Analysis of Underivatized Glyphosate and Other Polar Contaminants by LC-MS/MS

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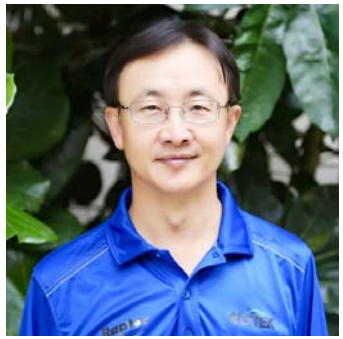
Senior Scientist



RESTEK®

Pure Chromatography

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Outline

- The Raptor Polar X Column
- Glyphosate and Polar Contaminants Analysis
- Novel Solution for Ultrashort-Chain PFAS Analysis
- Conclusions

Polar X – A Novel Phase Chemistry

Raptor LC Columns

Polar X

2.7 µm superficially porous particle (SPP) silica

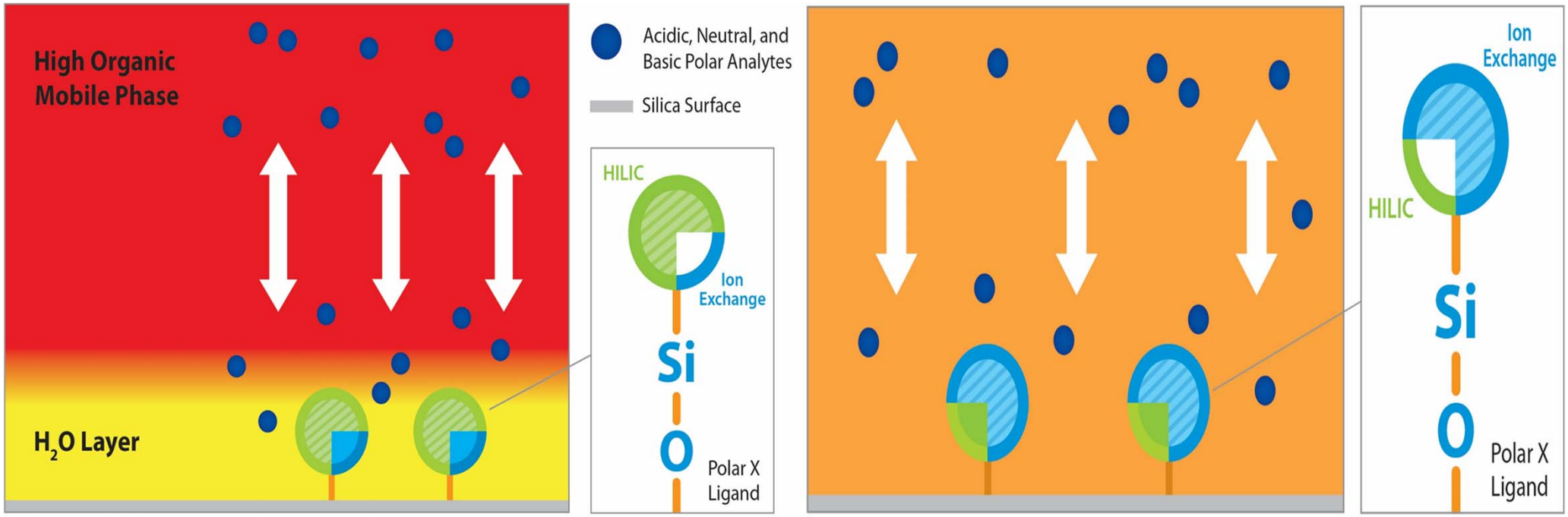
- A Single ligand capable of **HILIC** and **Ion Exchange** retention
- Proper retention for polar compounds

Polar X – A Novel Phase Chemistry

Tune Your Retention to What You Need

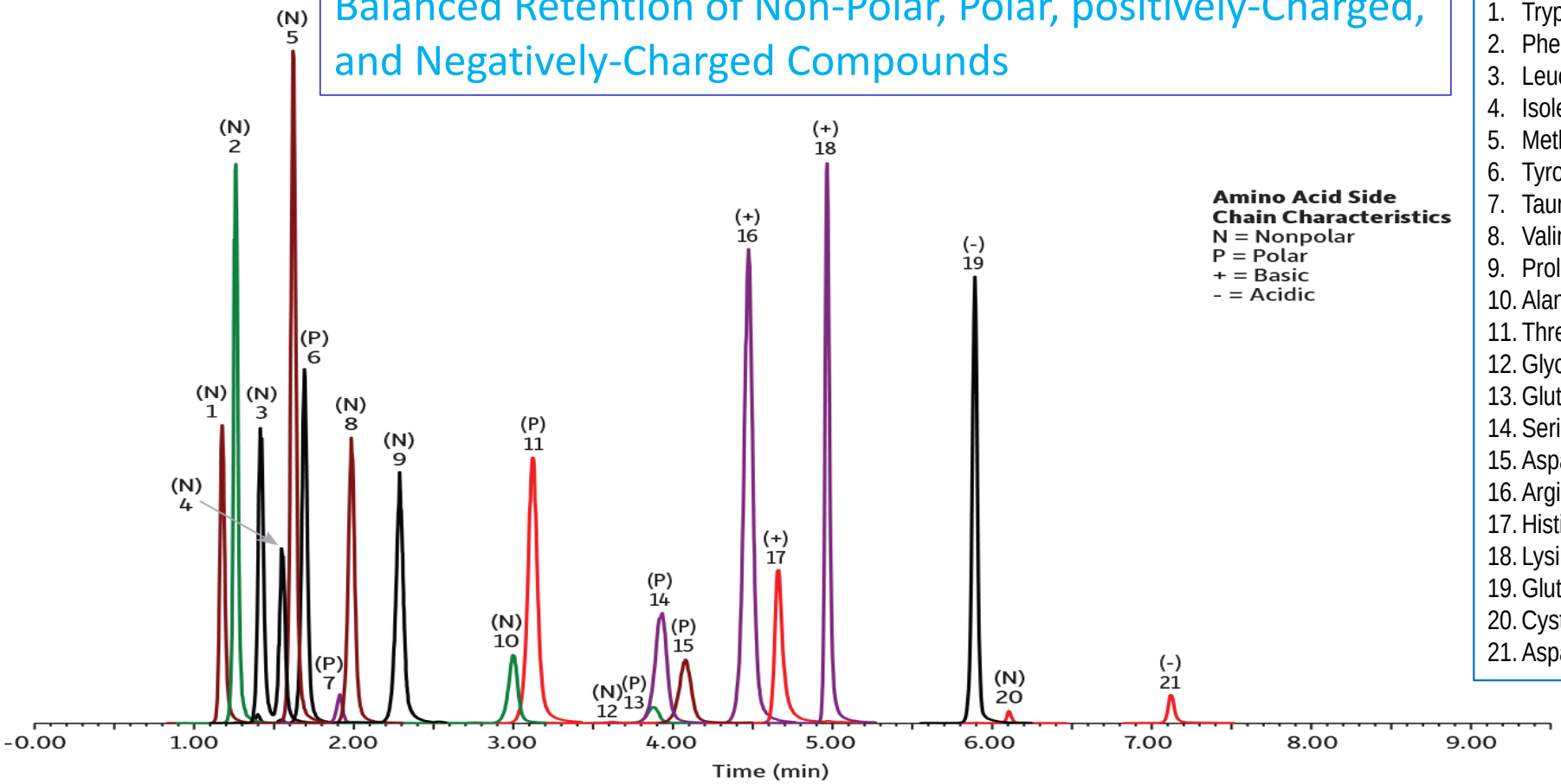
HILIC Retention

Ion Exchange Retention



Polar X – A Novel Phase Chemistry

Balanced Retention of Non-Polar, Polar, positively-Charged, and Negatively-Charged Compounds



