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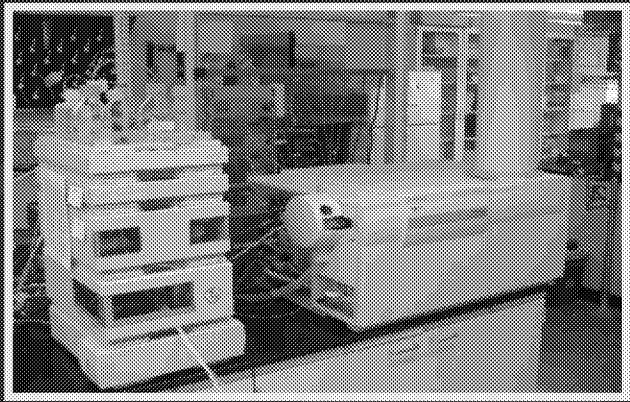
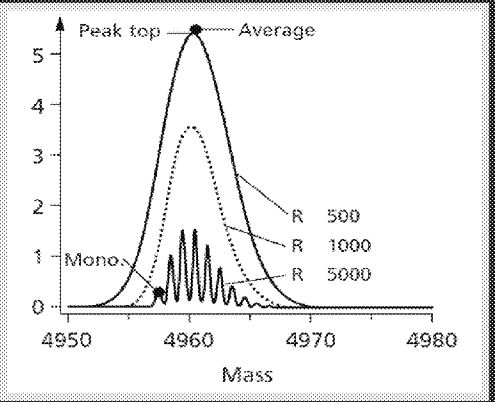
Determination of perfluoroalkyl ether carboxylic acids (PFECAs) and sulfonic acids (PFESAs) in North Carolina surface water using high resolution mass spectrometry

Mark Strynar, Rebecca McMahen, Shuang Liang, Sonia Dagnino, Andrew Lindstrom, Erik Andersen, Larry McMillan, Michael Thurman, Imma Ferrer, Carol Ball

35th SETAC N. America Annual Meeting
November 9-13th, 2014
Vancouver BC





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National Exposure Research Lab/ Human Exposure and Atmospheric Science Division

November 5, 2014

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Presentation Outline

- Background on TOF MS
- Water sampling effort;
  - routine analysis UPLC-MS/MS;
  - discovery TOFMS (SETAC 2012 Long Beach CA)
- Tools used for identification of novel polyfluorinated compound in surface water
  - Sequential water sampling locations
  - Non-targeted screening
  - Negative mass defect
  - Kendrick mass transformation plots

Ex. Mass Accuracy (Tune)

1633.9498 - 1633.9395 = 0.0103

0.0103 / 1633.9498 10^6 = 6.3 ppm

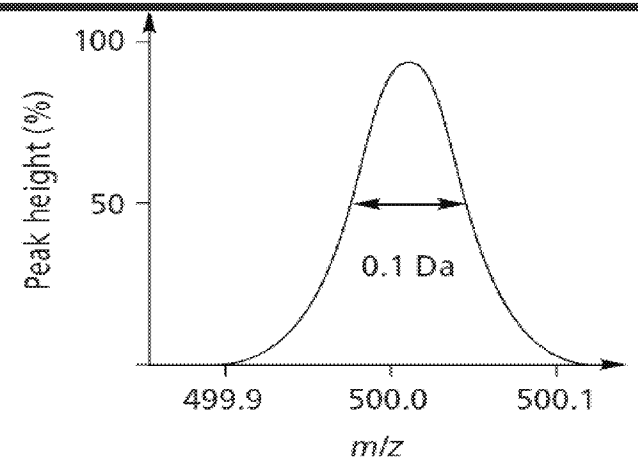
Ex Resolution (Tune)

1633.9395 / 0.0661 = 24, 720

FWHM = Full Width at Half-Height Maximum

Resolution in mass spectrometry is the ability to differentiate a detected ion from any other

True mass	400.0000
Measured mass	400.0020
Difference	0.0020 or 2 mmu
Error	$\frac{0.002}{400}$ 10^6 5 ppm



Mass	500
Peak width (at 50%)	0.1
Resolution (FWHM)	$\frac{500}{0.1}$ 5000

Figure 1: (Top) Mass accuracy determination and (bottom) the FWHM method for determining resolution for a mass spectrometer measured at a given ion.

LCGC North America Vol 22 Number 2 February 2004 MS The Practical Art
Michael Balough "Debating Resolution and Mass Accuracy"

Monoisotopic Mass (Accurate mass)

The monoisotopic mass is the sum of the masses of the atoms in a molecule using the unbound, ground-state, rest mass of the principal (most abundant) isotope for each element instead of the isotopic average mass.

Ex.	Carbon	^{12}C (98.9%), ^{13}C (1.1%),
	Hydrogen	^{1}H (99.99%), ^{2}H (0.001%),
	Nitrogen	^{14}N (99.63%), ^{15}N (0.37%)
	Oxygen	^{16}O (99.76%), ^{18}O (0.2%)
	Phosphorus	^{31}P (100%)
	Sulfur	^{32}S (95.02%), ^{34}S (4.21%)…(25 isotopes; 4 stable)
	Fluorine	^{19}F (100%)
	Chlorine	^{35}Cl (75%), ^{37}Cl (25%)
	Bromine	^{79}Br (50.7 %), ^{81}Br (49.3%)

Kind and Fiehn, BMC Bioinformatics 2007

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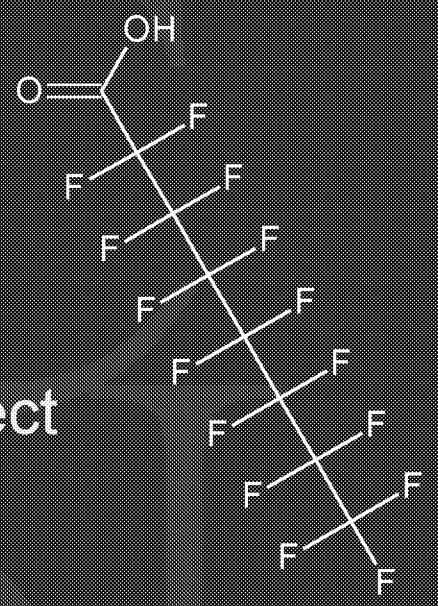
Mass Defect



Accurate Mass – Nominal Mass = Mass Defect

Ex. PFOA Nominal Mass: 414 Da
Accurate Mass: 413.973702 Da

413.973702 - 414 =

J Mass Spectrom. 2012 Feb;47(2):226-36. doi: 10.1002/jms.2953. **The use of mass defect in modern mass spectrometry.** Sleno L.

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Negative Mass Defect: Per- and Poly- fluorinated Compounds

Formula	Mass	C	H	N	O	P	S	Cl	F
		12.000000	1.007825	14.003074	15.994915	30.973762	31.972071	34.968853	18.998403
C_8H_{18}	114.1408506	8	18						
$C_8H_{16}O_2$	144.1150298	8	16		2				
$C_8HO_2F_{15}$	413.9737023	8	1		2				15
$C_8HO_3F_{15}$	429.9686169	8	1		3				15
$C_8HO_3SF_{17}$	499.937494	8	1		3		1		17

Octane	114.1408506
Octanoic acid	144.1150298
PFOA	413.9737023
Perfluoro-4-ether-octanoic acid	429.9686169
PFOS	499.937494

Mass fragments of per and poly fluorinated compounds conserve negative mass defect

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Water Sampling Cape Fear River, NC

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Nakayama et al. 2007 *ES&T* 41:5271-5276

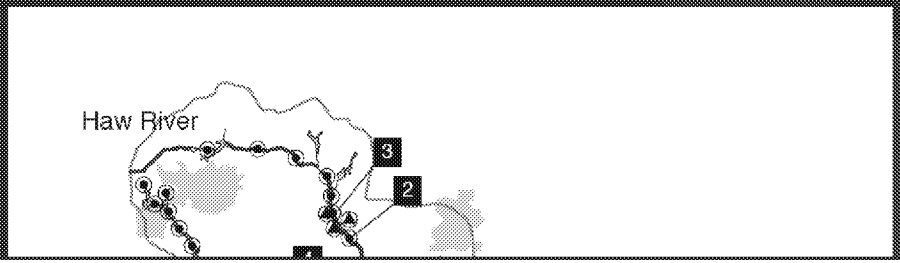
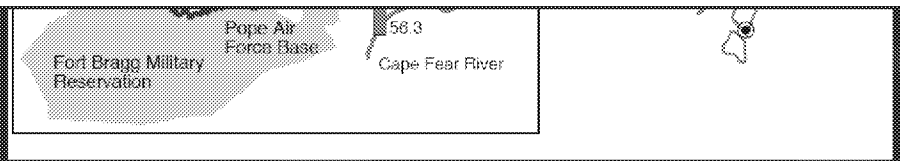


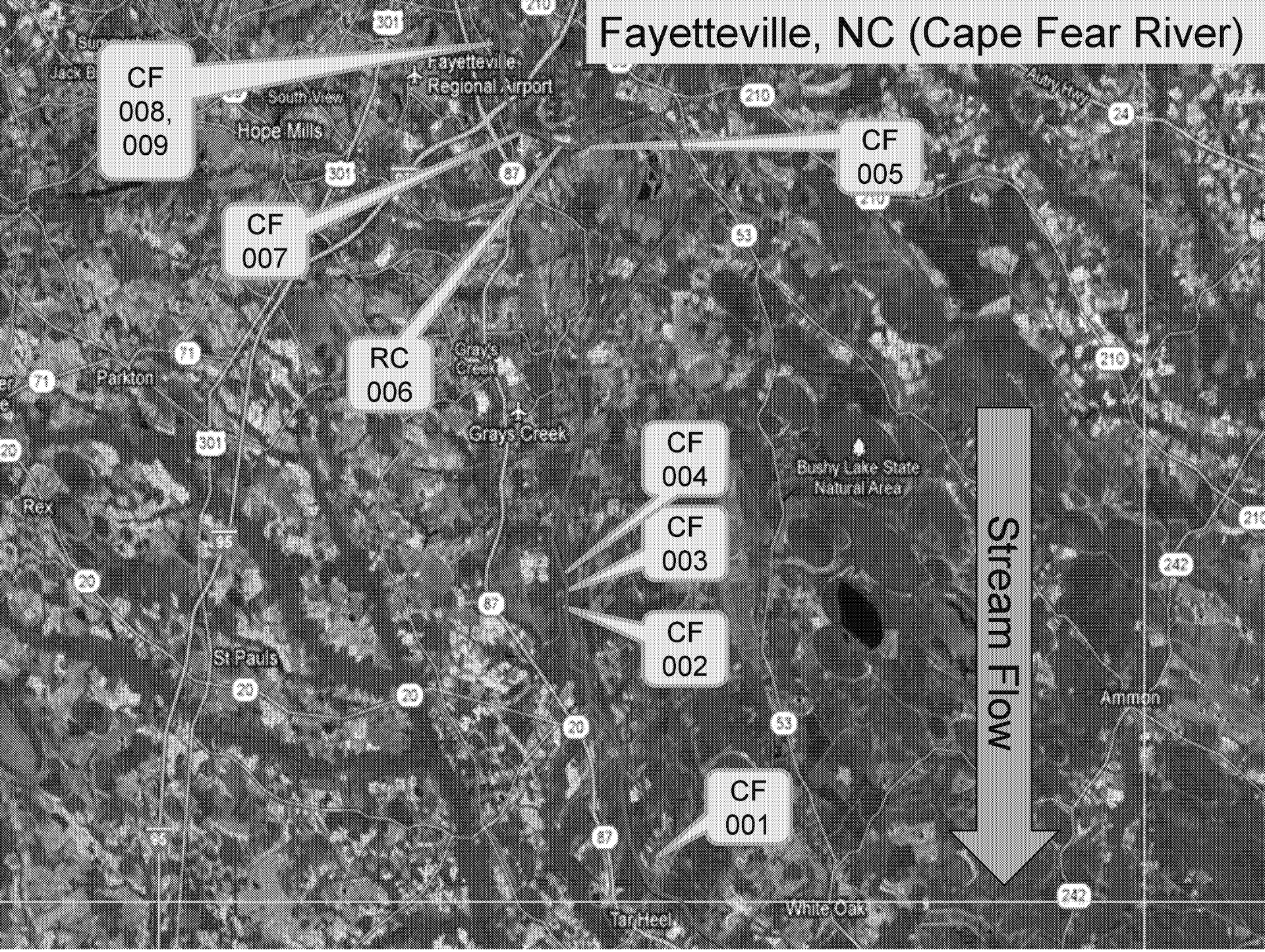
TABLE 3. Measured Concentrations at the Eleven Sites with the Highest Total Concentrations of PFCs in the Cape Fear River Basin[a] (See Figure 1 for locations)

