Quantitative comparison of perfluorinated active substances in drinking water between tandem triple quadrupole MS/MS and high resolution mass spectrometry using orbitrap technology – knowns and unknowns

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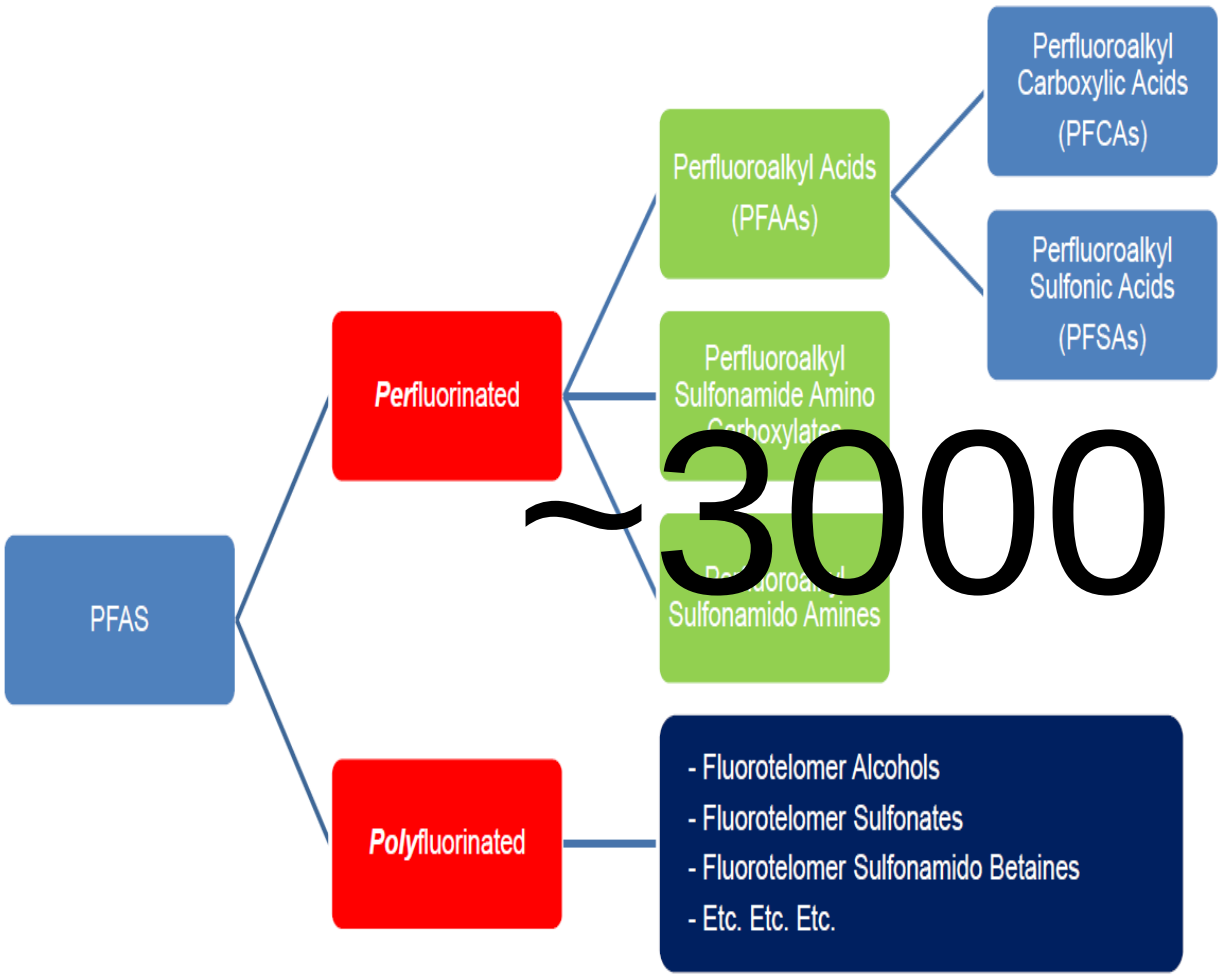
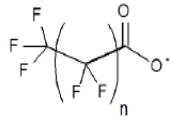
Andy Eaton, PhD, BCES (Eurofins Eaton Analytical)

Perfluorinated compounds as emerging contaminants



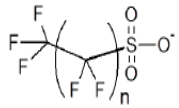


PFCAs incl. PFOA



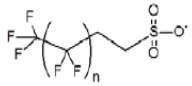
n=2, PFBA; n=3, PFPeA;
n=4, PFHxA; n=5, PFHpA;
n=6, PFOA; n=7, PFNA;
n=8, PFDA; n=9, PFUnDA;
n=10, PFDoDA;

PFSA incl. PFOS



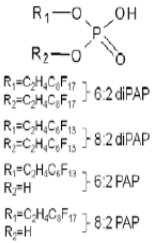
n=3, PFBS
n=5, PFHxS
n=7, PFOS

FTS

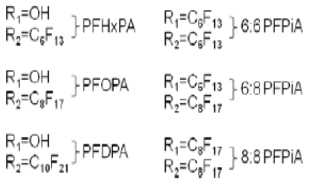


n=3, 4:2 FTS
n=5, 6:2 FTS
n=7, 8:2 FTS

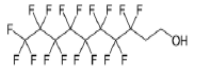
PAP, DiPAP



PFPA/PFPiA



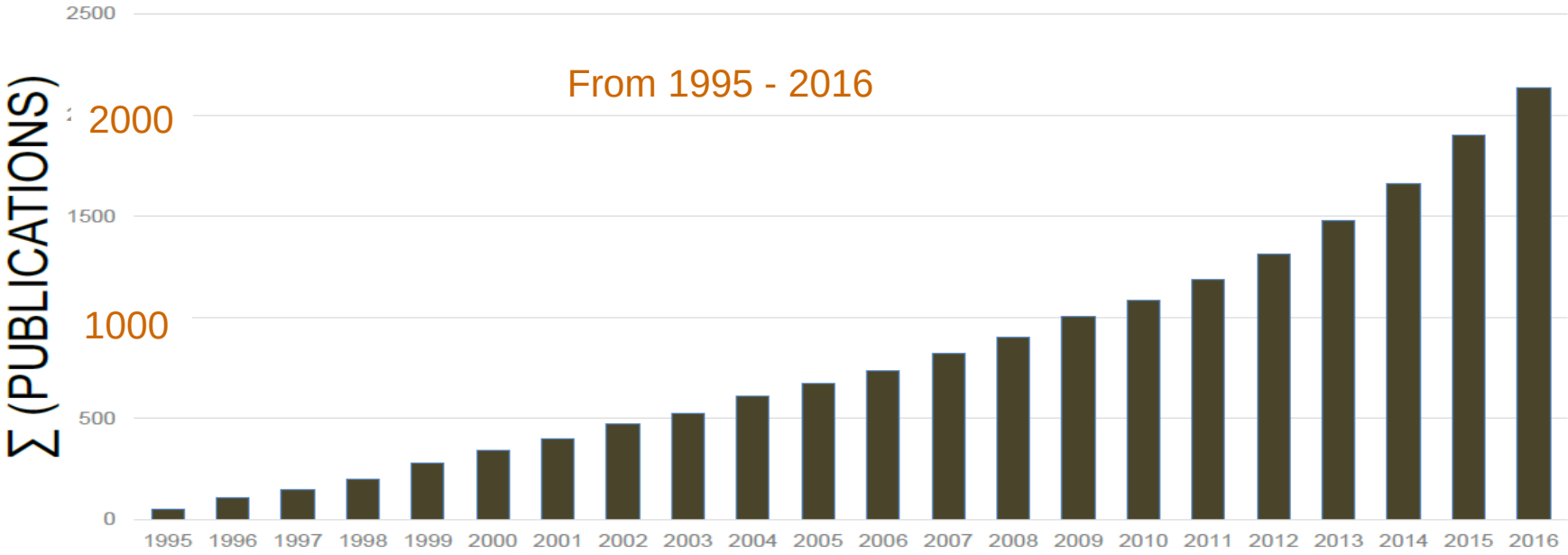
FTOH



Publications shows continued interest in PFAS.



- **Rapidly Evolving Science**



Google Scholar search of "Perfluoroalkyl" in title

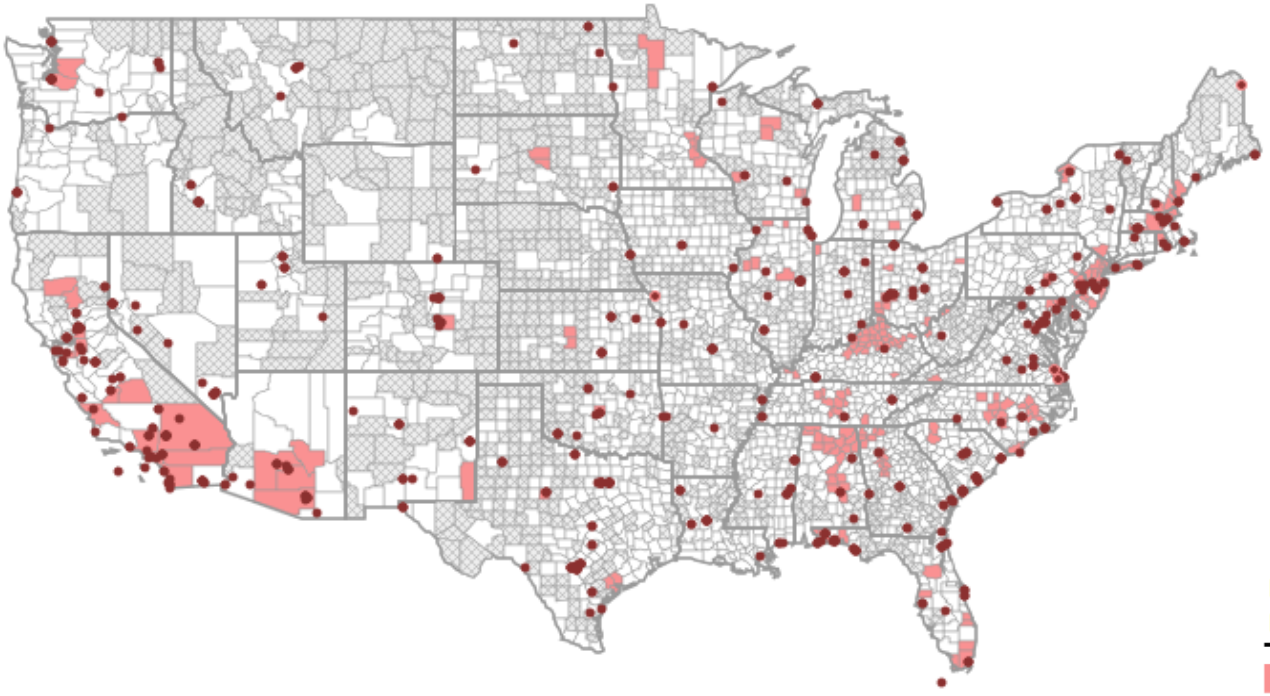
Dr. Richard (Hunter) Anderson
AFCEC/CZTE
February 2017

PFAS Occurrence – UCMR 3



2012: Six PFASs added to Unregulated Contaminant Monitoring Rule 3 (UCMR 3) list, including PFOS and PFOA using EPA 537 method.

October 2015 UCMR 3 data summary



Contaminant	Minimum Reporting Level
PFOS	0.04 µg/L
PFOA	0.02 µg/L
PFNA	0.02 µg/L
PFHxS	0.03 µg/L
PFHpA	0.01 µg/L
PFBS	0.09 µg/L

Data visualization: Moiz Syed. Sources: EPA and Department of Defense.
https://theintercept.com/2015/12/16/toxic-firefighting-foam-has-contaminated-u-s-drinking-water-with-pfcs/

EPA 537 Method Used – Summary: Extraction



- A 250-mL preserved water sample with Trizma is fortified with surrogates and passed through a solid phase extraction (SPE) cartridge containing **Solex HRPHS** in lieu of polystyrenedivinylbenzene (SDVB) to extract the method analytes and surrogates. The compounds are eluted from the solid phase with a small amount of methanol. The extract is concentrated to dryness with nitrogen in a heated water bath, and then adjusted to a 1-mL volume with 96:4% (vol/vol) methanol:water after adding the IS(s). A **5-µL** in lieu of 10-µL injection is made into an LC equipped with a C18 column.