1. Tryptophan (1.17)
2. Phenylalanine (1.26)
3. Leucine (1.41)
4. Isoleucine (1.55)
5. Methionine (1.62)
6. Tyrosine (1.69)
7. Taurine (1.91)
8. Valine (1.98)
9. Proline (2.29)
10. Alanine (3.00)
11. Threonine (3.12)
12. Glycine (3.62)
13. Glutamine (3.87)
14. Serine (3.93)
15. Asparagine (4.08)
16. Arginine (4.47)
17. Histidine (4.66)
18. Lysine (4.97)
19. Glutamic Acid (5.89)
20. Cystine (6.10)
21. Aspartic Acid (7.12)

Outline

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- Novel Solution for Ultrashort-Chain PFAS Analysis
- Conclusions

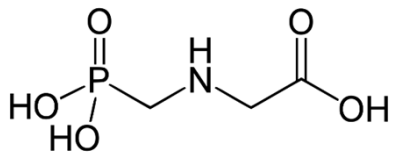
Glyphosate and Polar Contaminants Analysis in Waters

- Glyphosate is a herbicide and crop desiccant, and main ingredient in the broadleaf weed killer Roundup.
- By volume, it is one of the most widely used herbicides in the world.
- In 2015 the WHO International Agency for Research on Cancer classified glyphosate as a probable carcinogen in humans

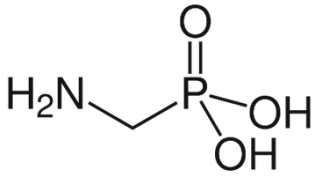
Glyphosate and Polar Contaminants Analysis in Waters

Analytical Challenges of Glyphosate Analysis

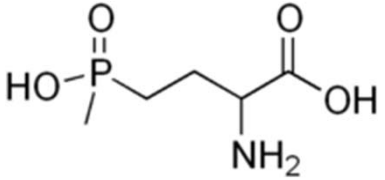
- Analysis of **glyphosate** in water often include its main metabolite, **AMPA**, and another commonly used herbicide **glufosinate**. The target detection limit is at low part-per-trillion (ppt) levels.
- They are small and very polar organophosphorus compounds and therefore pose a significant chromatographic challenge especially with conventional reversed-phase approaches.
- In addition to the chromatographic challenges, their phosphate functional groups makes them prone to metal chelation, which makes them susceptible to interact with any active metal in the sample flow path.
- Chelation will adversely affect peak shape and signal response regardless of the separation performance of the LC stationary phase.



Glyphosate



Aminomethylphosphonic acid (AMPA)



Glufosinate

Glyphosate and Polar Contaminants Analysis in Waters

New Hardware & System Passivation

- To ensure that Raptor Polar X Columns and Guard Column Cartridges are **Ready to Go** right out of the box, the columns have been treated with a passivation solution using a proprietary manufacturing process.

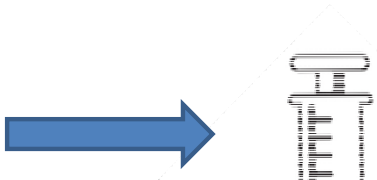
- However because most LCs have active metal in the flow path beyond the column, Restek developed a **LC Passivation Solution** to allow customers to quickly and easily passivate their LC instrument to enable them to achieve optimal analytical performance.

