

Application of Quadrupole-Orbitrap MS for Enhanced-Sensitivity in Targeted and Non-Targeted Analysis of PFAS in Environmental Waters

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San Jose, CA



Objectives

- Assess sensitivity and quantitative performance of Thermo Scientific™ Orbitrap Exploris™ 240 MS for 48 targeted PFAS compounds:
 - Direct injection of raw/finished drinking water via LC-HRAM
 - Analysis of NIST Standard Reference Material for PFCAs
- Perform AcquireX-mediated non-targeted analysis of AFFF formulations for PFAS discovery
 - Mixture of six AFFF formulations diluted 10,000x and measured using 5 injections for DDA MS²
 - ESI+ and ESI- acquisitions performed
- Analyze NC Haw river water extracts (WAX) for PFAS and other emerging pollutants
 - Thirteen water samples analyzed via AcquireX workflow (3 DDA MS² injections of two mixtures)

Orbitrap Exploris 240 Mass Spectrometer



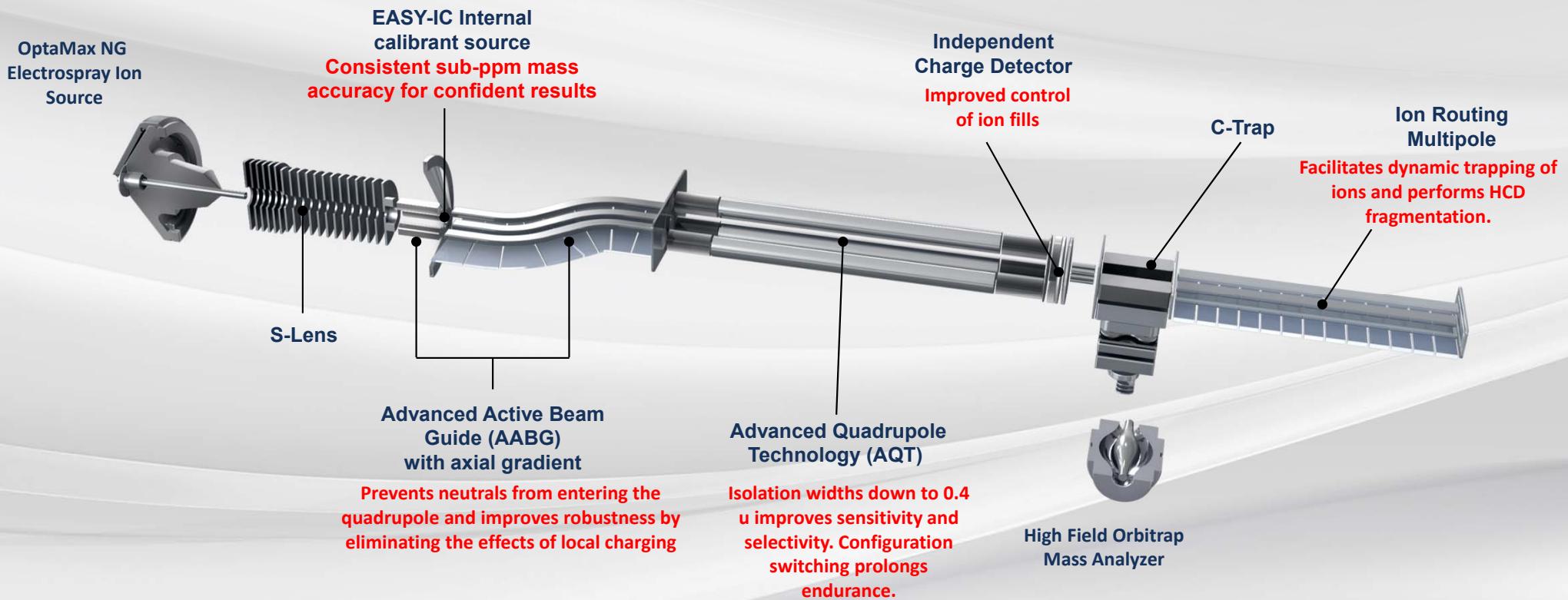
Leading Performance

With leading performance, application versatility, and operational simplicity, the Orbitrap Exploris 240 mass spectrometer fast tracks your path to high-confidence discovery and identification.

- Environmental testing
- Food Safety testing
- Academic Research analysis
- Clinical Proteomics Research analysis
- Metabolomics analysis
- Lipidomics analysis
- Biopharmaceutical analysis

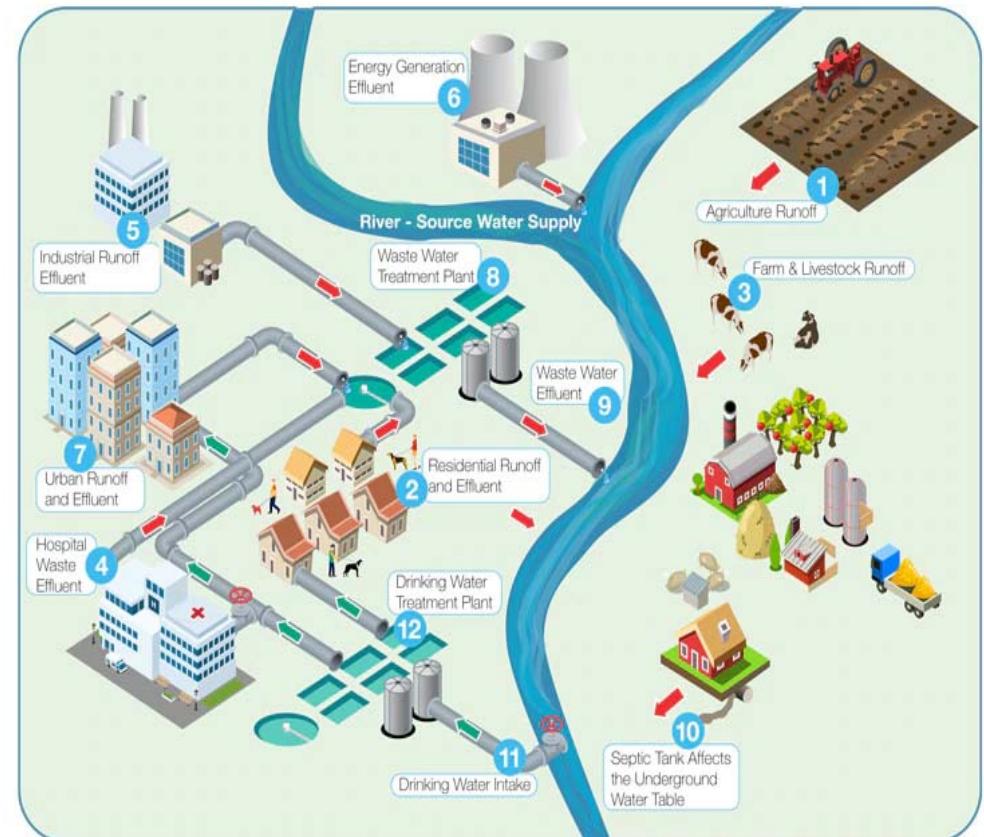
- **Mass range:** 40 - 6000 m/z (8000 m/z optional)
- **Quad isolation:** down to 0.4 u & up to 2500 u
- **Scan rate MS²:** up to 22 Hz
- **Max resolution:** 240,000 (FWHM) at m/z 200
- **Dynamic range:** > 5000:1
- **Mass Accuracy:** 3 ppm RMS ext., 1 ppm RMS int., sub 1 ppm EASY-IC
- **Polarity Switching:** 125 ms tSIM scan
- **Dissociation:** Higher energy Collisional Dissociation (HCD)
- **Scan Analysis:** dd-MS2 Top N, APD, Acquire X, TMT @ 45k res
- **Analyzer:** Quadrupole, High Field Orbitrap
- **Compact:** 530 x 760 x 700 mm (w,d,h)
- **Options:** BioPharma , FAIMS Pro interface

Thermo Scientific Orbitrap Exploris 240 MS Schematic

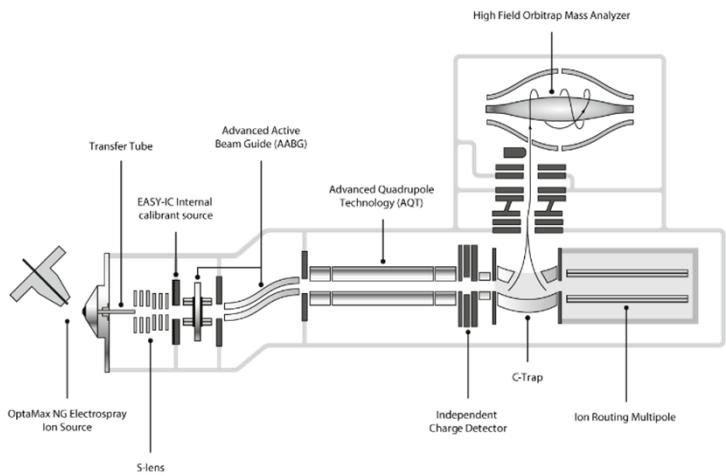


New Emerging Contaminates from Farm to Environmental to Fork

- Newly introduced or newly discovered compounds
- Improved knowledge of health effects from exposure
- Changes in occurrence data
- Observed concentrations have elevated or are now detectable due to changing environmental conditions (e.g., drought, bioaccumulation) or advances in sensitivity of monitoring technologies
- Feasibility of monitoring and/or treatment technologies
- Future exposure risks



Targeted Analysis Method



- Thermo Scientific Orbitrap Exploris 240 MS
 - ESI spray voltage: 3500 V - Legacy Compounds
1500 V - Ether Acids
 - Sheath Gas: 40 - Legacy compounds
30 - Ether acids
 - Auxiliary Gas: 10
 - Sweep Gas: 1
 - Ion Transfer Tube: 325 °C – Legacy Compounds
150 °C – Ether Acids
 - Vaporizer Temp: 300 °C
 - EASY-IC enabled
 - Full scan (120K resolution)

- Thermo Scientific™ Vanquish™ UHPLC System
 - Thermo Scientific™ Accucore™ RP-MS 2.6 µm, 100x2.1, analytical column
 - Thermo Scientific™ Acclaim™ RSLC 120 C18, 2.2 µm 120 Å 2.1 x 50mm delay column
 - 50 µL injection
 - Flow rate: 0.5 mL/min
 - Mobile phase gradient (A) 98:2 H₂O: MeOH, 2mM NH₄OAC, 0.1% CH₃COOH
(B) 98:2 MeOH: H₂O, 2mM NH₄OAC, 0.1% CH₃COOH

Time (min)	%A	%B
0	100	0
1	70	30
6	55	45
13	20	80
14	0	100
18-21	100	0

Estimated Detection limits for targeted PFAS compounds

Fluoroalkyl sulfonamides

Compound	TSQ Altis (ng/L)	Exploris 240 (full scan) (ng/L)
PFBSA	1	1
PFHxSA	1	2
PFOSA	1	20
NMeFOSAA	1	50
NEtFOSAA	5	50

Fluorotelomer sulfonates

Compound	TSQ Altis (ng/L)	Exploris 240 (full scan) (ng/L)
4:2 FTS	1	5
6:2 FTS	1	5
8:2 FTS	1	20
10:2 FTS	1	50

Perfluoroalkylcarboxylic acids

Compound	TSQ Altis (ng/L)	Exploris 240 (full scan) (ng/L)
PFBA	1	50
PFPeA	2	2
PFHxA	2	2
PFHpA	1	5
PFOA	1	1
PFNA	5	5
PFDA	5	20
PFUnDA	1	50
PFDoDA	1	50
PFTrDA	2	50
PFHxDA	10	50

Estimated Detection limits for targeted PFAS compounds (50 ul injections) can switch

Perfluoroalkylsulfonic acids

Compound	TSQ Altis (ng/L)	Exploris 240 (full scan) (ng/L)
PFBS	1	1
PPeS	1	1
PFHxS	2	2
PFHpS	1	2
PFOS	1	10
PFNS	1	10
PFDS	10	10
PFDoS	10	50

