Targeted and Non-Targeted Analysis LC-Orbitrap MS workflow for the analysis of more than 40,000 PFAS compounds

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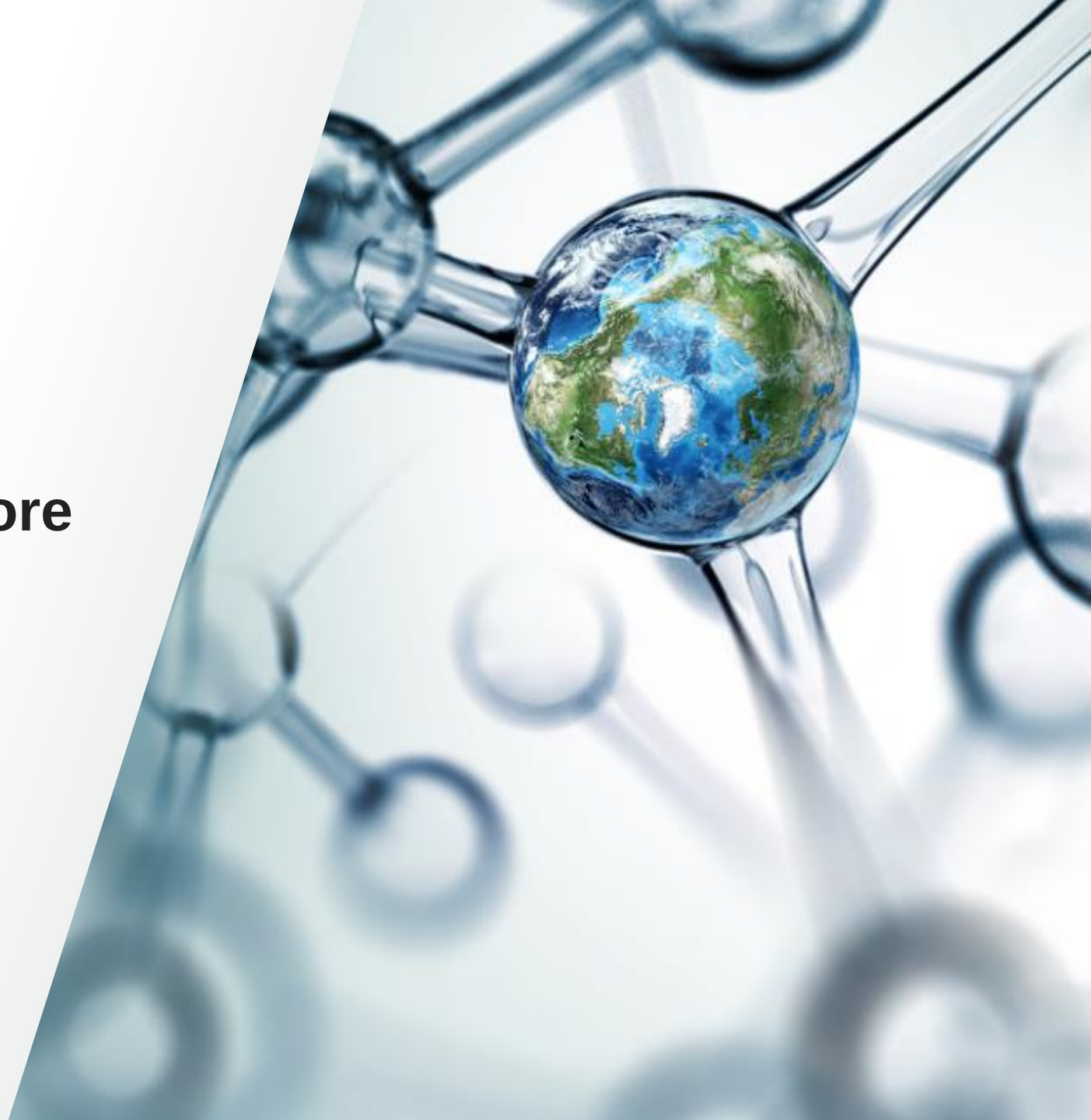

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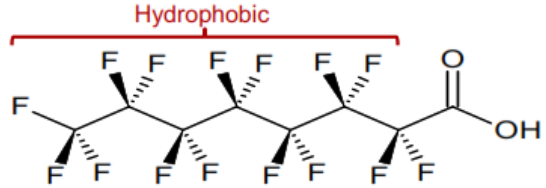
Agenda

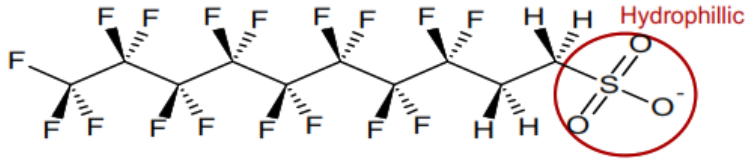
- What are PFAS and why study them?
- Considerations for selecting the right workflow for targeted and non-targeted analysis **in one injection**
- Summary of the capabilities of Compound Discoverer™ library to analyze PFAS in surface water, AFFF foam, and food contact material samples
- What's next?

Background – What are PFAS compounds?

- PFASs are **P**er- and **P**oly**F**luorinated Alkyl **S**ubstances. Exclusively anthropogenic.
- Structures contain a hydrophobic perfluoroalkyl backbone and a hydrophilic end group
- Include a diverse range of compounds with a variety of chain lengths and end groups
- **Industrial uses**
  - Industrial polymers
  - Stain repellants
  - Aqueous film forming foams (AFFF) – fire fighting applications
- **Areas of elevated concentrations and concern are:**
  - Airports
  - Run-off from incidents of fire
  - Landfill leachate
  - WWTP effluent



Perfluorooctanoic acid
- PFOA



8:2 Fluorotelomer sulfonate
- 8:2 FTS

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PFAS transport through the environment

Aqueous firefighting foam

Airports
Military bases

Household products

Cleaning products
Personal care products
Paints, waxes, polishes

Manufacturing processes

Commercial products
Electronics
Petrochemicals

Fabrics

Stain resistant carpets and fabrics
Waterproof or resistant clothing

Food contact materials

Non-stick cookware
Microwave popcorn bags
Fast food wrappers

Water	Drinking water	Surface water	Ground water	Wastewater
Soil	Leachate	Runoff	Ground water	Wastewater

>10,000 possible PFAS compounds present in the environment

Workflow strategies

Sample collection ▶ **Sample preparation** ▶ **Analysis** ▶ **Data Processing**

PFAS testing workflows

Adsorbable organic fluorine (AOF)

Sample Preparation Automation

Routine Targeted Testing

Non-Targeted Testing

Consumables – Validated Methods – Application Support and Service – PFAS Libraries – Compliance Software

Drinking Water

Wastewater

Biosolids

Soil

Air

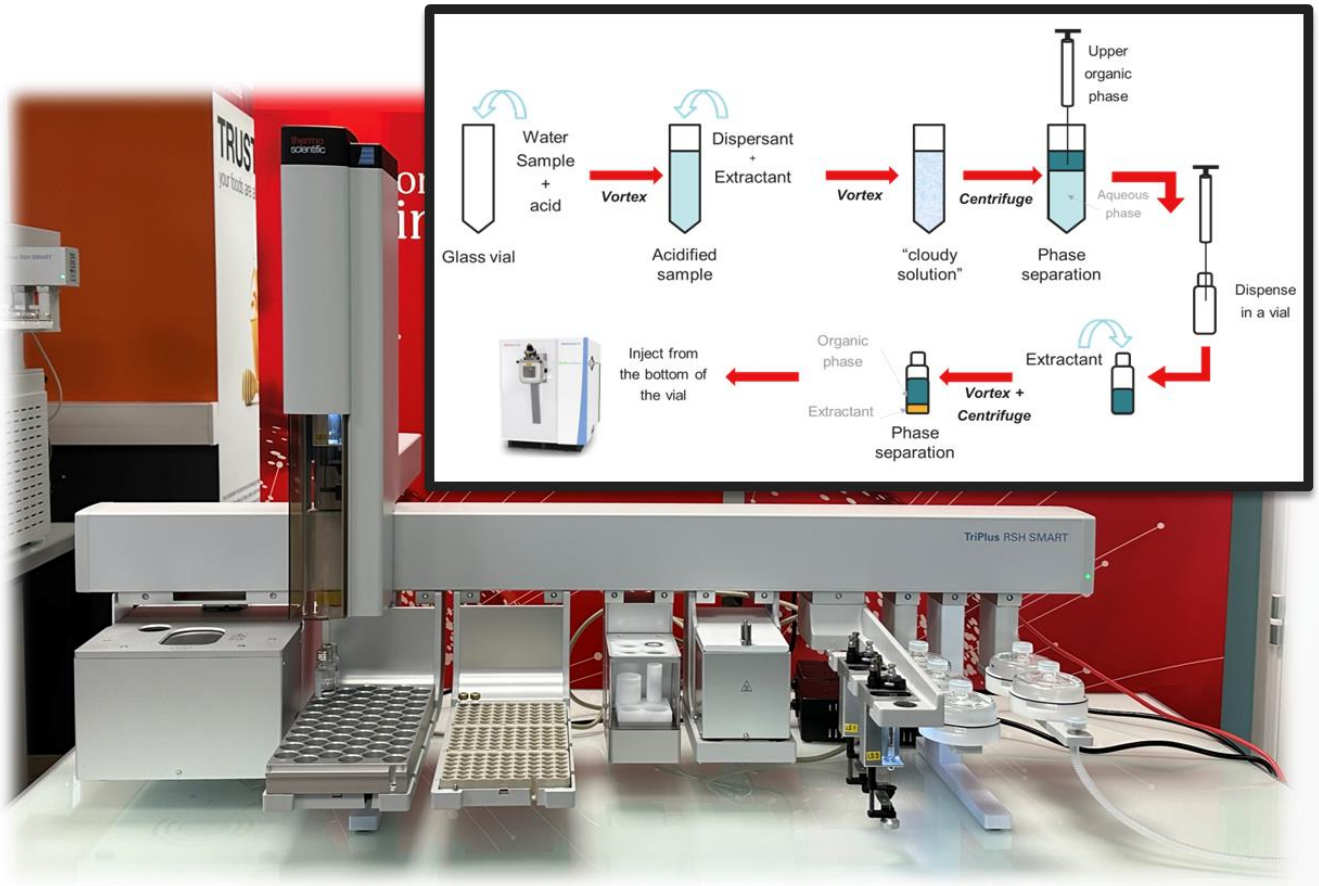
Food

Blood

Plasma

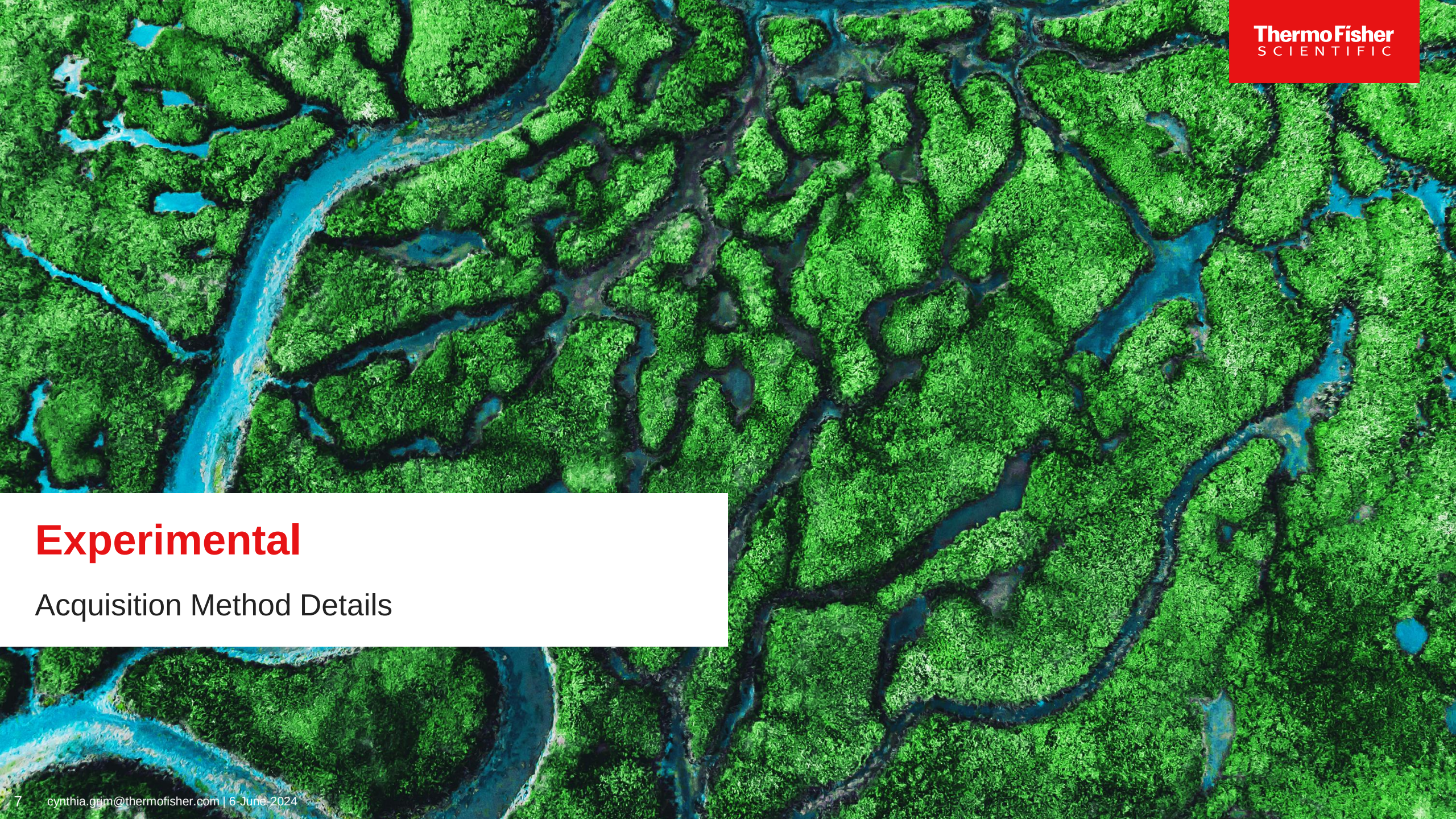
If you've missed our poster (TP068)…

Automated dispersive liquid liquid microextraction for PFAS analysis in drinking water



- 56 compounds – covering compounds of interest for EU and US regulations
- 18 minutes to extract two samples
- 15 mL sample, sub ppt quantitation
- Non-targeted analysis in a separate injection

Poster will be available on https://thermofisher.com/asms



Experimental

Acquisition Method Details

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Orbitrap Exploris 240 Mass Spectrometer

Leading performance

The Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer provides the performance and versatility needed to drive discovery and identification, and the quantitative precision and accuracy to confidently scale up for impact.