Glyphosate and Polar Contaminants Analysis in Waters

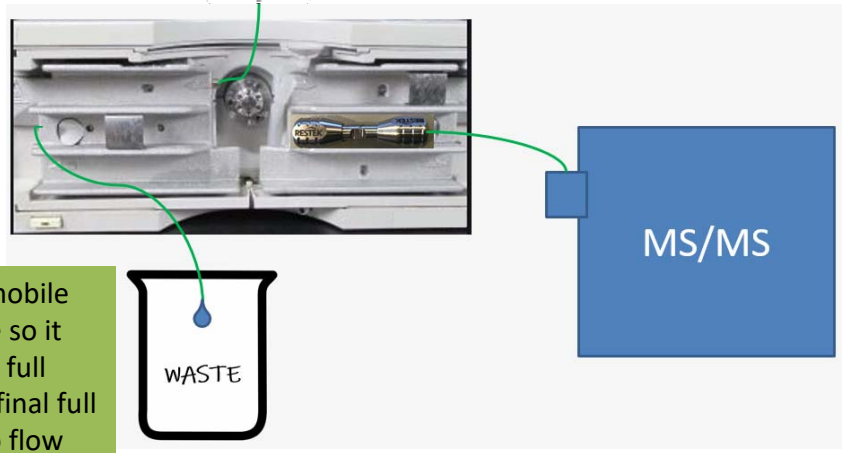
Simple System Passivation

Snap open the Ampule of Restek's LC Passivation Solution

Transfer to a Vial

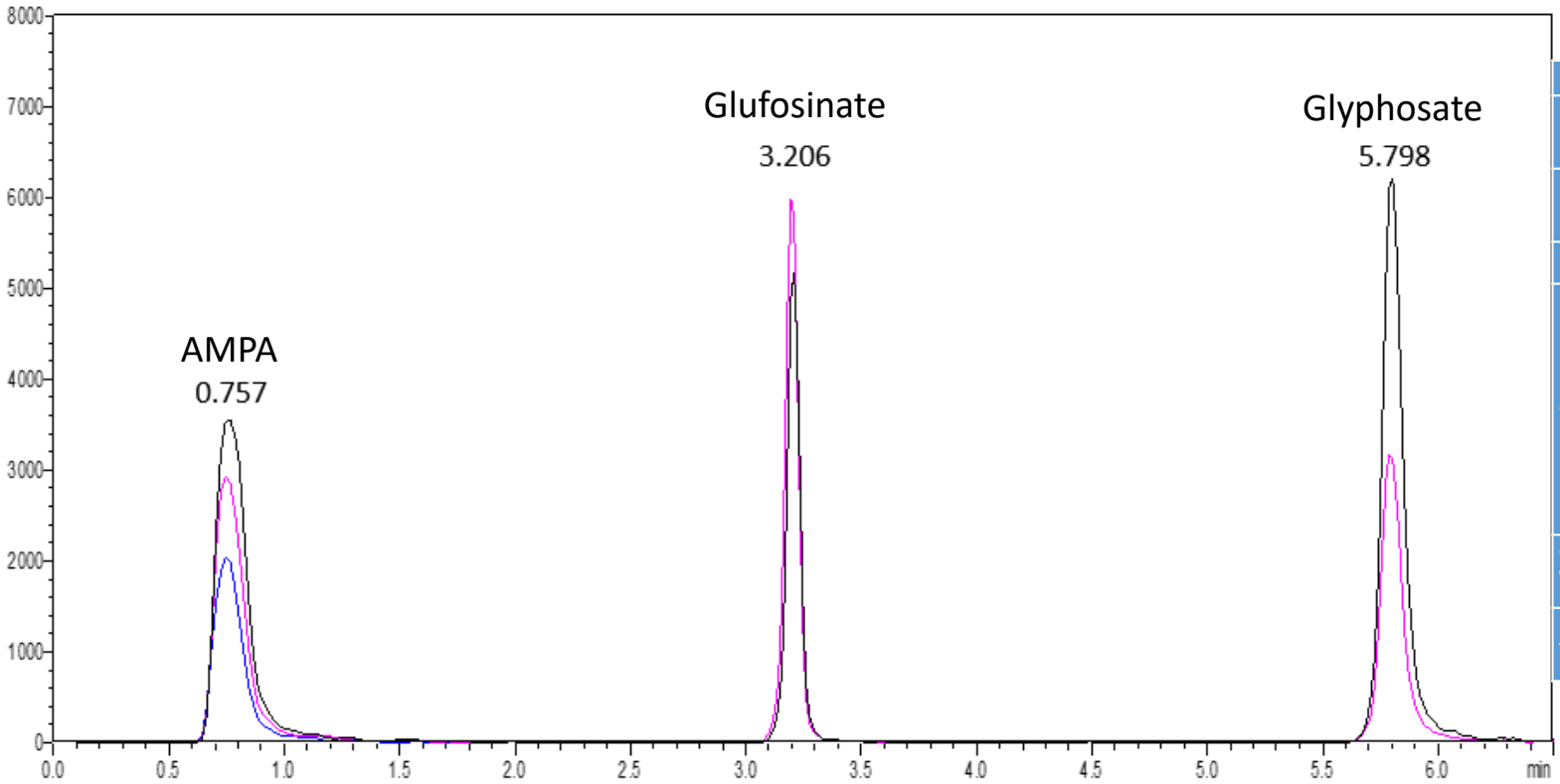


Inject several times through your injector and tubing but not including your column. Just let the flow goes to waste.



Set your mobile phase flow rate to 0.4 mL/min and mobile phase composition to 50%. Divert your flow to waste so it does not enter your detector, and then make 5 to 10 full loop injections of the passivation solution. After the final full loop injection, allow the mobile phase to continue to flow for 1 minute to allow the passivation solution to flow completely through your LC flow path.

Glyphosate and Polar Contaminants Analysis in Waters



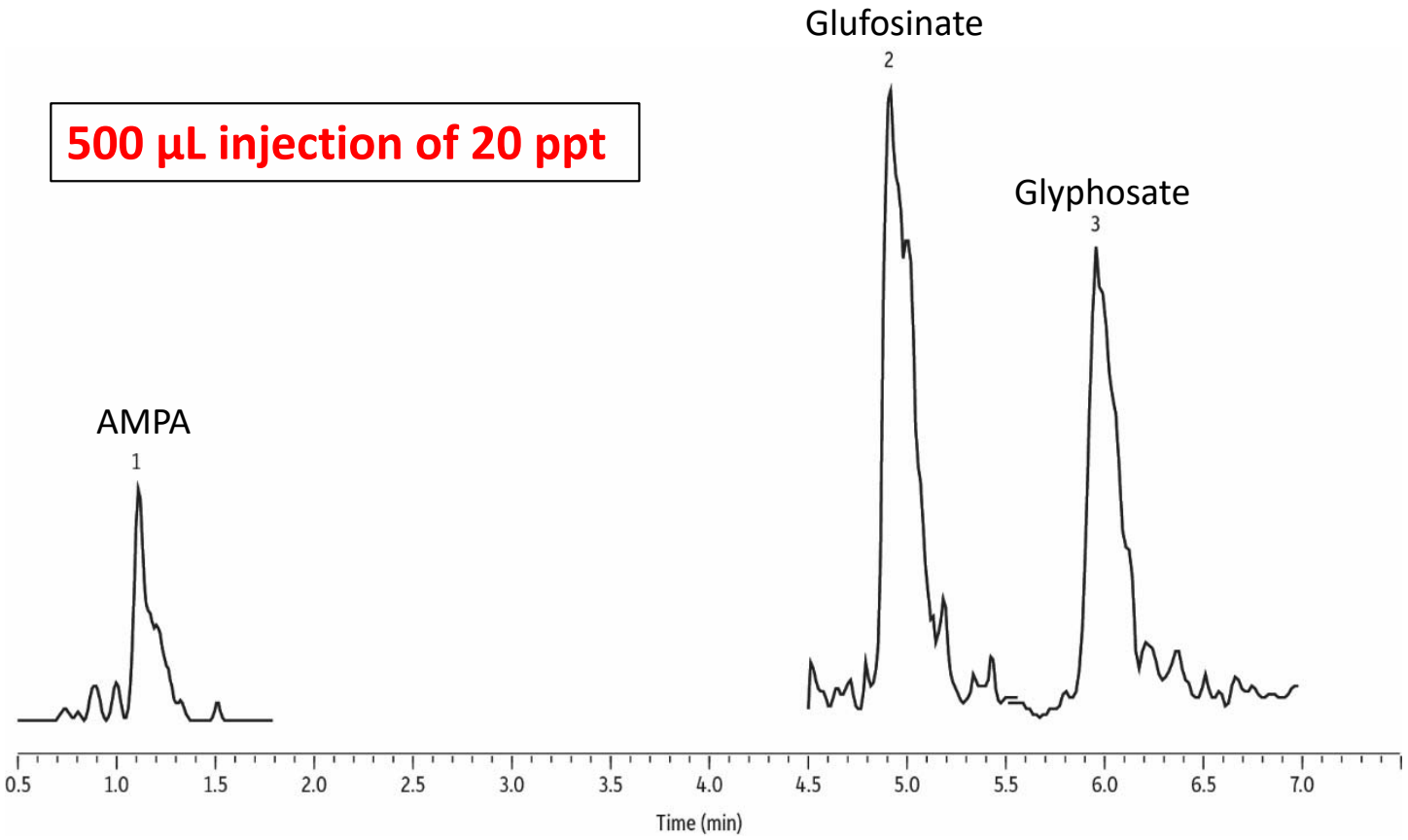
Polar X: 2.7µm 30x2.1 mm

Method Conditions:		
MPA:	0.5% formic acid in water	
MPB:	0.5% formic acid in acetonitrile	
Flow:	0.5 mL/min	
Gradient:	Time	%B
	0.0	65
	5.0	10
	6.5	10
	6.51	65
	8.0	65
Oven Temp:	35 °C	
Injection Vol:	5 µL	

