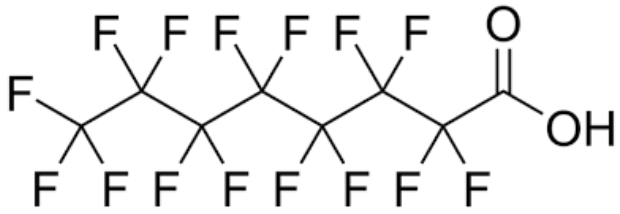
Comprehensive PFAS Analysis Using Diverse LC Column Types



HPLC 2024

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Shun-Hsin Liang, PhD; Sr. Principal Scientist
Jamie York, PhD; Principal Scientist
Justin Steimling, PhD; Sr. Manager Applications

Today's Agenda

- Background on PFAS sources, types, analysis
- Utility and Limitations of C18 for PFAS
- Ultrashort chain, short chain, and PFAS derivative analysis
  - Polar embedded C18 - Ultra IBD
  - Anion exchange and HILIC - Raptor Polar X
- Summary and Conclusions

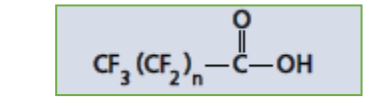
Human Exposure

Inhalation

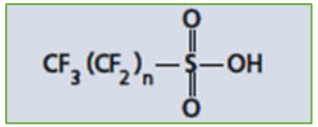
Ingestion

Dermal Contact

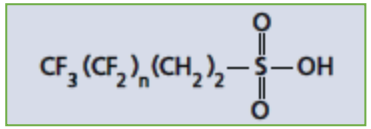
PFAS (Per- and Polyfluoroalkyl Substances)



Perfluoroalkylcarboxylic Acid (PFCA)



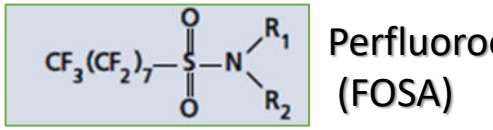
Perfluoroalkylsulfonic Acid (PFSA)



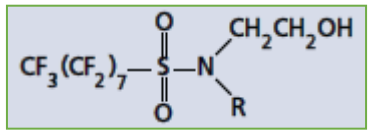
X:2 Telomer Sulfonic Acid (X:2 FTS)

Surfactant

Processing aid
Mist suppressant
Fire fighting foam
Cleaning Products

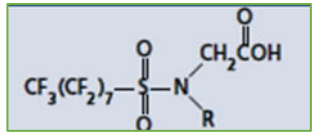


Perfluorooctanesulfonamide (FOSA)

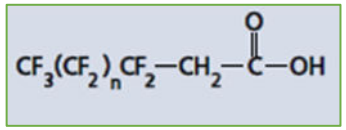


Perfluorooctanesulfonamido-ethanol (FOSE)

Raw Material



Perfluorooctanesulfonamido-acetic acid (FOSAA)



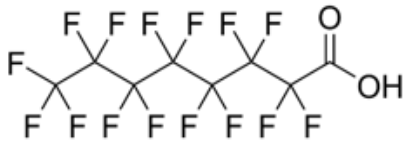
Telomer Carboxylic Acid (X:2 FTA)

Environmental Transformation Product

Ultrashort-Chain/Legacy/Alternative PFAS

Legacy PFAS

C8

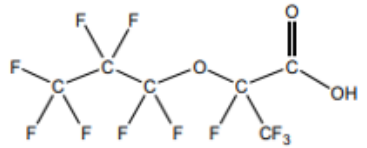


Perfluorooctanoic acid (PFOA)

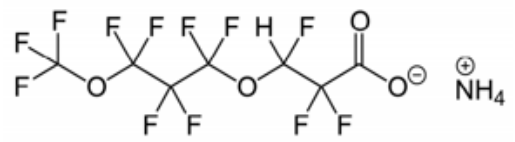
Perfluorooctanesulfonic acid (PFOS)

PFOA and PFOS Alternatives

Perfluoroalkyl ether carboxylic acids (PFECAs)

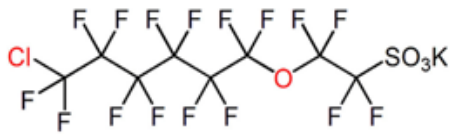


Hexafluoropropylene oxide dimer acid
(HFPO-DA)

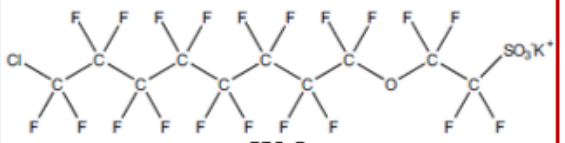


ammonium 4,8-dioxa-3H-perfluorononanoate
(ADONA)

Polyfluoroalkyl ether Sulfonates (PFESAs)

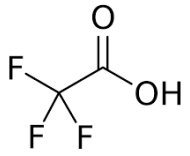


F53-B
(9-chlorohexadecafluoro-3-oxanonane-1-sulfonate)
(9Cl-PF3ONS)



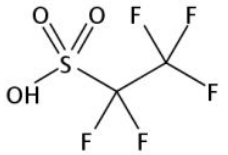
F53-B
(11-chloroeicosafluoro-3-oxanonane-1-sulfonate)
(11Cl-PF3OUdS)

UltraShort & Short-Chain PFAS

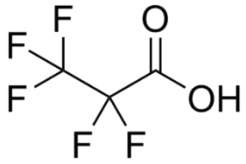


Trifluoroacetic acid (TFA)

C2

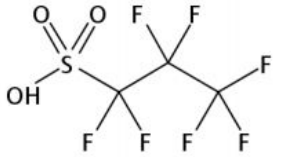


Perfluoroethane sulfonate (PFEtS)

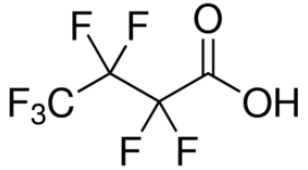


Perfluoropropionic acid (PFPrA)