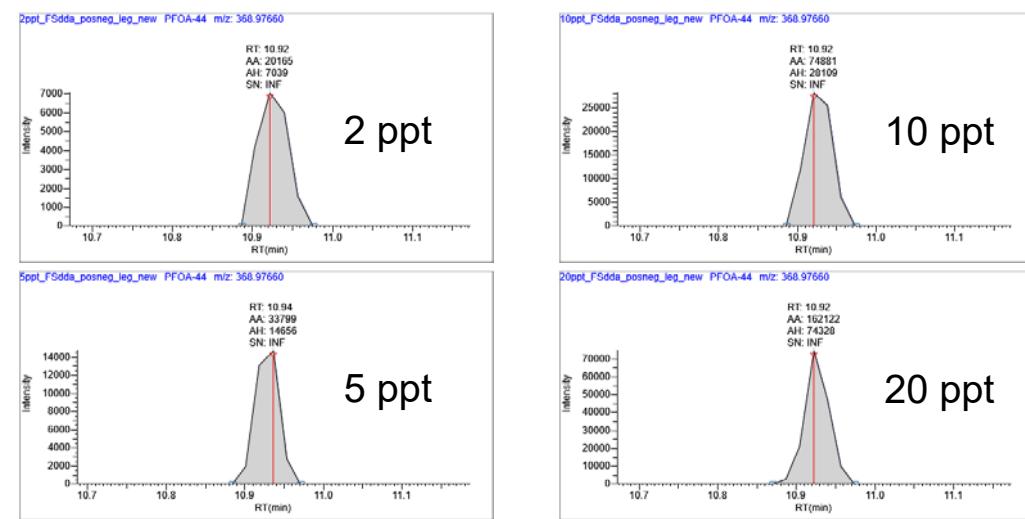
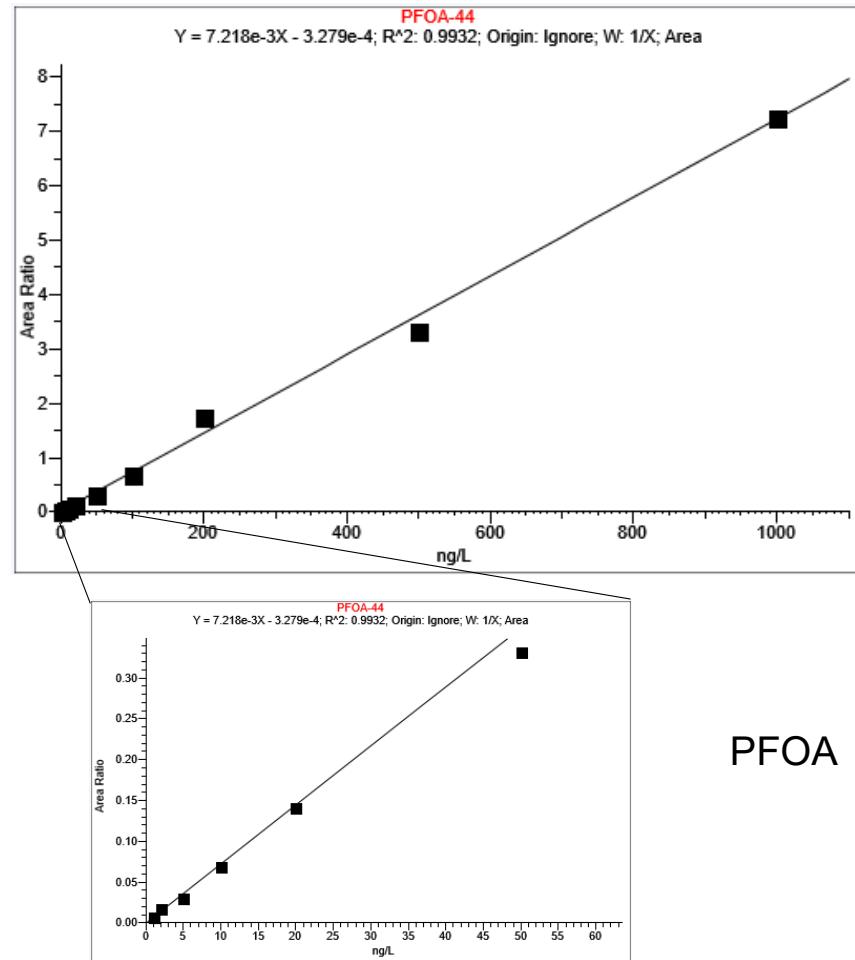
Fluorinated zwitterions

Compound	TSQ Altis (ng/L)	Exploris 240 (full scan) (ng/L)
N-AP-FHxSA	5	50
N-TAmP-FHxSA	1	50
N-CMAmP-6:2FOSA (6:2 FTAB)	2	200

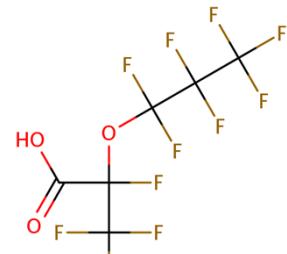
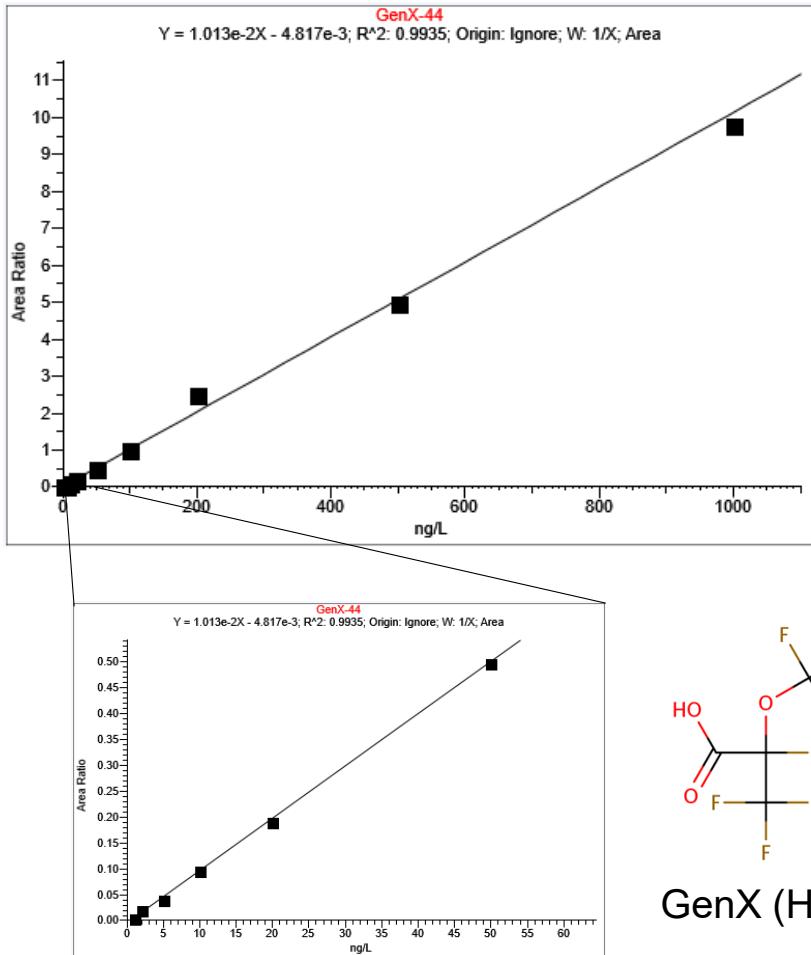
Perfluoroalkylether acids

Compound	TSQ Altis (ng/L)	Exploris 240 (full scan) (ng/L)
PFMOAA	5	50
PMPA	1	1
PFO2HxA	1	200
PEPA	1	5
NVHOS	1	1
PFO3OA	1	50
GenX	1	2
PFO4DA	1	20
ADONA	1	1
Hydro-EVE acid	1	1
Nafion by-product 1	1	1
PFO5DoDA	2	50
Nafion by-product 2	1	1
F-53B (Major)	1	5
F-53B (Minor)	1	10

Outstanding sensitivity for legacy PFAS (PFOA)

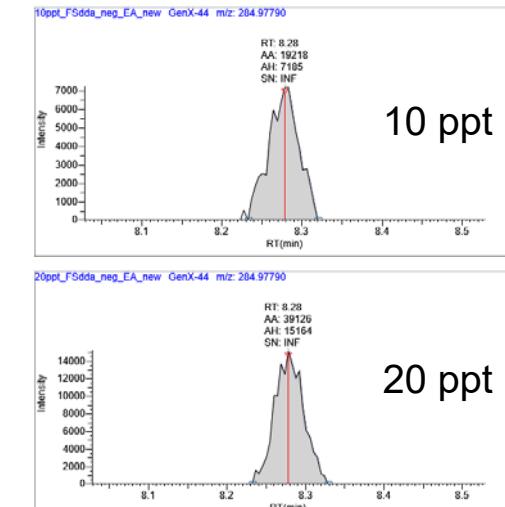
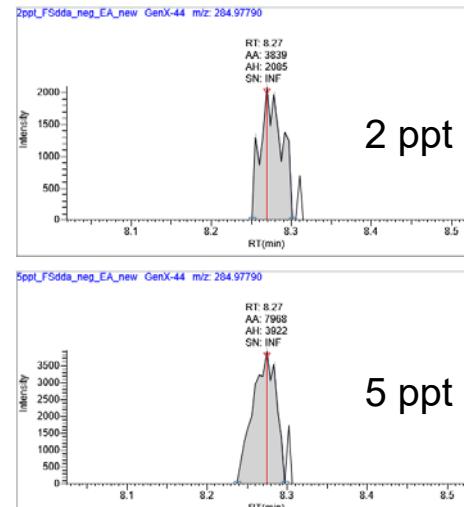


Outstanding sensitivity for emerging PFAS (GenX)



GenX (HFPO-DA)

