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

Mike Chang
mike.chang@restek.com
Market Development Manager

A Journey of PFAS Testing

2000's

PFOA
PFOS

Mostly water

2010's

C4 – C12

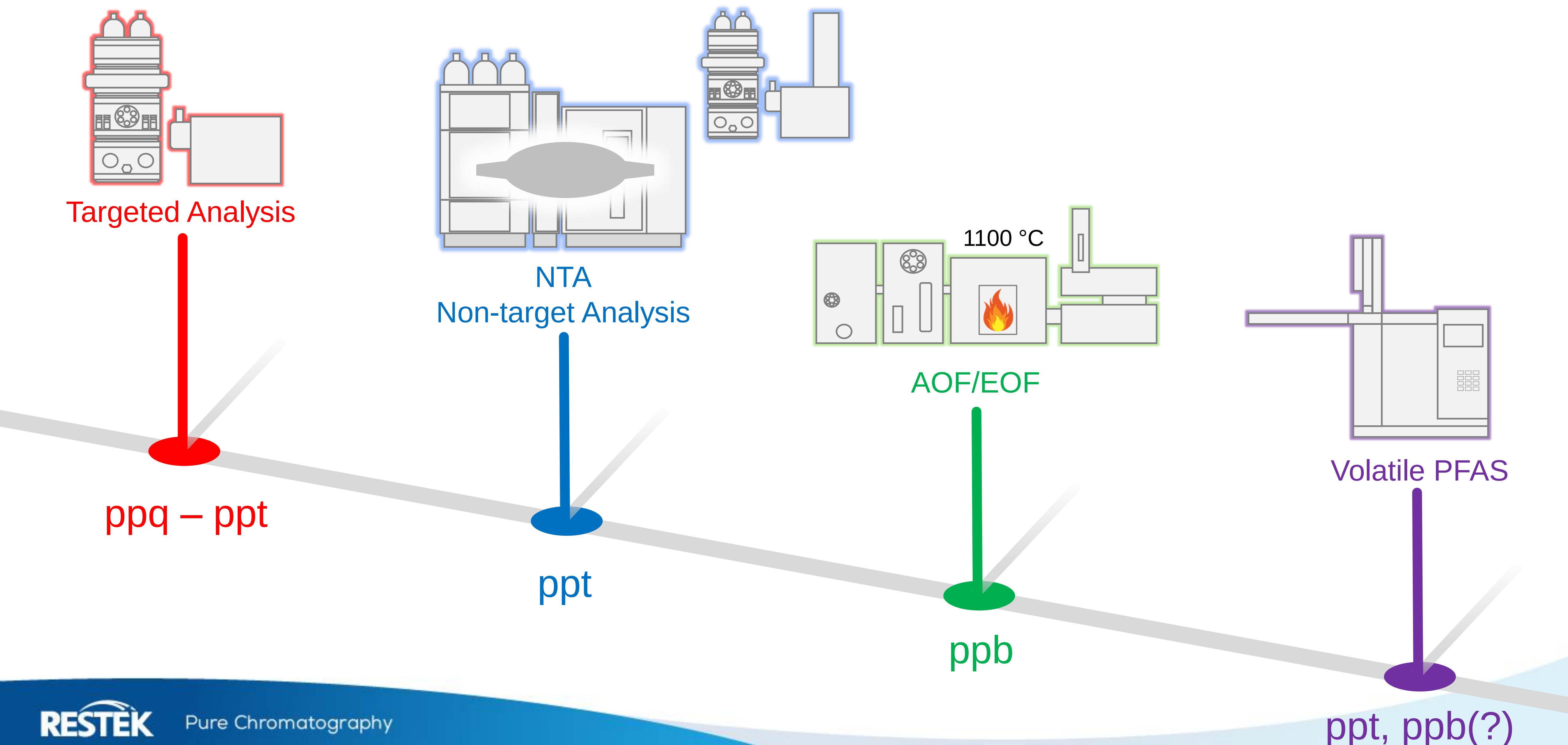
Water
Soil

2020's

C1 – C18

Water/Soil
Food
Food packaging
Blood/serum
Consumer products

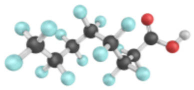
A Journey of Measurement Technologies



Remember the Basics
PFAS ≠ PFAS

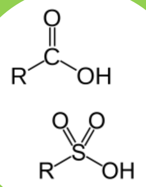
PFAS

Chain Length



C1 – C18 and beyond

Functional Groups

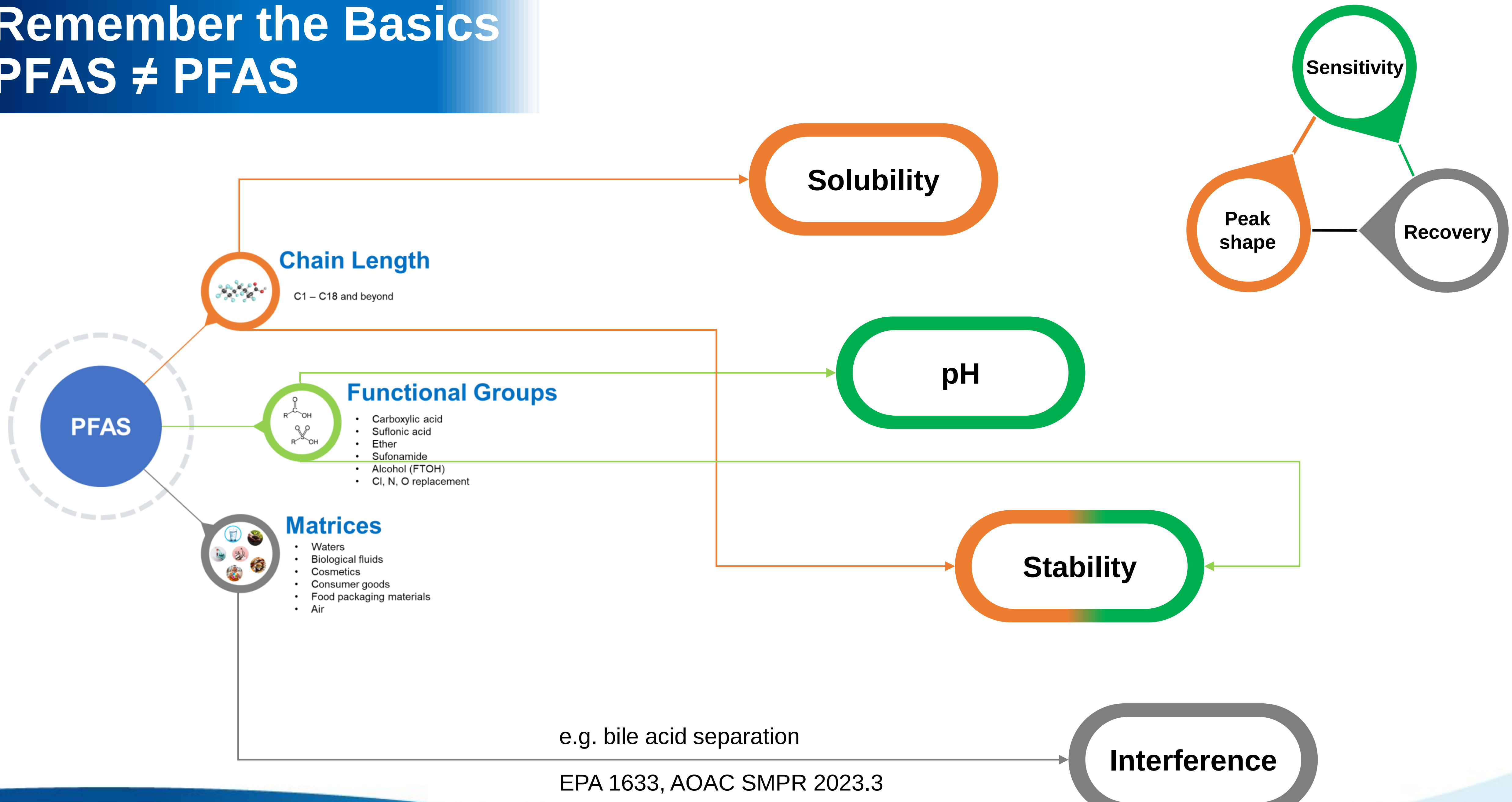


- Carboxylic acid
- Sulfonic acid
- Ether
- Sulfonamide
- Alcohol (FTOH)
- Cl, N, O, H replacement

