

# A journey from water samples to human plasma and serum finding ultrashort-chain and alternative PFAS (C1 – C10) simultaneously

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Pure Chromatography

# A Journey of PFAS Testing

2000's

PFOA  
PFOS

Mostly water



2010's

C4 – C12  
Water  
Soil

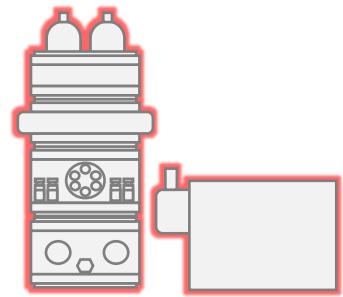


2020's

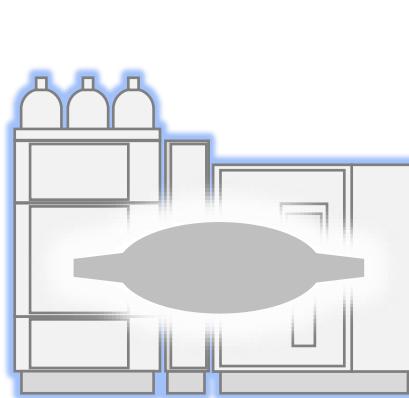
C1 – C18  
Water/Soil  
Food  
Food packaging  
Blood/serum  
Consumer products



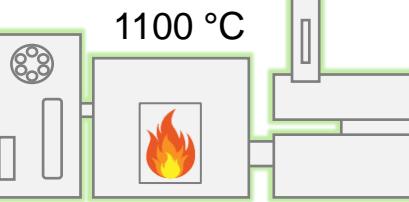
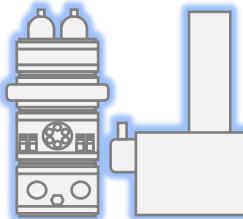
# A Journey of Measurement Technologies



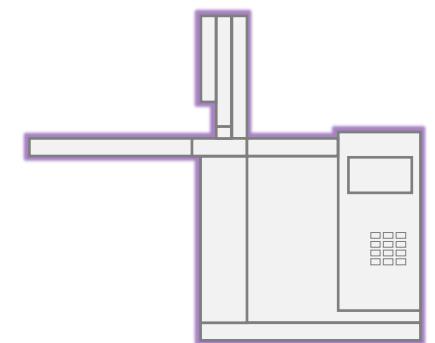
Targeted Analysis



NTA  
Non-target Analysis



1100 °C  
AOF/EOF



Volatile PFAS

ppq – ppt

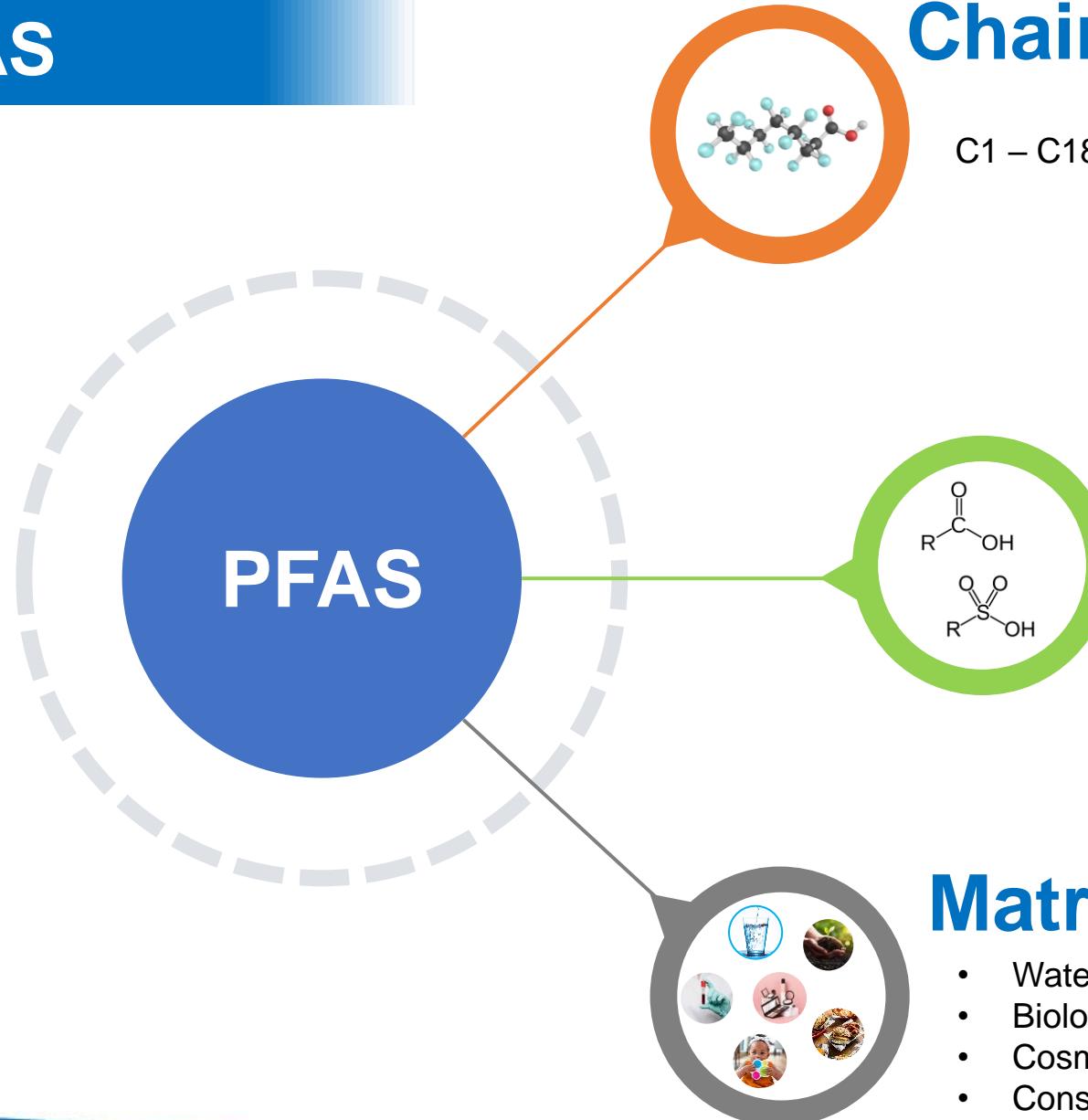
ppt

ppb

ppt, ppb(?)

# Remember the Basics

## PFAS ≠ PFAS



## Chain Length

C1 – C18 and beyond

## Functional Groups

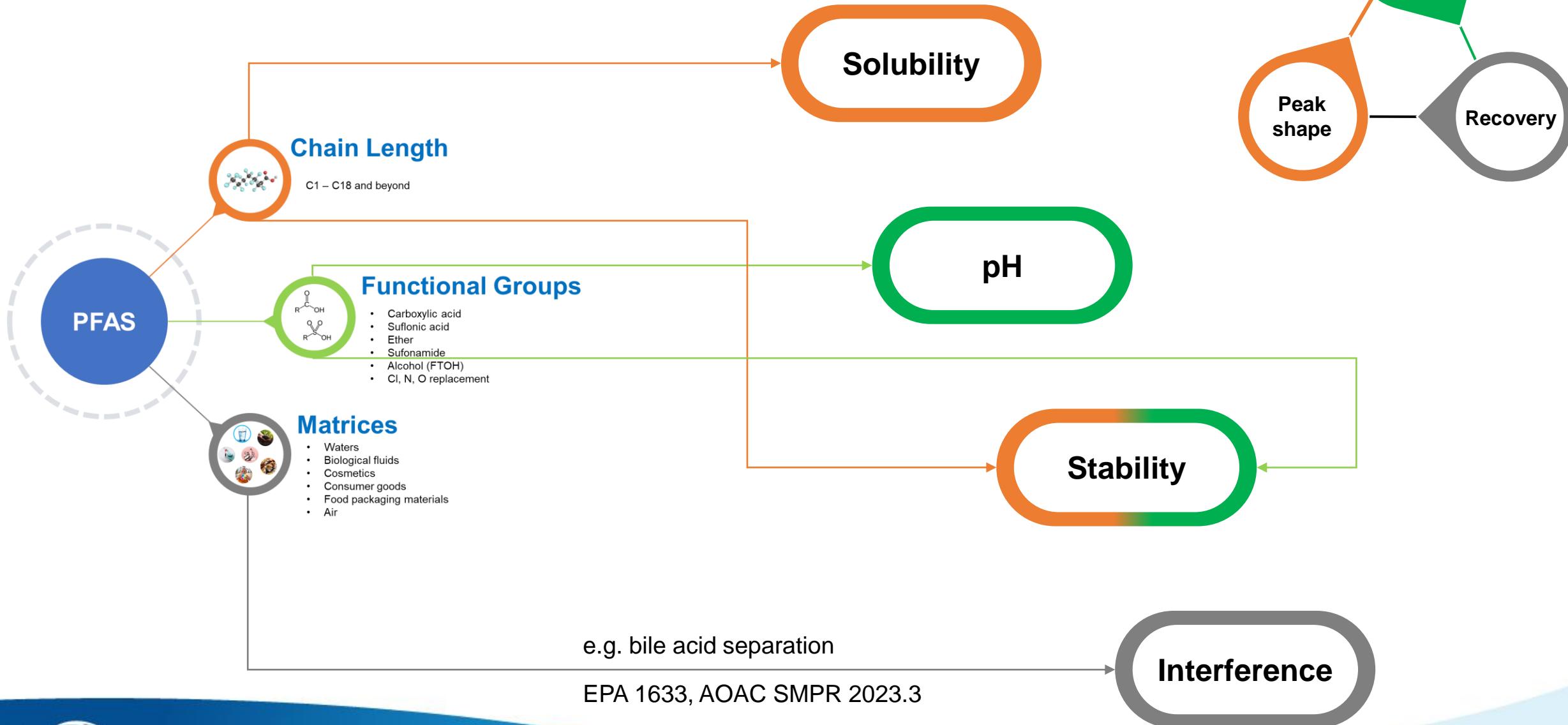
- Carboxylic acid
- Sulfonic acid
- Ether
- Sulfonamide
- Alcohol (FTOH)
- Cl, N, O, H replacement

## Matrices

- Waters
- Biological fluids
- Cosmetics
- Consumer goods
- Food packaging materials
- Air

