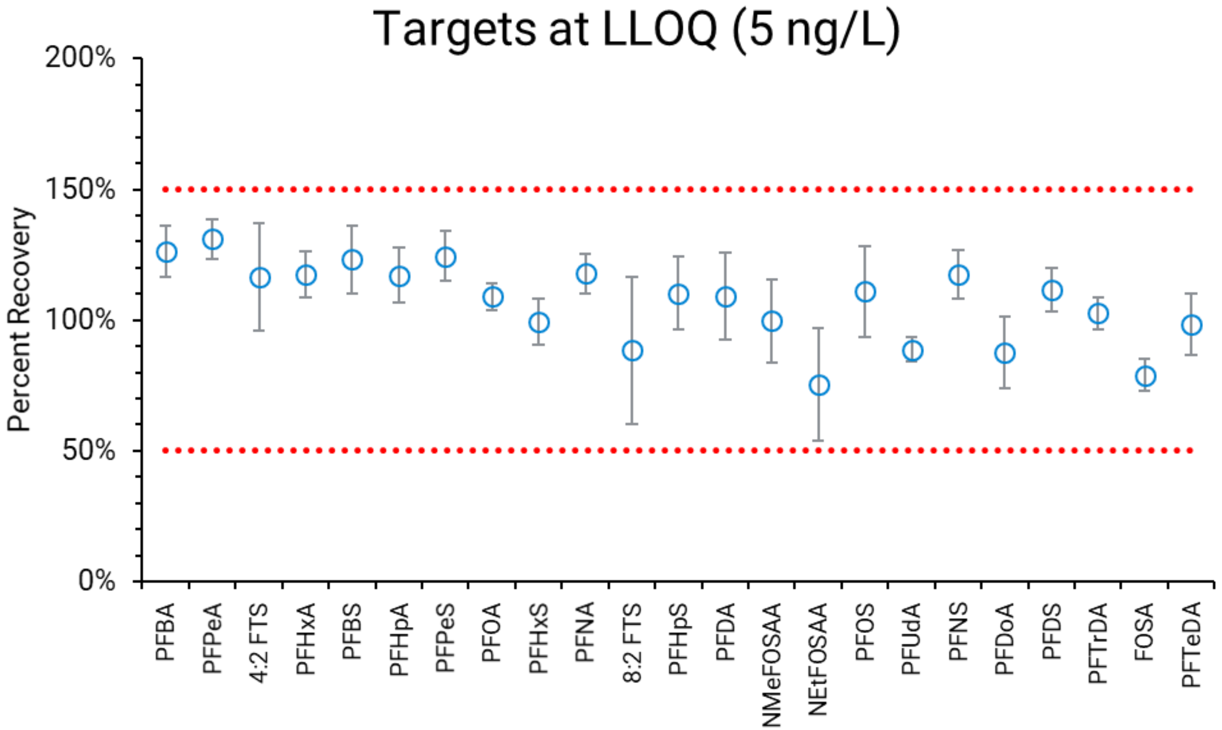
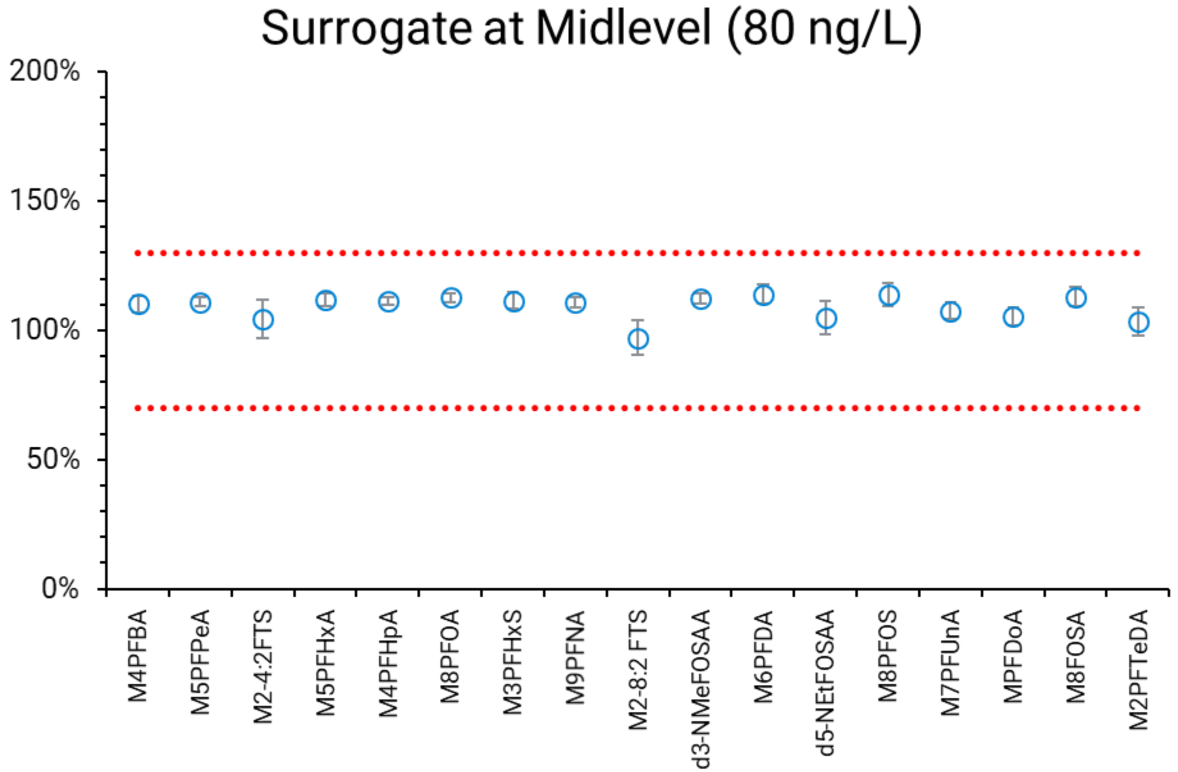
Measuring Low Level Spike Recoveries