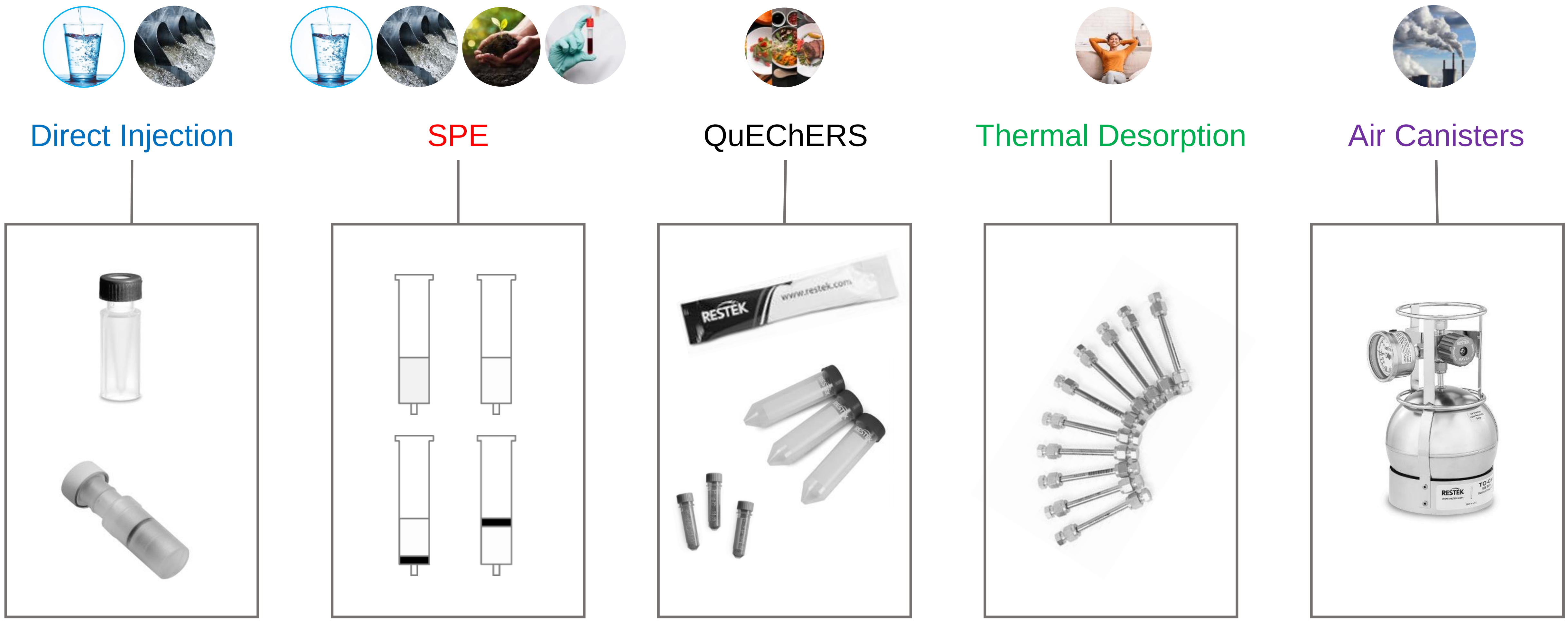
Matrices

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

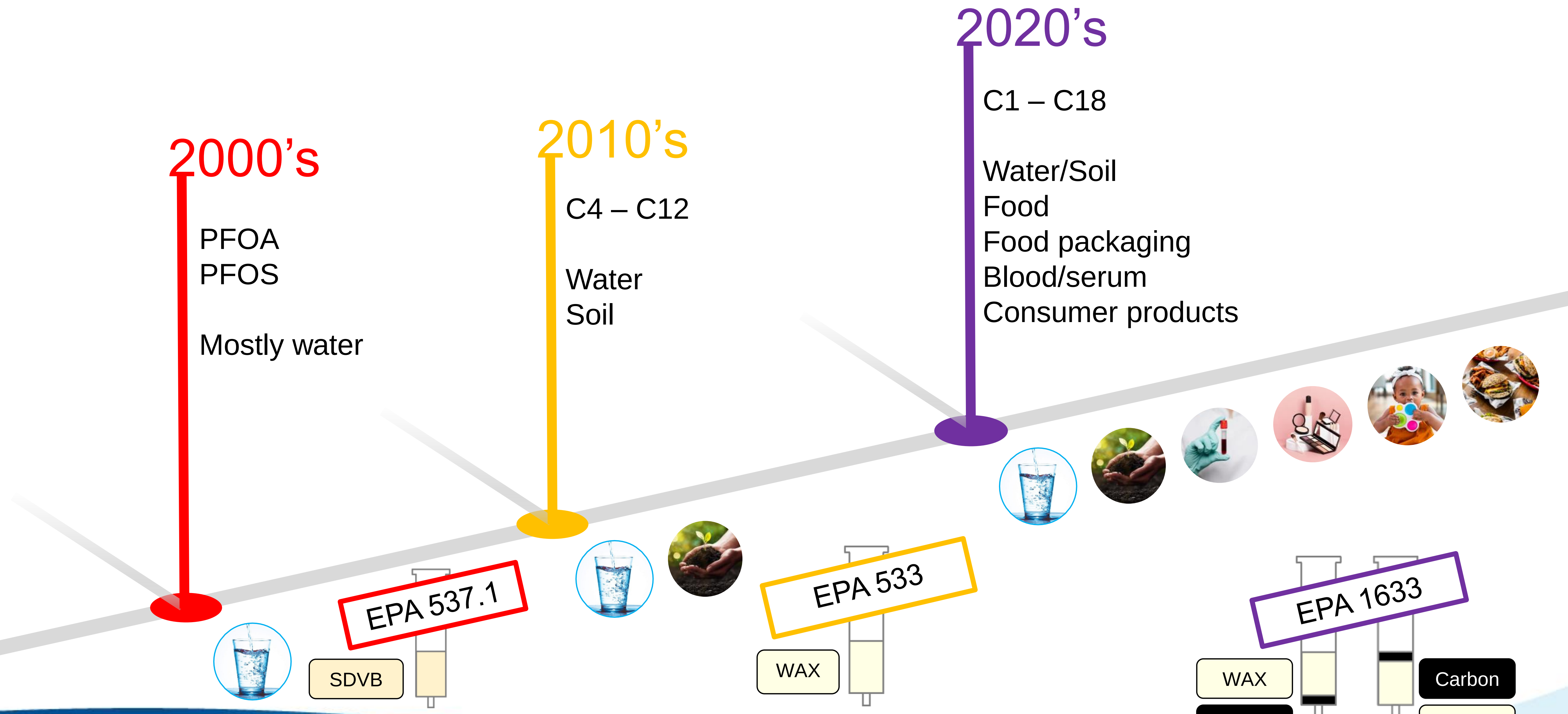
Remember the Basics
PFAS ≠ PFAS



A Journey of Sample Preparation Technologies



A Journey of Solid Phase Extraction



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

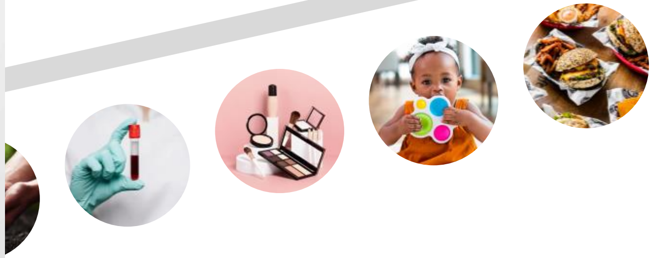
2000's

PFOA
PFOS

Mostly water

ging

roducts




C18 Column

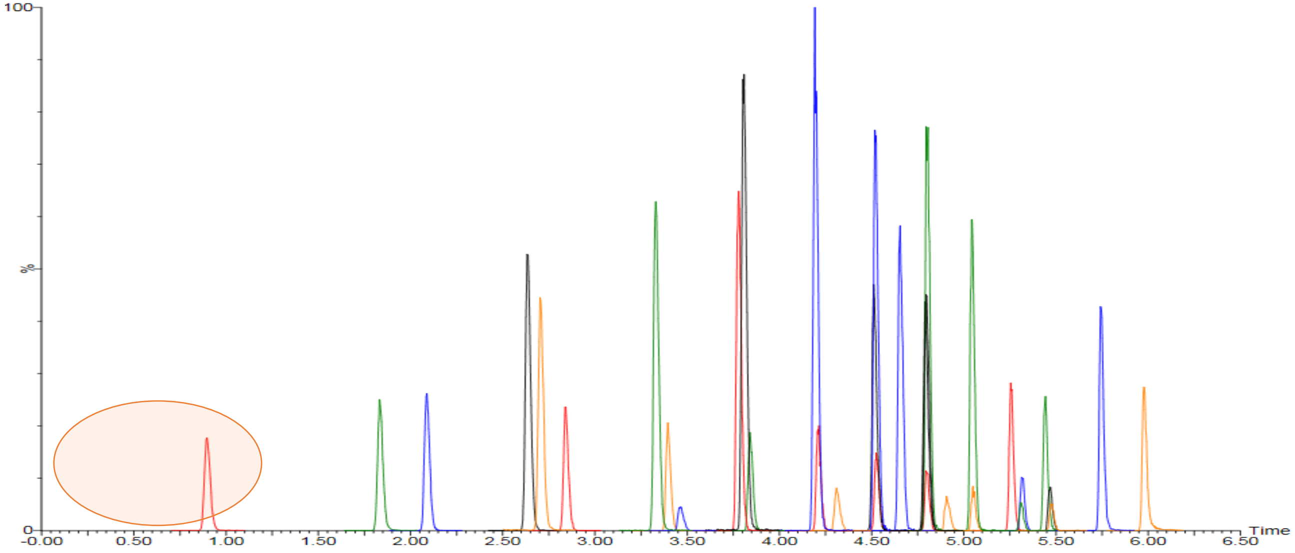

C18 Column


C18 Column

C18 ≠ C18

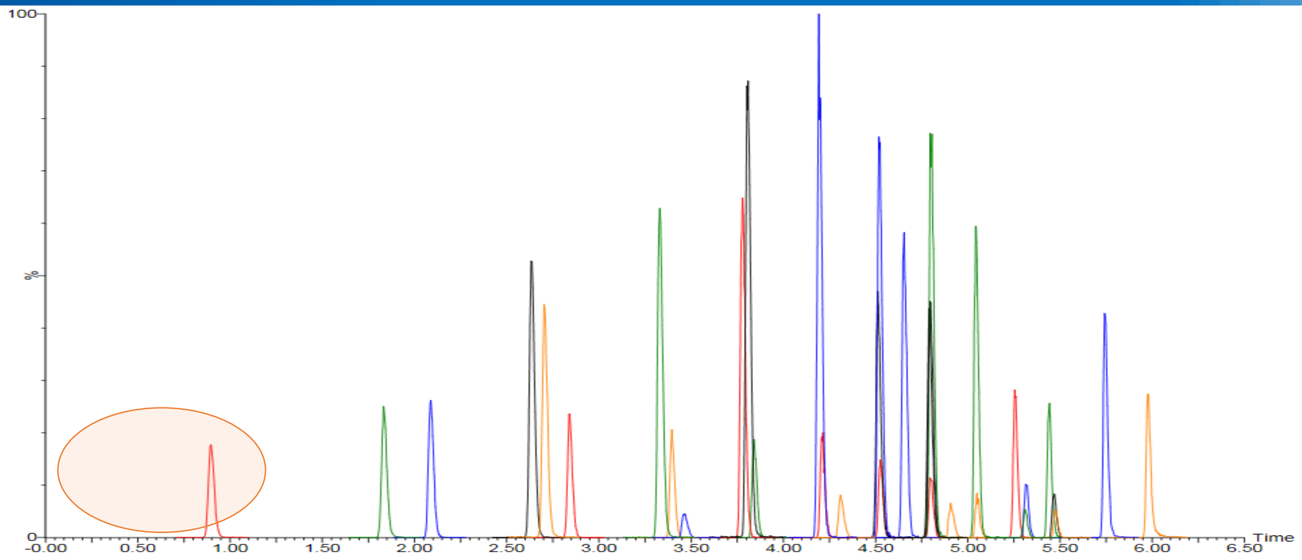
Raptor C18 **1.8um, 50x2.1mm**

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

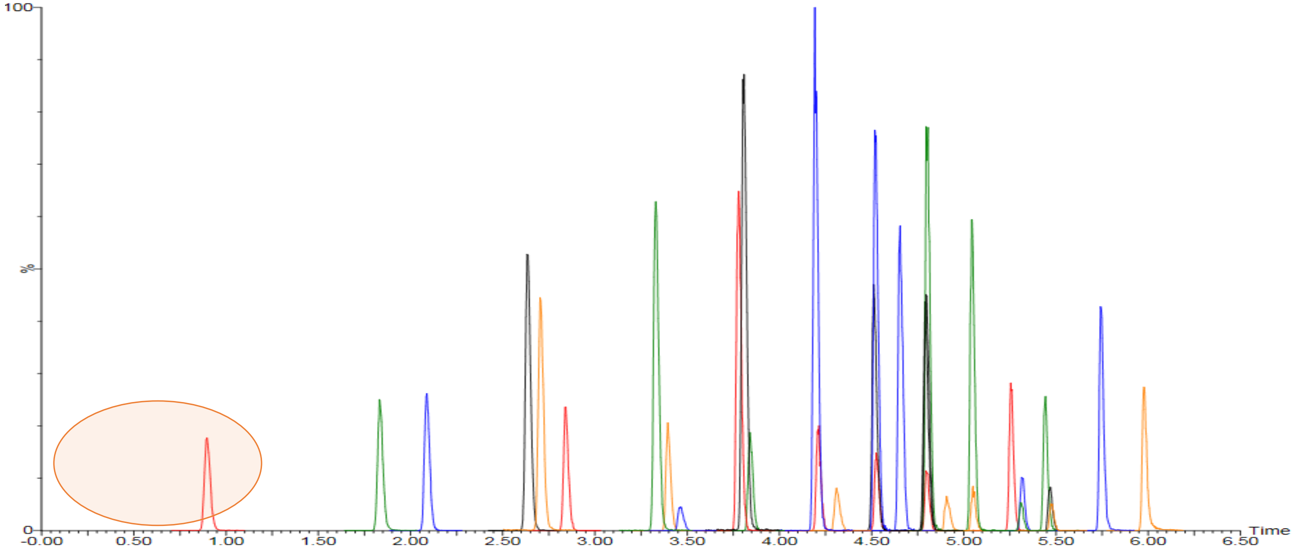
C18 ≠ C18

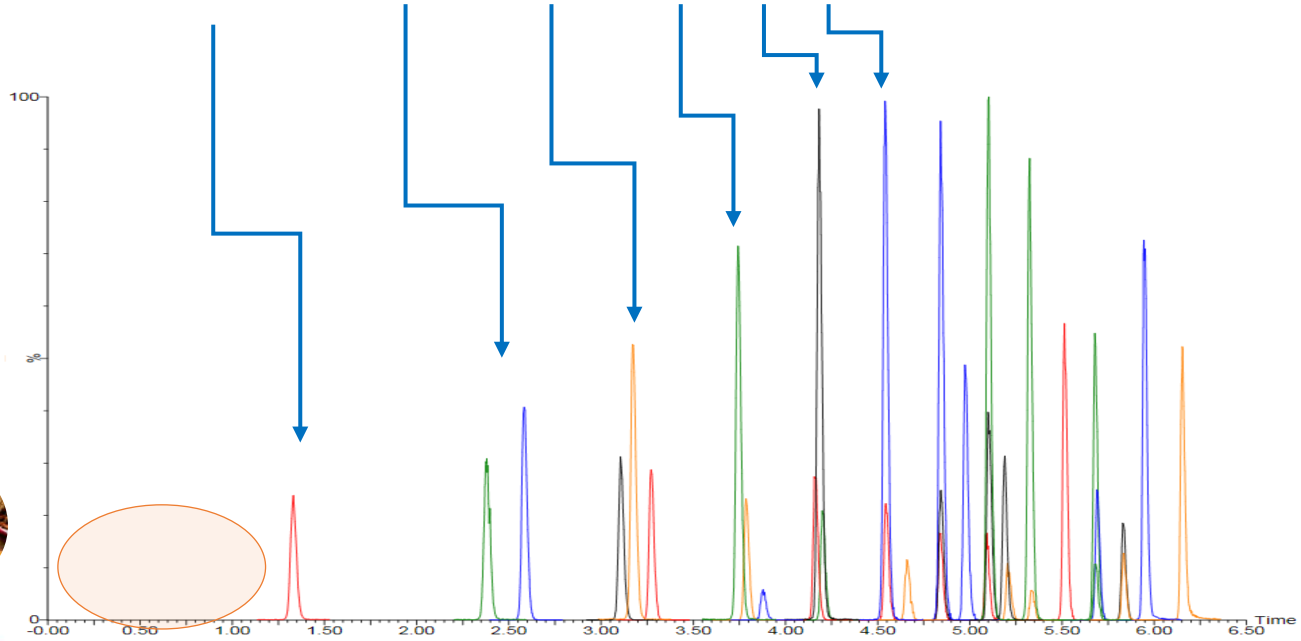
Raptor C18 **1.8um, 50x2.1mm**

OK for

Force C18 **1.8um, 50x2.1mm**

More ion suppression





Methodologies

1

Conventional
PFAS Methods

2

Exotic
PFAS Methods

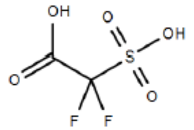
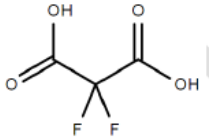
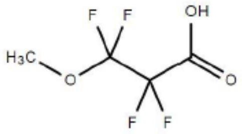
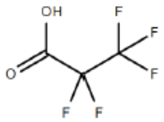
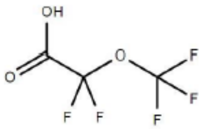
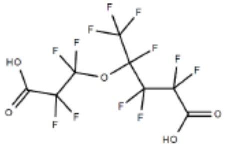
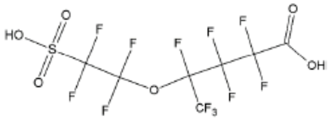
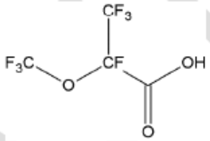
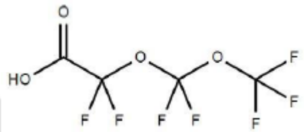
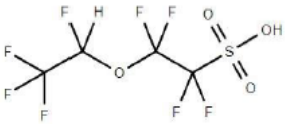
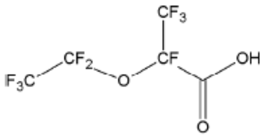
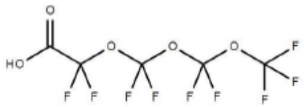
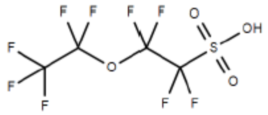
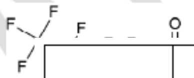
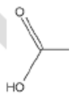
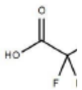
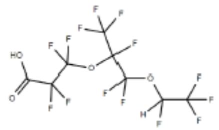
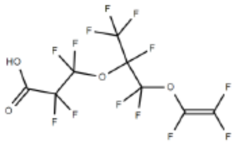
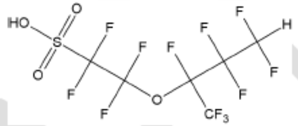
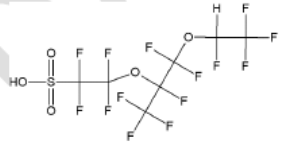
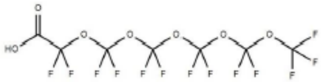
Table 3

3

Ultrashort-Chain
PFAS Methods

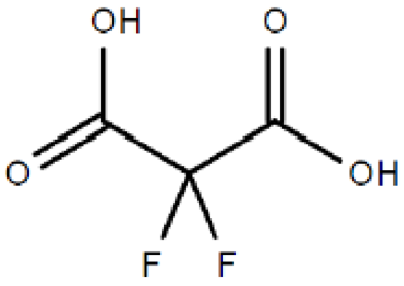
Table 3 Compounds

Chemours™

