Current LC/MS Approaches for PFAS Analysis with Short and Long Chain Mixtures

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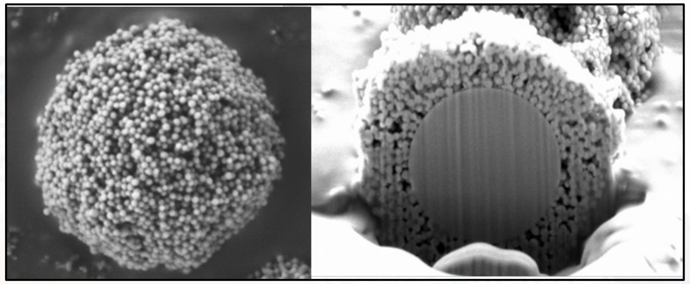
Presentation Outline

- Advanced Materials Technology
  - Superficially Porous Particles (SPP) vs. Fully Porous Particles (FPP)
  - HALO Enviroclass (PFAS Solution)
- Short and Long Chain PFAS
  - Existing EPA Methods
  - The Challenges
  - Charge Surface RP/AEX
- Method Development
  - Column Selection
  - Mobile Phase Optimization
  - Dry Lab Optimization
- Applications
  - Well Water Samples
  - Soil Samples (Collaboration w/ PFAS Solutions)
- Technical Resources/ Support



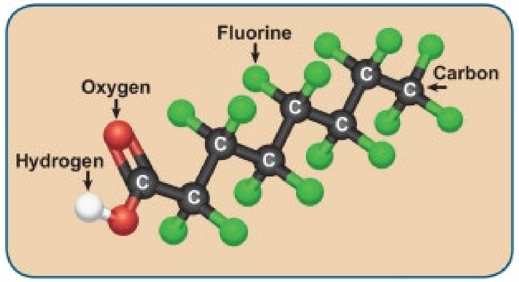
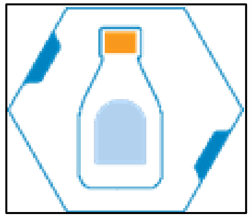
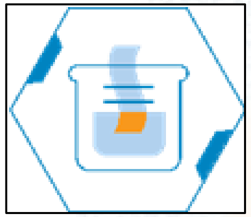
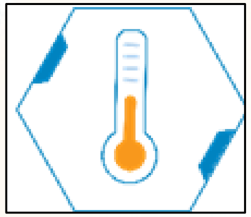
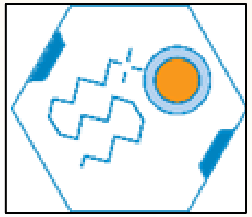


Image credit: NIEHS.









Founded in 2005 by Tim Langlois and Joe DeStefano

First company to commercially manufacture sub 3 µm superficially porous particles – *Fused-Core®*

CELEBRATING 20 YEARS advancedmaterialstechnology

Facility

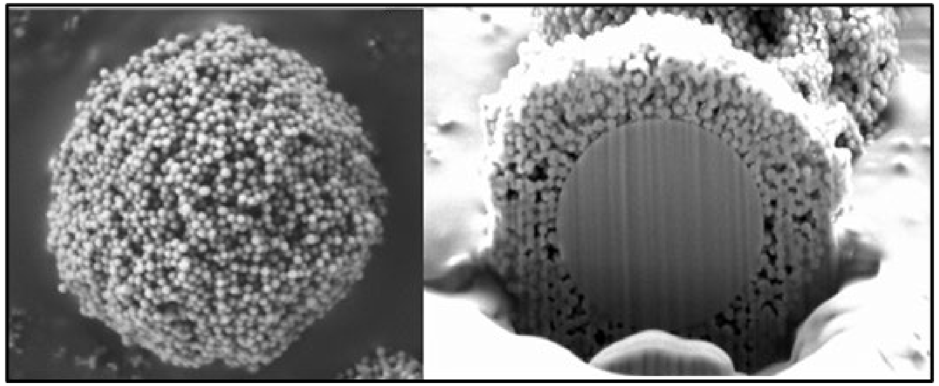
- ISO 9001 QMS certified company
- Fully equipped state of the art laboratories
- Own process: seed reaction
- All operations handled in Wilmington, DE
  - R&D, Applications, QA/QC, Manufacturing, Sales and Marketing
- Global distribution

AMT is a company of innovators and continues to grow and deliver enabling materials to market. Our incredible team is our greatest resource.

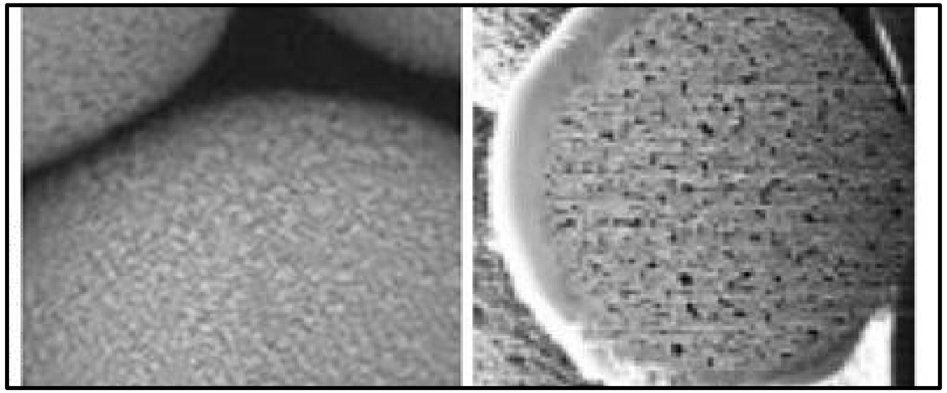
SPP vs. FPP

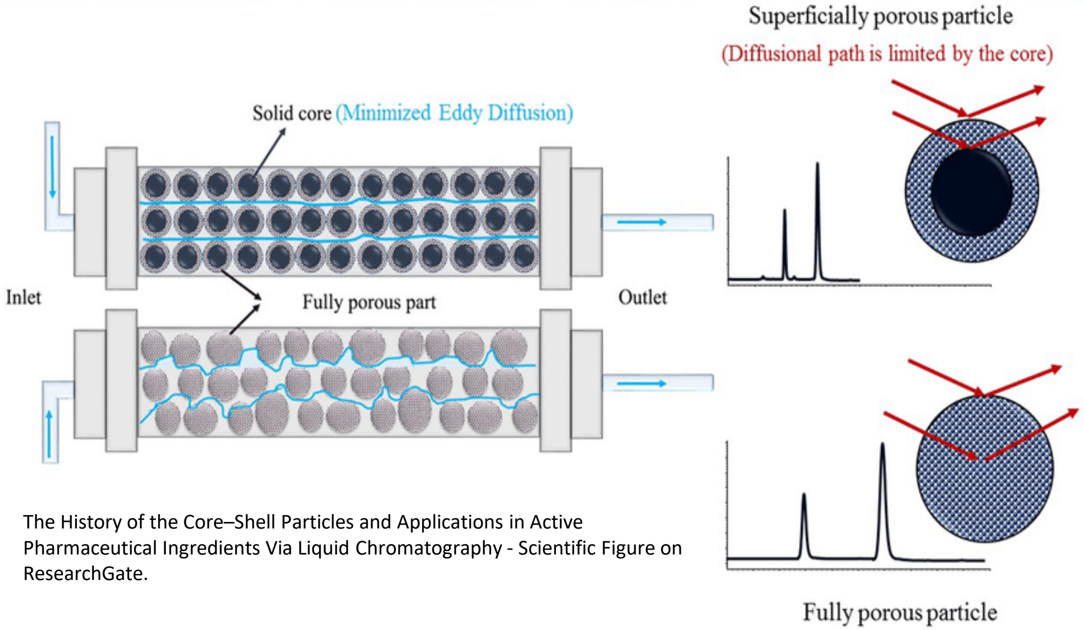
HALO®

Superficially Porous Particle (SPP)



Fully Porous Particle (FPP)

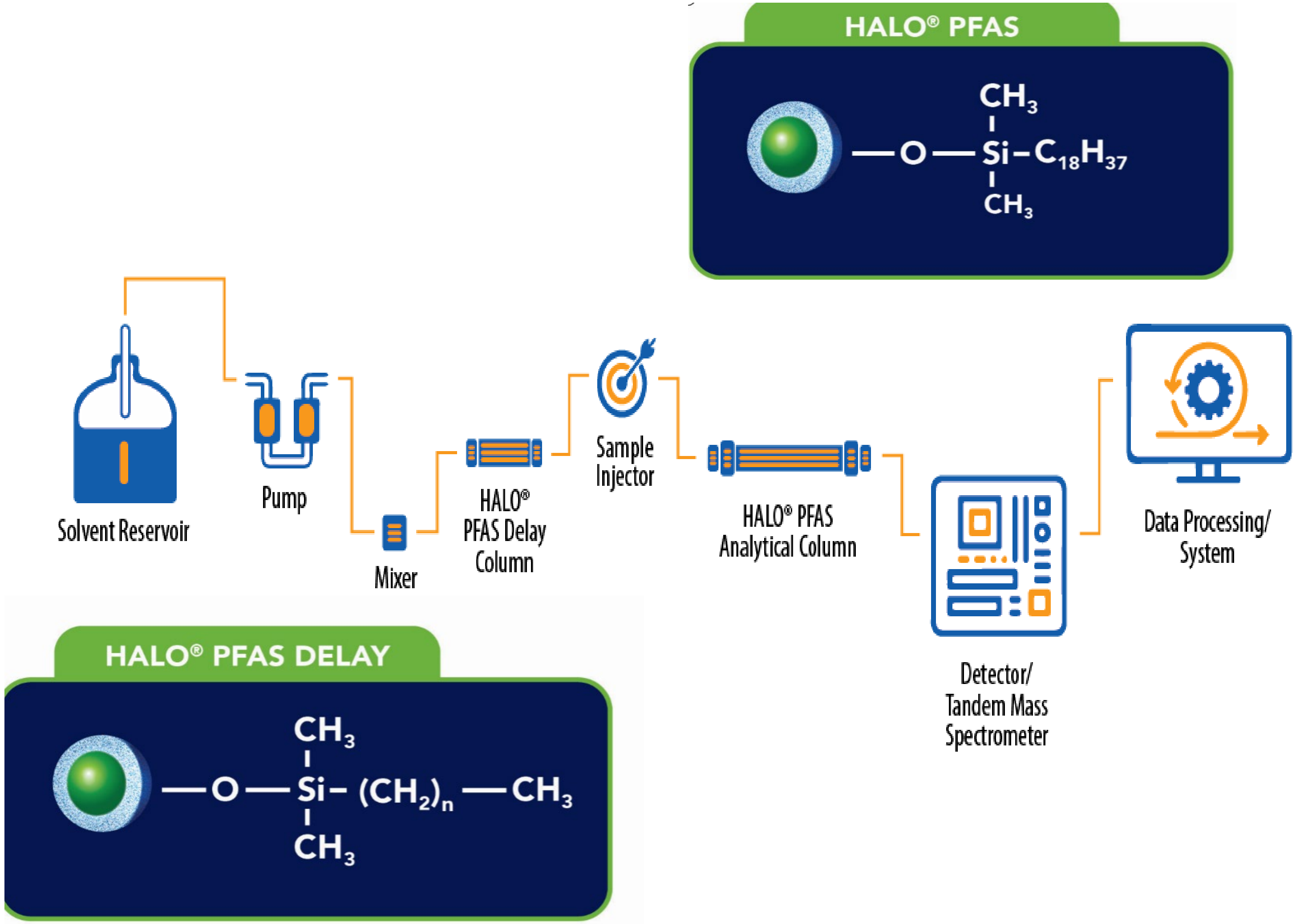




The History of the Core–Shell Particles and Applications in Active Pharmaceutical Ingredients Via Liquid Chromatography - Scientific Figure on ResearchGate.