no.	river	C12 (ng/L)	C11 (ng/L)	C10 (ng/L)	C9 (ng/L)	C8 (ng/L)	C7 (ng/L)	C6 (ng/L)	PFOS (ng/L)	PFHS (ng/L)	PFBS (ng/L)	total (ng/L)
1	Haw River	***4.46***	***52.1***	***120***	***194***	***287***	118	21.7	127	8.43	***9.41***	942
2	Haw River	3.20	28.7	112	157	200	66.8	14.5	33.4	7.87	2.61	626
3	Haw River	3.29	27.6	109	157	191	59.2	13.7	36.4	9.49	3.04	609
4	Haw River	1.98	20.0	88.2	151	201	58.2	13.2	31.5	7.49	2.88	574
5	tributary to Cape Fear	2.26	15.0	19.6	71.2	58.6	***329***	***23.0***	30.0	3.36	ND	531
6	Haw River	1.18	8.87	31.0	72.1	152	58.3	13.5	31.2	7.70	ND	376
7	Cape Fear River	< LOQ	3.34	13.2	34.8	70.3	24.0	7.84	66.7	5.59	ND	227
8	Cape Fear River	1.14	6.39	17.2	35.7	71.5	26.9	9.35	50.4	4.82	ND	223
9	Cape Fear River	1.23	6.75	17.1	38.0	72.7	23.7	7.05	40.7	4.10	ND	211
10	Cape Fear River	< LOQ	7.55	19.3	31.2	46.8	13.9	4.62	56.3	6.84	2.12	189
11	Little River	< LOQ	< LOQ	2.17	2.24	12.6	3.38	3.23	***132***	***26.4***	3.20	185

[a] Italicized values show maximal concentrations of each compound.



Fayetteville, NC (Cape Fear River)



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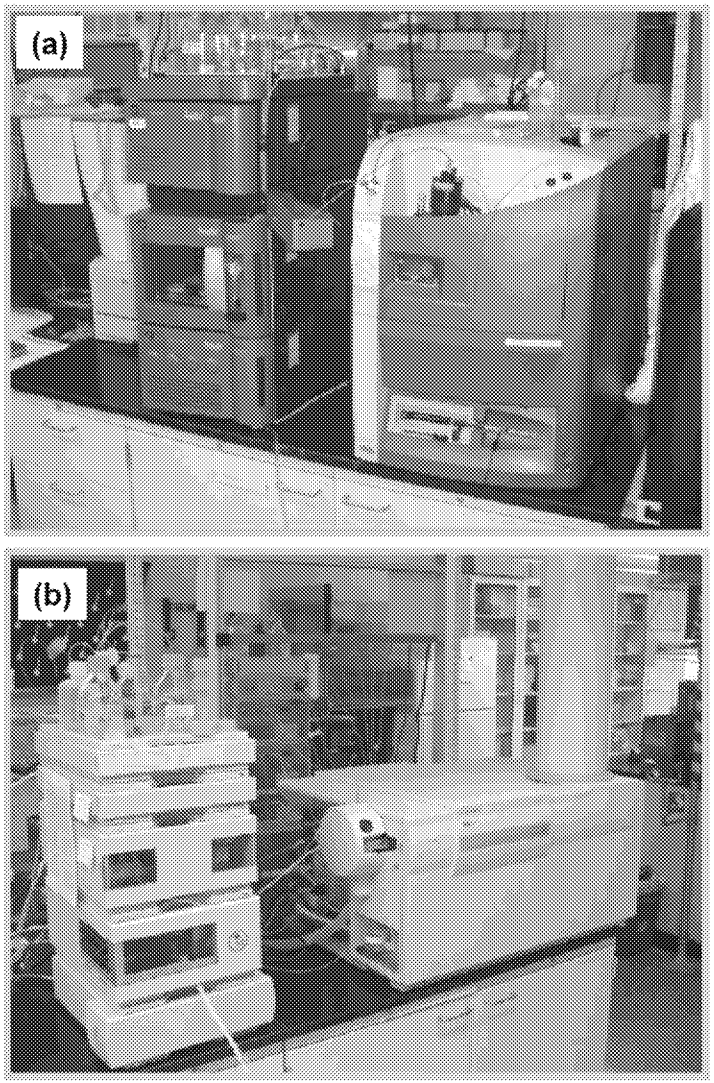
Water samples obtained February 2012

•Waters WAX SPE

Analysis by:

- (a) UPLC MS/MS routine monitoring PFCs
- (b)TOFMS discovery



UPLC MS/MS System
UPLC - Waters Acquity

MS/MS - Micromass Quatro Premier XE

Column - Acquity UPLC BEH C18 1.7 um 2.1x50 mm

Gradient separation
A 2mM Ammonium Acetate
B Methanol

HPLC/TOFMS System
HPLC - Agilent 1100

TOFMS - Agilent LC/MSD TOF

Column - Agilent Eclipse Plus C18 3.5 um 3.0x50 mm custom packed

Gradient separation
A 2mM Ammonium Acetate
B Methanol

Figure 2. Picture of the (a) UPLC/MS/MS and (b) LC/TOFMS systems used

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Traditional PFCs found in Cape Fear Water

Stream Flow

Analyte	001	002	003	004	005	006	007	008	008	009
C4	23	502	**3761**	6	4	0	8	7	5	3
C5	441	**5607**	**43590***	17	9	1	32	46	12	9
PFBS	4	5	3	4	5	2	9	5	6	4
C6	17	90	434	18	12	2	27	16	18	14
C7	37	599	**3873**	14	17	0	11	20	21	9
PFHS	7	12	10	9	7	4	9	10	9	22
C8	32	39	71	33	25	2	38	36	41	18
C9	13	34	127	7	11	1	6	8	11	5
PFOS	19	27	26	17	23	0	0	16	18	14
C10	10	17	12	11	0	3	3	8	10	5

items in red exceed the standard curve high end of 500 ng/L; 10x diluted and re analyzed; * still exceed curve and are estimated

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Workflow for TOFMS Discovery

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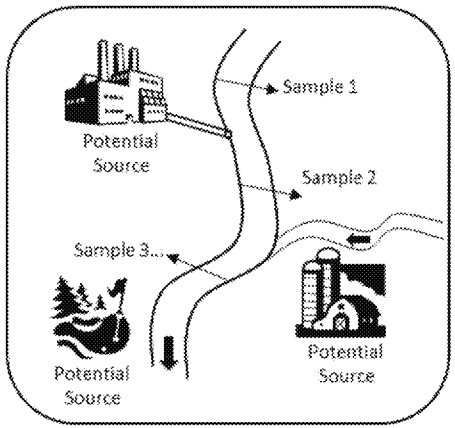


Sample prep.

Non-targeted TOFMS screening

Sample 1 (control)
Sample 2 (experiment)
Source input 1

Sample 2 (control)
Sample 3 (experiment)
Source input 2...

molecular feature extraction (MFE)

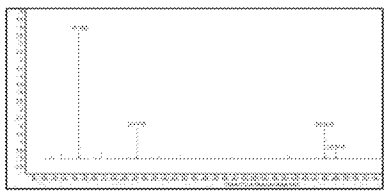
Visualization of features unique to experiment

Compound Exchange File (CEF) exportation

Reduction of data by extracted ion chromate-Gram (EIC) of traditional PFCs

Investigation of large, negative mass defect features

Extract Spectral features



Background subtracted for clean spectra

Formulae generation (C, H, O, N, S, F, Cl, P, Na...)

Advanced techniques

Investigation of co-eluting peaks (adducts - formate, acetate and dimers - Na+, K+)

Diagnostic fragment ions

Isotope peak matching (distribution, spacing)

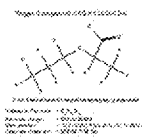
Exact mass caliper tool

Exact mass off sets (CF_2, CF_2O..) homologous series

Kendrick Mass Defect Plots

Advanced techniques

Proposed Novel Compounds



Acquisition of authentic standard for confirmation if possible

Chemical database searching

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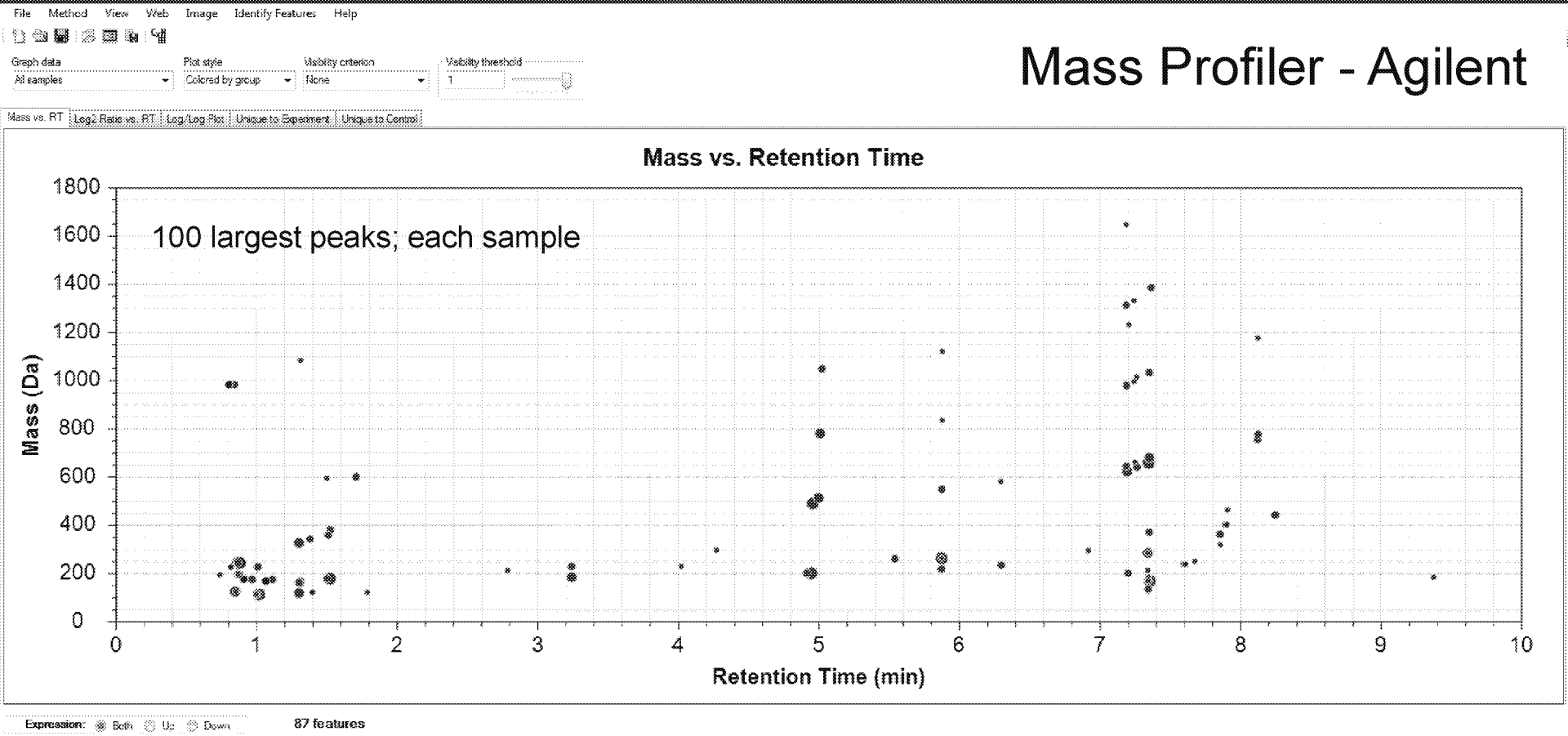
Control sample; CFR 004 Upstream

Experiment; CFR 002 Downstream

Mass Profiler - Agilent



Size of circle proportional to peak area; typical usage dosed vs. non-dosed animals