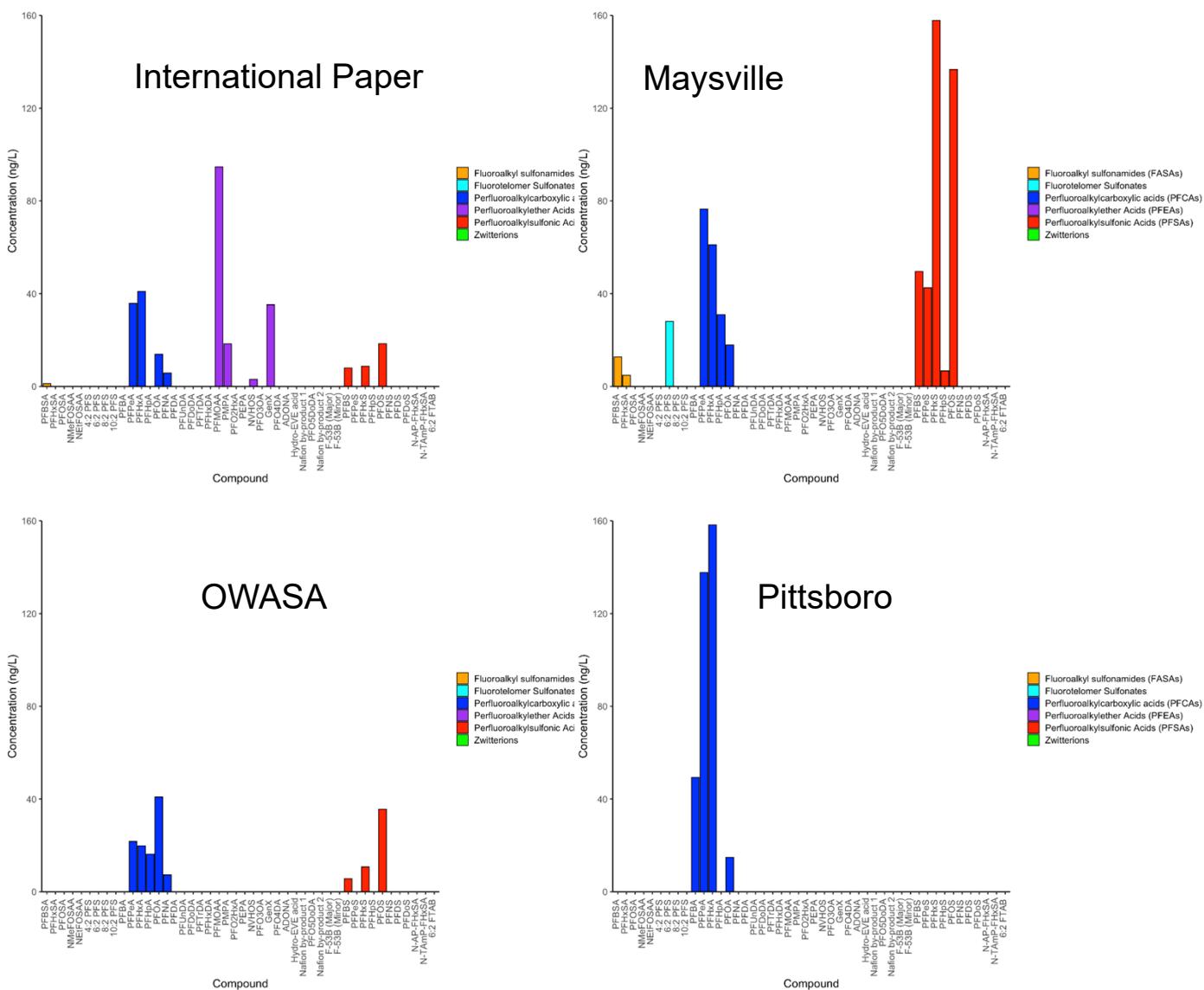
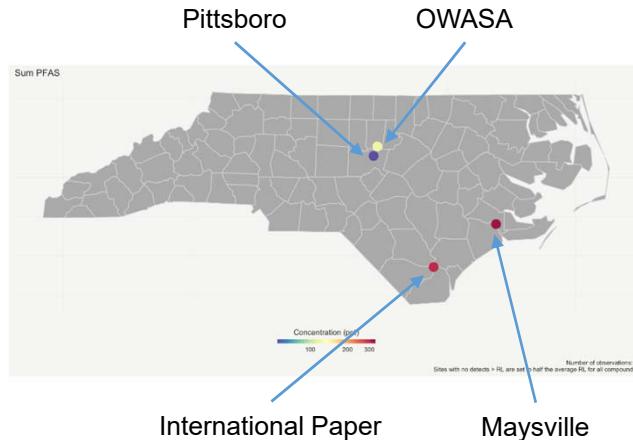
Full Scan



PFAS in North Carolina drinking water sources

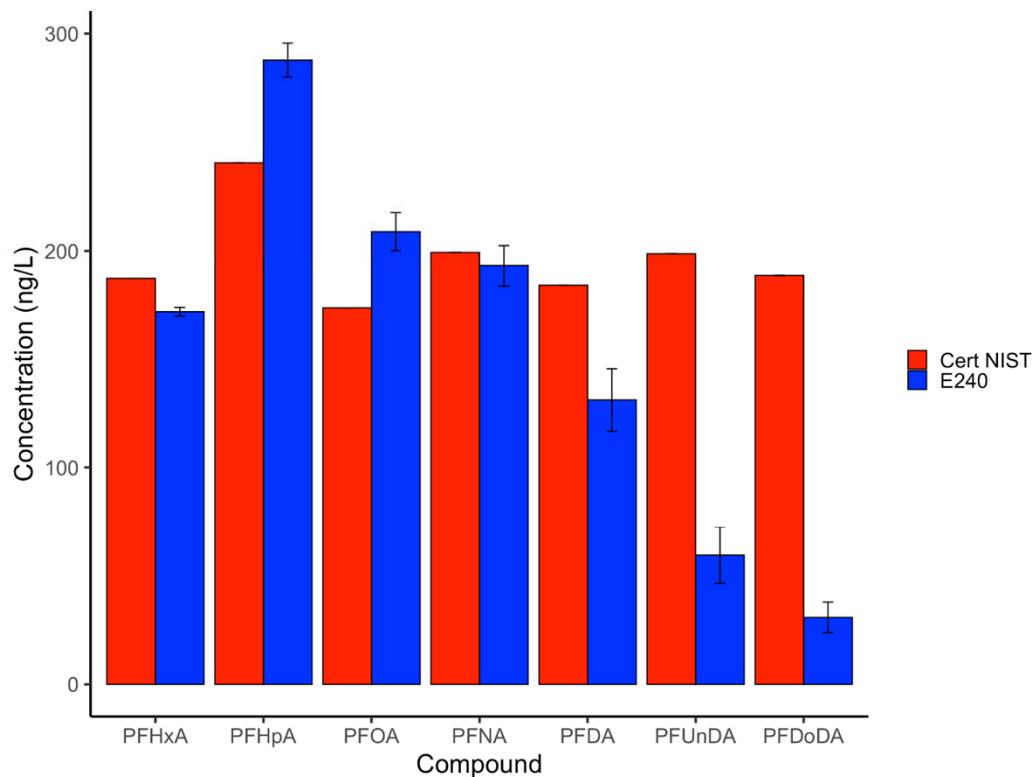
PFAS in drinking water sources around NC show evidence of different sources:

- AFFF (Maysville)
- Biosolid runoff (OWASA)
- Industrial discharge (Int. Paper, Pittsboro)

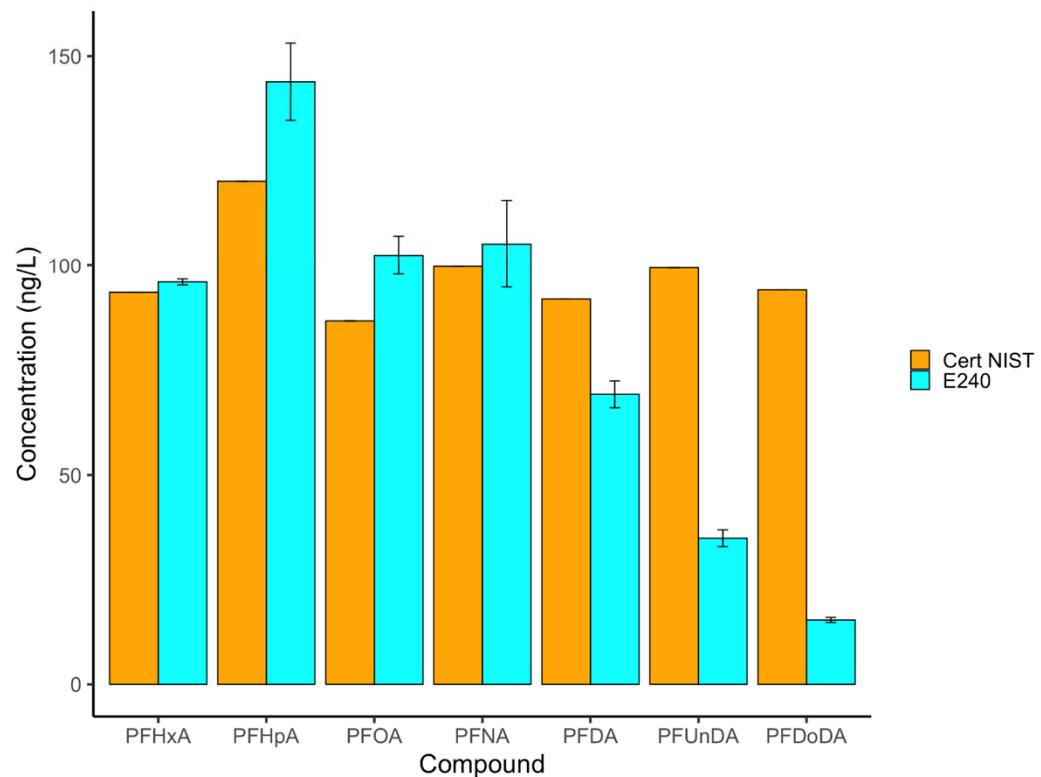


Quantitation performance for PFCAs in NIST SRM 8446

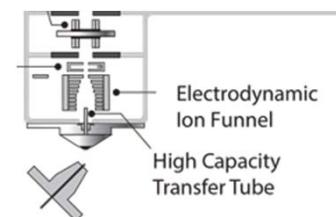
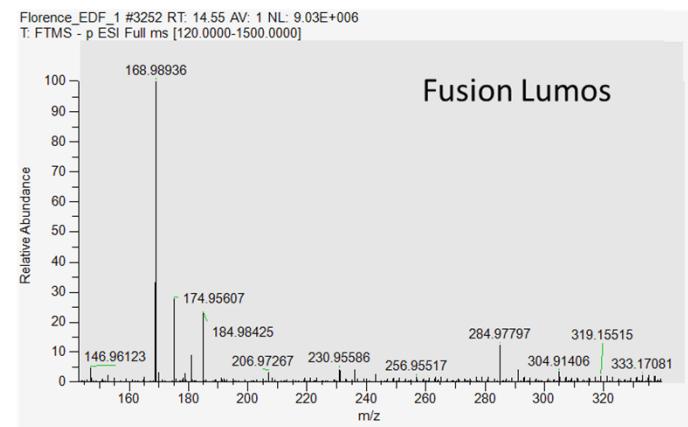
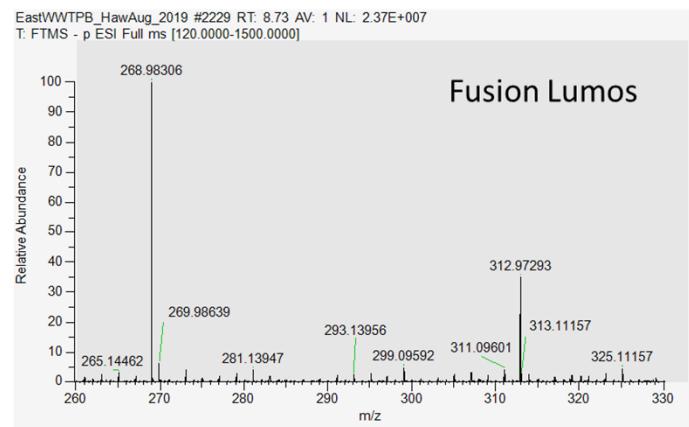
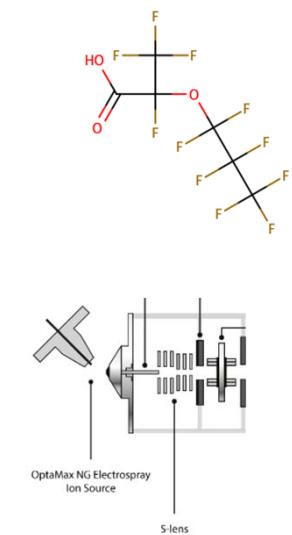
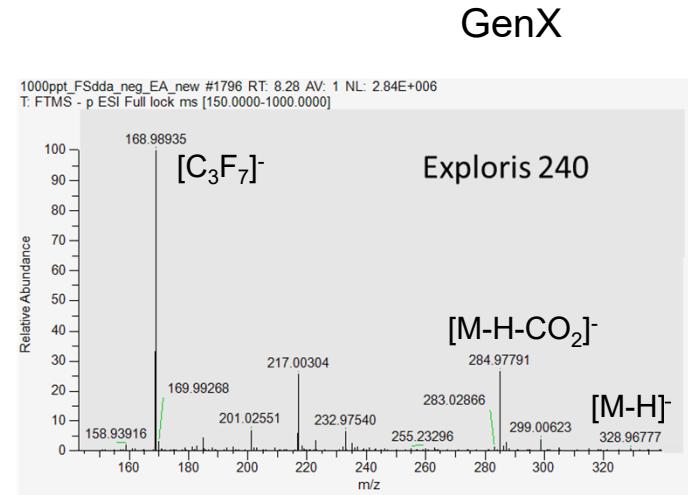
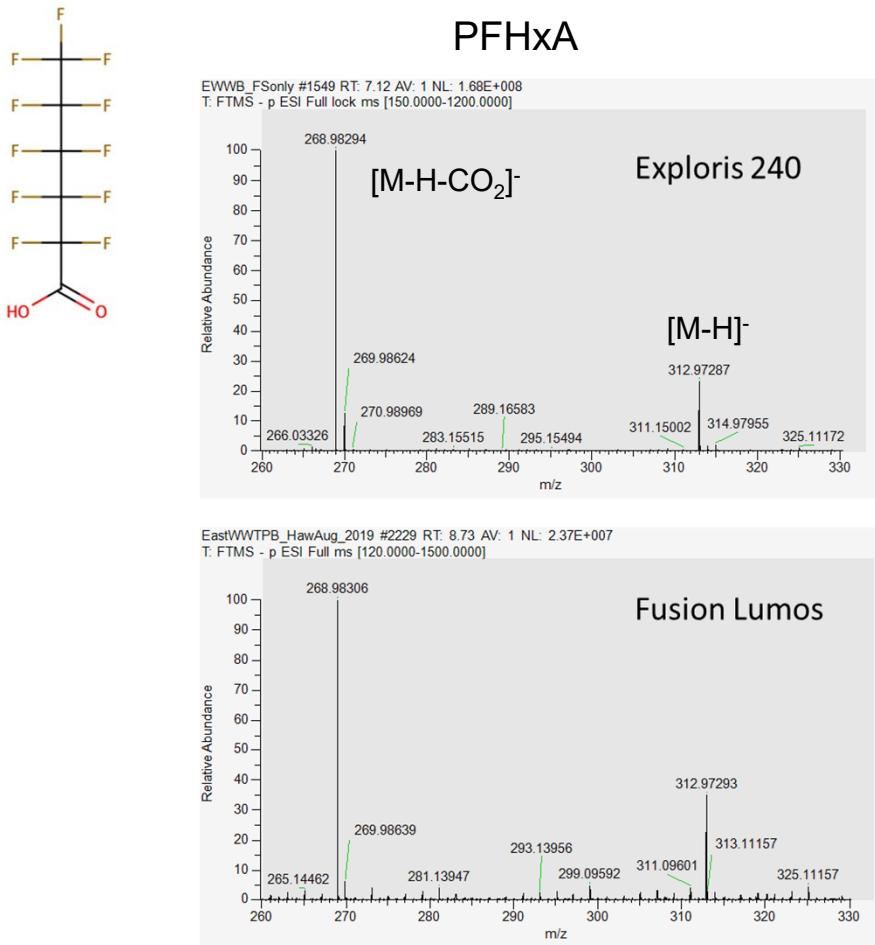
Dilution 1