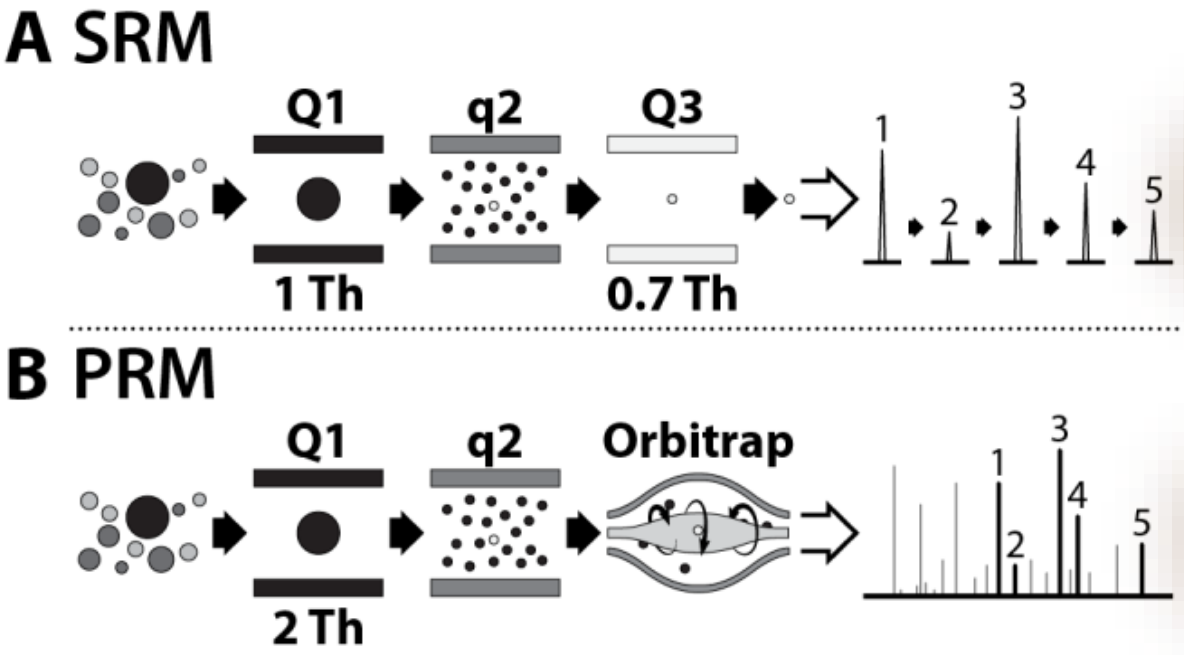
EPA 537 Method M. Used – Mas Spec

- **Q-Exactive hybrid HRAM capable of producing MS/MS data** in lieu of “low resolution triple” -MS/MS. The analytes are separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical LC/MS/MS conditions. The concentration of each analyte is determined by using the internal standard technique. Surrogate analytes are added to all Field and QC Samples to monitor the extraction efficiency of the method analytes.

Hybrid HRAM can be used for EPA LC-MS/MS Analysis



Using Parallel Reaction Monitoring (PRM) - Targeted MS2



Serial monitoring

Parallel monitoring

Parallel reaction monitoring for high resolution and high mass accuracy quantitative, targeted proteomics. *Peterson et al., MCP 2012,* ***O112.020131***

Q-Exactive tune page: the setup.



040216-IDOC-PRM-FS-006 04/02/16 02:11:23 ICAL-5
10

040216-IDOC-PRM-FS-006
Q Exactive - Orbitrap MS

Full Scan Set up

```
FULL MS - SIM
General
Runtime                                   0 to 19.2 min
Polarity                                     Negative
In-source CID                                 0.0 eV
Full MS - SIM
Microscans                                          1
Resolution                                     70,000
AGC target                                        1e6
Maximum IT                                      100 ms
Number of scan ranges                               1
Scan range                               100 to 1100 m/z
Spectrum data type                           Profile
```

70000 Resolution

100-1000 m/z

PRM Set up

```
PRM
General
Runtime                                   0 to 19.2 min
Polarity                                     Negative
In-source CID                                 0.0 eV
Default charge state                                1
Inclusion                                          on
MS²
Microscans                                          1
Resolution                                     35,000
AGC target                                        2e5
Maximum IT                                      100 ms
MSX count                                           1
MSX isochronous ITs                                on
Isolation window                                1.0 m/z
Isolation offset                                0.0 m/z
Fixed first mass                                    -
NCE / stepped NCE                              10, 55
Spectrum data type                           Profile
```

35,000 Resolution

Isolation window

Setup

```
TUNEFILES
General
Switch Count  0
Base Tunefile C:\Xcalibur\methods\600UL-EPA-537-TUNE.mstune

CONTACT CLOSURE
```

No need for optimization

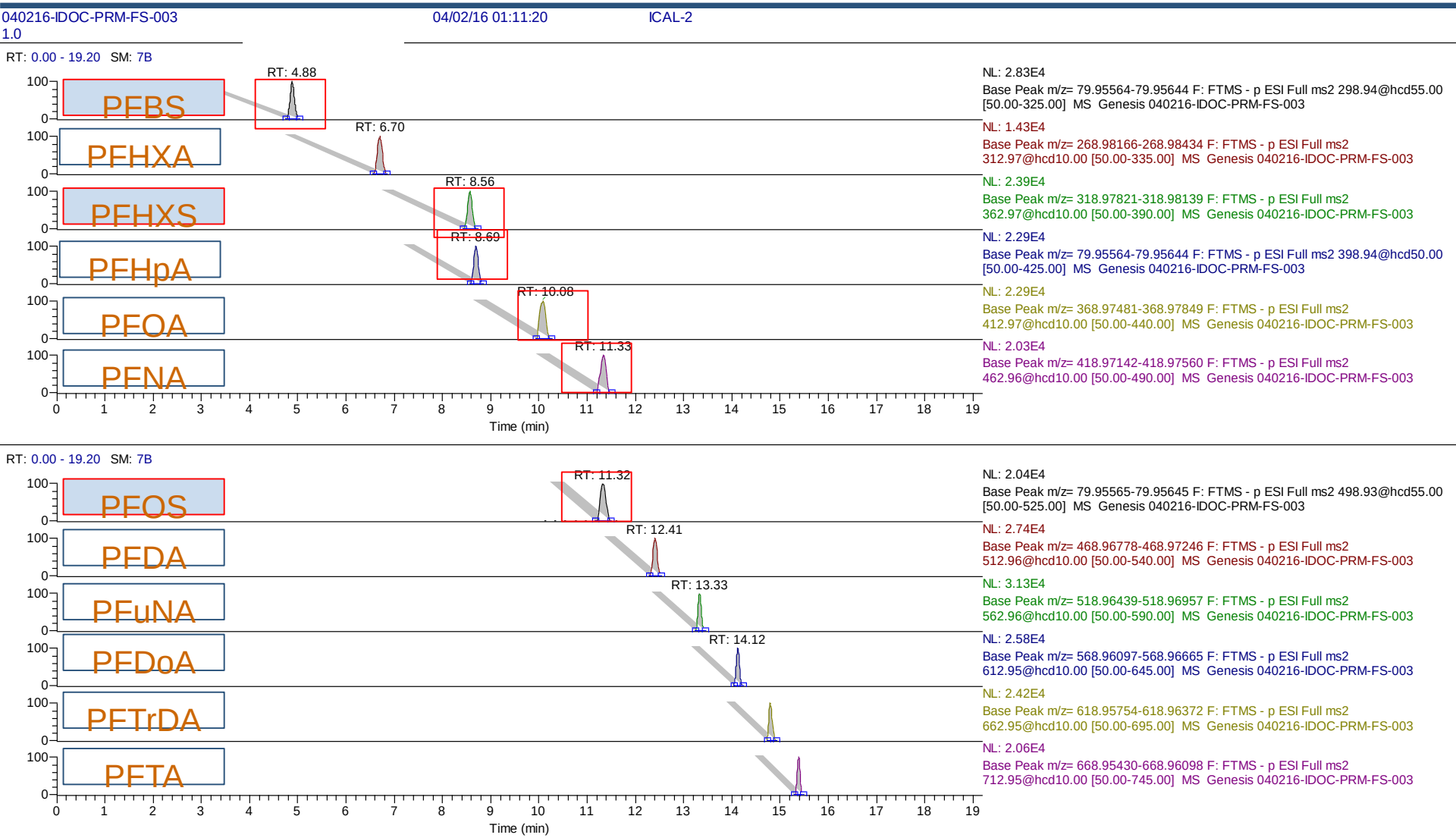
Targeted + Non Targeted

Targeted

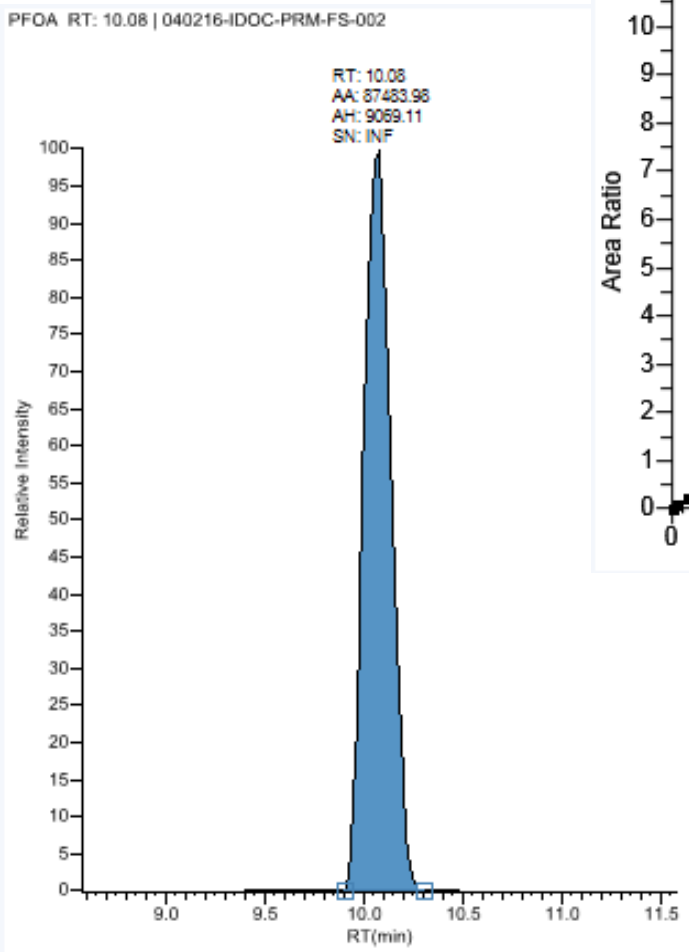
Needs RT and specific collision E.

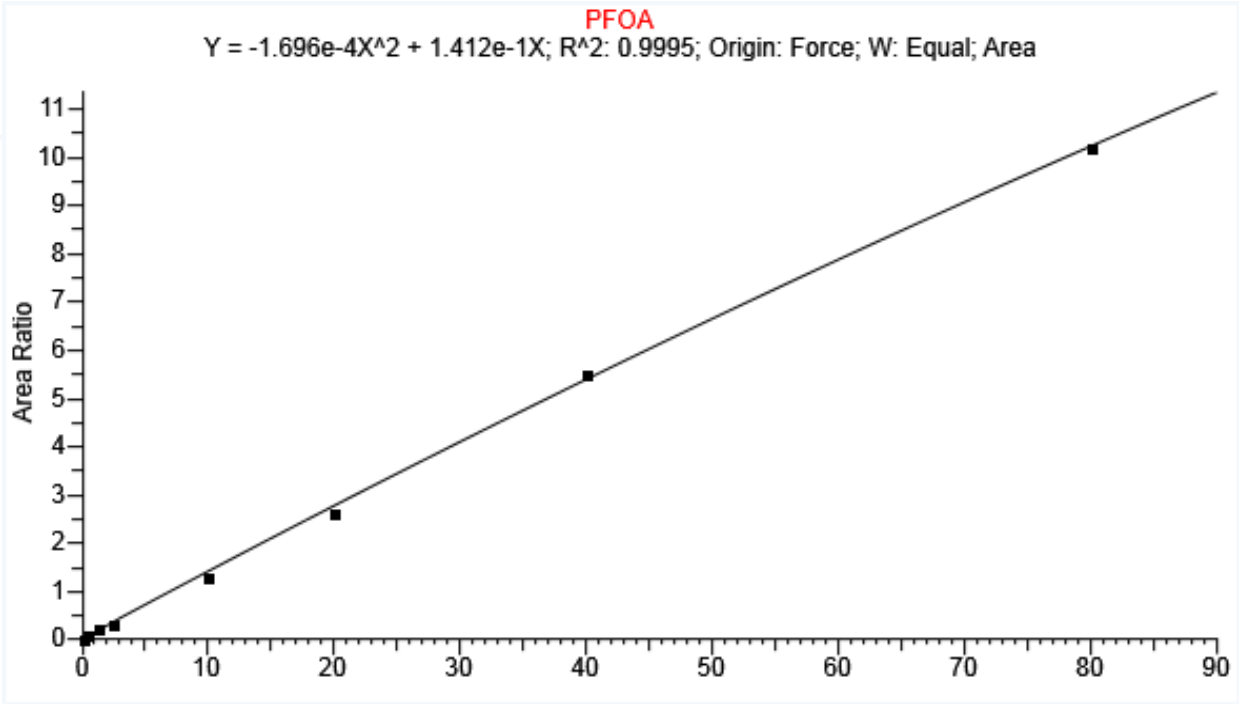
EPA 537 – FULL-MS at 70K resolution showing good peak shapes, and S/N for a 2.5ppt standard.