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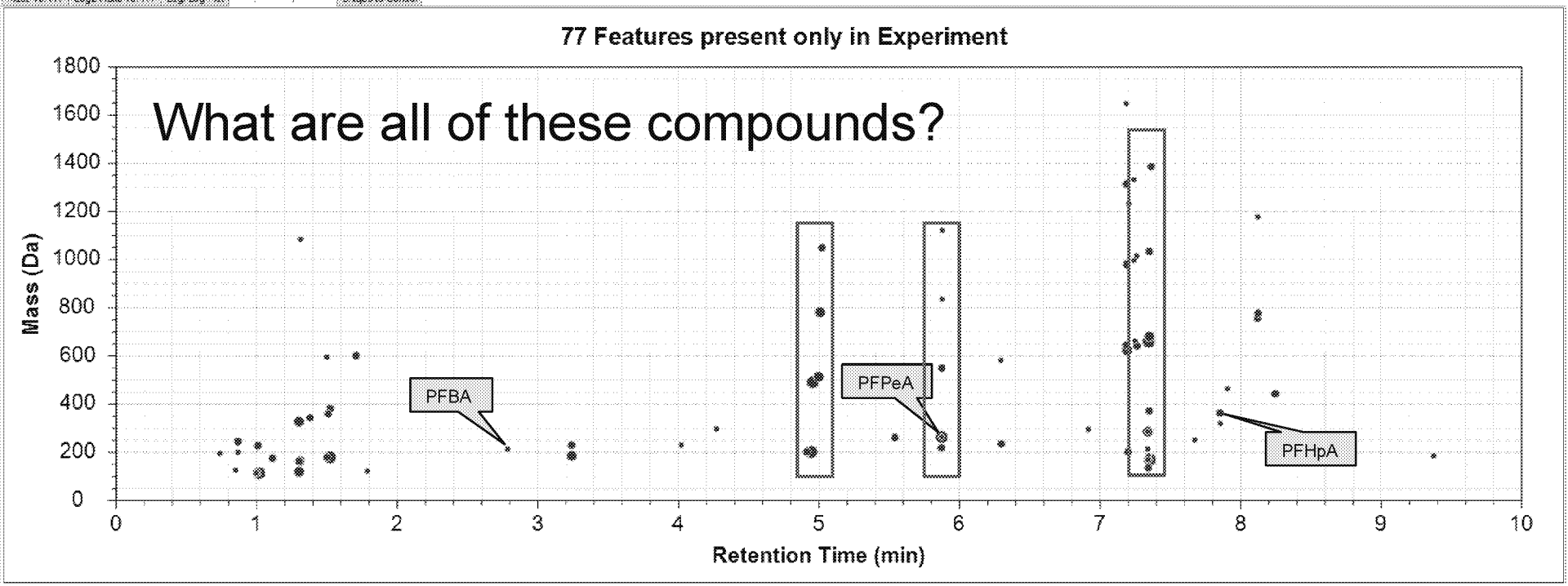
Experiment; CF 002 Downstream

File Method View Web Image Identify Features Help

Graph data: All samples | Plot style: Colored by group | Visibility criterion: None | Visibility threshold: 1

Mass vs. RT | Log2 Ratio vs. RT | Log/Log Plot | Unique to Experiment | Unique to Control



Expression: Both Up Down 87 features

Most Have Negative Mass Defect; some appear at same RT

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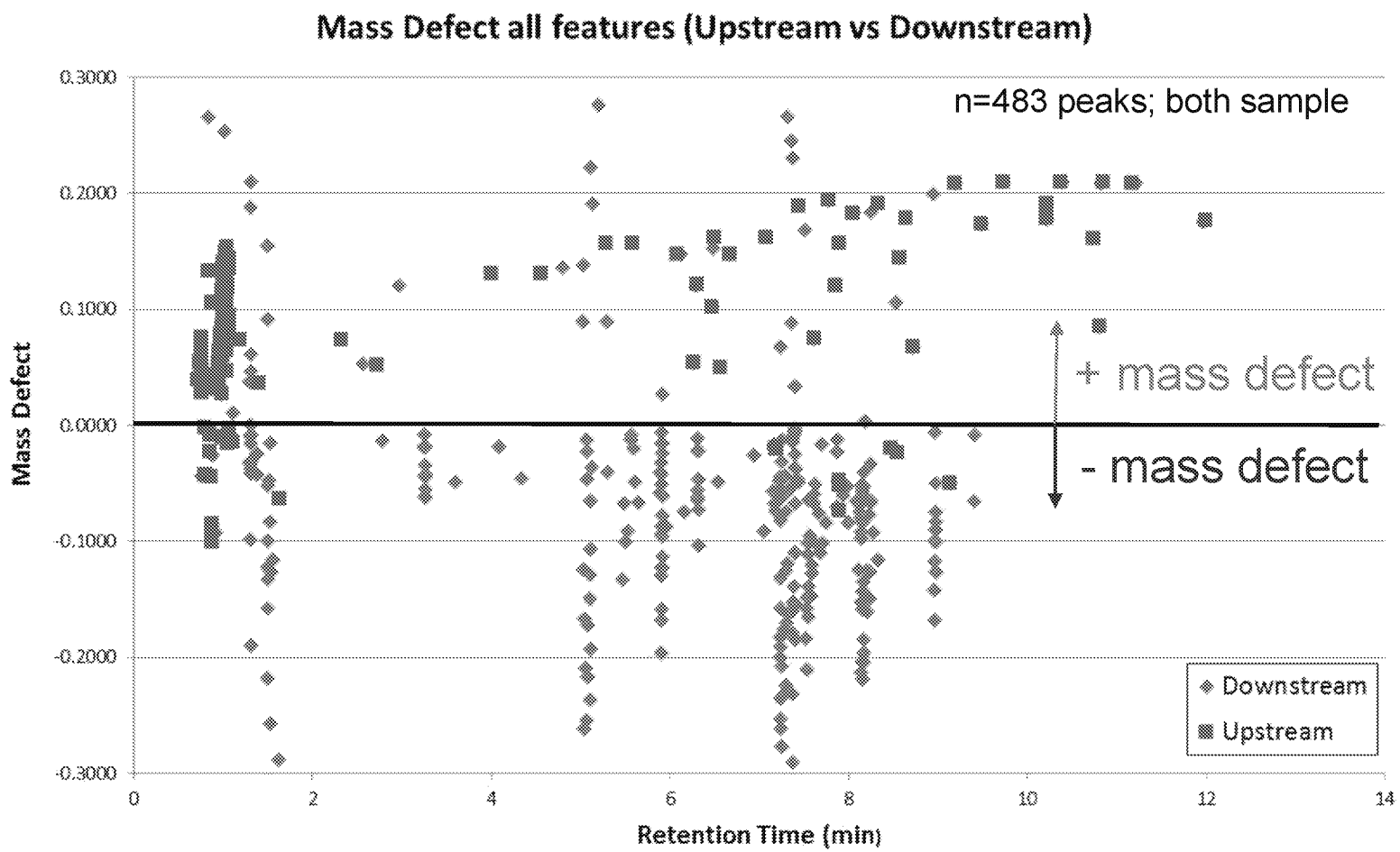
Mass Defect Plot TOFMS data



Identified polyfluorinated compound (U2M3OHxA)

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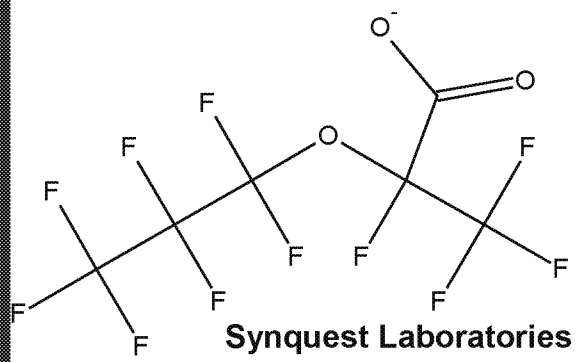
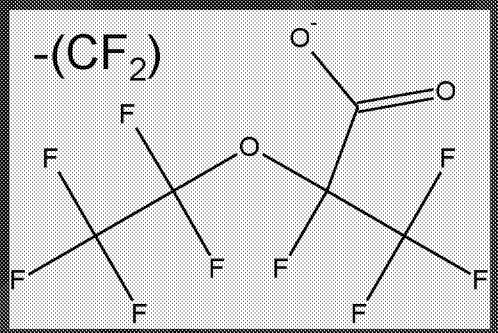


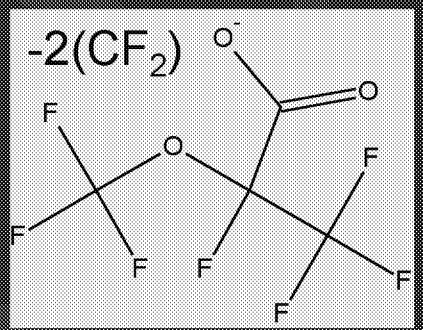
Target Compound CAS # 13252-13-6

Molecular Formula	= $C_6F_{11}O_3$
Formula Weight	= 329.0453838
Composition	= C(21.90%) F(63.51%) O(14.59%)
Monoisotopic Mass	= 328.967728 Da
[M-H]-	= 327.960451 Da

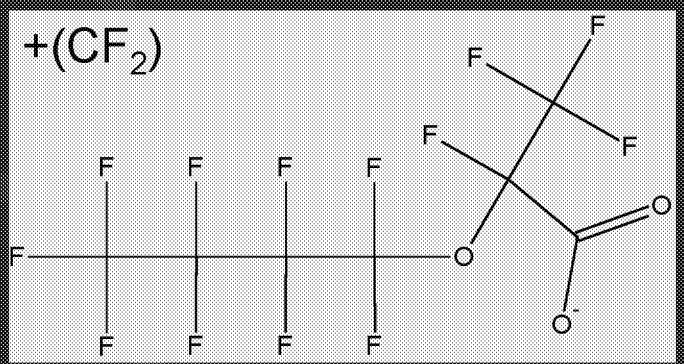
Synquest Laboratories - Undecafluoro-2-methyl-3-oxahexanoic acid

$-3(CF_2) = 178.9733$
$-2(CF_2) = 228.9741$
$-(CF_2) = 278.9709$
$+(CF_2) = 378.9645$



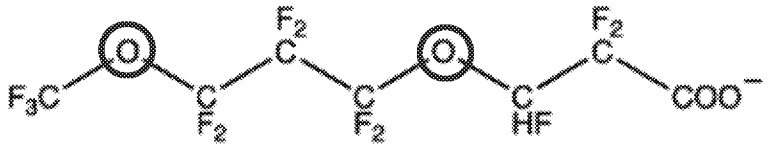


(Strynar et al., SETAC 2012 Long Beach CA)

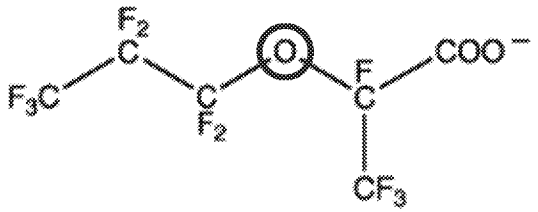


Fluoropolymer manufacture

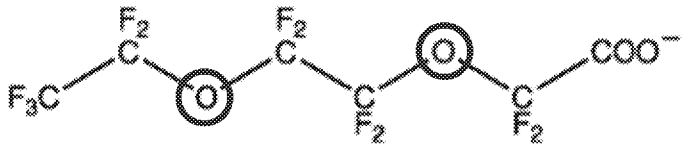
ADONA (CAS No. 958445-44-8)



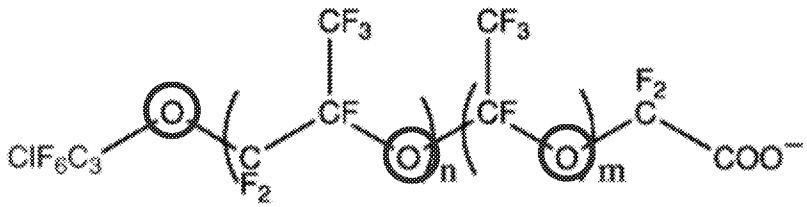
GenX (CAS No. 62037-80-3)



