Orbitrap Workflows for Non-targeted Analysis Using Dispersive Liquid-liquid Microextraction (DiLLME) Sample Preparation

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Vertical Marketing Manager
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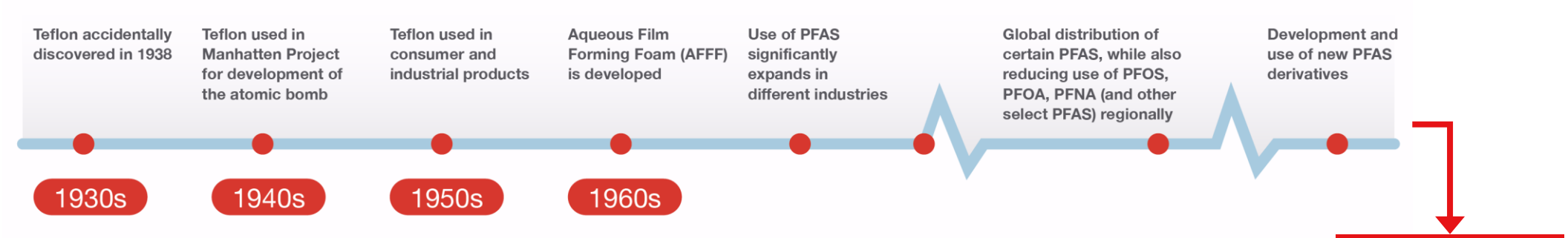
The world leader in serving science

History of PFAS and where we are today

PFAS →

"*fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it), i.e. with a few noted exceptions, any chemical with at least a perfluorinated methyl group (–CF3) or a perfluorinated methylene group (–CF2–)*"
Organization for Economic Co-operation and Development (OECD)

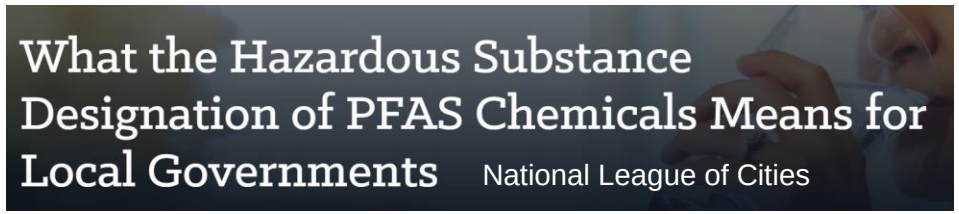


Current

WATERSHED | WHYY NEWS CLIMATE DESK

EPA finalizes new rules for companies producing toxic PFAS chemicals

Companies must now notify the EPA if they want to resume the production of certain PFAS chemicals.



PFAS transport through the environment

Aqueous firefighting foam

Airports
Military bases

Household products

Cleaning products
Personal care products
Paints, waxes, polishes

Manufacturing processes

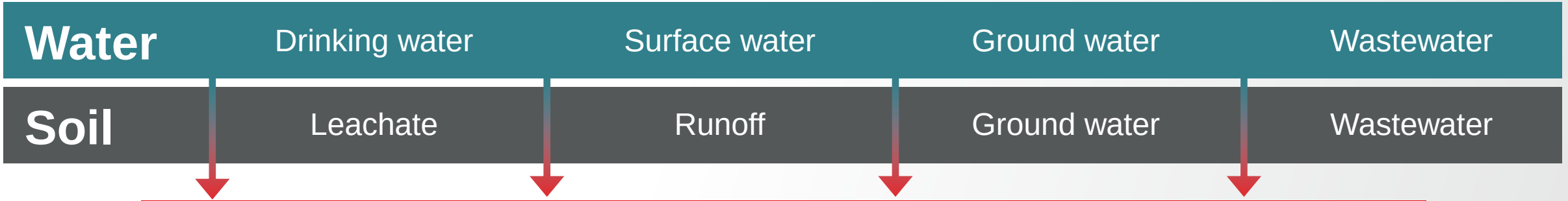
Commercial products
Electronics
Petrochemicals