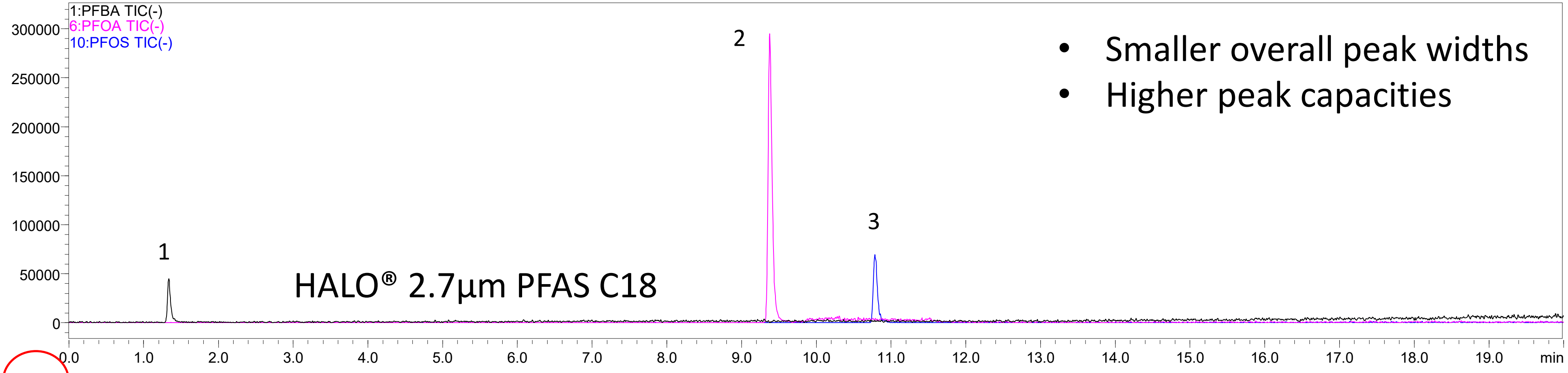
- Higher Efficiency/ Peak Capacities: SPPs provide a 30-50% increase in efficiency over FPPs of the same particle size
- Faster Analysis Times: Due to enhanced mass transfer
- Great Performance at Lower Back Pressures: SPPs allow for performance similar to smaller FPPs but at significantly lower back pressures

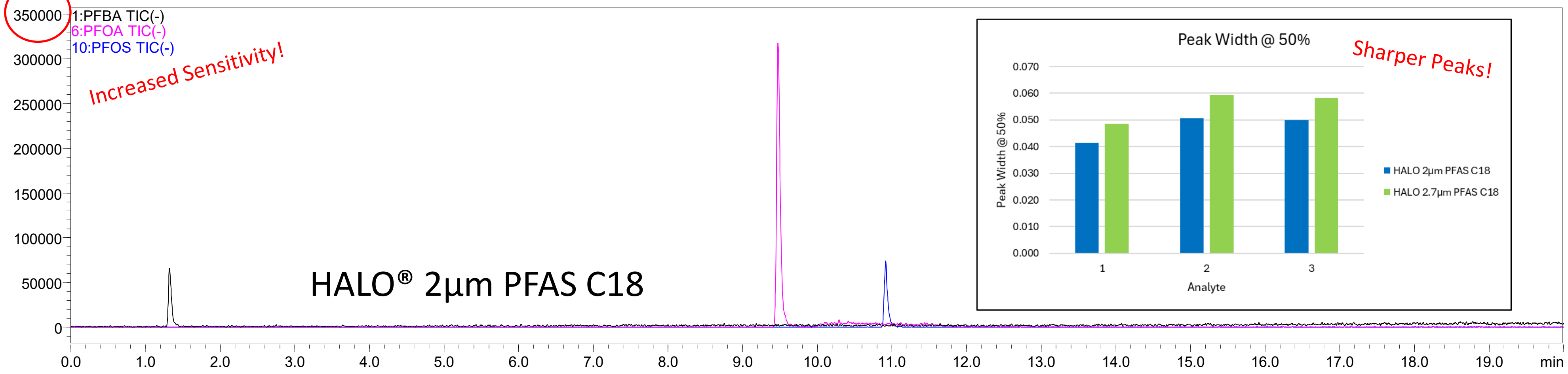
HALO® Enviroclass: PFAS Solution



HALO® 2µm PFAS C18

- Smaller overall peak widths
- Higher peak capacities





The PFAS Family

Panieri E, Baralic K, Djukic-Cosic D, Buha Djordjevic A, Saso L. PFAS Molecules: A Major Concern for the Human Health and the Environment. *Toxics*. 2022; 10(2):44. https://doi.org/10.3390/toxics10020044

HALO®

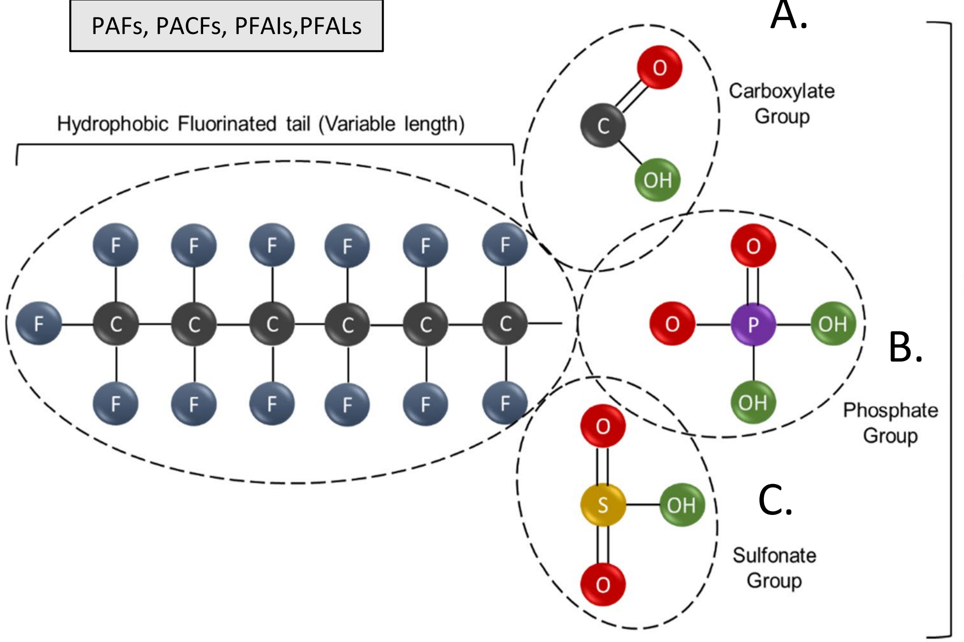
PFAS

Non-Polymeric

- **Per**fluoroalkyl substances
  - PFAAs (A, B, C)
  - PFEAs, PASFs, FASAs
  - PAFs, PACFs, PFAIs,PFALs
- **Poly**fluoroalkyl substances
  - Fluorotelomer substances
  - Polyfluoroalkane sufonamido substances
  - Chloropolyfluroalkyl either acids
  - Chloropolyfluroalkyl acids
  - PolyFCAs
  - PolyFEAs

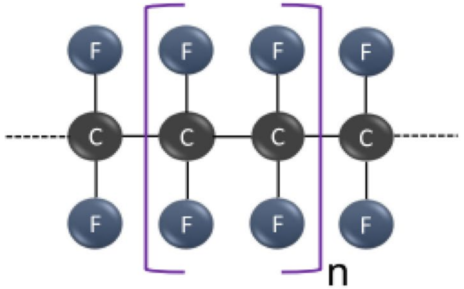
Polymeric

- Side-chain fluorinated polymers
- Fluoropolymers (D)
- PFPE (E)



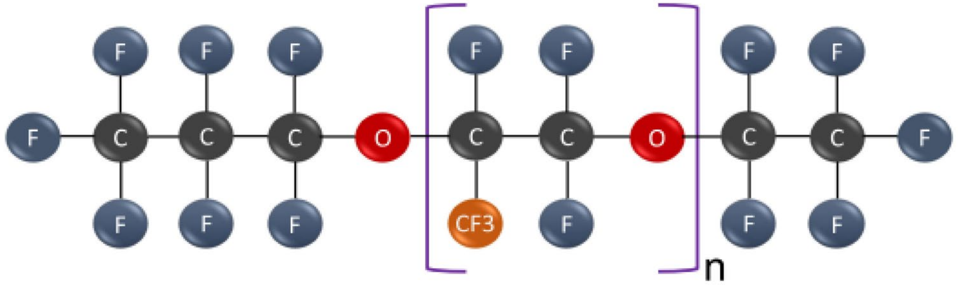
D.

FLUOROPOLYMERS (e.g. Polytetrafluoroethylene, PTFE)



E.