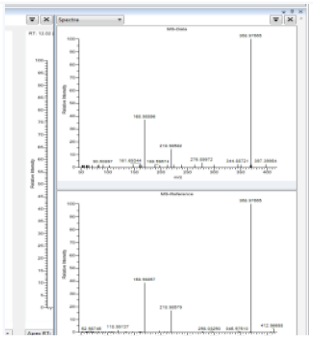




EPA 537M (PRM) – targeted-MS2 for PFOA at the lowest cal std 0.5ppt shown with spectra confirmation using Trace Finder 4.1





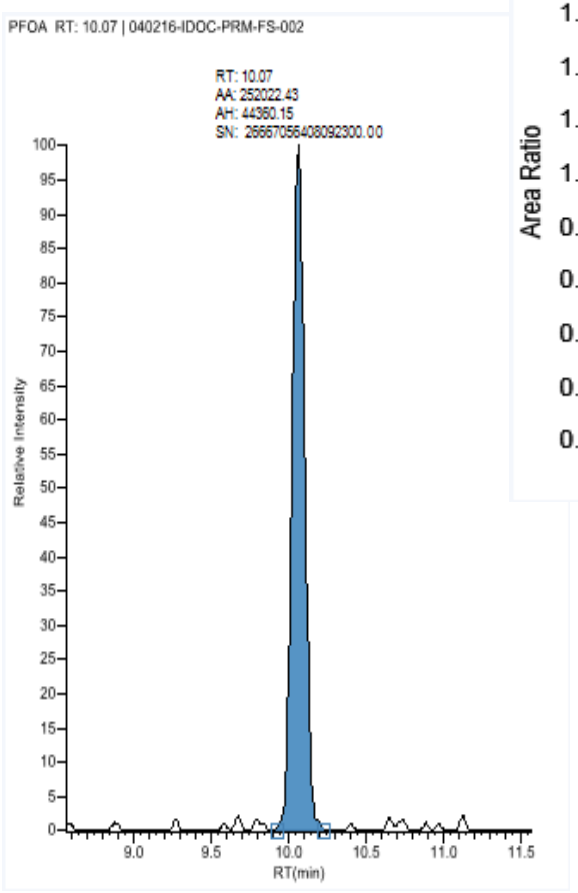


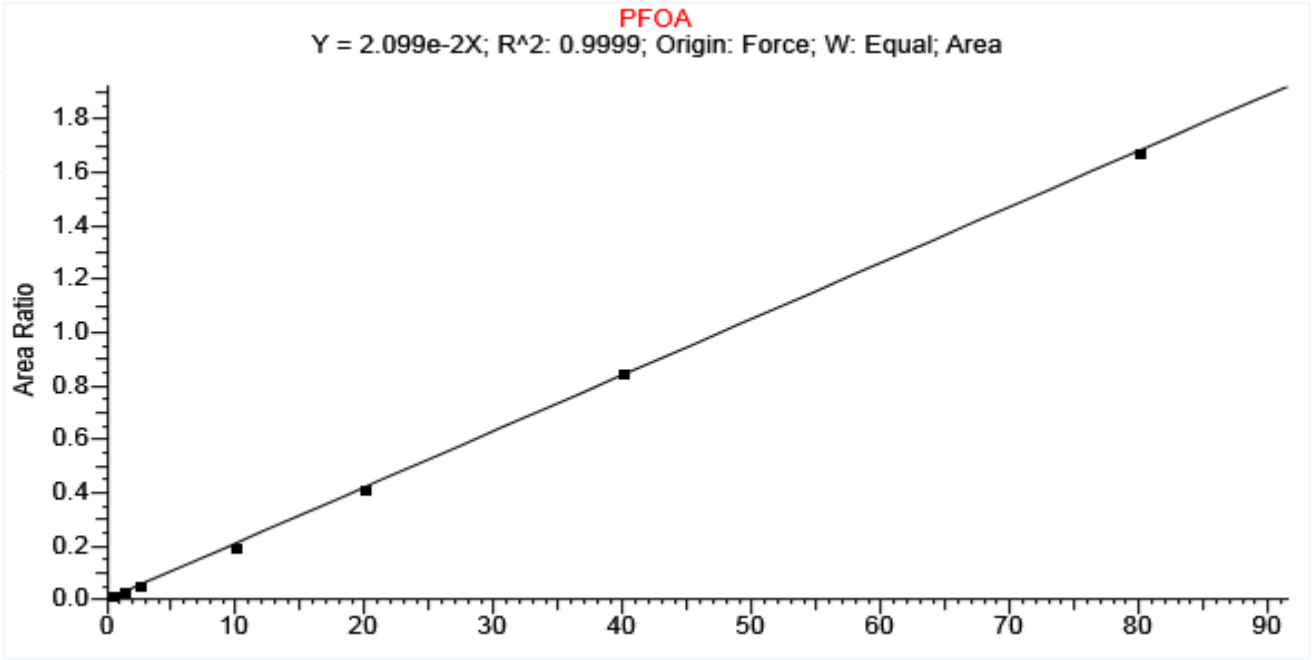
Sample MS2 Spectra

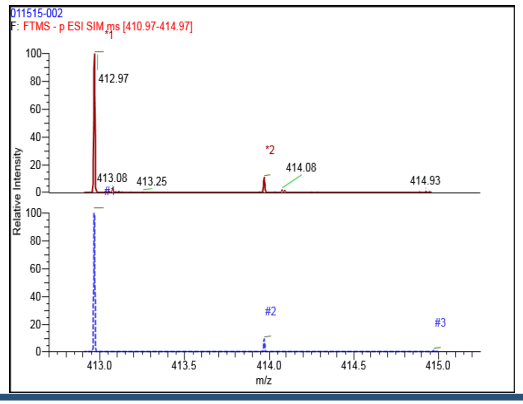
Library MS2 Spectra

By adding Full-Scan to PRM workflow – 0.5ppt PFOA shown









Isotopic pattern match

Determination of Minimum Reporting Limit Using LCMRL Using Regressional LOQ Calculation



Enter Test Data

Contaminant - 17 alpha-Ethynylestradiol

Method - EPA 539

Units for all measurements - Nanograms/Liter (ng/L)

****Important** Please do not truncate or round any of the results. If possible, results should have a minimum of 3 significant digits.**

Use the buttons below to add a concentration (column) or measured value (row). To remove a row or column check the box next to it and click the Remove button.

Add Concentration | Add Measurement | Remove

Concentrations

	Conc. 1	Conc. 2	Conc. 3	Conc. 4	Conc. 5	Conc. 6	Conc. 7	Conc. 8
	0.225	0.45	0.9	1.8	3.6	5.4	7.2	0
Measured Concentrations								
Value 1	0.184	0.347	0.684	1.361	2.657	4.469	6.07	0.003
Value 2	0.184	0.35	0.728	1.365	2.819	4.551	5.814	0.000
Value 3	0.202	0.36	0.705	1.363	2.66	4.323	6.046	0.000
Value 4	0.174	0.4	0.534	1.362	2.987	4.207	5.563	0.000

http://water.epa.gov/scitech/drinkingwater/labcert/analyticalmethods_ogwdw.cfm

<< Back | Next >>

NB – Calculator only works with 32 bit computers.

The LCMRL is defined as the lowest spiking concentration at which recovery of between 50 and 150 percent is expected 99 percent of the time by a single analyst. The procedure requires, at a minimum, four replicates at each of seven fortification levels. Four laboratory reagent blanks should also be included. All must be processed through the entire method procedure

Target & non targeted compounds EPA 537 plus.



PRM

EPA 537 PFCA's, and PFSA'S target list

LCMRL equal or better than high end - mid range triple quads – background contamination is the limiting factor.

	Critical level	DL	LCMRL
PFBS	0.077	0.12	<0.5
PFDA	0.18	<0.5	<0.5
PFDoA	0.14	0.29	<0.5
PFHpA		0.35	0.97
PFHxA	0.16	0.27	<0.5
PFHxS		0.52	0.77
PFNA	0.14	0.26	<0.5
PFOA		0.36	0.5
PFOS	0.14	0.21	<0.5
PFTA		0.48	0.71
PFTrDA	0.18	0.32	<0.5
PFuNA		0.31	0.72

Stock standard contained other compounds not part of EPA 537 target list which were identified and quantified using Full-MS

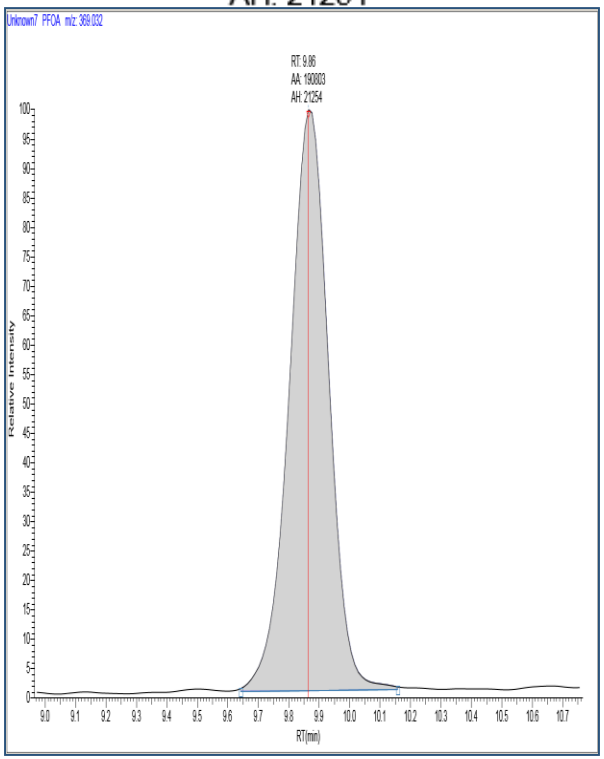
Full Scan

	Critical level	DL	LCMRL
PFBS	0.15	0.2	<0.5
PFDA	0.15	0.26	<0.5
PFDoA		0.47	0.73
PFHpA	0.09	0.15	<0.5
PFHxA	0.13	0.19	<0.5
PFHxS		1.7	2.4
PFNA	0.11	0.17	<0.5
PFOA		0.22	0.5
PFOS		0.26	0.5
PFTA	0.15	0.2	<0.5
PFTrDA		0.31	0.55
PFuNA		0.38	1
PFBA		0.19	0.64
PFODA		0.55	1
PFDS	0.13	0.19	<0.5
PFHxDA		0.12	0.5
PFPA	0.18	0.19	<0.5

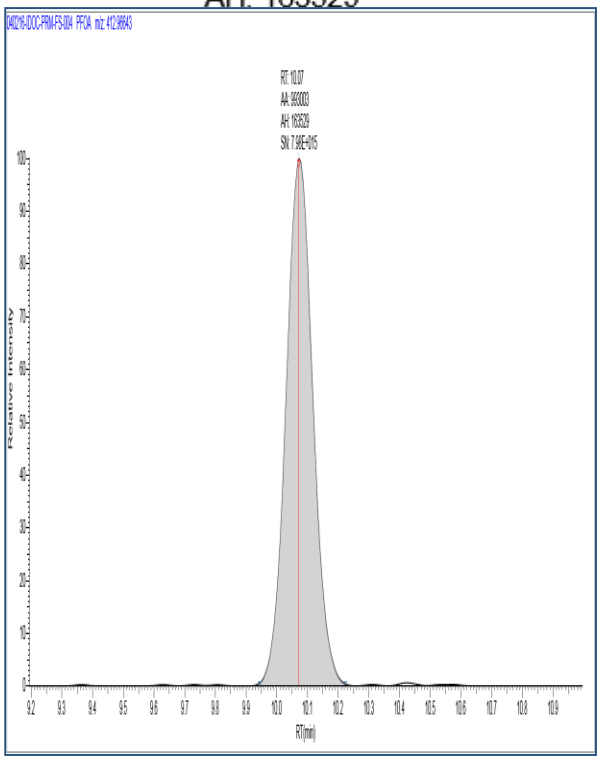
A 2.5ppt standard of PFOA. Excellent quantitation and sensitivity is obtained with HRAM in comparison to QQQ analysis.



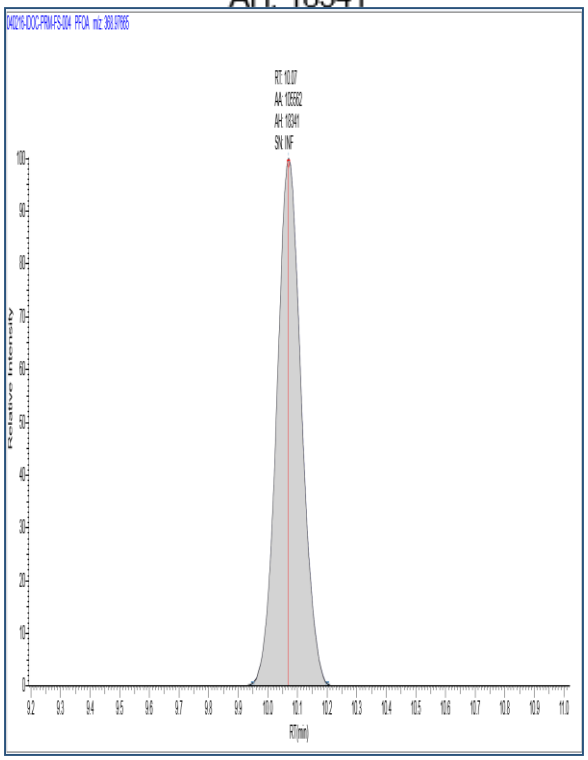
RT: 9.86
AA: 190803
AH: 21254



RT: 10.07
AA: 993003
AH: 163529



RT: 10.07
AA: 105562
AH: 18341



a) SRM Analysis-QQQ

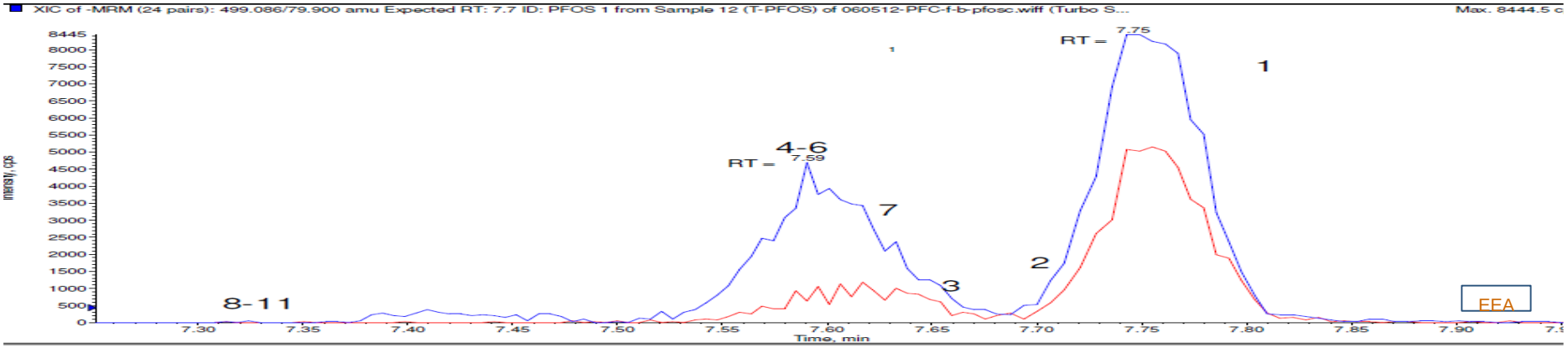
b) HRAM Full Scan

c) HRAM PRM

PFOS quantification is challenging: Showing a technical grade standard



Isomer	1	2	3	4	5	6	7	8	9	10	11
		MS/MS relative response factors									
m/z 499 m/z 99	100	117	97	49	39	43	78	10	0	0	19
m/z 499 m/z 80	100	0	78	135	241	142	123	113	118	220	90