Mass range: 40 - 6000 *m/z*

Quad isolation: down to 0.4 Da & up to *m/z* 2500

Max resolution: 240,000 at *m/z* 200

Mass Accuracy: 3 ppm RMS external, 1 ppm RMS internal with Thermo Scientific™ EASY-IC™ Source

Polarity Switching: one Full Cycle @ >1.4 Hz in Full MS, >1.6 Hz in tSIM (R = 60,000)

Dissociation: Higher energy Collisional Dissociation (HCD)

Scan Analysis: Full MS, tSIM, dd-MS2 (Top N), tMS2, AIF, AcquireX workflow

Multiplexing: up to 20 for tSIM, up to 2 for tMS2

Analytes

Compound	CAS
Perfluoroalkyl carboxylic acids	
PFBA	375-22-4
PFPeA	2706-90-3
PFHxA	307-24-4
PFHpA	375-85-9
PFOA	335-67-1
PFNA	375-95-1
PFDA	335-76-2
PFUnA; PFUdA	2058-94-8
PFDoA	307-55-1
PFTrDA; PFTriA	72629-94-8
PFTeA	376-06-7
PFHxDA*	67905-19-5
PFODA*	1763-23-1
Fluorotelomer sulfonic acids	
4:2 FTSA; 4:2 FTS	757124-72-4
6:2 FTSA; 6:2 FTS	27619-97-2
8:2 FTSA; 8:2 FTS	39108-34-4
10:2 FTSA; 10:2 FTS*	120226-60-0

Compound	CAS
Perfluoroalkyl sulfonic acids (acid form)	
PFBS	375-73-5
PFPeS	2706-91-4
PFHxS	355-46-4
PFHpS	375-92-8
PFOS	1763-23-1
PFNS	68259-12-1
PFDS	335-77-3
PFUnDS*	749786-16-1
PFDoS	79780-39-5
PFTrDS*	343629-46-9
Fluorotelomer carboxylic acids	
3:3 FTCA	356-02-5
5:3 FTCA	914637-49-3
7:3 FTCA	812-70-4
Per- and Polyfluoroether carboxylic acids	
HFPO-DA (Gen X)	13252-13-6
DONA; ADONA	919005-14-4
PFMPA	377-73-1
NFDHA	151772-58-6
PFMBA	863090-89-5

Compound	CAS
Per- and Polyfluoroether sulfonic acids	
6:2 Cl-PFESA; 9Cl- PF3ONS	756426-58-1
8:2 Cl-PFESA; 11Cl- PF3OUdS	763051-92-9
PFEESA	113507-82-7
Perfluoroalkyl sulfonamides	
FBSA; PFBSA*	30334-69-1
MeFBSA; MePFBSA*	68298-12-4
FHxSA*	41997-13-1
FOSA; PFOSA	754-91-6
MeFOSA; N-MeFOSA	31506-32-8
EtFOSA; N-EtFOSA	4151-50-2
Perfluorooctane sulfonamidoacetic acids	
NMeFOSAA; MeFOSAA	2355-31-9
NEtFOSAA; EtFOSAA	2991-50-6
Perfluorooctane sulfonamide ethanols	
MeFOSE	24448-09-7
EtFOSE	1691-99-2
Other	
PFECHS	646-83-3
6:2 diPAP*	57677-95-9
8:2 diPAP*	678-41-1
6:2/8:2 diPAP*	943913-15-3

* Non-EPA 1633 compounds were used for confirmation purposes only

Experimental – Liquid Chromatography

Thermo Scientific Vanquish Flex Binary UHPLC System with PFAS Upgrade Kit

- Mobile Phase A: UHPLC-MS grade water + 0.1% Optima™ LC/MS grade acetic acid
- Mobile Phase B: 78% ACN + 20% MeOH + 2 mM ammonium acetate (aq)
- Gradient: See table

- PFAS Delay Column: 3.0 x 50 mm, 1.9 µm Thermo Scientific™ Hypersil GOLD™
- Analytical Column: 2.1 x 100 mm, 2.2 µm Thermo Scientific™ Acclaim RSLC C18

- Column Temperature: 40 °C
- Injection Volume: 5 µL (with Strong Solvent Loop)
- Autosampler Temperature: 22 °C (to minimize PFAS adsorption losses)

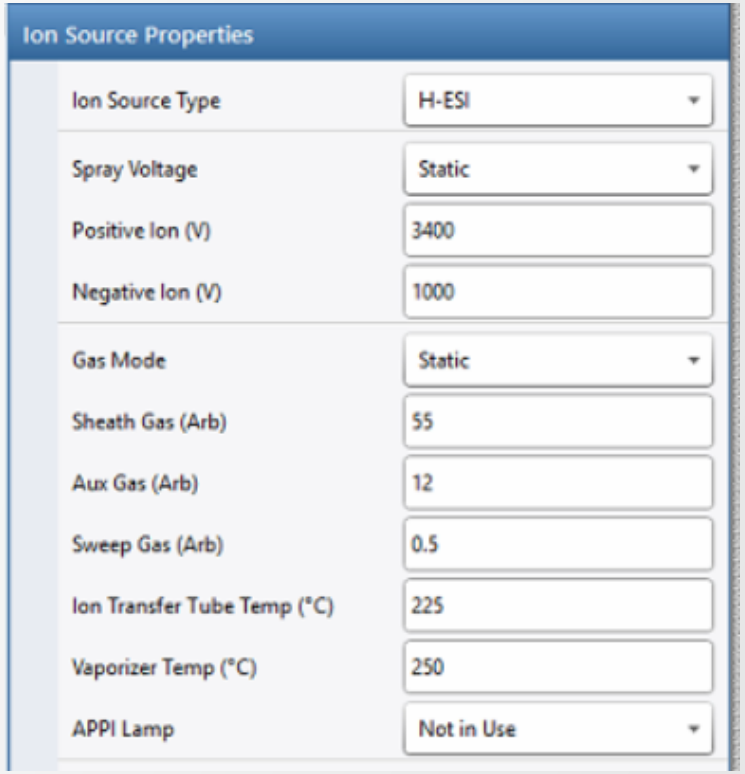
No	Time	Flow [ml/min]	%B	Curve
1	0.000	Run		
2	0.000	0.400	5.0	5
3	1.000	0.400	30.0	5
4	2.000	0.400	45.0	5
5	3.000	0.400	55.0	5
6	14.250	0.400	100.0	5
7	17.500	0.400	100.0	5
8	17.700	0.400	5.0	5
9	22.000	0.400	5.0	5
10	*New Row*			
11	22.000	Stop Run		

All chemicals listed are from Thermo Fisher Scientific

Experimental – Mass Spectrometry

Thermo Scientific Orbitrap Exploris 240 Mass Spectrometer

- **Ionization Mode:** HESI, Negative mode
  - HESI source parameters: see figure at right
- **MS Acquisition Modes:** Full-Scan MS, Data-Dependent MS2 (DDMS2)
  - Full-MS scan range: m/z 150-1100; RF Lens = 55%
- **DDMS2 Parameters:** Quad Isolation Width = 1.5 Da; Stepped CE (Absolute) = 2, 10, 25, 55 V; Maximum Ion Time = 50 ms; see more details on next slide
- **Resolution:** Full-Scan MS = 240,000 FWHM; DDMS2 = 30,000 FWHM
- **Mild Trapping:** On; to limit precursor ion fragmentation
- **EasyIC:** On (Scan-to-scan); for all acquisition modes (for best mass accuracy)



Experimental – Mass Spectrometry (2)

DDMS2 Parameters – Orbitrap Exploris 240 Mass Spectrometer

- **Intensity:** threshold for MS2 event (7.0E4)
- **Dynamic Exclusion:** excludes precursor ion from MS2 after N events and X sec
  - N = 1 events, X = 5 sec
- **Targeted Mass:** list of precursor ions for MS2 above Intensity and in RT Window (if set)
  - 102 PFAS targets (native and isotopically-labeled compounds)
  - Checked “Perform dependent scan on most intense ion if no targets found”
- **Targeted Mass Exclusion:** list of precursor ions to not consider for MS2 within RT Window (if set)
- **Apex Detection:** MS2 within % of half of user set Expected LC Peak Width (30%)

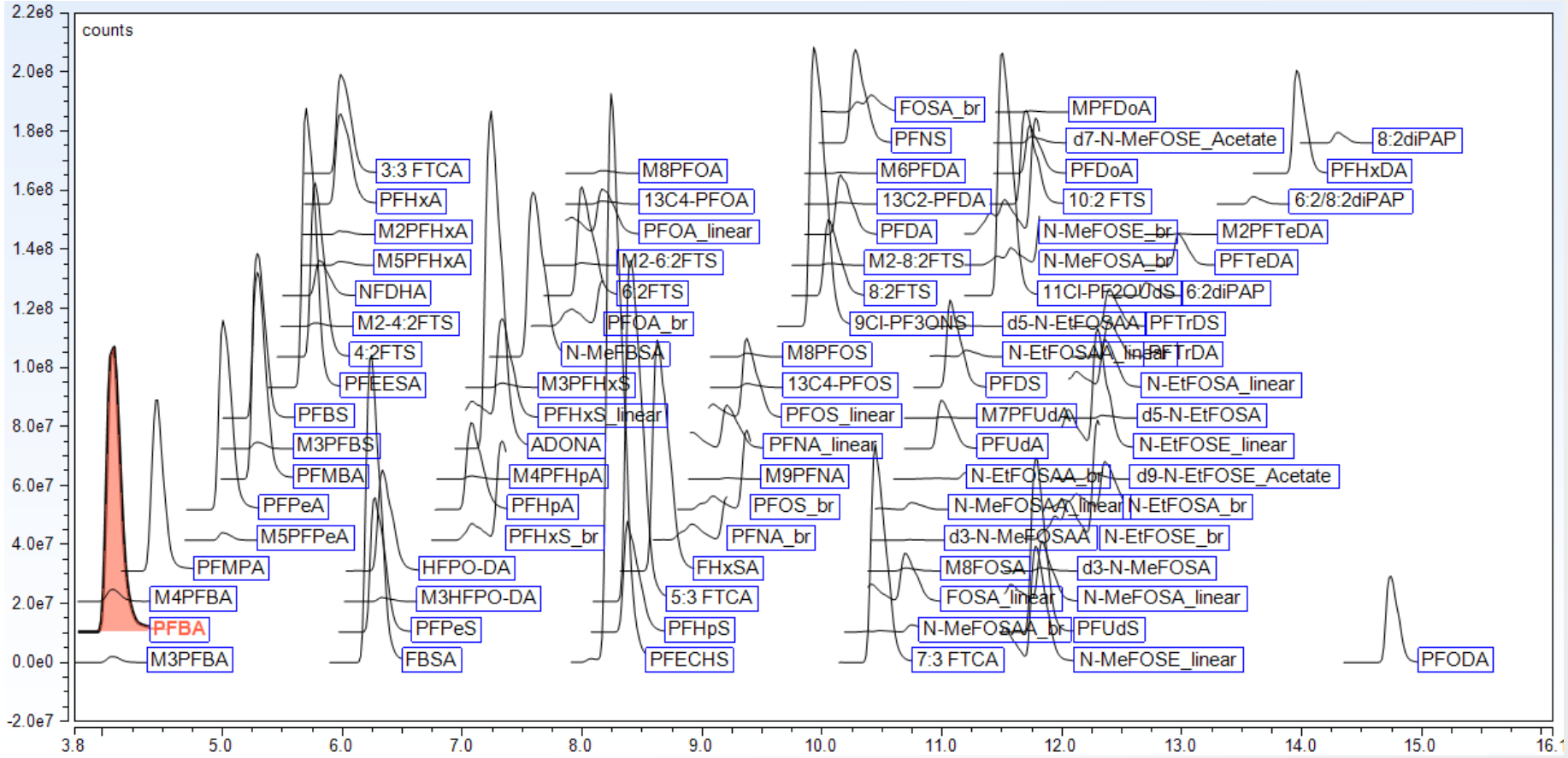
Note: integer (4) in “scans” box represents number of DDMS2 events after each Full Scan