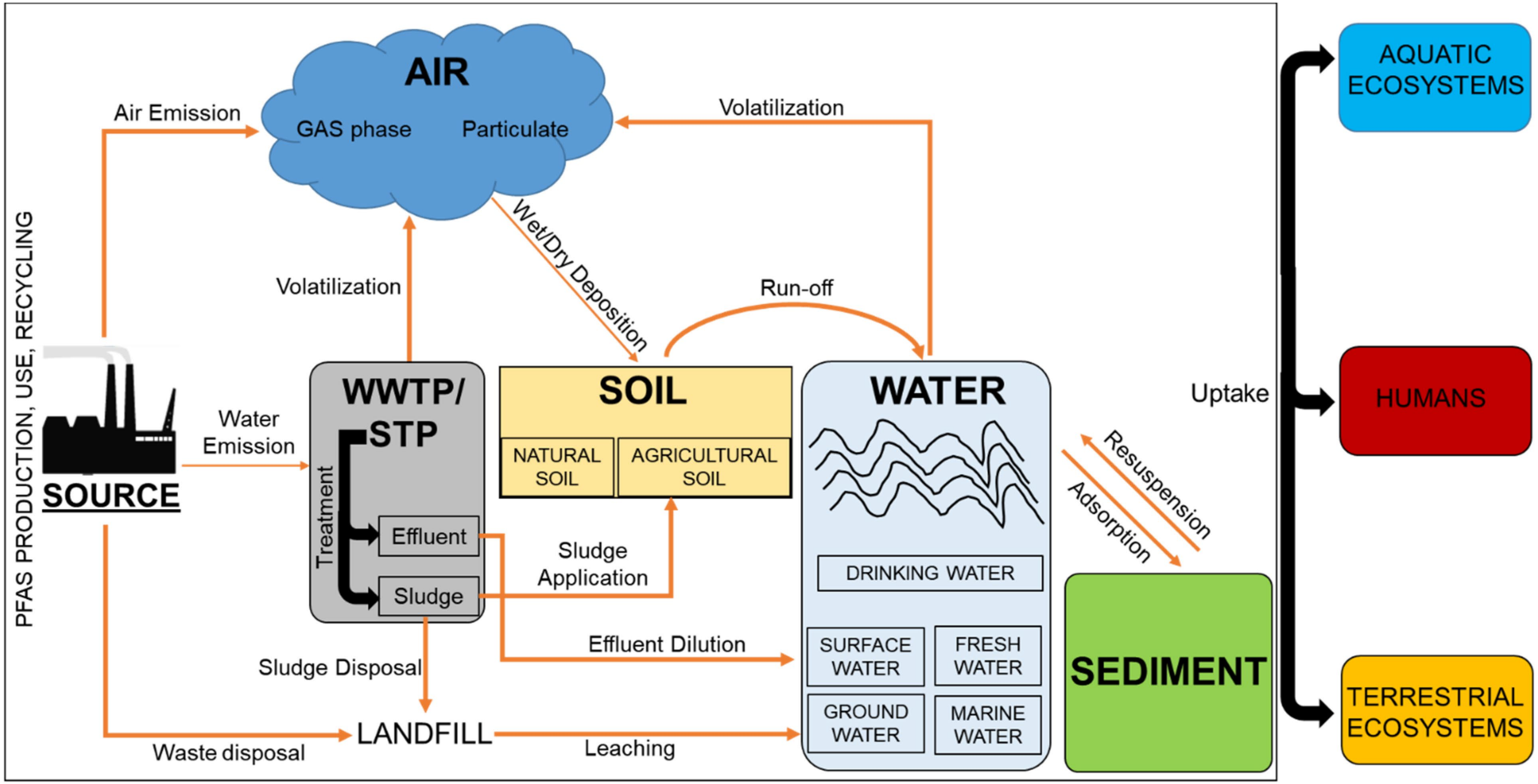
PERFLUOROPOLYETHERS (e.g. KRYTOX)



PFAS Environmental Distribution and Exposure Routes

Panieri E, Baralic K, Djukic-Cosic D, Buha Djordjevic A, Saso L. PFAS Molecules: A Major Concern for the Human Health and the Environment. *Toxics*. 2022; 10(2):44. https://doi.org/10.3390/toxics10020044

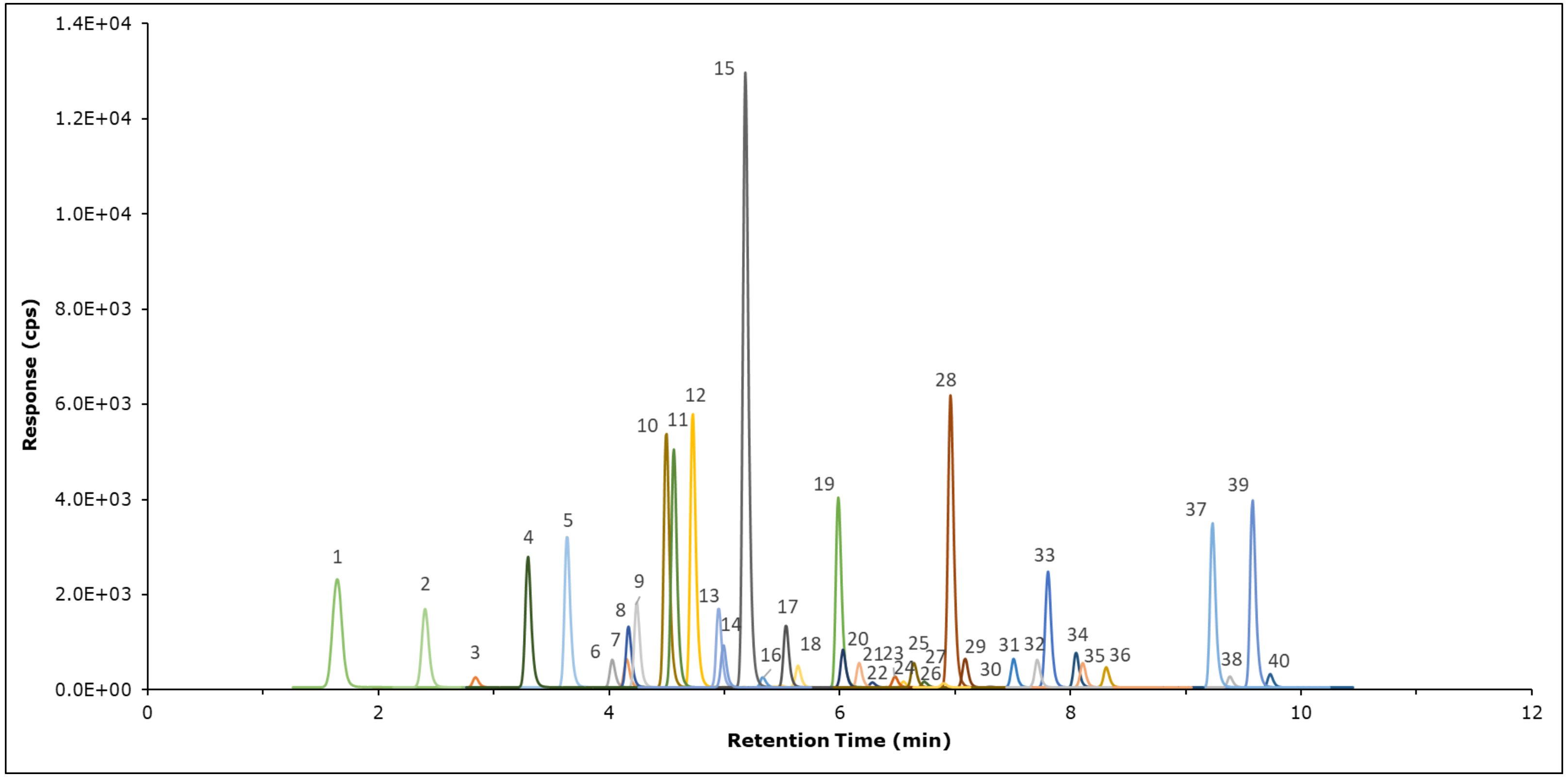
HALO®



EPA PFAS Drinking Water Laboratory Methods

Method	Matrix			Calibration Type		# of PFAS
	Drinking Water	Non-Potable Water	Solid	External	Isotope Dilution	
EPA 533	●				●	25
EPA 537.1	●			●		18
EPA 537.1 (modified)		●	●		●	Various
EPA 1633		●	●		●	40
EPA 8327		●		●		24
ASTM 7968			●	●		21–31
ASTM 7979		●		●		21–31
ASTM 8421		●		●	✱	44

EPA 1633: 40 PFAS Calibration Standard



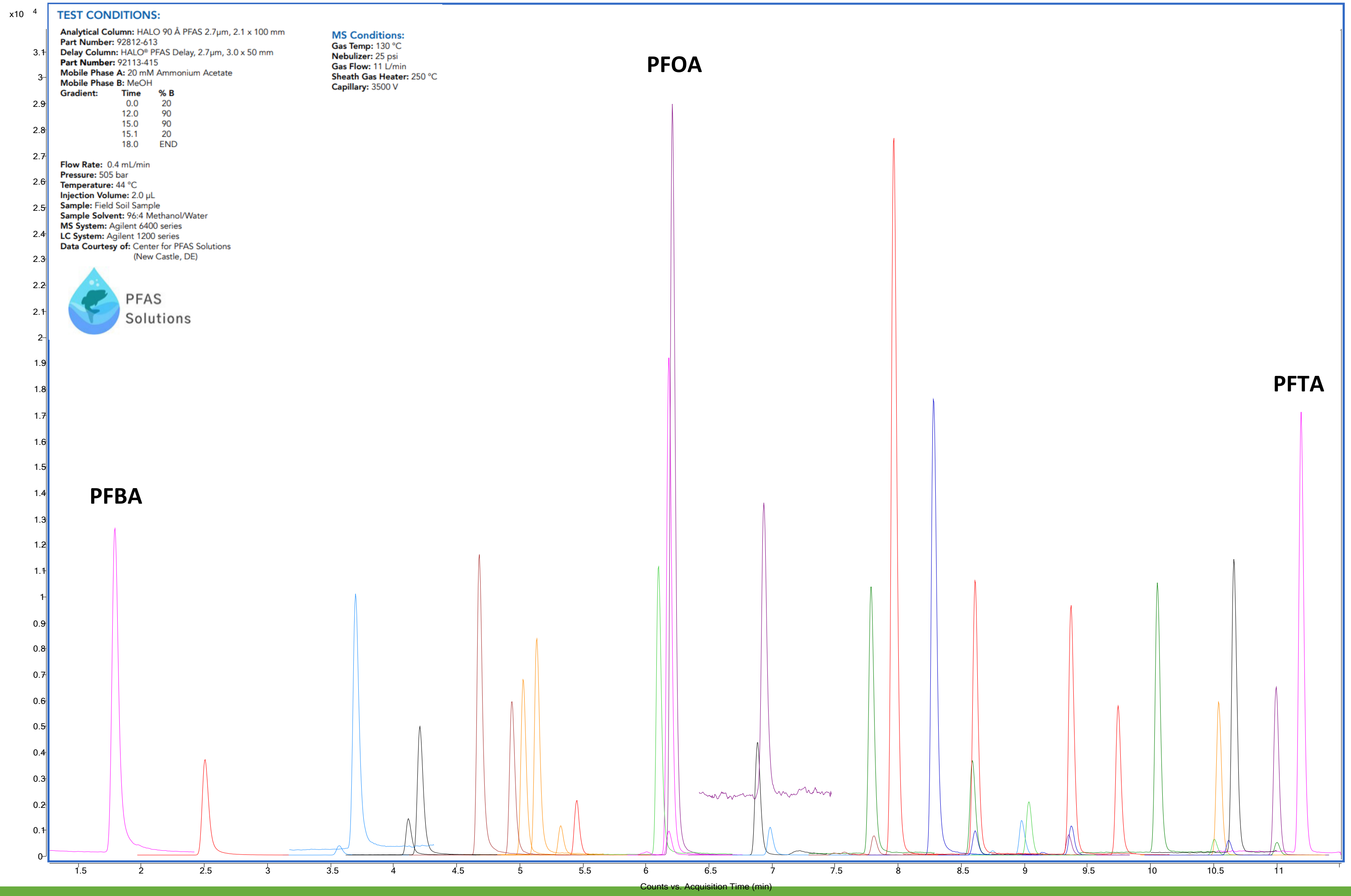
TEST CONDITIONS:

Column: HALO 90Å PFAS 2.7µm, 2.1 x 50 mm
Part Number: 92812-413
Delay Column: HALO® PFAS Delay, 2.7µm, 3.0 x 50 mm
Part Number: 92113-415
Mobile Phase A: 2 mM Ammonium Acetate in 95:5 water/acetonitrile (v/v)
Mobile Phase B: Acetonitrile
Gradient:

Time	%B	Flow Rate (mL/min)
0.0	2.0	0.35
0.2	2.0	0.35
4.0	30.0	0.40
7.0	55.0	0.40
9.0	75.0	0.40
10.0	95.0	0.40
10.4	2.0	0.40
11.8	2.0	0.40
12.0	2.0	0.35

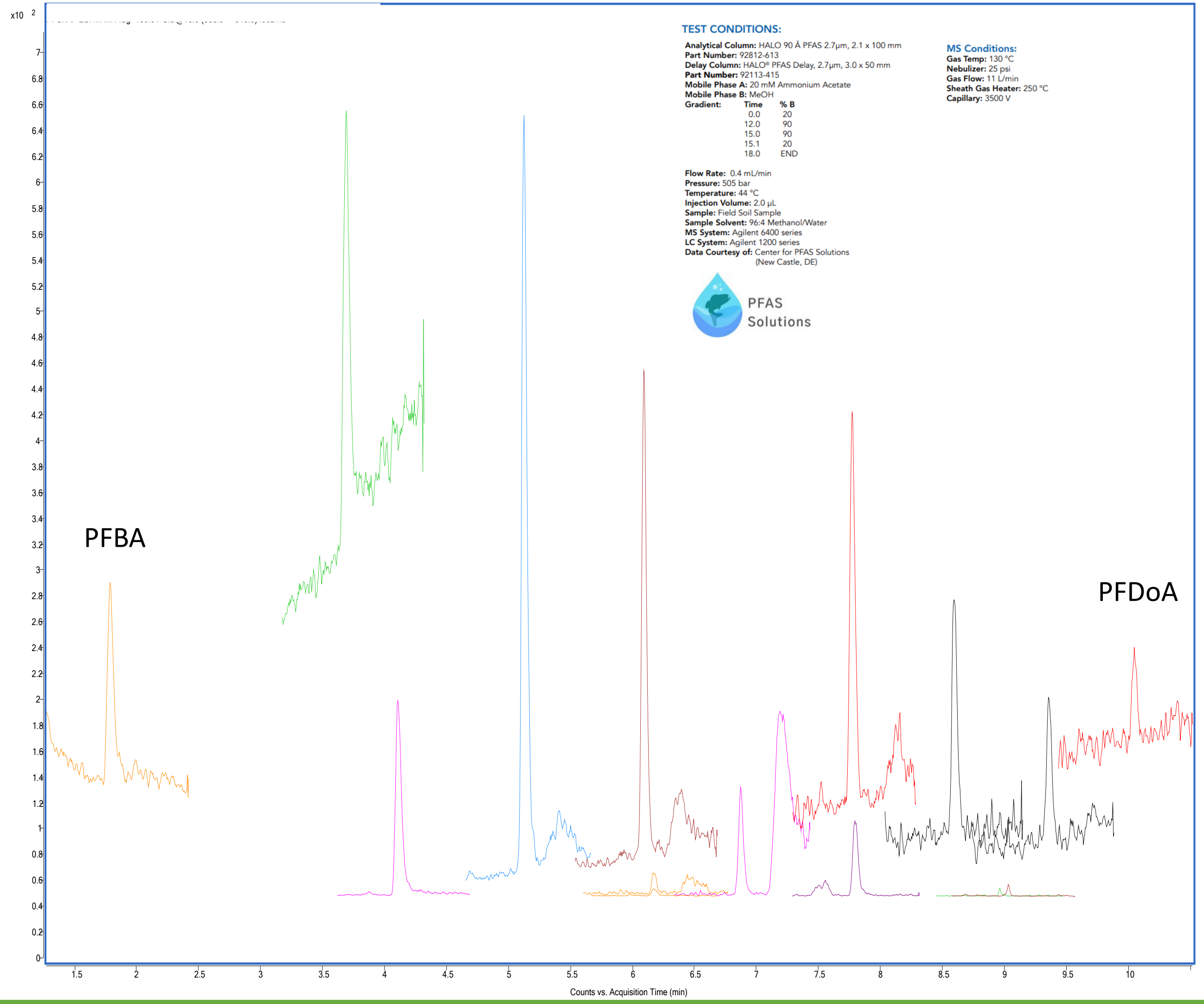
Pressure: 195 bar
Temperature: 40 °C
Injection Volume: 2.0 µL
Sample Solvent: 96:4 Methanol/Water
MS System: Agilent 6495C
LC System: Agilent 1290 Infinity II
Detector: MS (ESI-), MRM
Data Courtesy of: Lara Rosenberger

EPA 1633: 40 PFAS Calibration Standard



Analyte	Abbreviation	Retention Time (min)
Perfluorobutanoic acid	PFBA	1.79
Perfluoro-3-methoxypropanoic acid	PFMPA	2.51
3-perfluoropropyl propanoic acid	3:3 FTCA	3.57
Perfluoropentanoic acid	PFPeA	3.70
Perfluorobutanesulfonic acid	PFBS	4.12
Perfluoro-4-methoxybutanoic acid	PFMBA	4.21
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	4.68
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	4.94
1H, 1H, 2H, 2H-Perfluorohexane sulfonic acid	4:2FTS	5.03
Perfluorohexanoic acid	PFHxA	5.14
Perfluoropentanesulfonic acid	PFPeS	5.33
Hexafluoropropylene oxide dimer acid	HFPO-DA	5.45
Perfluoroheptanoic acid	PFHpA	6.10
Perfluorohexanesulfonic acid	PFHxS	6.18
2H,2H,3H,3H-Perfluorooctanoic acid	5:3 FTCA	6.18
4,8-dioxa-3H-perfluorononanoic acid	ADONA	6.21
1H, 1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2FTS	6.89
Perfluorooctanoic acid	PFOA	6.93
Perfluoroheptanesulfonic acid	PFHpS	6.99
Perfluorononanoic acid	PFNA	7.79
Perfluorooctanesulfonic acid	PFOS	7.81
3-perfluoroheptyl propanoic acid	7:3 FTCA	8.00
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	8.28
1H, 1H, 2H, 2H-Perfluorodecane sulfonic acid	8:2FTS	8.59
Perfluorononanesulfonic acid	PFNS	8.61
Perfluorodecanoic acid	PFDA	8.61
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	8.98
Perfluorooctanesulfonamide	PFOSA	9.04
Perfluorodecanesulfonic acid	PFDS	9.35
Perfluoroundecanoic acid	PFUnA	9.37
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	9.37
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	9.74
Perfluorododecanoic acid	PFDoA	10.05
N-Methyl Perfluorooctanesulfonamide	NMeFOSA	10.51
N-Methyl Perfluorooctanesulfonamidoethanol	NMeFOSE	10.54
Perfluorododecanesulfonic acid	PFDoS	10.62
Perfluorotridecanoic acid	PFTrDA	10.66
N-Ethyl Perfluorooctanesulfonamidoethanol	NEtFOSE	11.00
N-Ethyl Perfluorooctanesulfonamide	NEtFOSA	11.00
Perfluorotetradecanoic acid	PFTA	11.19

Field Soil Sample



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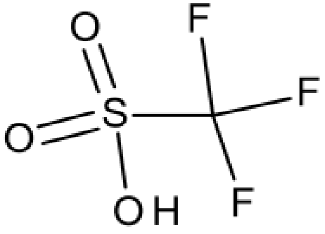
Perfluoropentanoic acid	PFPeA	3.70
Perfluorobutanesulfonic acid	PFBS	4.12
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	4.94
Perfluorohexanoic acid	PFHxA	5.14
Perfluoroheptanoic acid	PFHpA	6.10
Perfluorohexanesulfonic acid	PFHxS	6.18
2H,2H,3H,3H-Perfluorooctanoic acid	5:3 FTCA	6.18
1H, 1H, 2H, 2H-Perfluoroctane sulfonic acid	6:2FTS	6.89
Perfluorononanoic acid	PFNA	7.79
Perfluorooctanesulfonic acid	PFOS	7.81
Perfluorodecanoic acid	PFDA	8.61
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	8.98
Perfluorooctanesulfonamide	PFOSA	9.04
Perfluoroundecanoic acid	PFUnA	9.37
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	9.37
Perfluorododecanoic acid	PFDoA	10.05

Exemplary Ultra Short-Chain PFAS (USC)

HALO®

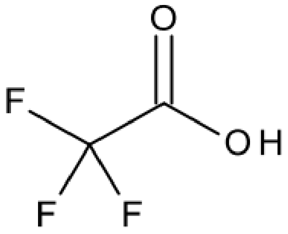
Challenges:
- Retention
- Sensitivity
- Peak Shape

C1

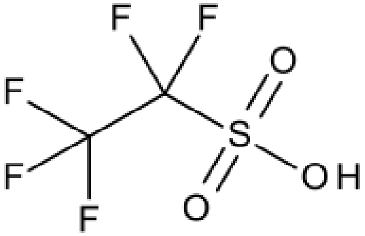


PFMtS
150.08 MW

TFA
114.02 MW

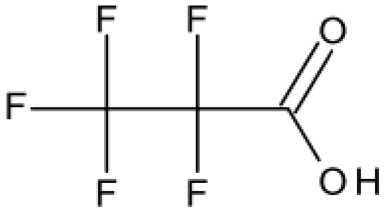


C2

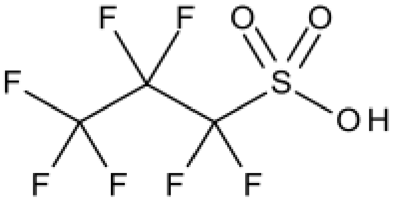


PFEtS
200.08 MW

PFPrA
164.03 MW

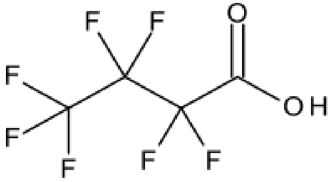


C3

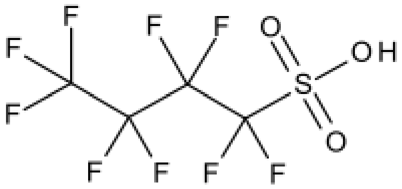


PFPrS
250.09 MW

PFBA
214.04 MW



C4



PFBS
300.10 MW

LGC

Ultrashort-chain-PFAS (C1-C4)
DRE-A30000064MW

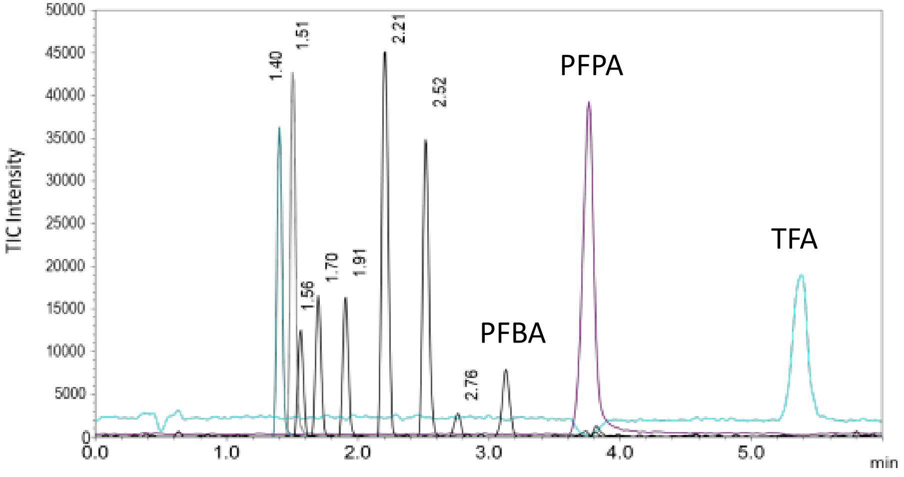
- Fluorine inductive effect causes low pKa's for compounds with an adjacent R-OH, R-COOH or R-SO_3H.
- Many compounds of interest are *fully ionized* under typical LC separation and ESI conditions.