1) $CF_3CF_2CF_2CF_2CF_2CF_2CF_2CF_2SO_3$

2) $CF_3CF_2CF_2CF_2CF_2CF_2CF(CF_3)SO_3$

3) $CF_3CF_2CF_2CF_2CF_2CF(CF_3)CF_2SO_3$

4) $CF_3CF_2CF_2CF_2CF_2CF(CF_3)CF_2CF_2SO_3$

5) $CF_3CF_2CF_2CF_2CF_2CF_2CF(CF_3)SO_3$

6) $CF_3CF_2CF_2CF_2CF_2CF_2CF(CF_3)CF_2SO_3$

7) $CF_3CF_2CF_2CF_2CF_2CF_2CF(CF_3)CF_2CF_2SO_3$

8) $(CF_3)_3CF_2CF_2CF_2CF_2CF_2CF_2SO_3$

9) $CF_3CF_2CF_2CF_2CF_2CF_2CF(CF_3)SO_3$

10) $CF_3CF_2CF_2CF_2CF_2CF_2CF(CF_3)CF_2SO_3$

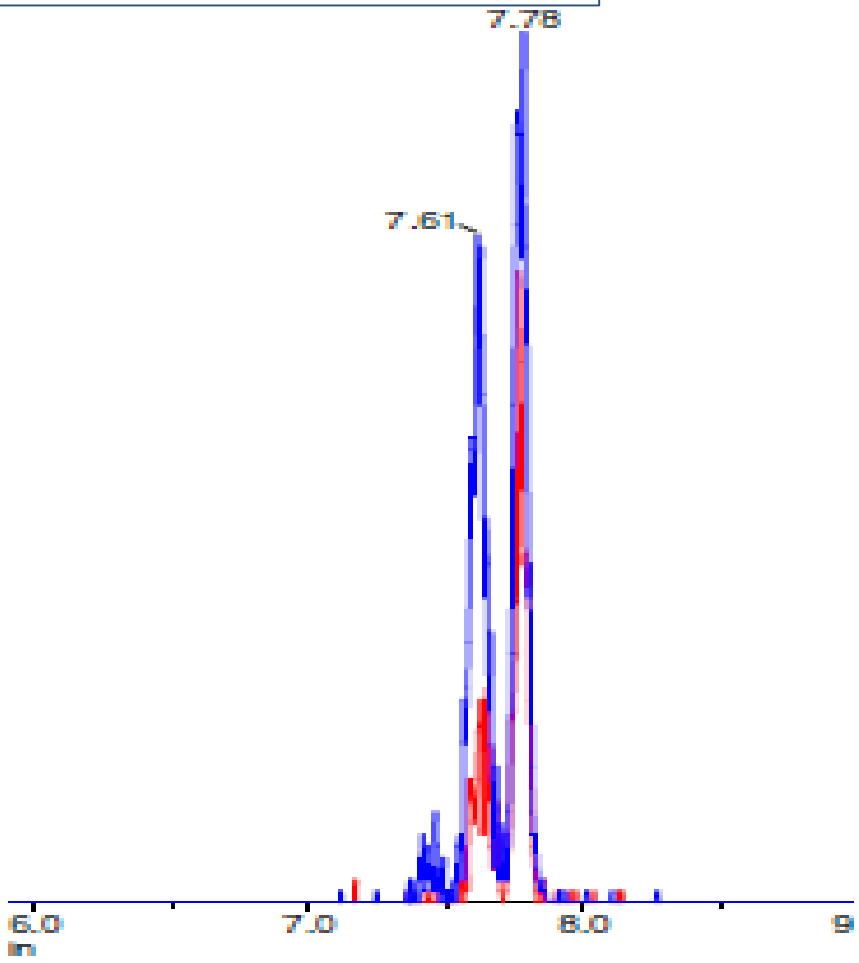
11) $CF_3CF_2CF_2CF_2CF_2CF(CF_3)CF_2CF_2SO_3$

Nicole Riddell etal 2009

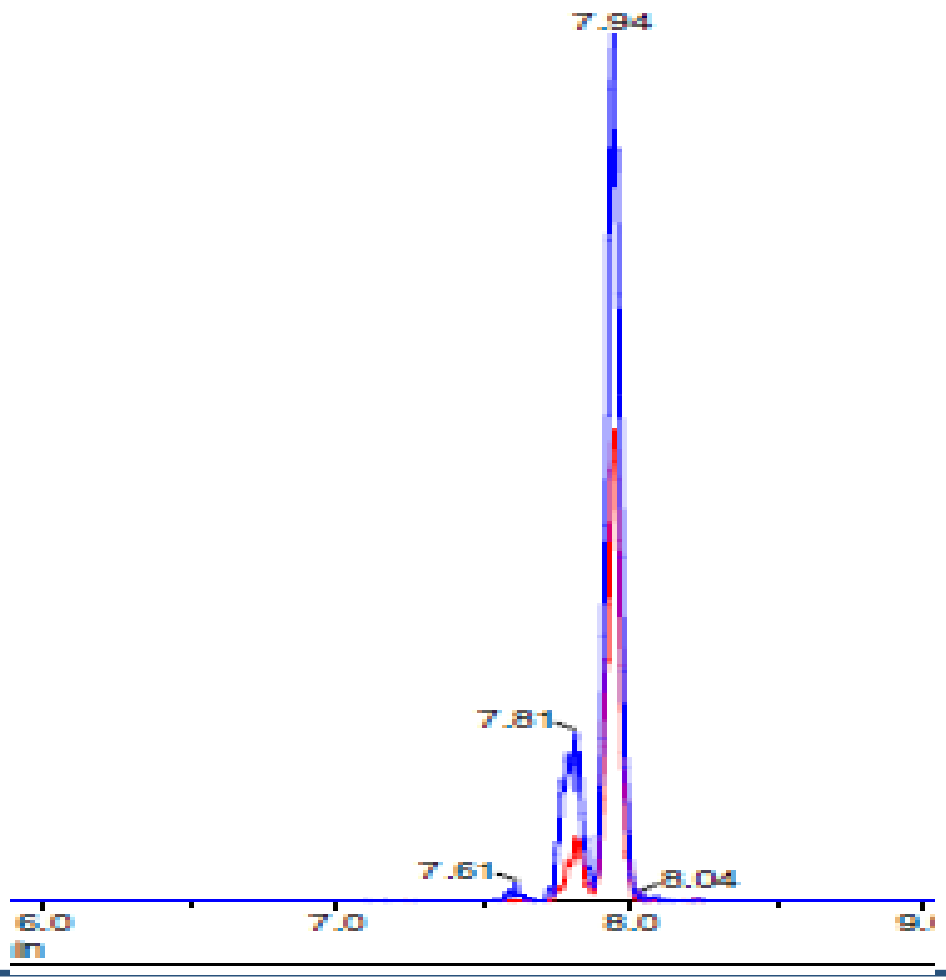
Samples from different locations can have different branch ratios



Field Sample

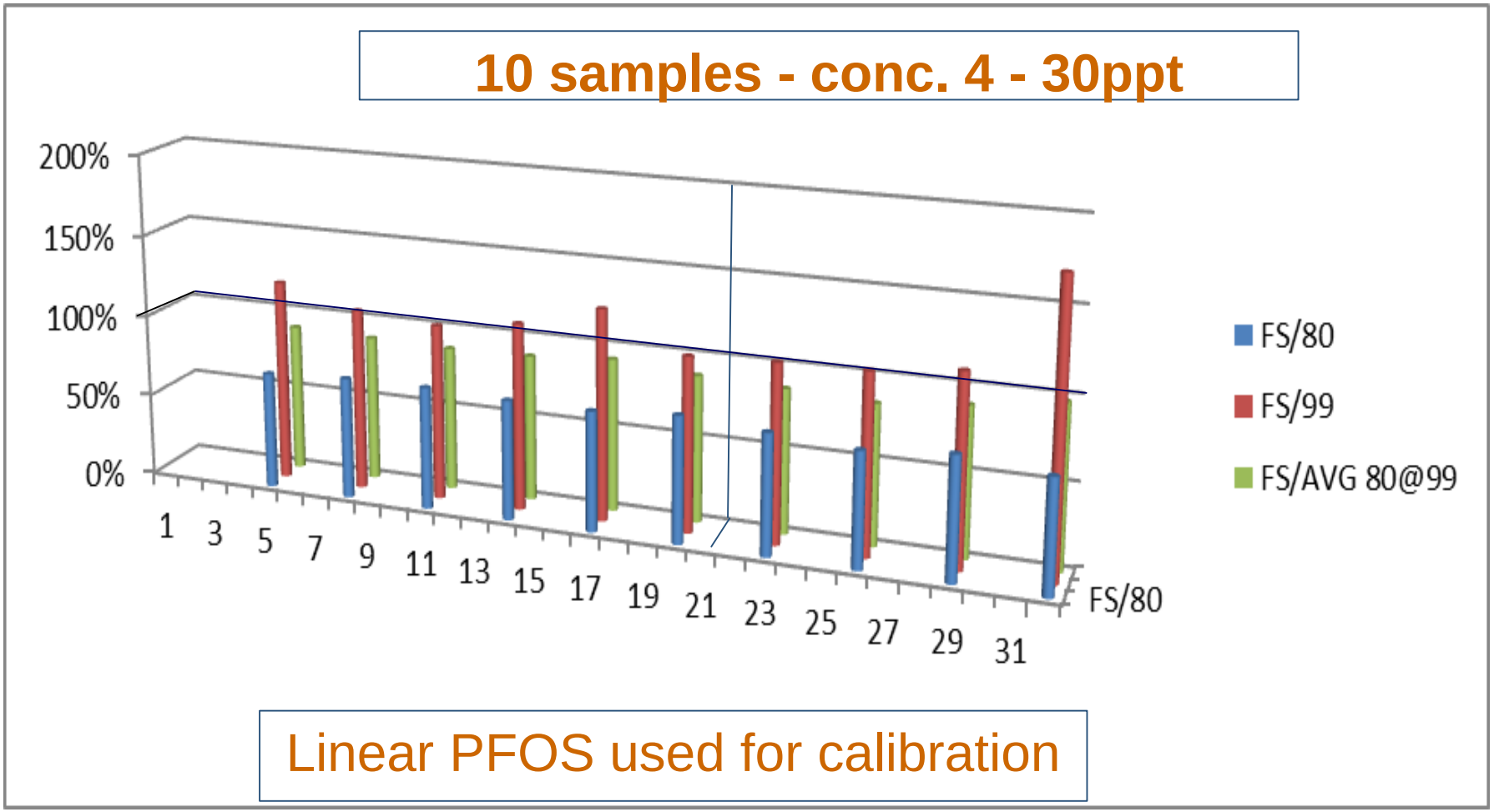


Std PFOS Branched



Fs scan covers all of the branches and looks to be more reliable for PFOS quantitation.