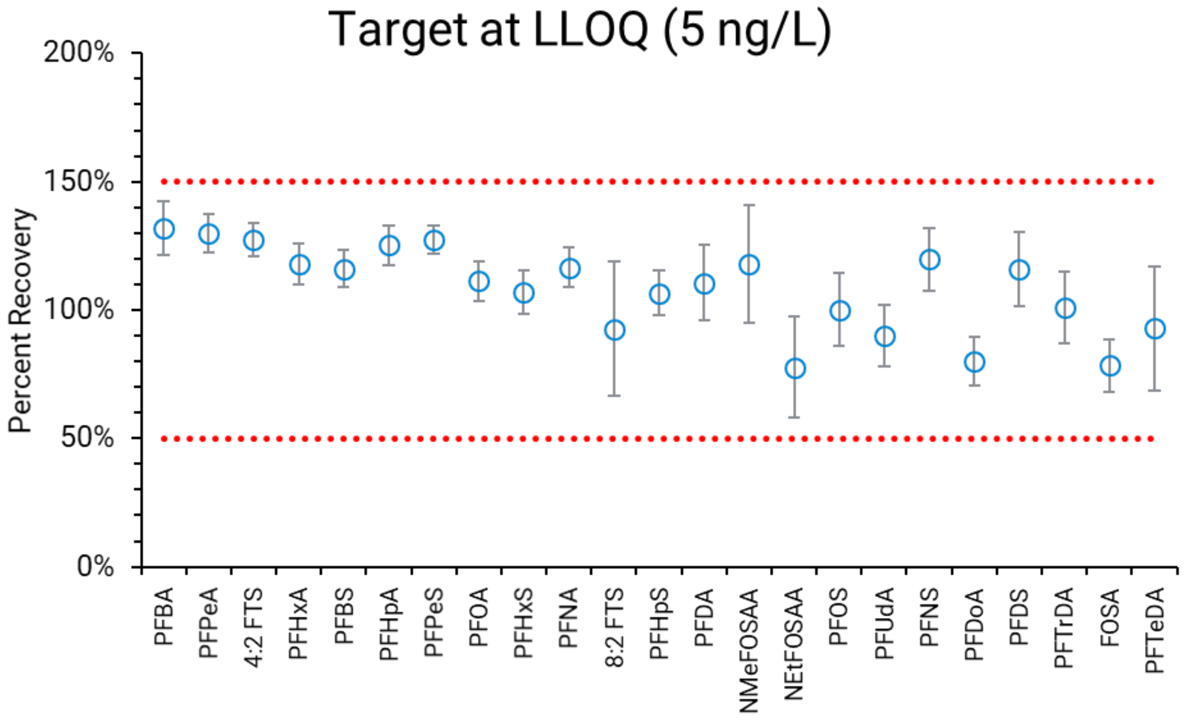
Results for polypropylene disposable syringes