Fabrics

Stain resistant carpets and fabrics
Waterproof or resistant clothing

Food contact materials

Non-stick cookware
Microwave popcorn bags
Fast food wrappers



>10,000 possible PFAS compounds present in the environment

Workflow strategies

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

Source of PFAS in the United States

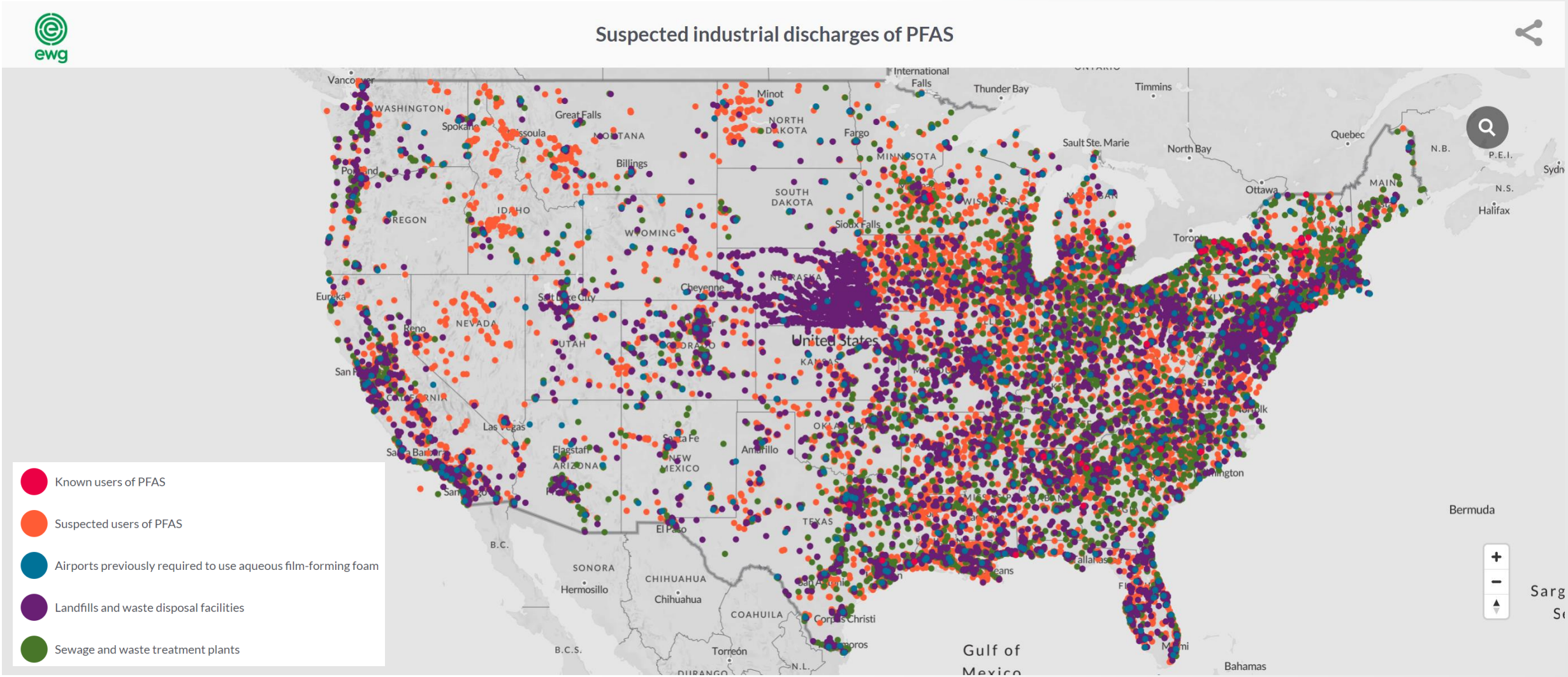


Image obtained from the Environmental Working Group (https://www.ewg.org/interactive-maps/2021_suspected_industrial_discharges_of_pfas/map/) on May 29, 2024.