Method Development USC PFAS
HILIC vs. Reverse Phase: Charged Surface Materials

HILIC: Charged Surface

EXAMPLE SEPARATION OF PFAS on HALO® HILIC-Plus Prototype Column

Conditions: 2.1 ID x 100 mm HALO HILIC-Plus Prototype, 0.5 mL/min; 35°C
85-65% B in 5 minutes, hold for 1 min., return to 85%;
B- 80% AcN/20%IPA/0.1% FA, A – 0.1% FA/10 mM AF in water



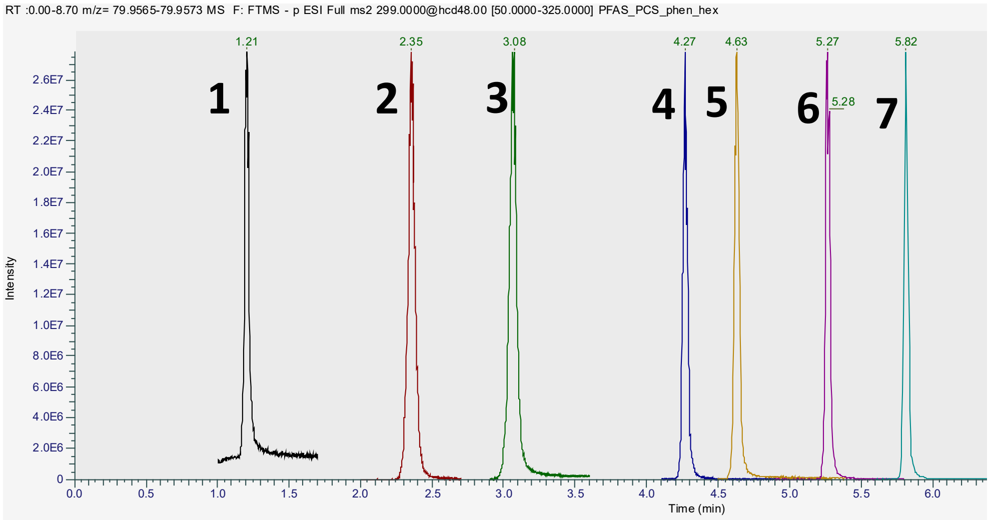
Rt and Compounds:

1.40 - 11-Chloroeicosafluoro-3-oxanonane-1-sulfonate
1.51 - 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonate
1.56 - Perfluorooctanesulfonic acid
1.70 - Perfluorohexanesulfonic acid
1.91 - Perfluorobutanesulfonic acid
2.21 - Perfluorooctanoic acid
2.52 - Perfluorohexanoic acid
2.76 - Perfluoropentanoic acid
3.13 – Perfluorobutanoic acid
3.76 – Perfluoropropionic acid
5.38 – Trifluoroacetic acid

Reverse Phase: Charged Surface

EXAMPLE SEPARATION OF PFAS on HALO® PCS-Phenyl-Hexyl

Conditions: 2.1 ID x 100 HALO PCS Phenyl Hexyl, 0.4 mL/min; 40°C
2-95% B in 7 minutes, hold for 1 min., return to 2%;
B- MeOH, A-5mM Amm. Formate, 0.05% FA in water
Thermo QE-HF in PRM mode

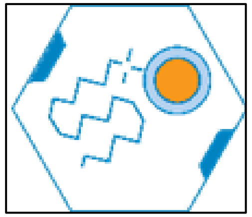


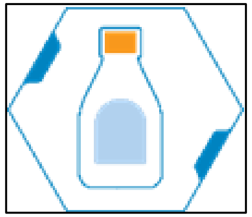
1. Trifluoroacetic acid
2. Perfluromethanesulfonic acid
3. Perflurpropionic acid
4. Perfluoroethanesulfonic acid
5. Perfluorobutanoic acid
6. Perfluoropropanesulfonic acid
7. Perfluorobutanesulfonic acid

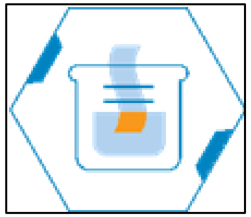
Method Development: Ultrashort Chain to Long Chain

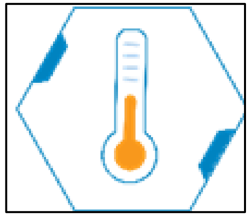
HALO®

Column Selection
- Reverse Phase vs. HILIC?
- Stationary Phase – Mixed
- Column Dimension
- SPP vs. FPP



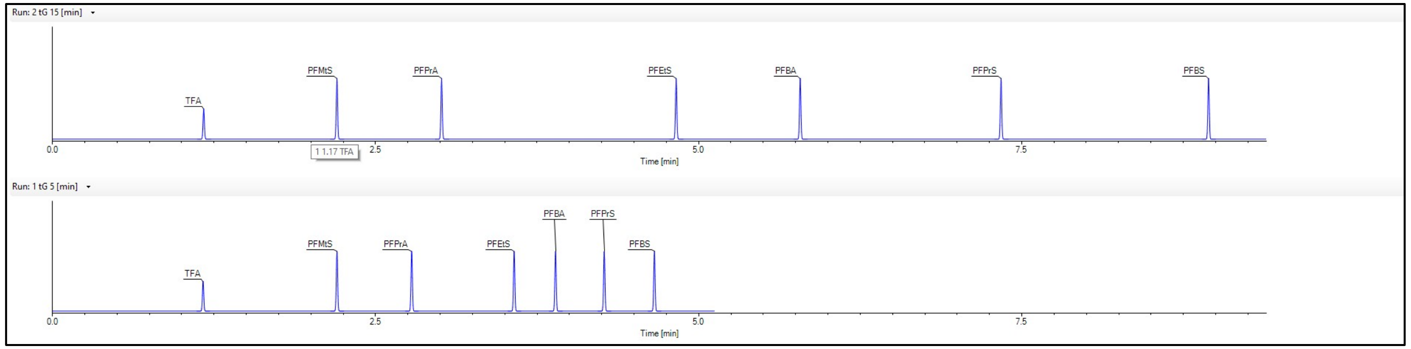






Mobile Phase Optimization
- Acidic Modifier/ Concentration
- Buffer Modifier/ Concentration
- pH, Temperature

Dry Lab Optimization



Method Development: USC to LC

Stationary Phase, Mobile Phase, Temperature, Gradient

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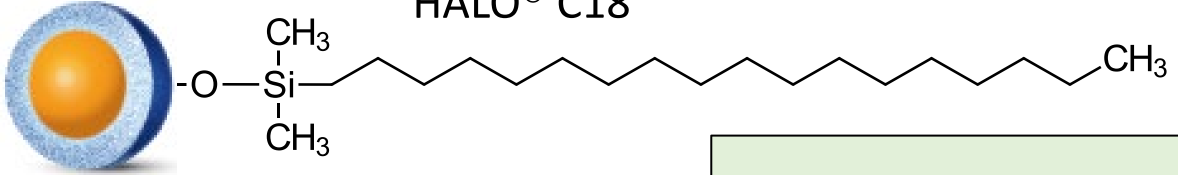
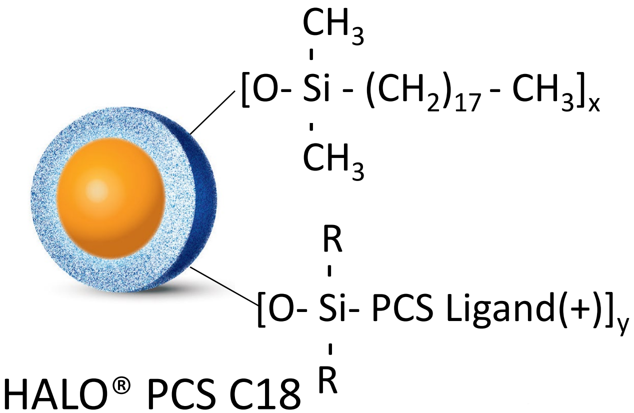
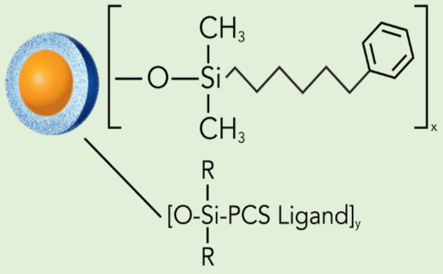
HALO® C18