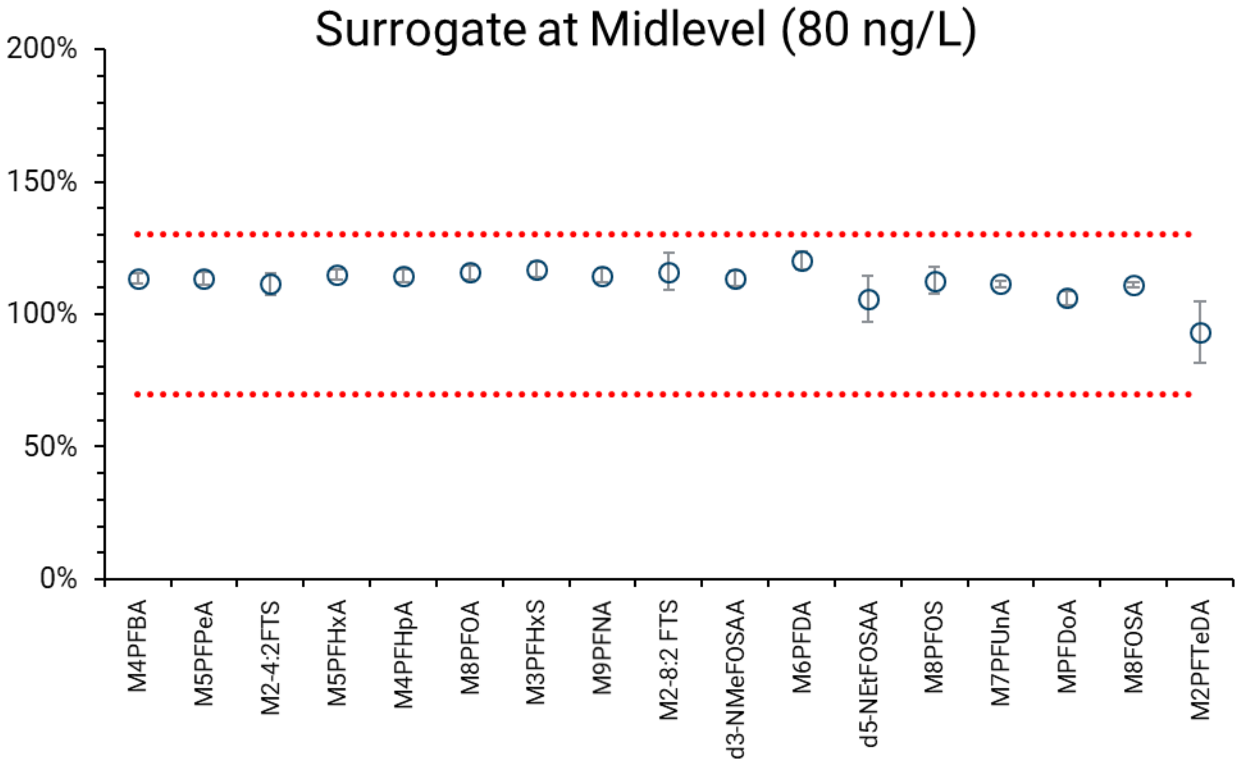




Measuring Low Level Spike Recoveries