Asahi's product (CAS No. 908020-52-0)

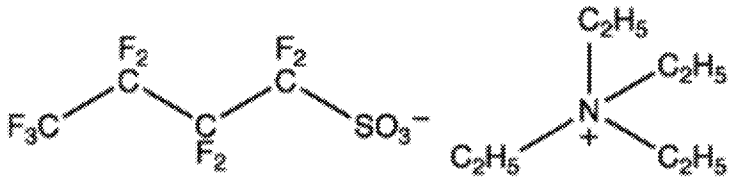


Solvay's product (CAS No. 329238-24-6)

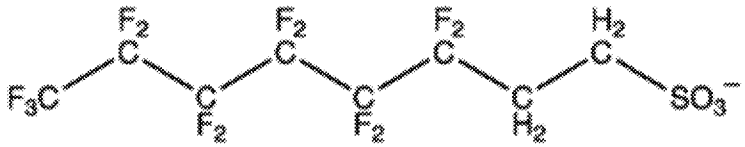


Metal plating

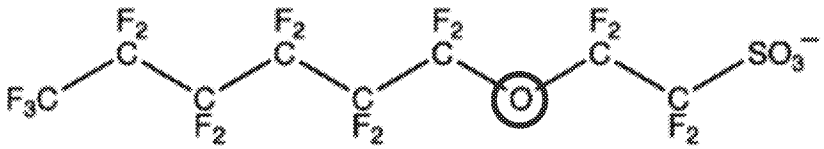
$N(Et)_4$-PFBS (CAS No. 25628-08-4)



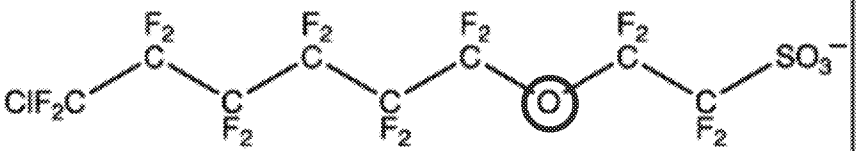
6:2 FTSA (CAS No. 27619-97-2)



F-53 (CAS No. 754925-54-7)



F-53B (CAS No. 73606-19-6)



Wang et al., 2013 Environment International 60 (2013) 242–248

m/z Extracted Ion Chromatograph (EIC) Suspected polyfluorinated compound homologous series

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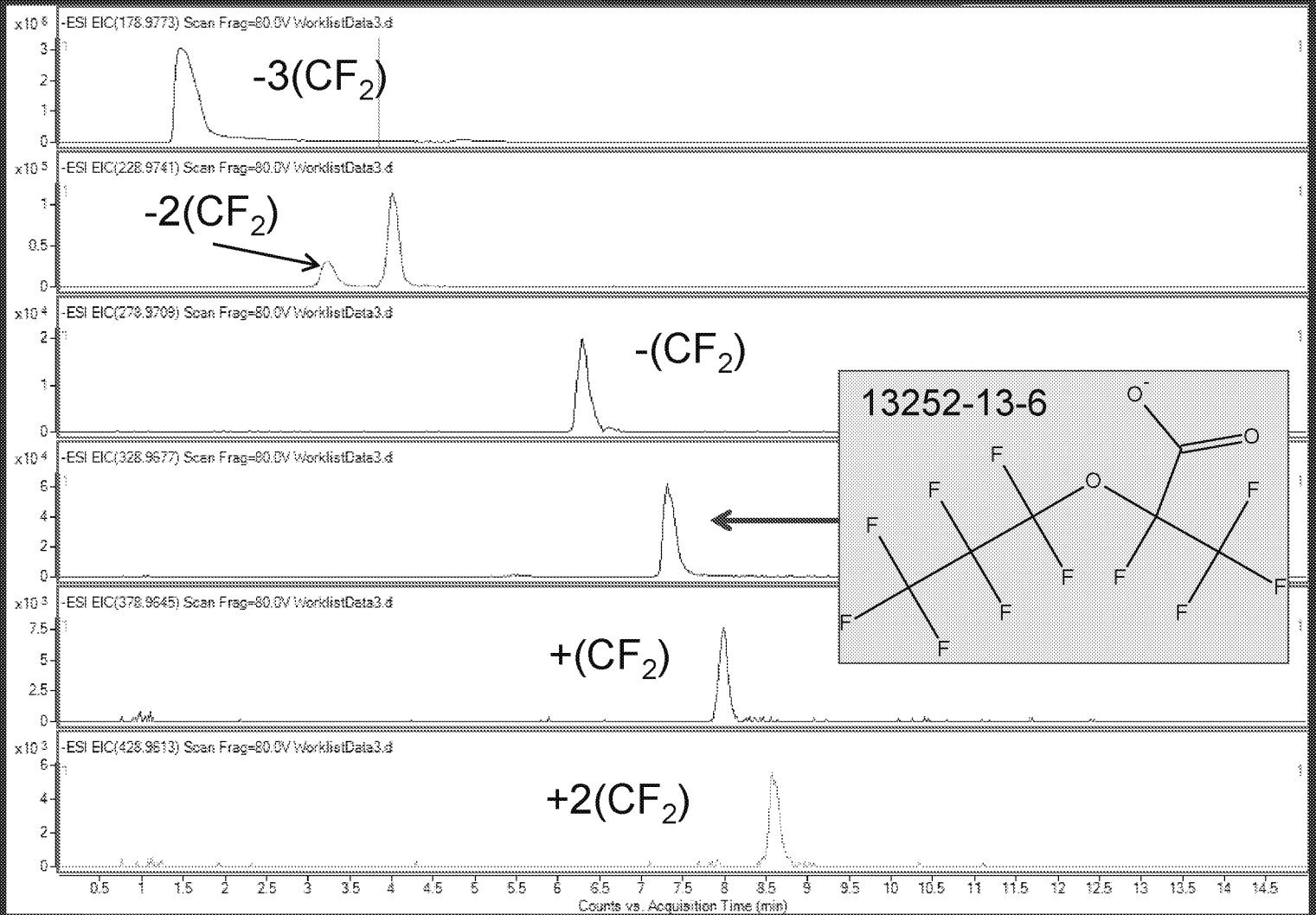
-150 amu

-100 amu

-50 amu

+50 amu

+100 amu



m/z Extracted Ion Chromatograph (EIC)

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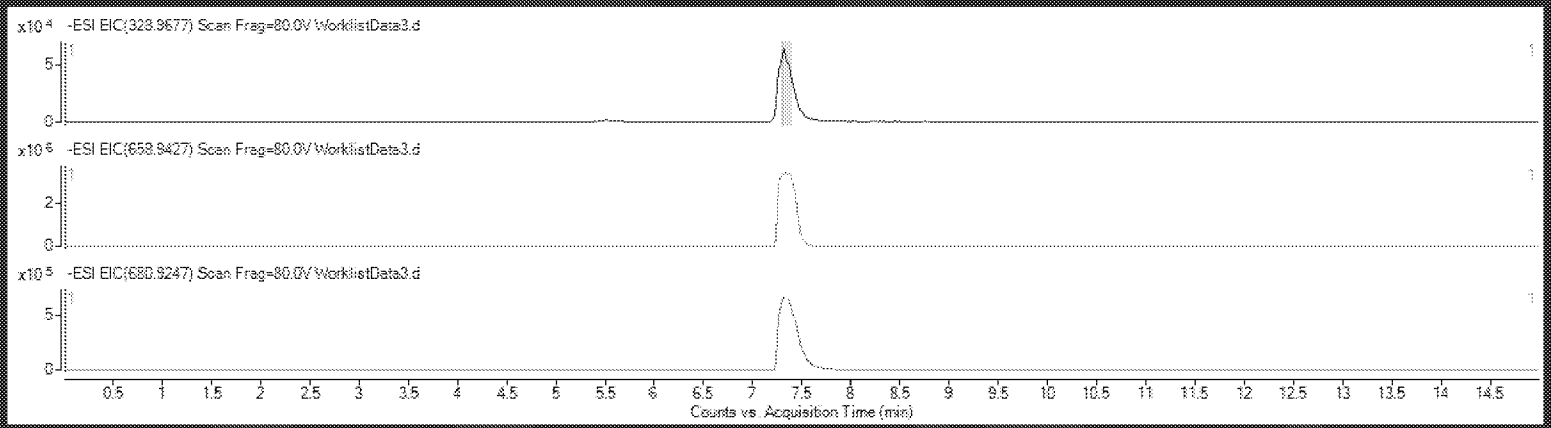
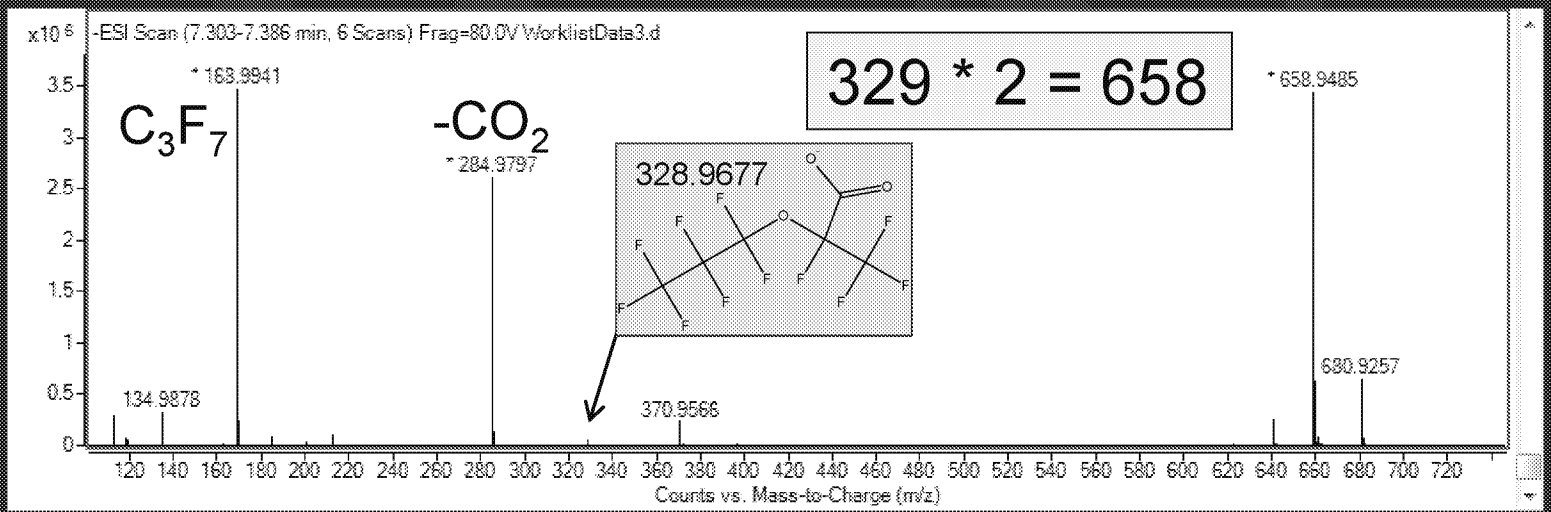