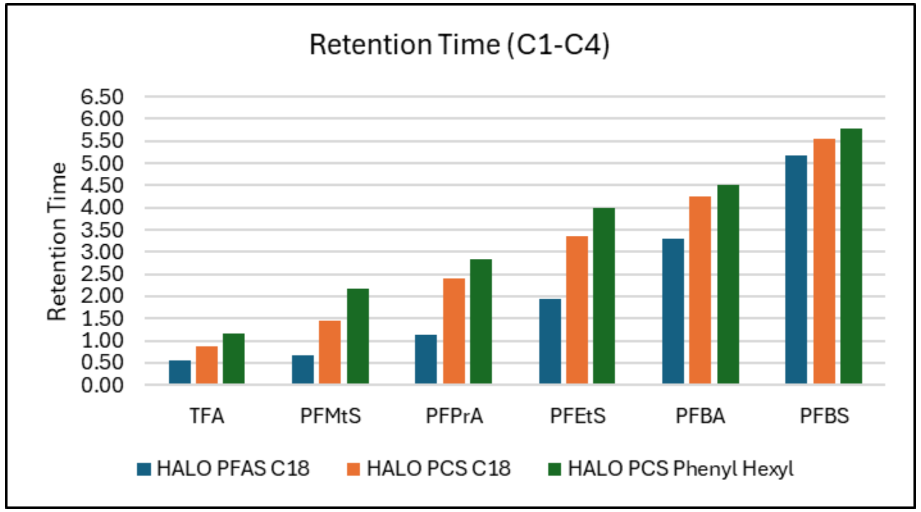




HALO® PCS C18

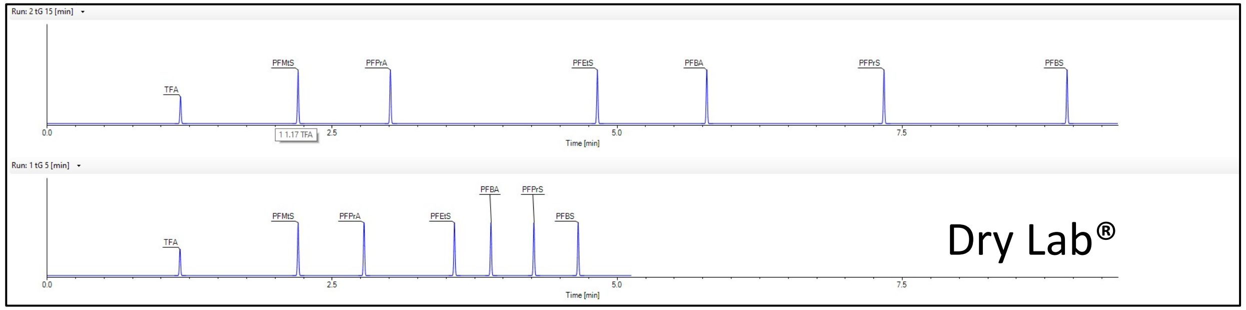


HALO® PCS Phenyl Hexyl

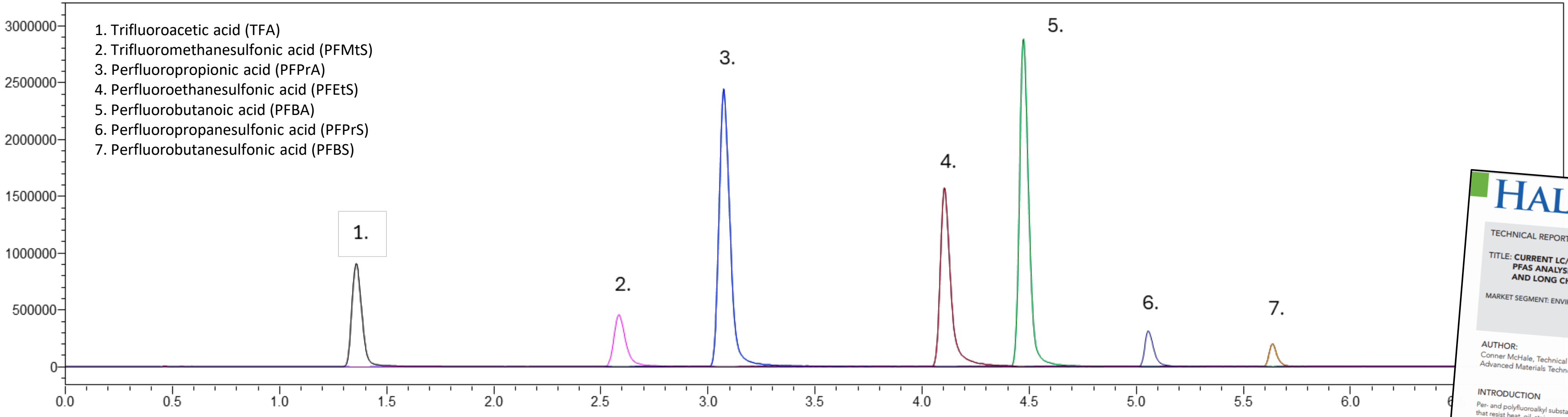


Aqueous Additive	Concentration
Formic Acid	0.01-0.1%
Acetic Acid	0.01-0.1%
Ammonium Formate	5mM
Ammonium Acetate	5mM
Ammonium Formate/ Formic Acid	5mM, 0.05%

Temperature
30°C
40°C
Organic Modifier
Acetonitrile
Methanol



HALO® PFAS PCS Phenyl-Hexyl: Separation of Ultrashort PFAS



Testing Conditions:

Mobile Phase: A: 5mM Ammonium Formate, 0.05% FA

B: Methanol

Gradient: 2-95%B in 7min

Instrument: Shimadzu 8060 NX

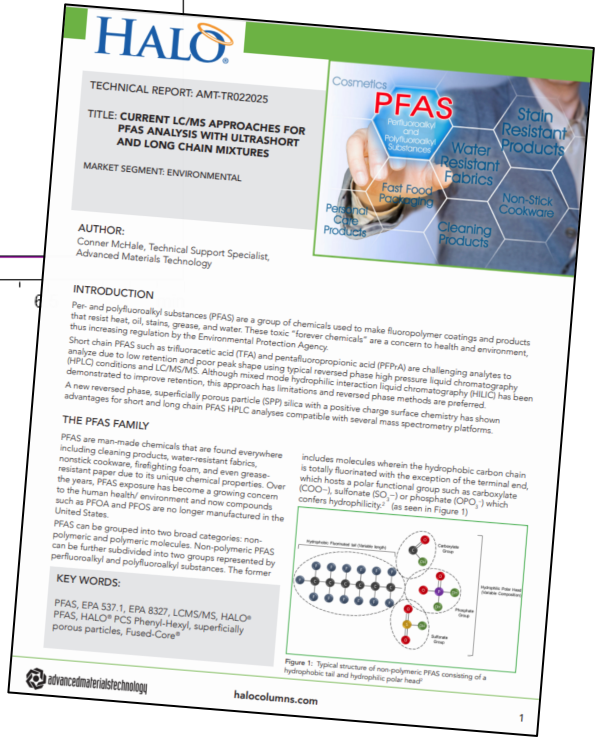
Injection: 2 µl (12.5µg/mL)

Temperature: 40 ^{o}C

Flow Rate: 0.4 mL/min.

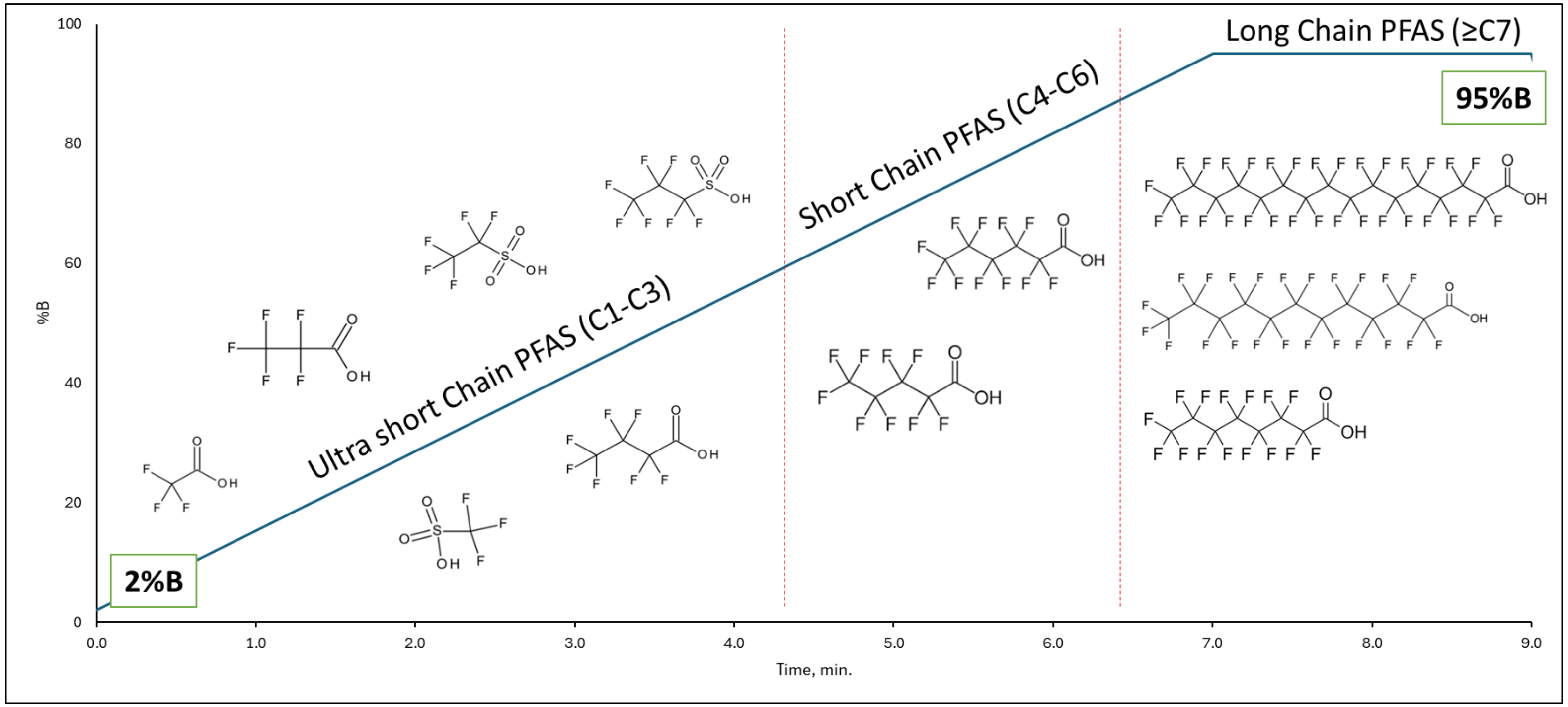
Column: HALO 90Å PCS Phenyl Hexyl, 2.7µm, 2.1 x 100mm

Delay Column: HALO 160Å PFAS Delay, 2.7µm, 3.0x50mm

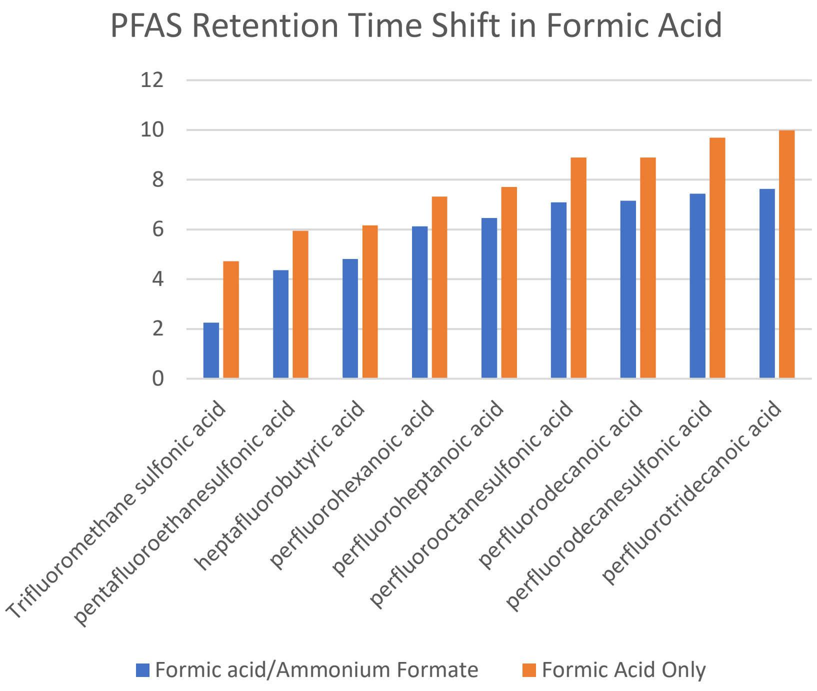


Scouting Conditions

Gradient Conditions: 2-95% B in 7min, 2 min. hold, Flow Rate 0.4 mL/min



Aqueous Component Comparisons



- Increase USC retention with good speak shape including elution of LC PFAS
- Minimize interference with MS signal
- Common reagents in use or similar to previous methods
- Maintain full system pressure below 500 bar