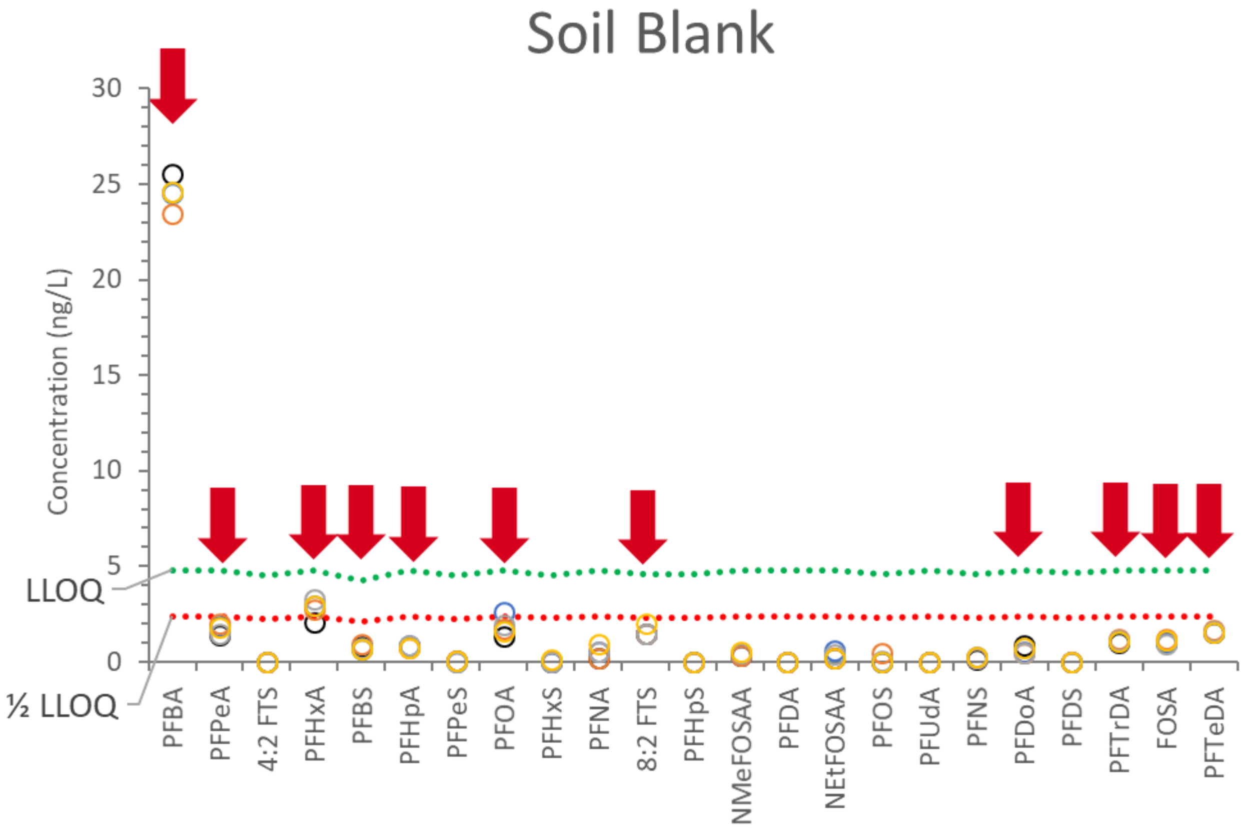
Results for regenerated cellulose syringe filters