# Remember the Basics

## PFAS ≠ PFAS



# A Journey of Sample Preparation Technologies



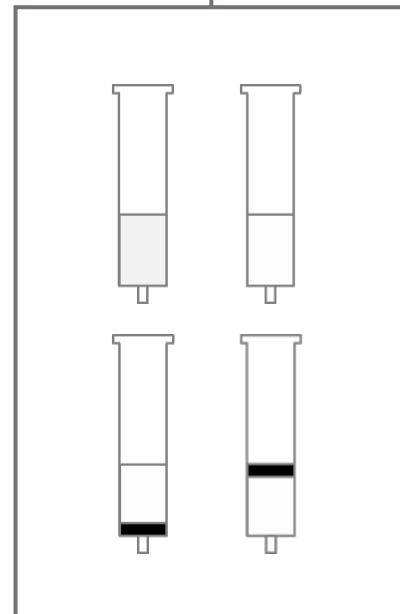
Direct Injection

SPE

QuEChERS

Thermal Desorption

Air Canisters



# A Journey of Solid Phase Extraction

2000's

PFOA  
PFOS

Mostly water



SDVB

EPA 537.1

2010's

C4 – C12  
Water  
Soil



WAX

EPA 533

2020's

C1 – C18  
Water/Soil  
Food  
Food packaging  
Blood/serum  
Consumer products



EPA 1633

WAX

Carbon

Carbon

WAX

# A Journey of LC Columns

2000's

PFOA  
PFOS

Mostly water



C18 Column

2010's

C4 – C12  
Water  
Soil



C18 Column

2020's

C1 – C18  
Water/Soil  
Food  
Food packaging  
Blood/serum  
Consumer products



C18 Column

# A Journey of LC Columns

2000's

PFOA  
PFOS

Mostly water



C18 Column



C18 Column

ing  
products

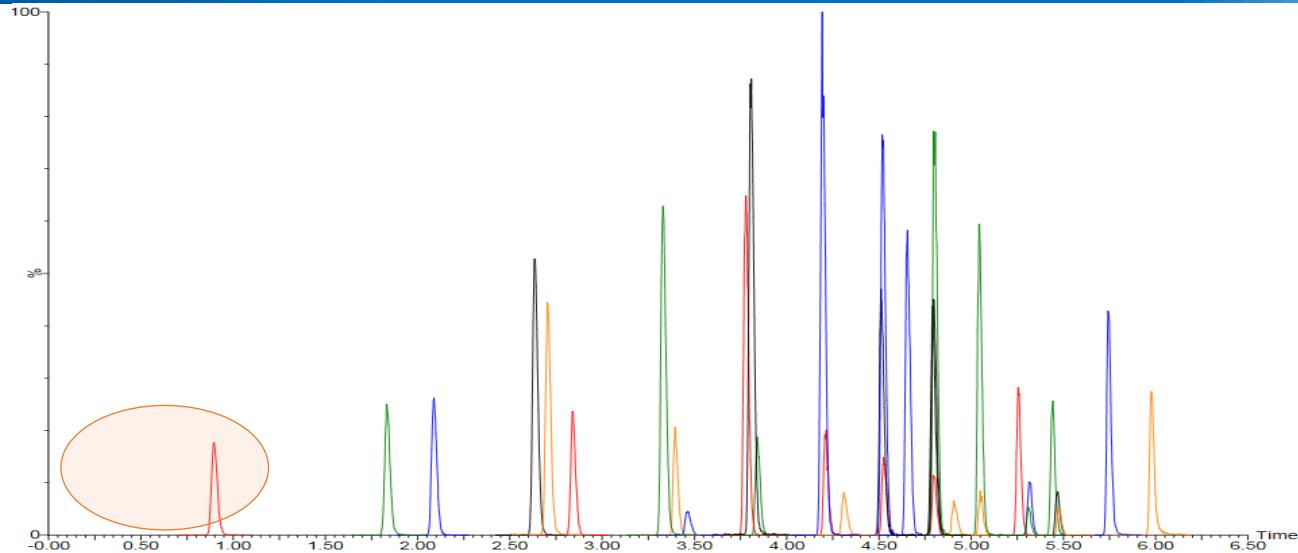


C18 Column

# C18 ≠ C18

Raptor C18 1.8um, 50x2.1mm

OK for

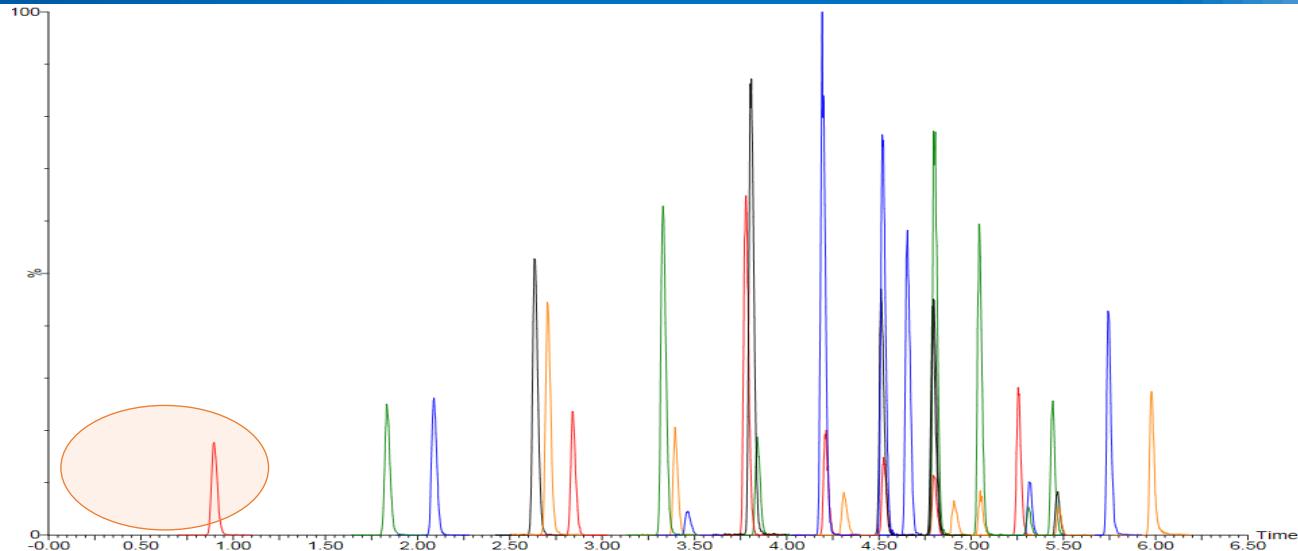


Force C18 1.8um, 50x2.1mm

# C18 ≠ C18

Raptor C18 1.8um, 50x2.1mm

OK for



Force C18 1.8um, 50x2.1mm

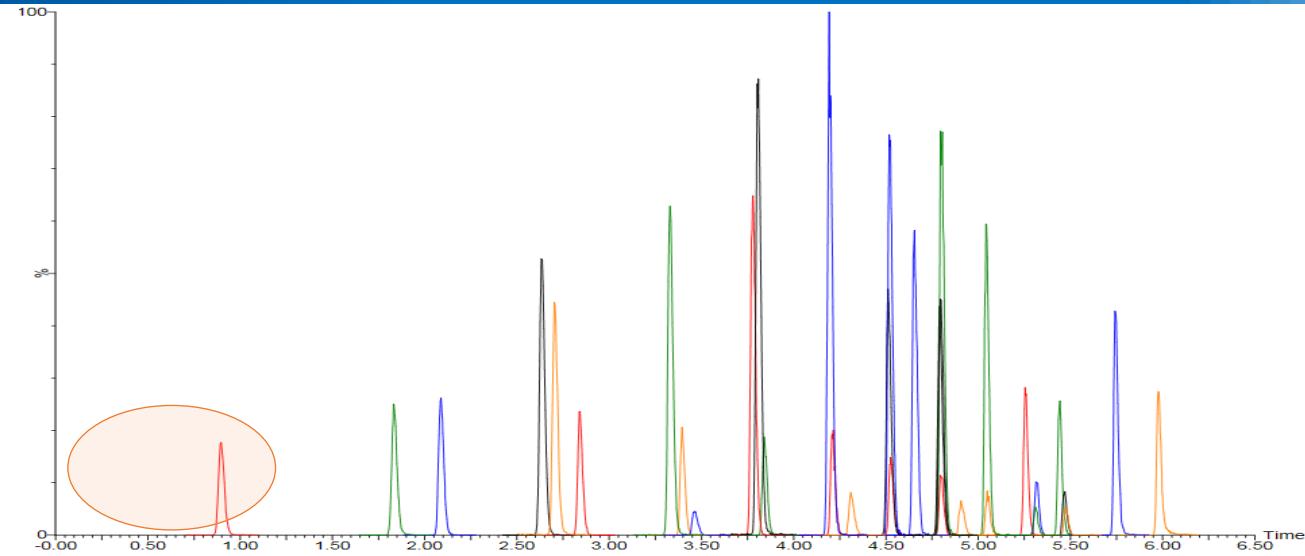
More ion suppression



# C18 ≠ C18

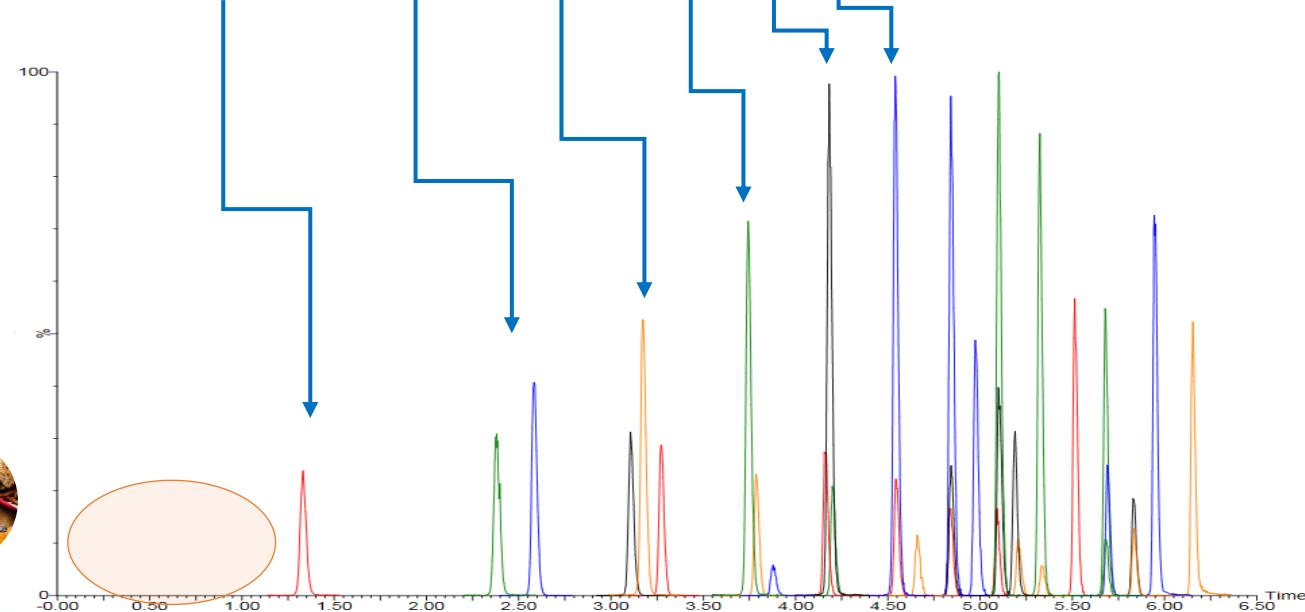
Raptor C18 1.8um, 50x2.1mm

OK for



Force C18 1.8um, 50x2.1mm

More ion suppression



# Methodologies

1

Conventional  
PFAS Methods



2

Exotic  
PFAS Methods



Table 3

3

Ultrashort-Chain  
PFAS Methods



# Table 3 Compounds

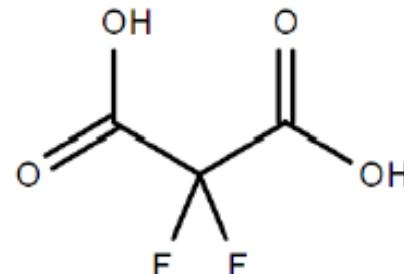


Compound Name	Molecular Formula	Structure	CASRN
DFSA	C <sub>2</sub> H <sub>2</sub> F <sub>2</sub> O <sub>5</sub> S		422-67-3
MMF	C <sub>3</sub> H <sub>2</sub> F <sub>2</sub> O <sub>4</sub>		
MTP	C <sub>4</sub> H <sub>4</sub> F <sub>4</sub> O <sub>3</sub>		
PPF Acid	C <sub>3</sub> HF <sub>5</sub> O <sub>2</sub>		
PFMOAA	C <sub>3</sub> HF <sub>5</sub> O <sub>3</sub>		
R-EVE	C <sub>8</sub> H <sub>12</sub> F <sub>12</sub> O <sub>5</sub>		

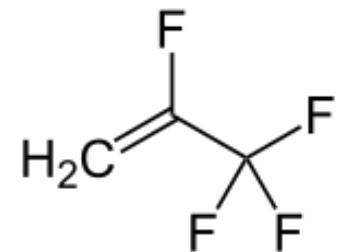
PFO3OA	C <sub>5</sub> HF <sub>9</sub> O <sub>5</sub>		39492-89-2
PES	C <sub>4</sub> HF <sub>9</sub> O <sub>4</sub> S		113507-82-7
HFPO-DA	C <sub>6</sub> HF <sub>11</sub> O <sub>3</sub>		
PFECA G	C <sub>7</sub> HF <sub>13</sub> O <sub>3</sub>		
PFO4DA	C <sub>6</sub> HF <sub>11</sub> O <sub>6</sub>		
Byproduct 4	C <sub>7</sub> H <sub>2</sub> F <sub>12</sub> O <sub>6</sub> S		N/A
Byproduct 5	C <sub>7</sub> H <sub>3</sub> F <sub>11</sub> O <sub>7</sub> S		N/A
PMPA	C <sub>4</sub> HF <sub>7</sub> O <sub>3</sub>		13140-25
PFO2HxA	C <sub>4</sub> HF <sub>7</sub> O <sub>4</sub>		39492-88-1
NVHOS	C <sub>4</sub> H <sub>2</sub> F <sub>8</sub> O <sub>4</sub> S		1132933-86-8
PEPA	C <sub>5</sub> HF <sub>9</sub> O <sub>3</sub>		267239-61-2
Hydro-EVE Acid	C <sub>8</sub> H <sub>2</sub> F <sub>14</sub> O <sub>4</sub>		773804-62-9
EVE Acid	C <sub>8</sub> HF <sub>13</sub> O <sub>4</sub>		69087-46-3
Byproduct 6	C <sub>6</sub> H <sub>2</sub> F <sub>12</sub> O <sub>4</sub> S		N/A
Byproduct 1	C <sub>7</sub> HF <sub>13</sub> O <sub>5</sub> S		29311-67-9
Byproduct 2	C <sub>7</sub> H <sub>2</sub> F <sub>14</sub> O <sub>5</sub> S		749836-20-2
PFO5DoA	C <sub>7</sub> HF <sub>13</sub> O <sub>7</sub>		39492-91-6

# New Replacement PFAS found by NTA (Non-Target Analysis)

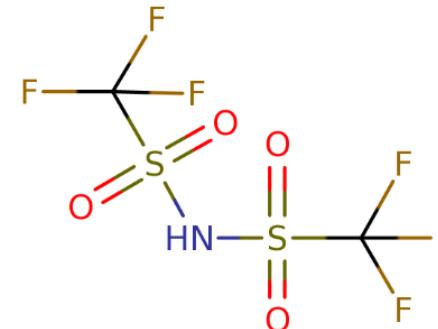
- Polar
- Ionic
- Short & Ultrashort



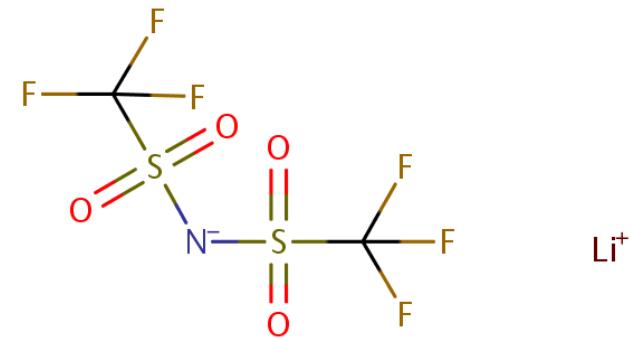
MMF



HFO-1234yf



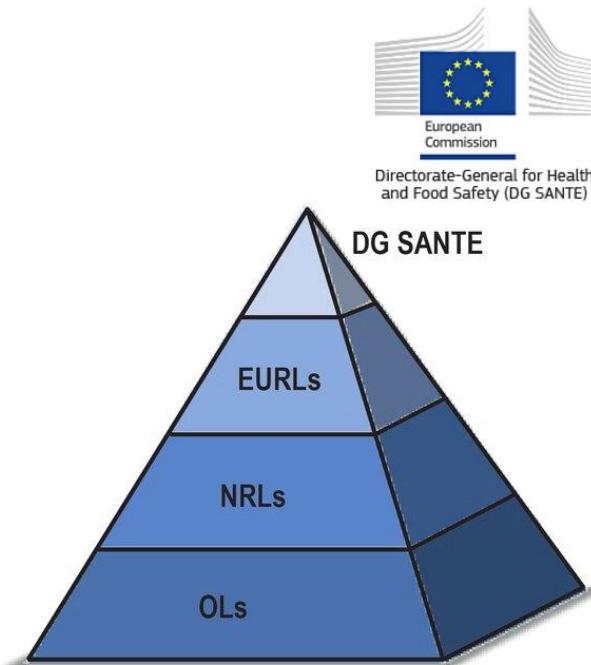
HQ-115



TFSI

# Confirmation Options

## Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed.