C3

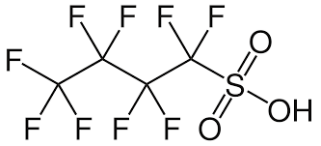


Perfluoropropane sulfonate (PFPrS)

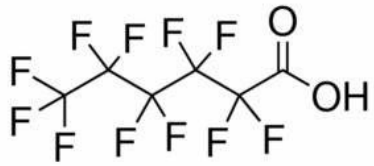


Perfluorobutanoic acid (PFBA)

C4

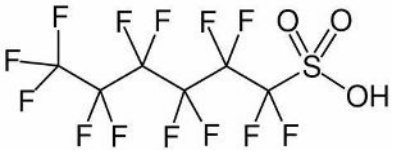


Perfluorobutane sulfonate (PFBS)



Perfluorohexanoic acid (PFHxA)

C6



Perfluorohexane sulfonate (PFHxS)

Measurement of Ultrashort-Chain PFAS

Reversed-phase liquid chromatography – insufficient retention/matrix effects

GC-MS for TFA and C4 – C6 carboxylic acid PFAS analysis – needs derivatization and is not suitable for simultaneous analysis of sulfonic acid PFAS

Anionic exchange LC column – extended retention (>20 minutes) and broader peak shapes for USC PFAS

Supercritical fluid chromatography – efficient analysis but needs to invest in SFC instrument

PFAS in Water Testing Standards

Test Method	US EPA 537.1	ISO 25101:2009	DIN 38407-42	ASTM D7968-17a	ASTM D7979-17	US EPA 533	US EPA 8327	ISO 21675
Sample Matrix	Drinking Water	All water types	All water types	soil	All water types (- drinking water)	Drinking Water	Non-potable water	All water types
# of Analytes	18	24	2	21	21	25	24	30
Sample Prep	SPE	Direct injection	SPE	Direct injection	Direct injection	SPE	Direct injection	SPE
Sample Volume	250 mL	1000 mL	50 mL	2 g	5 mL	250 mL	5 mL	50 – 1000 mL*
Detection limit	Optional	Not shown	• 0.01 ug/L • 0.025 ng/L for treated waste water	MDL (2.41 – 258.37 ng/kg)	MDL (0.7 – 106.8 ng/L)	LCMRL (1.7 – 20 ng/L)	• MDL (0.7 – 4.6 ng/L) • LLOQ is 10 ng/L	LOQ: 0.2 ng/L (loose term)

Sample Preparation Practices for PFAS

Several techniques/products. Dependent on method needs and sample type.

- Dilute and shoot/direct injection.
- QuEChERS – Milk, food
- SPE, polymeric wax
- Protein precipitation – Plasma

- Isotopically labeled internal standards required for improved quantitation robustness.

Example: Direct Injection Method Evaluation

Polar X Column
2.7µm, 50x2.1mm

Sample Preparation:

(polypropylene vial)

250 µL water sample or standard

+

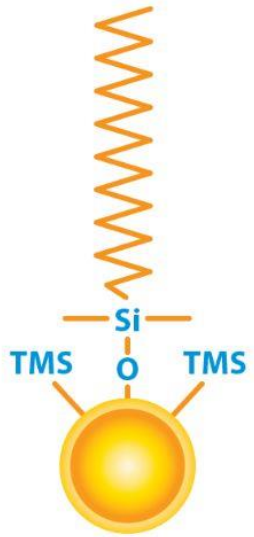
250 µL methanol

+

5 µL internal standard

(10 ng/mL $^{13}C_2$-*PFHxA*, $^{13}C_2$-*PFOA*, $^{13}C_3$-*PFBS*, $^{13}C_4$-*PFOS* in methanol)

Conventional Reverse Phase PFAS Analysis



Raptor, Ultra – C18

- C18 offers good separation of longer chain PFAS compounds; C6 and greater.
- Does not retain short chain PFAS well; dirty samples can cause unwanted matrix effects.
- Use the PFAS library in the EZLC tool to optimize your starting conditions.

FREE LC Modeling Software for PFAS

RESTEK

EZGC & EZLC Online Software Suite

My Account

Restek Home | Pro EZGC Chromatogram Modeler | Pro EZLC Chromatogram Modeler | EZGC Method Translator & Flow Calculator | Pro EZLC Method Translator

Pro EZLC Chromatogram Modeler

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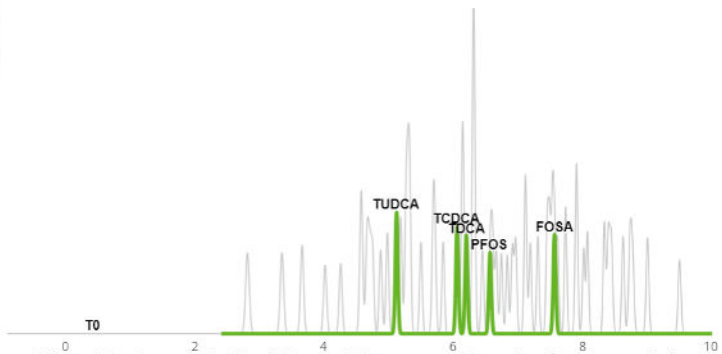
Generate accurate chromatograms in seconds without an injection.

Compounds | Conditions | My EZLC

Compound Class: PFAS

Search:

Compound Name	CAS
11Cl-PF3OUdS	763051-92-9
3:3 FTCA	356-02-5
4:2 FTS	757124-72-4
5:3 FTCA	914637-49-3
6:2 FTS	27619-97-2
6:2 diPAP	57677-95-9
6:2/8:2 diPAP	943913-15-3
7:3 FTCA	812-70-4



Click and drag to zoom; double-click to reset. Mouse over peak numbers for compound info.

Available Isobars: m/z 499 Critical Pair 23,26 Rs 1.56 Reset

Gradient inputs, flow, temperature, MS parameters, etc.