Results

Calibration curve results acquired and processed in Thermo Scientific™ Chromeleon™ Chromatography Data System

Calibration Level 8 – Chromatogram (XICs)

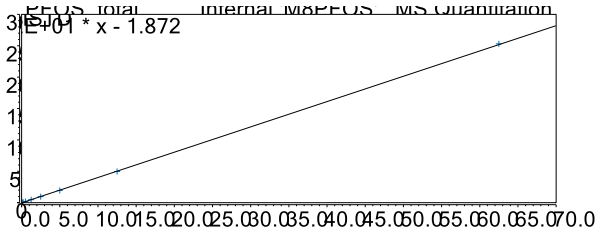
62.5 ng/mL – 1562.5 ng/mL (Methanol with 4% water, 1% NH4OH, 0.625% HOAc)



XIC windows set to ± 5 ppm

Calibration results: PFOS (sum, neat), Full-MS

0.1 – 62.5 ng/mL (sum, salt form), Linear, 1/x weighting, $r^2 > 0.9998$



Injection Name	Type	Level	RT	Quant Ion	Area	Amount	Accuracy
Selected Peak:			min	m/z	counts*min		%
Cal 1 - 5 uL	Calibration Standard	1	9.42	498.93020	4768	0.1126	111.1803
Cal 2 - 5 uL	Calibration Standard	2	9.40	498.93020	10229	0.1946	97.2156
Cal 3 - 5 uL	Calibration Standard	3	9.40	498.93020	30842	0.4823	96.3301
Cal 4 - 5 uL	Calibration Standard	4	9.38	498.93020	83377	1.2359	98.8580
Cal 5 - 5 uL	Calibration Standard	5	9.40	498.93020	168904	2.4349	97.3243
Cal 6 - 5 uL	Calibration Standard	6	9.38	498.93020	337439	4.8952	97.8583
Cal 7 - 5 uL	Calibration Standard	7	9.38	498.93020	883398	12.3721	98.9665
Cal 8 - 5 uL	Calibration Standard	8	9.37	498.93020	4615301	62.8225	100.5133

PFOS demonstrates high accuracy and linearity

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Calibration results: (additional)

3:3 FTCA
r^2 > 0.9996
0.5 – 310.25 ng/mL
Linear, 1/x weighting

Inj. No.	Injection Name Selected Peak:	Type	Level	RT min	Quant Ion m/z	Area counts*min	Amount	Accuracy %
6	Cal 1 - 5 uL	Calibration Standard	1	5.99	241.01050	5055	0.565	111.5903
7	Cal 2 - 5 uL	Calibration Standard	2	5.98	241.01050	10885	0.964	96.3385
8	Cal 3 - 5 uL	Calibration Standard	3	5.98	241.01050	29690	2.372	94.6357
9	Cal 4 - 5 uL	Calibration Standard	4	6.00	241.01050	80704	5.906	94.1904
10	Cal 5 - 5 uL	Calibration Standard	5	6.00	241.01050	171588	12.263	98.0705
11	Cal 6 - 5 uL	Calibration Standard	6	5.99	241.01050	347343	24.838	99.3508
12	Cal 7 - 5 uL	Calibration Standard	7	6.00	241.01050	923560	65.060	103.9362
13	Cal 8 - 5 uL	Calibration Standard	8	5.99	241.01050	4618092	308.527	99.4416

PFOA (sum)
r^2 > 0.9999
0.1 – 62.5 ng/mL
Linear, 1/x weighting

Inj. No.	Injection Name Selected Peak:	Type	Level	RT min	Quant Ion m/z	Area counts*min	Amount	Accuracy %
6	Cal 1 - 5 uL	Calibration Standard	1	8.22	412.96640	3767	0.094	94.2427
7	Cal 2 - 5 uL	Calibration Standard	2	8.21	412.96640	9536	0.205	102.7949
8	Cal 3 - 5 uL	Calibration Standard	3	8.21	412.96640	25416	0.507	101.4630
9	Cal 4 - 5 uL	Calibration Standard	4	8.22	412.96640	65812	1.242	99.3772
10	Cal 5 - 5 uL	Calibration Standard	5	8.18	412.96640	136699	2.545	101.7818
11	Cal 6 - 5 uL	Calibration Standard	6	8.17	412.96640	277080	5.052	101.0458
12	Cal 7 - 5 uL	Calibration Standard	7	8.15	412.96640	723101	12.342	98.7199
13	Cal 8 - 5 uL	Calibration Standard	8	8.17	412.96640	3581412	62.559	100.0956

4:2FTS
r^2 > 0.9999
0.4 – 250 ng/mL
Linear, 1/x weighting

Inj. No.	Injection Name Selected Peak:	Type	Level	RT min	Quant Ion m/z	Area counts*min	Amount	Accuracy %
6	Cal 1 - 5 uL	Calibration Standard	1	5.81	326.97430	9834	0.405	101.3158
7	Cal 2 - 5 uL	Calibration Standard	2	5.80	326.97430	21663	0.796	99.5989
8	Cal 3 - 5 uL	Calibration Standard	3	5.79	326.97430	58362	1.981	99.0594
9	Cal 4 - 5 uL	Calibration Standard	4	5.77	326.97430	145324	4.878	97.5133
10	Cal 5 - 5 uL	Calibration Standard	5	5.79	326.97430	306375	9.980	99.8055
11	Cal 6 - 5 uL	Calibration Standard	6	5.78	326.97430	615426	20.333	101.6414
12	Cal 7 - 5 uL	Calibration Standard	7	5.78	326.97430	1618371	50.637	101.2594
13	Cal 8 - 5 uL	Calibration Standard	8	5.77	326.97430	7669632	249.185	99.6732

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Calibration results: (additional, 2)

ADONA
r^2 > 0.9994
0.4 - 250 ng/mL
Linear, 1/x weighting

Inj. No.	Injection Name Selected Peak:	Type	Level	RT min	Quant Ion m/z	Area counts*min	Amount	Accuracy %
6	Cal 1 - 5 uL	Calibration Standard	1	7.27	376.96890	22550	0.484	117.4951
7	Cal 2 - 5 uL	Calibration Standard	2	7.26	376.96890	42483	0.773	96.5172
8	Cal 3 - 5 uL	Calibration Standard	3	7.25	376.96890	113394	1.947	97.2975
9	Cal 4 - 5 uL	Calibration Standard	4	7.27	376.96890	308976	4.739	94.4971
10	Cal 5 - 5 uL	Calibration Standard	5	7.25	376.96890	627213	9.678	96.6771
11	Cal 6 - 5 uL	Calibration Standard	6	7.24	376.96890	1325208	19.037	94.9439
12	Cal 7 - 5 uL	Calibration Standard	7	7.23	376.96890	3457916	48.388	96.6687
13	Cal 8 - 5 uL	Calibration Standard	8	7.24	376.96890	17447428	253.151	101.2450

N-MeFOSE (total)
r^2 > 0.9998
1.0 - 6250 ng/mL
Linear, 1/x weighting

Inj. No.	Injection Name Selected Peak:	Type	Level	RT min	Quant Ion m/z	Area counts*min	Amount	Accuracy %
6	Cal 1 - 5 uL	Calibration Standard	1	11.80	616.00920	9314	1.139	112.2727
7	Cal 2 - 5 uL	Calibration Standard	2	11.79	616.00920	18539	1.907	95.1230
8	Cal 3 - 5 uL	Calibration Standard	3	11.78	616.00920	53277	4.978	99.5673
9	Cal 4 - 5 uL	Calibration Standard	4	11.77	616.00920	144333	12.127	96.9290
10	Cal 5 - 5 uL	Calibration Standard	5	11.77	616.00920	284057	23.905	95.4230
11	Cal 6 - 5 uL	Calibration Standard	6	11.78	616.00920	605541	49.761	99.5200
12	Cal 7 - 5 uL	Calibration Standard	7	11.77	616.00920	1561127	122.900	98.2920
13	Cal 8 - 5 uL	Calibration Standard	8	11.78	616.00920	8507157	628.779	100.6010

9Cl-PF3ONS
r^2 > 0.9990
0.4 - 250 ng/mL
Linear, 1/x weighting

Inj. No.	Injection Name Selected Peak:	Type	Level	RT min	Quant Ion m/z	Area counts*min	Amount	Accuracy %
6	Cal 1 - 5 uL	Calibration Standard	1	9.96	530.89560	17044	0.353	86.9510
7	Cal 2 - 5 uL	Calibration Standard	2	9.95	530.89560	35974	0.775	96.7699
8	Cal 3 - 5 uL	Calibration Standard	3	9.96	530.89560	95399	2.051	102.4930
9	Cal 4 - 5 uL	Calibration Standard	4	9.94	530.89560	248439	5.030	100.6077
10	Cal 5 - 5 uL	Calibration Standard	5	9.95	530.89560	510642	10.335	103.2496
11	Cal 6 - 5 uL	Calibration Standard	6	9.94	530.89560	1050031	20.757	103.6476
12	Cal 7 - 5 uL	Calibration Standard	7	9.93	530.89560	2782595	53.000	105.6610
13	Cal 8 - 5 uL	Calibration Standard	8	9.93	530.89560	14228015	245.896	98.3310

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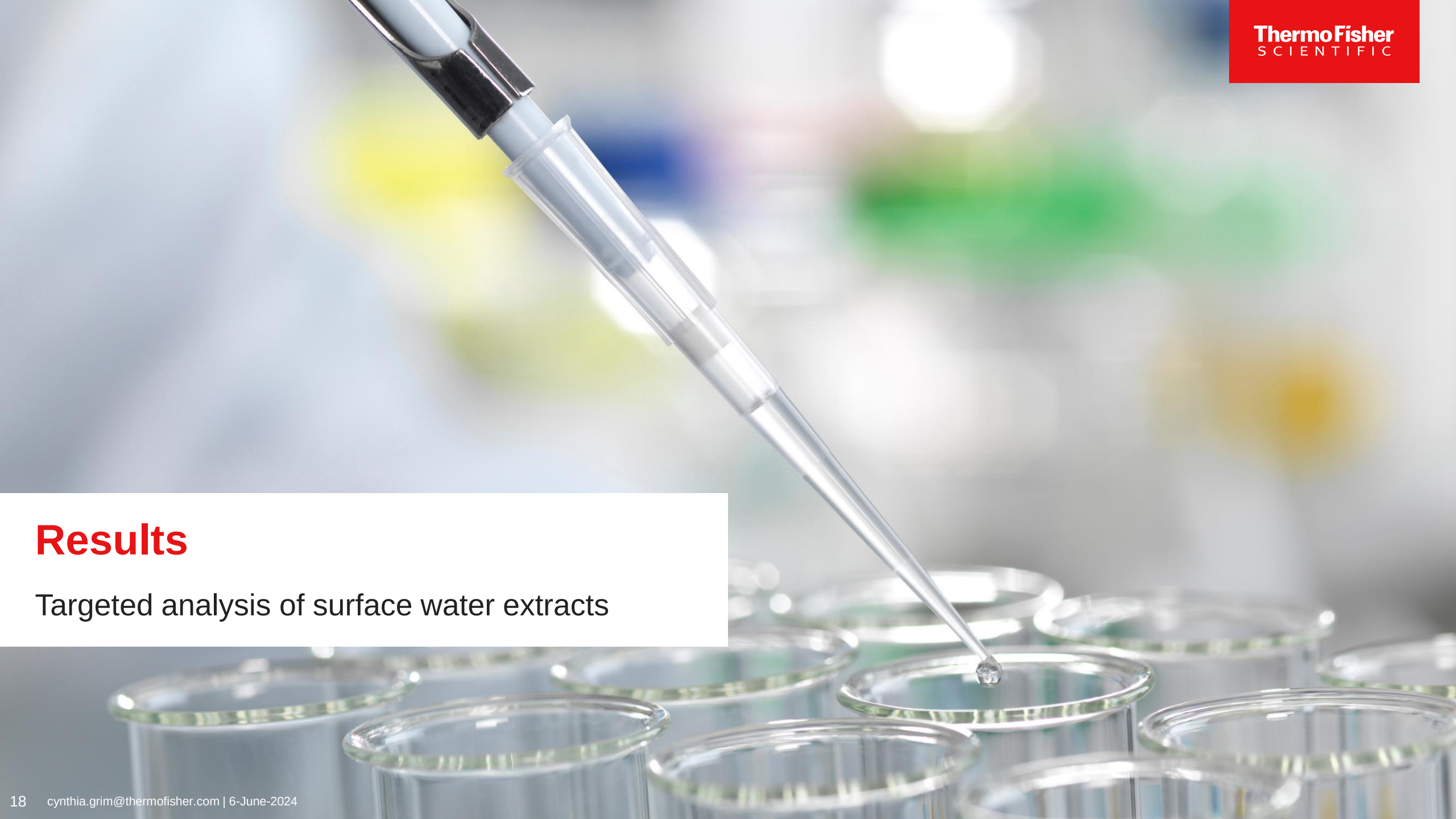