Dilution 2

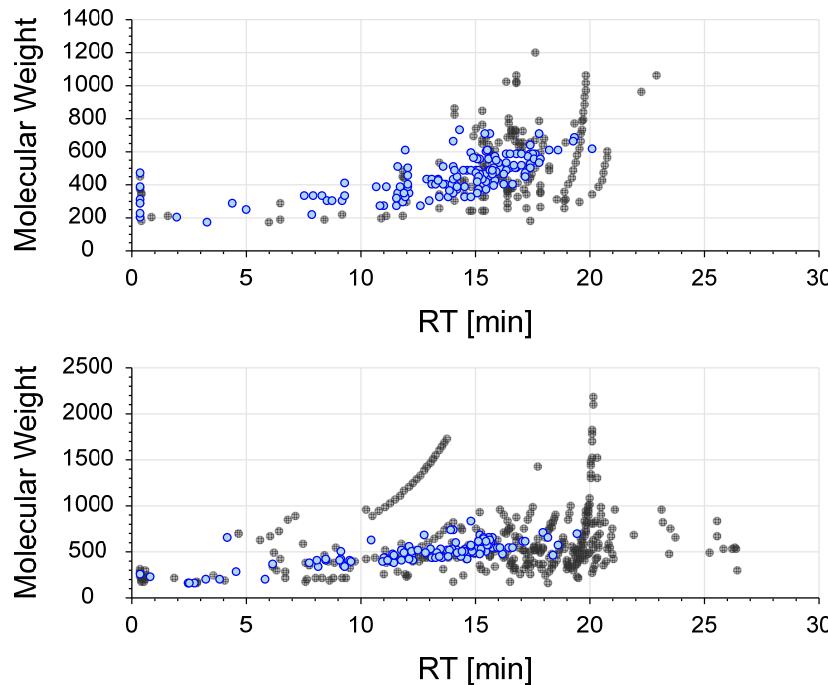


In-source fragmentation for PFAS: comparison of Orbitrap Exploris 240 MS & Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ MS



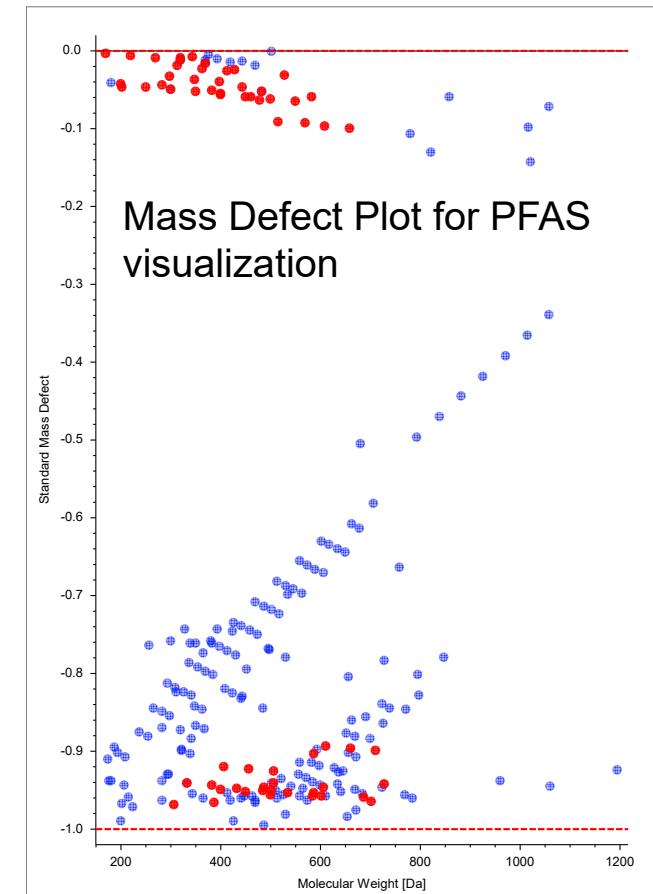
Non-targeted analysis of PFAS in Aqueous Film-Forming Foams

- 307 Total compounds detected from Six AFFF formulations in ESI(-)
- 449 Total compounds detected in ESI(+)
- AcquireX analysis: 100% DDA MS2 acquisition coverage for both polarities (5 injections)
- 140 putative PFAS compounds detected in ESI(-), 116 PFAS in ESI(+)
- Note: Many “redundant” detections because of branched isomers



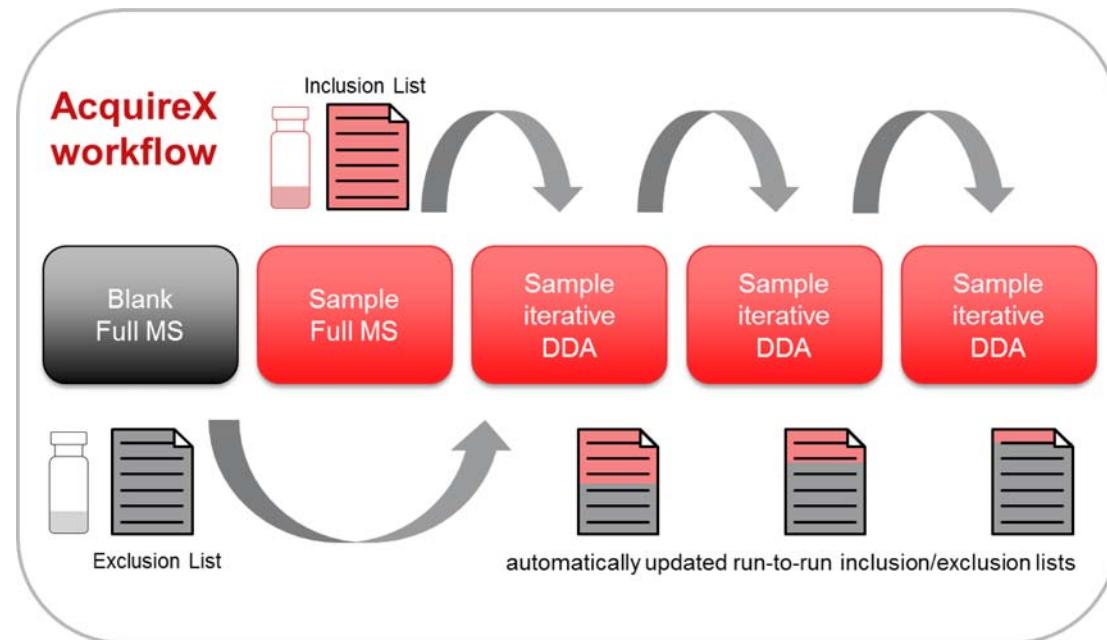
ESI(-)

ESI(+)



Mass Defect Plot for PFAS visualization

Enhanced data acquisition methods for increased DDA MS² coverage

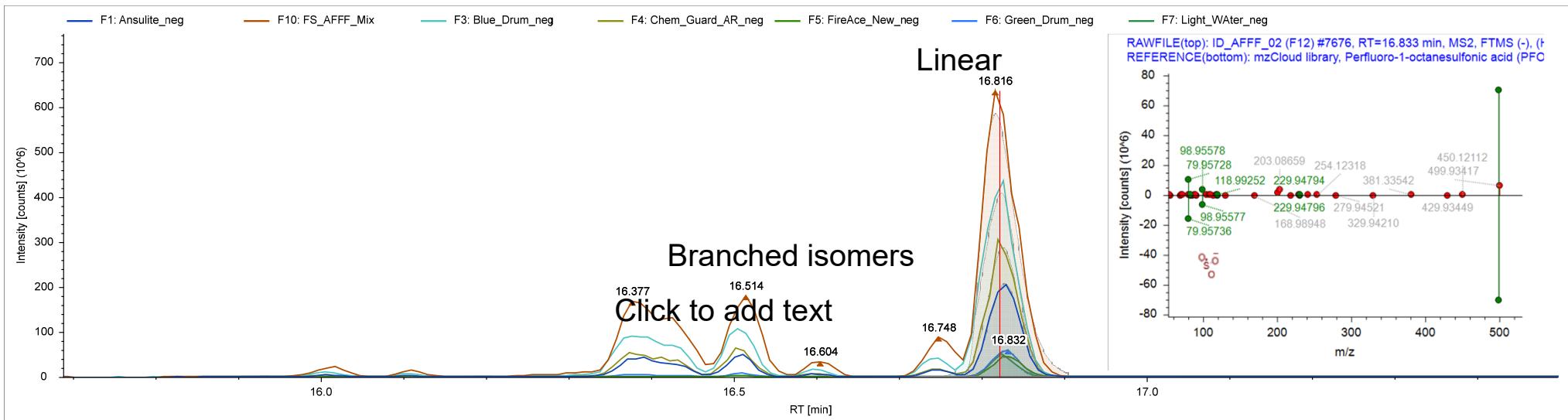


Results for analysis of EPA ENTACT samples:

1. Standard DDA (three replicate injections) with intensity-dependent precursor selection – **< 60% MS² coverage (typical)**
2. AcquireX DDA (three sequential injections) with updated exclusion/inclusion lists – **85–100% MS² coverage**