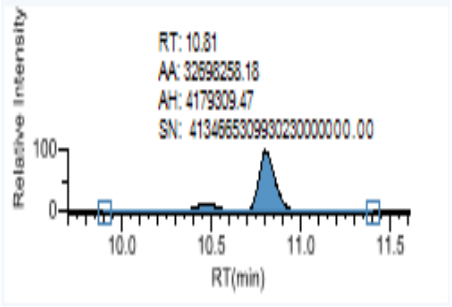


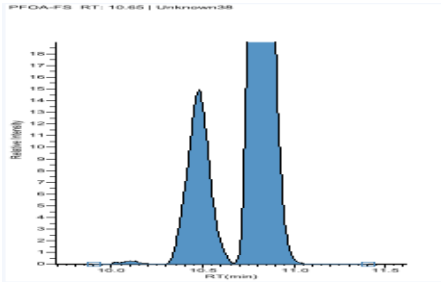




United States Environmental Protection Agency

Technical Advisory- Laboratory Analysis of Drinking Water Samples for Perfluorooctanoic Acid (PFOA) Using EPA Method 537 Rev. 1.1



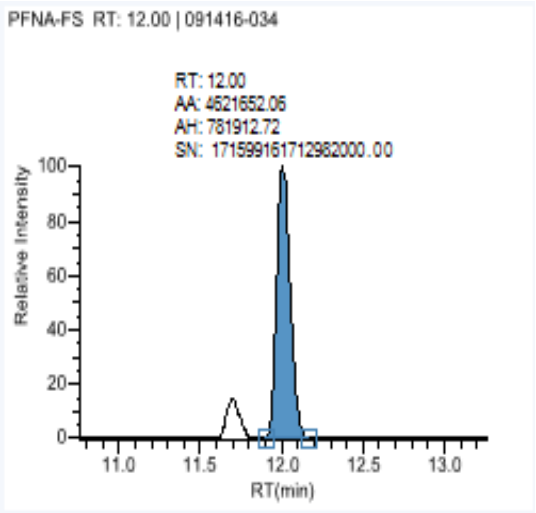


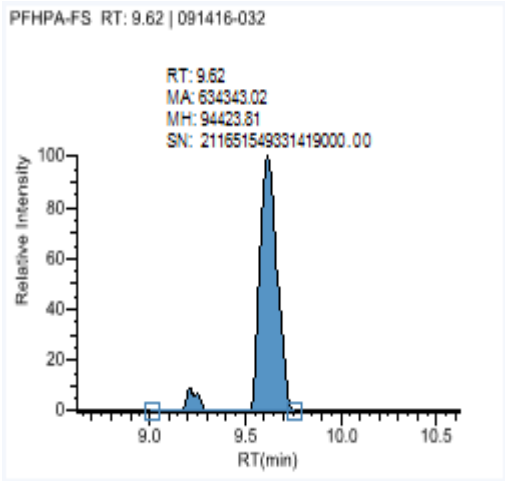
Office of Water (MS-140) EPA 815-B-16-021 September 2016

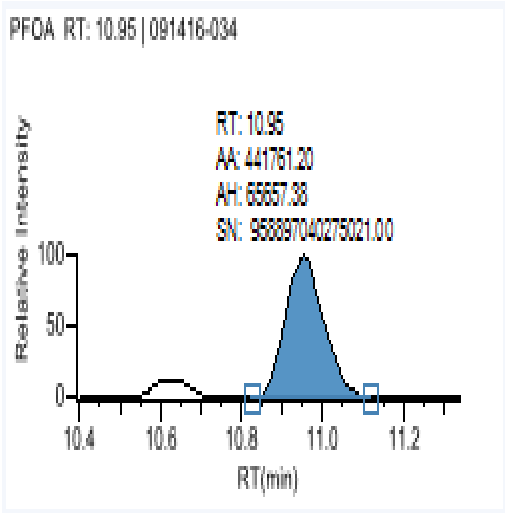
Till around 1970 PFOA was also produced by Electrochemical fluorination (ECF) process creating branched isomers which can still be detected occasionally so it is important to also integrate the branch isomers.

Full Scan 70000 resolution









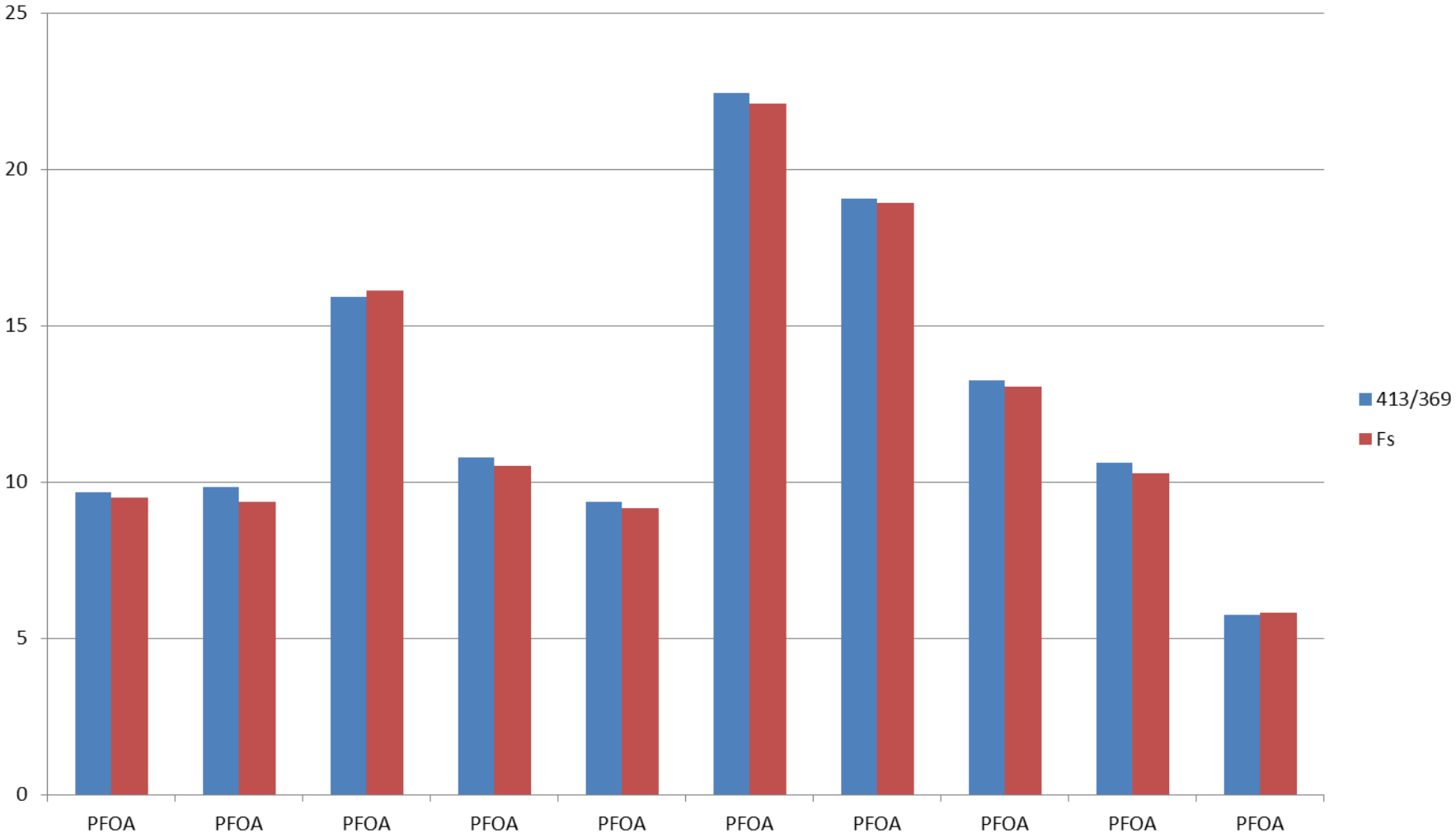
PFNA

PFHpA

PFOA

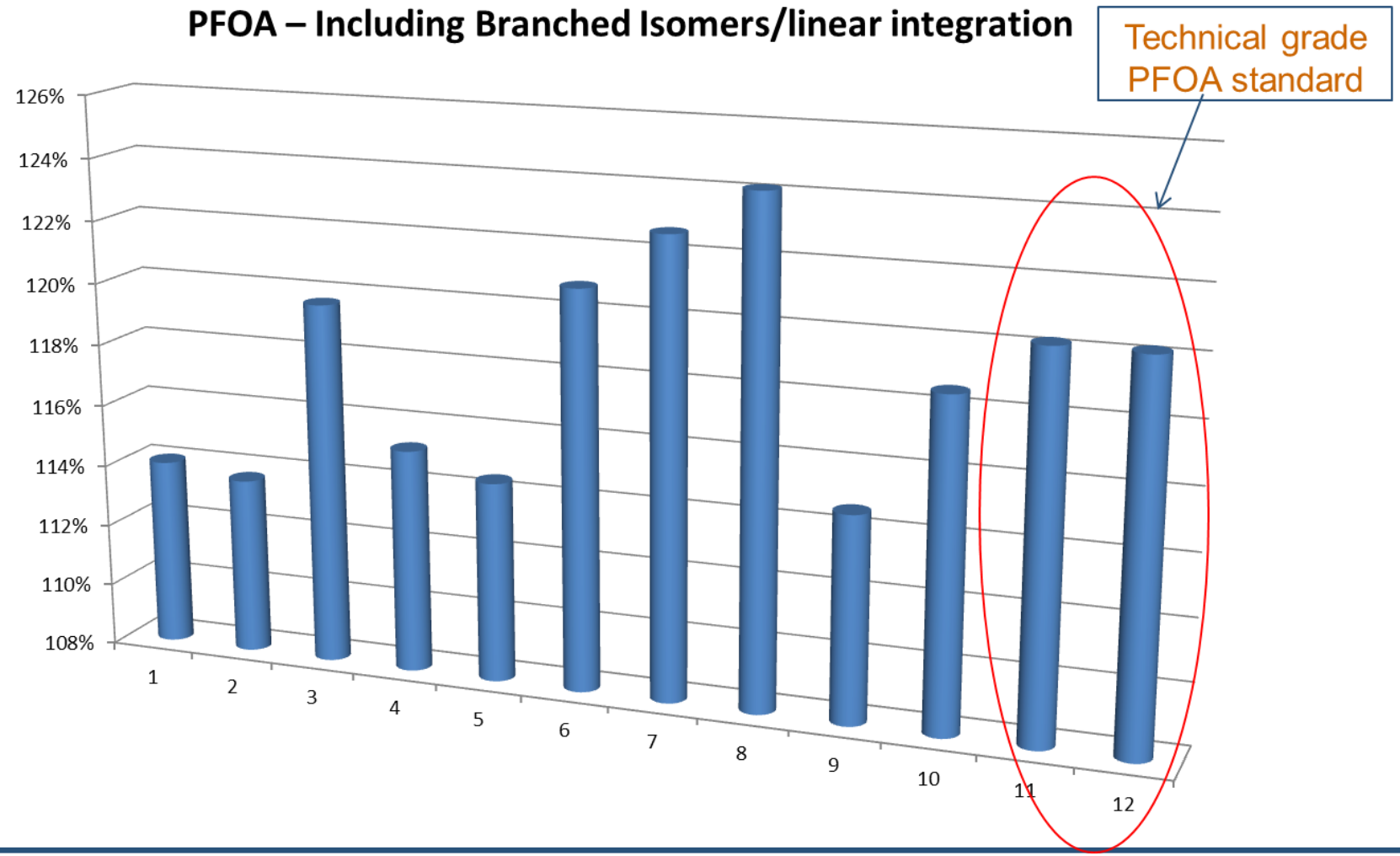
FS and 413/369 compares well in real field samples having branched PFOA - ppt





Including branched isomers when present about 20% difference in a technical grade

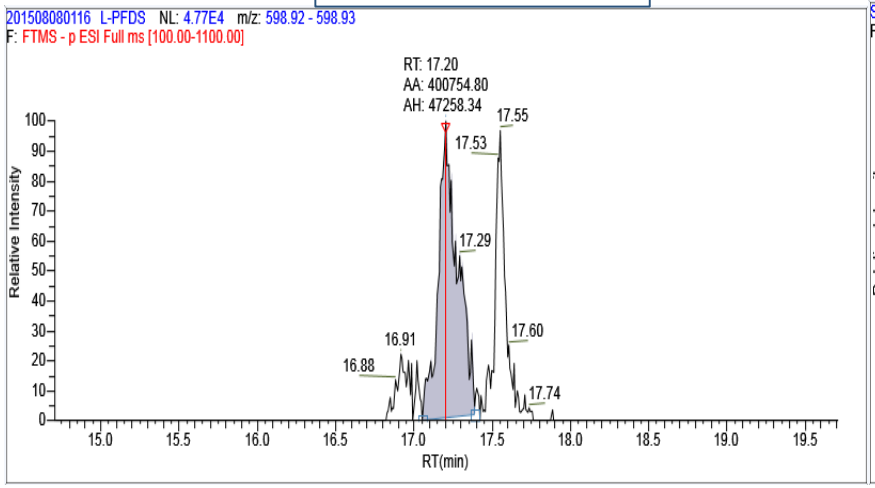




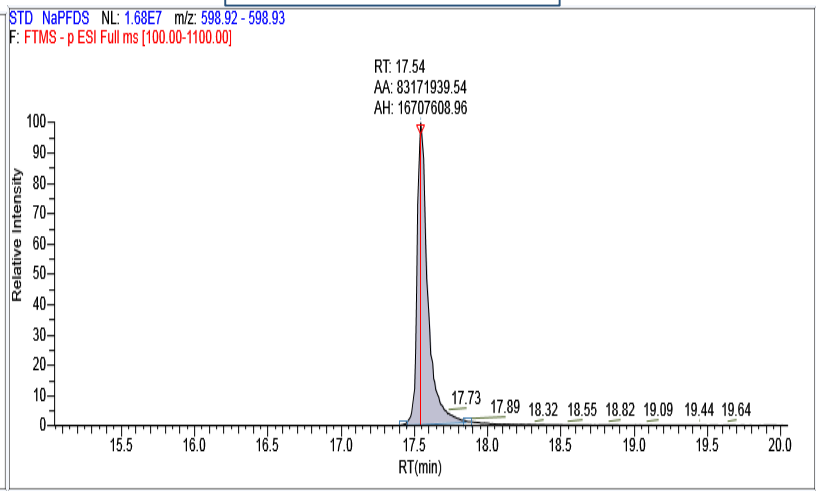
A UCMR3 sample shown having a trace hit for non-targeted known compound: PFDS