Results

Targeted analysis of surface water extracts

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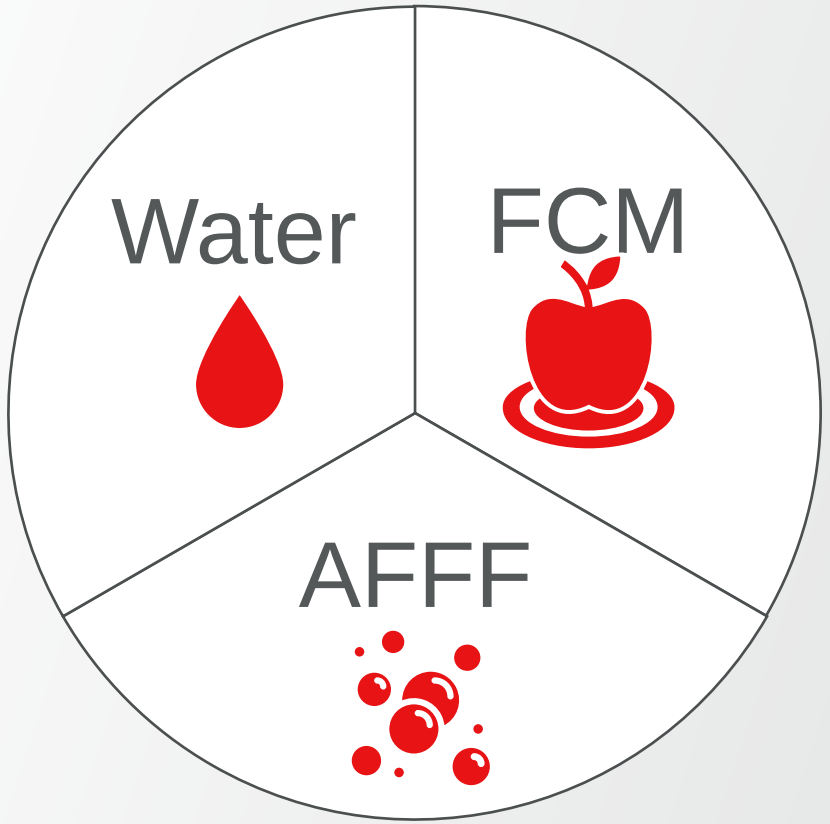
Sample processing

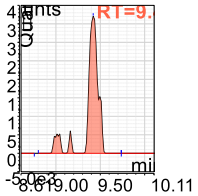
- Surface water samples
  - 250 mL volume, two different locations
  - Spiked with additional compounds to demonstrate performance
  - Extracted following EPA 1633 using WAX/GCB cartridges (50x concentration factor)
- Food contact materials
  - Old paper plates
  - Extracted by automated solvent extraction
- Diluted AFFF samples
  - Provided by collaborator Dr. Lee Ferguson
  - Reconstituted in 96% MeOH, 4% water, 1% NH4OH, 0.625% HOAc



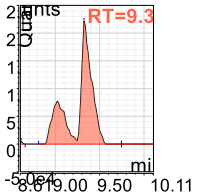
Chromatograms – PFOS, total

Branched and linear isomers

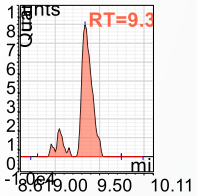
Standard - 0.1 ng/mL



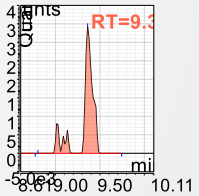
River water – 10.7 ppt



AFFF



Food Contact Mat'l



PFOS retains high signal to noise and good peak shape across matrix types

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Quantitative results – Surface water

Concentrations reported in the salt form, sum of branched and linear isomers where relevant

Compound	250 mL River water – San Jose (ppt)	250 mL River water – Alviso (ppt)
PFBA	67.2	17.3
PFPeA	43.9	8.9
PFHxA	9.1	8.1
PFHpA	5.4	3.2
PFOA (total)	11.2	27.9
PFBS	10.7	4.4
PFHxS (total)	10.8	25.8
PFOS (total)	36.6	10.7
HFPO-DA	N.D.	90.5
ADONA	19.1	N.D.
NFDHA	30.5	N.D.
4:2FTS	N.D.	25.0
6:2FTS	30.2	19.5
8:2FTS	19.5	N.D.
7:3FTCA	17.2	N.D.

Results

NTA of PFAS in food contact materials, surface water, and AFFF foams

Compound Discoverer™ 3.3 SP3

PFAS Unknown ID w Database Searches and Molecular Networks

Each detected compound with a MS^2 spectrum is searched against the in-silico generated PFAS spectral library from Duke University and 2023 NIST MS/MS library

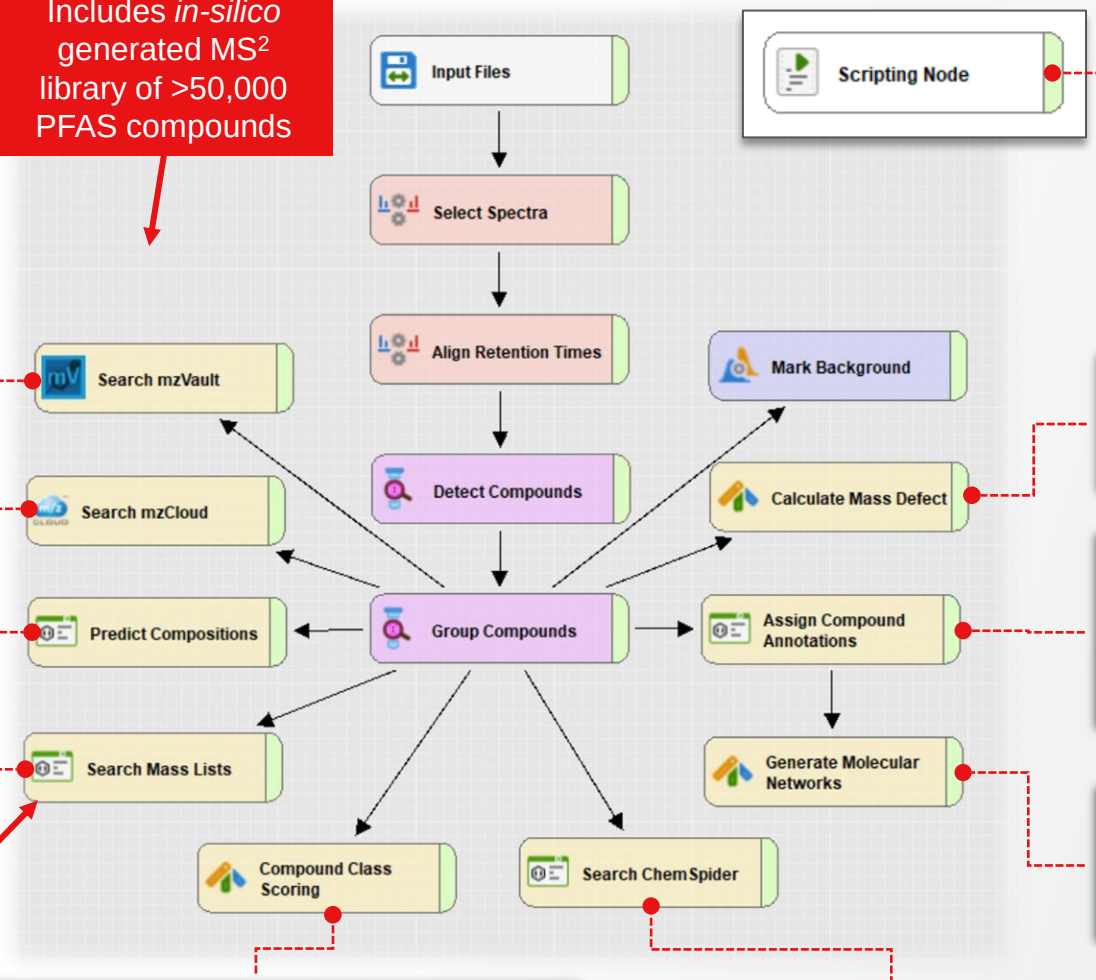
Includes *in-silico* generated MS^2 library of >50,000 PFAS compounds

Each detected compound with a MS^2 spectrum is searched against the mzCloud™ HRAM spectral database

Determine possible empirical formulas with monoisotopic masses similar to the measured mass in the Full Scan spectrum

Compares detected compounds to the EPA PFAS Structure List, NIST Suspect List of PFAS

Also includes Duke Research PFAS Database of >50,000 PFAS compounds



Addition calculations, including number of fluorines in the annotated empirical formula, m/C, md/C, etc.

Calculates the mass defect (including Kendrick) for each detected compound.

Annotations and metadata from all resources used in the workflow are imported to each of the detected compounds

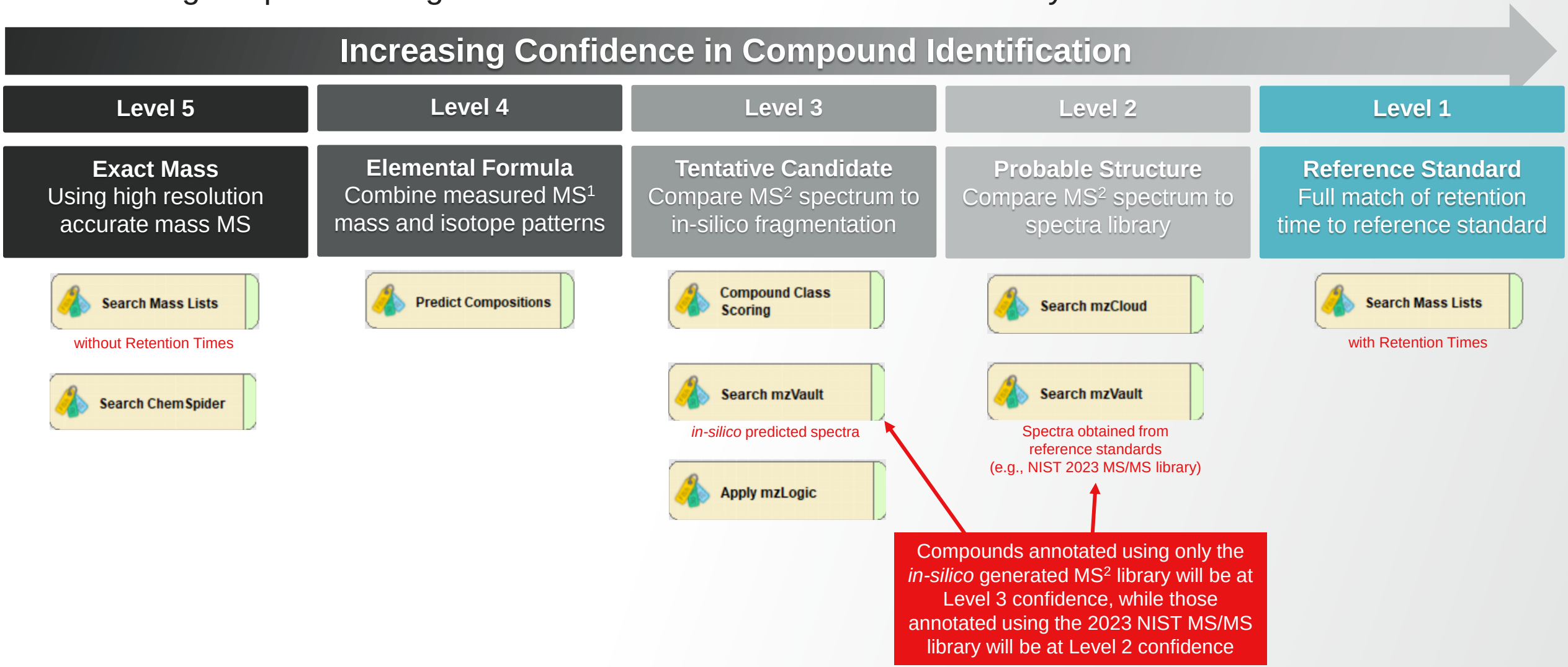
Determines and visualizes the similarity between various compounds across your samples

Compares MS^2 fragments of detected compounds to the FluoroMatch™ fragment library (InnovativeOmics) and PFAS fine structure fragment library