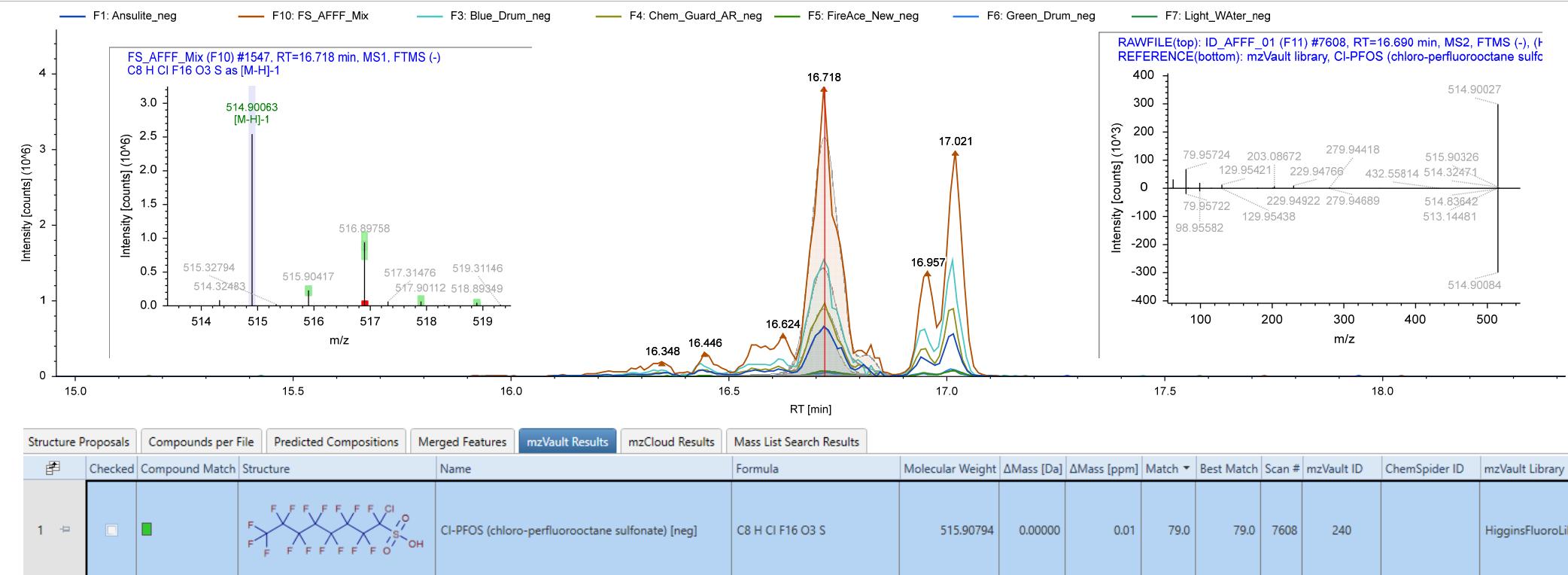
Ionna Ntai et al. (2018) Improved Metabolome Coverage and Increased Confidence in Unknown Identification Through Novel Automated Acquisition Strategy Combining Sequential Injections and MS². Poster presented at the 66th ASMS Conference on Mass Spectrometry and Allied Topics, San Diego, CA, June 3 – 7, 2018.

mzCloud annotation example: PFOS



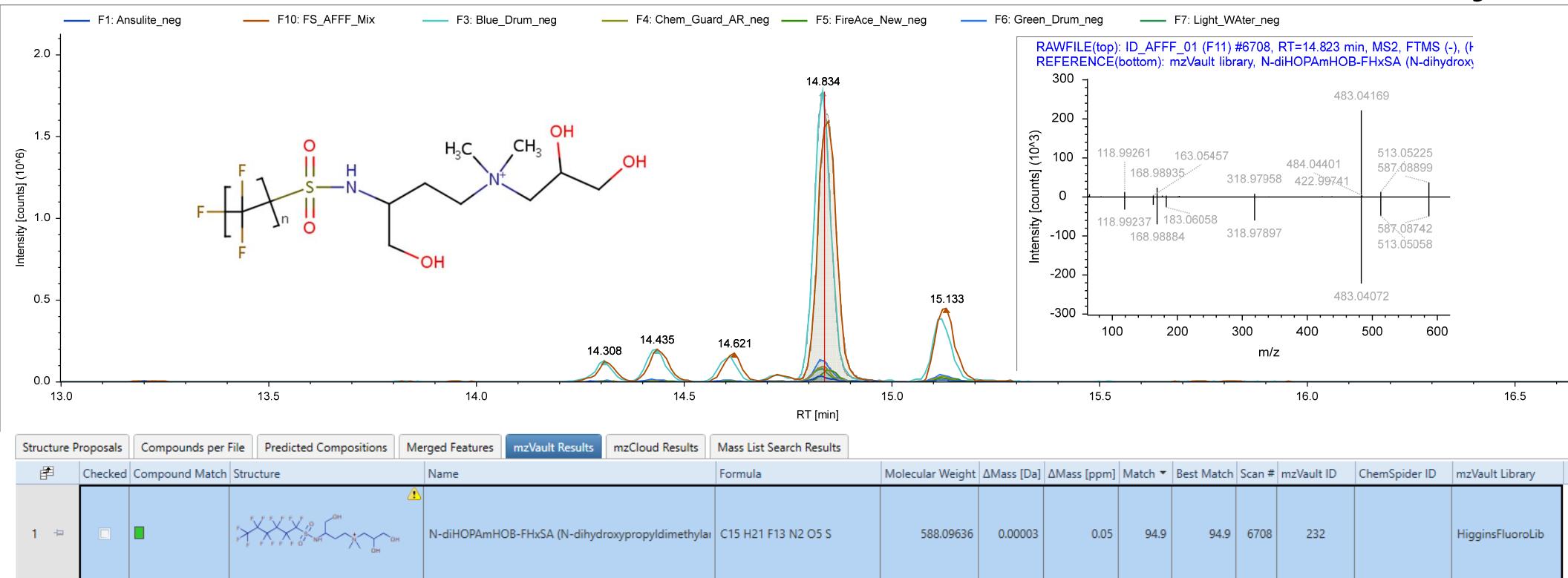
	Structure Proposals	Compounds per File	Predicted Compositions	Merged Features	mzVault Results	mzCloud Results	Mass List Search Results
1	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Compound Match	Structure	Name	Formula	Molecular Weight
					Perfluoro-1-octanesulfonic acid (PFOS)	C8 H F17 O3 S	499.93749
						ΔMass [Da]	-0.00019
						ΔMass [ppm]	-0.38
						Match ▾	87.3
						Best Match	95.0
						Best Sim. Match	98.3

Novel PFAS annotation via custom mzVault library



Note: this Thermo Scientific mzVault library is custom-translated from a colleague's PFAS HRAM spectral library collected on a Sciex QTOF. Total # compounds: 320 compounds, 633 spectra.

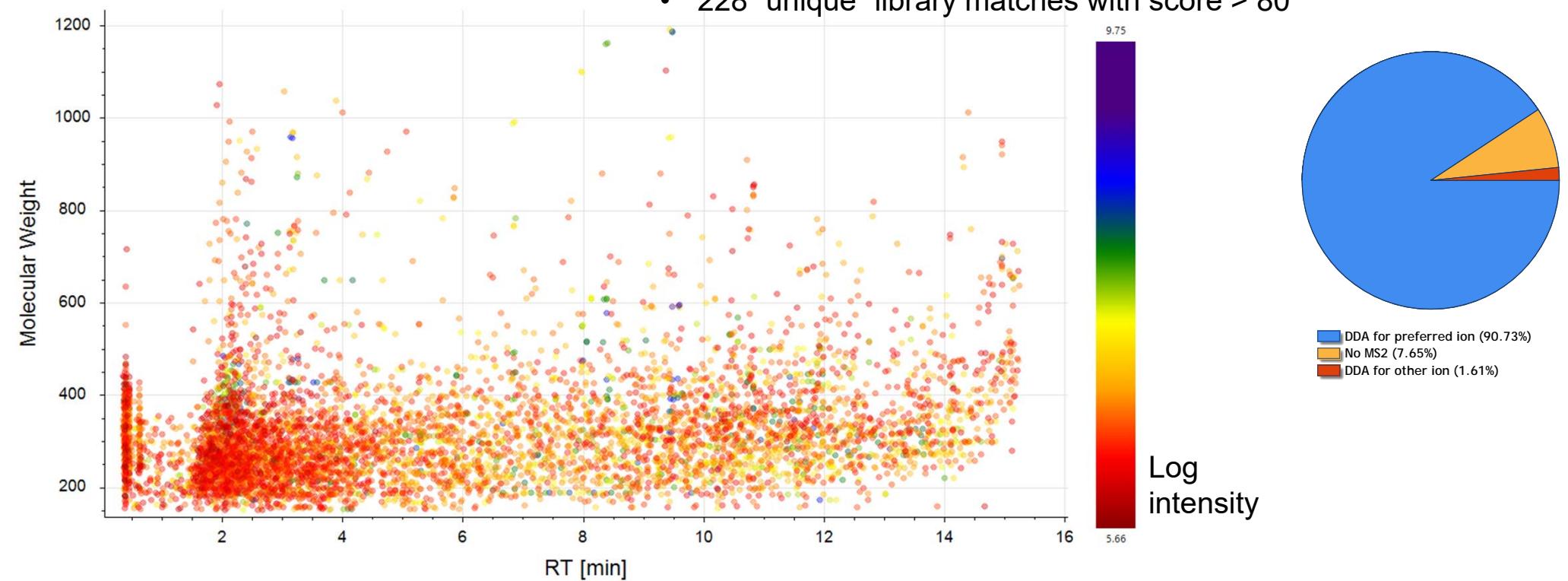
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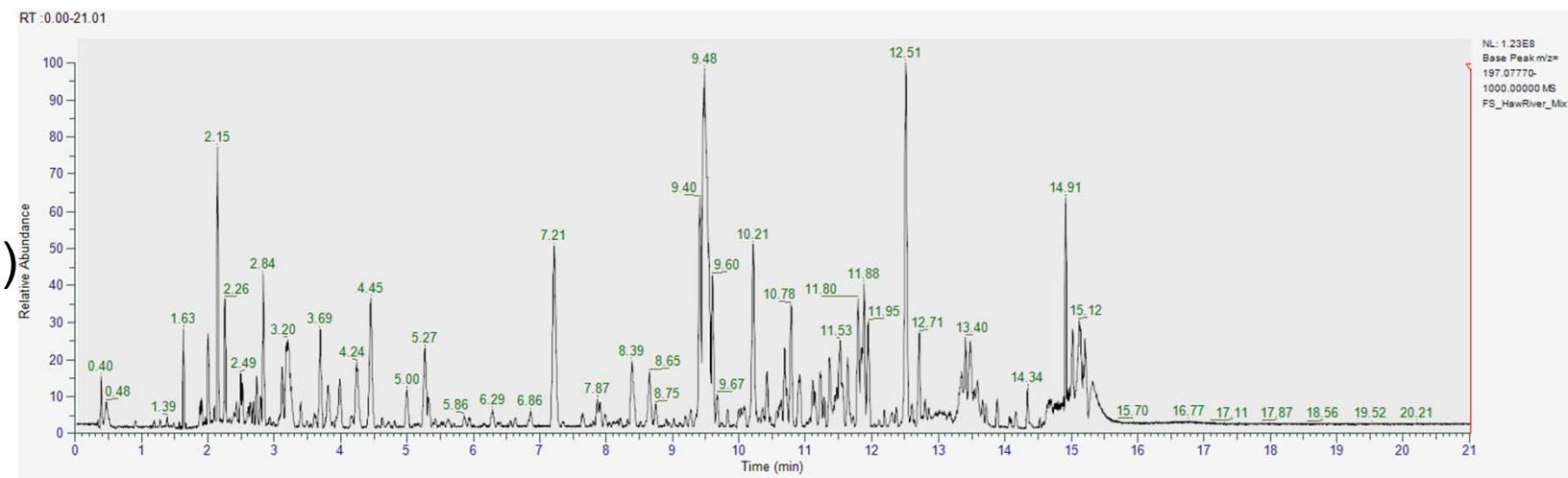
PFAS and other emerging pollutants in NC river water