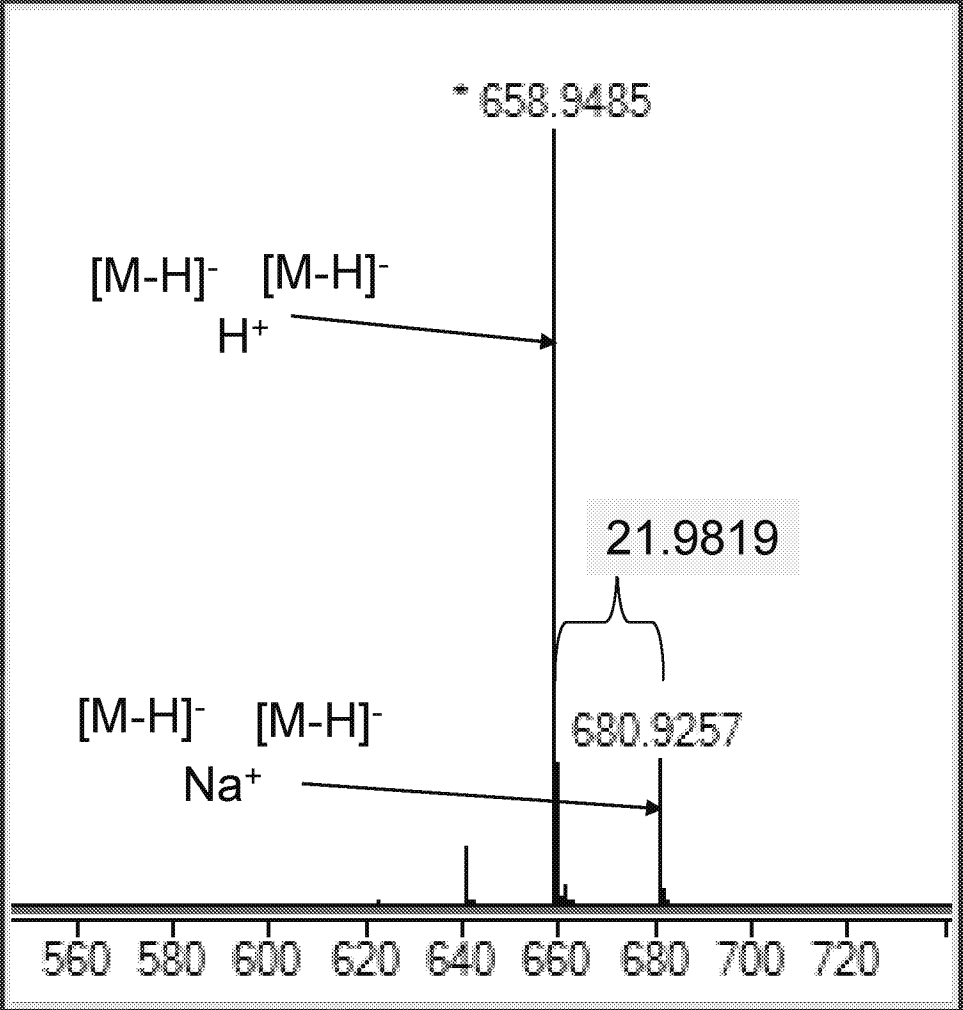


Standard or extracted water, same spectra. m/z 328 LOW

Key TOFMS Information

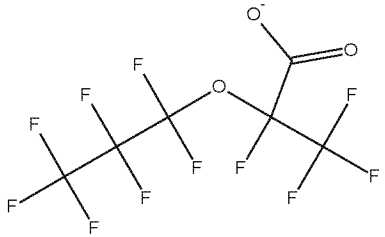
Difference Between Peaks

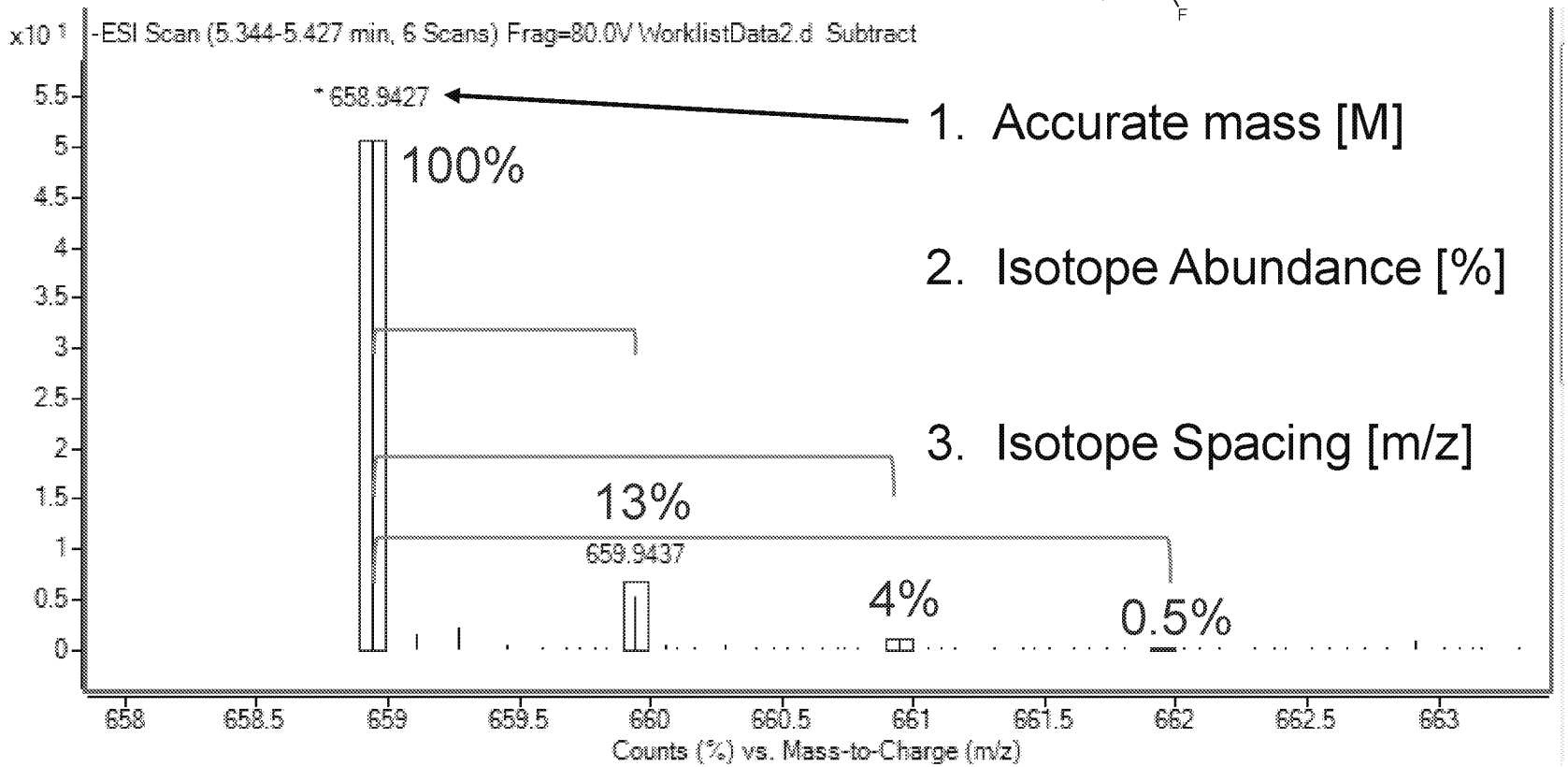
Na^+ (22.9892)
$-H^+$ (1.0073)
= **21.9819**

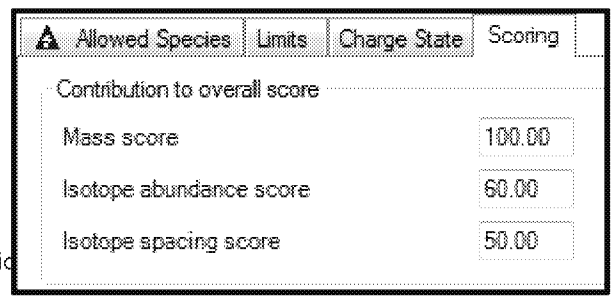


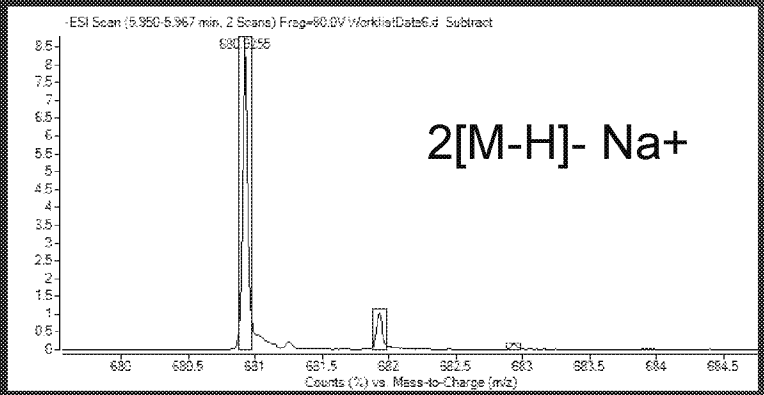
Isotope Cluster Scoring

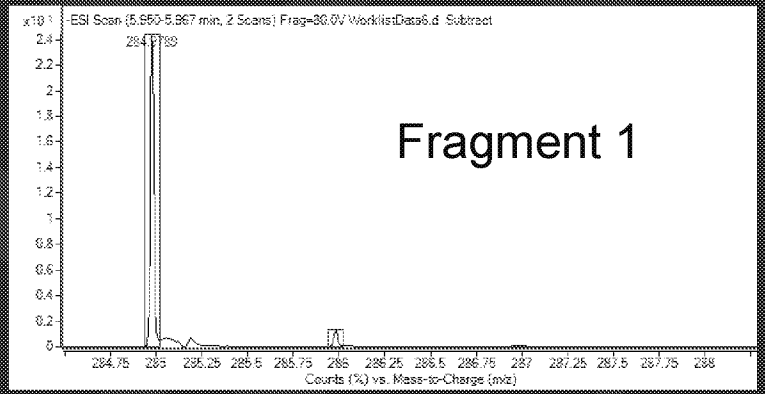
Ex. $C_{12}HF^{22}O_6$

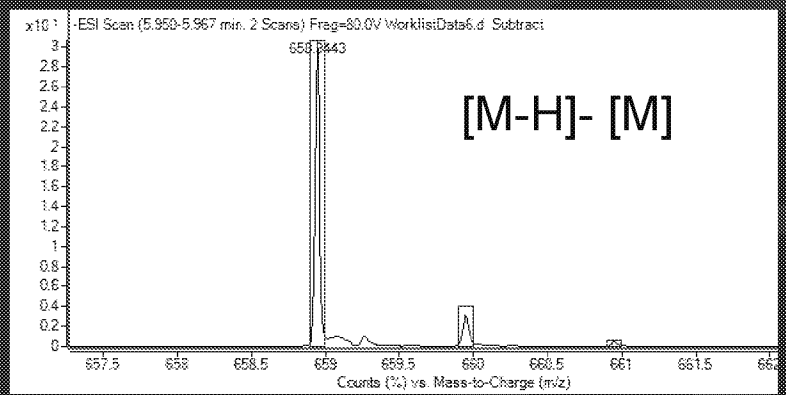


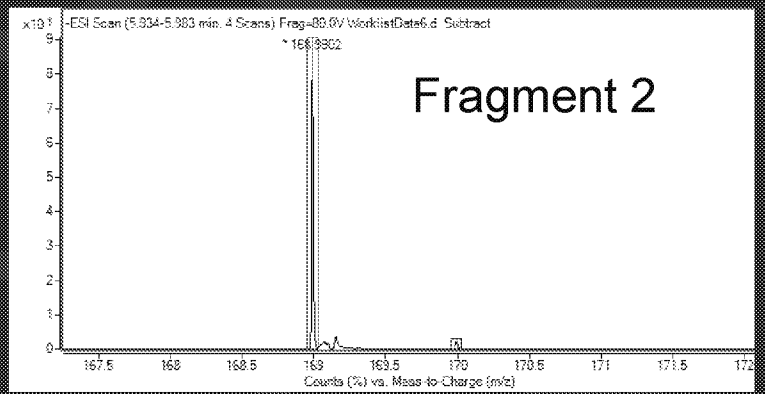


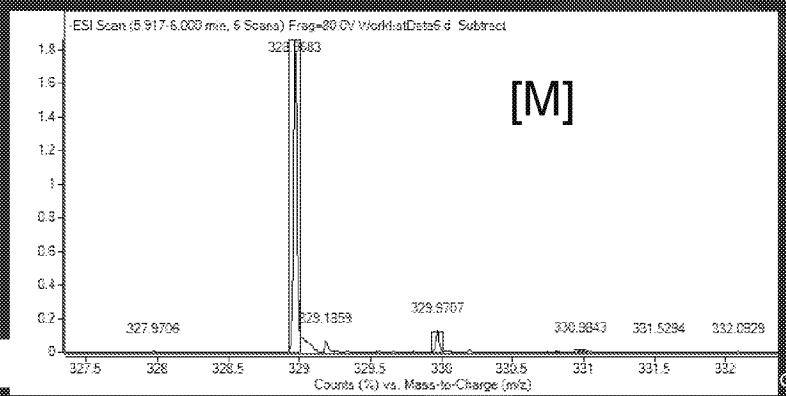












	Ion Formula	m/z [M-H]-	m/z (calc)	Diff (ppm)	Score
2[M-H]- Na+	$C_{12}F_{22}NaO_6$	680.9255	680.9247	-1.2	98.11
[M-H]- [M]	$C_{12}HF_{22}O_6$	658.9433	658.9427	-2.36	92.96
[M-H]-	$C_6F_{11}O_3$	328.9683	328.9677	-1.8	97.85
Fragment 1	$C_5F_{11}O$	284.9789	284.9779	-3.49	96.35
Fragment 2	C_3F_{17}	168.9902	168.9894	-4.75	84.41