Each detected compound is searched against the ChemSpider chemical database

Increasing confidence in identifying PFAS compounds

Harnessing the power of high resolution accurate mass and MS^2 Analysis



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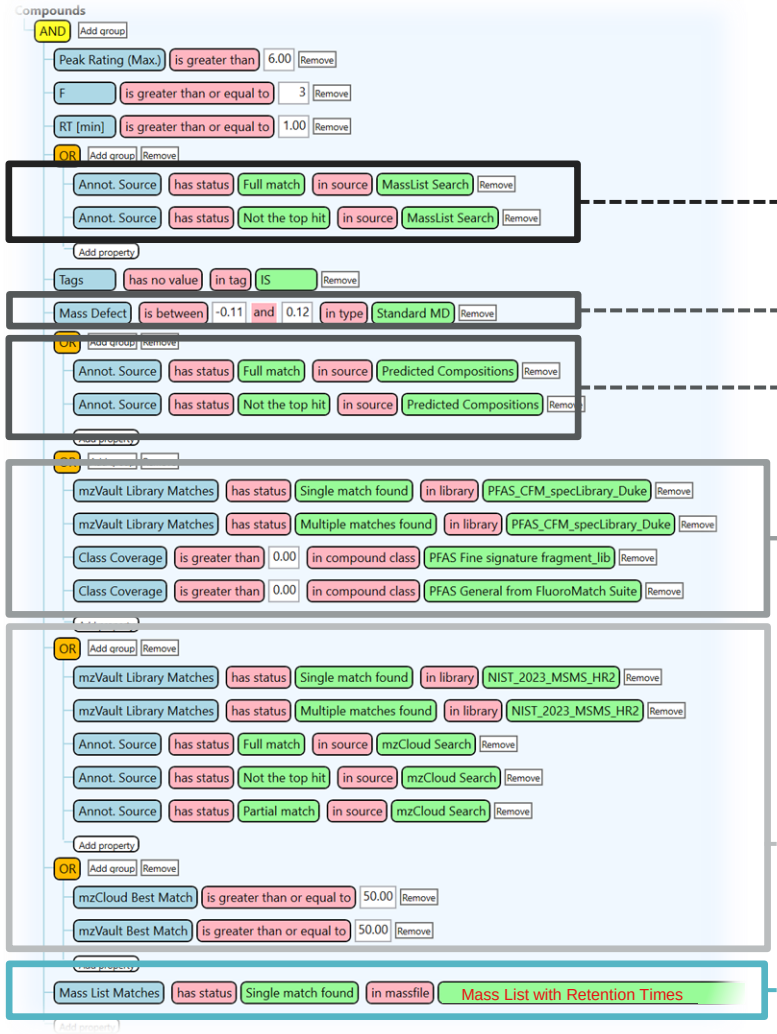
See additional details on confidence levels in identifying PFAS compounds in Charbonnet et al. *Environ. Sci. Technol. Lett.* **2022**, *9*, 473–481.

Data reduction approach

Moving towards a finalized list of compounds annotated with Level 1 confidence



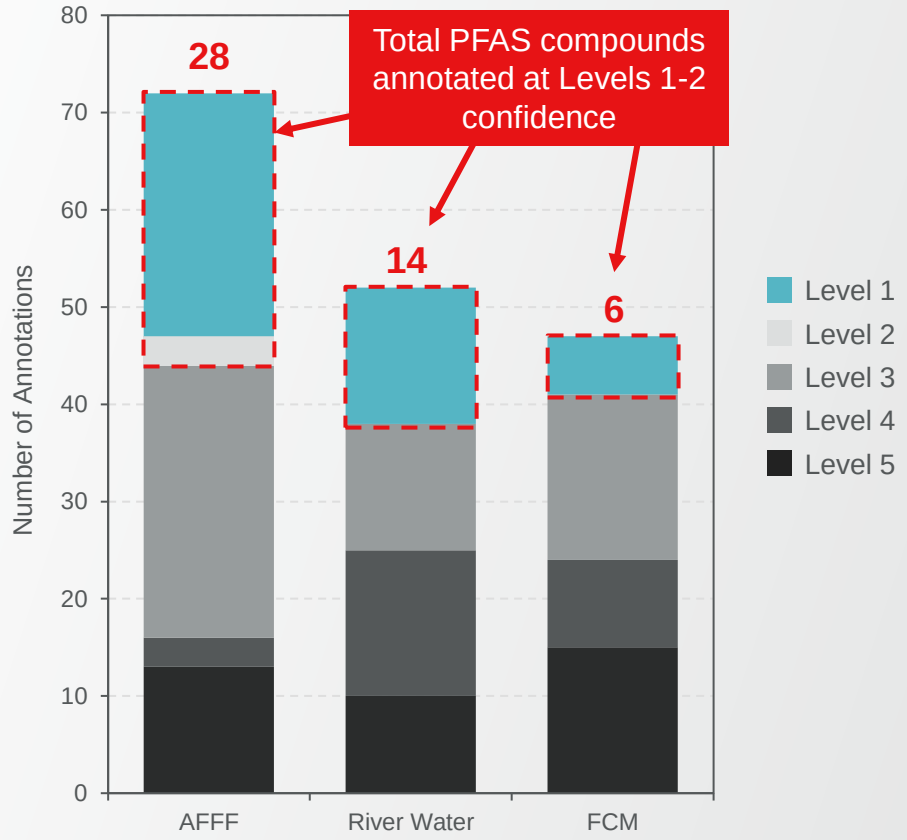
Annotation Criteria	Annotation Confidence Level 5	4	3	2	1
Measured mass ±2 ppm of at least one PFAS Mass List	✓	✓	✓	✓	✓
Standard mass defect is between -0.11–0.12		✓	✓	✓	✓
Isotopic pattern match		✓	✓	✓	✓
≥1 MS^2 fragment with match to FluoroMatch database			✓ or		
>50% similarity match to in-silico PFAS spectral libraries			✓		
>50% similarity match to mzCloud™ or 2023 NIST MS/MS spectral libraries				✓	✓
Retention time match to Reference Standard					✓

See additional details on confidence levels in identifying PFAS compounds in Charbonnet et al. *Environ. Sci. Technol. Lett.* **2022**, *9*, 473–481.

Data reduction approach

Moving towards a finalized list of compounds annotated with Levels 1–5 confidence

Annotation Criteria	Annotation Confidence Level 5	4	3	2	1
Measured mass ±2 ppm of at least one PFAS Mass List	✓	✓	✓	✓	✓
Standard mass defect is between -0.11–0.12		✓	✓	✓	✓
Isotopic pattern match		✓	✓	✓	✓
≥1 MS^2 fragment with match to FluoroMatch database			✓ or		
>50% similarity match to in-silico PFAS spectral libraries			✓		
>50% similarity match to mzCloud™ or 2023 NIST MS/MS spectral libraries				✓	✓
Retention time match to Reference Standard					✓



See additional details on confidence levels in identifying PFAS compounds in Charbonnet et al. *Environ. Sci. Technol. Lett.* **2022**, *9*, 473–481.

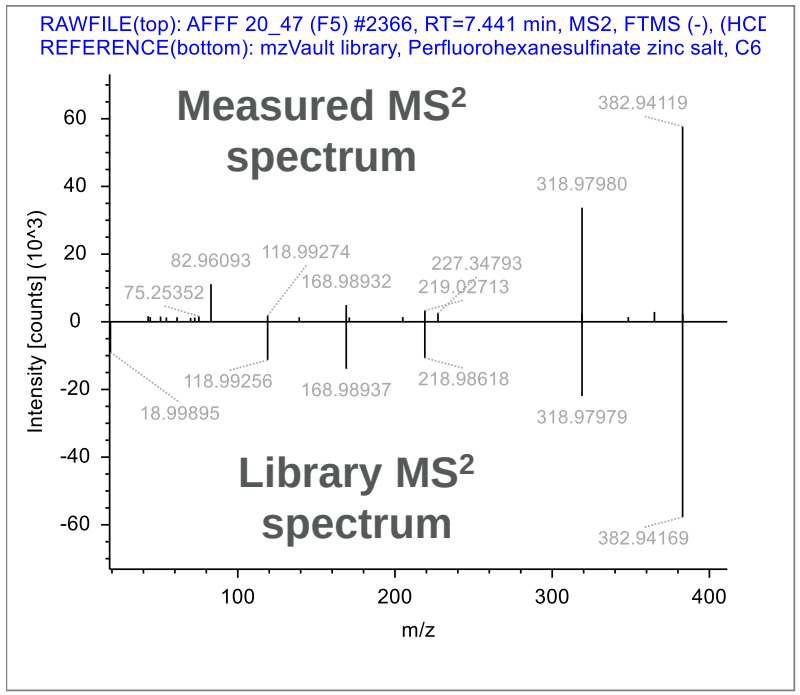
Annotating at Level 3 confidence

Utilizing multiple fragmentation libraries to gain confidence in structure elucidation

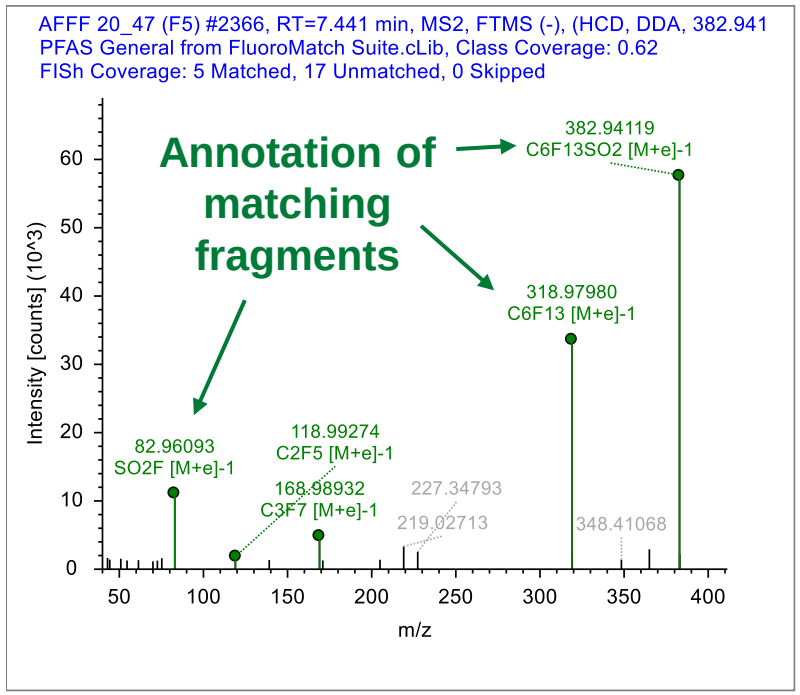
Compounds | Compounds per File | Features per File | mzCloud Results | mzVault Results | ChemSpider Results | Input Files | Study Info

	Name	Formula	m/C	md/C	F	Annot. ΔMass [ppm]	Calc. MW
18	PFHxSi	C6 H F13 O2 S	56.42550	-0.00758	13	-1.48	383.94840
19	PFOS_br	C8 H F17 O3 S	66.83765	-0.00842	17	-0.96	499.93701

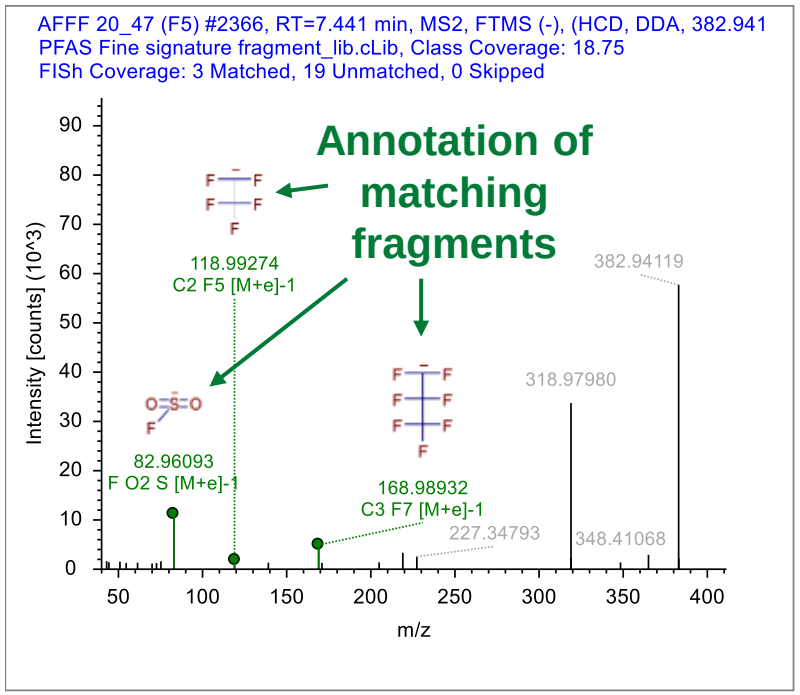
In-silico PFAS Spectral Library (Duke University)



PFAS Fragments Library (FluoroMatch)



PFAS Fragments Library (PFAS Signature Fragments)