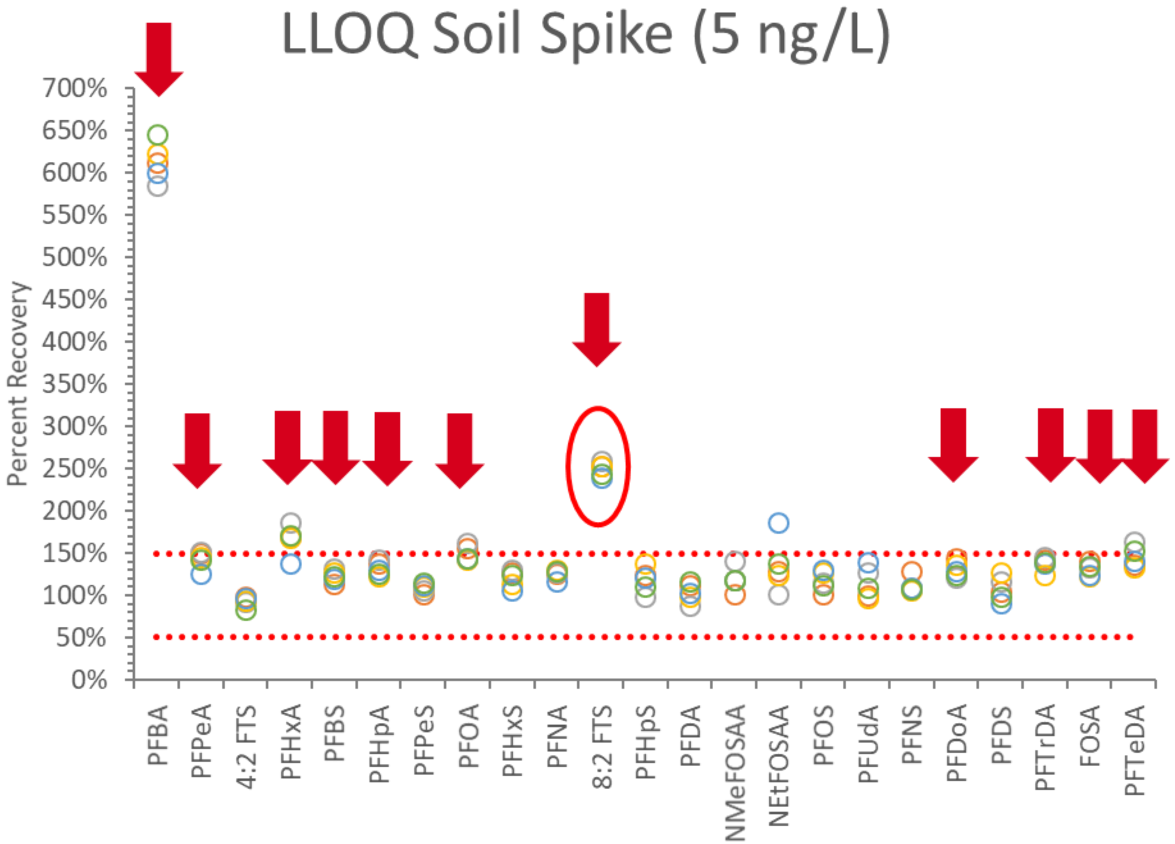




Soil Extract

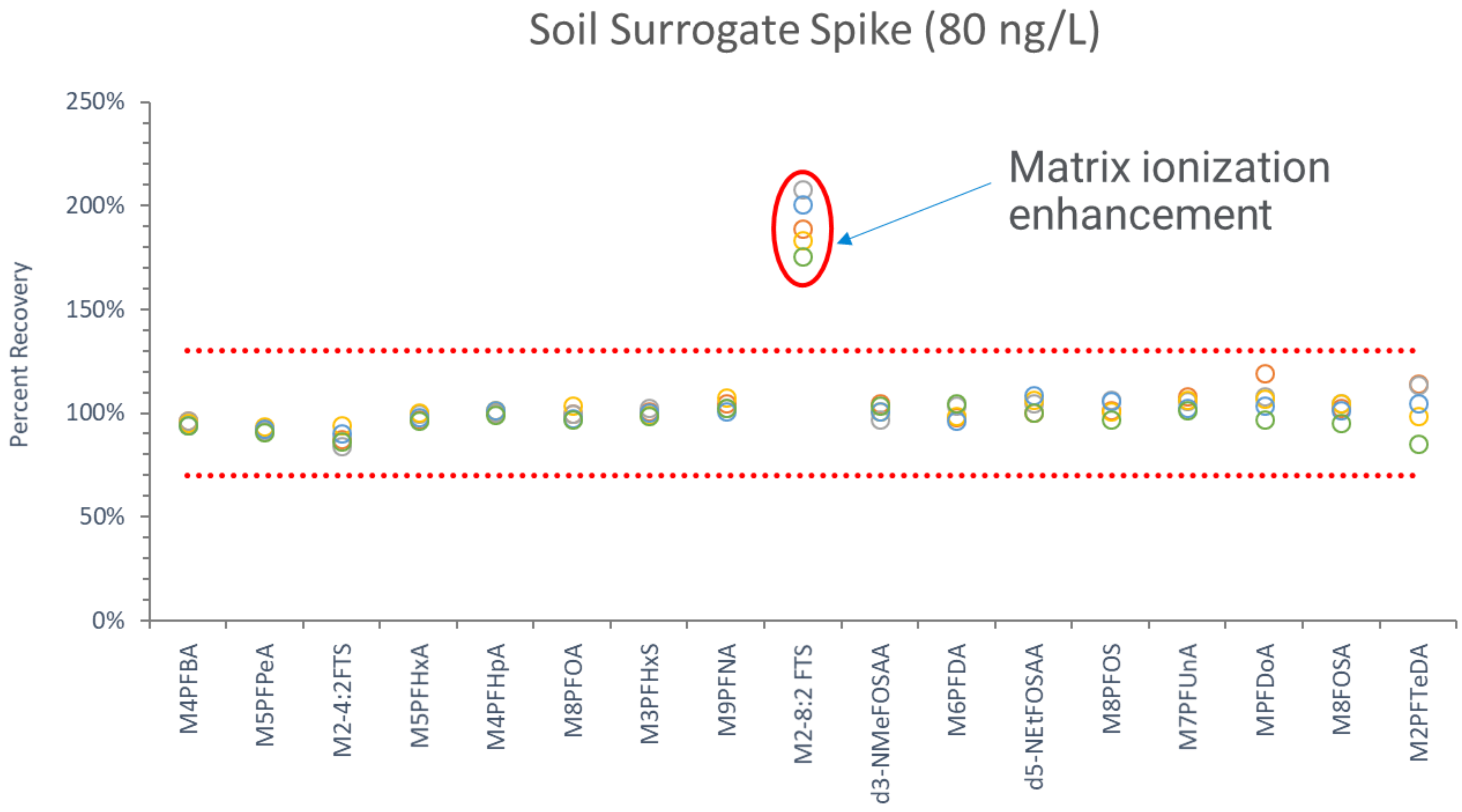
- Clean sandy loam – 6 replicates
- Low levels of PFAS present