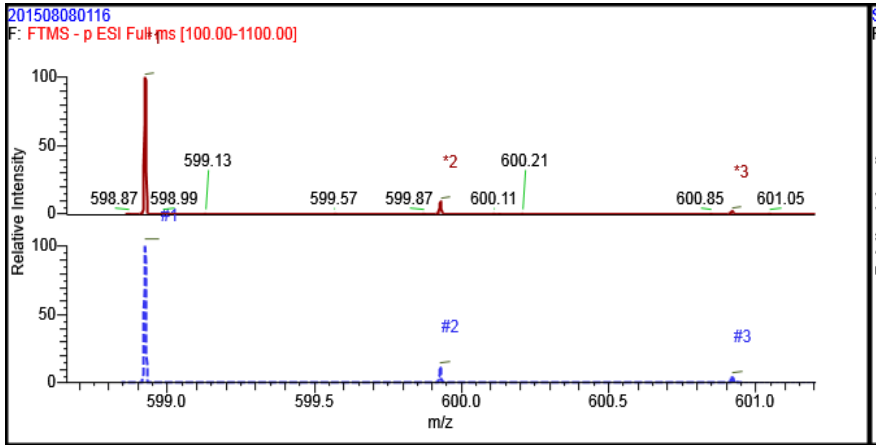


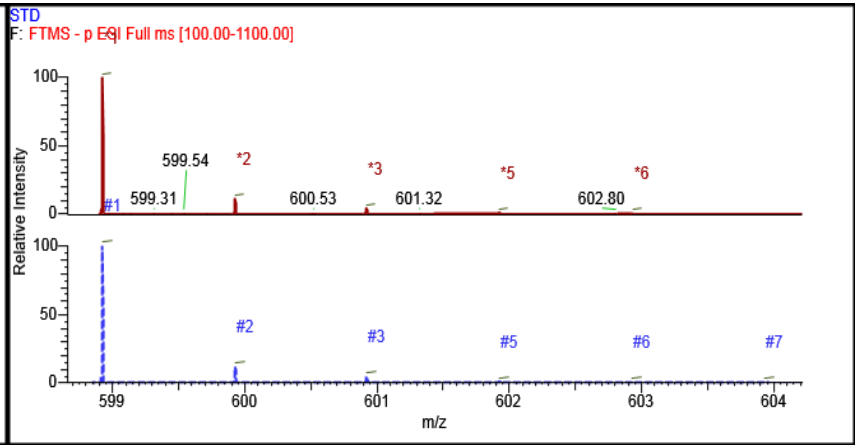
Sample



80 ppt STD

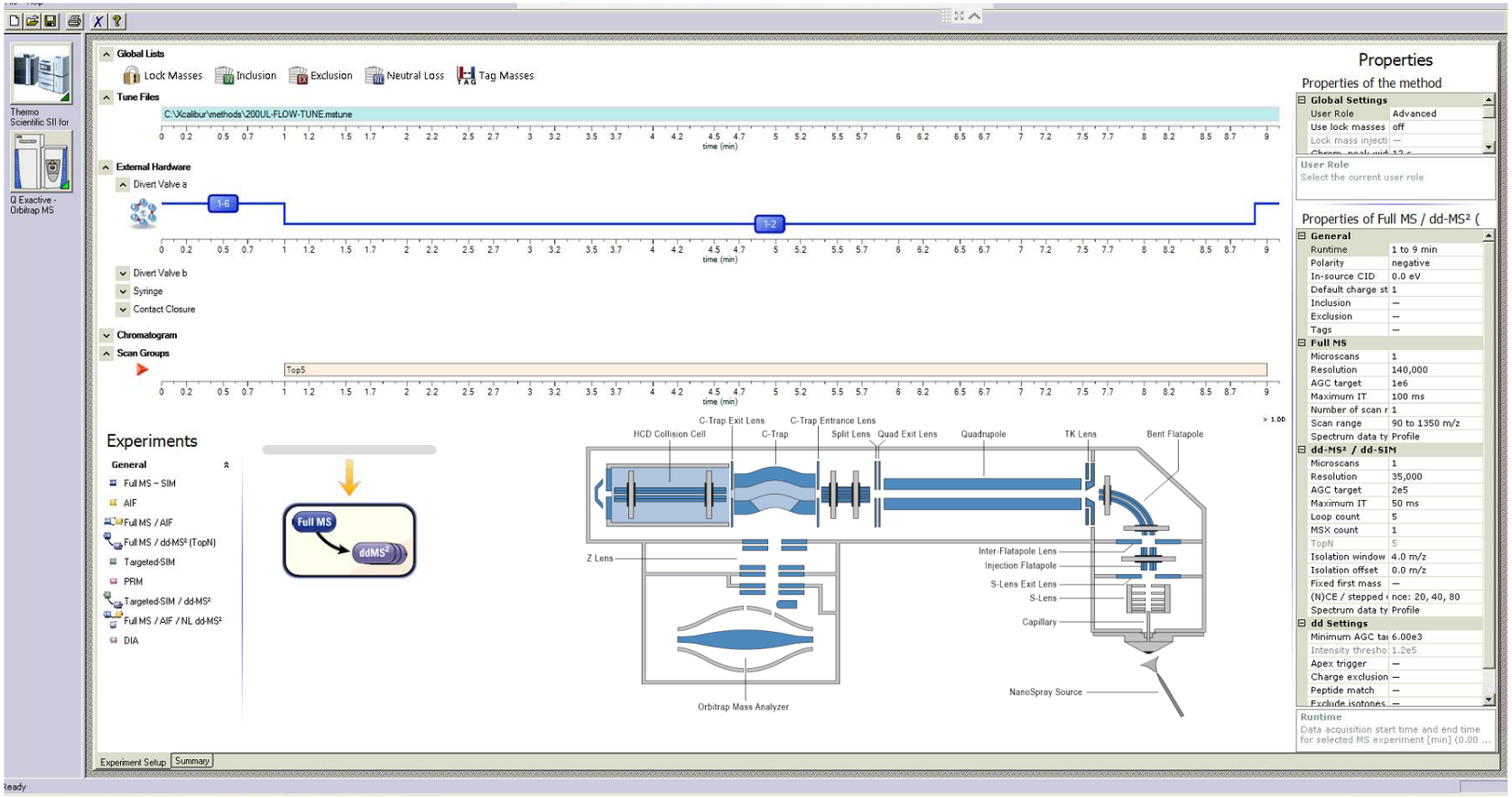






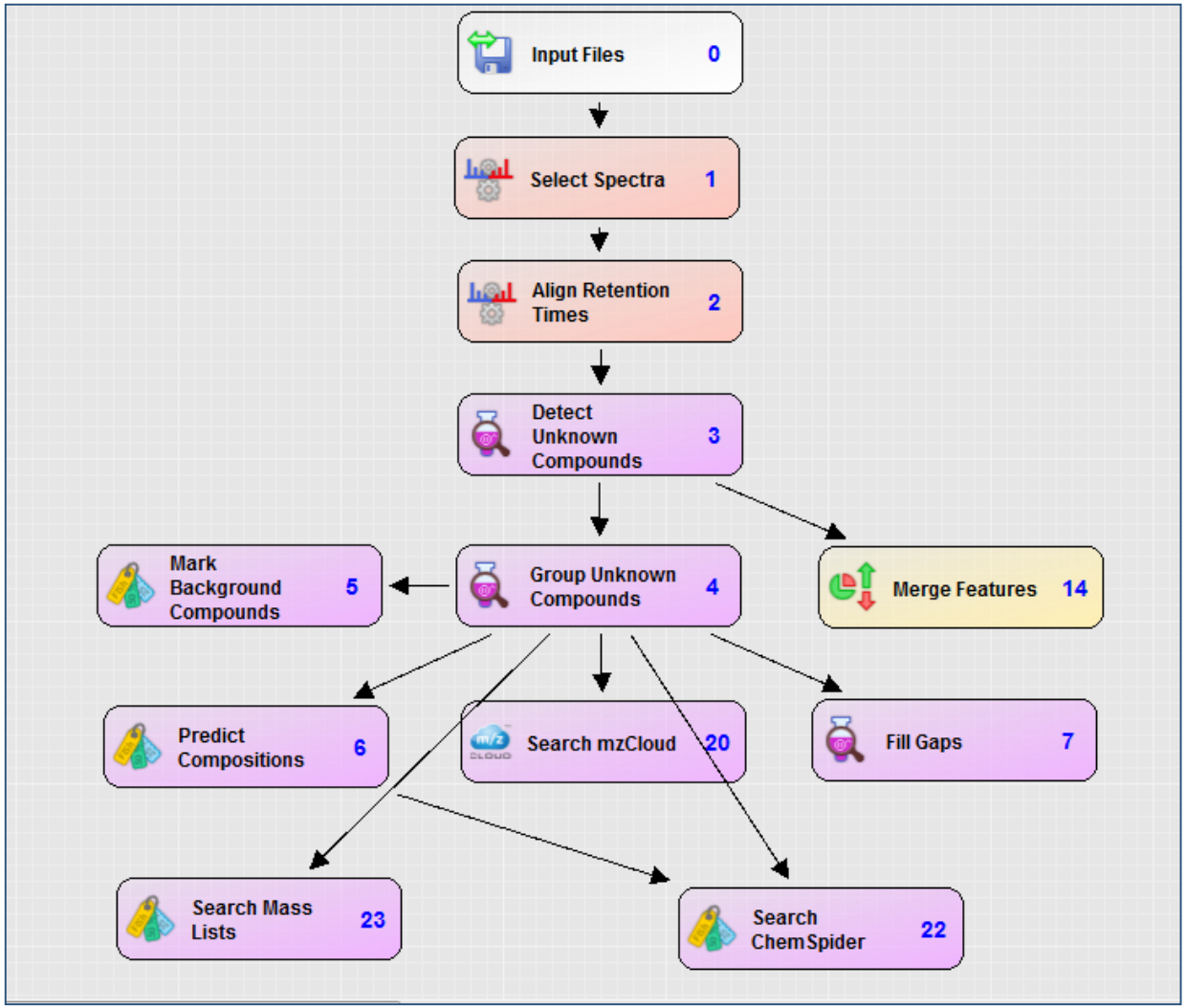
Looking for Unknowns : Contaminated GW in a AFFF site: On-line SPE UHPLC / Fs-ddms2, top 5





Data mining software using “Compound Discoverer”

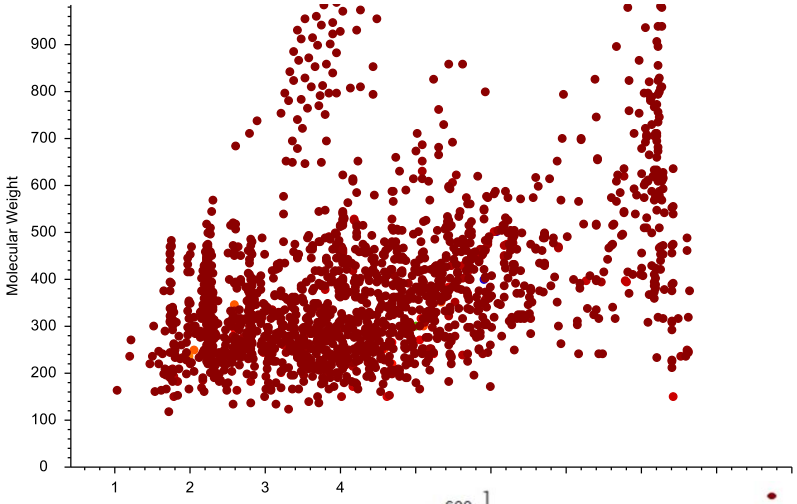




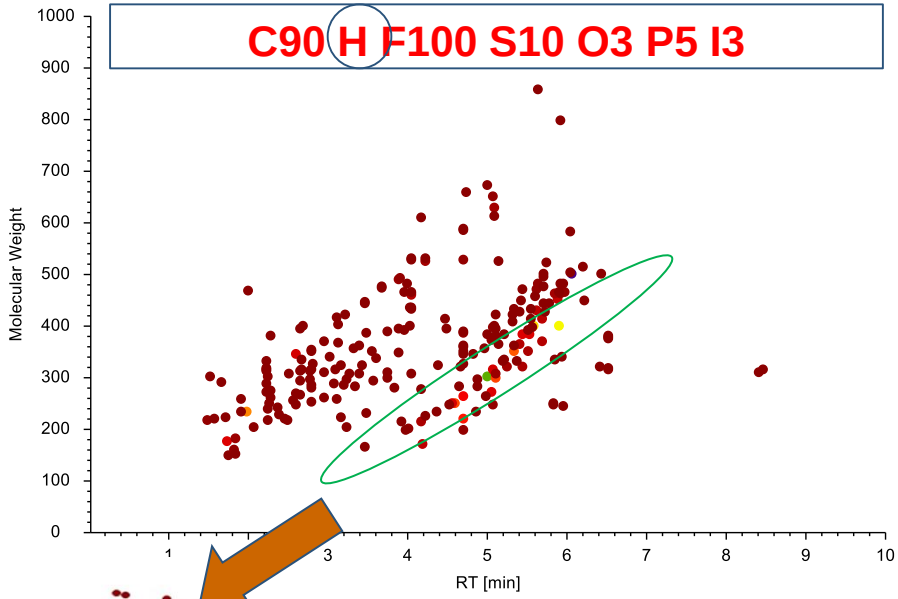
Refining data to explore known classes of compounds