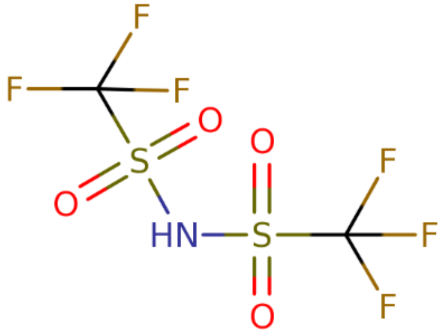
Compound Name	Molecular Formula	Structure	CASRN
DFSA	$C_2H_2F_2O_5S$		422-67-3
MMF	$C_3H_2F_2O_4$		
MTP	$C_4H_4F_4O_3$		
PPF Acid	$C_3HF_5O_2$		
PFMOAA	$C_3HF_5O_3$		
R-EVE	$C_8H_2F_{12}O_5$		
Byproduct 4	$C_7H_2F_{12}O_6S$		N/A
Byproduct 5	$C_7H_3F_{11}O_7S$		N/A
PMPA	$C_4HF_7O_3$		13140-29
PFO2HxA	$C_4HF_7O_4$		39492-88-1
NVHOS	$C_4H_2F_8O_4S$		1132933-86-8
PEPA	$C_5HF_9O_3$		267239-61-2
PFO3OA	$C_5HF_9O_5$		39492-89-2
PES	$C_4HF_9O_4S$		113507-82-7
HFPO-DA	$C_6HF_{11}O_3$		
PFECA G	$C_7HF_{13}O_3$		
PFO4DA	$C_6HF_{11}O_6$		
Hydro-EVE Acid	$C_8H_2F_{14}O_4$		773804-62-9
EVE Acid	$C_8HF_{13}O_4$		69087-46-3
Byproduct 6	$C_6H_2F_{12}O_4S$		N/A
Byproduct 1	$C_7HF_{13}O_5S$		29311-67-9
Byproduct 2	$C_7H_2F_{14}O_5S$		749836-20-2
PFO5DoA	$C_7HF_{13}O_7$		39492-91-6

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

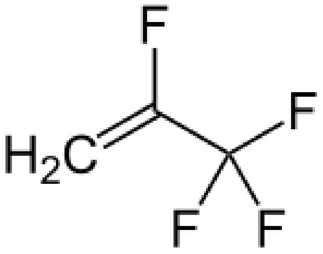
- **Polar**
- **Ionic**
- **Short & Ultrashort**



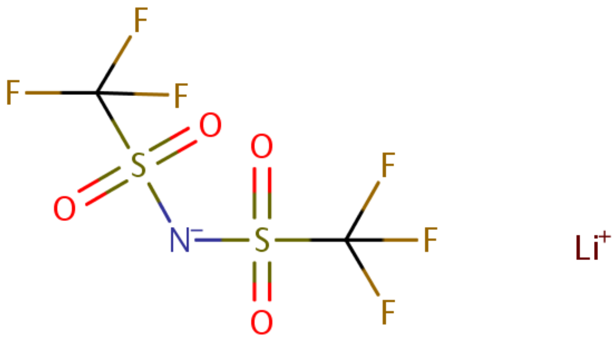
MMF



HQ-115



HFO-1234yf

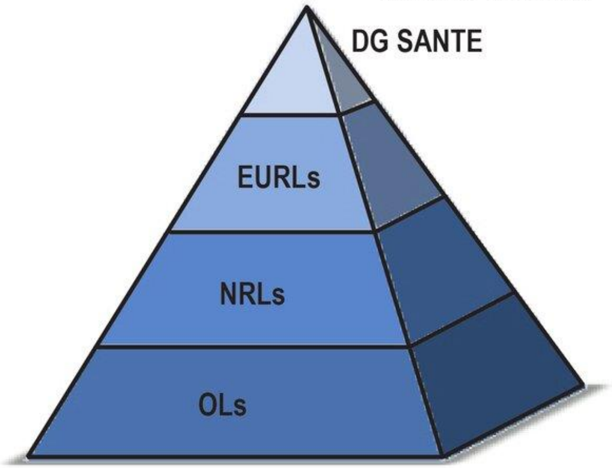


TFSI

Confirmation Options

European Commission

Directorate-General for Health and Food Safety (DG SANTE)



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*[2]

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[d)] 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[a, b, c)]	S/N ≥ 3[d)] Analyte peaks from precursor and/or product ion(s) in the extracted ion chromatograms must fully overlap. Ion ratio: see D12

US FDA C-010.03

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^{TM} 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**

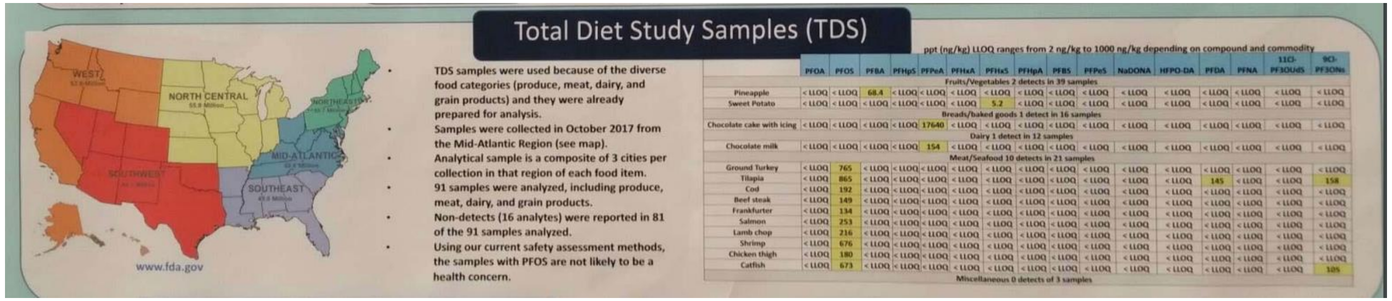


Table 1: Comparison of preliminary and revised reported concentrations of PFOS

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

AOAC SMPR 2023.003

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.