100 ppb in water

Glyphosate and Polar Contaminants Analysis in Waters

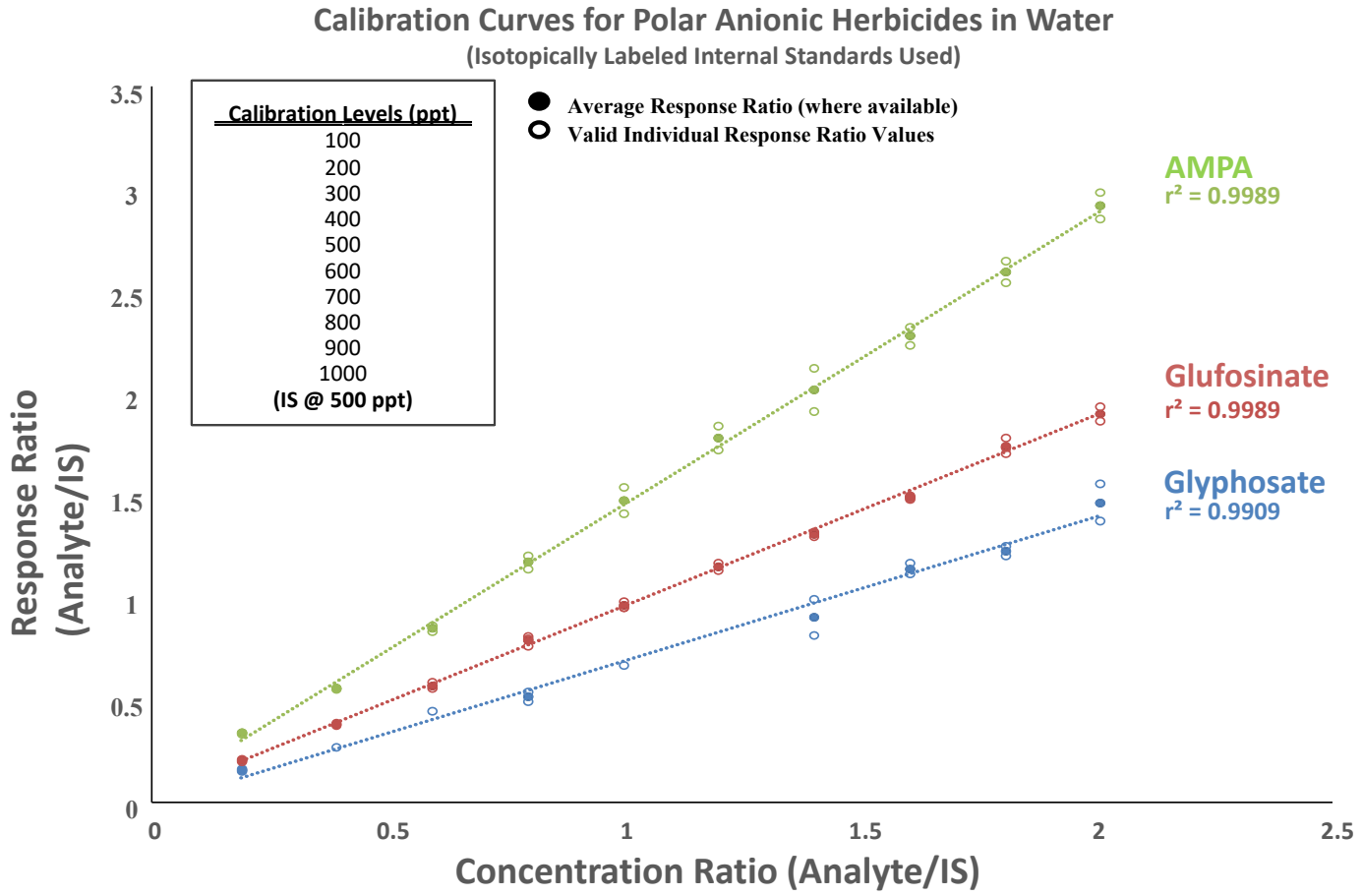
500 µL injection of 20 ppt



Polar X: 2.7µm 30x2.1 mm

Method Conditions:		
MPA:	0.5% formic acid in water	
MPB:	0.5% formic acid in acetonitrile	
Flow:	0.5 mL/min	
Gradient:	Time	%B
	0.0	95
	1.0	95
	3.0	10
	9.0	10
	9.01	95
	10.5	95
Oven Temp:	30 °C	
Injection Vol:	500 µL	