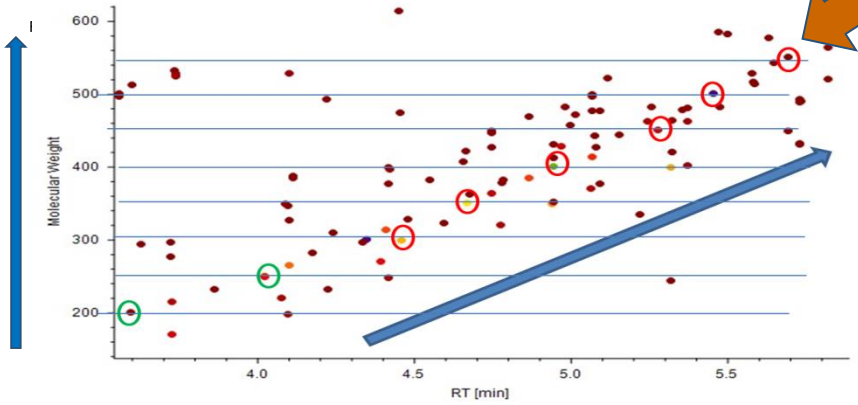


C90 H100 F100 S10 O10 P5 I3



C90 H F100 S10 O3 P5 I3





Pattern MW vs RT

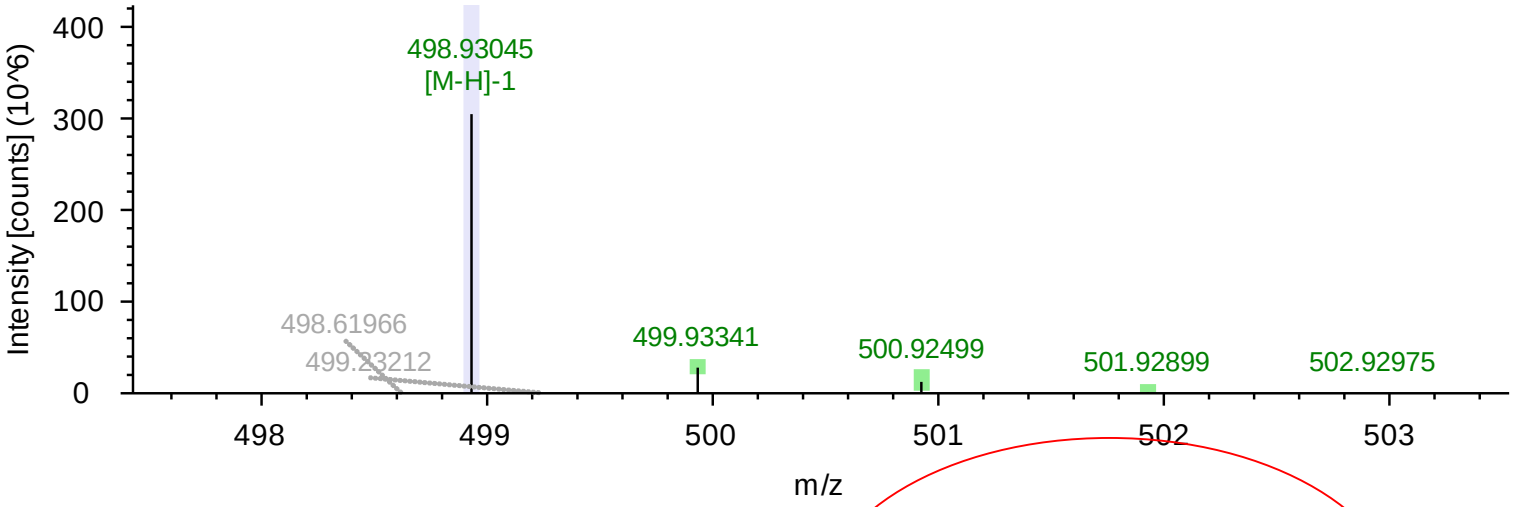
- RDB
- Isotopic pattern
- Possible formula
- Mass defect
- Mass range
- Etc.

At higher resolutions more trace isotopic patterns can be used for MS scans.

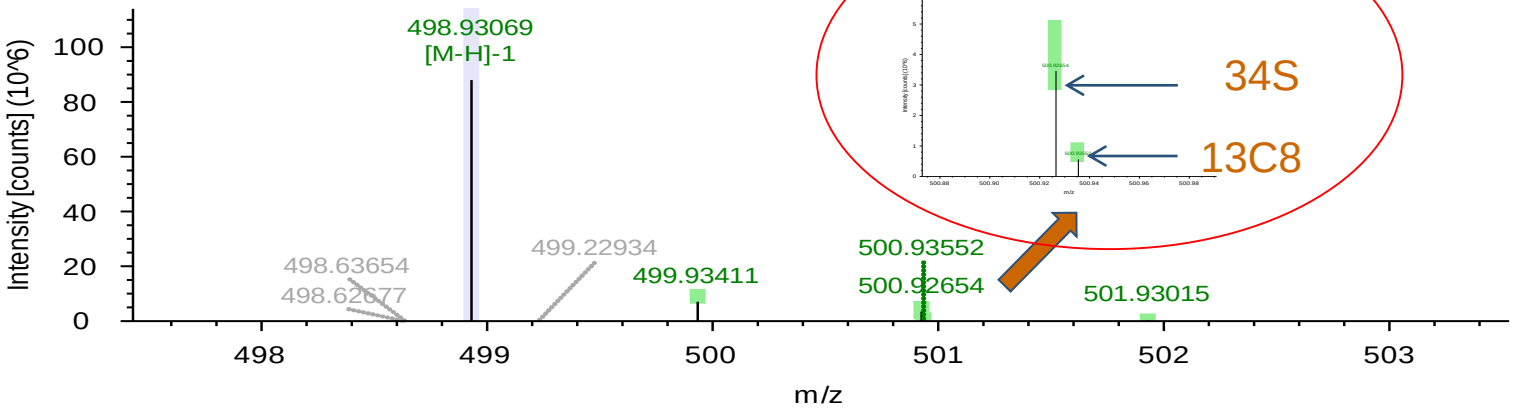


201605240377R, #2094, RT=5.457 min, FTMS (-)
C8 H F17 O3 S as [M-H]-1

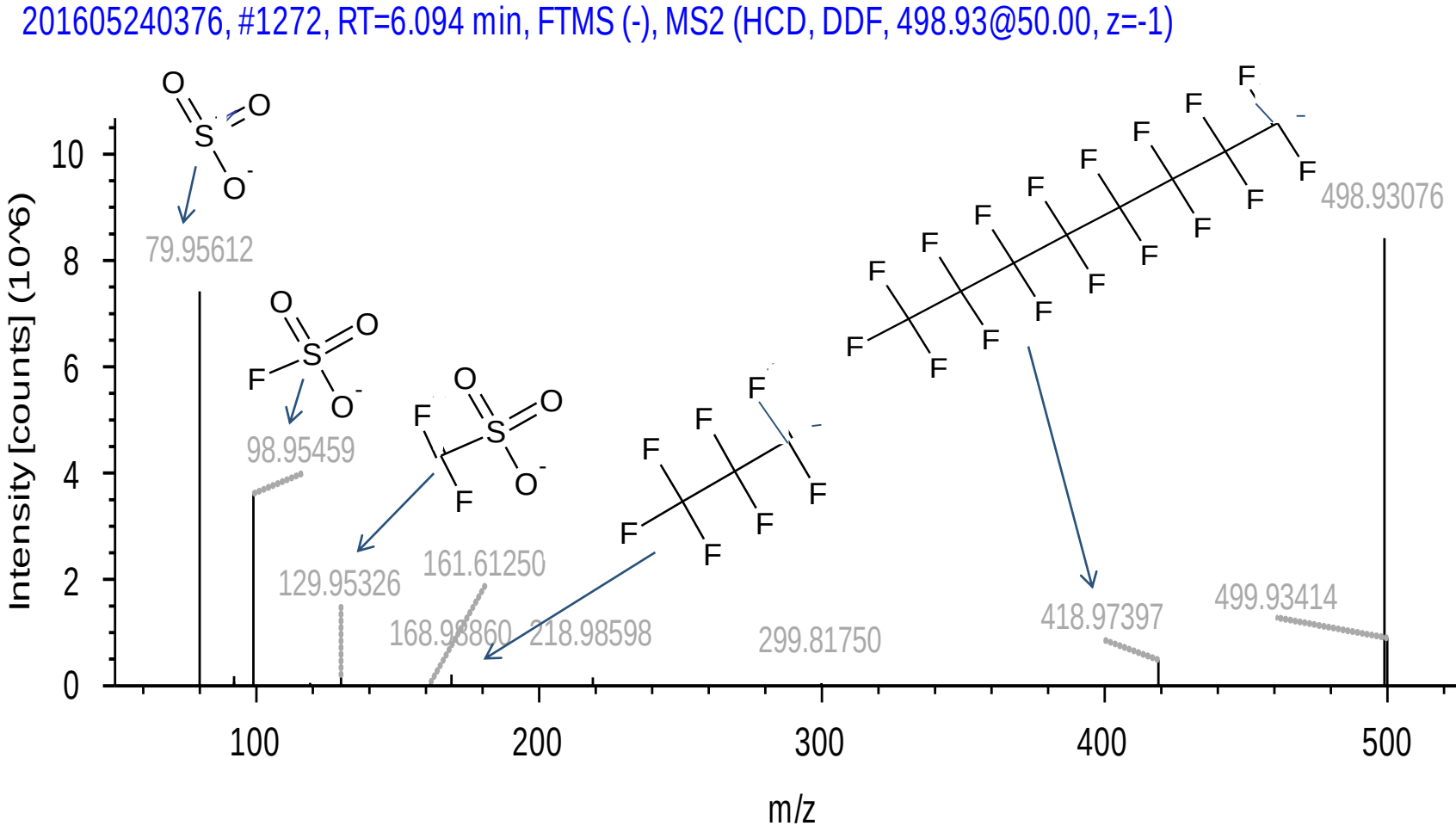
70K Res.



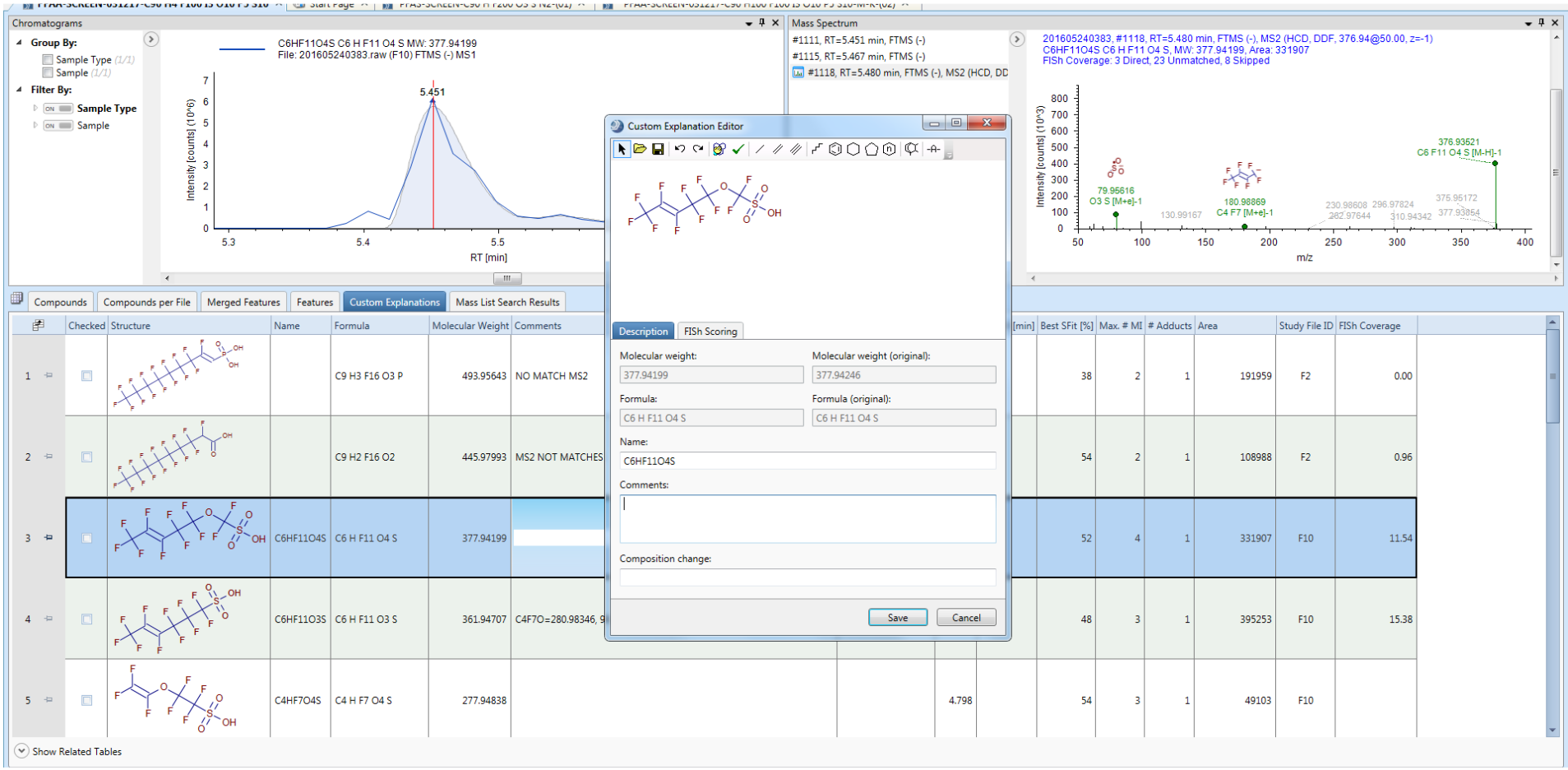
140K Res.