- 6,767 Total compounds detected from 13 water samples in ESI(-)
- AcquireX analysis: > 90 % DDA MS2 acquisition coverage (3 injections)
- 139 mzCloud matches with score > 80
- 145 mzVault matches with score > 80
- 228 “unique” library matches with score > 80

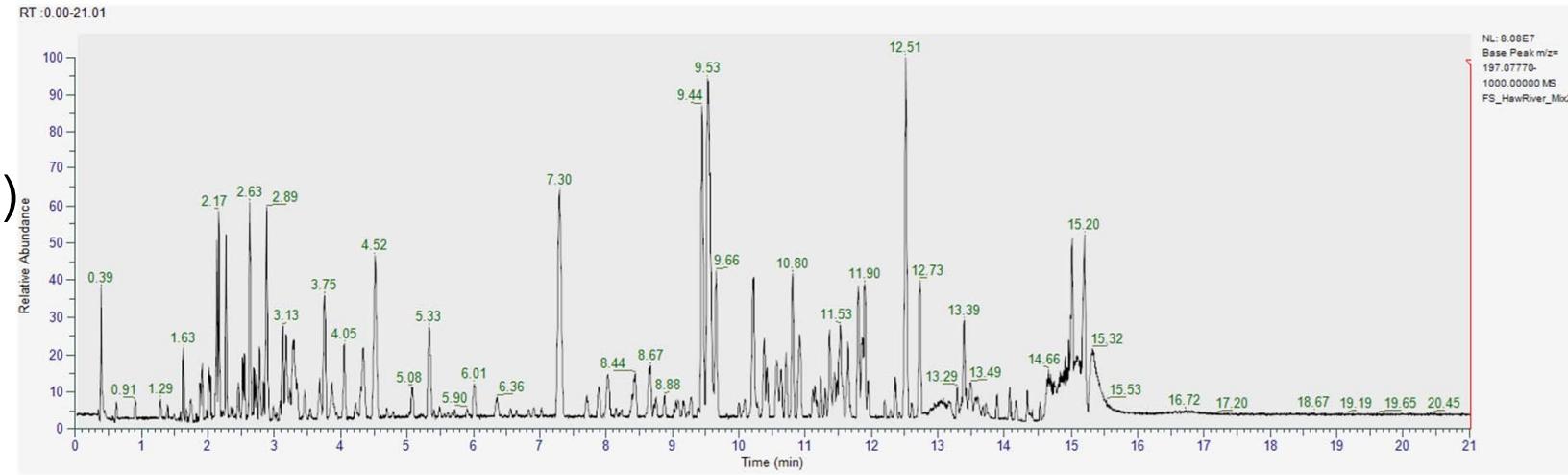


Two chimeric mixtures were analyzed via AcquireX

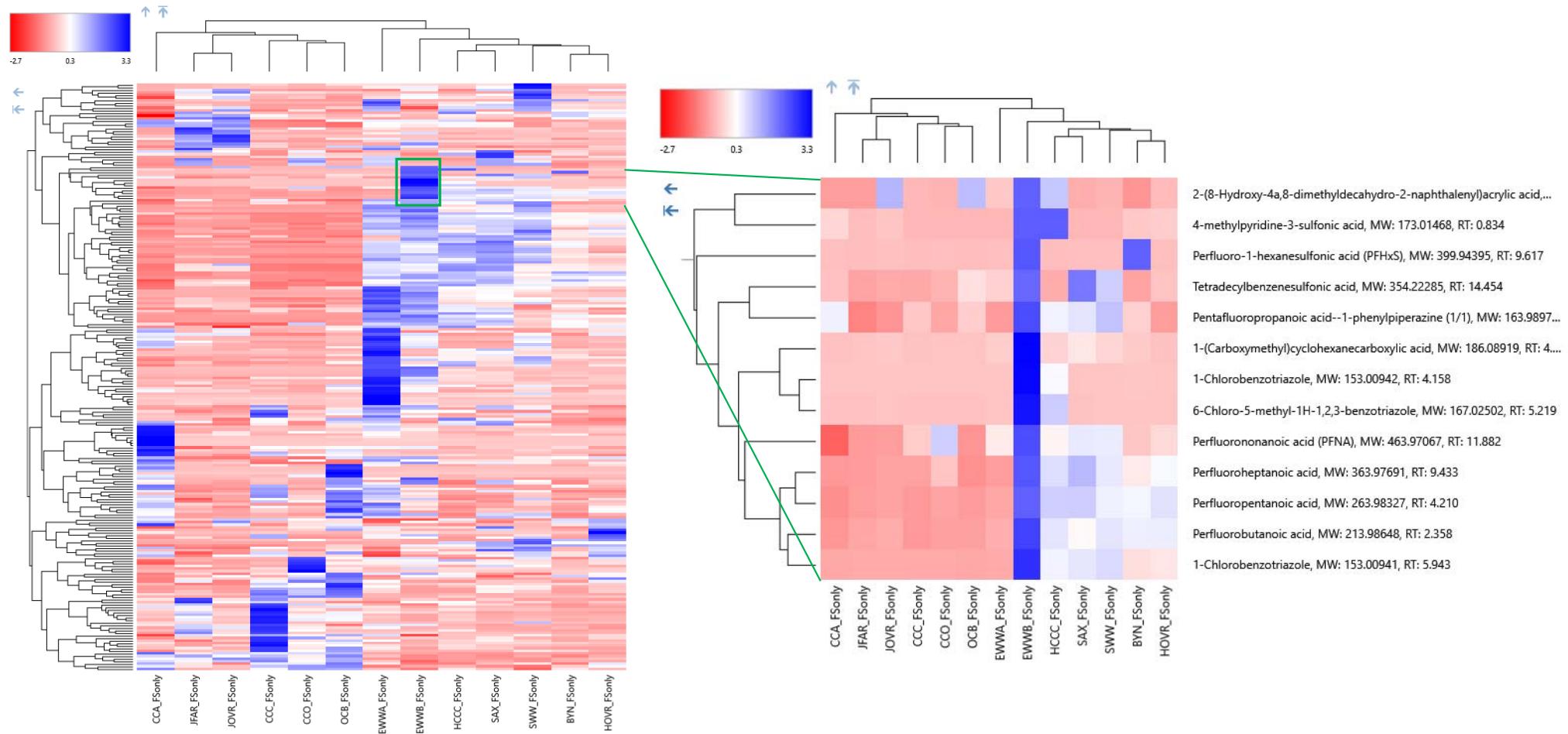
Mix 1 (6 samples)



Mix 2 (7 samples)

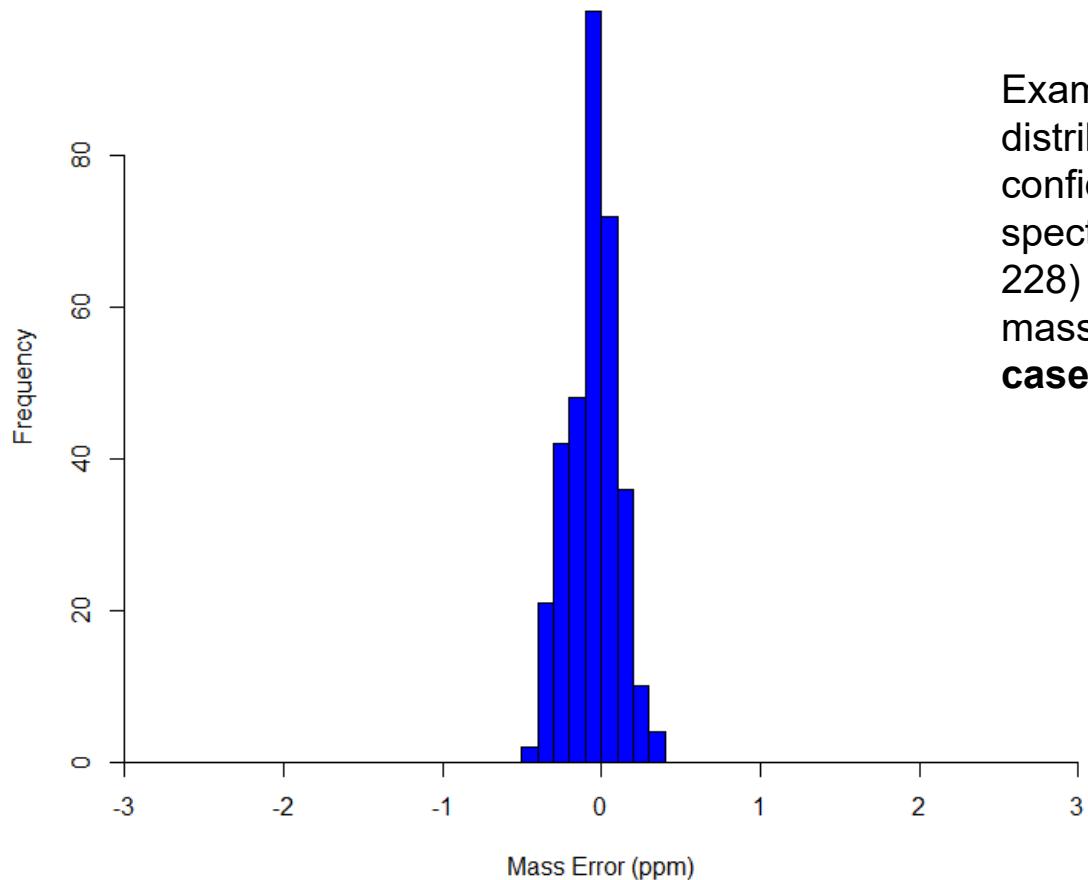


Hierarchical clustering of 228 library matches



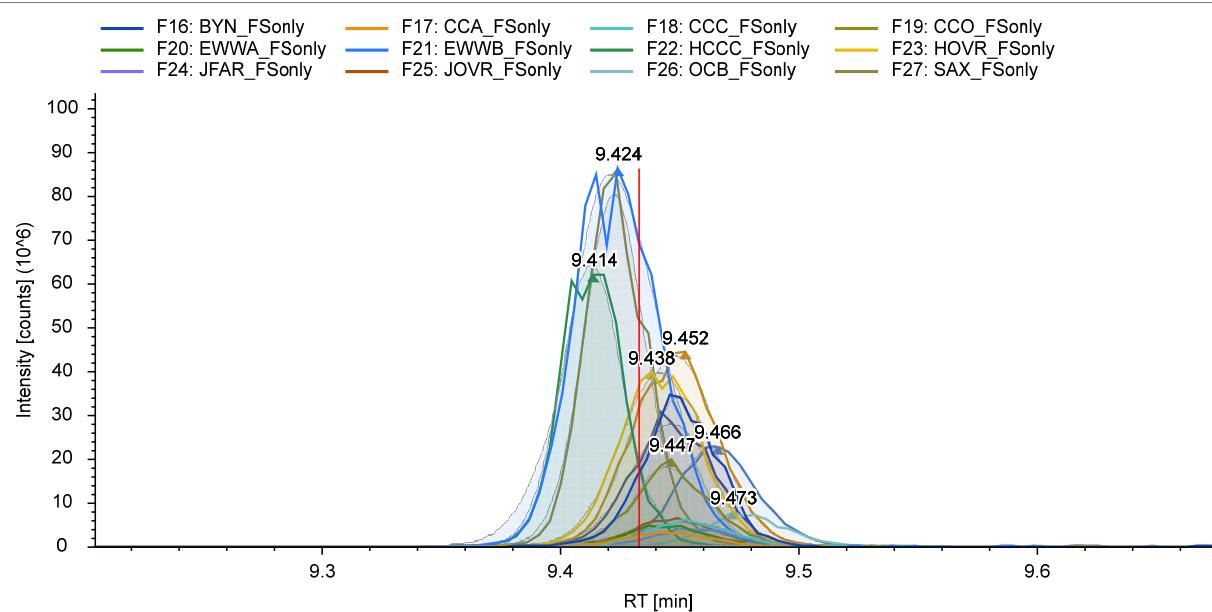
EASY-IC ion source yields exceptional mass accuracy

Library match mass error distribution

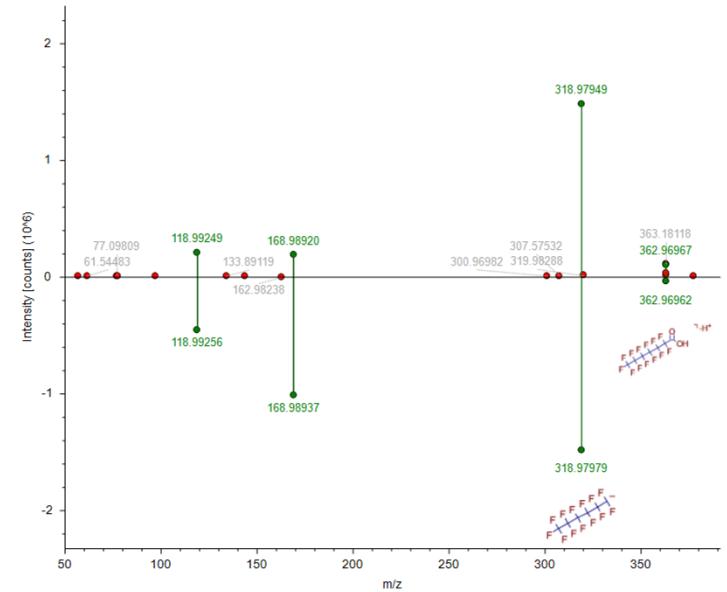


Examination of mass accuracy distribution of compounds having confident mzCloud/mzVault spectral library matches ($n = 228$) reveals extremely high mass accuracy (**< 0.5 ppm in all cases**).