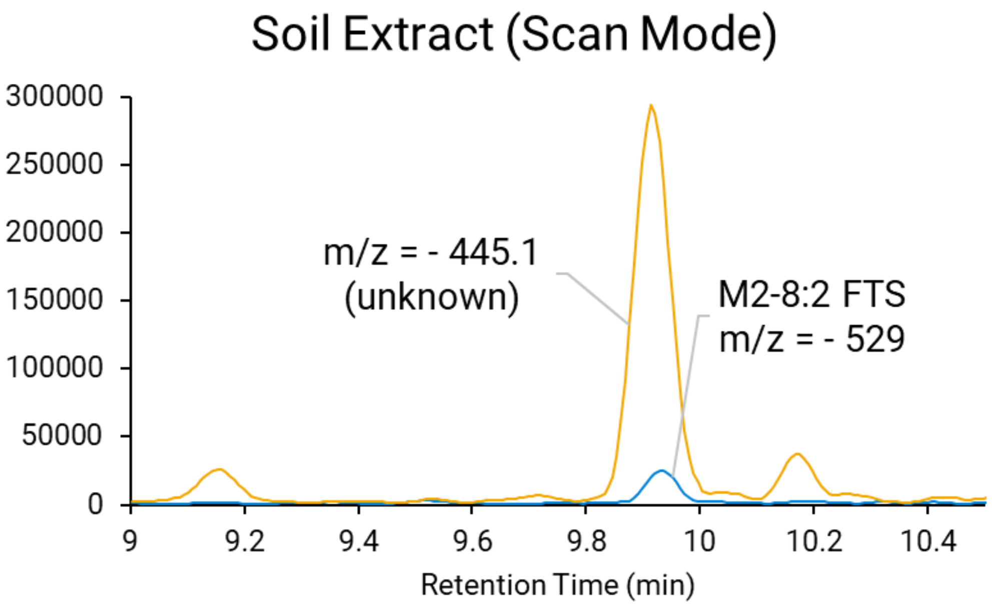
Soil Extract

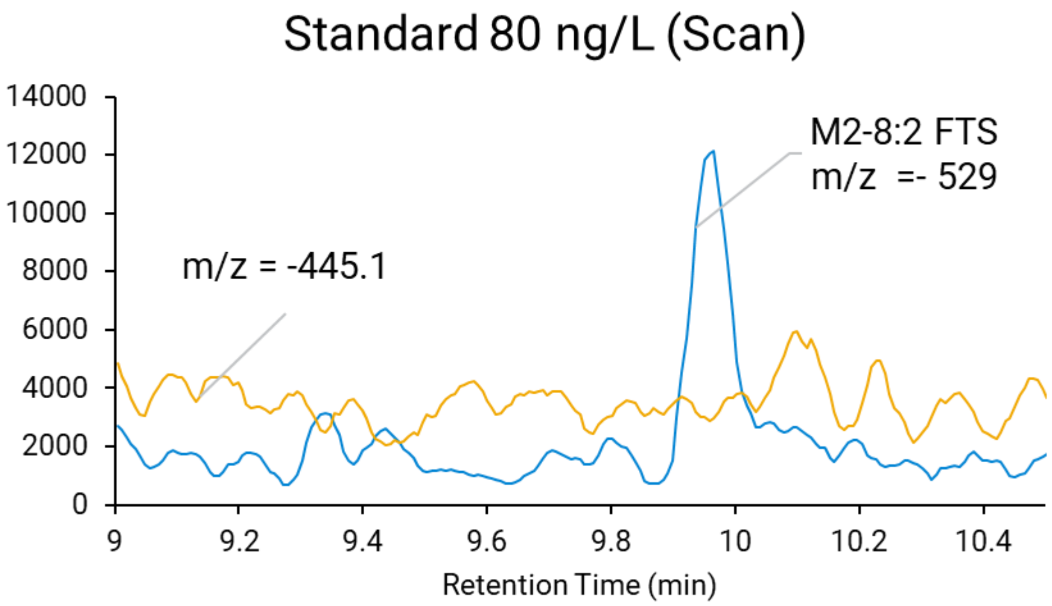
Clean sandy loam – 6 replicates



Soil Extract

Matrix Ionization Enhancement – approximately 2x response increase

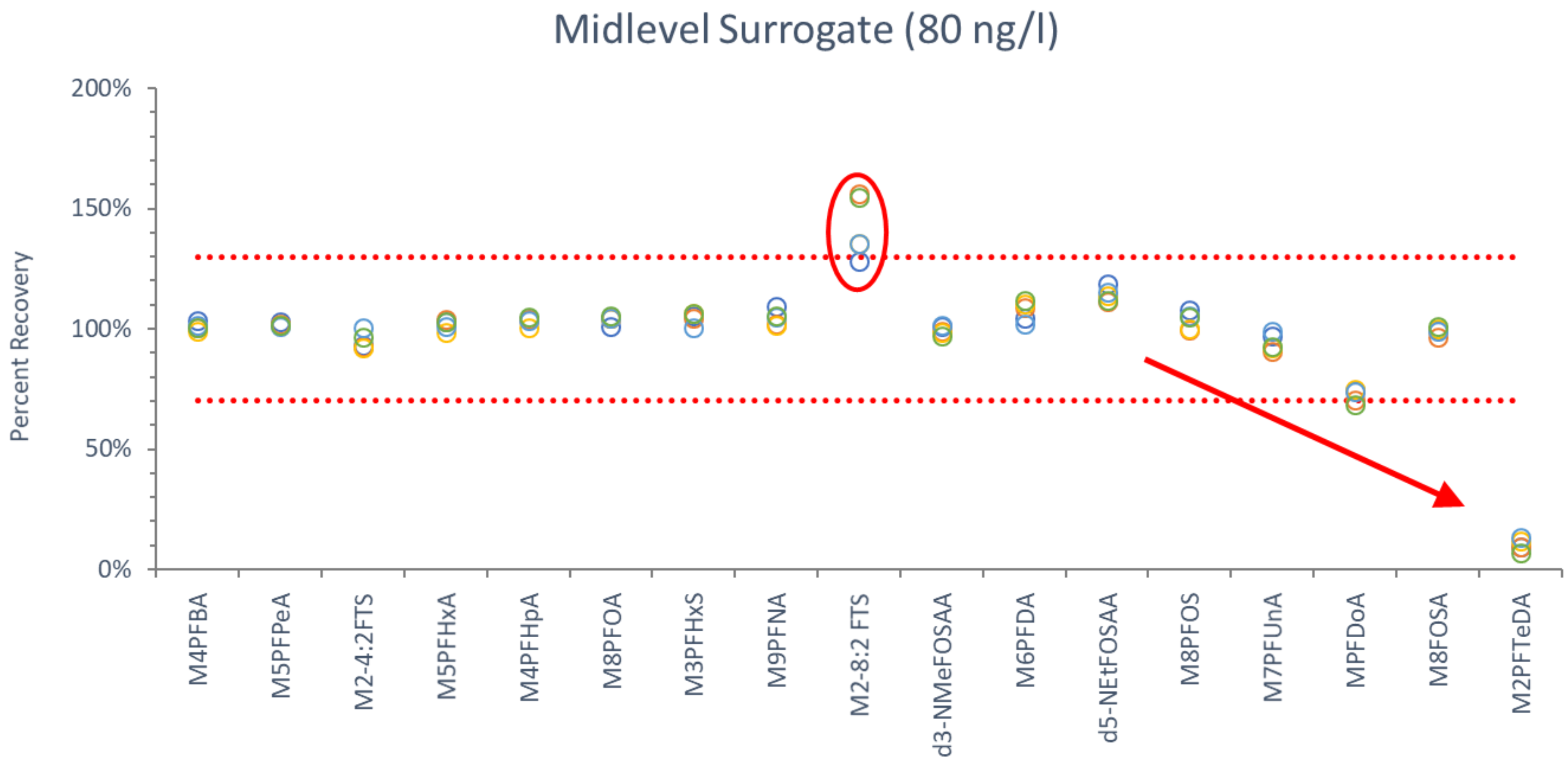




m/z -445.1 not yet identified

Filter Membrane

Matrix Ionization Enhancement – also observed PP filter media



Equipment & Supplies

Description	Agilent Part Number
Polypropylene autosampler vials and snap caps	5182-0567 and 5182-054
50 mL polypropylene volumetric flask	9301-1424
Captiva disposable syringes (10 mL)	9301-6474
Captiva premium syringe filters, regenerated cellulose	5190-5110
Zorbax RR Eclipse Plus C18, 2.1 x 100 mm, 1.8 µm	959758-902
HPLC	1290 Infinity II LC System
MS/MS	6470 Triple Quadrupole
Ion Source	Jet Stream ESI

Available September 1, 2020!	
InfinityLab PFC Delay Column	5062-8100
InfinityLab PFC-free HPLC conversion kit	5004-0006

Conclusion

- Workflow approach to method optimization is key for creating robust methods for PFAS analysis
- Establishing best practices for each step in the sample extraction, handling, separation, and detection is necessary to maximize recoveries and obtain low-level detection limits
- Selection of sample containers, syringes, filters, columns, and instrumentation suitable for PFAS analysis is critical to workflow optimization

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