Ms2 Data Shows CmF2m+1- “9 series” and CnF2nSO3- radical “0 series” as expected.



The structure of the selected compound can be drawn in “Custom Explanation” using Mass Frontier to check against MS and MS2 collected data



Summary of all PFAS's found for the studied group.



Compound Discoverer 2.0.0.303

File Reporting Libraries View Help

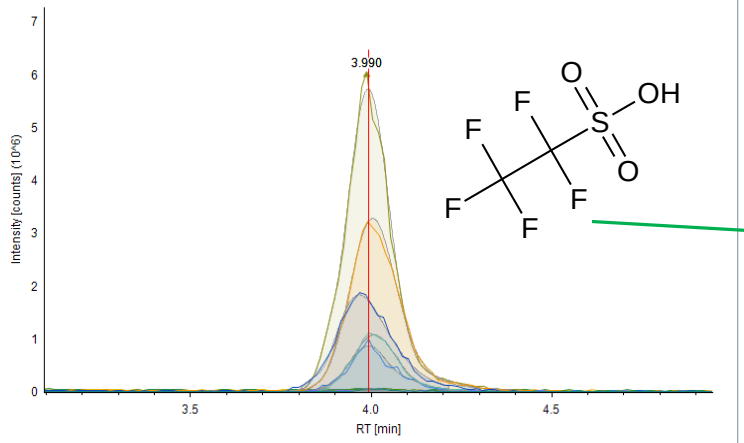
PFAA-SCREEN-031217-C90 H4 F100 I3 O10 P5 S10 | PFAS-SCREEN-(01) | PFAA-SCREEN-031217-C90 H100 F100 I3 O10 P5 S10-M-K-(02) | PFAA-SCREEN-031217-C90 H100 F100 I3 O10 P5 S10 | Start Page | PFAS-SCREEN-C90 H F200 O3 S N2-(01) | PFAS-SCREEN-C90 H F200 O3 S N2

Chromatograms

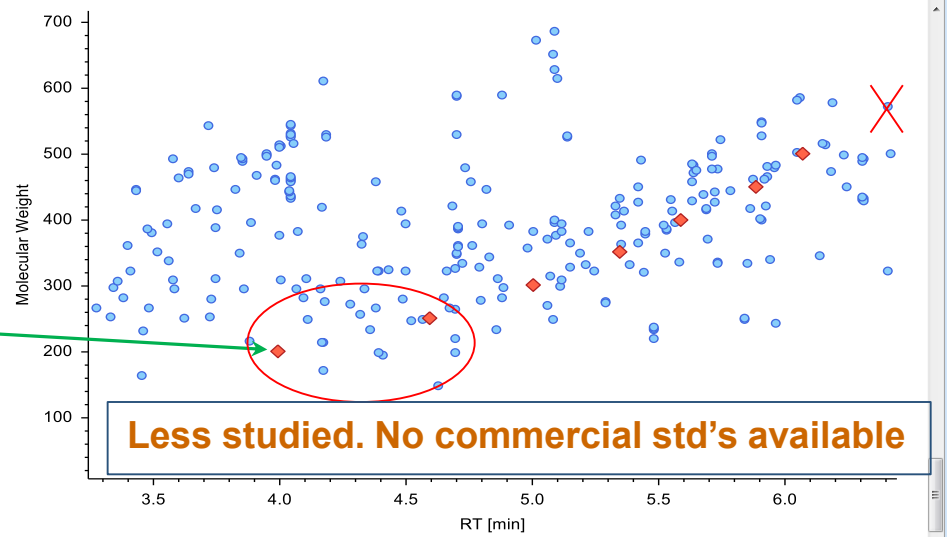
Group By: Sample Type (2/3) Sample (17/18)

Filter By: Sample Type Sample

MW: 199.95624 File: 201603160389.raw (F1) FTMS (-) MS1
MW: 199.95624 File: 201605240375.raw (F2) FTMS (-) MS1
MW: 199.95624 File: 201605240376.raw (F3) FTMS (-) MS1
MW: 199.95624 File: 201605240377.raw (F4) FTMS (-) MS1
MW: 199.95624 File: 201605240378.raw (F5) FTMS (-) MS1
MW: 199.95624 File: 201605240379.raw (F6) FTMS (-) MS1



Mass Spectrum



Less studied. No commercial std's available

Compounds | Compounds per File | Merged Features | Features | Custom Explanations | Mass List Search Results

	Checked	Name	Predicted Formula	Molecular Weight	RT [min]	Area (Max.)	Mass List Matches	Pattern Matches	Group Areas F1*	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F18
1	☑	pentafluoroethane-1-sulfonic acid	C2 H F5 O3 S	199.95624	3.993	897880			6.56e3	3.77e3	3.53e5	5.62e5	3.78e3	1.71e5	3.52e3	6.60e3	1.34e5	8.98e5	9.75e3	1.41e3	2.24e3	1.69e3	3.92e3	6.65e3	5.20e2
2	☑	heptafluoropropane-1-sulfonic acid	C3 H F7 O3 S	249.95345	4.594	9850756			1.46e5	2.79e4	2.23e6	4.73e6	4.43e2	1.41e6	9.97e3	6.62e4	1.62e6	9.85e6	3.19e4	6.06e4	1.90e4	5.49e4	8.90e4	1.08e3	1.90e2
3	☑	nonafluorobutane-1-sulfonic acid	C4 H F9 O3 S	299.95035	5.009	25056204			9.37e5	2.25e7	2.32e7	1.06e7	3.07e5	1.80e7	4.32e5	9.04e5	4.45e6	2.51e7	5.36e5	8.50e5	4.78e5	7.48e5	9.34e5	3.64e5	7.82e5
4	☑	undecafluoropentane-1-sulfonic acid	C5 H F11 O3 S	349.94728	5.347	10657640			6.02e5	8.68e4	3.04e6	1.02e7	2.69e3	2.77e6	5.95e4	1.96e5	2.67e6	1.07e7	2.43e5	5.02e5	1.76e5	4.40e5	6.24e5	3.15e2	7.94e2
5	☑	tridecafluorohexane-1-sulfonic acid	C6 H F13 O3 S	399.94405	5.589	18042640			2.33e6	2.89e6	1.69e7	1.66e7	3.68e4	3.66e6	1.45e6	8.08e5	4.00e6	1.80e7	4.16e5	2.82e6	6.21e5	1.59e6	2.97e6	1.04e4	4.74e5
6	☑	pentadecafluoroheptane-1-sulfonic acid	C7 H F15 O3 S	449.94102	5.887	3384802			1.41e5	1.34e5	1.03e6	3.38e6	3.80e3	8.97e5	2.89e4	2.56e3	2.16e4	1.94e6	2.78e3	2.11e5	1.42e4	8.98e4	2.62e5	3.03e2	1.93e2
7	☑	heptadecafluorooctane-1-sulfonic acid	C8 H F17 O3 S	499.93788	6.075	47989212			1.83e6	3.96e6	4.80e7	2.48e7	8.57e4	8.80e6	7.04e5	8.21e3	1.19e4	2.11e7	1.76e4	3.39e6	2.21e5	1.36e6	3.54e6	1.27e4	2.17e5

Show Related Tables

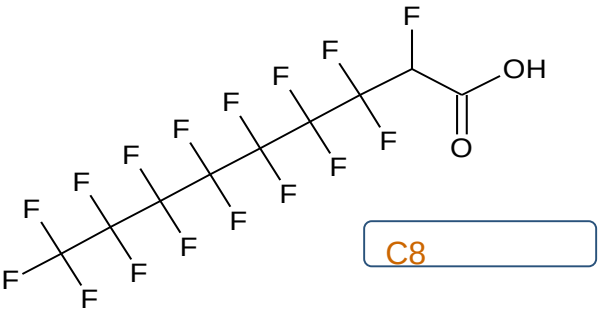
C2 TO C8 PFSA's

Study sample / area

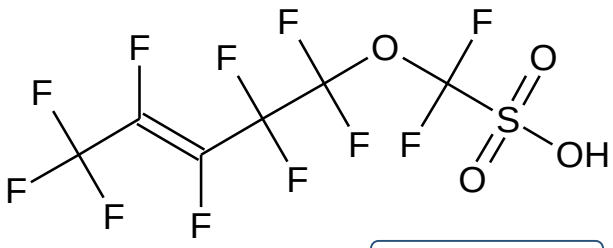
Std column / area

A few identified suspects using prescribed workflow which were missing from the built in library. Some need additional confirmations.

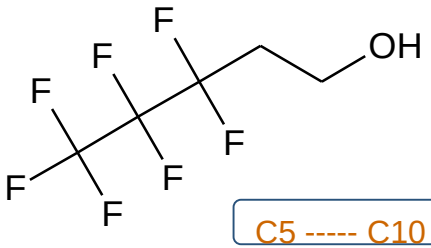



C8

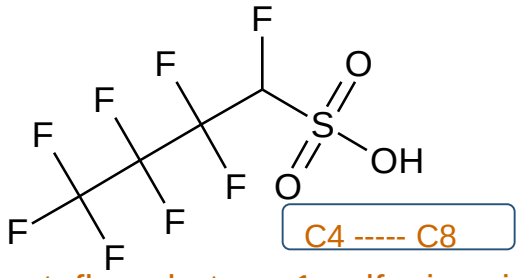
hexadecafluorononanoic acid


C5 ----- C8

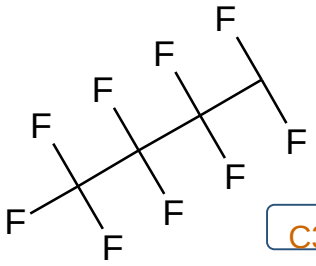
difluoro{[(3E)-1,1,2,2,3,4,5,5,5-nonafluoropent-3-en-1-yl]oxy}methanesulfonic acid


C5 ----- C10

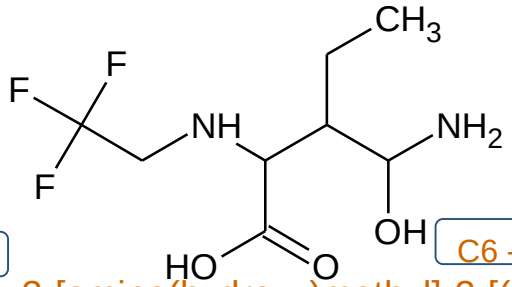
3:2 FTOH


C4 ----- C8

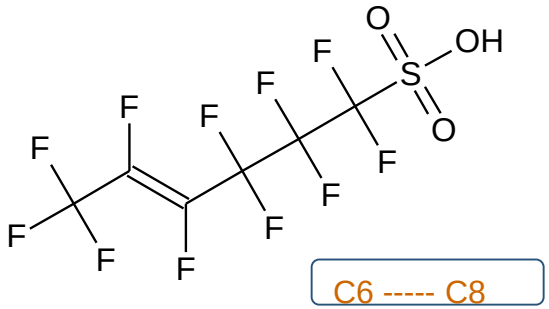
octafluorobutane-1-sulfonic acid


C3 ----- C8

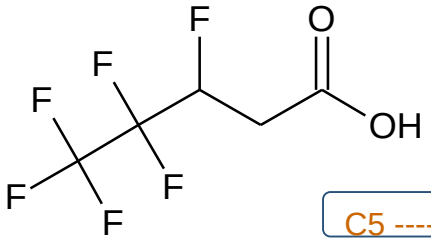
nonafluorobutane


C6 ----- C8

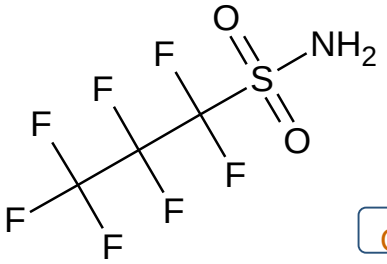
3-[amino(hydroxy)methyl]-2-[(2,2,2-trifluoroethyl)amino]pentanoic acid


C6 ----- C8

undecafluorohex-4-ene-1-sulfonic acid


C5 ----- C8

3:2 FTA


C2 ----- C8

heptafluoropropane-1-sulfonamide

Conclusions

- Q-Exactive HRAM instrumentation in the PRM scan mode can be used for quantitation with performance like a triple quadrupole in SRM mode with added specificity, selectivity and comparable sensitivity.
- Full scan HRAM can likely produce more accurate quantitative data for compounds that contain branched isomers such as PFOS.
- Routine quantitative workflows and non-target analysis can be performed in a single analysis.
- HRAM data processing using Thermo Fisher Scientific Compound Discoverer software can simplify complex data reduction/save time.
- Other techniques may be necessary for further confirmation of suspects/unknowns structures such as MS^n, 13C and 19F NMR, when standards are not commercially available.

Any Questions?

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