Column dimensions, particle size, chemistry, Mobile phase

Compounds | Conditions | My EZLC

Column

Length	100.00 mm
Inner Diameter	2.10 mm
Particle Size	2.70 µm
Available Columns	100, 2.1, 2.7

Volume Effects

Dwell Volume	0.25 mL
Extra-Column Volume	25.00 µL

Mobile Phase

Eluent A	Water
	5.0 mM Ammonium Acetate
Eluent B	ACN:MeOH

Column Raptor C18 (cat.# 9304A12)
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 2.7 µm
Temp.: 30°C

Mobile Phase
A: Water, 5.0 mM Ammonium Acetate
B: 50:50 Acetonitrile:Methanol

Time (min)	Flow (mL/min)	%A	%B
0	0.4	96	4
7	0.4	20	80
14	0.4	0	100

Detector MS
Notes T0: 0.41 min
Dwell Volume: 0.25 mL

Mobile Phase

Eluent A	Water
	5.0 mM Ammonium Acetate
Eluent B	ACN:MeOH
	Acetonitrile 50 % Methanol 50 %
Temperature	30.00 °C
Back Pressure	psi 4379 psi

Gradient Program

	Time (min)	%B	Flow (mL/min)
Add Start Isocratic Hold	0	4	0.4
2 # of Gradient Steps	7	80	0.4
Add Final Isocratic Hold	14	100	0.4
Add Re-equilibration Time			

Target Resolution: 1.50

Optimize Gradient Slope

Results

Gradient Time + Delay / Run Time	15.04 / 15.04 min
T0	0.41 min
Isobaric Compounds Separated	7
Critical Pair	33,34
Critical Pair Resolution	0.64 (0.22 - 0.68) Rs

Undo | Redo

Detector MS
Notes T0: 0.41 min
Dwell Volume: 0.25 mL
Extra-Column Volume: 25 µL
Back Pressure: 4379+ psi
Learn how to simulate delay column effects on t_R and pressure

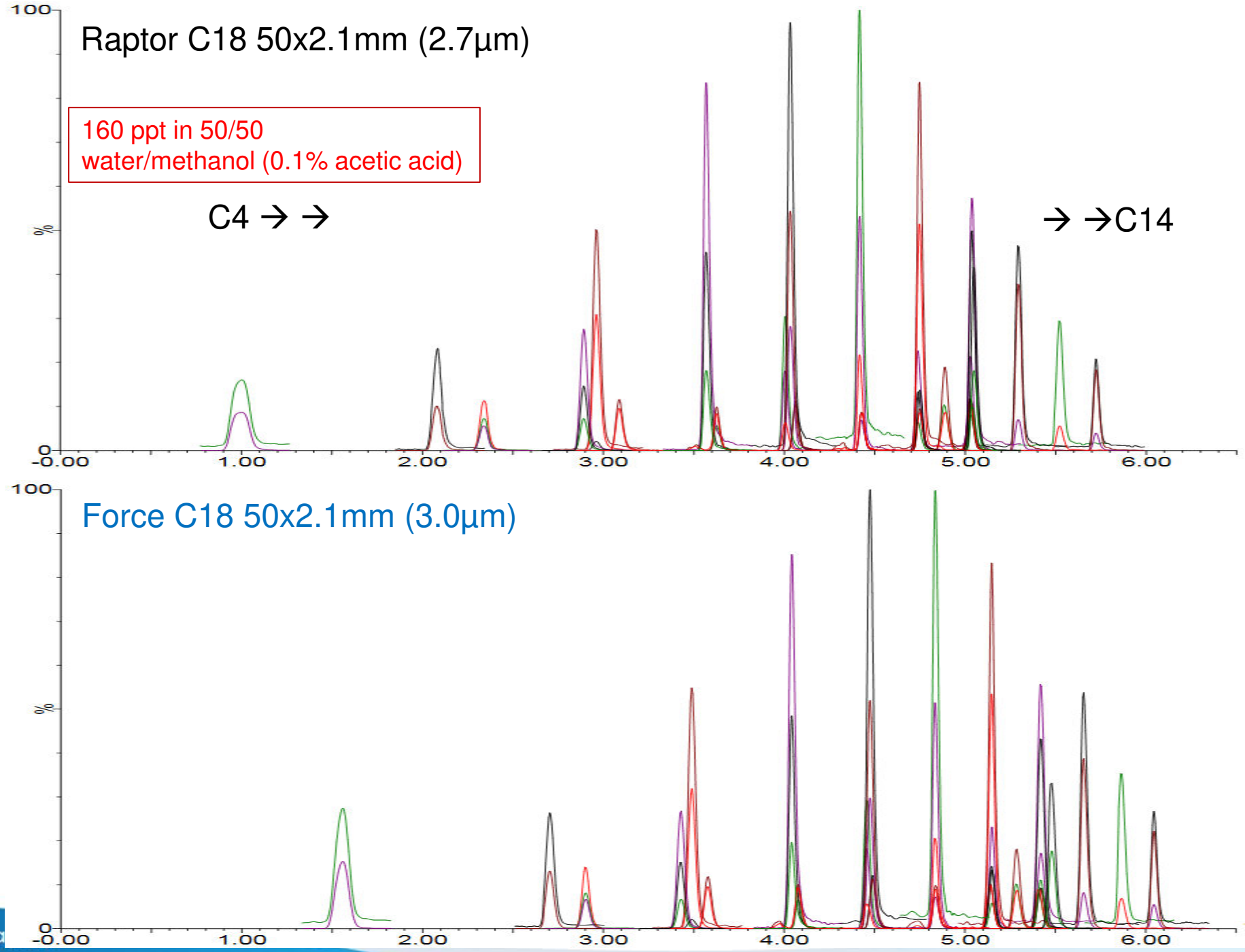
Peaks	t_R (min)	R_s	Peak Width (min)	Exit %B	Mode	Precursor Ion	Quantifier Product Ion
1. PFBA	2.81	--	0.131	23.9	-	213.0	168.7
2. PFMPA	3.35	--	0.121	29.4	-	229.0	84.9
3. 3:3 FTCA	3.66	--	0.112	32.6	-	241.0	176.7
4. PFPeA	4.01	--	0.110	36.5	-	262.9	218.7
5. PFMBA	4.25	--	0.106	39.0	-	279.0	84.9
6. PFBS	4.57	--	0.102	42.4	-	299.0	79.9
7. 4:2 FTS	4.58	--	0.099	42.5	-	327.0	307.0
8. NFDHA	4.66	--	0.100	43.4	-	294.9	200.7
9. LiTFSA	4.70	--	0.112	43.9	-	286.1	169.7
10. PFHxA	4.75	--	0.099	44.4	-	313.1	268.8
11. PFEESA	4.87	--	0.099	45.7	-	315.0	134.8
12. HFPO-DA	4.98	--	0.097	46.8	-	285.0	168.8
13. TUDCA	5.12	10.2	0.090	48.4	-	498.5	79.9