PFAS contamination of drinking water in the United States

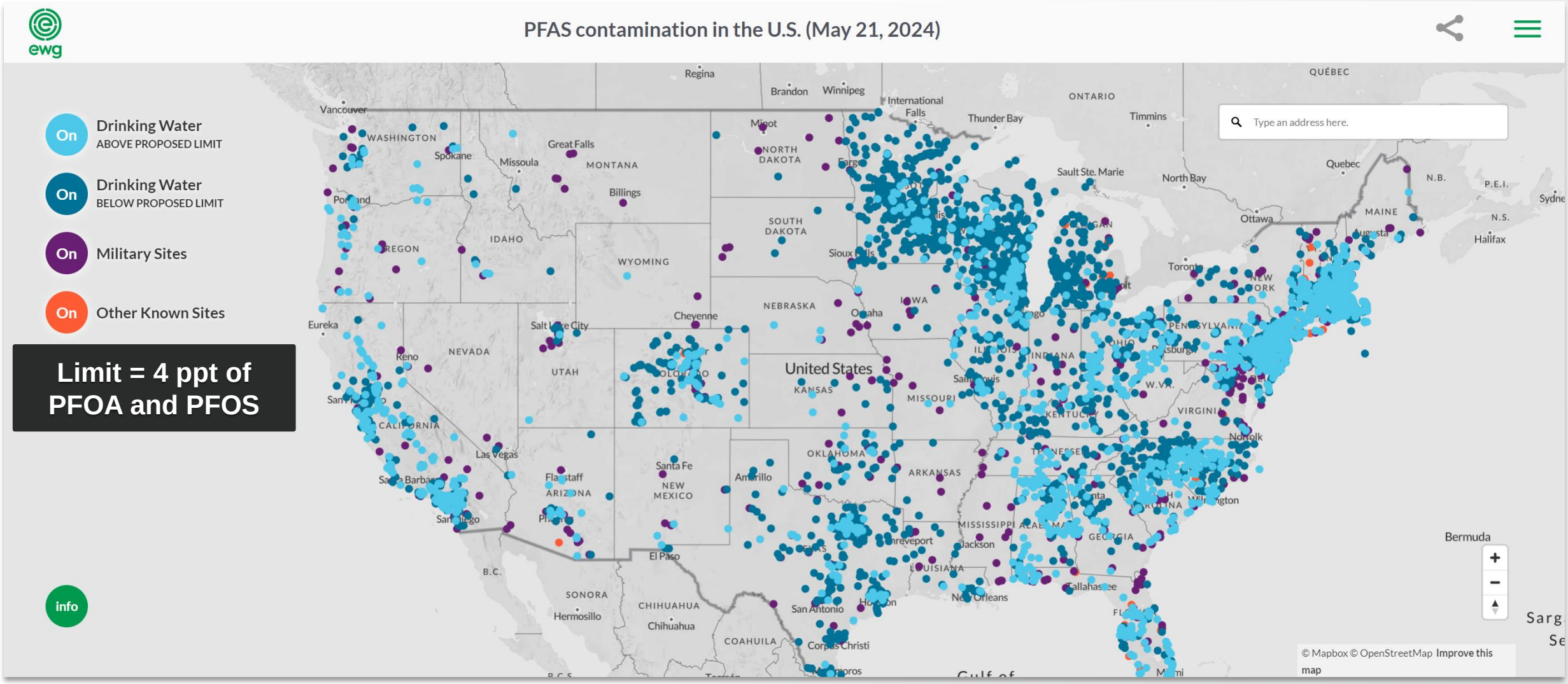


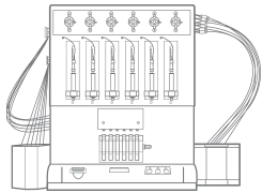
Image obtained from the Environmental Working Group (https://www.ewg.org/interactive-maps/pfas_contamination/map/) on May 29, 2024.