**Table 3.** Identification requirements for different MS techniques<sup>2</sup>

MS detector/Characteristics		Acquisition	Requirements for identification	
Resolution	Typical systems (examples)		minimum number of ions	additionally
Unit mass resolution	Single MS Quadrupole, ion trap, TOF	Full scan, limited m/z range, SIM	3 ions	S/N ≥ 3 <sup>d)</sup>  Analyte peaks from both product ions in the extracted ion chromatograms must fully overlap.
	MS/MS Triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	Selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 product ions	Ion ratio from sample extracts should be within ±30 % (relative) of average of calibration standards from same sequence
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap	Full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	2 ions with mass accuracy ≤ 5 ppm <sup>a, b, c)</sup>	S/N ≥ 3 <sup>d)</sup>  Analyte peaks from precursor and/or product ion(s) in the extracted ion chromatograms must fully overlap.

2024.8.6 Verification of PFBA and PFPeA using liquid chromatography/high resolution mass spectrometry (LC-HRMS)

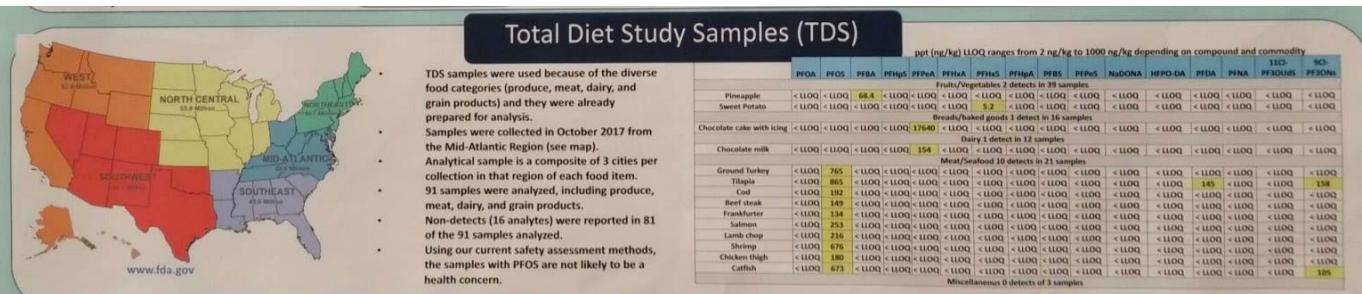
Due to matrix interferences and the potential for false positives for PFBA and PFPeA given that they only have one MS/MS transition, any positive detection of PFBA or PFPeA must be confirmed by LC-HRMS.

The LC-HRMS instrument includes a Nexera LC (Shimadzu, Columbia, MD) coupled to a Q-Exactive Orbitrap™ mass spectrometer (Thermo Fisher Scientific, Waltham, MA). The LC separation is performed using the same conditions as described for the LC-MS/MS analysis (2024.8.4).

## Confirm using HRMS.

## PFPeA false positive for Total Diet Study

## Chocolate cake with icing: PFPeA at 17,640 ppt



**Table 1: Comparison of preliminary and revised reported concentrations of PFOS**

Food type	June 2019 Report	October 2019 Report	Change
<b>Ground turkey</b>	765 ppt	85.7 ppt	8.9 times lower
<b>Tilapia</b>	865 ppt	87 ppt	8.8 times lower



For PFAS with only one specific MS/MS transition (e.g. PFBA and PFPeA), a second confirmation of identity (e.g., high-resolution mass spectrometry) is needed if reporting results from the analysis of food samples.

**Confirm using HRMS.**



- Parent ion
- Quantitation ion
- Confirmation ion

Table 10. Analyte Ions Monitored, Extracted Internal Standard, and Non-extracted Internal Standard Used for Quantification

Abbreviation	Example Retention Time <sup>1</sup>	Parent Ion Mass	Quantification Ion Mass	Confirmation Ion Mass <sup>2</sup>	Typical Ion Ratio	Quantification Reference Compound
<b>Target Analytes</b>						
PFBA	1.96	212.8	168.9	NA	NA	<sup>13</sup> C <sub>4</sub> -PFBA
PFPeA	4.18	263.0	219.0	68.9	NA	<sup>13</sup> C <sub>5</sub> -PFPeA
PFHxA	4.81	313.0	269.0	118.9	13	<sup>13</sup> C <sub>5</sub> -PFHxA
PFHpA	5.32	363.1	319.0	169.0	3.5	<sup>13</sup> C <sub>5</sub> -PFHpA
PFOA	6.16	413.0	369.0	169.0	3.0	<sup>13</sup> C <sub>6</sub> -PFOA
PFNA	6.99	463.0	419.0	219.0	4.9	<sup>13</sup> C <sub>6</sub> -PFNA
PFDA	7.47	512.9	469.0	219.0	5.5	<sup>13</sup> C <sub>6</sub> -PFDA
PFUnA	7.81	563.1	519.0	269.1	6.9	<sup>13</sup> C <sub>7</sub> -PFUnA
PFDoA	8.13	613.1	569.0	319.0	10	<sup>13</sup> C <sub>7</sub> -PFDoA
PFTrDA <sup>3</sup>	8.53	663.0	619.0	168.9	6.7	avg. <sup>13</sup> C <sub>2</sub> -PFTeDA and <sup>13</sup> C <sub>2</sub> -PFDoA
PFTeDA	8.96	713.1	669.0	168.9	6.0	<sup>13</sup> C <sub>2</sub> -PFTeDA
PFBS	4.79	298.7	79.9	98.8	2.1	<sup>13</sup> C <sub>3</sub> -PFBS
PFPeS	5.38	349.1	79.9	98.9	1.8	<sup>13</sup> C <sub>3</sub> -PFHxS
PFHxS	6.31	398.7	79.9	98.9	1.9	<sup>13</sup> C <sub>3</sub> -PFHxS
PFHpS	7.11	449.0	79.9	98.8	1.7	<sup>13</sup> C <sub>8</sub> -PFOS
PFOS	7.59	498.9	79.9	98.8	2.3	<sup>13</sup> C <sub>8</sub> -PFOS
PFNS	7.92	548.8	79.9	98.8	1.9	<sup>13</sup> C <sub>8</sub> -PFOS
PFDS	8.28	599.0	79.9	98.8	1.9	<sup>13</sup> C <sub>8</sub> -PFOS
PFDoS	9.14	699.1	79.9	98.8	1.9	<sup>13</sup> C <sub>8</sub> -PFOS
4:2FTS	4.67	327.1	307.0	80.9	1.7	<sup>13</sup> C <sub>2</sub> -4:2FTS
6:2FTS	5.81	427.1	407.0	80.9	1.9	<sup>13</sup> C <sub>2</sub> -6:2FTS
8:2FTS	7.28	527.1	507.0	80.8	3.0	<sup>13</sup> C <sub>2</sub> -8:2FTS
PFOSA	8.41	498.1	77.9	478.0	47	<sup>13</sup> C <sub>8</sub> -PFOSA
NMeFOSA	9.70	511.9	219.0	169.0	0.66	D <sub>1</sub> -NMeFOSA
NEtFOSA	9.94	526.0	219.0	169.0	0.63	D <sub>3</sub> -NEtFOSA
NMeFOSAA	7.51	570.1	419.0	483.0	2.0	D <sub>1</sub> -NMeFOSAA
NEtFOSAA	7.65	584.2	419.1	526.0	1.2	D <sub>3</sub> -NEtFOSAA
NMeFOSE	9.57	616.1	58.9	NA	NA	D <sub>1</sub> -NMeFOSE
NEtFOSE	9.85	630.0	58.9	NA	NA	D <sub>3</sub> -NEtFOSE
HFPO-DA	4.97	284.9	168.9	184.9	1.95	<sup>13</sup> C <sub>3</sub> -HFPO-DA
ADONA	5.79	376.9	250.9	84.8	2.8	<sup>13</sup> C <sub>3</sub> -HFPO-DA
PFMPA	3.21	229.0	84.9	NA	NA	<sup>13</sup> C <sub>5</sub> -PFPeA
PFMBA	4.53	279.0	85.1	NA	NA	<sup>13</sup> C <sub>5</sub> -PFPeA
NFDHA	4.84	295.0	201.0	84.9	1.46	<sup>13</sup> C <sub>5</sub> -PFHxA
9Cl-PF3ONS	7.82	530.8	351.0	532.8→533.0	3.2	<sup>13</sup> C <sub>3</sub> -HFPO-DA
11Cl-PF3OUDs	8.62	630.9	450.9	632.9→452.9	3.0	<sup>13</sup> C <sub>3</sub> -HFPO-DA
PFEESA	5.08	314.8	134.9	82.9	9.22	<sup>13</sup> C <sub>5</sub> -PFHxA
3:3FTCA	3.89	241.0	177.0	117.0	1.70	<sup>13</sup> C <sub>2</sub> -PFPeA
5:3FTCA	5.14	341.0	237.1	217.0	1.16	<sup>13</sup> C <sub>5</sub> -PFHxA
7:3FTCA	6.76	441.0	316.9	336.9	0.69	<sup>13</sup> C <sub>5</sub> -PFHxA
<b>EIS Compounds</b>						
<sup>13</sup> C <sub>4</sub> -PFBA	1.95	216.8	171.9	NA	NA	<sup>13</sup> C <sub>4</sub> -PFBA
<sup>13</sup> C <sub>5</sub> -PFPeA	4.18	268.3	223.0	NA	NA	<sup>13</sup> C <sub>5</sub> -PFHxA
<sup>13</sup> C <sub>5</sub> -PFHxA	4.80	318.0	273.0	120.3	NA	<sup>13</sup> C <sub>5</sub> -PFHxA
<sup>13</sup> C <sub>4</sub> -PFHpA	5.32	367.1	322.0	NA	NA	<sup>13</sup> C <sub>4</sub> -PFHxA
<sup>13</sup> C <sub>6</sub> -PFOA	6.16	421.1	376.0	NA	NA	<sup>13</sup> C <sub>6</sub> -PFOA
<sup>13</sup> C <sub>6</sub> -PFNA	6.99	472.1	427.0	NA	NA	<sup>13</sup> C <sub>6</sub> -PFNA

# EPA 1633



Table 10. Analyte Ions Monitored, Extracted Internal Standard, and Non-extracted Internal Standard Used for Quantification

Abbreviation Target Analytes	Example Retention Time <sup>1</sup>	Parent Ion Mass	Quantification Ion Mass	Confirmation Ion Mass <sup>2</sup>	Typical Ion Ratio	Quantification Reference Compound
PFBA	1.96	212.8	168.9	NA	NA	<sup>13</sup> C <sub>4</sub> -PFBA
PFPeA	4.18	263.0	219.0	68.9	NA	<sup>13</sup> C <sub>5</sub> -PFPeA
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PFOA	6.16	413.0	369.0	169.0	3.0	<sup>13</sup> C <sub>6</sub> -PFOA
PFNA	6.99	463.0	419.0	219.0	4.9	<sup>13</sup> C <sub>6</sub> -PFNA
PFDA	7.47	512.9	469.0	219.0	5.5	<sup>13</sup> C <sub>6</sub> -PFDA
PFUnA	7.81	563.1	519.0	269.1	6.9	<sup>13</sup> C <sub>7</sub> -PFUnA
			569.0	319.0	10	<sup>13</sup> C <sub>2</sub> -PFDoA
			619.0	168.9	6.7	avg. <sup>13</sup> C <sub>2</sub> -PFTeDA and <sup>13</sup> C <sub>2</sub> -PFDoA

	Parent ion	Quantitation ion	Confirmation ion	Ion ratio		
PFBA	1.96	212.8	168.9	NA	NA	<sup>13</sup> C <sub>4</sub> -PFBA
PFPeA	4.18	263.0	219.0	68.9	NA	<sup>13</sup> C <sub>5</sub> -PFPeA

PFNS	7.92	548.8	79.9	98.8	1.9	<sup>13</sup> C <sub>8</sub> -PFOS
PFDS	8.28	599.0	79.9	98.8	1.9	<sup>13</sup> C <sub>8</sub> -PFOS

PFMPA	3.21	229.0	84.9	NA	NA	<sup>13</sup> C <sub>5</sub> -PFPeA
PFMBA	4.53	279.0	85.1	NA	NA	<sup>13</sup> C <sub>5</sub> -PFPeA