LC-MS/MS Conditions : (Waters TQ-S)	
Mobile Phase A	5mM ammonium acetate in water
Mobile Phase B	methanol

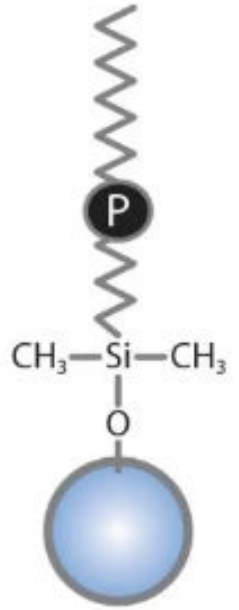
Gradient	Time (min)	%B
	0.00	20
	6.00	95
	6.50	95
	6.51	20
	8.50	20

Flow Rate	0.4 mL/min
Run Time	8.5 min
Column Temp.	40°C

Ion Mode	Negative ESI
Capillary Voltage	0.9 kV
Gas Flow	1000 (L/Hr) Desolvation 150 (L/Hr) Cone 7.0 (bar) Nebuliser
Desolvation Temp.	450°C
Injection	10 uL
Concentration	160 ppt (analyte) 80 ppt (surrogate) In 50/50 water/methanol (0.1% acetic acid)



Solution for Ultrashort-Chain PFAS Analysis in dirty samples

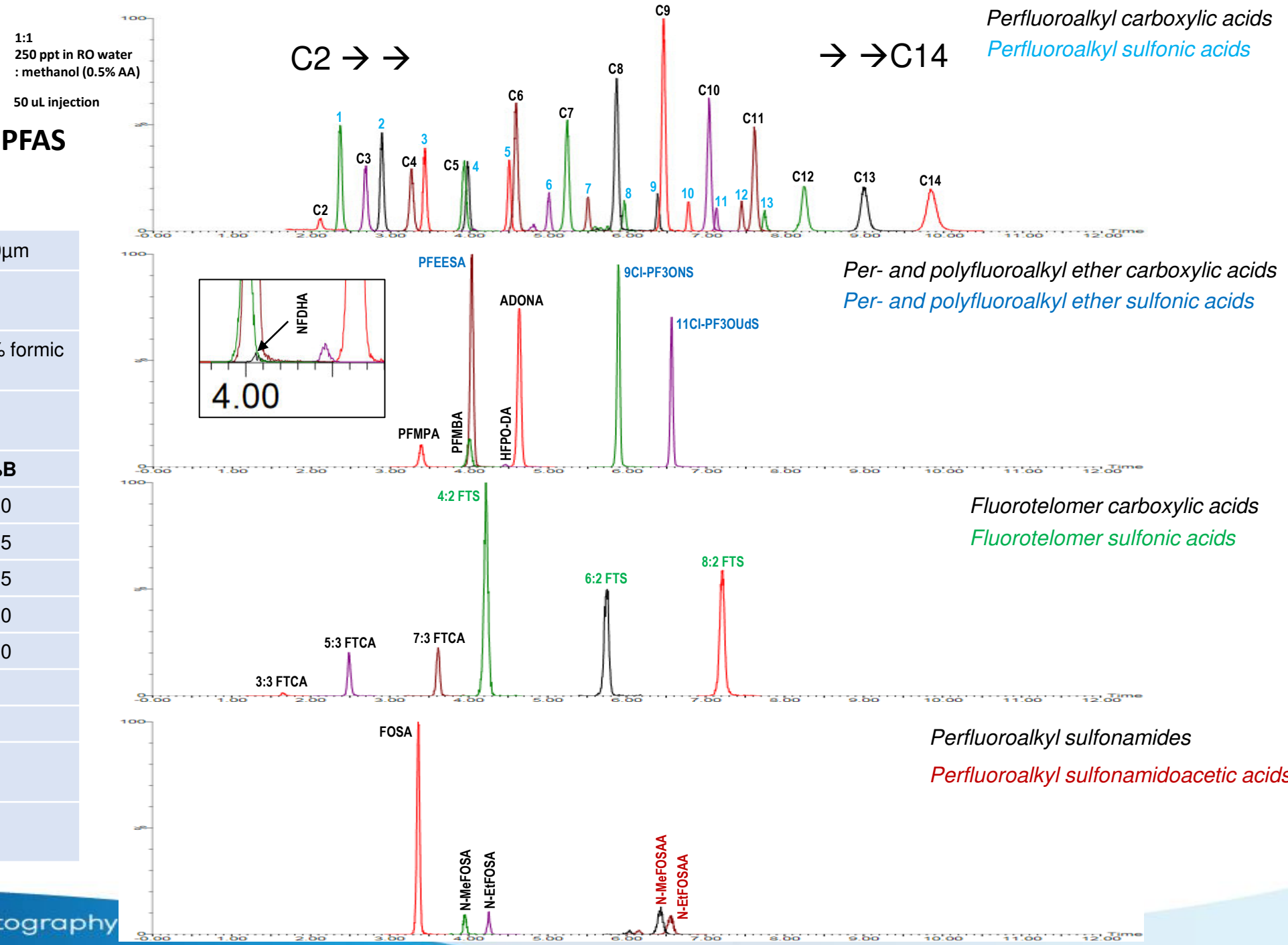


Ultra IBD

- A polar-embedded alkyl phase provides versatile retention characteristics.
- Provides good separations for USC and long chain PFAS under reversed-phase conditions.
- Recommended in recent applications for comprehensive PFAS analysis in plasma and milk.
- Favorable retention leads to reduced matrix interferences for USC PFAS versus C18.