EPA 1633

- 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 [1]	Parent Ion Mass	Quantification Ion Mass	Confirmation Ion Mass[2]	Typical Ion Ratio	Quantification Reference Compound
Target Analytes						
PFBA	1.96	212.8	168.9	NA	NA	$^{13}C_4$-PFBA
PFPeA	4.18	263.0	219.0	68.9	NA	$^{13}C_5$-PFPeA
PFHxA	4.81	313.0	269.0	118.9	13	$^{13}C_5$-PFHxA
PFHpA	5.32	363.1	319.0	169.0	3.5	$^{13}C_4$-PFHpA
PFOA	6.16	413.0	369.0	169.0	3.0	$^{13}C_8$-PFOA
PFNA	6.99	463.0	419.0	219.0	4.9	$^{13}C_9$-PFNA
PFDA	7.47	512.9	469.0	219.0	5.5	$^{13}C_6$-PFDA
PFUnA	7.81	563.1	519.0	269.1	6.9	$^{13}C_7$-PFUnA
PFDoA	8.13	613.1	569.0	319.0	10	$^{13}C_2$-PFDoA
PFTrDA[3]	8.53	663.0	619.0	168.9	6.7	avg. $^{13}C_2$-PFTeDA and $^{13}C_2$-PFDoA
PFTeDA	8.96	713.1	669.0	168.9	6.0	$^{13}C_2$-PFTeDA
PFBS	4.79	298.7	79.9	98.8	2.1	$^{13}C_3$-PFBS
PFPeS	5.38	349.1	79.9	98.9	1.8	$^{13}C_3$-PFHxS
PFHxS	6.31	398.7	79.9	98.9	1.9	$^{13}C_3$-PFHxS
PFHpS	7.11	449.0	79.9	98.8	1.7	$^{13}C_8$-PFOS
PFOS	7.59	498.9	79.9	98.8	2.3	$^{13}C_8$-PFOS
PFNS	7.92	548.8	79.9	98.8	1.9	$^{13}C_8$-PFOS
PFDS	8.28	599.0	79.9	98.8	1.9	$^{13}C_8$-PFOS
PFDoS	9.14	699.1	79.9	98.8	1.9	$^{13}C_8$-PFOS
4:2FTS	4.67	327.1	307.0	80.9	1.7	$^{13}C_2$-4:2FTS
6:2FTS	5.81	427.1	407.0	80.9	1.9	$^{13}C_2$-6:2FTS
8:2FTS	7.28	527.1	507.0	80.8	3.0	$^{13}C_2$-8:2FTS
PFOSA	8.41	498.1	77.9	478.0	47	$^{13}C_8$-PFOSA
NMeFOSA	9.70	511.9	219.0	169.0	0.66	D_3-NMeFOSA
NEtFOSA	9.94	526.0	219.0	169.0	0.63	D_5-NEtFOSA
NMeFOSAA	7.51	570.1	419.0	483.0	2.0	D_3-NMeFOSAA
NEtFOSAA	7.65	584.2	419.1	526.0	1.2	D_5-N-EtFOSAA
NMeFOSE	9.57	616.1	58.9	NA	NA	D_7-NMeFOSE
NEtFOSE	9.85	630.0	58.9	NA	NA	D_9-NEtFOSE
HFPO-DA	4.97	284.9	168.9	184.9	1.95	$^{13}C_3$-HFPO-DA
ADONA	5.79	376.9	250.9	84.8	2.8	$^{13}C_3$-HFPO-DA
PFMPA	3.21	229.0	84.9	NA	NA	$^{13}C_5$-PFPeA
PFMBA	4.53	279.0	85.1	NA	NA	$^{13}C_5$-PFPeA
NFDHA	4.84	295.0	201.0	84.9	1.46	$^{13}C_5$-PFHxA
9Cl-PF3ONS	7.82	530.8	351.0	532.8→353.0	3.2	$^{13}C_3$-HFPO-DA
11Cl-PF3OUdS	8.62	630.9	450.9	632.9→452.9	3.0	$^{13}C_3$-HFPO-DA
PFEESA	5.08	314.8	134.9	82.9	9.22	$^{13}C_5$-PFHxA
3:3FTCA	3.89	241.0	177.0	117.0	1.70	$^{13}C_5$-PFPeA
5:3FTCA	5.14	341.0	237.1	217.0	1.16	$^{13}C_5$-PFHxA
7:3FTCA	6.76	441.0	316.9	336.9	0.69	$^{13}C_5$-PFHxA
EIS Compounds						
$^{13}C_4$-PFBA	1.95	216.8	171.9	NA		$^{13}C_3$-PFBA
$^{13}C_5$-PFPeA	4.18	268.3	223.0	NA		$^{13}C_2$-PFHxA
$^{13}C_5$-PFHxA	4.80	318.0	273.0	120.3		$^{13}C_2$-PFHxA
$^{13}C_4$-PFHpA	5.32	367.1	322.0	NA		$^{13}C_2$-PFHxA
$^{13}C_8$-PFOA	6.16	421.1	376.0	NA		$^{13}C_4$-PFOA
$^{13}C_9$-PFNA	6.99	472.1	427.0	NA		$^{13}C_5$-PFNA

EPA 1633

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PFNA	6.99	463.0	419.0	219.0	4.9	$^{13}C_9$-PFNA
PFDA	7.47	512.9	469.0	219.0	5.5	$^{13}C_6$-PFDA
PFUnA	7.81	563.1	519.0	269.1	6.9	$^{13}C_7$-PFUnA
	8		569.0	319.0	10	$^{13}C_2$-PFDoA
	8		619.0	168.9	6.7	avg. $^{13}C_2$-PFTeDA and $^{13}C_2$-PFDoA
PFNS	7.92	548.8	79.9	98.8	1.9	$^{13}C_8$-PFOS
PFDS	8.28	599.0	79.9	98.8	1.9	$^{13}C_8$-PFOS
NEtFOSA	9.94	526.0	219.0	169.0	0.63	D_5-NEtFOSA
NMeFOSAA	7.51	570.1	419.0	483.0	2.0	D_3-NMeFOSAA
NEtFOSAA	7.65	584.2	419.1	526.0	1.2	D_5-N-EtFOSAA
NMeFOSE	9.57	616.1	58.9	NA	NA	D_7-NMeFOSE
NEtFOSE	9.85	630.0	58.9	NA	NA	D_9-NEtFOSE
HFPO-DA	4.97	284.9	168.9	184.9	1.95	$^{13}C_3$-HFPO-DA
ADONA	5.79	376.9	250.9	84.8	2.8	$^{13}C_3$-HFPO-DA
PFMPA	3.21	229.0	84.9	NA	NA	$^{13}C_5$-PFPeA
PFMBA	4.53	279.0	85.1	NA	NA	$^{13}C_5$-PFPeA
NFDHA	4.84	295.0	201.0	84.9	1.46	$^{13}C_5$-PFHxA
9Cl-PF3ONS	7.82	530.8	351.0	532.8→353.0	3.2	$^{13}C_3$-HFPO-DA
11Cl-PF3OUdS	8.62	630.9	450.9	632.9→452.9	3.0	$^{13}C_3$-HFPO-DA
$^{13}C_4$-PFBA	1.95	216.8	171.9	NA		$^{13}C_3$-PFBA
$^{13}C_5$-PFPeA	4.18	268.3	223.0	NA		$^{13}C_2$-PFHxA
$^{13}C_5$-PFHxA	4.80	318.0	273.0	120.3		$^{13}C_2$-PFHxA
$^{13}C_4$-PFHpA	5.32	367.1	322.0	NA		$^{13}C_2$-PFHxA
$^{13}C_8$-PFOA	6.16	421.1	376.0	NA		$^{13}C_4$-PFOA
$^{13}C_9$-PFNA	6.99	472.1	427.0	NA		$^{13}C_5$-PFNA

Parent ion | Quantitation ion | Confirmation ion | Ion ratio

PFBA	1.96	212.8	168.9	NA	NA	$^{13}C_4$-PFBA
PFPeA	4.18	263.0	219.0	68.9	NA	$^{13}C_5$-PFPeA

PFMPA	3.21	229.0	84.9	NA	NA	$^{13}C_5$-PFPeA
PFMBA	4.53	279.0	85.1	NA	NA	$^{13}C_5$-PFPeA

NMeFOSE	9.57	616.1	58.9	NA	NA	D_7-NMeFOSE
NEtFOSE	9.85	630.0	58.9	NA	NA	D_9-NEtFOSE

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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.

EPA 8327

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)[1]
		Primary	Secondary	
PFBA[2]	213	169	-	3.07
PFPeA[2]	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[3]	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[3]	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[2]	498	78	-	9.77
PFTeDA	713	669	169	10.12
Isotopically labeled surrogates[4]:				
M4PFBA	217	172		3.06
M5PFPeA	268	223		4.15
M2-4:2 FTS[5]	329	309	81	5.12
M5PFHxA	318	273		5.44
M3PFBS	302	80		5.72
M2PFHpA	367	322		6.38
M2-6:2 FTS[5]	429	409	81	6.8
M8PFOA	421	376		7.08
M3PFHxS	402	80		7.46
M9PFNA	472	427		7.66
M2-8:2 FTS[5]	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)

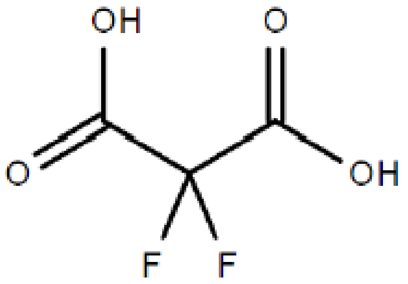
EPA OTM-45

Analyte	Precursor Ion (m/z)	Primary Product Ion (m/z)	Secondary Product Ion (m/z)
PFBA	213	169	
$^{13}C_3$-PFBA	216	172	
$^{13}C_4$-PFBA	217	172	
PFPeA	263	219	
$^{13}C_5$-PFPeA	268	223	
PFHxA	313	269	119
$^{13}C_5$-PFHxA	318	273	
PFHpA	363	319	169
$^{13}C_4$-PFHpA	367	322	
PFOA	413	369	169
$^{13}C_2$-PFOA	415	370	
$^{13}C_8$-PFOA	421	376	
PFNA	463	419	169
$^{13}C_9$-PFNA	472	427	
PFDA	513	469	169
$^{13}C_6$-PFDA	519	474	
PFUnA	563	519	169
$^{13}C_7$-PFUnA	570	525	
PFDoA	613	569	169
$^{13}C_2$-PFDoA	615	570	
PFTrDA	663	619	169
PFTeDA	713	169	219
PFHxDA	813	769	169
PFODA	913	869	169
PFBS	299	80	99
$^{13}C_3$-PFBS	302	80	83
PFPeS	349	80	99
PFHxS	399	80	99
$^{13}C_3$-PFHxS	402	80	
PFHpS	449	80	99
PFOS	499	80	99
$^{13}C_4$-PFOS	503	80	
$^{13}C_8$-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	
$^{13}C_2$-4:2FTS	329	309	81
6:2FTS	427	407	
$^{13}C_2$-6:2FTS	429	409	81
8:2FTS	527	507	
$^{13}C_2$-8:2FTS	529	509	81
10:2FTS	627	607	80
ADONA	377	251	
HFPO-DA	285	169	
$^{13}C_3$-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

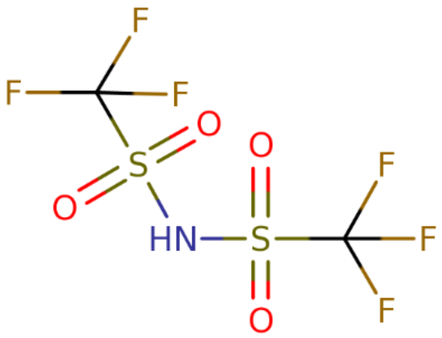
Confirmation Options?

Ion Ratio is NOT available.