Glyphosate and Polar Contaminants Analysis in Waters



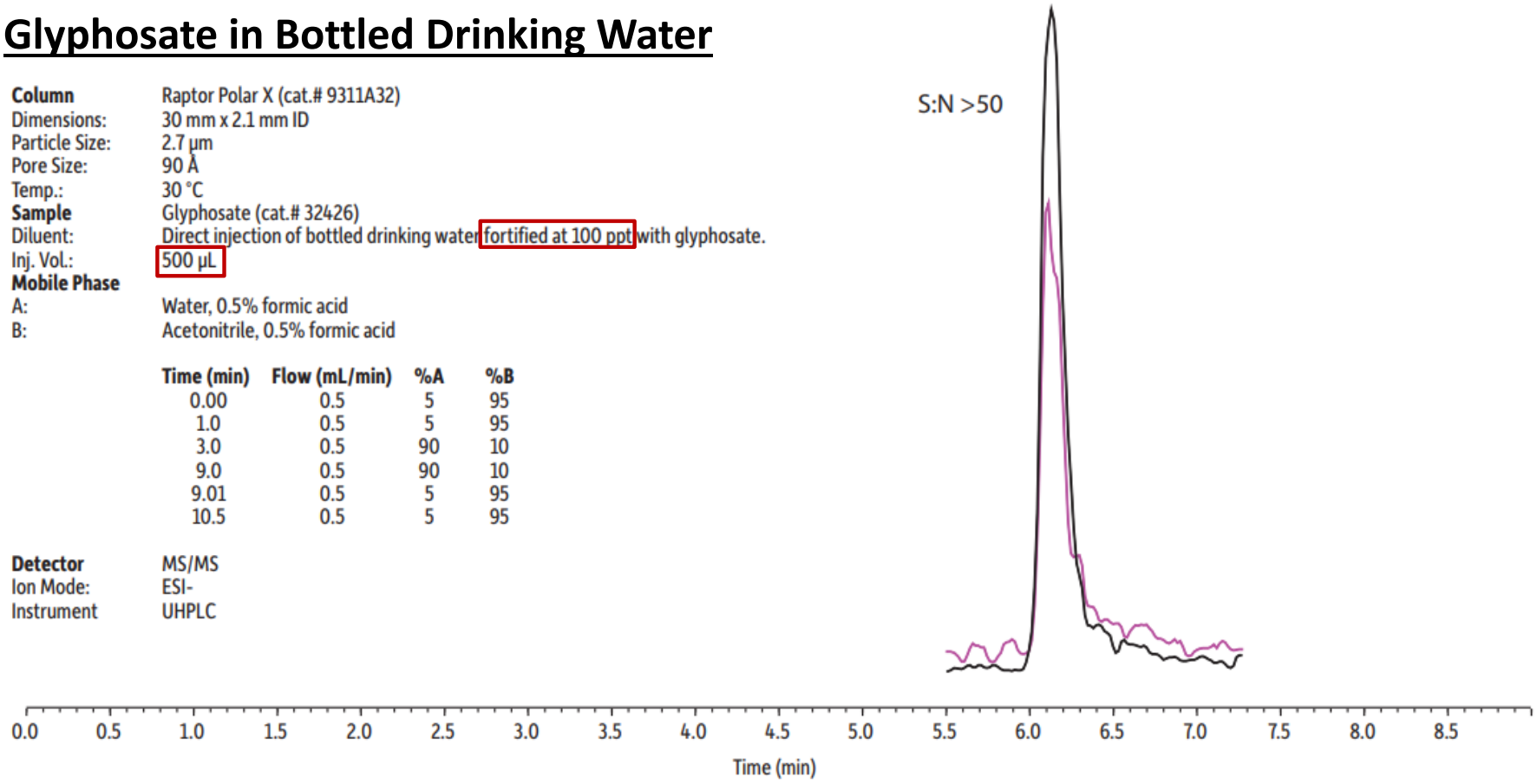
Glyphosate and Polar Contaminants Analysis in Waters

Glyphosate in Bottled Drinking Water

Column	Raptor Polar X (cat.# 9311A32)
Dimensions:	30 mm x 2.1 mm ID
Particle Size:	2.7 µm
Pore Size:	90 Å
Temp.:	30 °C
Sample	Glyphosate (cat.# 32426)
Diluent:	Direct injection of bottled drinking water fortified at 100 ppt with glyphosate.
Inj. Vol.:	500 µL
Mobile Phase	
A:	Water, 0.5% formic acid
B:	Acetonitrile, 0.5% formic acid

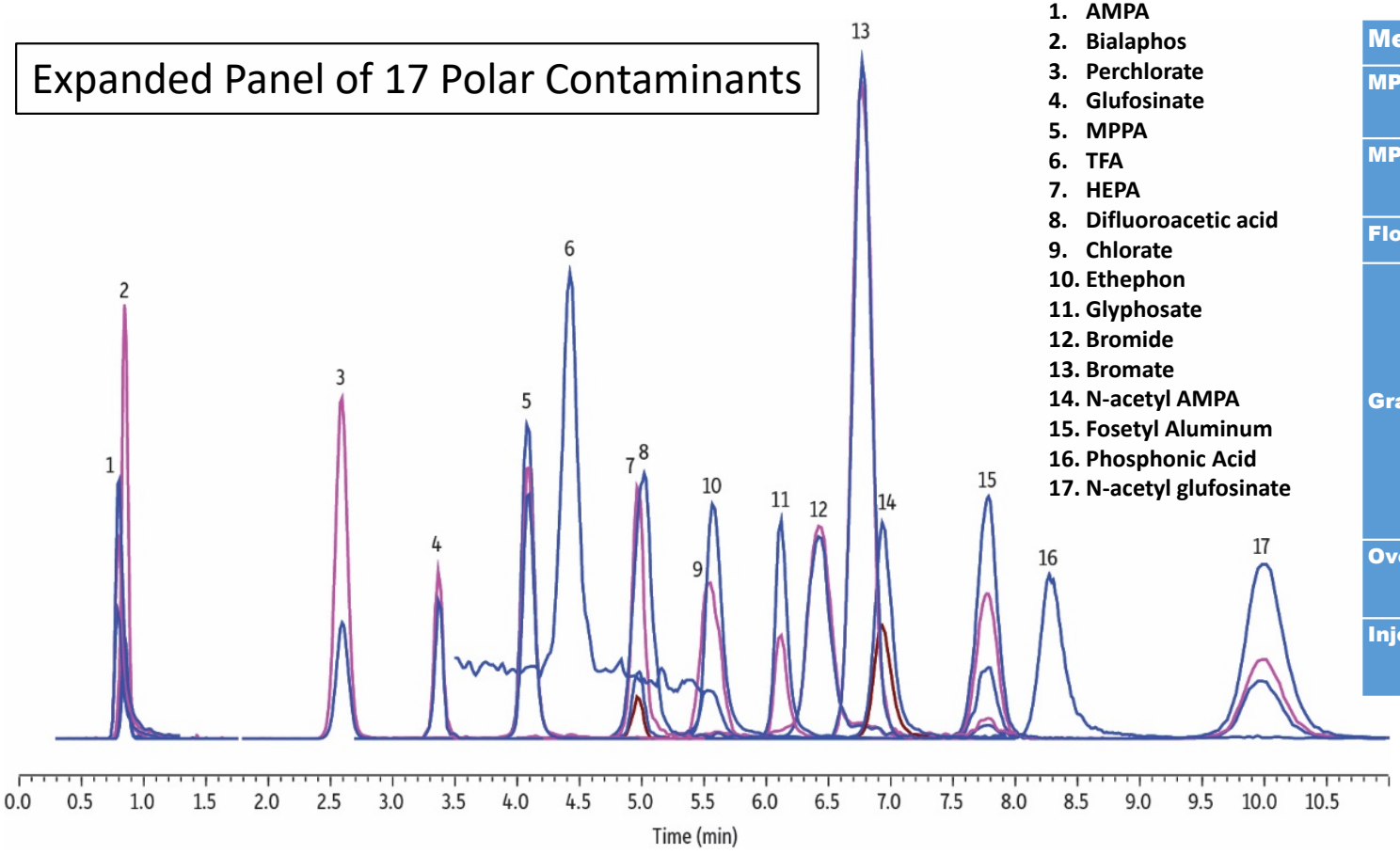
Time (min)	Flow (mL/min)	%A	%B
0.00	0.5	5	95
1.0	0.5	5	95
3.0	0.5	90	10
9.0	0.5	90	10
9.01	0.5	5	95
10.5	0.5	5	95