LC method for comprehensive PFAS analysis with Ultra IBD

Column	Ultra Inert IBD 100x2.1mm, 3.0µm	
Delay Column	PFAS Delay Column	
Mobile Phase A	5mM ammonium formate, 0.1% formic acid in water	
Mobile Phase B	Acetonitrile	
Gradient	**Time (min)**	**%B**
	0.00	50
	7.00	95
	10.00	95
	10.01	50
	12.00	50
Flow Rate	0.4 mL/min	
Injection Volume	50 µL	
Column Temp.	40°C	



Plasma, Serum Method Accuracy & Precision

Analytes

Analytes	Linearity Range (ng/mL)	LOD (ng/mL)	Average Recovery (RSD, %) Fortified Concentration (ng/mL) 0.4	2	10	30
^{13}C-TFA	0.50 - 40*	0.1250*	-	90.6 (9.28)	98.0 (2.99)	100 (10.7)
PFPrA	0.25 - 40	0.0102	108 (2.53)	115 (0.965)	105 (6.99)	-
PFBA	0.10 - 40	0.0222	104 (5.57)	109 (1.75)	104 (1.51)	-
PFPeA	0.10 - 40	0.0125	97.4 (3.25)	93.3 (4.19)	89.1 (5.57)	-
PFHxA	0.10 - 40	0.0098	99.2 (2.46)	109 (4.65)	102 (6.31)	-
PFHpA	0.10 - 40	0.0050	86.7 (5.37)	99.2 (2.04)	89.2 (1.80)	-
PFOA	0.10 - 40	0.0051	94.8 (8.24)	107 (5.30)	95.6 (3.35)	-
PFNA	0.10 - 40	0.0012	96.2 (1.44)	111 (1.87)	99.1 (2.62)	-
PFDA	0.10 - 40	0.0083	93.6 (1.93)	102 (2.03)	95.5 (3.64)	-
TFMS	0.05 - 40	0.0070	89.4 (7.33)	88.8 (3.80)	91.9 (3.19)	-
PFEtS	0.05 - 40	0.0020	98.0 (2.62)	103 (1.47)	99.3 (2.26)	-
PFPrS	0.05 - 40	0.0030	98.1 (8.18)	108 (4.23)	98.9 (4.15)	-
PFBS	0.05 - 40	0.0124	88.0 (8.84)	94.1 (4.95)	86.5 (6.17)	-
PFPeS	0.10 - 40	0.0031	94.8 (4.57)	100 (8.05)	94.3 (5.43)	-
PFHxS	0.10 - 40	0.0115	85.8 (7.88)	96.0 (10.0)	92.2 (8.98)	-
PFHpS	0.10 - 40	0.0088	92.5 (6.75)	99.8 (6.45)	93.4 (5.74)	-
PFOS	0.10 - 40	0.0200	97.8 (8.66)	97.9 (7.01)	95.9 (3.37)	-
PFNS	0.10 - 40	0.0129	92.1 (7.98)	94.2 (4.43)	91.9 (2.78)	-
PFDS	0.10 - 40	0.0111	92.6 (8.20)	82.3 (3.48)	87.6 (3.55)	-
HFPO-DA	0.50 - 40	0.1875	-	99.9 (11.3)	91.1 (8.95)	90.4 (6.13)
ADONA	0.10 - 40	0.0035	90.4 (7.14)	106 (4.67)	95.7 (4.25)	-
9Cl-PF3ONS	0.10 - 40	0.0031	95.8 (4.20)	93.7 (5.12)	93.8 (6.71)	-
11Cl-PF3OUdS	0.10 - 40	0.0023	106 (5.10)	84.5 (4.20)	97.2 (4.86)	-

* For non-labeled TFA

Extracted Internal Standard

Compounds	Recovery Range (%)	RSD (%)
$^{13}C_3$-PFPrA	113 - 117	1.42
$^{13}C_4$-PFBA	107 - 116	2.62
$^{13}C_5$-PFPeA	81.0 - 93.8	5.60
$^{13}C_5$-PFHxA	87.0 - 102	5.97
$^{13}C_4$-PFHpA	81.2 - 87.2	2.52
$^{13}C_8$-PFOA	92.5 - 106	4.54
$^{13}C_9$-PFNA	98.0 - 105	2.30
$^{13}C_6$-PFDA	95.6 - 113	6.28
$^{13}C_3$-PFHxS	80.7 - 87.8	2.89
$^{13}C_8$-PFOS	80.3 - 98.0	7.48

- 3 batches of analyses (n=9 for each fortified level)
  - *Analytes Recovery : 82.3 – 115%*
  - *Analytes % RSD : 1.51 – 11.3%*
  - *EIS Recovery: 80.3 – 117%*
  - $r^2 > 0.995$, deviations < 20%

LC Method Development with IBD

- Salty condition and chromatographic performance
  - Early eluting compounds (**TFA, TFMS, PFPrA**) displayed broader peak shapes for the analysis of plasma/serum.
  - Incorporating **phosphate-buffered saline** into standard solution (osmosis water) to gain uniform peak shapes between standard and sample solutions.
- Ammonium formate/formic acid solution is the most satisfactory aqueous mobile phase coupled to an acetonitrile organic mobile phase
  - Usage of ammonium acetate alone resulted in less detection sensitivity.
  - Addition of formic acid alone resulted in prolonged retention and weaker signals.
  - Acetonitrile was superior to methanol to yield sharper peaks and enhanced detection signals.
  - The most favorable condition for retention, separation, and detection sensitivity:

    (A) 5 mM ammonium formate, 0.1% formic acid in water
    (B) Acetonitrile

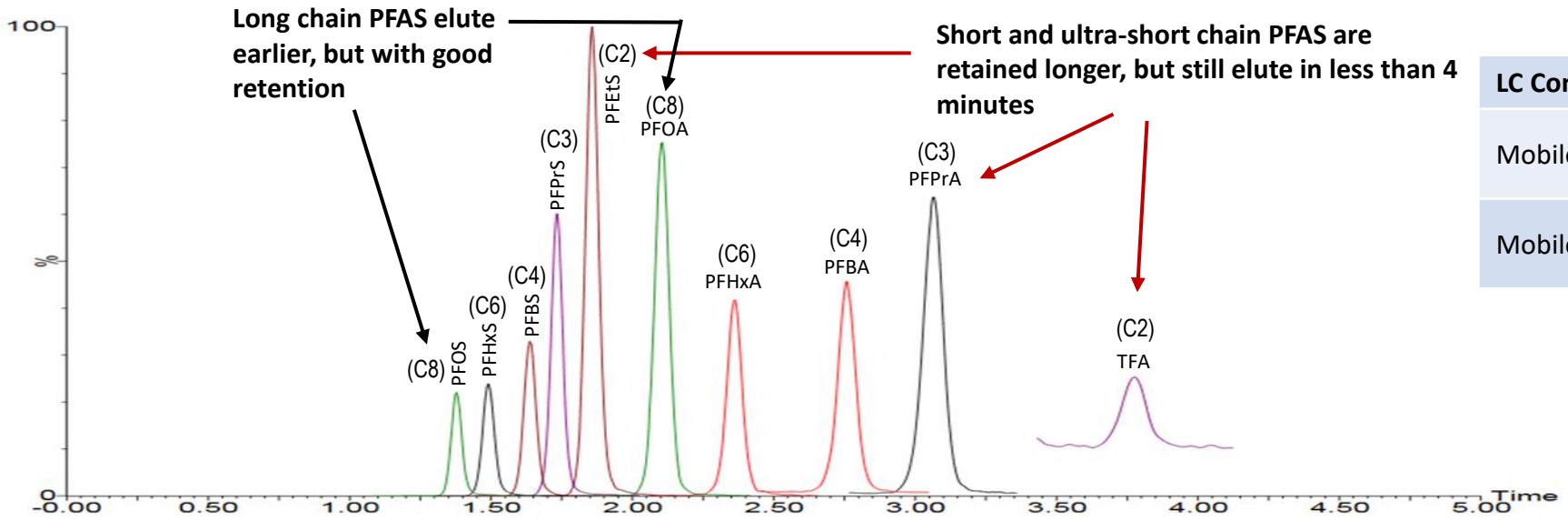
Solution for Ultrashort-Chain PFAS Analysis

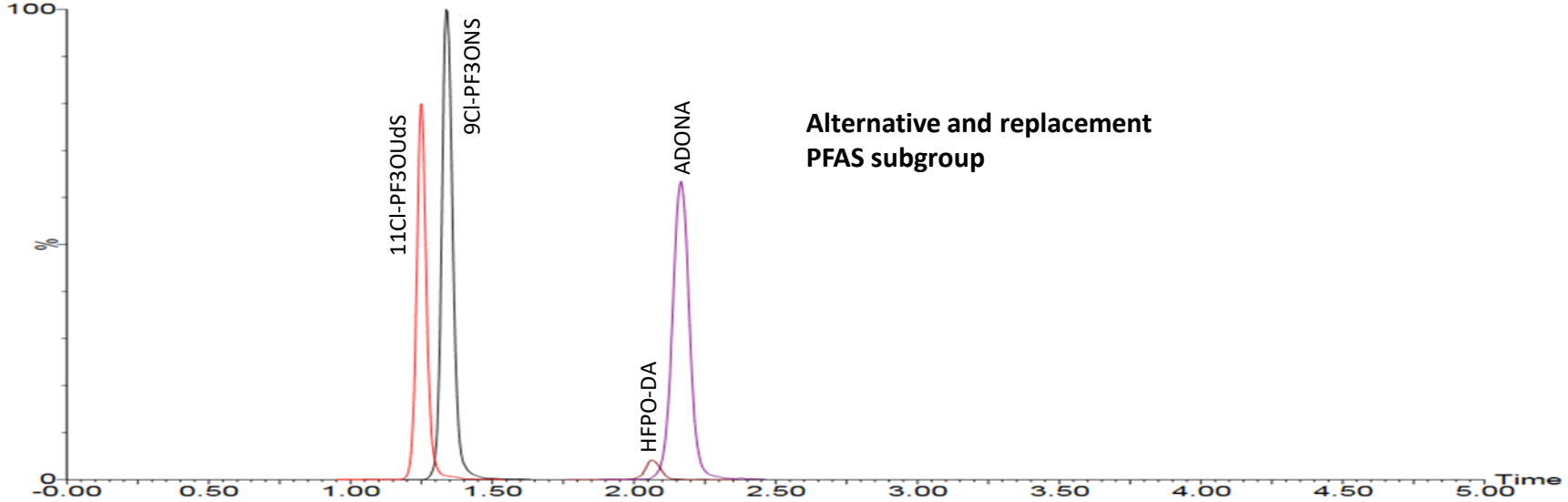
Raptor LC Columns

Polar X

- Ligand chemistry capable of **HILIC** and **anion Exchange** retention.
- Strong retention of polar compounds using isocratic LC method.
- Ideal for ultrashort chain and highly acidic PFAS alternatives in water samples.