- **1 MRM Transition Only**
- **Weak 2nd Transition**

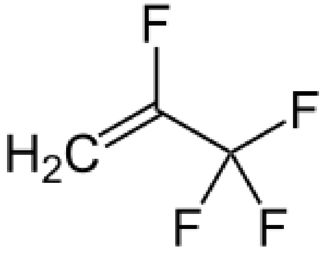


MMF

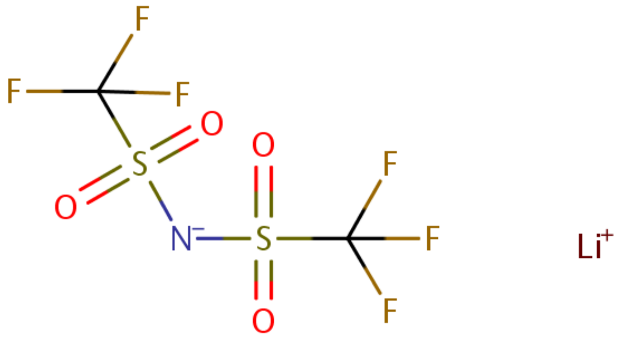


HQ-115

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



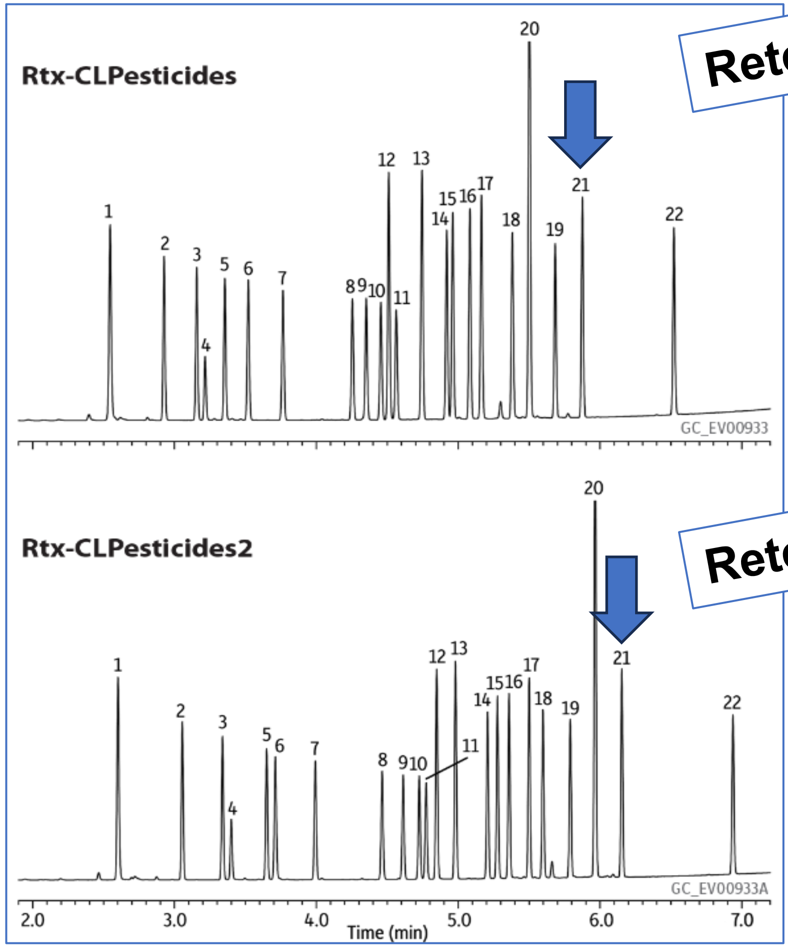
HFO-1234yf



TFSI

Hint from EPA 8081B

Dual ECD



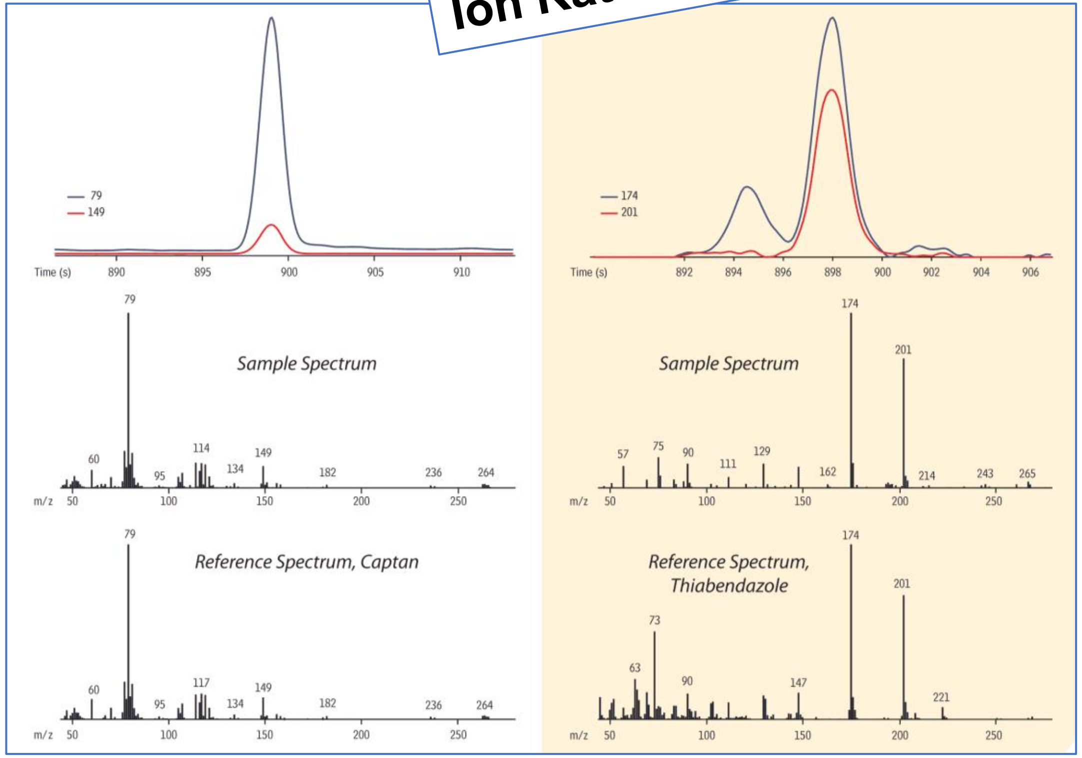
Retention time 1

Retention time 2

GC-MS/MS

Retention time

Ion Ratio



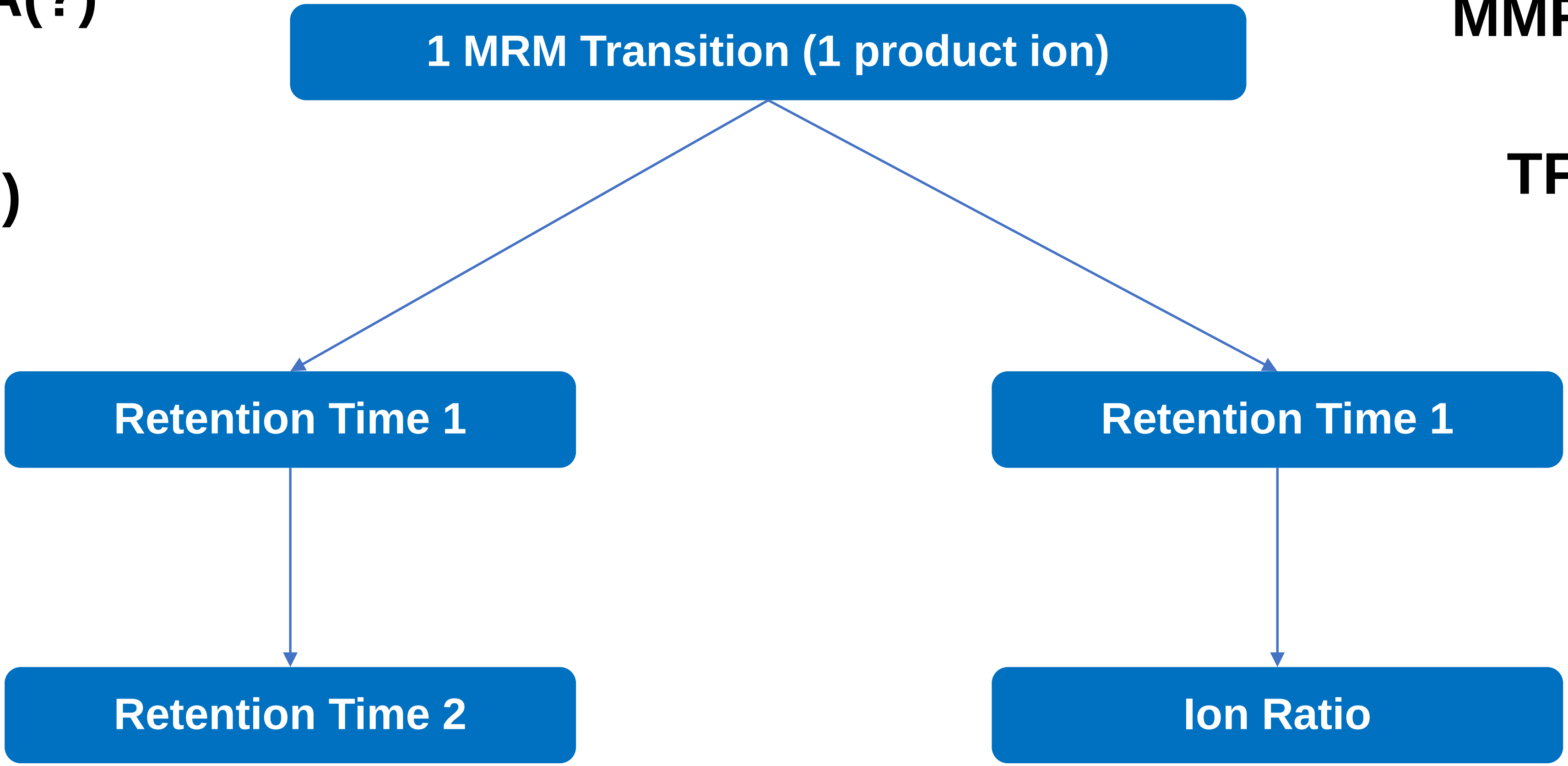
Revisiting Problems of Short Chain PFAS Confirmation

PFBA(?)

MMF(?)

PFPeA(?)

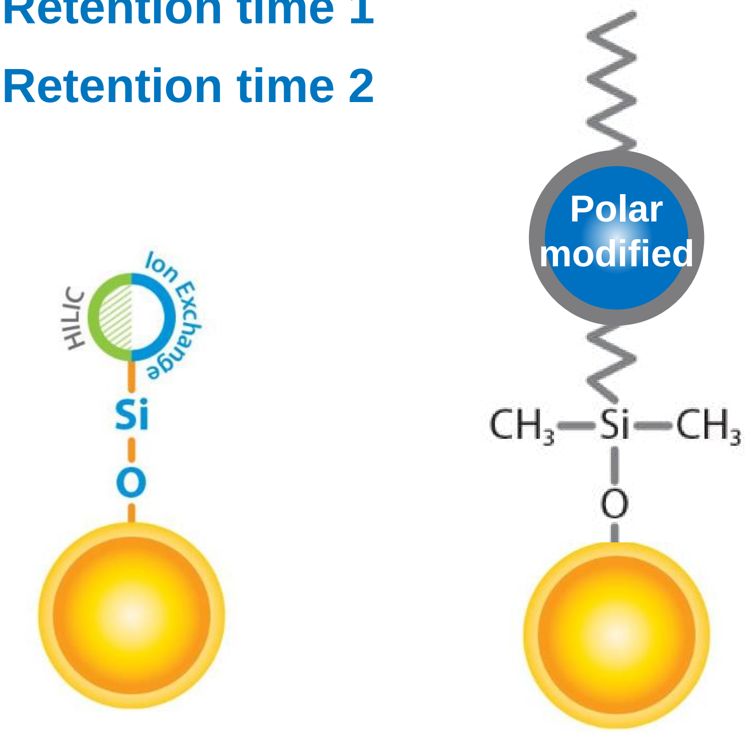
TFSI(?)



Short-Chain PFAS Confirmation Tools

Two different retention mechanisms for short chain PFAS analysis

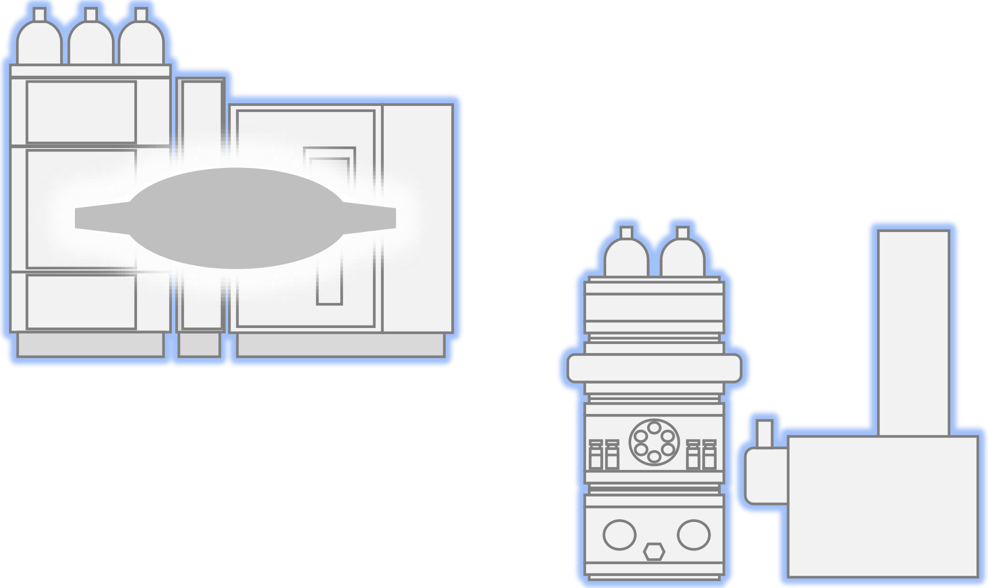
- **Retention time 1**
- **Retention time 2**