Detector	MS/MS
Ion Mode:	ESI-
Instrument	UHPLC



Glyphosate and Polar Contaminants Analysis in Waters

Expanded Panel of 17 Polar Contaminants



1. AMPA
2. Bialaphos
3. Perchlorate
4. Glufosinate
5. MPPA
6. TFA
7. HEPA
8. Difluoroacetic acid
9. Chlorate
10. Ethephon
11. Glyphosate
12. Bromide
13. Bromate
14. N-acetyl AMPA
15. Fosetyl Aluminum
16. Phosphonic Acid
17. N-acetyl glufosinate

Polar X: 2.7µm 30x2.1 mm

Method Conditions:		
MPA:	0.5% formic acid in water	
MPB:	0.5% formic acid in acetonitrile	
Flow:	0.5 mL/min	
Gradient:	Time	%B
	0.0	65
	5.0	10
	11.5	10
	11.51	65
	13	65
Oven Temp:	35 °C	
Injection Vol:	1 µL	

Outline

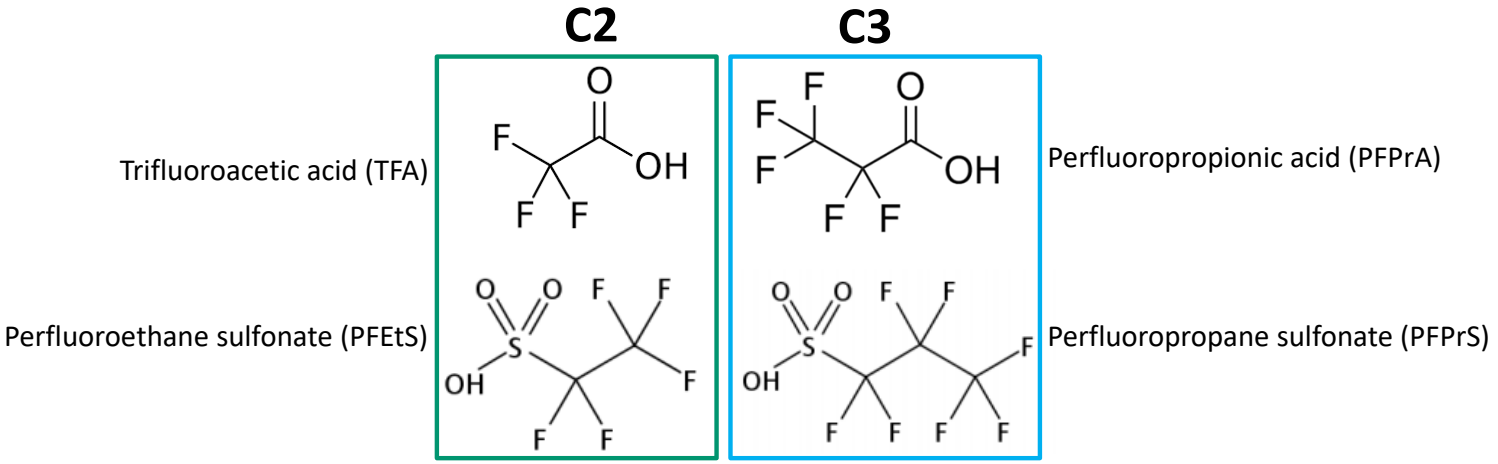
- The Raptor Polar X Column
- Glyphosate and Polar Contaminants Analysis
- Novel Solution for Ultrashort-Chain PFAS Analysis
- Conclusions

Novel Solution for Ultrashort-Chain PFAS Analysis

- Ubiquitous presence of ultrashort-chain PFAS in environmental waters has become a major concern in parallel to long-chain PFAS contamination.

- The highly polar ultrashort-chain PFAS poses a challenge to the current reversed-phase approach to PFAS chromatographic analysis.

Novel Solution for Ultrashort-Chain PFAS Analysis



Detected in different water matrices (snow, rain, river, and tap water)

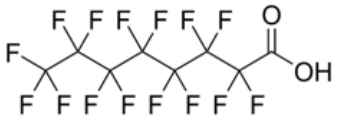
1. **PFPrA is up to 45% of the total PFAS in rain and snow samples (USA, France, Japan)**
2. **C2-C3 accounted for >40% of the total PFAS in rain samples (Canada)**
3. **TFA is the most abundant PFAS in rainwater (Japan)**
4. **PFEtS and PFPrS are detected in aqueous film-formimg foam (AFFF)**

Analytical methods for investigation on the sources and environmental levels

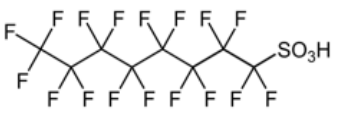
Novel Solution for Ultrashort-Chain PFAS Analysis

Legacy PFAS

C8



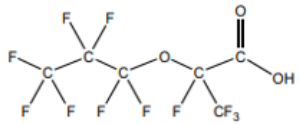
Perfluorooctanoic acid (PFOA)



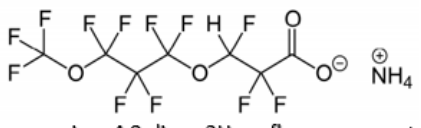
Perfluorooctanesulfonic acid (PFOS)

PFOA and PFOS Alternatives

Perfluoroalkyl ether carboxylic acids (PFECAs)

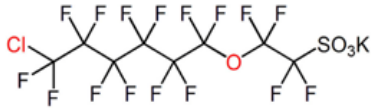


Hexafluoropropylene oxide dimer acid
(HFPO-DA)



ammonium 4,8-dioxa-3H-perfluorononanoate
(ADONA)

Polyfluoroalkyl ether Sulfonates (PFESAs)

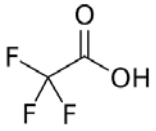


F53-B
(9-chlorohexadecafluoro-3-oxanonane-1-sulfonate)
(9Cl-PF3ONS)

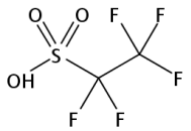
F53-B
(11-chloroeicosafluoro-3-oxanonane-1-sulfonate)
(11Cl-PF3OUdS)

UltraShort & Short-Chain PFAS

C2

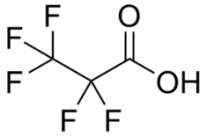


Trifluoroacetic acid (TFA)

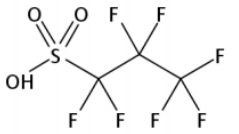


Perfluoroethane sulfonate (PFEtS)

C3

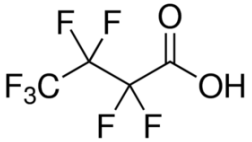


Perfluoropropionic acid (PFPrA)

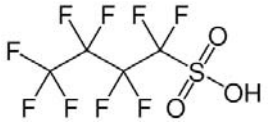


Perfluoropropane sulfonate (PFPrS)

C4

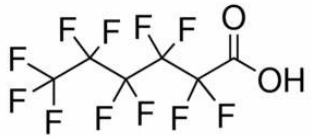


Perfluorobutanoic acid (PFBA)