Homologous series: $[M-H]^-$, $[M_2-H]^-$, $[M-H]^-_2Na^+$, All have 21.9819 m/z offset peaks

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-3(CF_2)

-2(CF_2)

-(CF_2)

-ESI EIC(178.9773) Scan Frag=80.0V WorklistData3.d

-ESI Scan (1.488-1.587 min, 7 Scans) Frag=80.0V WorklistData3.d

* 178.9789

* 358.9669

-ESI EIC(228.9741) Scan Frag=80.0V WorklistData3.d

-ESI Scan (3.178-3.294 min, 8 Scans) Frag=80.0V WorklistData3.d

184.9852

112.9859

-ESI EIC(458.9555) Scan Frag=80.0V WorklistData3.d

-ESI EIC(278.9709) Scan Frag=80.0V WorklistData3.d

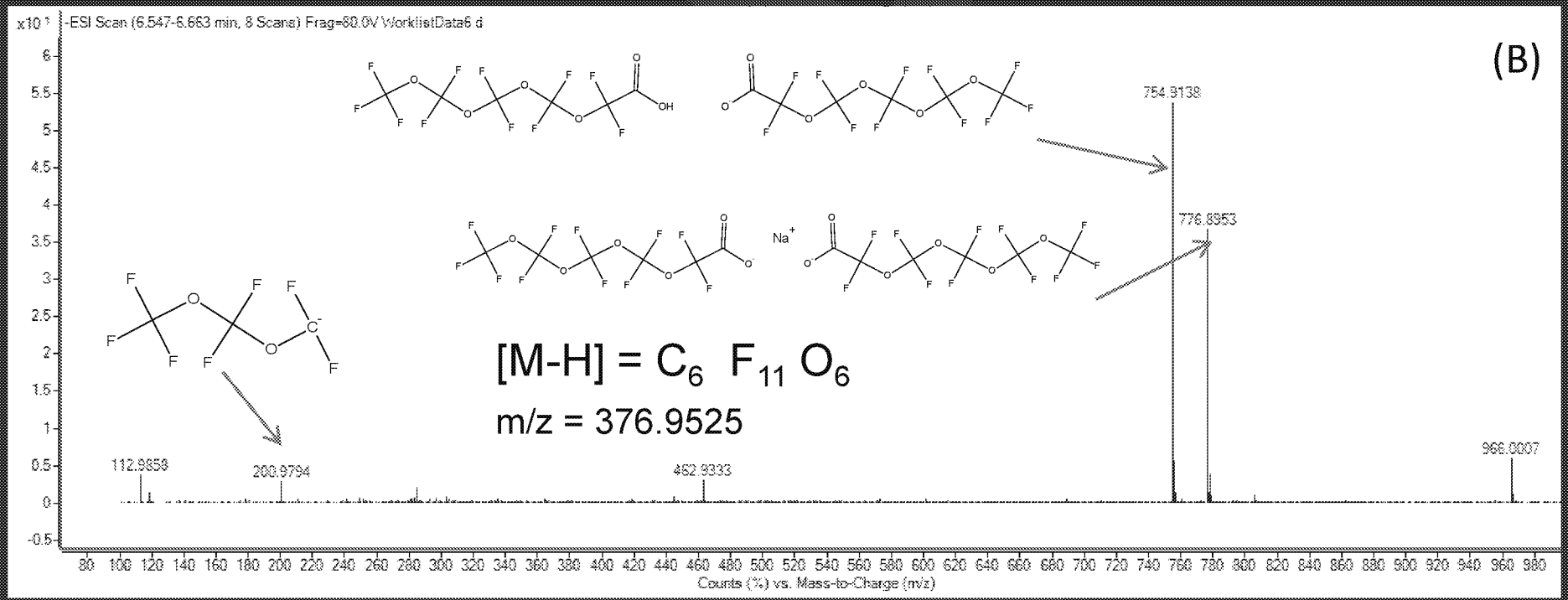
-ESI EIC(558.9491) Scan Frag=80.0V WorklistData3.d

-ESI EIC(580.9311) Scan Frag=80.0V WorklistData3.d

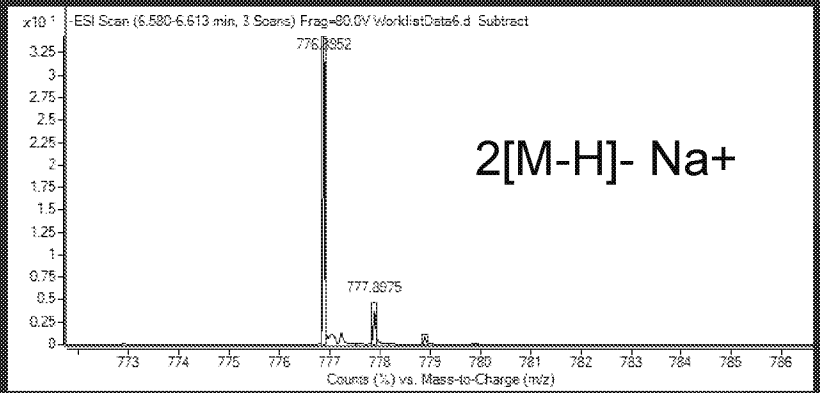
Counts vs. Acquisition Time (min)

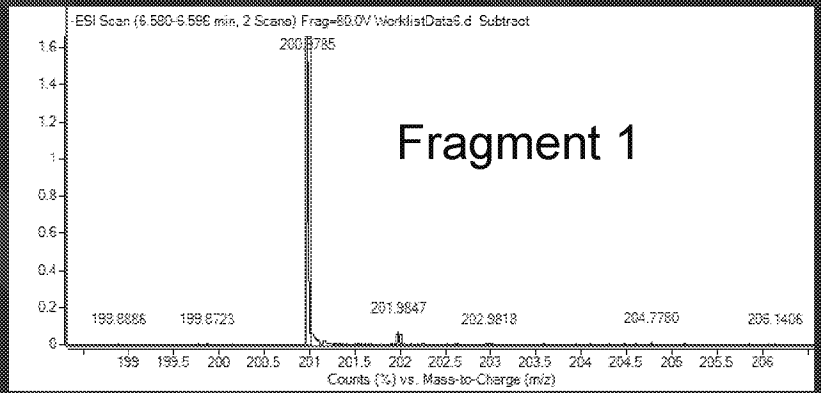
Counts vs. Mass-to-Charge (m/z)

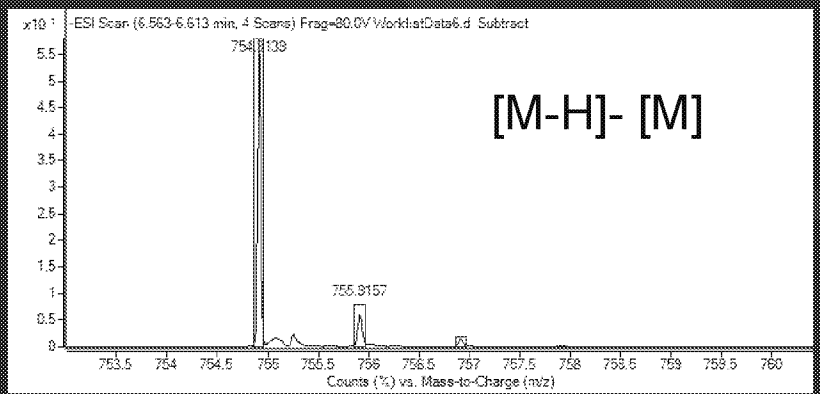
Additional Polyfluorinated Compounds

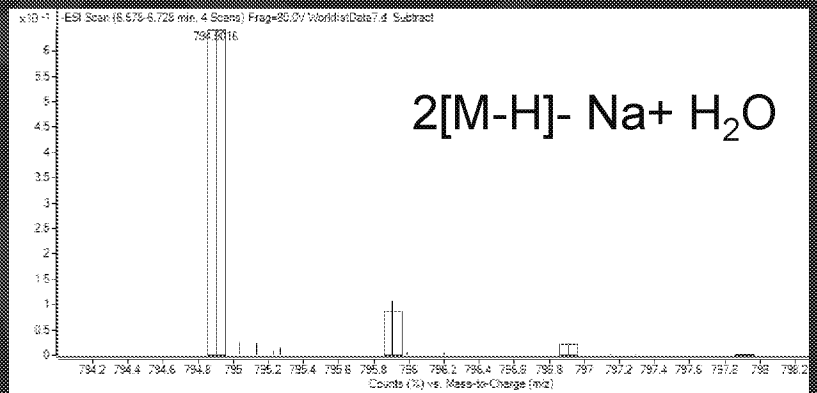


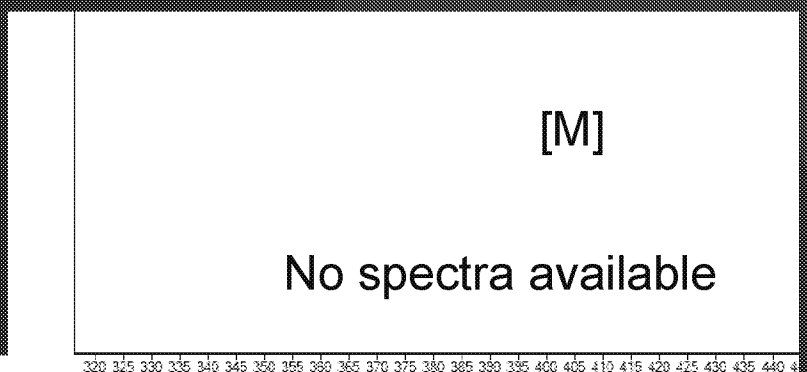
Continued to see 21.9819 m/z offset peaks





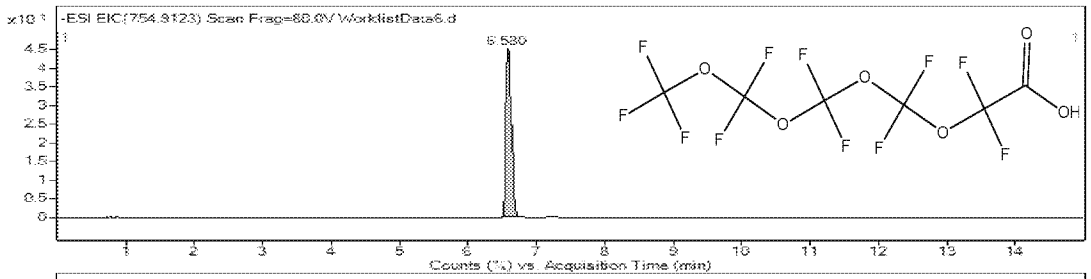




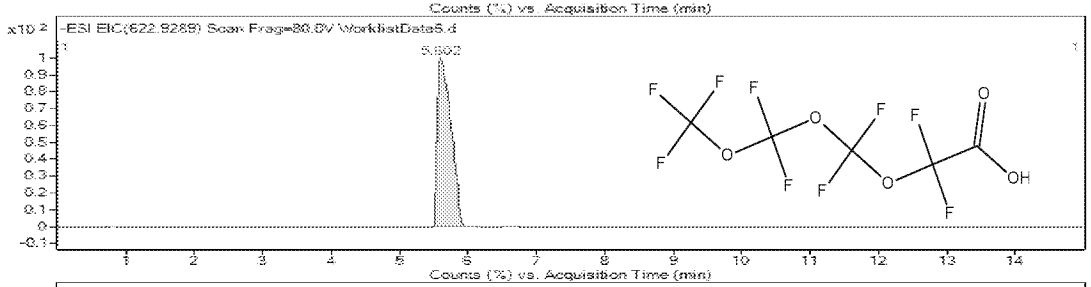


	Ion Formula	m/z (M-H)-	m/z (calc)	Diff (ppm)	Score
2[M-H]- Na+	$C_{12}F_{22}NaO_{12}$	776.8952	776.8942	-1.31	95.92
[M-H]- [M]	$C_{12}HF_{22}O_{12}$	754.9139	754.9122	-2.22	92.66
[M]	$C_6F_{11}O_6$	Not Observed	376.9525	---	---
Fragment 1	$C_3F_7O_2$	200.9785	200.9792	3.71	95.18
2[M-H]- Na+ H_2O	$C_{12}H_3F_{22}NaO_{13}$	794.9016	794.9047	3.95	84.15