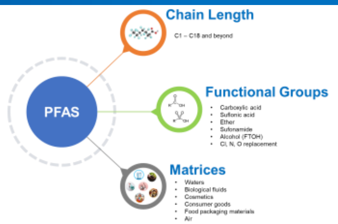
Polar X **Ultra IBD**

OR

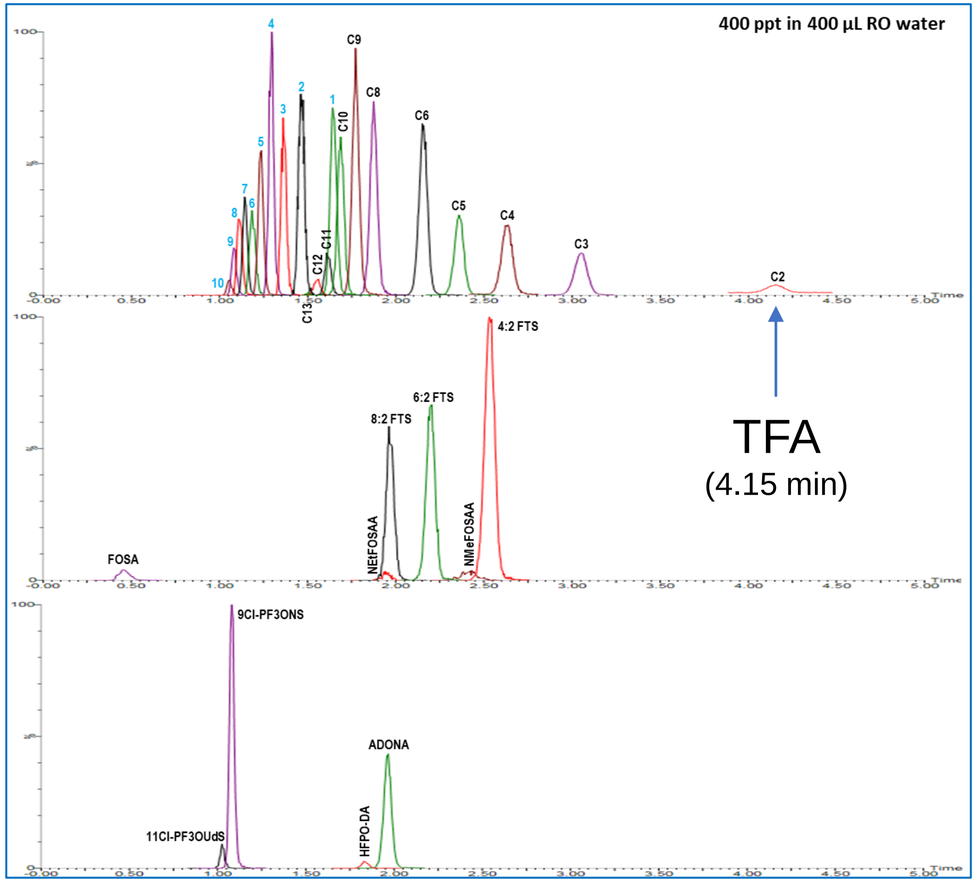
High-res MS



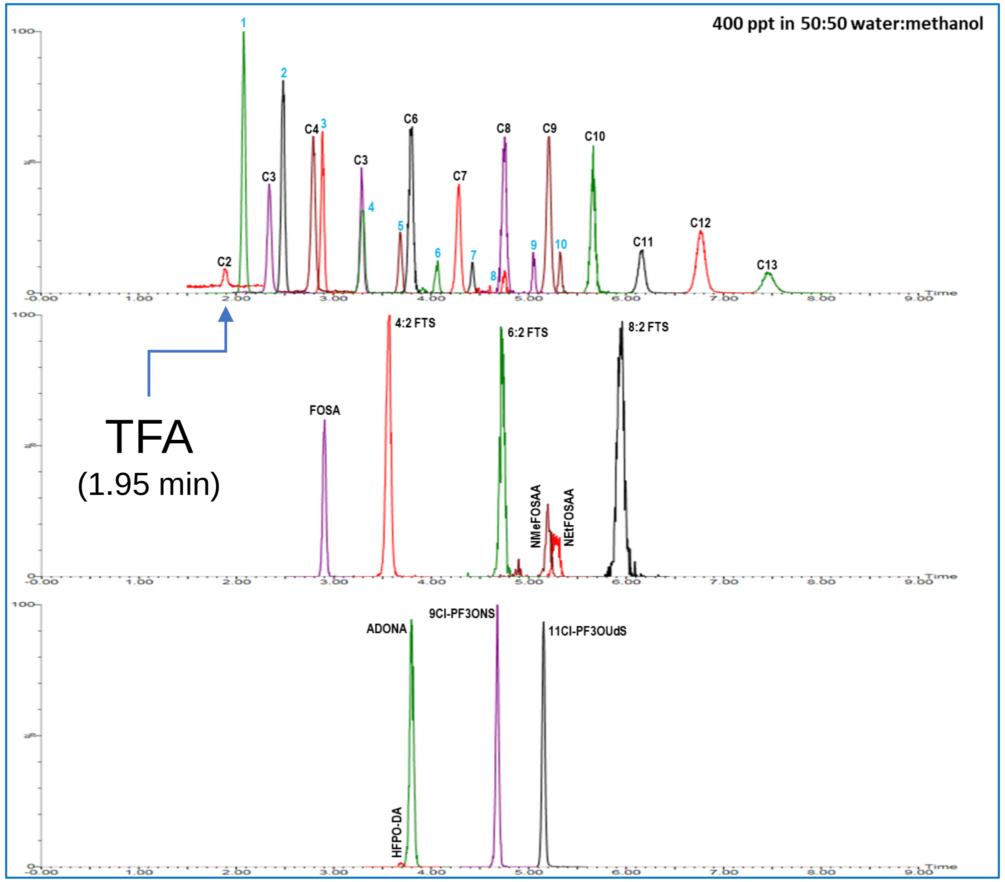
Two Polar Columns for PFAS

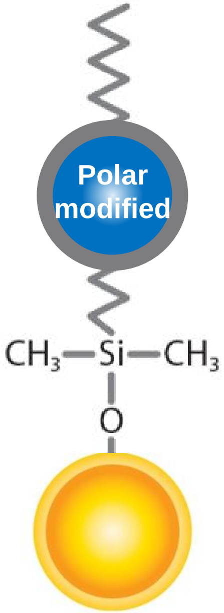


Polar X



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 # ❶ (Polar X)

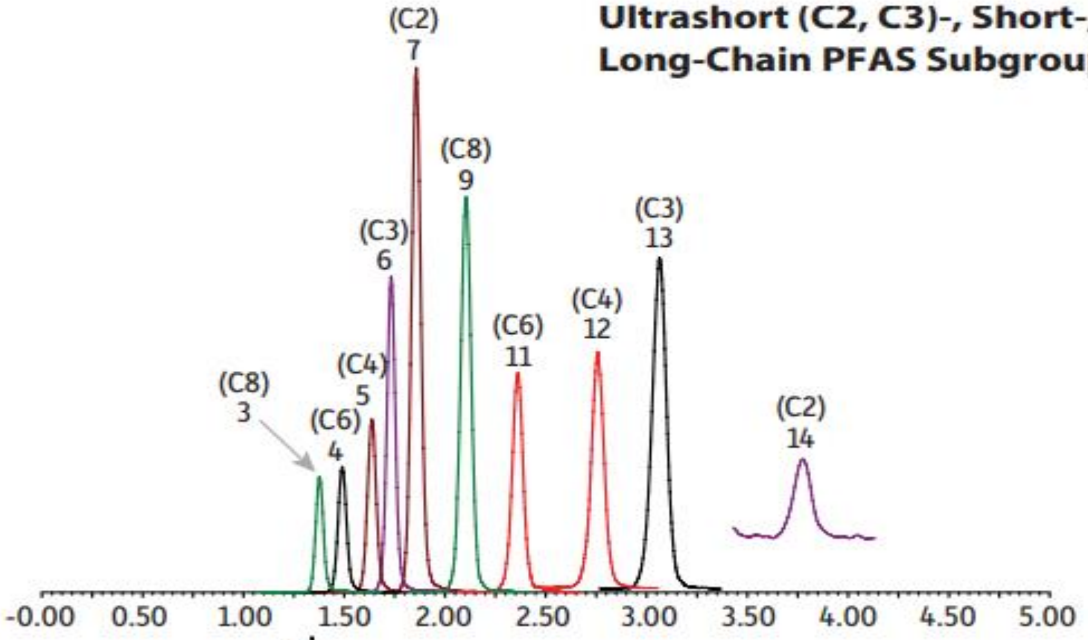
Peaks	tR (min)	Conc. (ng/L)	Precursor Ion	Product Ion
1. 11-Chloroeicosafluoro-3-oxanonane-1-sulfonate (11CL-PF3OUdS)	1.25	400	630.78	450.80
2. 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonate9-Chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS)	1.34	400	530.78	350.85
3. Perfluorooctanesulfonic acid (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

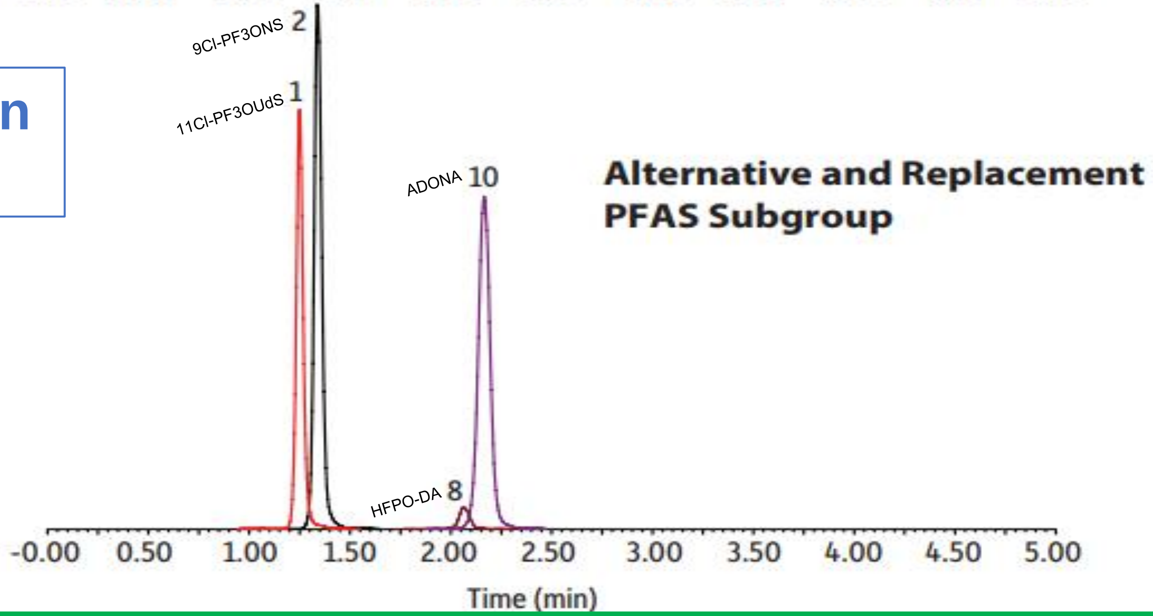
Column Raptor Polar X (cat.# 9311A52)
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 2.7 µm
Temp.: 40 °C
Sample
Diluent: 50:50 Water:methanol
Conc.: 400 ng/L
Inj. Vol.: 10 µL
Mobile Phase
A: Water, 10 mM ammonium formate, 0.05% formic acid
B: 60:40 Acetonitrile:methanol, 0.05% formic acid