Annotating at Level 2 confidence

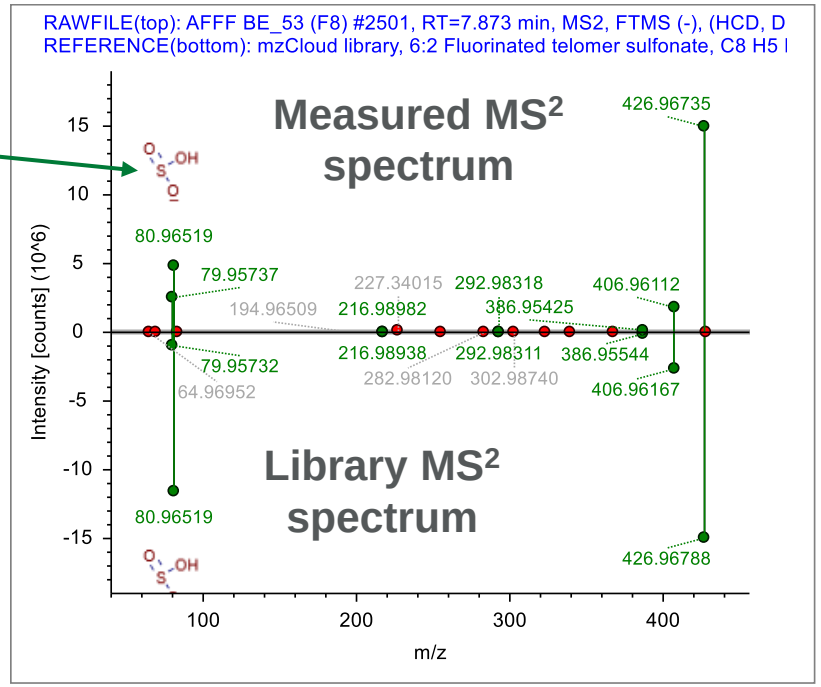
Utilizing multiple fragmentation libraries to gain confidence in structure elucidation

Compounds | Compounds per File | Features per File | mzCloud Results | mzVault Results | ChemSpider Results | Input Files | Study

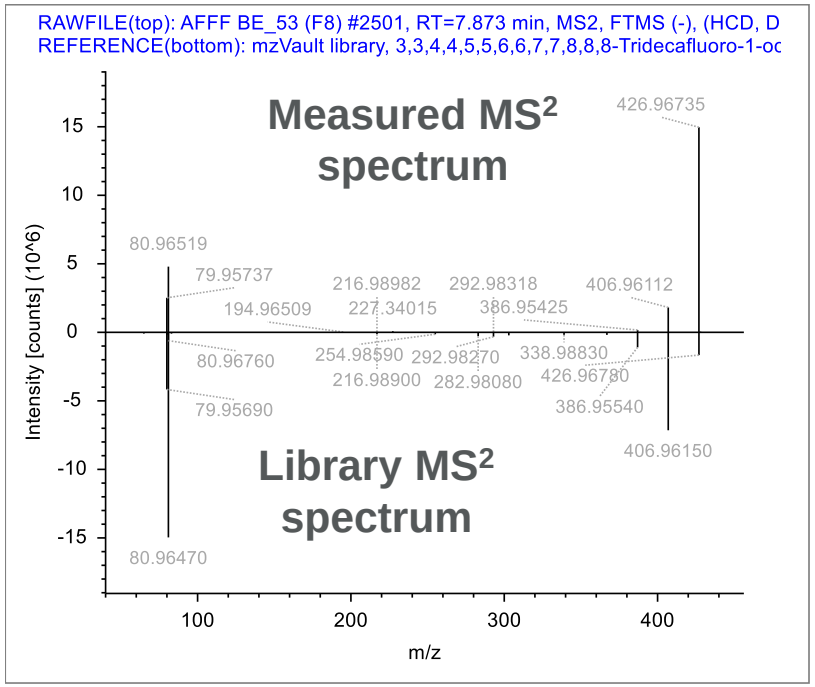
	Name	Formula	m/C	md/C	F	Annot. ΔMass [ppm]	Calc. M
10	6:2FTS	C8 H5 F13 O3 S	58.18858	-0.00345	13	-1.30	427.974
11	PFBS	C4 H F9 O3 S	76.63501	-0.01272	9	-0.20	299.950
12	PFTrDA	C13 H F25 O2	47.80965	-0.00306	25	-0.36	663.957

Annotation of matching fragments

mzCloud™ Spectral Library
(Thermo Scientific)



2023 HRAM MS² Spectral Library
(NIST)



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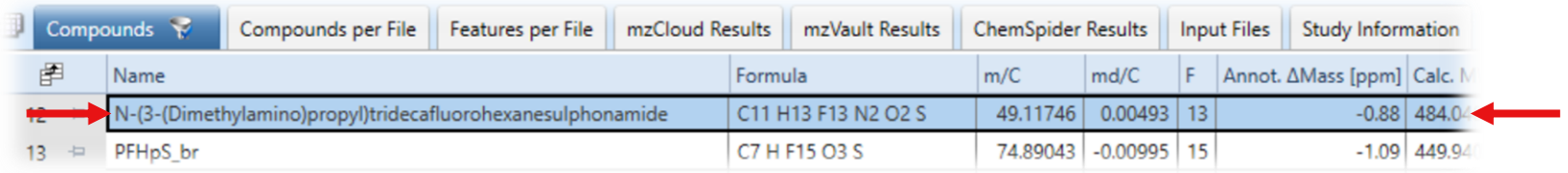
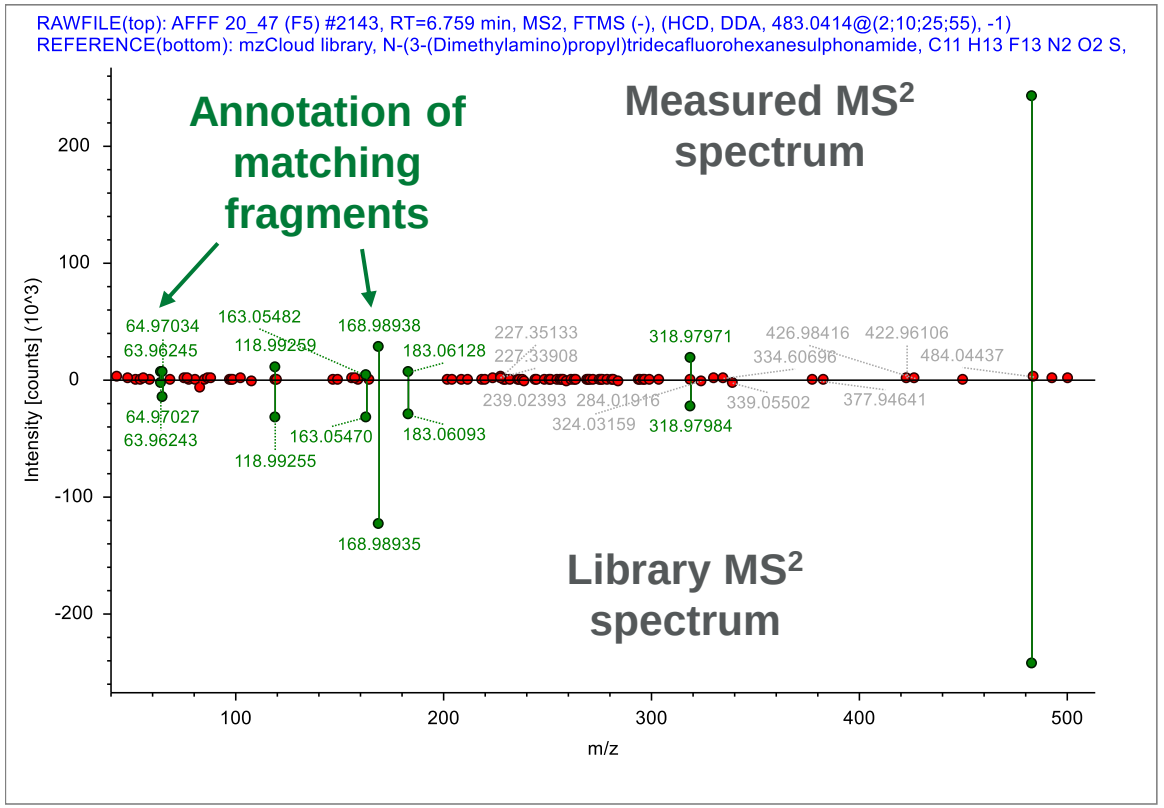
Annotating at Level 2 confidence

Utilizing multiple fragmentation libraries to gain confidence in structure elucidation



mzCloud™ Spectral Library
(Thermo Scientific)



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Final list of compounds with Level 1–2 annotations

ThermoFisher SCIENTIFIC

Comparisons across three samples matrices

AFFF

Name	Formula	Area (Ma	Annot. ΔMass [ppm]
PFOS_linear	C8 H F17 O3 S	638266907	-1.08
PFOS_br	C8 H F17 O3 S	249094514	-1.02
PFHxS_linear	C6 H F13 O3 S	183756035	-1.58
6:2FTS	C8 H5 F13 O3 S	448601124	-1.30
PFBS	C4 H F9 O3 S	282373294	-0.20
PFHxS_br	C6 H F13 O3 S	243808319	-1.50
PFOS_br	C8 H F17 O3 S	141604652	-0.96
PFHpS	C7 H F15 O3 S	107661270	-1.11
PFHxA	C6 H F11 O2	93260107	-0.02
PFNA_linear	C9 H F17 O2	58049781	-1.11
PFOA_linear	C8 H F15 O2	44722196	-1.37
N-(3-(Dimethylamino)propyl)tridecafluorohexanesulphonamide	C11 H13 F13 N2 O2 S	30709050	-0.88
PFHpS_br	C7 H F15 O3 S	30040380	-1.09
PFPeA	C5 H F9 O2	27332045	-0.12
PFPeS	C5 H F11 O3 S	25143343	-1.95
PFBA	C4 H F7 O2	24618112	0.00
PFHpA	C7 H F13 O2	23512367	-1.41
PFNS	C9 H F19 O3 S	21465603	-0.58
PFUdA	C11 H F21 O2	14983329	-0.46
PFOA_br	C8 H F15 O2	12686102	-1.34
PFHxA_br	C6 H F11 O2	11346096	-0.04
8:2FTS	C10 H5 F17 O3 S	7113691	-0.52
PFNS	C9 H F19 O3 S	6123853	-0.59
PFNS_br	C9 H F19 O3 S	6000881	-0.48
PFDS_br	C10 H F21 O3 S	5434435	-0.41
PFHpA_br	C7 H F13 O2	4887134	-1.47
PFTrDA	C13 H F25 O2	3561276	-0.36
PFDS	C10 H F21 O3 S	2439703	-0.27

River Water

Name	Formula	Area (Ma	Annot. ΔMass [ppm]
PFBA	C4 H F7 O2	32425087	0.02
PFOA_linear	C8 H F15 O2	21846717	-1.31
PFPeA	C5 H F9 O2	18854941	-0.02
PFHxS_linear	C6 H F13 O3 S	18232927	-1.32
PFOS_linear	C8 H F17 O3 S	11199510	-0.70
ADONA	C7 H2 F12 O4	9583458	-1.56
PFOS_br	C8 H F17 O3 S	9031595	-0.75
PFBS	C4 H F9 O3 S	6925799	0.06
4:2FTS	C6 H5 F9 O3 S	5914067	0.07
6:2FTS	C8 H5 F13 O3 S	4946624	-1.13
PFHxA	C6 H F11 O2	4616720	0.14
PFHxS_br	C6 H F13 O3 S	3112051	-1.23
NFDHA	C5 H F9 O4	2832775	0.05
8:2FTS	C10 H5 F17 O3 S	1850560	-0.63

FCM

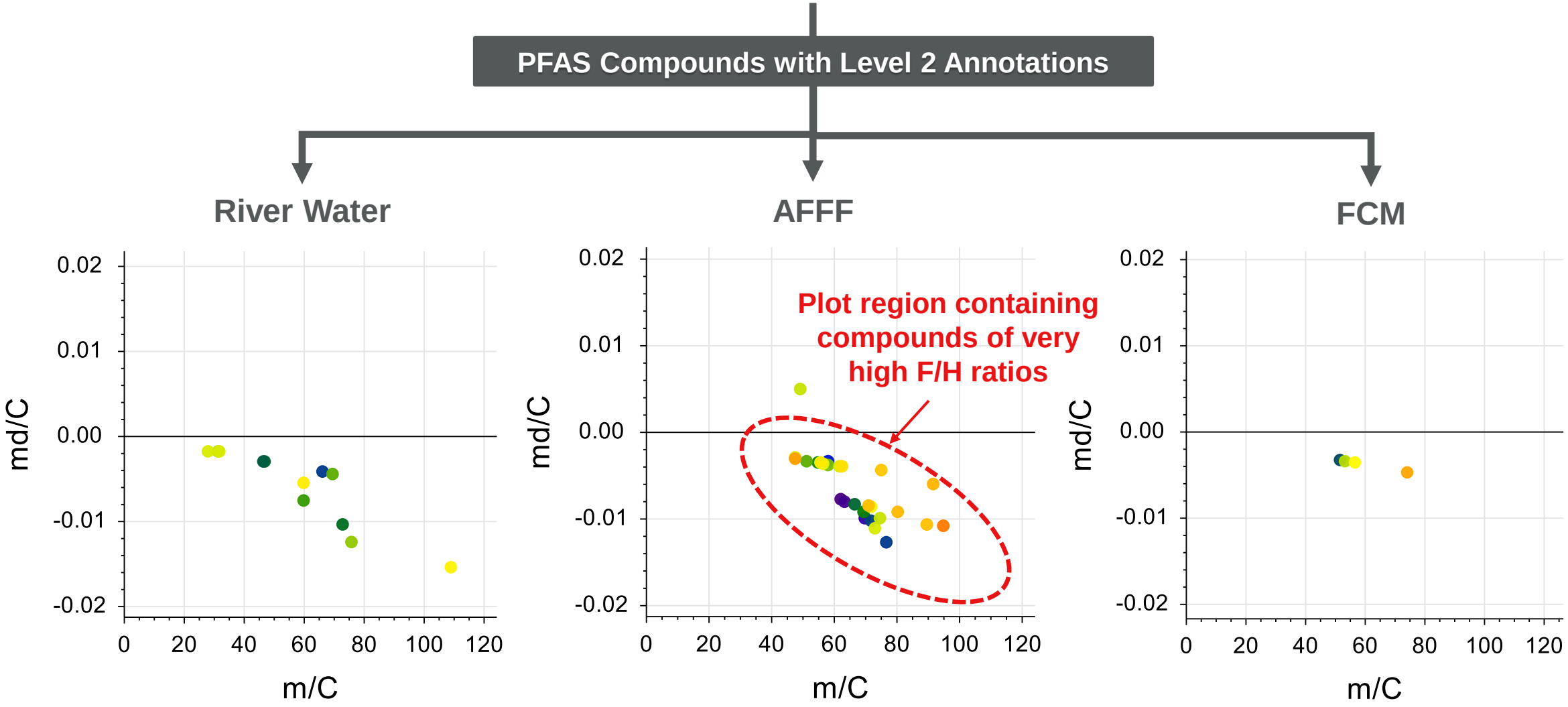
Name	Formula	Area (Max.)	Annot. ΔMass [ppm]
PFHxA	C6 H F11 O2	168232727	0.06
PFBS	C4 H F9 O3 S	23377752	0.06
PFBA	C4 H F7 O2	25163446	0.09
PFHpA	C7 H F13 O2	23503653	-1.33
PFPeA	C5 H F9 O2	13615545	-0.02
PFOA_linear	C8 H F15 O2	2927556	-1.14