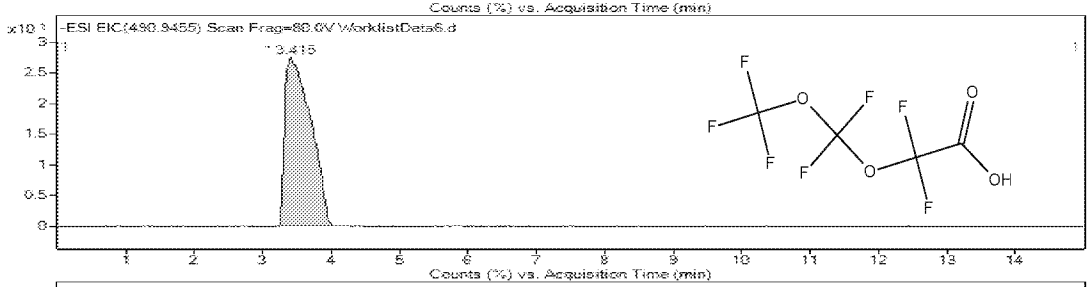
Homologous series NOT offset CF_2 BUT CF_2O



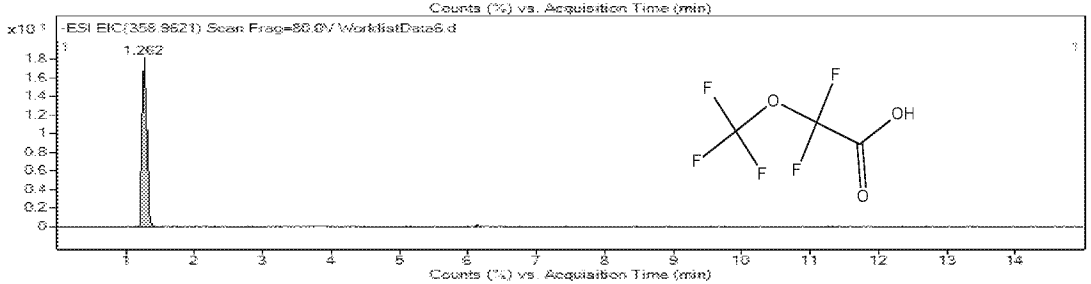
Molecular Formula = $C_6HF_{11}O_6$
Monoisotopic Mass = 377.959748 Da
[M-H]- = 376.952472 Da



Molecular Formula = $C_5HF_9O_5$
Monoisotopic Mass = 311.968027 Da
[M-H]- = 310.96075 Da



Molecular Formula = $C_4HF_7O_4$
Monoisotopic Mass = 245.976306 Da
[M-H]- = 244.969029 Da



Molecular Formula = $C_3HF_5O_3$
Monoisotopic Mass = 179.984585 Da
[M-H]- = 178.977308 Da

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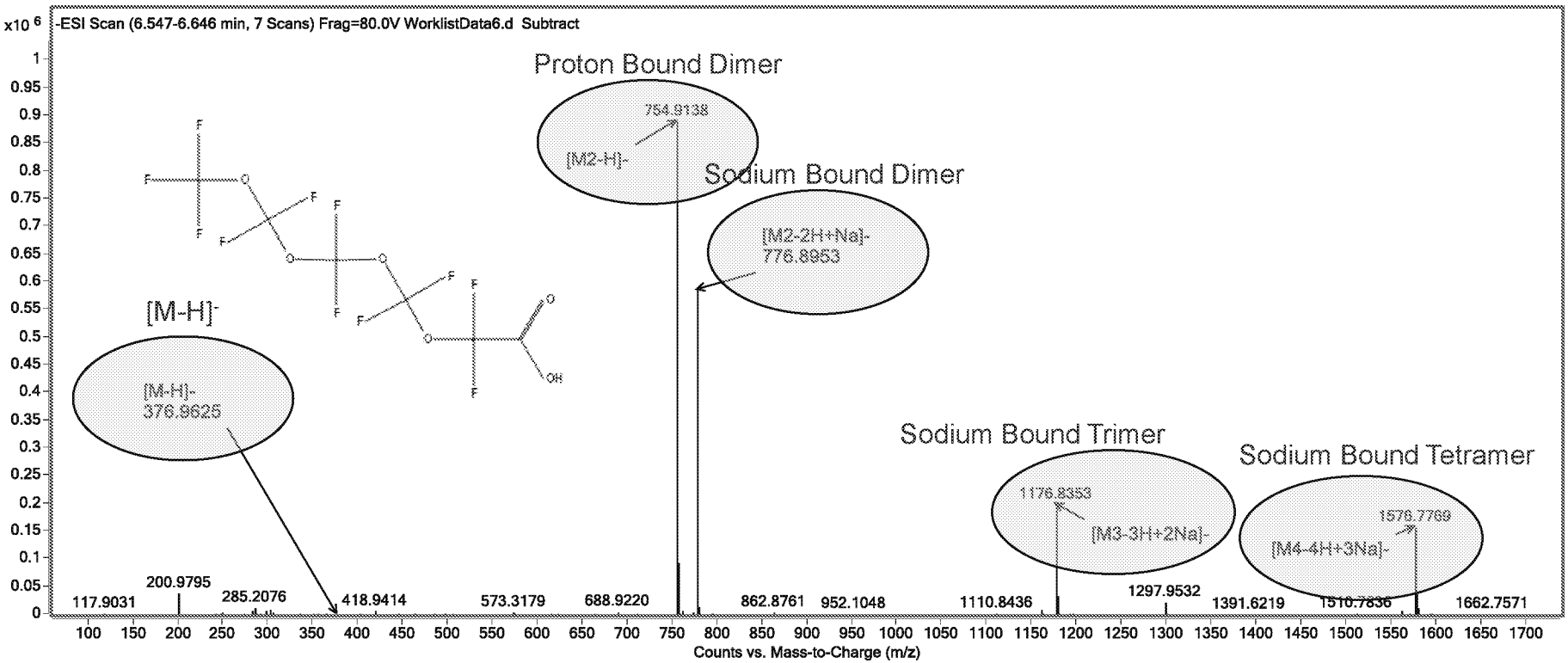
Additional *n*-mers*



* Trier, X.; Granby, K.; Christensen, J. H., Tools to discover anionic and nonionic polyfluorinated alkyl surfactants by liquid chromatography electrospray ionisation mass spectrometry. *J. Chromatogr. A* **2011,** *1218*, (40), 7094-104.

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https://edm.nc.gov/DENR-Portal/

Sample ID (Monitoring Well Description)	Monitoring Date	PFOA (µg/L)
MW-14D Onsite monitoring well located on the west side of the inactive Biosludge Lagoon area in the surficial groundwater beneath a local clay feature; South of the PPA Manufacturing Facility.	06-06-2013	0.045
MW-15D Onsite monitoring well located on the west side of the River Sediment Basins in the surficial groundwater beneath a local clay feature; South of the PPA Manufacturing Facility.	06-06-2013	0.052
MW-16D Onsite monitoring well located northeast of the Fire House in the surficial groundwater beneath a local clay feature; Southwest of the PPA Manufacturing Facility.	06-12-2013	0.016
MW-17D Onsite monitoring well located west of the Administration Building in the surficial groundwater beneath a local clay feature; West of the PPA Manufacturing Facility.	06-19-2013	< 0.004
MW-16D Onsite monitoring well located upgradient of the Nafion® Common Sump in the perched groundwater above a local clay feature; Southeast of the PPA Manufacturing Facility.	06-06-2013	0.052

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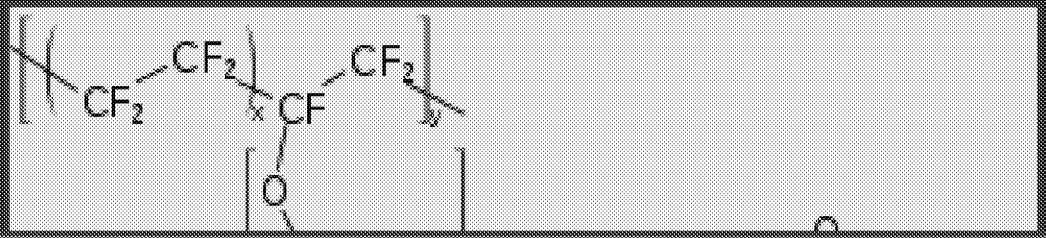

Nafion

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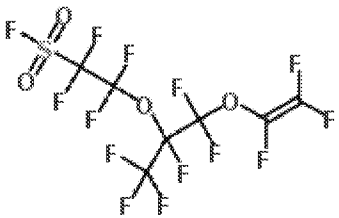


2D 3D Save Zoom

1,1,2,2-tetrafluoro-2-{{1,1,1,2,3,3-hexafluoro-3-[(trifluoro vinyl)oxy]-2-propanyl}oxy)ethanesulfonyl fluoride

ChemSpider ID: **77539**

Molecular Formula: $C_7F_{14}O_4S$

Average mass: 446.115112 Da

Monoisotopic mass: 445.929382 Da

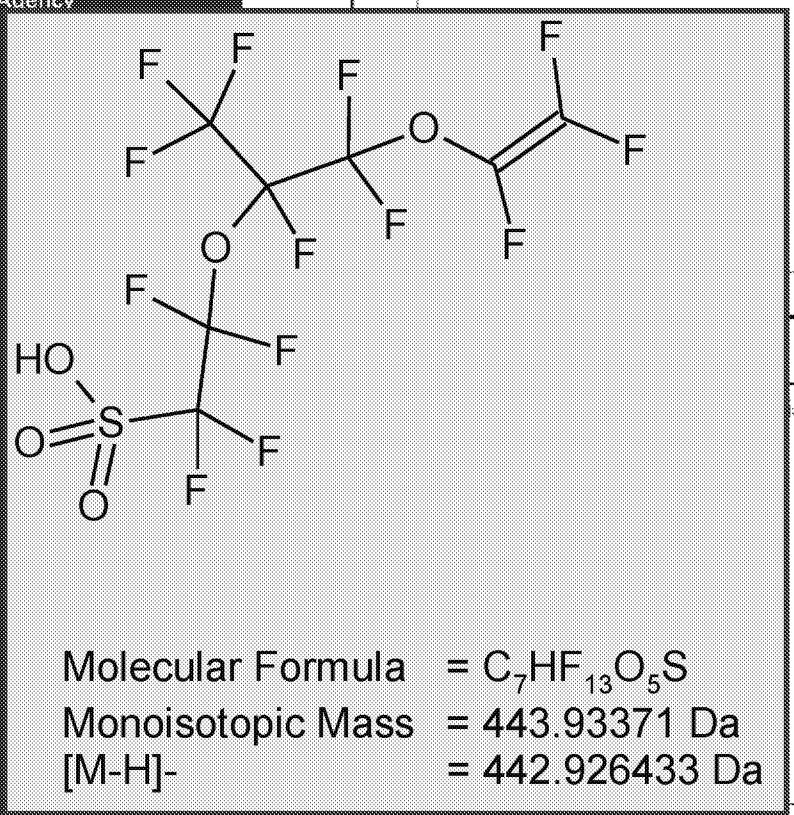
Systematic name

1,1,2,2-Tetrafluoro-2-({1,1,1,2,3,3-hexafluoro-3-[(trifluorovinyl)oxy]-2-propanyl}oxy)ethanesulfonyl fluoride