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

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

- Isocratic Condition
- No Delay Column

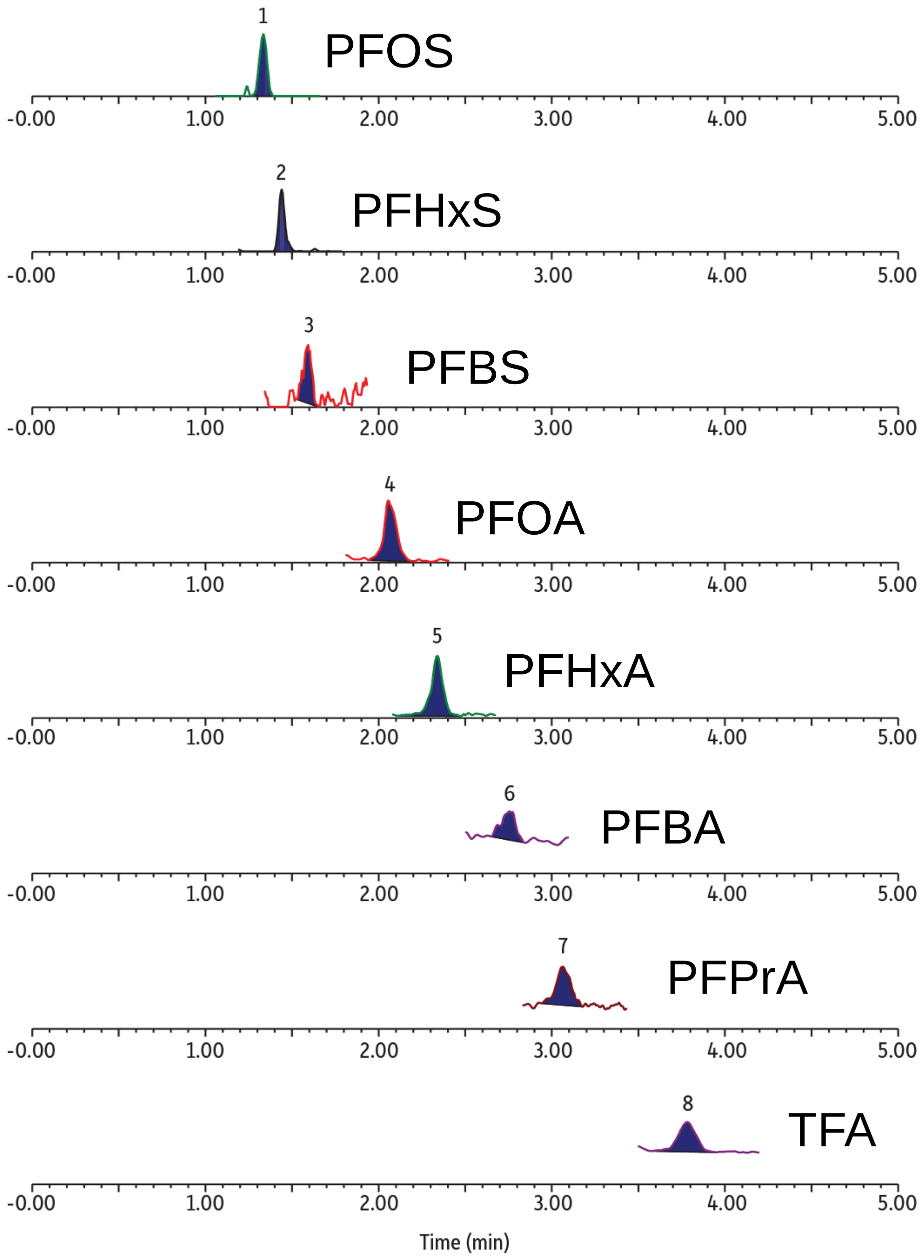




RESTEK Pure Chromatography

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.	Perfluorooctanoic 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

1

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
Potable Waters							
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
Non-Potable Waters							
POTW water (treated seweage 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

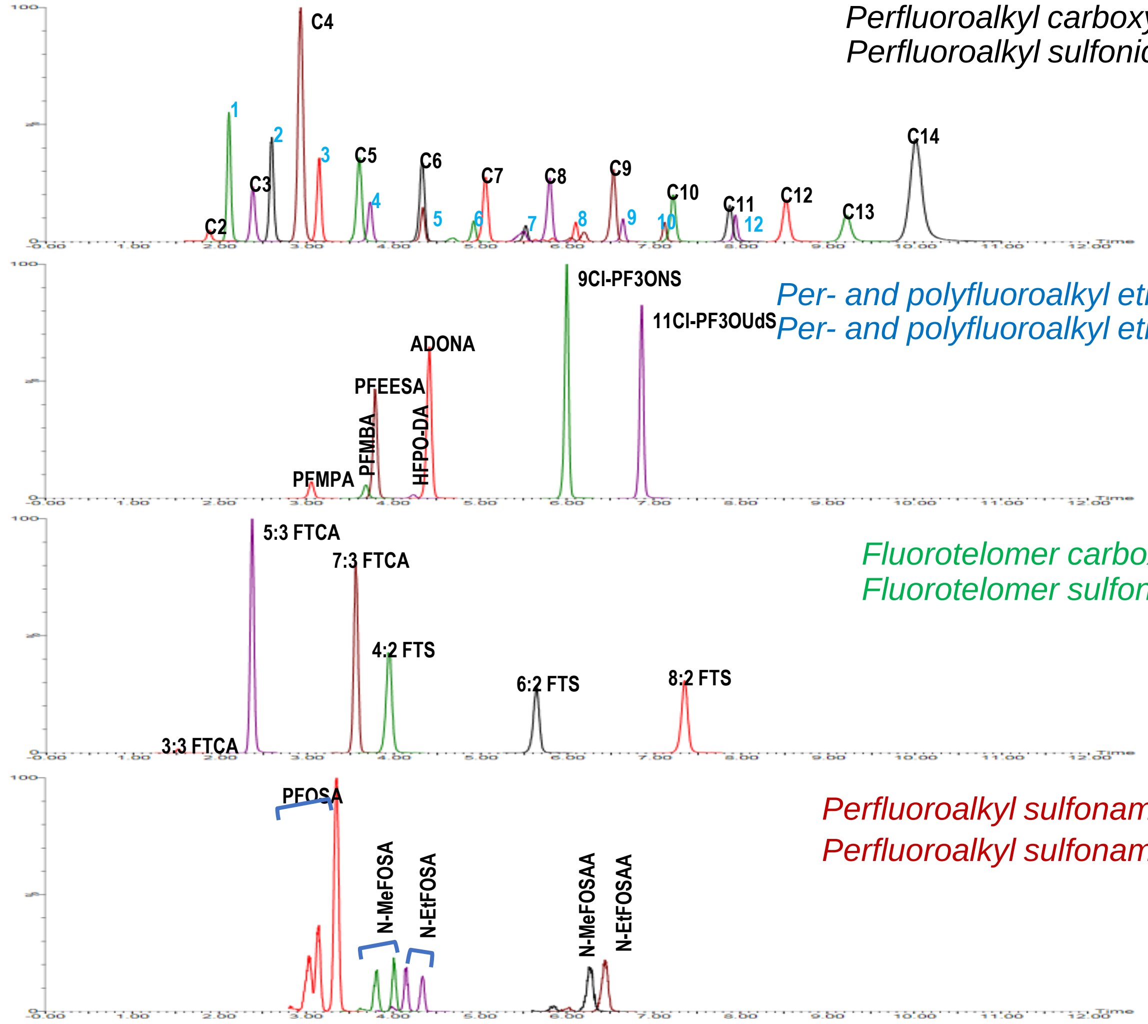
* non-detected

Residential and commercial (Tap Water #1–#10) · Store bought (Bottled Water #1–#3, Natural Spring Water) · EPA (Non-Potable Waters)

Polar Column #2 (Ultra IBD)

Column	Ultra 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
	8.00	95
	10.00	95
	10.01	50
	12.00	50
Flow Rate	0.4 mL/min	
Injection Volume	5 µL	
Column Temp.	40°C	

- **Gradient**
- **With Delay Column**



Perfluoroalkyl carboxylic acids
Perfluoroalkyl sulfonic acids

Per- and polyfluoroalkyl ether carboxylic acids
Per- and polyfluoroalkyl ether sulfonic acids