NEtFOSA	9.94	526.0	219.0	169.0	0.63	D <sub>7</sub> -NEtFOSA
NMeFOSAA	7.51	570.1	419.0	483.0	2.0	D <sub>7</sub> -NMeFOSAA
NEtFOSAA	7.65	584.2	419.1	526.0	1.2	D <sub>7</sub> -NEtFOSAA
NMeFOSE	9.57	616.1	58.9	NA	NA	D <sub>7</sub> -NMeFOSE
NEtFOSE	9.85	630.0	58.9	NA	NA	D <sub>9</sub> -NEtFOSE
HFPO-DA	4.97	284.9	168.9	184.9	1.95	<sup>13</sup> C <sub>3</sub> -HFPO-DA
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9Cl-PF3ONS	7.82	530.8	351.0	532.8→353.0	3.2	<sup>13</sup> C <sub>3</sub> -HFPO-DA
11Cl-PF3Ouds	8.62	630.9	450.9	632.9→452.9	3.0	<sup>13</sup> C <sub>3</sub> -HFPO-DA

NMeFOSE	9.57	616.1	58.9	NA	NA	D <sub>7</sub> -NMeFOSE
NEtFOSE	9.85	630.0	58.9	NA	NA	D <sub>9</sub> -NEtFOSE

<sup>13</sup> C <sub>4</sub> -PFBA	1.95	216.8	171.9	NA	<sup>13</sup> C <sub>5</sub> -PFBA
<sup>13</sup> C <sub>5</sub> -PFPeA	4.18	268.3	223.0	NA	<sup>13</sup> C <sub>2</sub> -PFHxA
<sup>13</sup> C <sub>5</sub> -PFHxA	4.80	318.0	273.0	120.3	<sup>13</sup> C <sub>2</sub> -PFHxA
<sup>13</sup> C <sub>4</sub> -PFHpA	5.32	367.1	322.0	NA	<sup>13</sup> C <sub>2</sub> -PFHxA
<sup>13</sup> C <sub>6</sub> -PFOA	6.16	421.1	376.0	NA	<sup>13</sup> C <sub>6</sub> -PFOA
<sup>13</sup> C <sub>6</sub> -PFNA	6.99	472.1	427.0	NA	<sup>13</sup> C <sub>6</sub> -PFNA

**RESTEK**

Pure Chromatography



**Note:** Some of the target analytes in Table 9 do not produce confirmation ions or produce confirmation ions with very low relative abundances; therefore, for those analytes, the IAR does not apply.

### IAR (Ion Abundance Ratio) does not apply.

The total response of all isomers (branched and linear) in the quantitative standards must be used to define the IAR. In samples, the total response should include only the branched isomer peaks that have been identified in either the quantitative or qualitative standard (see Section 7.3 regarding records of traceability of all standards). If standards (either quantitative or qualitative) are not available for purchase, only the linear isomer can be identified and quantitated in samples. The ratio requirement does not apply for PFBA, PFPeA, NMeFOSE, NEtFOSE, PFMPA, and PFMBA because suitable (not detectable or inadequate S/N) secondary transitions (Q2) are not available.

- PFBA
- PFPeA
- NMeFOSE
- NEtFOSE
- PFMPA
- PFMBA

**Ignore ion ratio for confirmation on these analytes.**



TABLE 2. RECOMMENDED PRECURSOR IONS AND PRODUCT IONS AND EXAMPLE CHROMATOGRAPHIC RETENTION TIMES

Target Analyte	Precursor Ion m/z	Product Ion m/z		Retention Time (min) <sup>1</sup>
		Primary	Secondary	
PFBA <sup>2</sup>	213	169	-	3.07
PFPeA <sup>2</sup>	263	219	-	4.16
4:2 FTS	327	307	81	5.13
PFHxA	313	269	119	5.46
PFBS	299	80	99	5.72
PFHpA	363	319	169	6.39
PFPeS	349	80	99	6.74
6:2 FTS	427	407	81	6.81
PFOA	413	369	169	7.08
PFHxS <sup>3</sup>	399	80	99	7.45
PFNA	463	419	219	7.68
8:2 FTS	527	507	81	7.92
PFHpS	449	80	99	8.08
PFDA	513	469	219	8.2
N-MeFOSAA	570	419	483	8.22
N-EtFOSAA	584	419	483	8.43
PFOS <sup>3</sup>	499	80	99	8.6
PFUnDA	563	519	269	8.7
PFNS	549	80	99	9.15
PFDoDA	613	569	169	9.17
PFTrDA	663	619	169	9.66
PFDS	599	80	99	9.67
PFOSA <sup>2</sup>	498	78	-	9.77
PFTeDA	713	669	169	10.12
Isotopically labeled surrogates <sup>4</sup> :				
M4PFBA	217	172		3.06
M5PFPeA	268	223		4.15
M2-4:2 FTS <sup>5</sup>	329	309	81	5.12
M5PFHxA	318	273		5.44
M3PFBS	302	80		5.72
M2PFHpA	367	322		6.38
M2-6:2 FTS <sup>5</sup>	429	409	81	6.8
M8PFOA	421	376		7.08
M3PFHxS	402	80		7.46
M9PFNA	472	427		7.66
M2-8:2 FTS <sup>5</sup>	529	509	81	7.92
M6PFDA	519	474		8.18
d3-NMeFOSAA	573	419		8.21
d5-NEtFOSAA	589	419		8.41
M8PFOS	507	80		8.63
M7PFUnDA	570	525		8.68
M2PFDoDA	615	570		9.17
M8PFOSA	506	78		9.77
M2PFTeDA	715	670		10.12

**1.3.2 The following compounds lack or have low abundance of secondary product ions, and interferences may make qualitative identification more difficult.**

Perfluorobutanoic acid (PFBA).

Perfluoropentanoic acid (PFPeA)

Perfluorohexanoic acid (PFHxA)

Perfluoro-1-octanesulfonamide (PFOSA)

# EPA OTM-45



Analyte	Precursor Ion (m/z)	Primary Product Ion (m/z)	Secondary Product Ion (m/z)
PFBA	213	169	
<sup>13</sup> C <sub>3</sub> -PFBA	216	172	
<sup>13</sup> C <sub>4</sub> -PFBA	217	172	
PFPeA	263	219	
<sup>13</sup> C <sub>5</sub> -PFPeA	268	223	
PFHxA	313	269	119
<sup>13</sup> C <sub>5</sub> -PFHxA	318	273	
PFHpA	363	319	169
<sup>13</sup> C <sub>4</sub> -PFHpA	367	322	
PFOA	413	369	169
<sup>13</sup> C <sub>2</sub> -PFOA	415	370	
<sup>13</sup> C <sub>8</sub> -PFOA	421	376	
PFNA	463	419	169
<sup>13</sup> C <sub>9</sub> -PFNA	472	427	
PFDA	513	469	169
<sup>13</sup> C <sub>6</sub> -PFDA	519	474	
PFUnA	563	519	169
<sup>13</sup> C <sub>7</sub> -PFUnA	570	525	
PFDoA	613	569	169
<sup>13</sup> C <sub>2</sub> -PFDoA	615	570	
PFTrDA	663	619	169
PTeDA	713	169	219
PFHxDA	813	769	169
PFODA	913	869	169
PFBS	299	80	99
<sup>13</sup> C <sub>3</sub> -PFBS	302	80	83
PFPeS	349	80	99
PFHxS	399	80	99
<sup>13</sup> C <sub>3</sub> -PFHxS	402	80	
PFHpS	449	80	99
PFOS	499	80	99
<sup>13</sup> C <sub>4</sub> -PFOS	503	80	
<sup>13</sup> C <sub>8</sub> -PFOS	507	80	
PFNS	549	80	99
PFDS	599	80	99
PFDoS	699	80	99
FOSA	498	78	
MeFOSA	512	169	
EtFOSA	526	169	
N-MeFOSE	616	59	
N-EtFOSE	630	59	
MeFOSAA	570	419	512
EtFOSAA	584	419	526
4:2FTS	327	307	

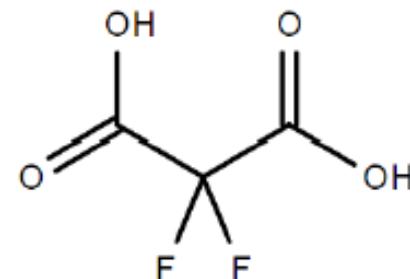
<sup>13</sup> C <sub>2</sub> -4:2FTS	329	309	81
6:2FTS	427	407	
<sup>13</sup> C <sub>2</sub> -6:2FTS	429	409	81
8:2FTS	527	507	
<sup>13</sup> C <sub>2</sub> -8:2FTS	529	509	81
10:2FTS	627	607	80
ADONA	377	251	
HFPO-DA	285	169	
<sup>13</sup> C <sub>3</sub> -HFPO-DA	287	169	
9Cl-PF3ONS	531	351	
11Cl-PF3OUdS	631	451	
NFDHA	295	201	
PFEESA	315	135	
PFDoS	699	80	99
PFMBA	279	85	
PFMPA	229	85	
PFecHS	461	381	99
8:2 FTUCA or FOUEA	457	393	
10:2 FDEA	577	493	
8:2 FTA or FOEA	477	393	
6:2 FHUEA	357	293	
6:2FTCA or 6:2 FHEA	377	243	
3:3 FTCA	241	177	117
5:3 FTCA	341	237	217
7:3 FTCA or FHpPA	441	337	317

# Confirmation Options?

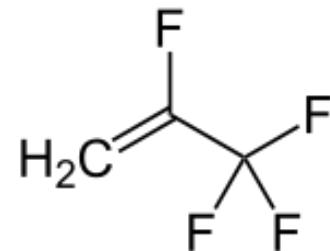
**Ion Ratio is NOT available.**

- **1 MRM Transition Only**
- **Weak 2<sup>nd</sup> Transition**

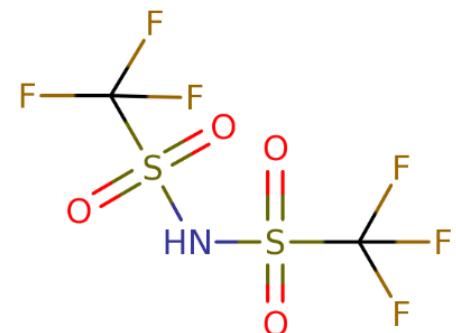
- **PFBA**
- **PFPeA**
- **NMeFOSE**
- **NEtFOSE**
- **PFMPA**
- **PFMBA**