PFAS PCS Phenyl-Hexyl

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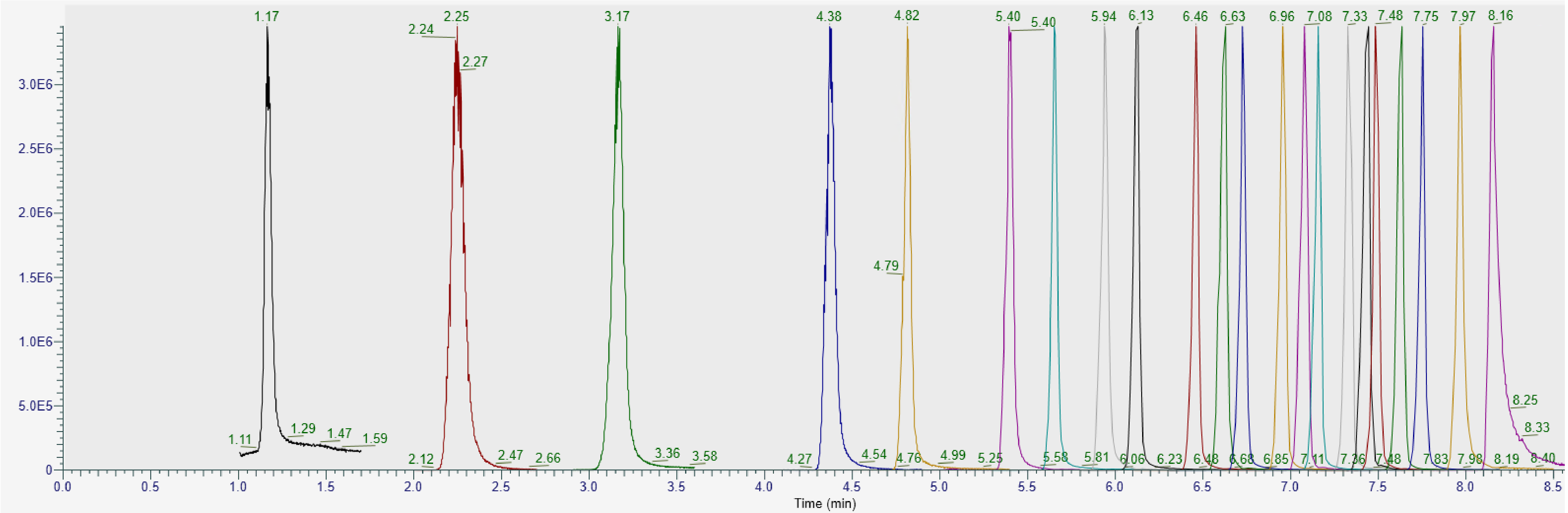

- TFA improved distance from V_0
- Peak shapes are otherwise acceptable, although PFDoDeA somewhat broadened

PFAS PCS Phenyl-Hexyl un-normalized

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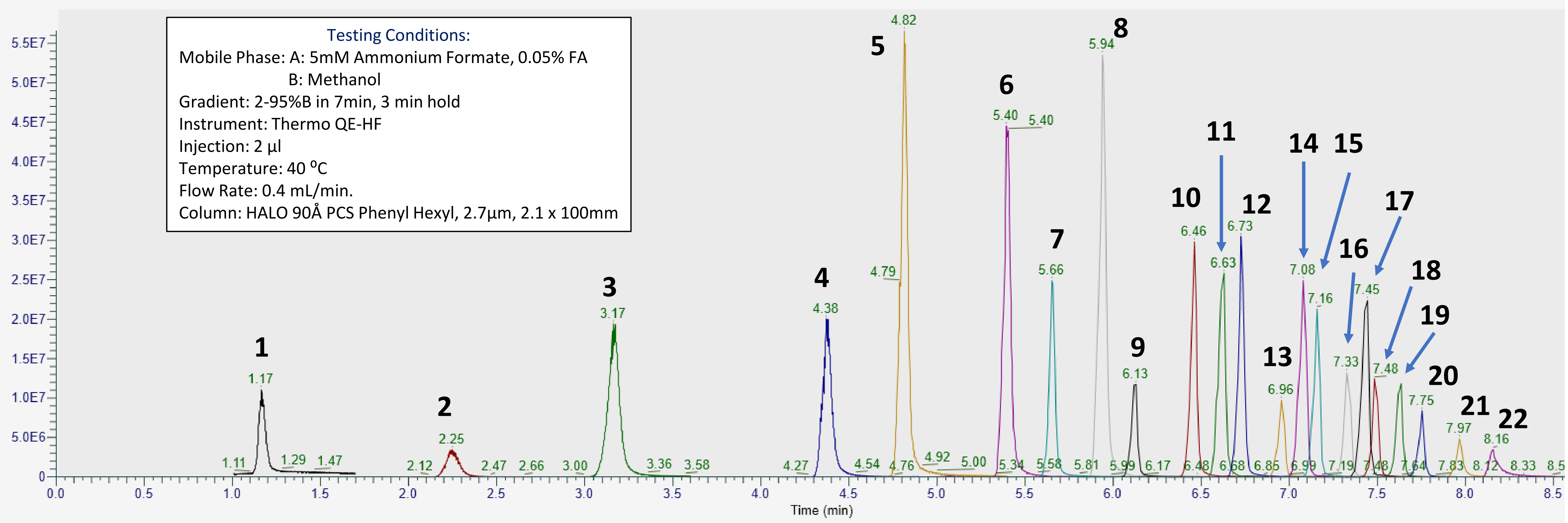

1. Trifluoro acetic acid
2. Trifluoromethane sulfonic acid
3. pentafluoropropionic acid
4. pentafluoroethanesulfonic acid
5. heptafluorobutyric acid
6. perfluoropropanesulfonic acid
7. perfluoropentanoic acid
8. perfluorobutanesulfonic acid
9. perfluorohexanoic acid
10. perfluoroheptanoic acid
11. perfluorohexanesulfonic acid
12. perfluorooctanoic acid
13. perfluorononanoic acid
14. perfluorooctanesulfonic acid
15. perfluorodecanoic acid
16. perfluoroundecanoic acid
17. perfluorodecanesulfonic acid
18. perfluorododecanoic acid
19. perfluorotridecanoic acid
20. perfluorotetradecanoic acid
21. perfluorohexadecanoic acid
22. perfluorooctadecanoic acid

Direct Sample Analysis: Short Chain

HALO®

- NEW -

EPA
United States
Environmental Protection
Agency

METHOD 534: DETERMINATION OF SELECTED PER- AND POLYFLUOROALKYL SUBSTANCES IN DRINKING WATER BY SOLVENT DILUTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY

1.1 Analyte List

Analyte[a]	Abbreviation	CASRN
Hexafluoropropylene oxide dimer acid[b]	HFPO-DA	13252-13-6[b]
Perfluorobutanesulfonic acid	PFBS	375-73-5
Perfluorodecanoic acid	PFDA	335-76-2
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorohexanesulfonic acid	PFHxS	355-46-4
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluorononanoic acid	PFNA	375-95-1
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluorooctanoic acid	PFOA	335-67-1

2 Method Summary

A 3–6 mL drinking water sample is collected in a tared polypropylene tube. Isotopically substituted analogues are added as internal standards. The sample is dechlorinated in the laboratory by the addition 10 µg ascorbic acid. The sample is diluted with methanol to a final concentration of 50% methanol/50% aqueous, then 10 µL acetic acid is added. The sample is mixed, and an aliquot is transferred to an autosampler vial. The diluted sample is analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) using a mixed-mode column with high retentivity for anions. Analytes and their internal standards are detected in the MRM mode. The concentration of each analyte is calculated using internal standard calibration.

17 Tables, Figures and Method Performance Data

Table 1. HPLC Method Conditions[a]

Time (min)	% 2 mM aq. Ammonium acetate	% 2 mM ammonium acetate in methanol
Initial	100	0
2.0	100	0
3.0	50	50
15.0	5	95
17.0	5	95
18	100	0
20	100	0

a. Atlantis Premier BEH C18 AX VanGuard FIT Column, 2.5 µm, 2.1 x 50 mm, column temperature 50 °C. Flow rate of 0.4 mL/min; 200 µL injection into a 250 µL loop (while the loop was set to inject 200 uL, due to specific characteristics of the instrument sample loop the actual injected volume was 199 uL). The chromatogram in Figure 1 was obtained under these conditions.

Table 2. ESI-MS Method Conditions

ESI Conditions for Waters Absolute	
Polarity	Negative ion
Capillary needle voltage	0.90 kV
Cone gas flow	150 L/hour
Nitrogen desolvation gas	1000 L/hour
Desolvation gas temperature	400 °C

USC PFAS in Well Water ~10-100 ng/L

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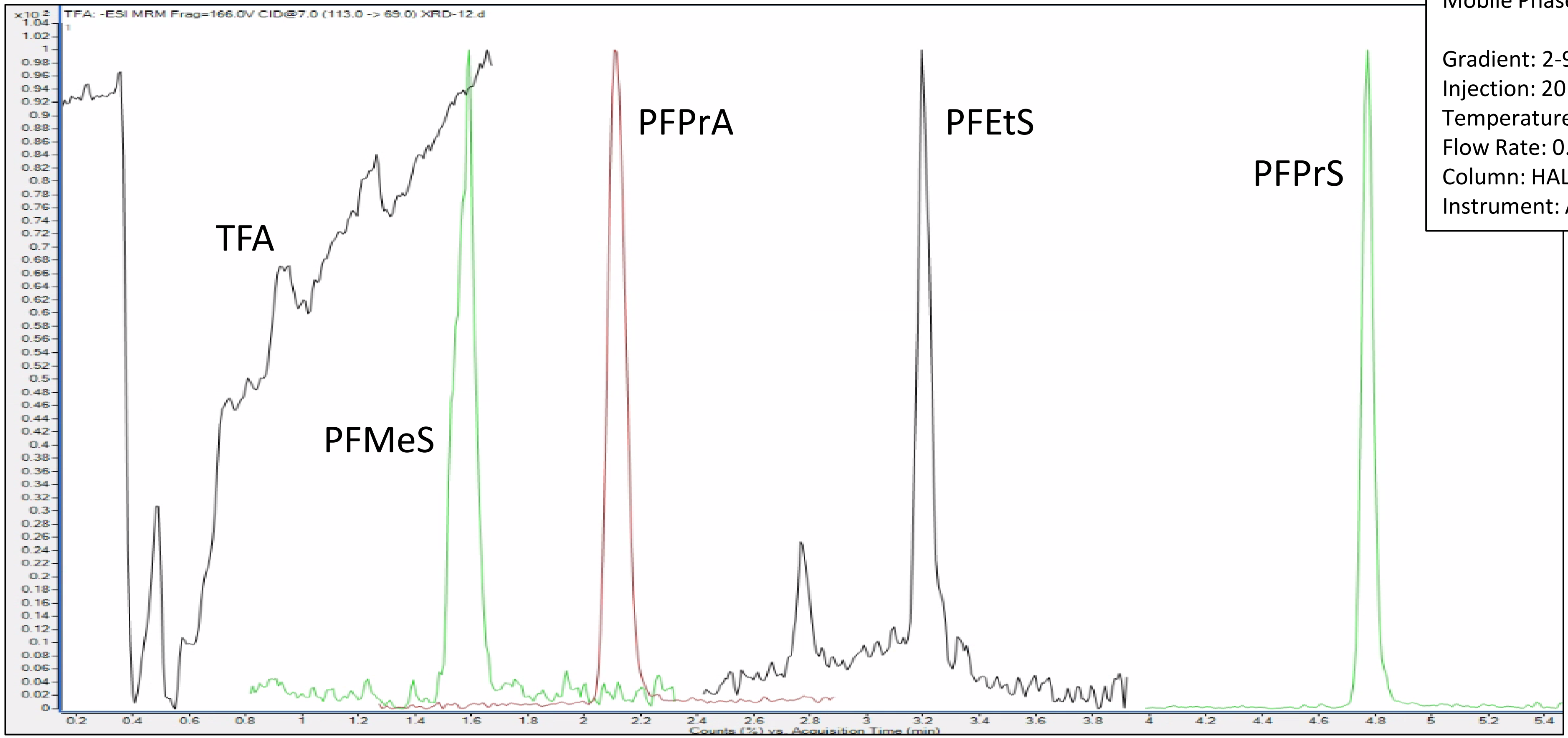