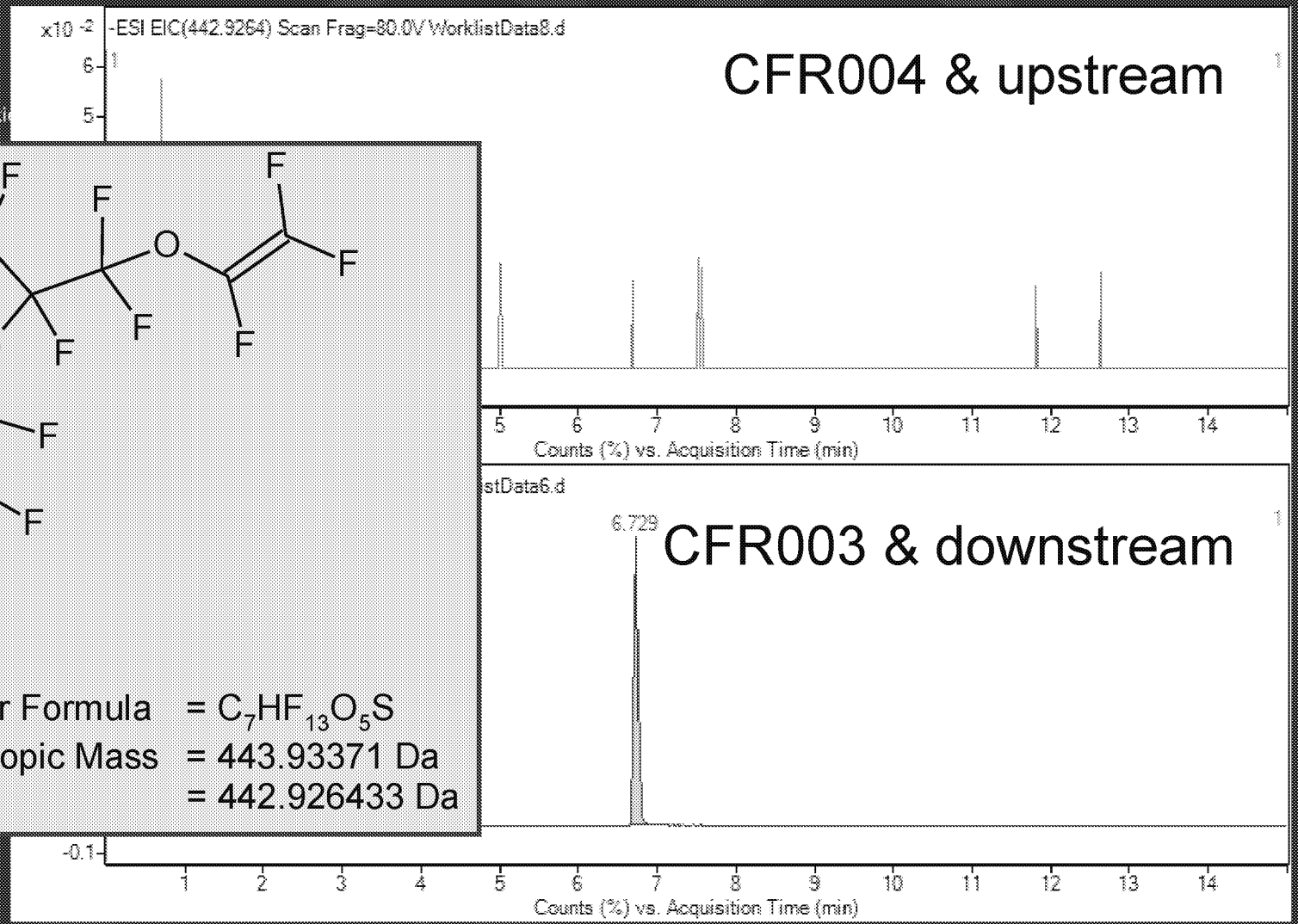
SMILES and InChIs

Cite this record

EPA
United States
Environmental Protecti
Agency



Molecular Formula = $C_7HF_{13}O_5S$
Monoisotopic Mass = 443.93371 Da
[M-H]- = 442.926433 Da



This polyfluoro sulfonates does not show H^+ and Na^+ bound dimers

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Kendrick Mass Defect Transformations

$$\text{Kendrick Mass (F)} = (\text{observed mass}) \times \frac{\text{Nominal mass F}}{\text{Exact mass F}}$$

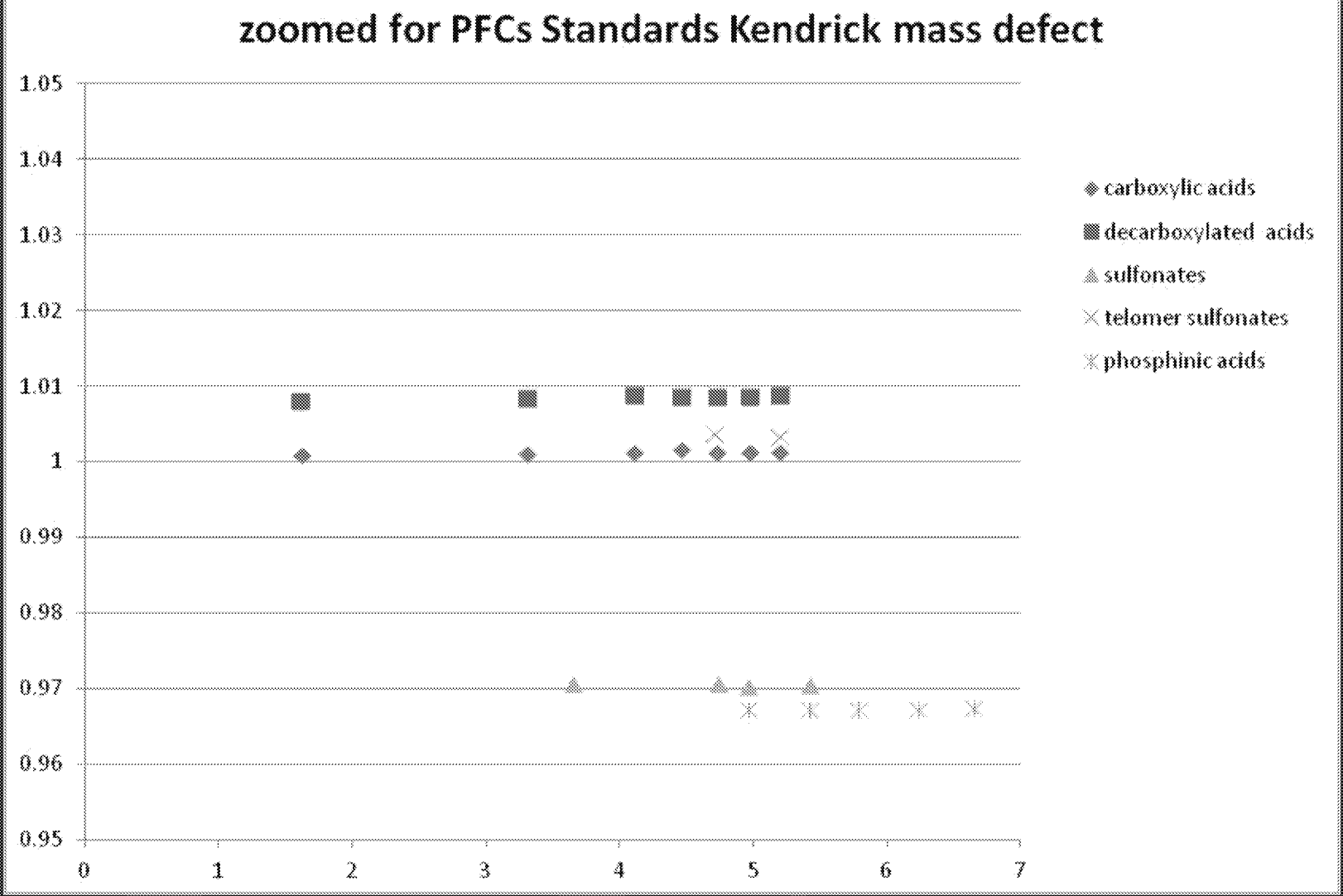
$$\text{If (F)} = CF_2 \qquad \frac{\text{Nominal mass F}}{\text{Exact mass F}} = \frac{50}{49.99681}$$

http://en.wikipedia.org/wiki/Kendrick_mass

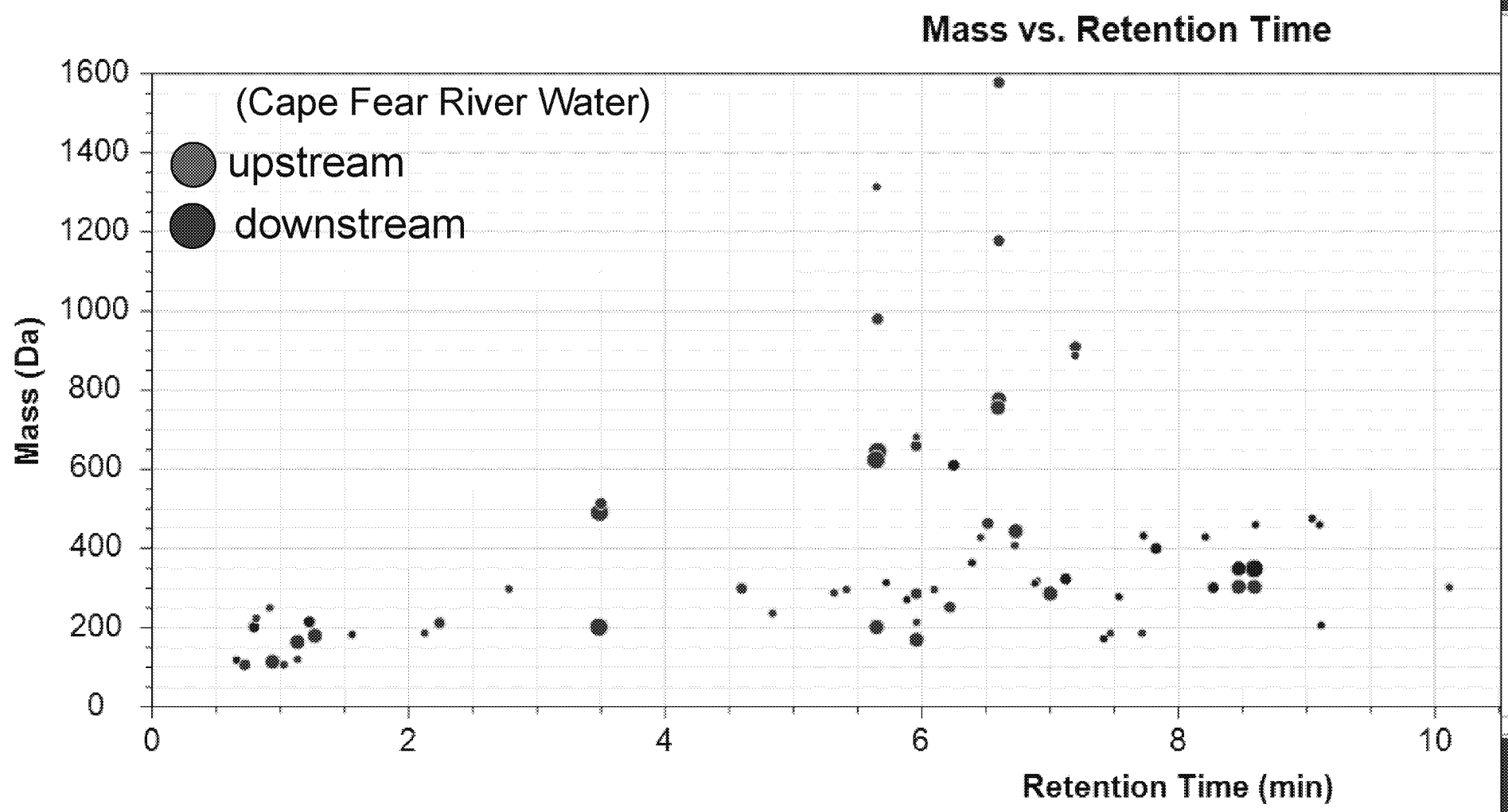
Kendrick, Edward (1963) Anal. Chem. 35 (13) 2146-2154

ED_002878_00003619-00032

Kendrick Mass Defect Standards Example



Kendrick Mass Defect Water Example



1.268	179.9849	4011200	178.97706	179	179.9963838	180.0075373	0.996383769	1.007537312

Aknowledgements

United States Environmental Protection Agency

U.S. EPA RTP Team

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