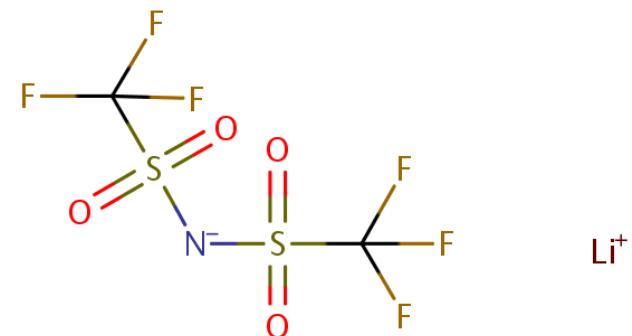
MMF



HFO-1234yf



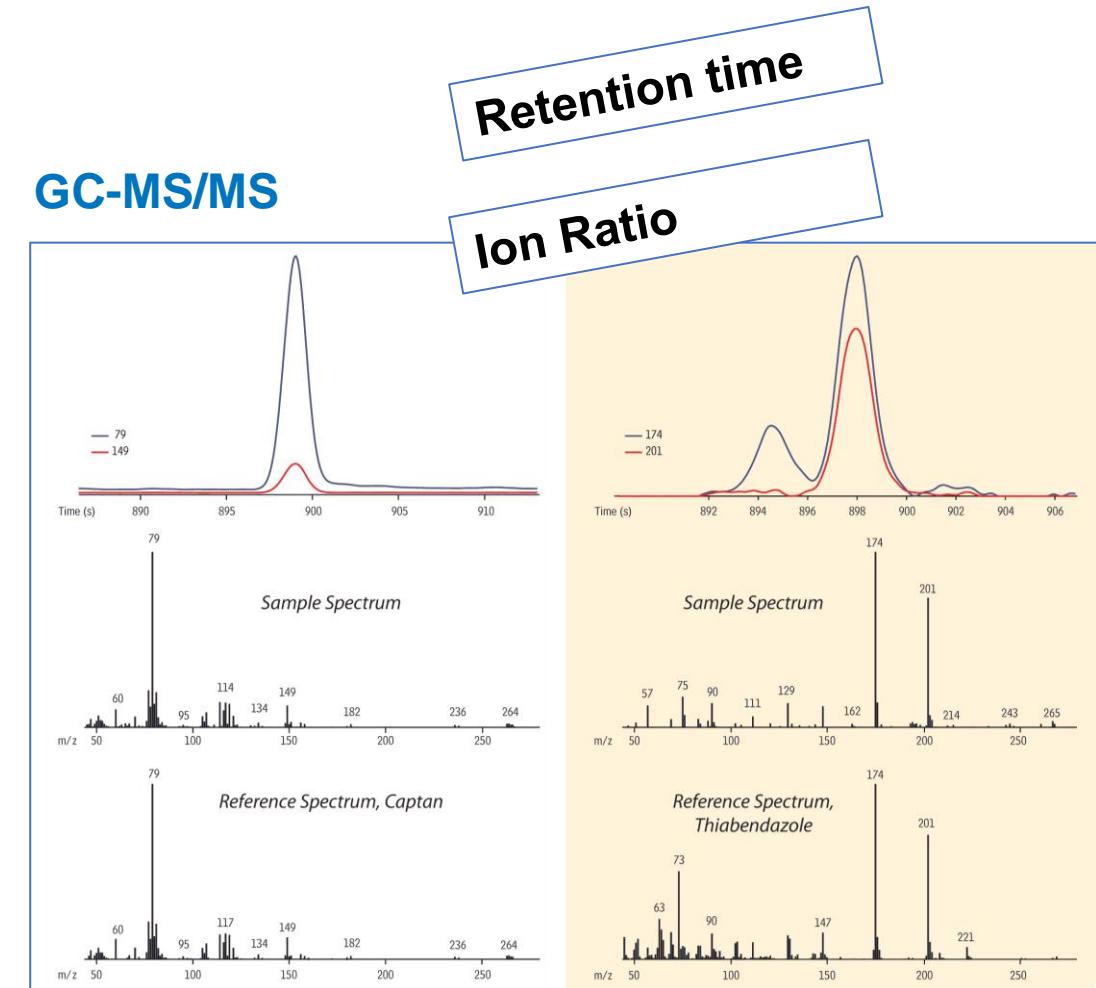
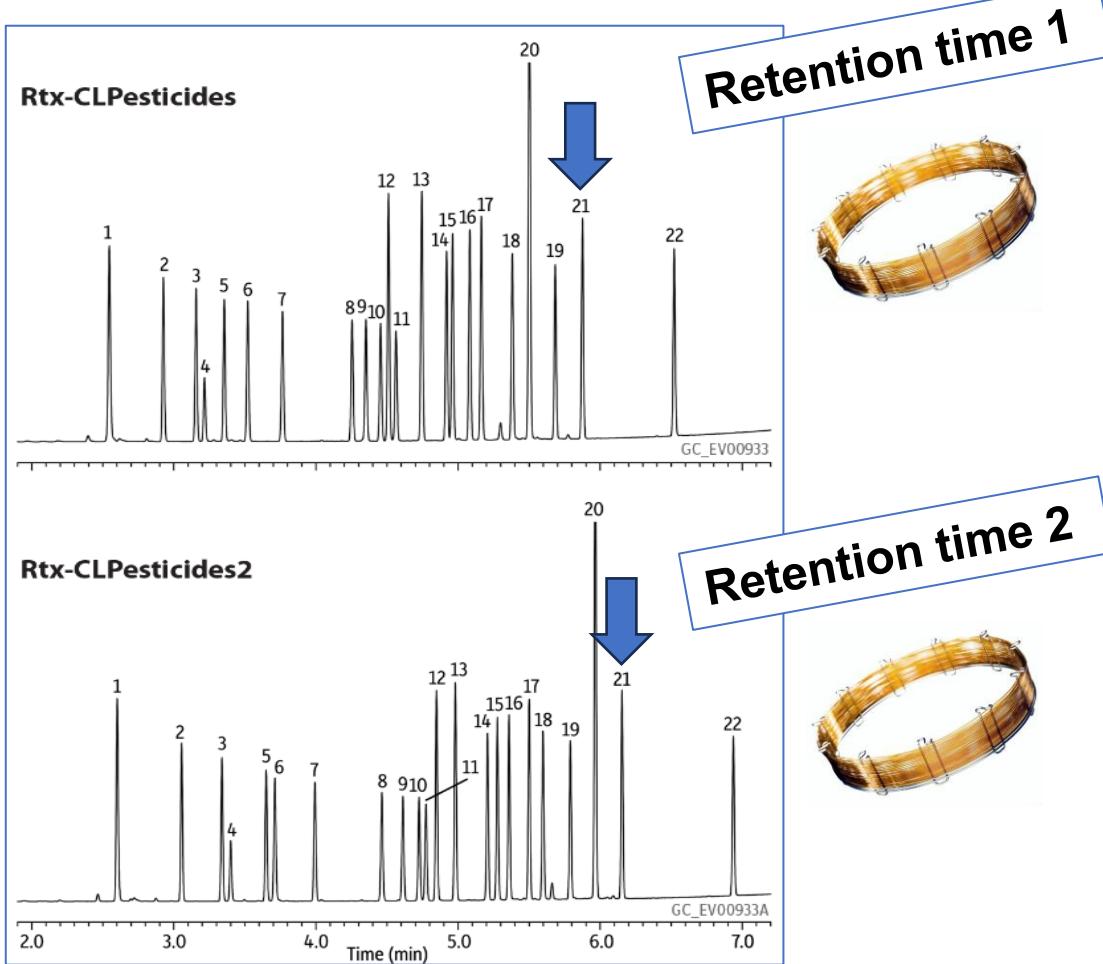
HQ-115



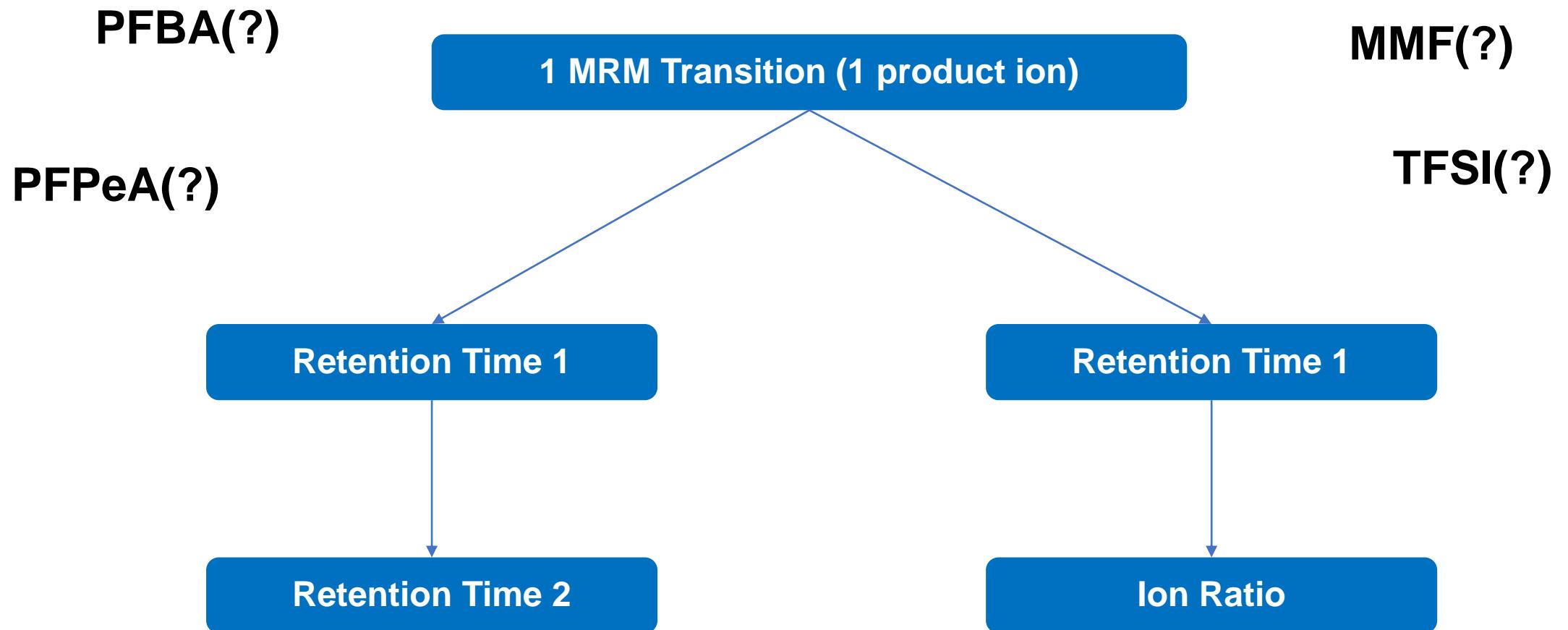
TFSI

# Hint from EPA 8081B

## Dual ECD



# Revisiting Problems of Short Chain PFAS Confirmation



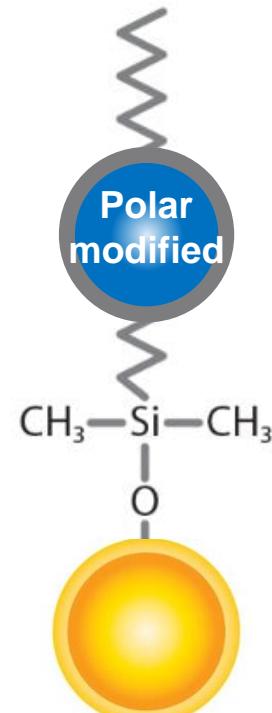
# Short-Chain PFAS Confirmation Tools

Two different retention mechanisms for short chain PFAS analysis

- Retention time 1
- Retention time 2



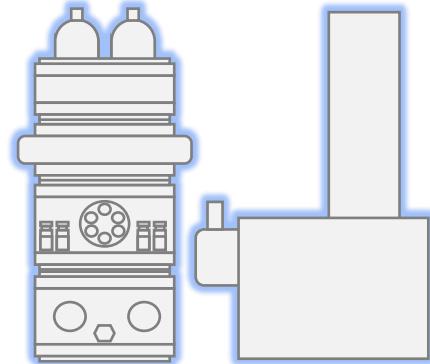
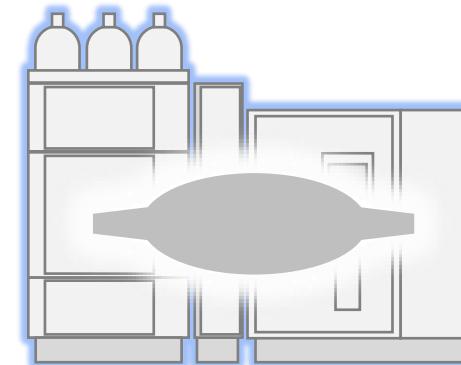
Polar X



Ultra IBD

OR

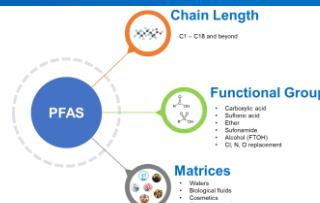
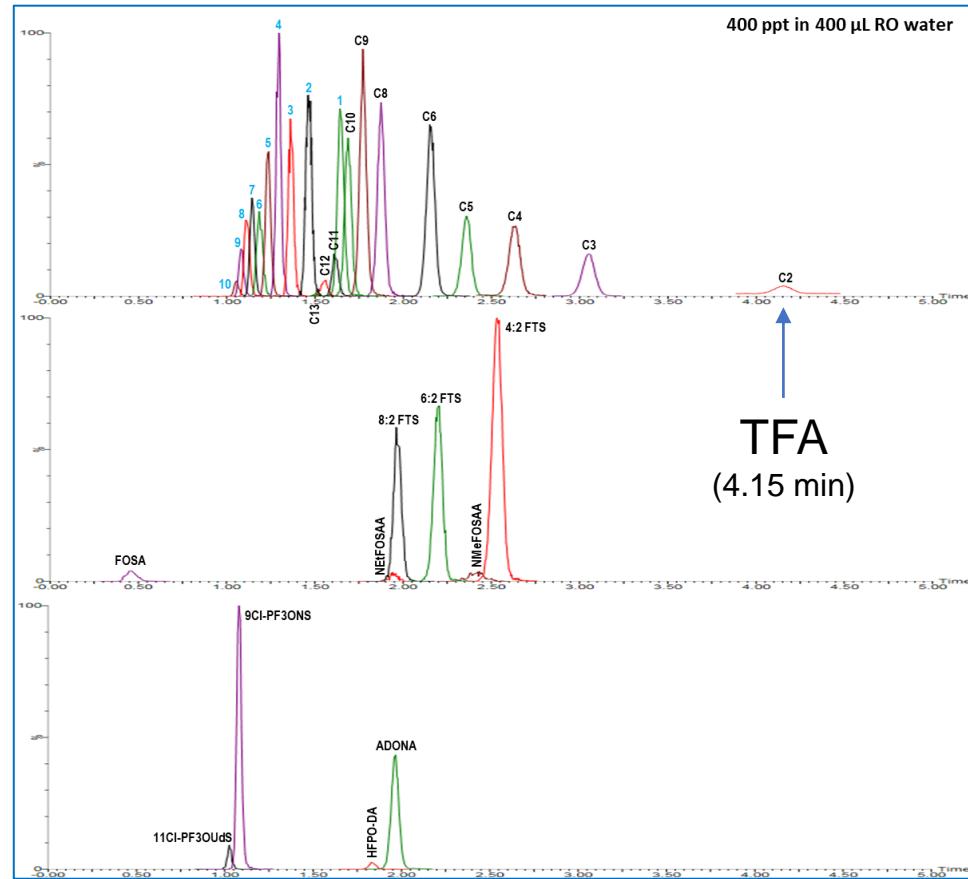
High-res MS