Perfluoroheptanoic acid in Haw river water

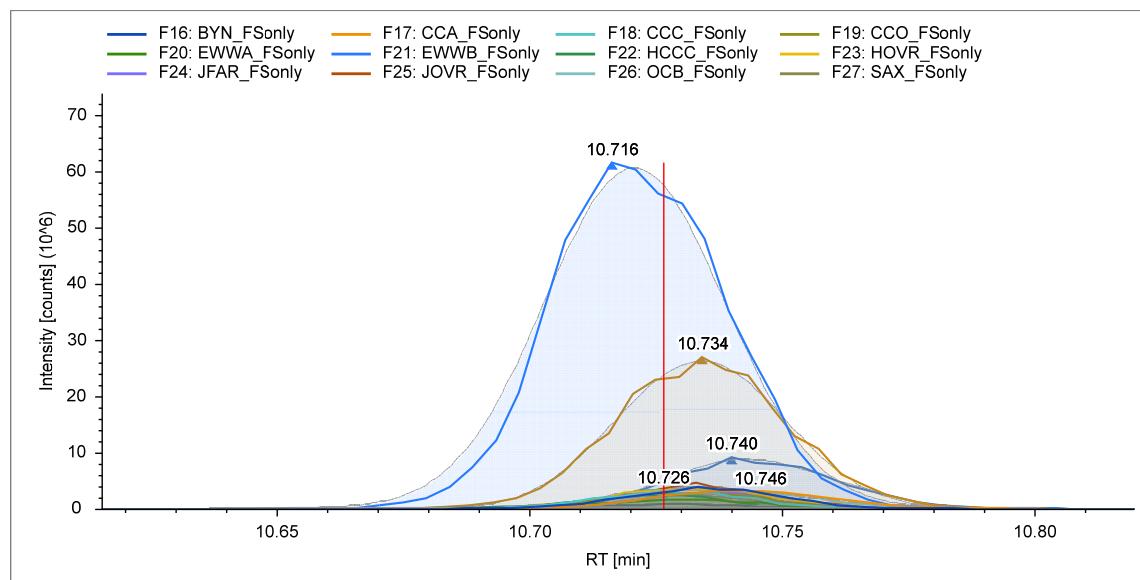


RAWFILE(top) ID_HawRiver_Mix2_02 (F39) #4886, RT=9.442 min, MS2, FTMS (-), (HCD, DDA, 363.1813@{15,45,60}, -1)
REFERENCE(bottom): mzCloud library, Perfluoroheptanoic acid, C7 H13 O2, MS2, FTMS, (HCD, 362.9696@{10,30,40})

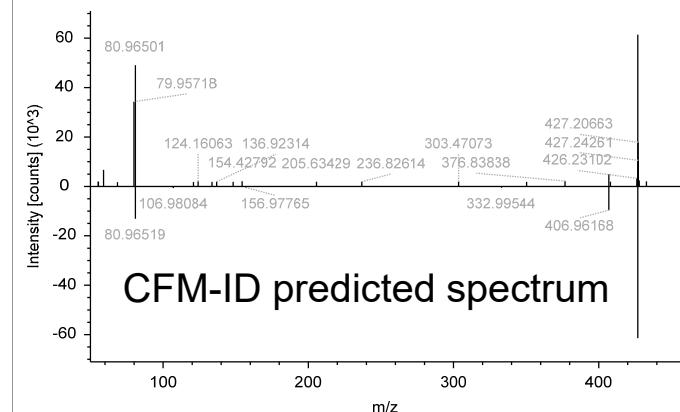


Structure Proposals		Compounds per File		Predicted Compositions		Merged Features		mzVault Results		mzCloud Results		Mass List Search Results				
		Checked	Compound Match	Structure		Name		Formula	Molecular Weight	ΔMass [Da]	ΔMass [ppm]	Match				
1	+		□			Perfluoroheptanoic acid		C7 H13 O2	363.97690	0.00001	0.04	91.7				

An example of in silico MS/MS library match for PFAS ID: 6:2 FTS

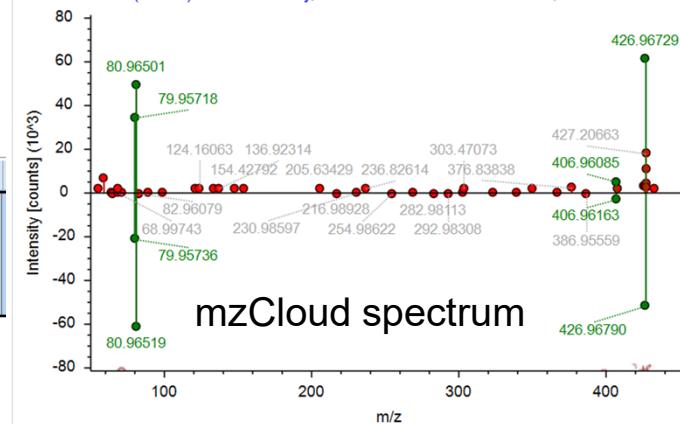


RAWFILE(top): ID_HawRiver_Mix1_01 (F32) #5492, RT=10.718 min, MS2, FTMS (-), (HCD, DD, REFERENCE(bottom): mzVault library, 1-Octanesulfonic acid, 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctane)

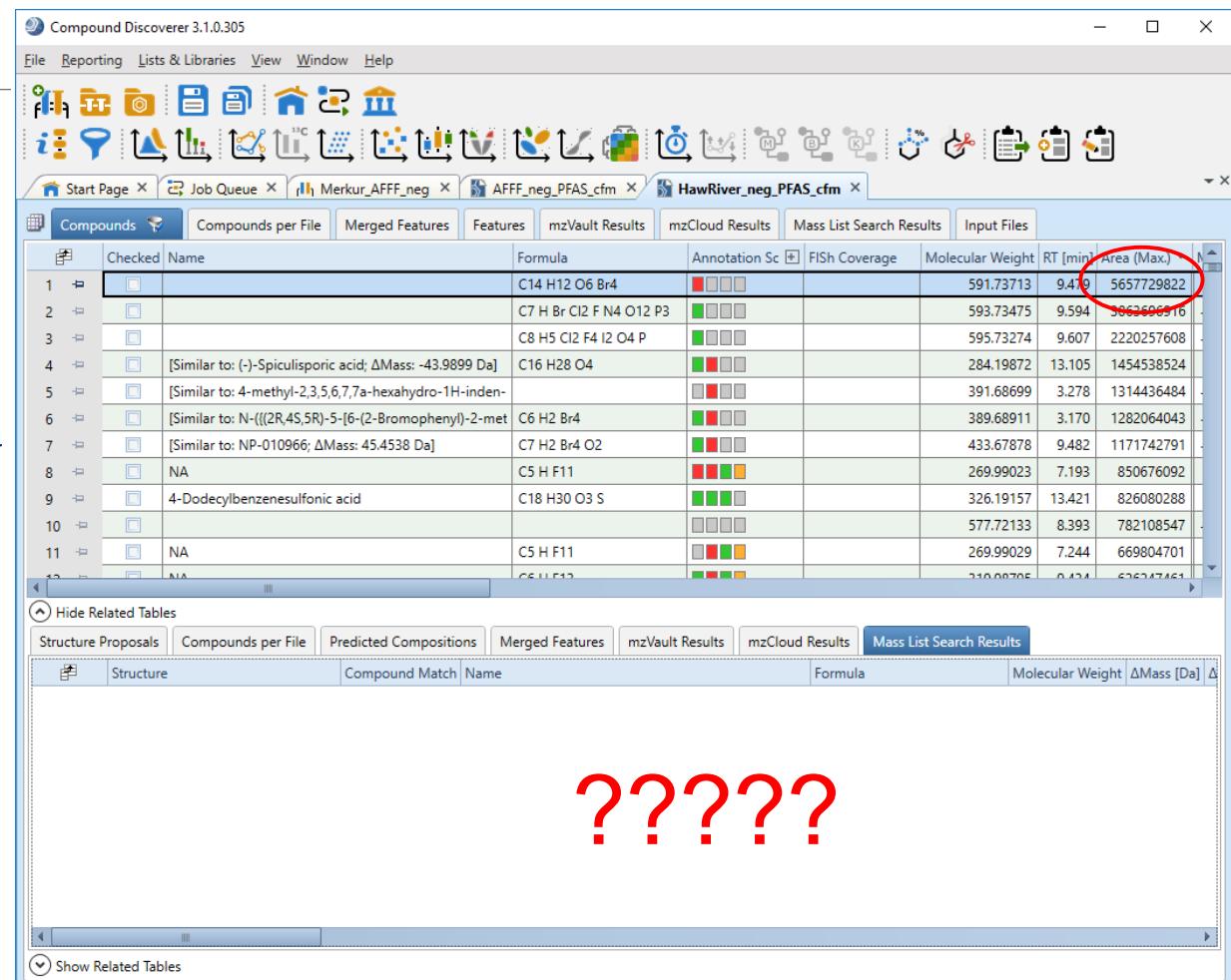
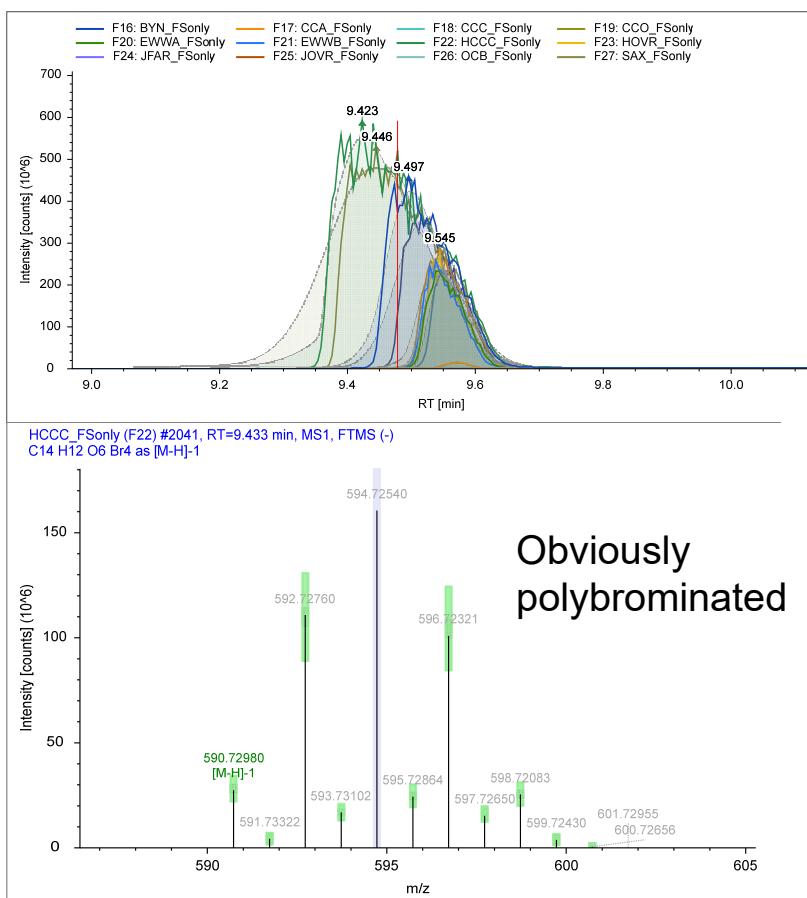