Comparing finalized results to quantitative analysis

	Quantitative Results from Chromeleon™		Results from Compound Discoverer™	
Compound	River water – San Jose (ppt)	River water – Alviso (ppt)	Detected?	Annotation Level
PFBA	67.2	17.3	✓	1
PFPeA	43.9	8.9	✓	1
PFHxA	9.1	8.1	✓	1
PFHpA	5.4	3.2	N.D.	
PFOA (total)	11.2	27.9	✓ linear isomer	1
PFBS	10.7	4.4	✓	1
PFHxS (total)	10.8	25.8	✓ br. & lin. isomers	1
PFOS (total)	36.6	10.7	✓ br. & lin. isomers	1
HFPO-DA	N.D.	90.5	✓	3
ADONA	19.1	N.D.	✓	1
NFDHA	30.5	N.D.	✓	1
4:2FTS	N.D.	25.0	✓	1
6:2FTS	30.2	19.5	✓	1
8:2FTS	19.5	N.D.	✓	1
7:3FTCA	17.2	N.D.	N.D.	

Data visualization tools – m/C vs/ md/C plots

Comparisons of three different sample matrices: AFFF, FCM and River Water



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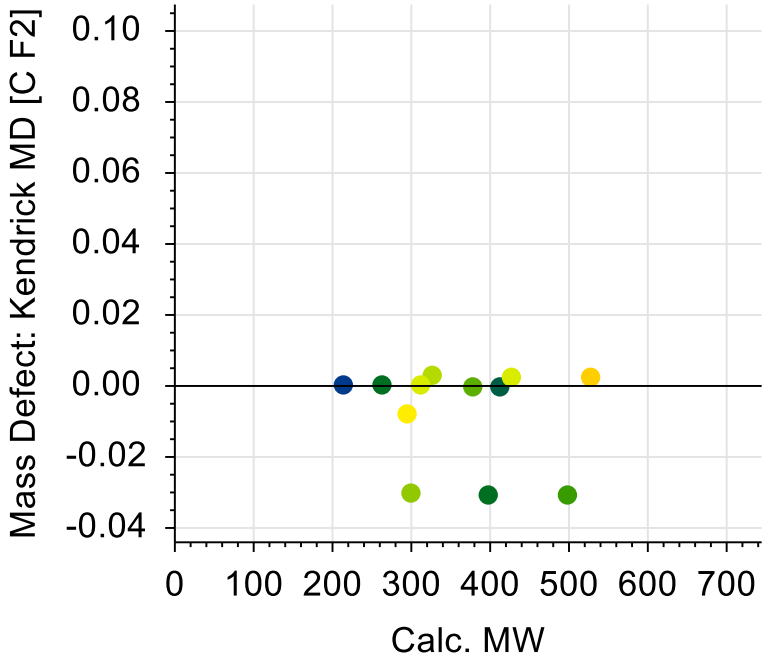
See additional details on m/C vs. md/C plots in Kaufmann et al. *J. AOAC Inter.* **2022**, 105, 1280–1287.

Data visualization tools – Kendrick Mass Defect plots

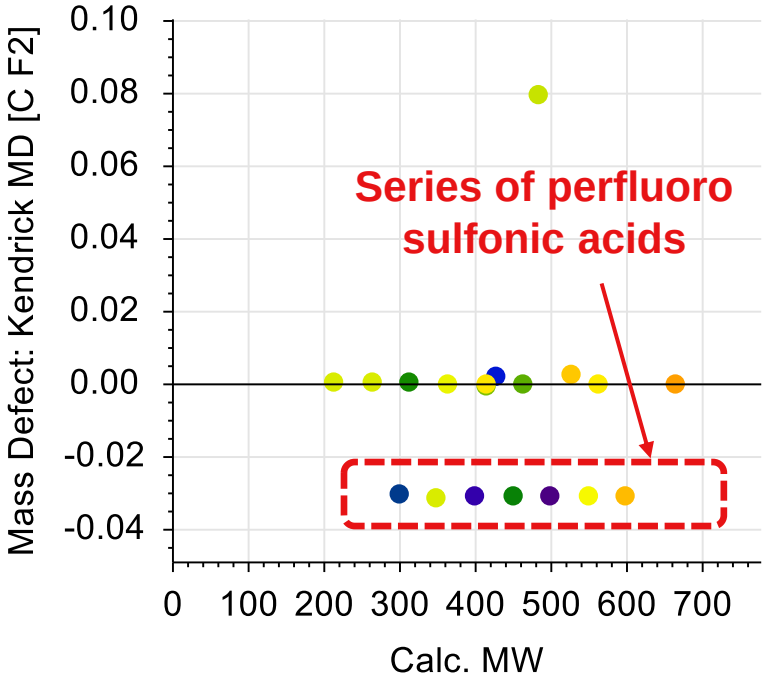
Comparisons of three different sample matrices: AFFF, FCM and River Water

PFAS Compounds with Level 2 Annotations

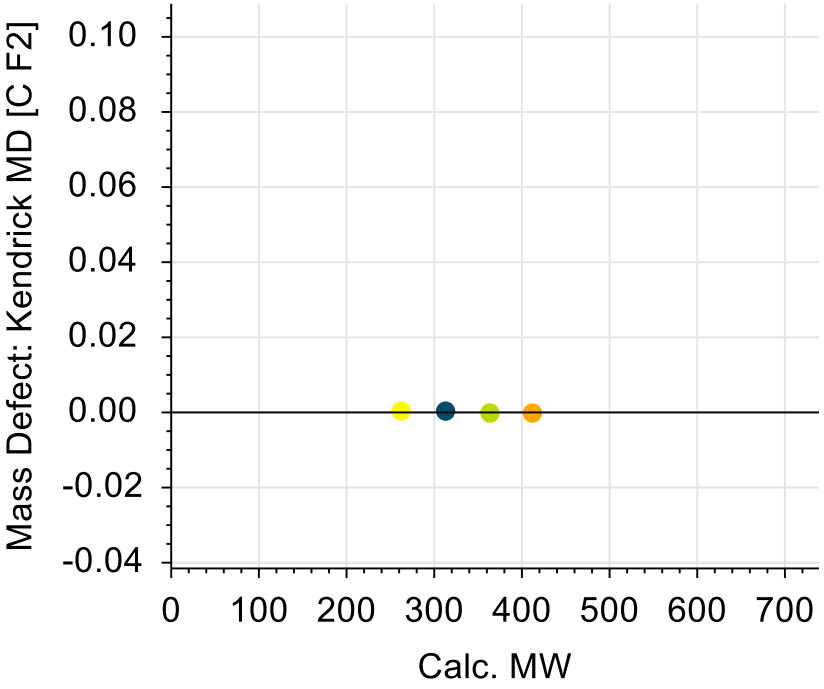
River Water



AFFF



FCM

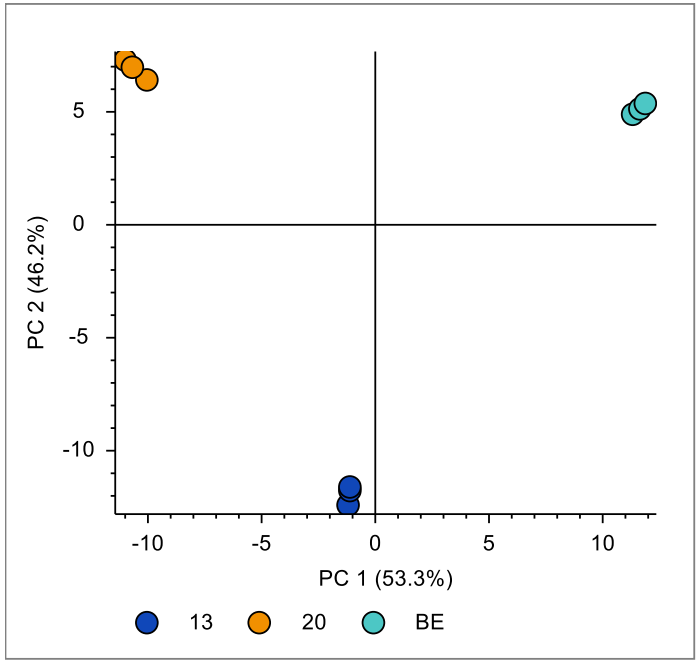


Additional data evaluation tools

Gaining insight into the statistical differences between samples

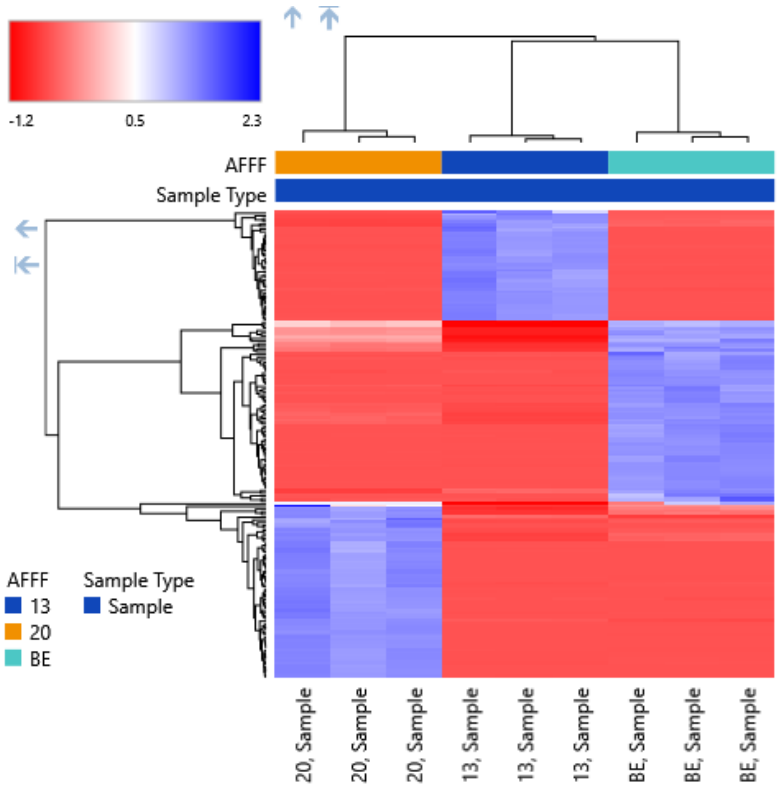
Principal Component Analysis (PCA)