Workflows for Targeted and Non-targeted Analysis

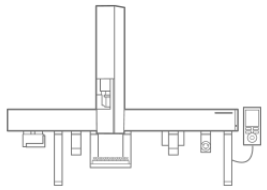
Thousands of possible PFAS compounds present in the environment

Workflow strategies

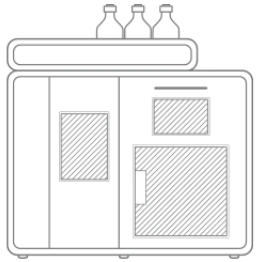
Sample collection ▶ **Sample preparation** ▶ **Analysis** ▶ **Data Processing**



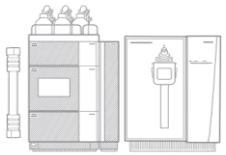
Thermo Scientific™ AutoTrace 280 PFAS
Automated SPE



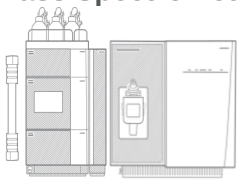
Thermo Scientific™ TriPlus RSH SMART
Automated DLLME



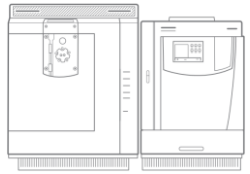
Thermo Scientific™ EXTREVA ASE
Accelerated Solvent Extractor



Thermo Scientific™ Vanquish UHPLC + Orbitrap Exploris™ Mass Spectrometer



Thermo Scientific™ Vanquish UHPLC + TSQ Plus Series Triple Quadrupole Mass Spectrometer



Thermo Scientific™ TSQ 9610 and Orbitrap™ Exploris™ GC Mass Sepctrometers

Chromeleon™ CDS
- Targeted quantification

TraceFinder™
- Targeted quantification
- Targeted Screening

Compound Discoverer™
- Targeted screening
- Non-targeted analysis

Mass Frontier™
- Interpretation of mass spectral data

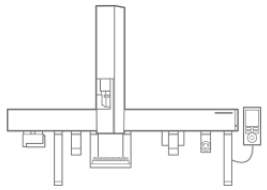
Workflows for Targeted and Non-targeted Analysis

Thousands of possible PFAS compounds present in the environment

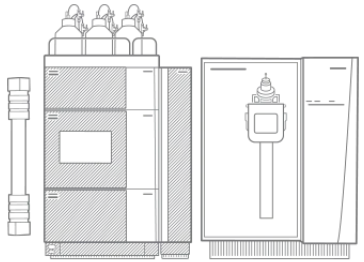
Workflow strategies

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

Thermo Scientific™
AutoTrace 280 PFAS
Automated SPE



Thermo Scientific™ TriPlus RSH SMART
Automated DLLME



Thermo Scientific™ Vanquish UHPLC + Orbitrap Exploris 240 Mass Spectrometer

Chromeleon™ CDS
- Targeted quantification

TraceFinder™
- Targeted quantification
- Targeted Screening

Compound Discoverer™
- Targeted screening
- Non-targeted analysis

Mass Frontier™
- Interpretation of mass spectral data

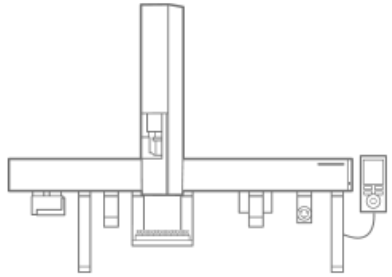
Objective

Demonstrate the comprehensive workflow for PFAS in drinking water samples with a fully automated novel extraction technique followed by targeted quantitation and non-targeted analysis.

Accelerated Solvent Extractor

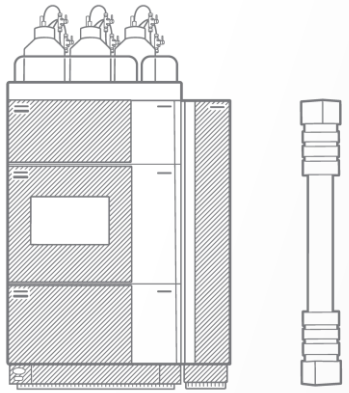
Thermo Scientific™ Vanquish
UHPLC + TSQ Plus Series Triple
Quadrupole Mass Spectrometer