	Structure Proposals	Compounds per File	Predicted Compositions	Merged Features	mzVault Results	mzCloud Results	Mass List Search Results
1	Checked	Compound Match	Structure	Name	Formula	Molecular Weight	ΔMass [Da]
				6:2 Fluorinated telomer sulfonate	C ₈ H ₅ F ₁₃ O ₃ S	427.97518	-0.00006

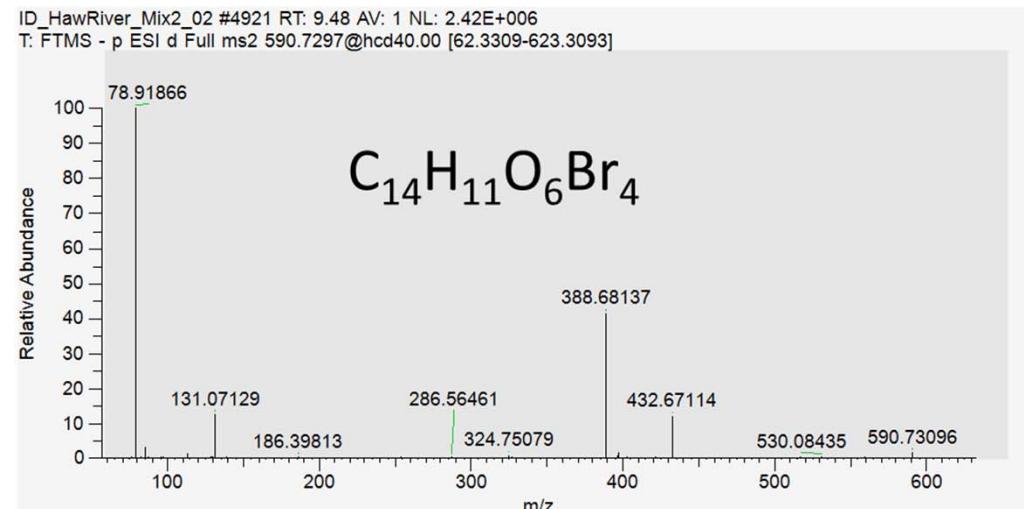
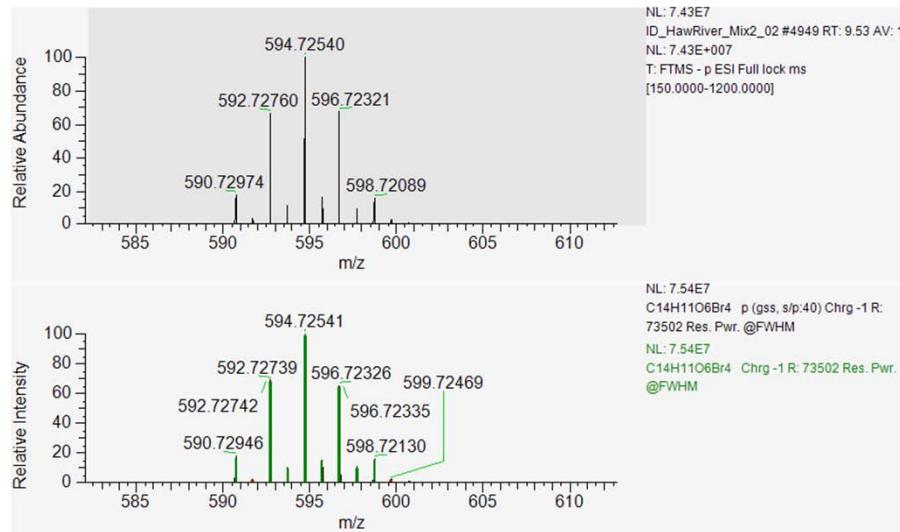
RAWFILE(top): ID_HawRiver_Mix1_01 (F32) #5492, RT=10.718 min, MS2, FTMS (-), (HCD, DD, REFERENCE(bottom): mzCloud library, 6:2 Fluorinated telomer sulfonate, C₈H₅F₁₃O₃S, MS:



The most abundant compound in the samples is a mystery.



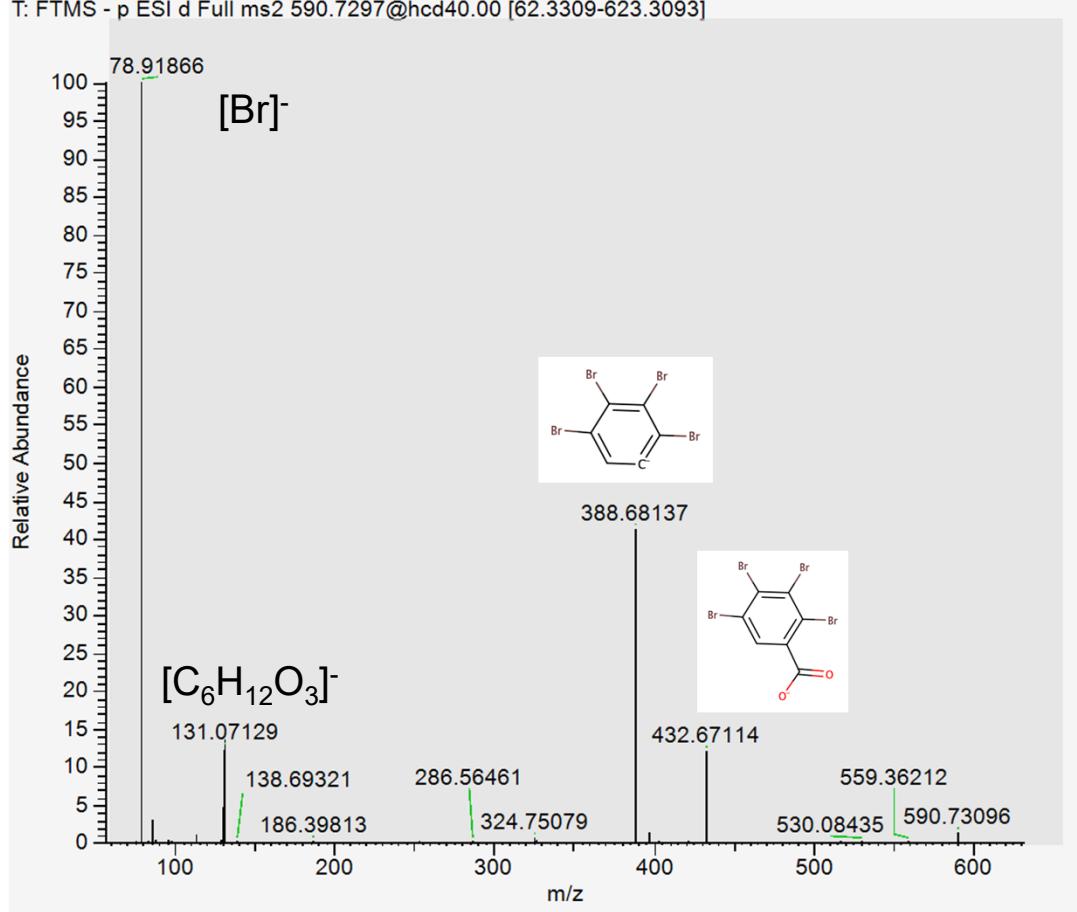
Something unexpected and unknown...



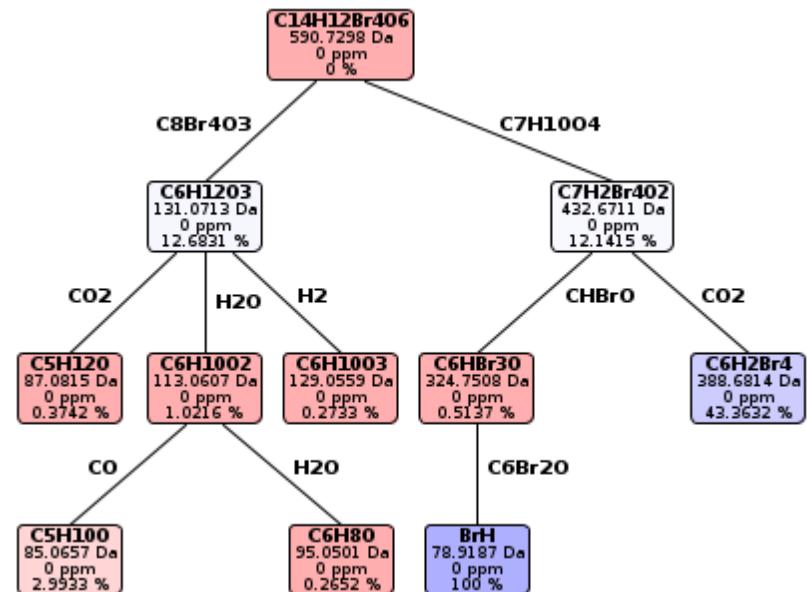
Elemental Composition Results														
Peak Mass	Display Formula	Combined Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined Score	# Matched Iso.	# Missed Iso.	MS Cov. [%]	Pattern Cov. [%]	MSMS Cov. [%]	MSMS Shift Measure	MSMS Matched Peaks
590.72974	C ₁₄ H ₁₁ O ₆ ⁷⁹ Br ₄	76.74	7.50	0.46	590.72946	1	98.79	11	0	99.96	100.00	99.85	60.56	12
Fragment														Mass
⁷⁹ Br								78.9187						
C ₅ H ₉ O								85.0657						
C ₅ H ₁₁ O								87.0815						
C ₆ H ₇ O								95.0501						
C ₆ H ₉ O ₂								113.0607						
C ₆ H ₉ O ₃								129.0559						
C ₆ H ₁₁ O ₃								131.0713						
C ₆ O ⁷⁹ Br ₃								324.7508						
C ₆ H ⁷⁹ Br ₄								388.6814						
C ₆ O ₄ ⁷⁹ Br ₃								396.7348						
C-HO ₂ ⁷⁹ Br ₄								432.6711						
C ₁₄ H ₁₁ O ₆ ⁷⁹ Br ₄								590.731						

Still trying to solve the structure...

ID_HawRiver_Mix2_02 #4921 RT: 9.48 AV: 1 NL: 2.42E+006
T: FTMS - p ESI d Full ms2 590.7297@hcd40.00 [62.3309-623.3093]



- Nothing in CAS, PubChem, or the EPA DSSTox databases with the formula $C_{14}H_{11}O_6Br_4$
- In-silico MS/MS is of limited value (fragmentation tree only)
- Best guess is a brominated phthalate flame retardant that's not been seen before



Conclusions

- Thermo Scientific Orbitrap Exploris 240 MS provides high sensitivity for quantitative analysis of PFAS in drinking water sources through direct-injection LC-HRAM
- The combination of this instrument with AcquireX data acquisition software provides complete MS² coverage for analysis of PFAS in firefighting foam
- Exceptional mass accuracy (< 0.5 ppm achieved in practice) coupled with expanded HRAM MS² spectral libraries allows high-confidence compound annotation in non-targeted analysis of water samples.