Testing Conditions:

Mobile Phase: A: 5 mM AF, 0.05% FA

B: MeOH

Gradient: 2-95%B in 7 min, 2 min hold

Injection: 20 µL, 0.05% HOAc

Temperature: 40°C

Flow Rate: 0.5 mL/min

Column: HALO 90Å PCS Phenyl Hexyl, 2.7 µm, 2.1 x 100 mm

Instrument: Agilent 1290 UPLC, 6495C QQQ

PFAS Solutions

Short and Long PFAS in Soil Extract ~0.1-1 ng/g

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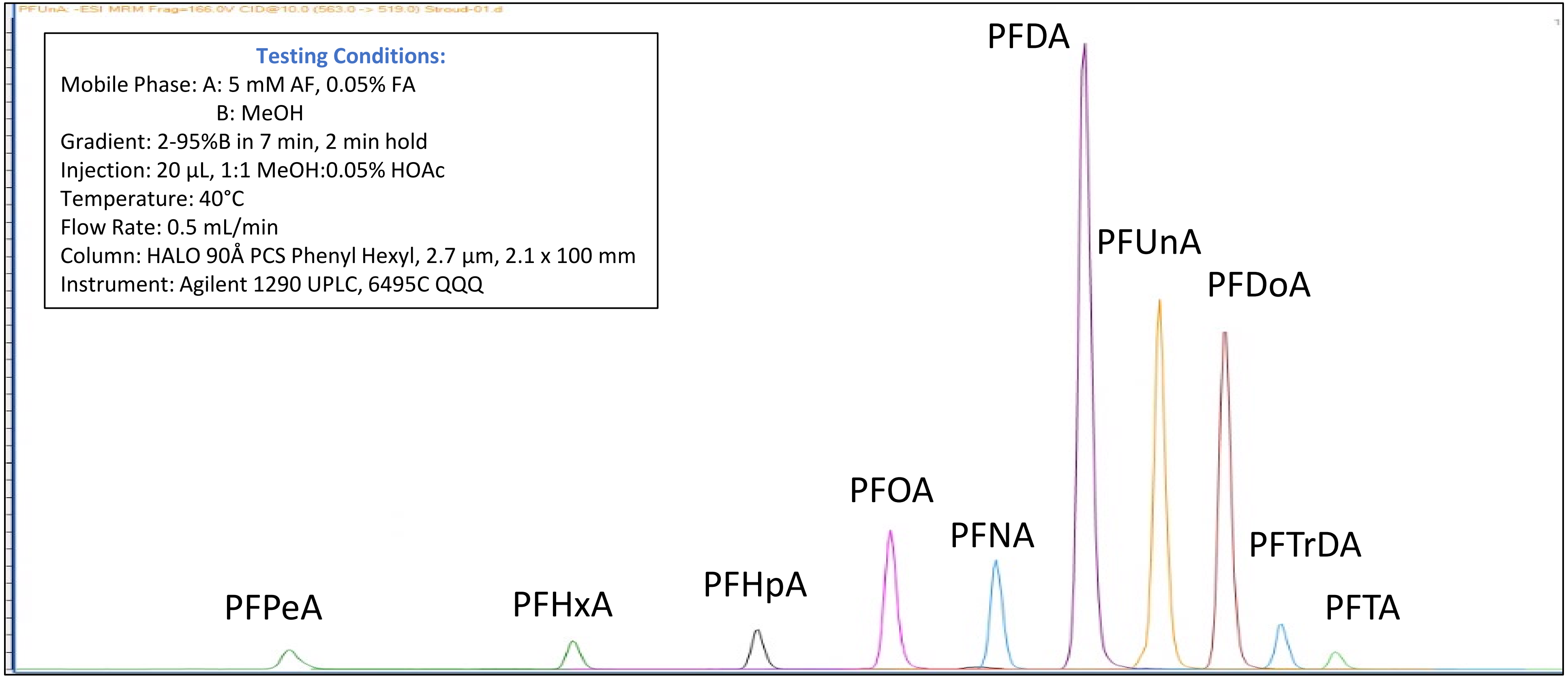
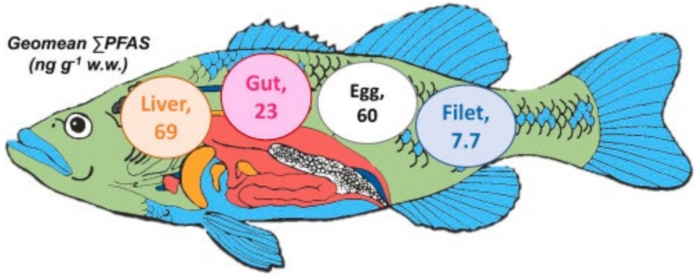


PFAS Solutions

Looking Forward: More!

- Capillary work/ Sensitivity Gains
  - Scale internal diameter (1.5, 1.0 mm, etc.)
  - Responding to an emerging Public Health/Regulatory need
  - Increased analysis demand will favor decreasing analysis time, solvent use, waste generation and overall cost

- Sample Prep
  - Isotopic Standards are great, but a real need is automating or eliminating sample preparation and quantification
  - Either large samples, or effective concentration, but Simpler and Cheaper

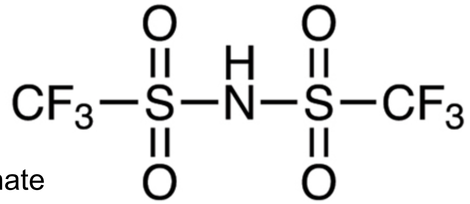


Staci L. Capozzi, Chunjie Xia, Matthew Shuwal, Gillian Zaharias Miller, Jeff Gearhart, Erica Bloom, Lennart Gehrenkemper, Marta Venier,
From watersheds to dinner plates: Evaluating PFAS exposure through fish consumption in Southeast Michigan,Chemosphere,Volume 345,2023,

- Materials Development to enable higher sample throughput via mixed mode and multimodal Workflows:
  - One size fits all is appealing, but potentially not realistic. HILIC/AEX and HILIC/AEX with 2D approaches may offer the highest sensitivity, but a more complex workflow

- More Analytes, More Matrices: The more we look, the more we find!

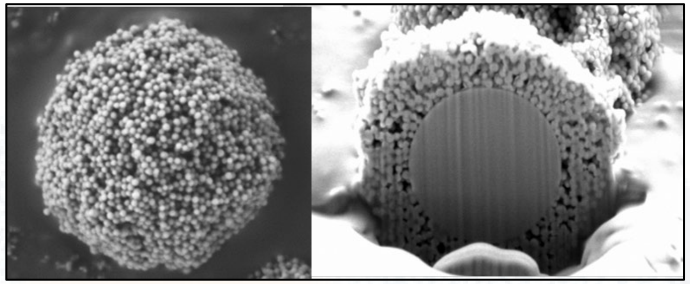
ex. Bis(trifluoromethanesulfonyl)imide: used as an electrolyte in lithium-ion batteries and is showing up especially in landfill leachate

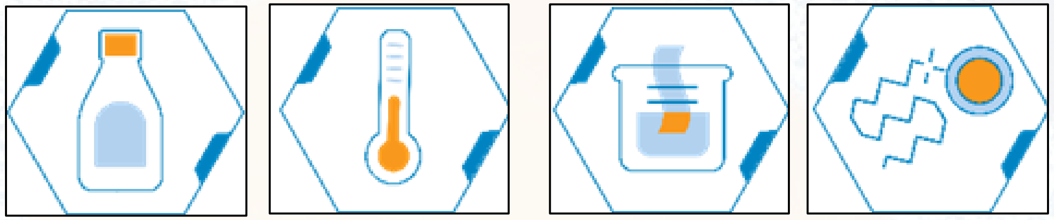


Conclusions/ Acknowledgements

HALO®

- Advanced Materials Technology
  - Superficially Porous Particle Technology
  - Current EPA Methods in place and challenges for the Ultrashort Chain
  - HALO PFAS PCS Phenyl Hexyl



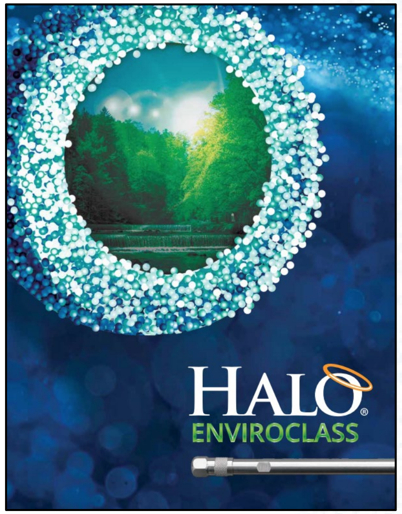


- Special Thanks to…

LGC

PFAS Solutions

- Technical Resources/ Support
  - halocolumns.com



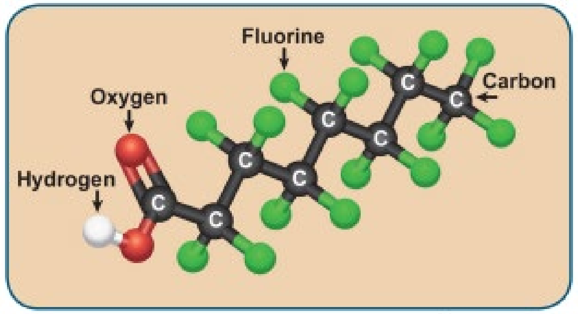


Image credit: NIEHS.

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