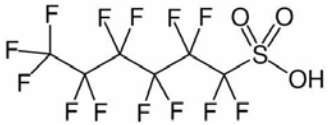


Perfluorobutane sulfonate (PFBS)

C6

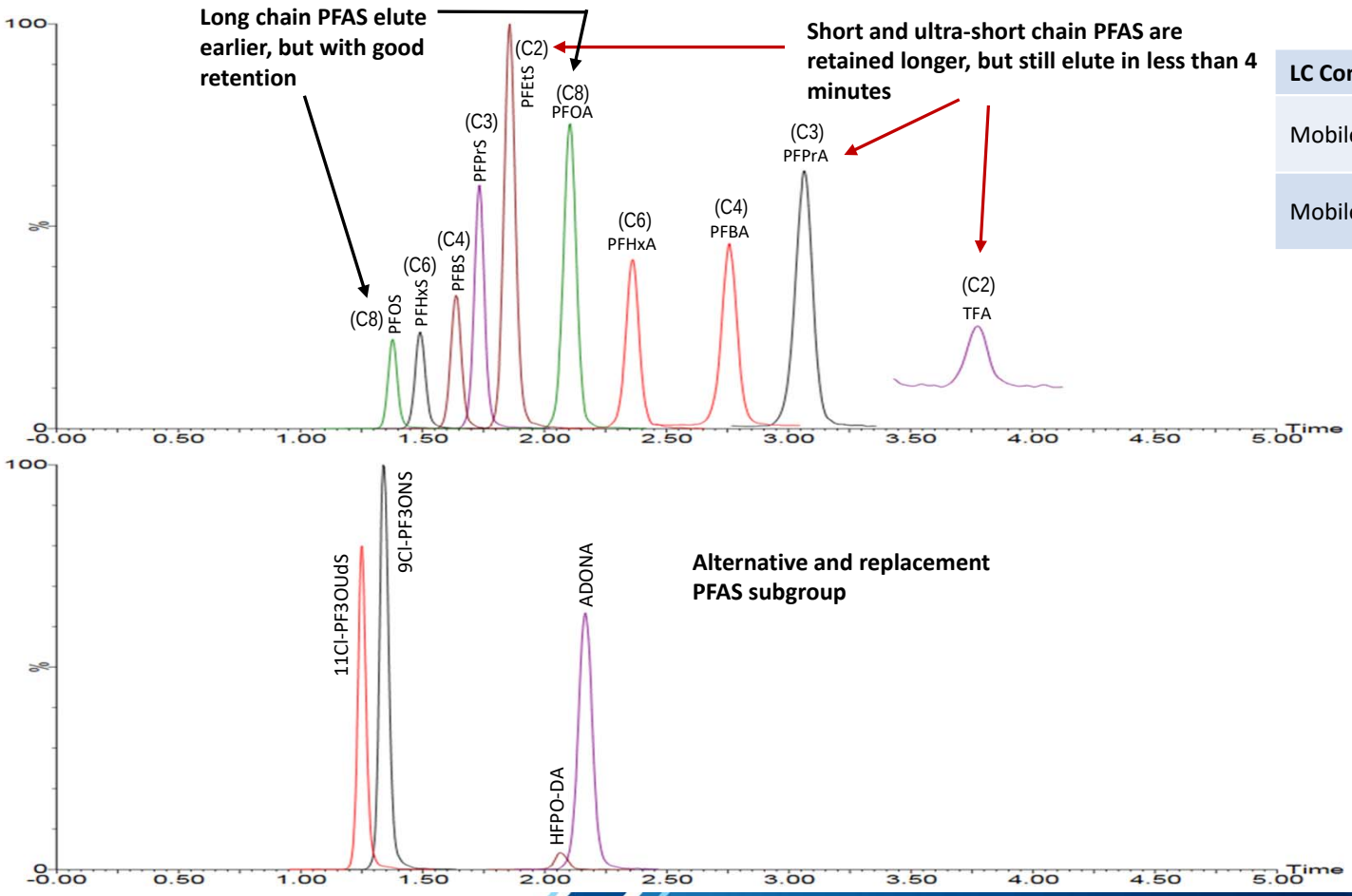


Perfluorohexanoic acid (PFHxA)



Perfluorohexane sulfonate (PFHxS)

Novel Solution for Ultrashort-Chain PFAS Analysis



Polar X: 2.7µm 50x2.1 mm

LC Conditions : (Waters Acquity UPLC)	
Mobile Phase A	10mM ammonium formate, 0.05% formic acid in water
Mobile Phase B	0.05% formic acid in 60:40 acetonitrile:methanol

Gradient	Time (min)	%B
	0.00	85
	5.00	85
Injection	10 µL	
Flow Rate	0.5 mL/min	
Run Time	5 min	
Column Temp.	40°C	

400 ppt in 50:50 water:methanol

Novel Solution for Ultrashort-Chain PFAS Analysis

Thursday, August 20

Polyfluoroalkyl Substances (PFAS) in the Environment – Session 3

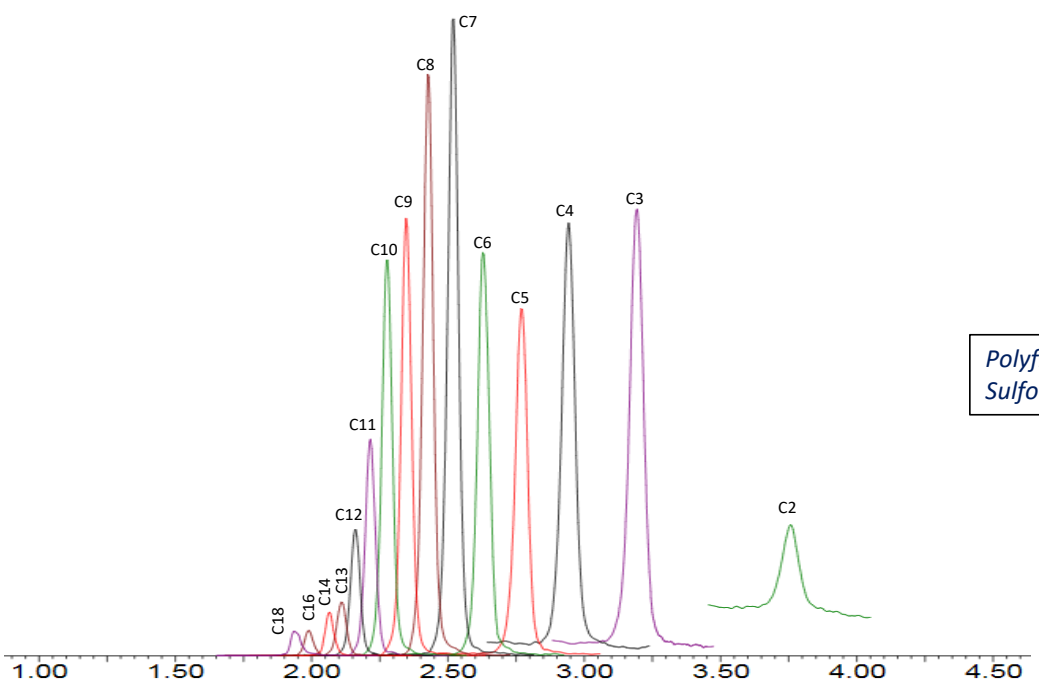
2:00 pm

"Novel Stationary Phase for Simultaneous Analysis of Ultrashort-Chain (C2, C3), Alternative, and Legacy PFAS"