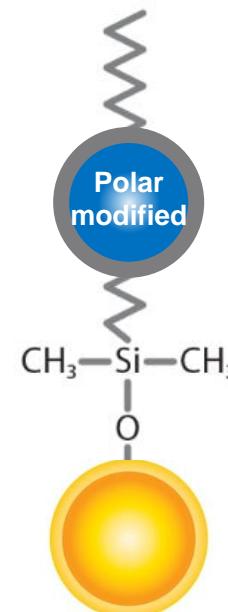
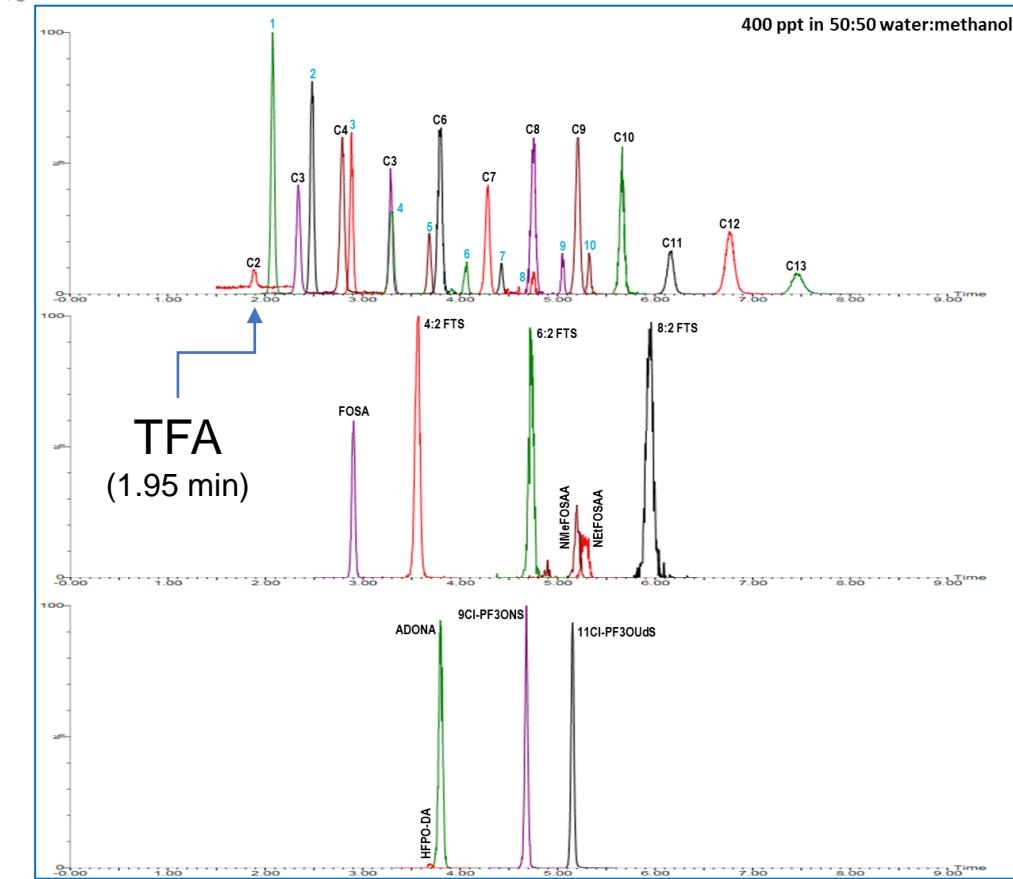
# Two Polar Columns for PFAS



Polar X



Ultra IBD



1 MRM transition problem solved for short chain & ultrashort-chain PFAS  
by having 2 different retention times on two polar LC columns

# Polar Column # 1 (Polar X)

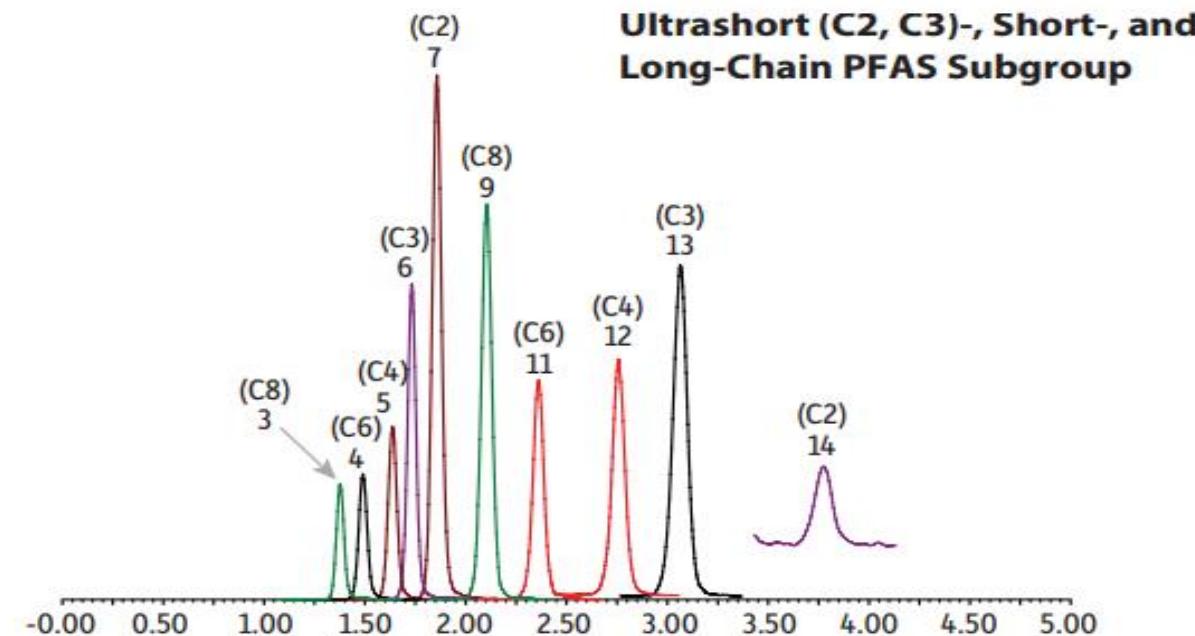
Peaks		t <sub>R</sub> (min)	Conc. (ng/L)	Precursor Ion	Product Ion
1. 11-Chloroeicosfluoro-3-oxanonane-1-sulfonate (11CL-PF3OUdS)		1.25	400	630.78	450.80
2. 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonate9-Chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS)		1.34	400	530.78	350.85
3. Perfluorooctanesulfonic acid (PFOS)		1.38	400	498.84	79.97
4. Perfluorohexanesulfonic acid (PFHxS)		1.49	400	398.90	79.97
5. Perfluorobutanesulfonic acid (PFBS)		1.64	400	298.97	79.97
6. Perfluoropropanesulfonic acid (PFPrS)		1.73	400	248.97	79.98
7. Perfluoroethanesulfonic acid (PFEtS)		1.86	400	198.98	79.92
8. Hexafluoropropylene oxide dimer acid (HFPO-DA)		2.06	400	284.97	168.92
9. Perfluorooctanoic acid (PFOA)		2.11	400	412.90	368.91
10. Ammonium 4,8-dioxa-3H-perfluorononanoate (ADONA)		2.15	400	376.90	250.93
11. Perfluorohexanoic acid (PFHxA)		2.36	400	312.97	268.90
12. Perfluorobutanoic acid (PFBA)		2.76	400	212.97	168.97
13. Perfluoropropionic acid (PFPrA)		3.06	400	163.03	119.01
14. Trifluoroacetic acid (TFA)		3.77	400	113.03	69.01

**Column** Raptor Polar X (cat.# 9311A52)  
**Dimensions:** 50 mm x 2.1 mm ID  
**Particle Size:** 2.7 µm  
**Temp.:** 40 °C  
**Sample**  
**Diluent:** 50:50 Water:methanol  
**Conc.:** 400 ng/L  
**Inj. Vol.:** 10 µL  
**Mobile Phase**  
**A:** Water, 10 mM ammonium formate, 0.05% formic acid  
**B:** 60:40 Acetonitrile:methanol, 0.05% formic acid

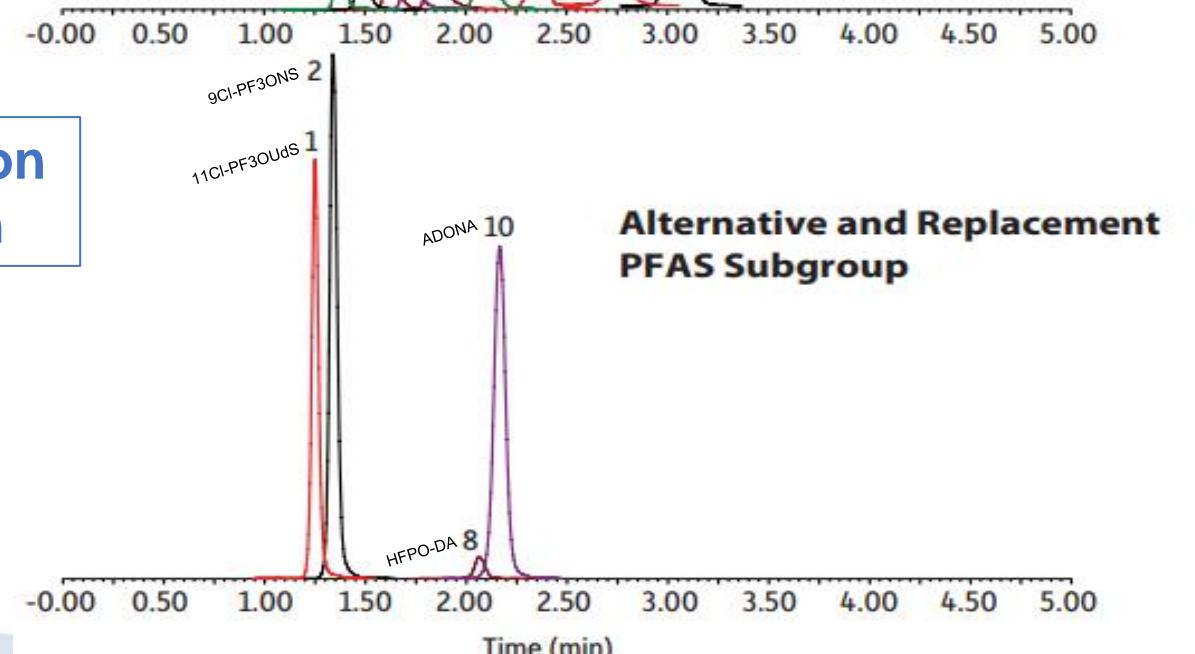
Time (min)	Flow (mL/min)	%A	%B
0.00	0.5	15	85
8.00	0.5	15	85

**Detector**  
**Ion Mode:** MS/MS  
**Mode:** ESI-  
**Instrument** MRM  
UHPLC

- Isocratic Condition
- No Delay Column



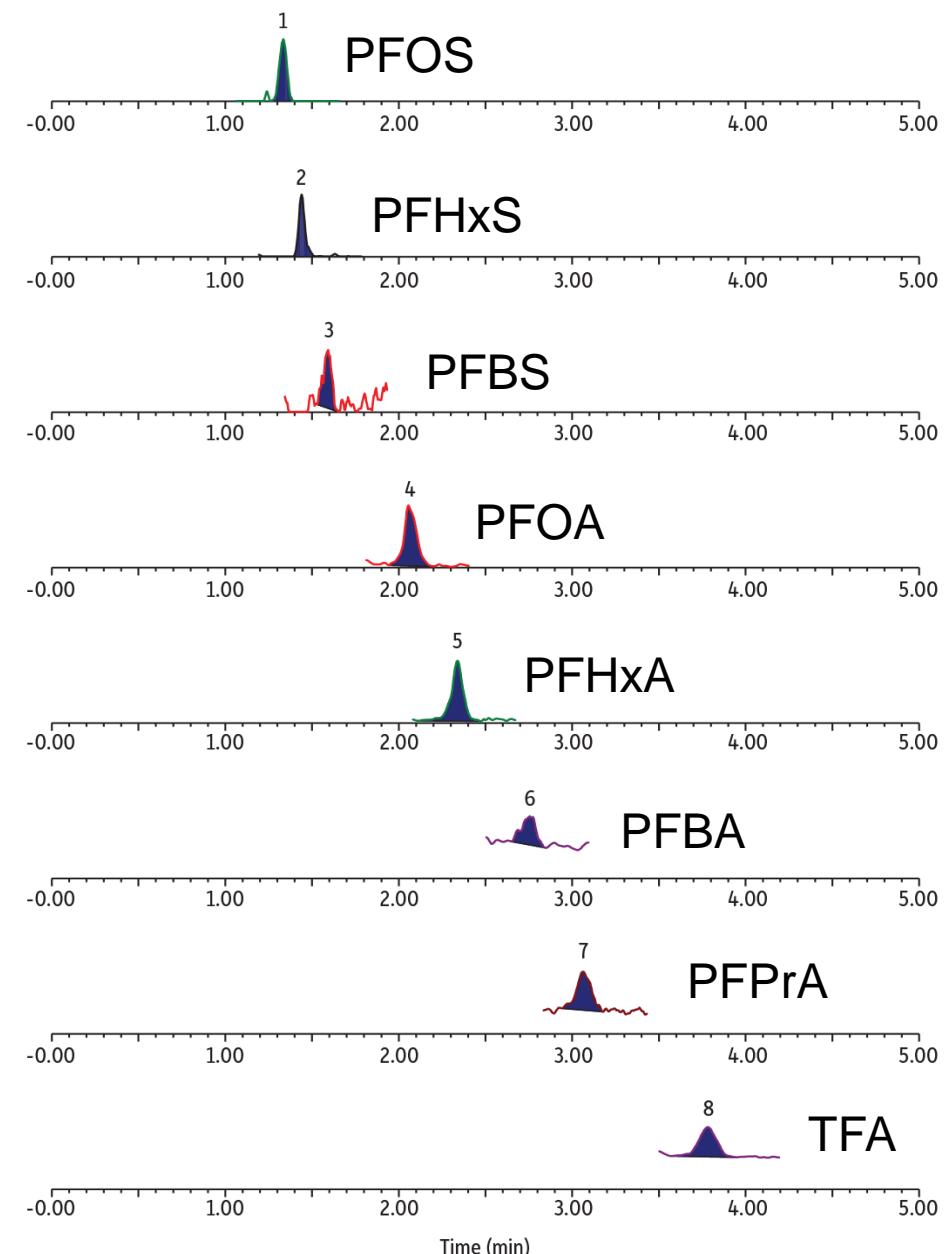
**Ultrashort (C2, C3)-, Short-, and Long-Chain PFAS Subgroup**