Analysis of Ultrashort-Chain/Legacy/Alternative PFAS





Polar X: 2.7µm 50x2.1 mm

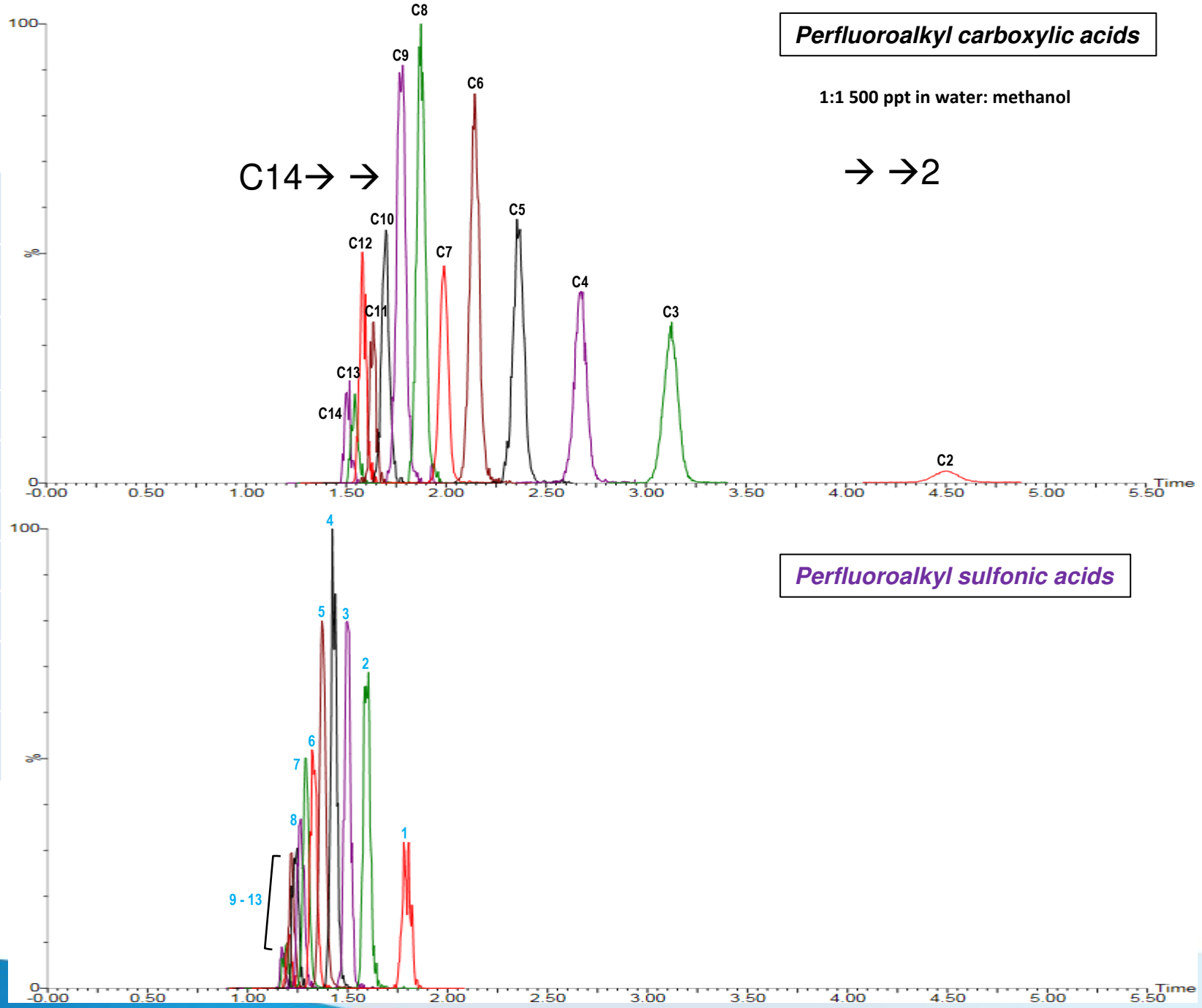
LC Conditions : (Waters Acquity UPLC)	
Mobile Phase A	10mM ammonium formate, 0.05% formic acid in water
Mobile Phase B	0.05% formic acid in 60:40 acetonitrile:methanol

Gradient	Time (min)	%B
	0.00	85
	5.00	85
Injection	10 µL	
Flow Rate	0.5 mL/min	
Run Time	8 min	
Column Temp.	40°C	