Fluorotelomer carboxylic acids
Fluorotelomer sulfonic acids

Perfluoroalkyl sulfonamides
Perfluoroalkyl sulfonamidoacetic acids

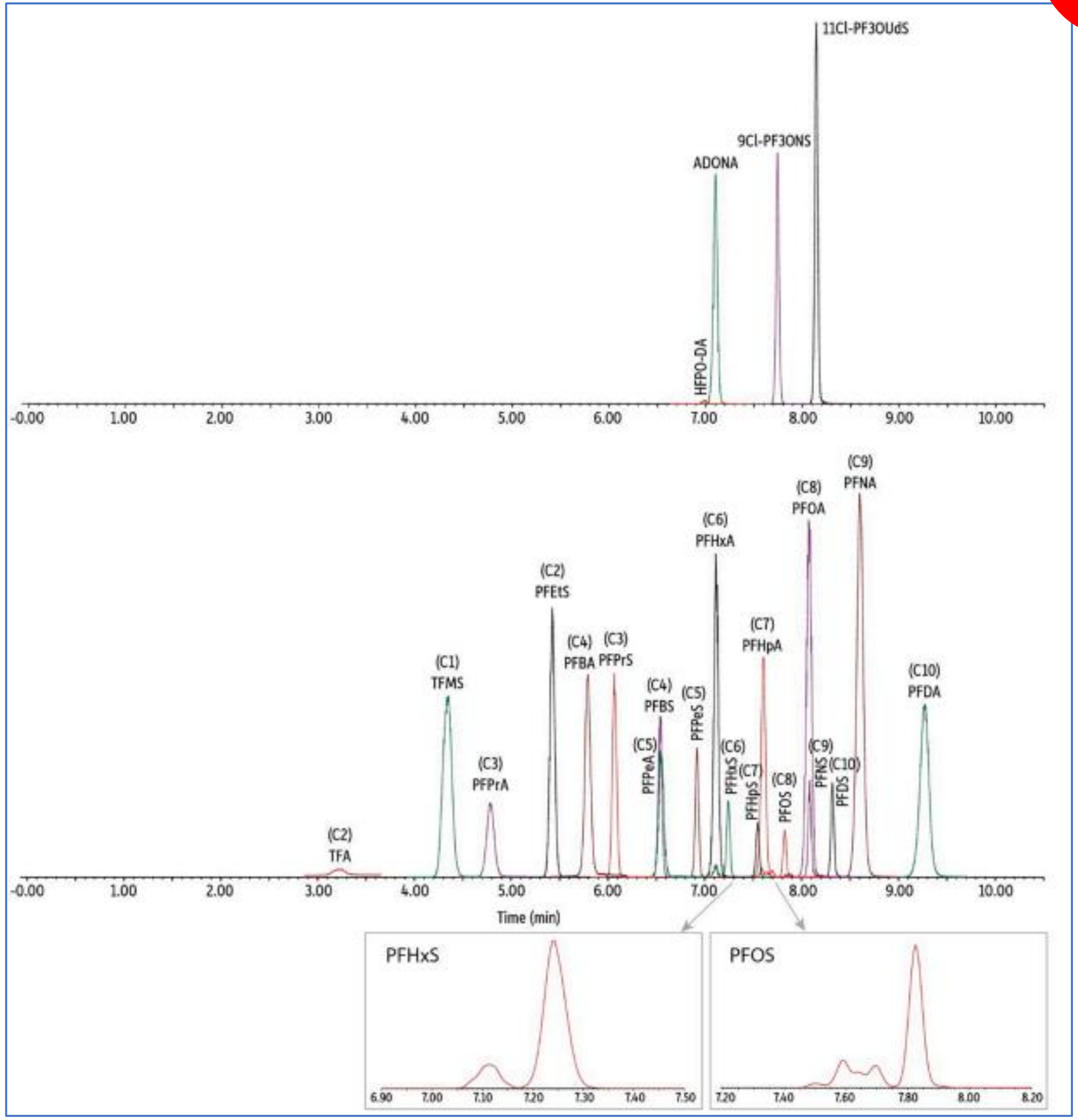
Human Plasma & Serum

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
Target Analytes						
Trifluoroacetic acid (TFA)	3.25	113.03 [M-H]-	69.01	10	10	$^{13}C_3$-PFBA
Perfluoropropanoic acid (PFPrA)	4.81	162.97 [M-H]-	119.02	10	8	$^{13}C_3$-PFBA
Perfluorobutanoic acid (PFBA)	5.80	213.03 [M-H]-	168.98	14	8	$^{13}C_3$-PFBA
Perfluoropentanoic acid (PFPeA)	6.56	262.97 [M-H]-	218.97	2	6	$^{13}C_2$-PFHxA
Perfluorohexanoic acid (PFHxA)	7.13	313.10 [M-H]-	268.97/118.99	2	8/20	$^{13}C_2$-PFHxA
Perfluoroheptanoic acid (PFHpA)	7.62	363.16 [M-H]-	319.09/169.06	8	10/18	$^{13}C_4$-PFOA
Perfluorooctanoic acid (PFOA)	8.10	413.10 [M-H]-	368.96/168.90	2	10/16	$^{13}C_4$-PFOA
Perfluorononanoic acid (PFNA)	8.62	463.10 [M-H]-	419.01/219.02	4	10/16	$^{13}C_5$-PFNA
Perfluorodecanoic acid (PFDA)	9.29	513.17 [M-H]-	469.16/219.06	4	12/16	$^{13}C_2$-PFDA
Trifluoromethanesulfonic acid (TFMS)	4.37	148.97 [M-H]-	79.93/98.92	62	18/18	$^{13}C_3$-PFBA
Perfluoroethanesulfonic acid (PFEtS)	5.44	198.90 [M-H]-	79.92/98.91	38	22/22	$^{13}C_3$-PFBA
Perfluoropropanesulfonic acid (PFPrS)	6.08	248.97 [M-H]-	79.92/98.91	2	24/24	$^{13}C_3$-PFBA
Perfluorobutanesulfonic acid (PFBS)	6.55	298.97 [M-H]-	79.97/98.89	2	26/26	$^{13}C_2$-PFHxA
Perfluoropentanesulfonic acid (PFPeS)	6.93	349.10 [M-H]-	79.98/98.98	6	32/30	$^{13}C_2$-PFHxA
Perfluorohexanesulfonic acid (PFHxS)	7.24	398.90 [M-H]-	79.97/98.89	56	32/34	$^{13}C_2$-PFHxA
Perfluoroheptanesulfonic acid (PFHpS)	7.56	449.17 [M-H]-	79.98/98.97	4	42/38	$^{13}C_2$-PFHxA
Perfluorooctanesulfonic acid (PFOS)	7.82	499.03 [M-H]-	79.92/98.90	8	40/40	$^{13}C_4$-PFOA
Perfluorononanesulfonic acid (PFNS)	8.09	549.10 [M-H]-	79.92/98.83	12	42/40	$^{13}C_4$-PFOA
Perfluorodecanesulfonic acid (PFDS)	8.32	599.17 [M-H]-	79.98/98.83	8	44/46	$^{13}C_5$-PFNA
Hexafluoropropylene oxide dimer acid (HFPO-DA)	7.01	285.03 [M-COOH]-	169.02/185.02	2	6/16	$^{13}C_2$-PFHxA
4,8-dioxa-3H-perfluorononoic acid (ADONA)	7.11	376.90 [M-H]-	250.93/84.97	22	12/26	$^{13}C_2$-PFHxA
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic aicd (9Cl-PF3ONS)	7.75	530.78 [M-H]-	350.85/82.96	12	26/24	$^{13}C_2$-PFHxA
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	8.15	630.78 [M-H]-	450.80/82.95	8	26/32	$^{13}C_5$-PFNA
Extracted Internal Standards						
^{13}C-TFA	3.25	114.03 [M-H]-	69.03	10	8	$^{13}C_3$-PFBA
$^{13}C_3$-PFPrA	4.81	165.97 [M-H]-	120.96	10	11	$^{13}C_3$-PFBA
$^{13}C_4$-PFBA	5.80	217.03 [M-H]-	171.98	2	8	$^{13}C_3$-PFBA
$^{13}C_5$-PFPeA	6.56	267.97 [M-H]-	222.99	2	6	$^{13}C_2$-PFHxA
$^{13}C_5$-PFHxA	7.13	318.03 [M-H]-	272.93	2	7	$^{13}C_2$-PFHxA
$^{13}C_4$-PFHpA	7.62	366.90 [M-H]-	321.93	2	10	$^{13}C_4$-PFOA
$^{13}C_8$-PFOA	8.10	420.97 [M-H]-	375.94	2	10	$^{13}C_4$-PFOA
$^{13}C_9$-PFNA	8.62	471.97 [M-H]-	426.87	4	8	$^{13}C_5$-PFNA
$^{13}C_6$-PFDA	9.29	518.90 [M-H]-	473.87	4	13	$^{13}C_2$-PFDA
$^{13}C_3$-PFHxS	7.24	401.90 [M-H]-	79.97	2	32	$^{13}C_2$-PFHxA
$^{13}C_8$-PFOS	7.82	506.84 [M-H]-	79.97	4	48	$^{13}C_4$-PFOA
Non-Extracted Internal Standards						
$^{13}C_3$-PFBA	5.80	215.97 [M-H]-	171.97	10	8	
$^{13}C_2$-PFHxA	7.13	314.97 [M-H]-	269.93	8	8	
$^{13}C_4$-PFOA	8.10	416.87 [M-H]-	371.88	2	8	
$^{13}C_5$-PFNA	8.62	467.87 [M-H]-	422.89	16	10	
$^{13}C_2$-PFDA	9.29	514.87 [M-H]-	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

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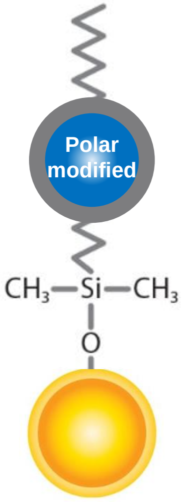


Conclusion

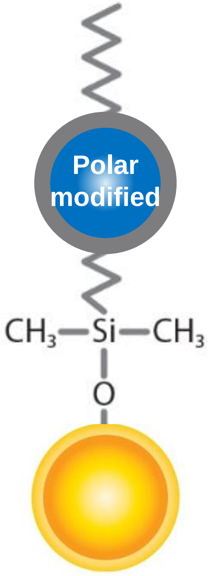
- PFAS testing has been evolving from # of analytes, functional groups of PFAS, and technologies across sample preparation, measurement systems.

- 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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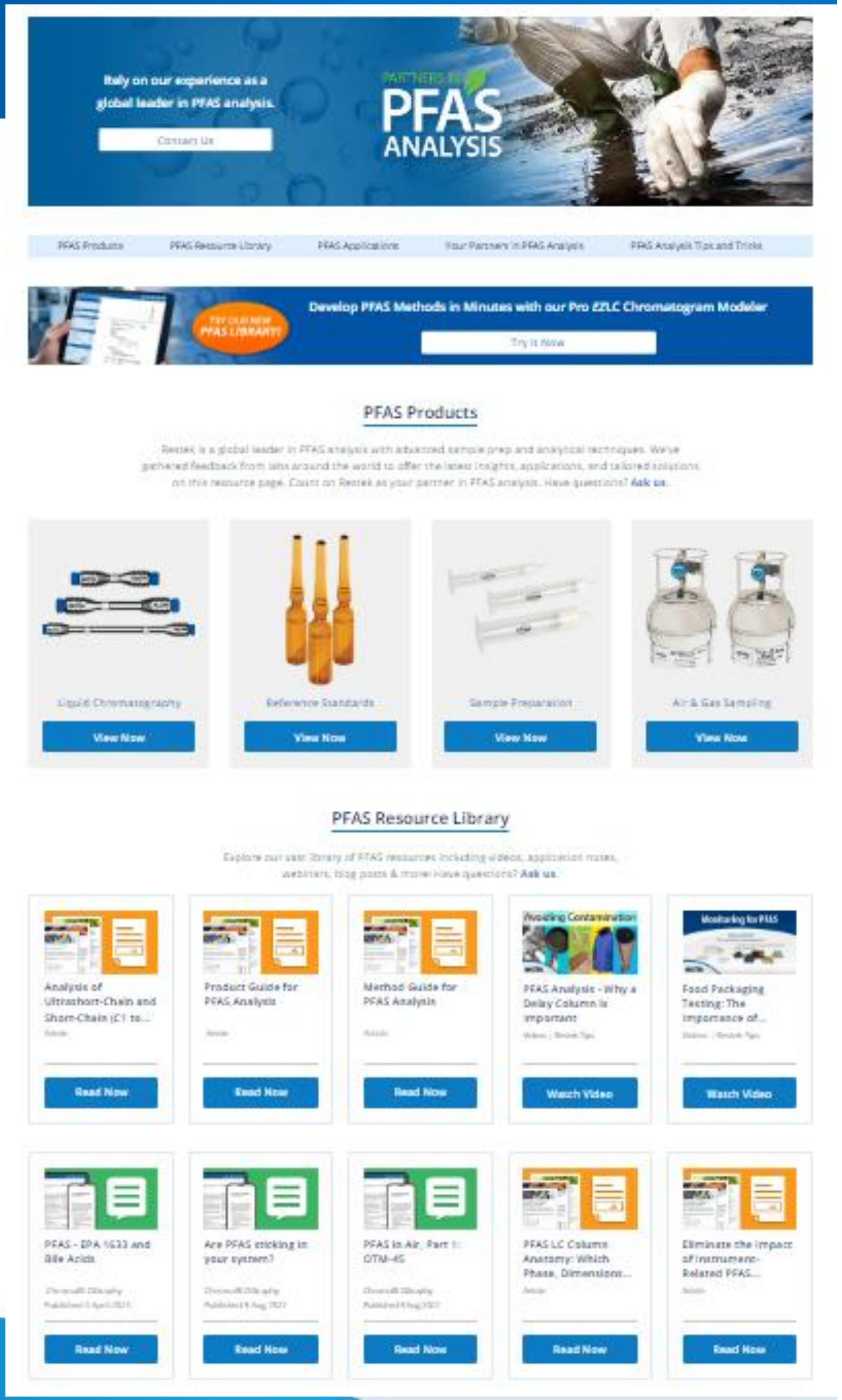

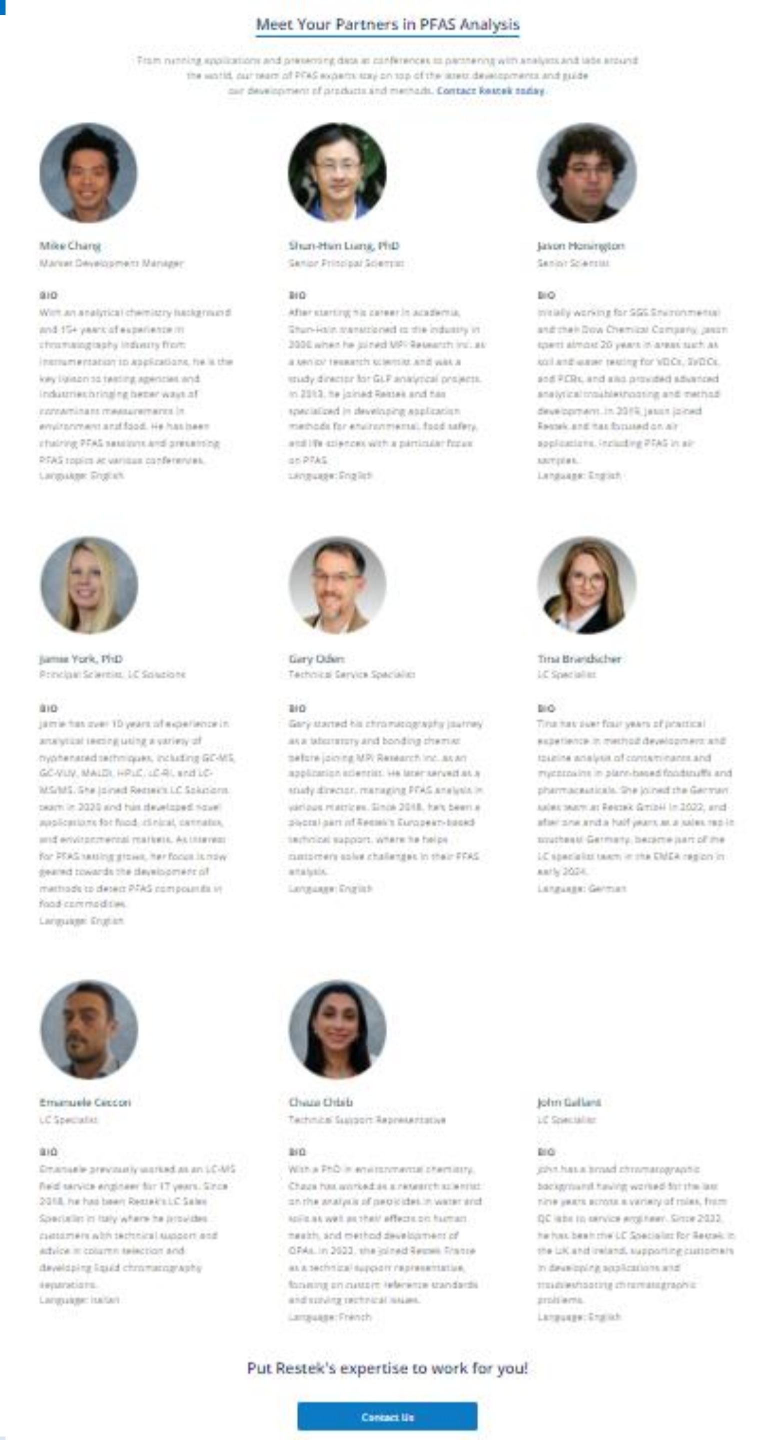

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Mike Chang
mike.chang@restek.com