**Alternative and Replacement PFAS Subgroup**

# POTW Sample by Polar X

Peaks	$t_R$ (min)	Precursor Ion	Product Ion
1. Perfluorooctanesulfonic acid (PFOS)	1.35	498.84	79.97
2. Perfluorohexanesulfonic acid (PFHxS)	1.45	398.90	79.97
3. Perfluorobutanesulfonic acid (PFBS)	1.58	298.97	79.97
4. Perfluoroctanoic acid (PFOA)	2.05	412.90	368.91
5. Perfluorohexanoic acid (PFHxA)	2.34	312.97	268.90
6. Perfluorobutanoic acid (PFBA)	2.76	212.97	168.97
7. Perfluoropropionic acid (PFPrA)	3.06	163.03	119.01
8. Trifluoroacetic acid (TFA)	3.78	113.03	69.01



# Ultrashort-Chain PFAS in Real Samples by Polar X

**Table 3**

The quantification of C1 to C4 PFAS in various potable and non-potable waters

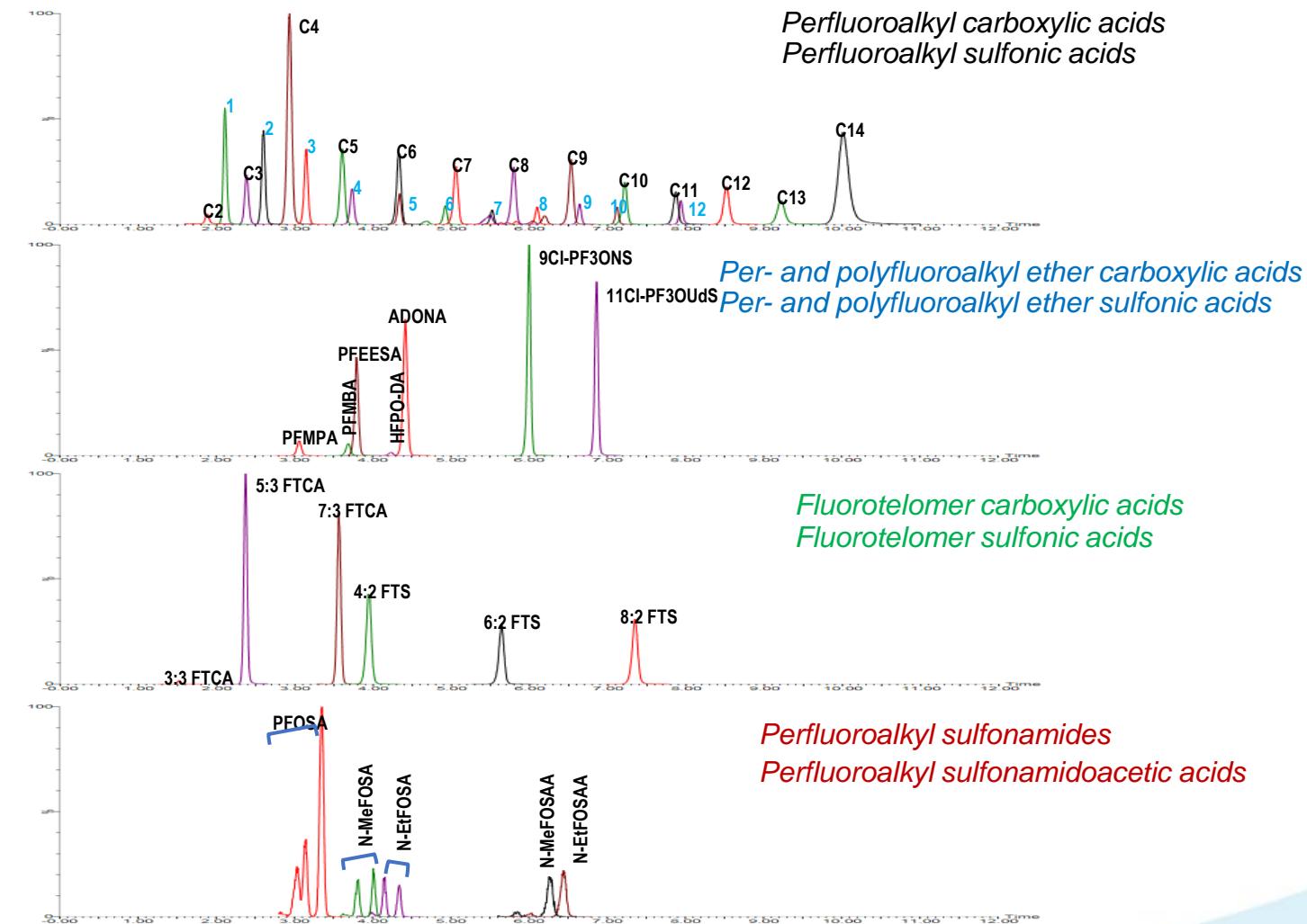
Water Samples	Averaged Concentration (ng/L; ppt)						
	TFA	PFPrA	PFBA	TFMS	PFEtS	PFPrS	PFBS
<b>Potable Waters</b>							
Tap Water #1	230	nd*	nd	5.58	nd	nd	nd
Tap Water #2	520	nd	nd	6.88	nd	nd	nd
Tap Water #3	450	< 5.00	nd	3.20	nd	nd	nd
Tap Water #4 (filtrated well water)	267	nd	nd	nd	nd	nd	nd
Tap Water #5	297	< 5.00	nd	4.68	nd	nd	nd
Tap Water #6	428	< 5.00	nd	< 2.5	nd	nd	nd
Tap Water #7 (RO filtrated tap water #6)	nd	nd	nd	nd	nd	nd	nd
Tap Water #8	400	< 5.00	nd	nd	nd	nd	nd
Tap Water #9	228	nd	nd	5.22	nd	nd	nd
Tap Water #10	117	nd	nd	nd	nd	nd	nd
Bottled Water #1 (RO purified)	nd	nd	nd	nd	nd	nd	nd
Bottled Water #2 (spring water)	102	nd	nd	nd	nd	nd	nd
Bottled Water #3 (spring water)	368	nd	nd	< 2.5	nd	nd	nd
Natural Spring Water	527	< 5.00	nd	< 2.5	nd	nd	nd
Well Water (non-filtrated)	342	nd	nd	15.6	nd	nd	nd
<b>Non-Potable Waters</b>							
POTW water (treated sewage wastewater, effluent)	1113	36.6	< 5.00	8.53	nd	nd	4.35
Hospital Effluent	1363	24.6	< 5.00	4.67	nd	nd	nd
Metal Finisher	741	11.4	< 5.00	5.16	nd	nd	2.77
Chemical Manufacturer Effluent	131200	11084	52.0	4.02	nd	nd	nd

\* non-detected

# Polar Column # 2 (Ultra IBD)

Column	Ultra IBD 100x2.1mm, 3.0 $\mu$ m												
Delay Column	PFAS Delay Column												
Mobile Phase A	5mM ammonium formate, 0.1% formic acid in water												
Mobile Phase B	Acetonitrile												
Gradient	<table border="1"> <thead> <tr> <th>Time (min)</th> <th>%B</th> </tr> </thead> <tbody> <tr> <td>0.00</td> <td>50</td> </tr> <tr> <td>8.00</td> <td>95</td> </tr> <tr> <td>10.00</td> <td>95</td> </tr> <tr> <td>10.01</td> <td>50</td> </tr> <tr> <td>12.00</td> <td>50</td> </tr> </tbody> </table>	Time (min)	%B	0.00	50	8.00	95	10.00	95	10.01	50	12.00	50
Time (min)	%B												
0.00	50												
8.00	95												
10.00	95												
10.01	50												
12.00	50												
Flow Rate	0.4 mL/min												
Injection Volume	5 $\mu$ L												
Column Temp.	40°C												

- Gradient
- With Delay Column



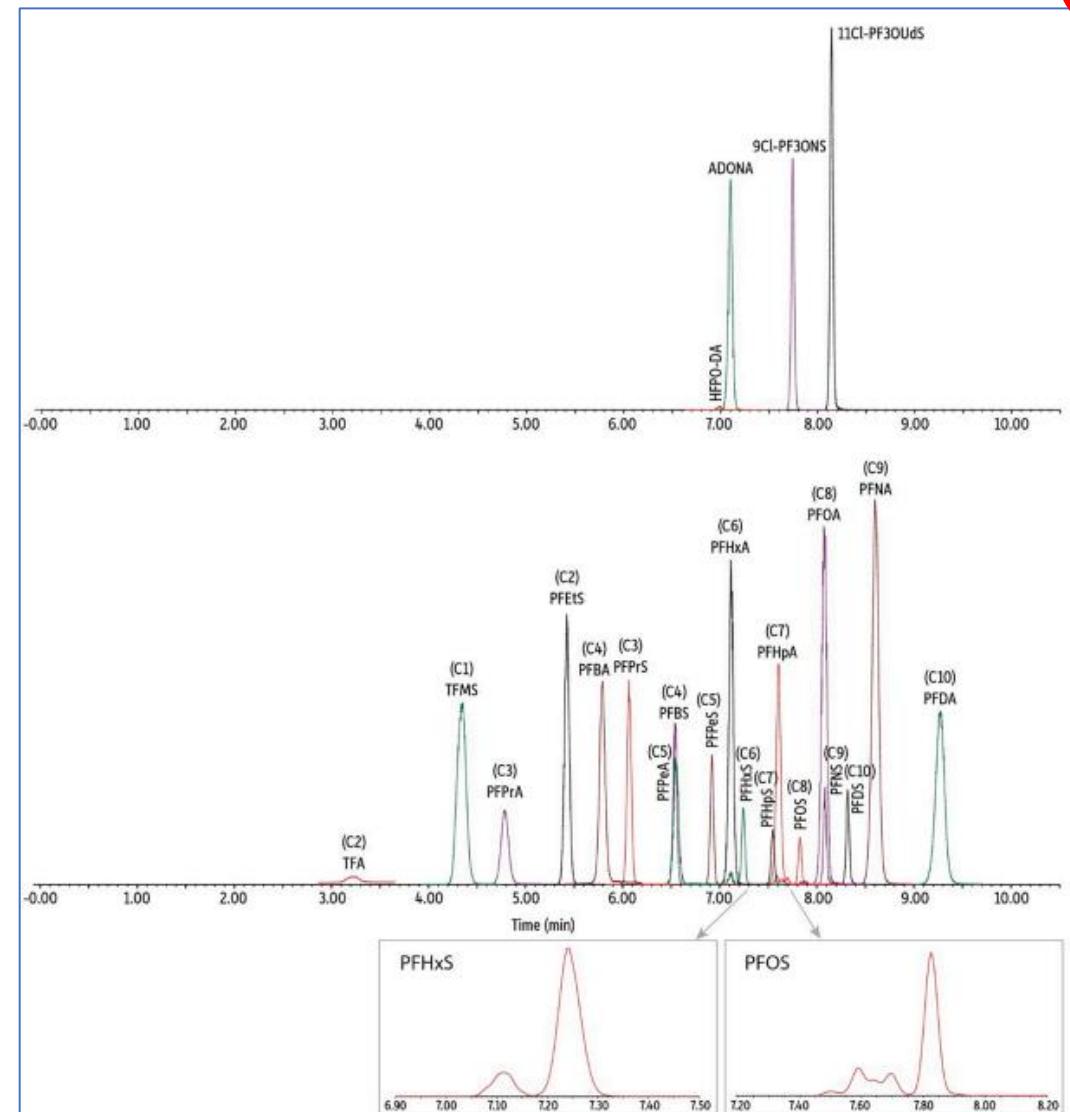
# Human Plasma & Serum

## By Ultra IBD

MS ion transitions, parameters, and chromatographic retention time of analytes.