400 ppt in 50:50 water:methanol

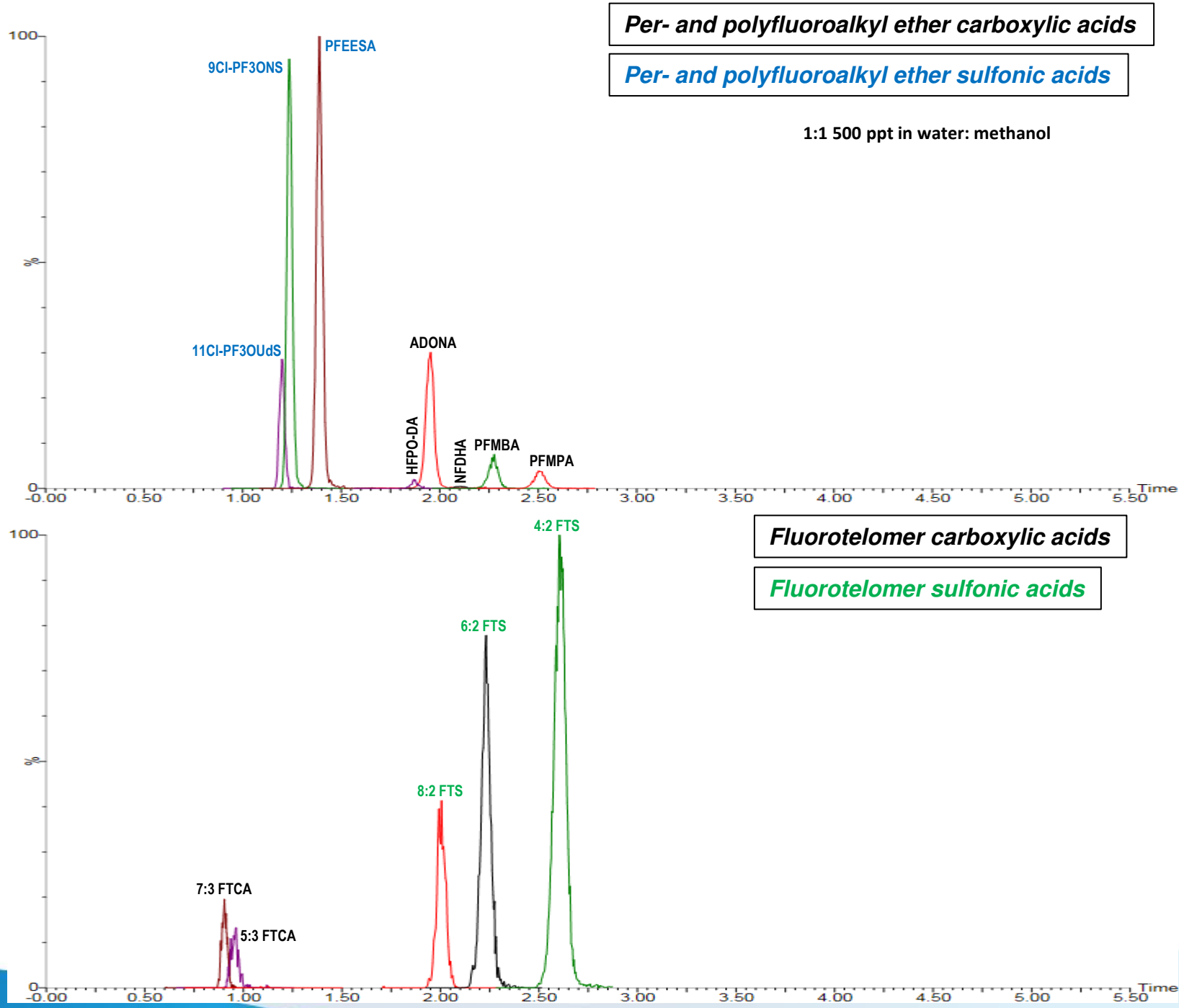
LC method for PFAS analysis with Polar X

Column	Polar X 50x2.1mm, 2.7µm	
Mobile Phase A	5mM ammonium formate, 0.1% formic acid in water	
Mobile Phase B	Acetonitrile	
Gradient	**Time (min)**	**%B**
	0.00	85
	7.00	85
Flow Rate	0.25 mL/min	
Injection Volume	40 µL	
Column Temp.	40°C	



LC method for PFAS analysis with Polar X

Column	Polar X 50x2.1mm, 2.7µm	
Mobile Phase A	5mM ammonium formate, 0.1% formic acid in water	
Mobile Phase B	Acetonitrile	
Gradient	**Time (min)**	**%B**
	0.00	85
	7.00	85
Flow Rate	0.25 mL/min	
Injection Volume	40 µL	
Column Temp.	40°C	



ASTM WK80687 Method Development (C1 to C4 PFAS in Potable and Non-Potable Waters)

Accuracy % Recovery: 86.6 – 107 %

Precision % RSD: 1.62 – 10.7 %

	Average Recovery (RSD, %)								
Samples	Tap Water			Spring Bottled Water			POTW Water		
Concentration (ng/L)	25	50	175	25	50	175	25	50	175
TFA	-	98.2 (7.63)	97.4 (6.68)	-	107 (5.92)	97.1 (4.27)	-	96.7 (10.7)	106 (4.02)
PFPrA	106 (3.49)	107 (2.26)	103 (2.19)	96.6 (4.10)	107 (4.29)	102 (2.19)	102 (3.08)	102 (3.02)	101 (1.71)
PFBA	99.5 (4.61)	100 (5.09)	101 (1.72)	94.4 (9.17)	101 (5.08)	99.6 (3.12)	100 (6.36)	95.2 (5.25)	97.4 (1.62)
TFMS	87.5 (1.62)	95.8 (5.66)	96.4 (3.02)	86.6 (5.99)	95.5 (5.74)	94.6 (3.99)	92.6 (7.42)	94.5 (7.94)	93.8 (5.25)
PFEtS	96.2 (5.68)	100 (7.62)	96.9 (3.93)	92.0 (6.18)	101 (6.24)	95.1 (6.77)	93.8 (6.54)	97.2 (7.75)	95.7 (7.48)
PFPrS	94.2 (4.80)	99.8 (5.38)	97.3 (3.60)	92.5 (7.94)	99.4 (6.31)	96.1 (4.50)	97.6 (4.47)	97.6 (6.52)	96.8 (5.78)
PFBS	98.7 (4.02)	102 (4.92)	101 (3.79)	95.5 (8.10)	104 (7.03)	98.6 (5.09)	99.8 (6.97)	103 (5.99)	100 (3.58)

ASTM WK80687 Method Development (C1 to C4 PFAS in Potable and Non-Potable Waters)

Analytes in Unspiked Water Samples

Samples	Detected Concentration (ng/L)						
	TFA	PFPrA	PFBA	TFMS	PFEtS	PFPrS	PFBS
Tap Water	230	ND	ND	5.58	ND	ND	ND
Bottled Spring Water	102	ND	ND	ND	ND	ND	ND
POTW Water	1113	36.6	<5.00	8.53	ND	ND	4.35

Summary and Conclusions

- TFA contamination controlled through identification of preferred consumables: reagent waters and solvents, pipette tips, vials, syringe filters, glass wool.
  - Workflows often benefit from delay column setup along with use of PFAS free consumables.
- Ultrashort chain PFAS and acid alternatives benefit from ionic stationary phase.
- Raptor Polar X and Ultra IBD show favorable retention of polar PFAS analytes that struggle on conventional C18.
  - Inert column hardware appears to promote superior peak shape, response.
- Selection depends on method needs including sample type, detection limits, and analyte list.
- For more information, please visit the Retek resource library for detailed methods and results, the EZLC tool, or contact the LC applications team.

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