Comprehensive & Simultaneous Analysis of Different Classes of PFAS on the Raptor Polar X

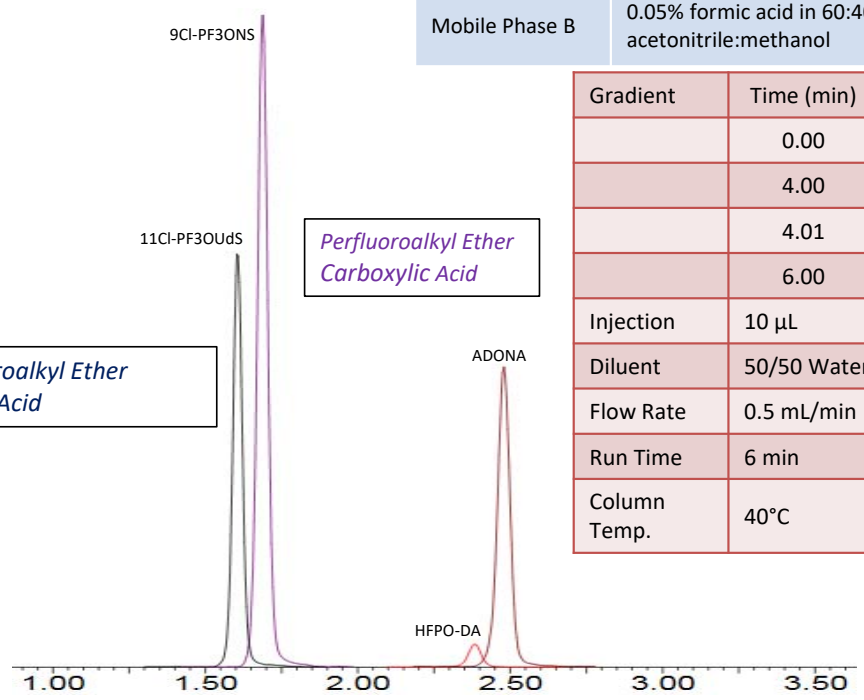
Polar X: 2.7µm 50 x 2.1 mm

Perfluoroalkylcarboxylic Acid



Polyfluoroalkyl Ether Sulfonic Acid

Perfluoroalkyl Ether Carboxylic Acid

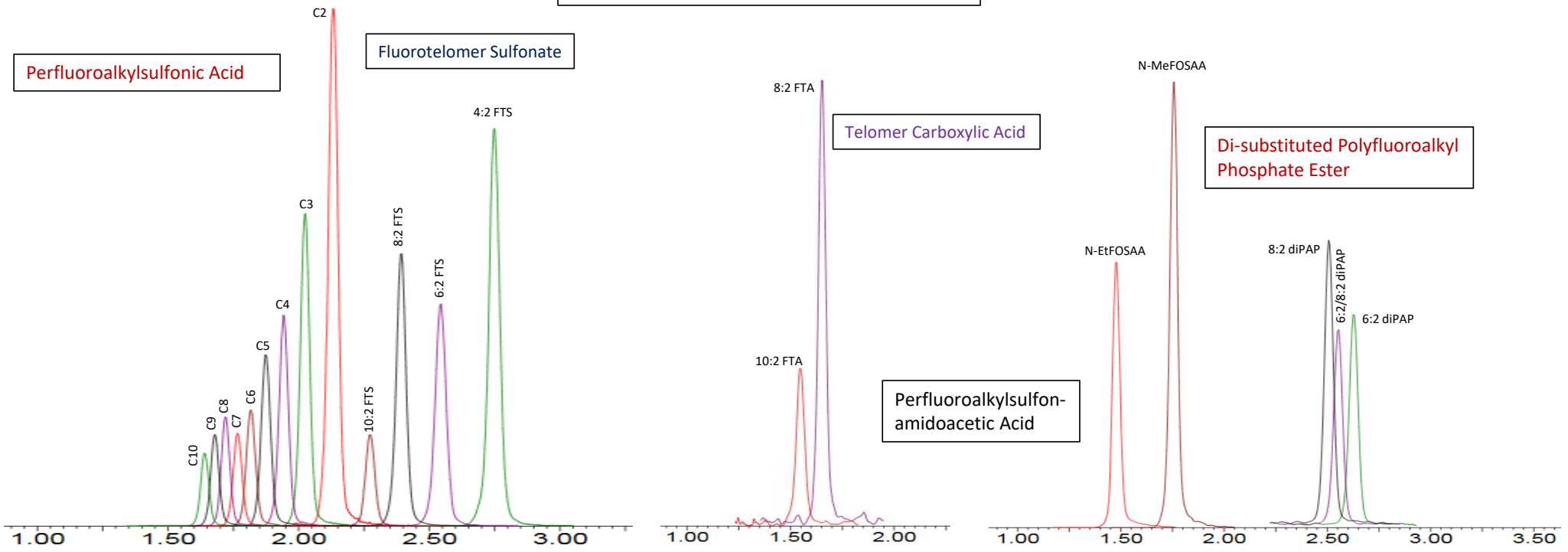


LC Conditions : (Waters Acquity UPLC)	
Mobile Phase A	10mM ammonium formate, 0.05% formic acid in water
Mobile Phase B	0.05% formic acid in 60:40 acetonitrile:methanol

Gradient	Time (min)	%B
	0.00	95
	4.00	65
	4.01	95
	6.00	95
Injection	10 µL	
Diluent	50/50 Water/MeOH	
Flow Rate	0.5 mL/min	
Run Time	6 min	
Column Temp.	40°C	

Comprehensive & Simultaneous Analysis of Different Classes of PFAS on the Raptor Polar X

Polar X: 2.7µm 50 x 2.1 mm



Conclusions

- The Raptor Polar X is an unique stationary phase specifically designed for balanced retention and selectivity of a wide range of polar compounds.
- HILIC methods are simplified by rapid equilibration that increases productivity.
- Proper chromatographic retention allows the development of fast and simple LC-MS/MS method for high-throughput analysis of glyphosate and ultrashort-chain PFAS in waters.
- Sensitive glyphosate analysis in water can be achieved by direct large volume injection on the Polar X column.
- Ultrashort-chain and long-chain PFAS can be simultaneously analyzed on the Polar X column.

Thanks for Your Attention

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