Compounds	Retention Time (min)	Precursor Ion	Product ions*	Cone (V)	Collision (V)	Quantification Internal Standard
<b>Target Analytes</b>						
Trifluoroacetic acid (TFA)	3.25	113.03 [M-H] <sup>-</sup>	69.01	10	10	<sup>13</sup> C <sub>3</sub> -PFBA
Perfluoropropanoic acid (PFPrA)	4.81	162.97 [M-H] <sup>-</sup>	119.02	10	8	<sup>13</sup> C <sub>3</sub> -PFBA
Perfluorobutanoic acid (PFBA)	5.80	213.03 [M-H] <sup>-</sup>	168.98	14	8	<sup>13</sup> C <sub>3</sub> -PFBA
Perfluoropentanoic acid (PFPeA)	6.56	262.97 [M-H] <sup>-</sup>	218.97	2	6	<sup>13</sup> C <sub>2</sub> -PFHxA
Perfluorohexanoic acid (PFHxA)	7.13	313.10 [M-H] <sup>-</sup>	268.97/118.99	2	8/20	<sup>13</sup> C <sub>2</sub> -PFHxA
Perfluoroheptanoic acid (PFHpA)	7.62	363.16 [M-H] <sup>-</sup>	319.09/169.06	8	10/18	<sup>13</sup> C <sub>4</sub> -PFOA
Perfluooctanoic acid (PFOA)	8.10	413.10 [M-H] <sup>-</sup>	368.96/168.90	2	10/16	<sup>13</sup> C <sub>4</sub> -PFOA
Perfluorononanoic acid (PFNA)	8.62	463.10 [M-H] <sup>-</sup>	419.01/219.02	4	10/16	<sup>13</sup> C <sub>5</sub> -PFNA
Perfluorodecanoic acid (PFDA)	9.29	513.17 [M-H] <sup>-</sup>	469.16/219.06	4	12/16	<sup>13</sup> C <sub>2</sub> -PFDA
Trifluoromethanesulfonic acid (TFMS)	4.37	148.97 [M-H] <sup>-</sup>	79.93/98.92	62	18/18	<sup>13</sup> C <sub>3</sub> -PFBA
Perfluoroethanesulfonic acid (PFEtS)	5.44	198.90 [M-H] <sup>-</sup>	79.92/98.91	38	22/22	<sup>13</sup> C <sub>3</sub> -PFBA
Perfluoropropanesulfonic acid (PFPrS)	6.08	248.97 [M-H] <sup>-</sup>	79.92/98.91	2	24/24	<sup>13</sup> C <sub>3</sub> -PFBA
Perfluorobutanesulfonic acid (PFBS)	6.55	298.97 [M-H] <sup>-</sup>	79.97/98.99	2	26/26	<sup>13</sup> C <sub>2</sub> -PFHxA
Perfluoropentanesulfonic acid (PFPeS)	6.93	349.10 [M-H] <sup>-</sup>	79.98/98.98	6	32/30	<sup>13</sup> C <sub>2</sub> -PFHxA
Perfluorohexanesulfonic acid (PFHxS)	7.24	398.90 [M-H] <sup>-</sup>	79.97/98.89	56	32/34	<sup>13</sup> C <sub>2</sub> -PFHxA
Perfluoroheptanesulfonic acid (PFHps)	7.56	449.17 [M-H] <sup>-</sup>	79.98/98.97	4	42/38	<sup>13</sup> C <sub>2</sub> -PFHxA
Perfluoroctanesulfonic acid (PFOS)	7.82	499.03 [M-H] <sup>-</sup>	79.92/98.90	8	40/40	<sup>13</sup> C <sub>4</sub> -PFOA
Perfluorononanesulfonic acid (PFNS)	8.09	549.10 [M-H] <sup>-</sup>	79.92/98.83	12	42/40	<sup>13</sup> C <sub>4</sub> -PFOA
Perfluorodecanesulfonic acid (PFDS)	8.32	599.17 [M-H] <sup>-</sup>	79.98/98.83	8	44/46	<sup>13</sup> C <sub>5</sub> -PFNA
Hexafluoropropylene oxide dimer acid (HFPO-DA)	7.01	285.03 [M-COOH] <sup>-</sup>	169.02/185.02	2	6/16	<sup>13</sup> C <sub>2</sub> -PFHxA
4,8-dioxa-3H-perfluorononanoic acid (ADONA)	7.11	376.90 [M-H] <sup>-</sup>	250.93/84.97	22	12/26	<sup>13</sup> C <sub>2</sub> -PFHxA
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	7.75	530.78 [M-H] <sup>-</sup>	350.85/82.96	12	26/24	<sup>13</sup> C <sub>2</sub> -PFHxA
11-Chloroeicosafauro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	8.15	630.78 [M-H] <sup>-</sup>	450.80/82.95	8	26/32	<sup>13</sup> C <sub>5</sub> -PFNA
<b>Extracted Internal Standards</b>						
<sup>13</sup> C-TFA	3.25	114.03 [M-H] <sup>-</sup>	69.03	10	8	<sup>13</sup> C <sub>3</sub> -PFBA
<sup>13</sup> C <sub>3</sub> -PFPrA	4.81	165.97 [M-H] <sup>-</sup>	120.96	10	11	<sup>13</sup> C <sub>3</sub> -PFBA
<sup>13</sup> C <sub>4</sub> -PFBA	5.80	217.03 [M-H] <sup>-</sup>	171.98	2	8	<sup>13</sup> C <sub>3</sub> -PFBA
<sup>13</sup> C <sub>5</sub> -PFPeA	6.56	267.97 [M-H] <sup>-</sup>	222.99	2	6	<sup>13</sup> C <sub>2</sub> -PFHxA
<sup>13</sup> C <sub>5</sub> -PFHxA	7.13	318.03 [M-H] <sup>-</sup>	272.93	2	7	<sup>13</sup> C <sub>2</sub> -PFHxA
<sup>13</sup> C <sub>4</sub> -PFHxA	7.62	366.90 [M-H] <sup>-</sup>	321.93	2	10	<sup>13</sup> C <sub>4</sub> -PFOA
<sup>13</sup> C <sub>6</sub> -PFOA	8.10	420.97 [M-H] <sup>-</sup>	375.94	2	10	<sup>13</sup> C <sub>4</sub> -PFOA
<sup>13</sup> C <sub>7</sub> -PFNA	8.62	471.97 [M-H] <sup>-</sup>	426.87	4	8	<sup>13</sup> C <sub>5</sub> -PFNA
<sup>13</sup> C <sub>6</sub> -PFDA	9.29	518.90 [M-H] <sup>-</sup>	473.87	4	13	<sup>13</sup> C <sub>2</sub> -PFDA
<sup>13</sup> C <sub>3</sub> -PFHxS	7.24	401.90 [M-H] <sup>-</sup>	79.97	2	32	<sup>13</sup> C <sub>2</sub> -PFHxA
<sup>13</sup> C <sub>8</sub> -PFOS	7.82	506.84 [M-H] <sup>-</sup>	79.97	4	48	<sup>13</sup> C <sub>4</sub> -PFOA
<b>Non-Extracted Internal Standards</b>						
<sup>13</sup> C <sub>3</sub> -PFBA	5.80	215.97 [M-H] <sup>-</sup>	171.97	10	8	
<sup>13</sup> C <sub>2</sub> -PFHxA	7.13	314.97 [M-H] <sup>-</sup>	269.93	8	8	
<sup>13</sup> C <sub>4</sub> -PFOA	8.10	416.87 [M-H] <sup>-</sup>	371.88	2	8	
<sup>13</sup> C <sub>5</sub> -PFNA	8.62	467.87 [M-H] <sup>-</sup>	422.89	16	10	
<sup>13</sup> C <sub>2</sub> -PFDA	9.29	514.87 [M-H] <sup>-</sup>	469.84	8	10	

\* Quantifier Ion/Qualifier Ion.



# NIST SRM 1950 & SRM 1957

**Ultra IBD is an excellent tool for PFAS analysis in human plasma & serum samples.**

- Ultrashort Chain
- Short Chain
- Long Chain

**Table 5**

The quantification of PFAS in NIST 1950 and 1957.

Analytes	Reference Concentration (ng/mL)	Experimental Avg. Concentration (ng/mL)	Experimental Precision (%RSD, n = 6)	Concentration Ratio
NIST 1950				
PFOA	3.27 ± 0.06	3.12	3.70	95.4
PFNA	0.720 ± 0.028	0.85	0.74	117
PFDA	0.322 ± 0.007	0.30	3.47	91.6
PFHxS	3.25 ± 0.08	2.91	6.26	89.5
PFOS	10.64 ± 0.13	12.57	2.92	118
TFA	—	5.74	4.45	—
PFPrA	—	0.26	6.90	—
PFHpA	—	0.23	3.04	—
TFMS	—	0.08	3.95	—
PFPeS	—	0.15	4.31	—
PFHpS	—	0.36	4.90	—
PFDS	—	0.10	4.64	—
NIST 1957*				
PFHpA	0.305 ± 0.051	0.28	2.16	92.1
PFOA	5.00 ± 0.44	4.21	1.91	84.2
PFNA	0.878 ± 0.077	0.77	1.38	87.9
PFDA	0.39 ± 0.12	0.29	2.69	74.4
PFHxS	4.00 ± 0.83	3.35	9.88	83.8
PFOS	21.1 ± 1.3	20.46	3.81	97.0
TFA	—	3.22	3.79	—
TFMS	—	0.07	0.10	—
PFPeS	—	0.10	7.97	—
PFHpS	—	0.48	6.20	—
PFDS	—	0.10	4.23	—

\* The reference concentration for NIST 1957 is present as mass fraction (µg/kg).

# Publications



Journal of Chromatography Open

Volume 4, 27 November 2023, 100098



Analysis of ultrashort-chain and short-chain (C1 to C4) per- and polyfluorinated substances in potable and non-potable waters

Shun-Hsin Liang , Justin A. Steimling , Mike Chang

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Journal of Chromatography Open

Volume 5, May 2024, 100132



Integration of ultrashort-chain compounds into the biomonitoring of per- and polyfluorinated substances in human plasma and serum

Shun-Hsin Liang , Justin A. Steimling

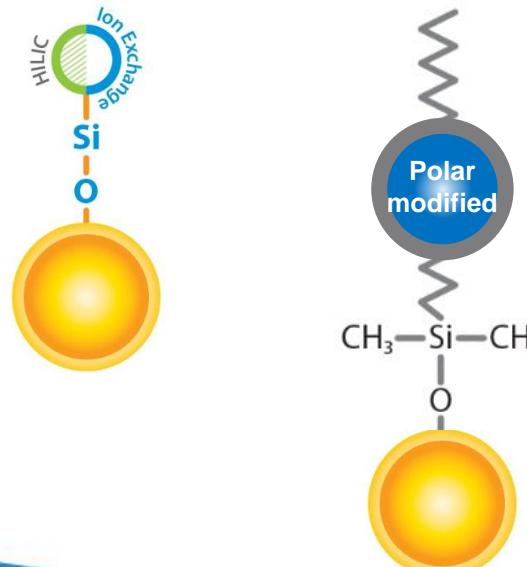
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# Conclusion

- PFAS testing has been evolving from # of analytes, functional groups of PFAS, and technologies across sample preparation, measurement systems.
- Polar, ionic, short-chain PFAS need a pair of innovative polar LC columns for confirmation.
- New approach on wider range of PFAS compounds including ultrashort and short-chain PFAS has shown excellent results on various matrices (drinking water, wastewater, river water, bottled water, blood) by \_\_\_\_\_ and \_\_\_\_\_ columns.

# Conclusion

- PFAS testing has been evolving from # of analytes, functional groups of PFAS, and technologies across sample preparation, measurement systems.
- Polar, ionic, short-chain PFAS need a pair of innovative polar LC columns for confirmation.
- New approach on wider range of PFAS compounds including ultrashort and short-chain PFAS has shown excellent results on various matrices (drinking water, wastewater, river water, bottled water, blood) by **Polar X** and **Ultra IBD** columns.



Complementary column set for challenging PFAS producing only 1 MRM

Both quantitation and confirmation are available on this column set.

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From running applications and preserving data at conferences, to partnering with analysts and labs around the world, our team of PFAS experts stay on top of the latest developments and guide our development of products and methods. Contact Restek today.



**Mike Chang**  
Market Development Manager



**Shun-Han Liung, PhD**  
Senior Principal Scientist



**Jason Monington**  
Senior Scientist

**BIO**  
With an analytical chemistry background and 15+ years of experience in chromatography industry from instrumentation to applications, he is the key liaison to testing agencies and industry on bringing better ways of contaminants measurement in environment and food. He has been sharing PFAS sessions and presenting PFAS topics at various conferences.  
Language: English

**BIO**  
After starting his career in academia, Shun-Han transitioned to the industry in 2006 when he joined MPA Research Inc., as a senior research scientist and was a study director for GLP analytical projects. In 2012, he joined Restek and has specialized in developing application methods for environmental, food safety, and life sciences with a particular focus on PFAS. and has focused on PFAS in all applications, including PFAS in air samples.  
Language: English

**BIO**  
Initially working for SGS Environmental and then Dow Chemical Company, Jason spent almost 20 years in areas such as soil and water testing for VOCs, SVOCs, and PCBs, and provided advanced analytical troubleshooting and method development. In 2016, Jason joined Restek and has focused on PFAS in all applications, including PFAS in air samples.  
Language: English



**Jamie York, PhD**  
Principal Scientist, LC Solutions



**Gary Olden**  
Technical Service Specialist



**Tina Brandtner**  
LC Specialist

**BIO**  
Jamie has over 10 years of experience in analytical testing using a variety of hydrolyzed techniques, including GC-MS, GC-VIN, MALDI, HPLC, LC-RI, and LC-MS/MS. She joined Restek's LC Solutions team in 2020 and has developed new applications for food, clinical, cannabis, and environmental markets. As a member for PFAS testing group, her focus has now geared towards the development of methods to detect PFAS compounds in food commodities.  
Language: English

**BIO**  
Gary started his chromatography journey as a laboratory and bonding chemist before joining MPA Research Inc. as an application scientist. He later served as a study director, managing PFAS analysis in various matrices. Since 2018, he's been a global part of Restek's European-based technical support, where he helps customers solve challenges in their PFAS analysis.  
Language: English

**BIO**  
Tina has over four years of practical experience in method development and routine analysis of contaminants and mycotoxins in plant-based foodstuffs and pharmaceuticals. She joined the German sales team at Restek GmbH in 2022, and after one and a half years as a sales rep in southern Germany, became part of the LC specialist team in the EMEA region in early 2024.  
Language: German



**Emanuele Cicconi**  
LC Specialist



**Chava Orsh**  
Technical Support Representative



**John Gallant**  
LC Specialist

**BIO**  
Emanuele previously worked as an LC/MS field service engineer for 17 years. Since 2018, he has been Restek's Sales Specialist in Italy where he provides customers with technical support and advice in column selection and developing liquid chromatographic separations.  
Language: Italian

**BIO**  
With a PhD in environmental chemistry, Chava has worked as a research scientist on the analysis of pesticides in water and soils as well as their effects on human health, and method development of OPA. In 2022, she joined Restek's team as a technical support representative, focusing on custom reference standards and solving technical issues.  
Language: English

**BIO**  
John has a broad chromatographic background having worked for the last nine years across a variety of roles, from QC lab to service engineer. Since 2022, he has been the LC Specialist for Restek in the UK and Ireland, supporting customers in developing applications and troubleshooting chromatographic problems.  
Language: English

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