Comparing Three AFFF Samples



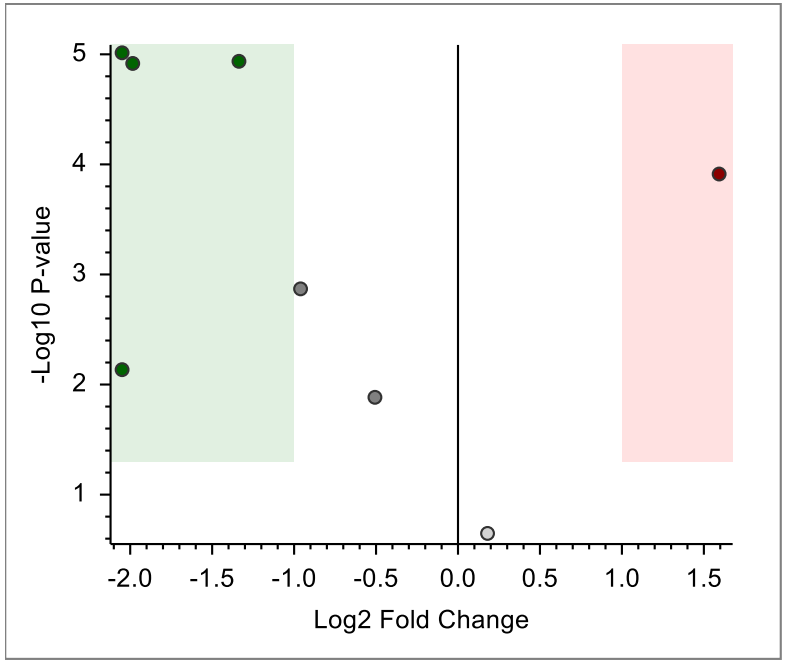
Hierarchical Cluster Analysis

Comparing Three AFFF Samples



Differential Analysis

Comparing River Water Samples
Alviso vs. San Jose



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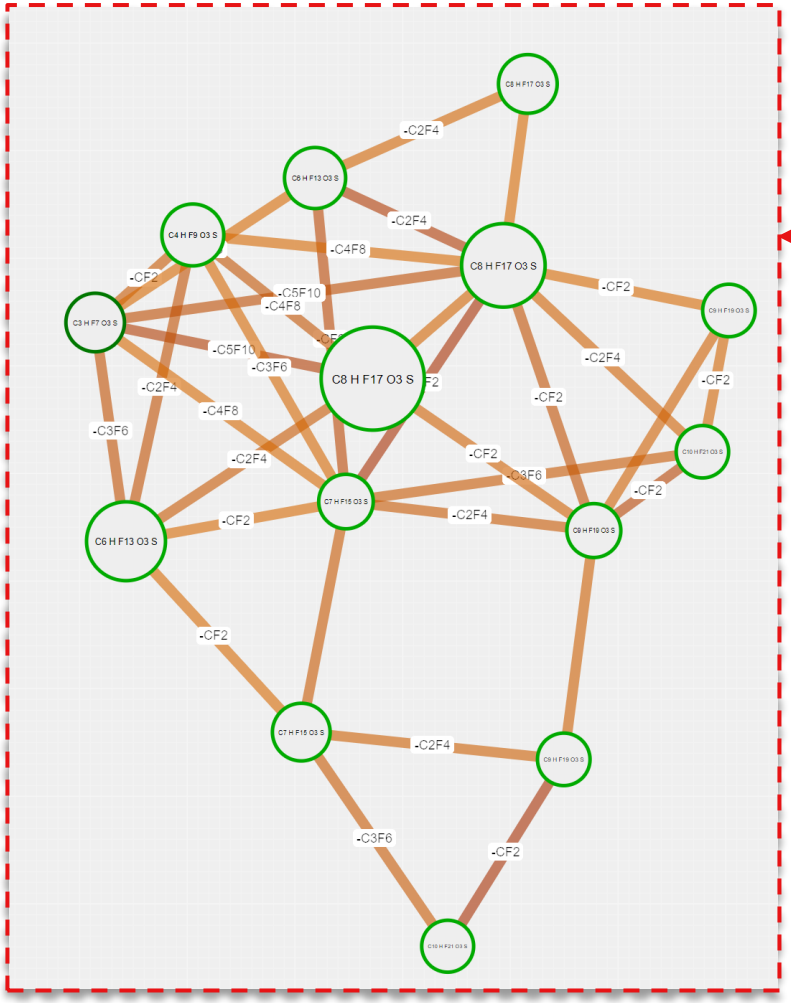
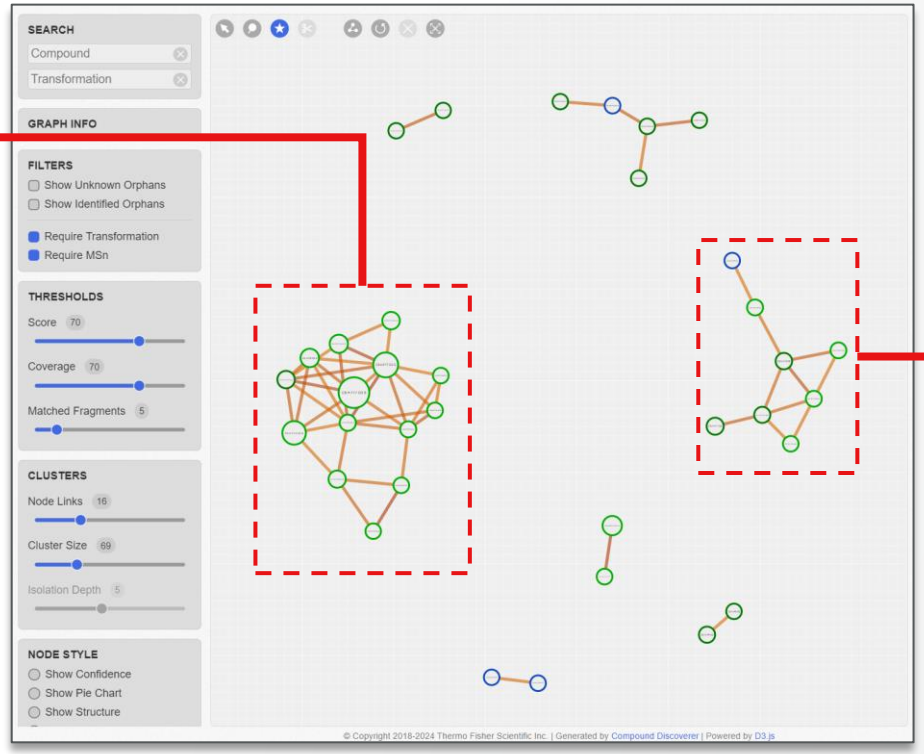
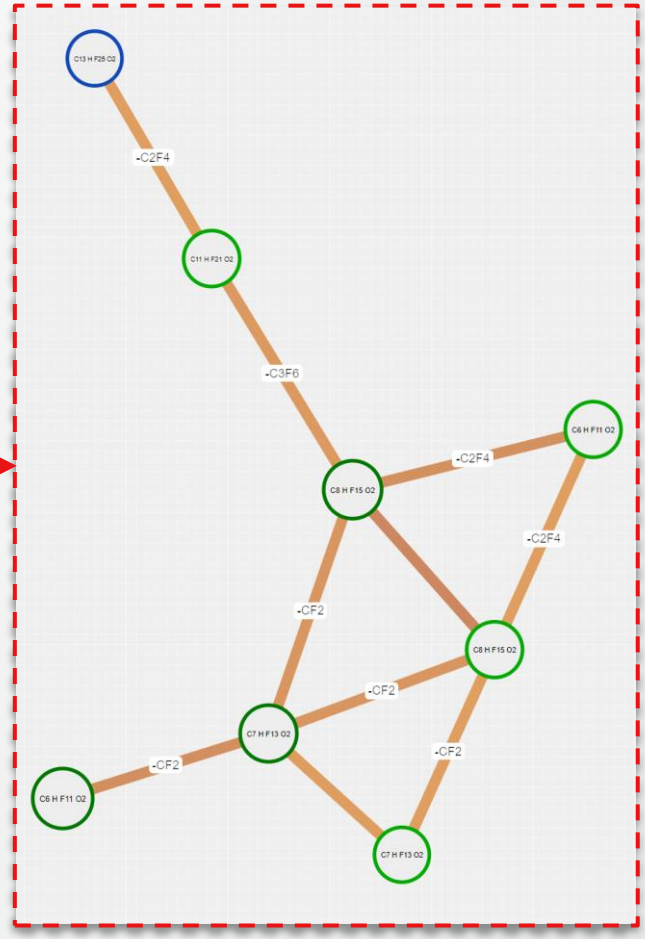
Additional data evaluation tools

Using the Molecular Network tool in Compound Discoverer™ to identify structurally-related compounds

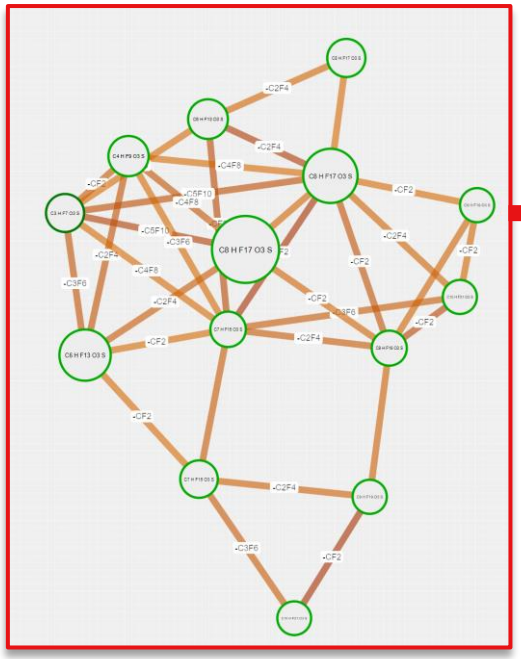




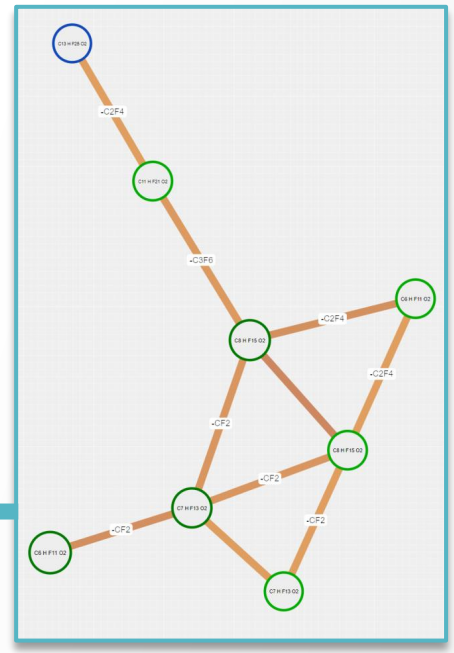


Additional data evaluation tools

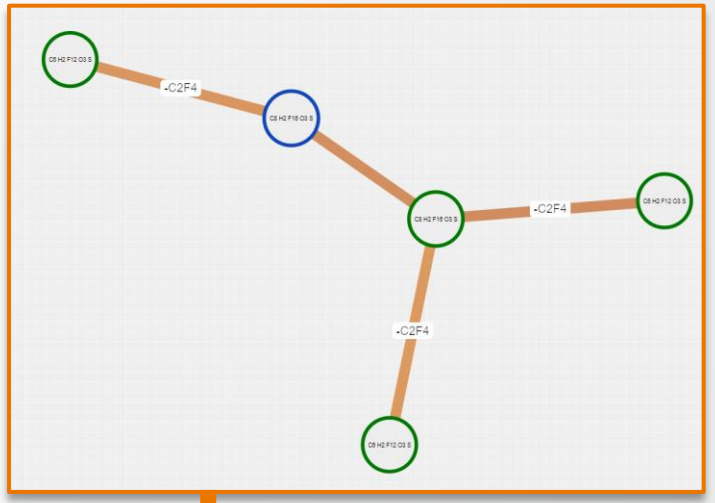
Using the Molecular Network tool in Compound Discoverer™ to identify structurally-related compounds



Name	Formula
PFOS_linear	C8 H F17 O3 S
PFDS	C10 H F21 O3 S
PFNS	C9 H F19 O3 S
PFHpS	C7 H F15 O3 S
PFOS_br	C8 H F17 O3 S
PFDS_br	C10 H F21 O3 S
PFPrS	C3 H F7 O3 S
PFNS_br	C9 H F19 O3 S
PFHpS_br	C7 H F15 O3 S
PFBS	C4 H F9 O3 S
PFNS	C9 H F19 O3 S
PFHxS_linear	C6 H F13 O3 S
PFOS_br	C8 H F17 O3 S
PFHxS_br	C6 H F13 O3 S



Name	Formula
PFTrDA	C13 H F25 O2
PFHxA_br	C6 H F11 O2
PFHpA	C7 H F13 O2
PFHpA_br	C7 H F13 O2
PFOA_linear	C8 H F15 O2
PFOA_br	C8 H F15 O2
PFHxA	C6 H F11 O2
PFUdA	C11 H F21 O2



Name	Formula
5H-Perfluorohexanesulfonic acid	C6 H2 F12 O3 S
Hydrido-PerFluoroOctane Sulfonate	C8 H2 F16 O3 S
7H-Perfluorooctanesulfonic acid	C8 H2 F16 O3 S
SKZFCJKAAJTRQC-UHFFFAOYSA-N	C6 H2 F12 O3 S
Hydrido-PerFluoroHexane Sulfonate	C6 H2 F12 O3 S

Summary

Summary

- By combining Thermo Scientific™ Orbitrap™ MS technology with powerful software packages (Chromeleon™ 7.3.2 CDS and Compound Discoverer™ 3.3 SP3), a single, simple-to-use workflow that includes both targeted and non-targeted PFAS analysis was shown.
- Chromeleon™ 7.3.2 CDS provides a flexible and effective solution for quantifying targeted PFAS compounds using high resolution accurate mass (HRAM) data.
- Utilizing comprehensive tools within Compound Discoverer™, multiple PFAS compounds were detected and annotated at between Level 1–3 confidence across three different matrices: AFFF, River Water and Food Contact Materials, and many more annotated at Levels 4 and 5.

Acknowledgements

Collaborators

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Thermo Fisher Scientific

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- Neil Rumachik
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- Ed George
- Ralf Tautenhahn

Thank you

Visit https://thermofisher.com/pfas and https://thermofisher.com/asms for more information