Key method conditions for NTA LC-Orbitrap Workflow



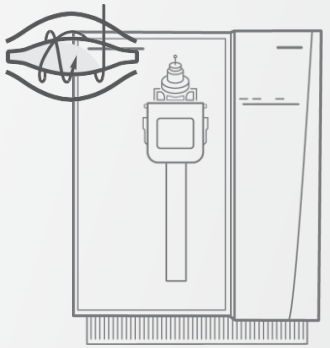
Automate DLLME with the Thermo Scientific™ TriPlus RSH SMART

- Samples were obtained from multiple drinking water sources, including tap water and drinking water from the area of Villebon, France.
- Prior to DLLME samples were spiked with 56 PFAS compounds at two different levels: 1 and 75 ng/L



Thermo Scientific™ Vanquish Flex UHPLC

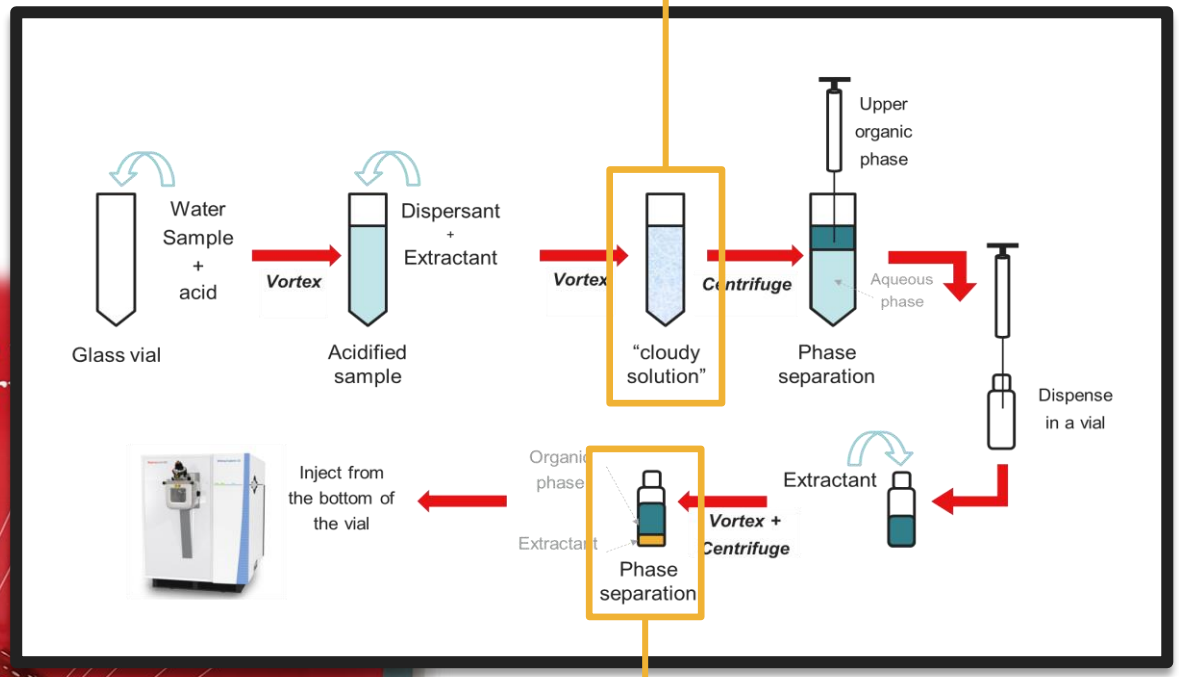
- Delay Column: Thermo Scientific™ Hypersil Gold C18
- Analytical column: Thermo Scientific™ Acclaim™ 120 C18 column
- Flow rate: 0.4 mL/min



Thermo Scientific™ Orbitrap Exploris™ 240

- Heated electrospray ionization (HESI)
- Negative mode
- Combination of Full Scan (MS1) and data-dependent MS2 (ddMS2)
- Mass resolution:
  - Full Scan = 240,000
  - ddMS2 Scan = 30,000
- Stepped HCD fragmentation at 10, 25, 55% (normalized collision energy)

Dispersive Liquid-Liquid Microextraction (DLLME) for PFAS



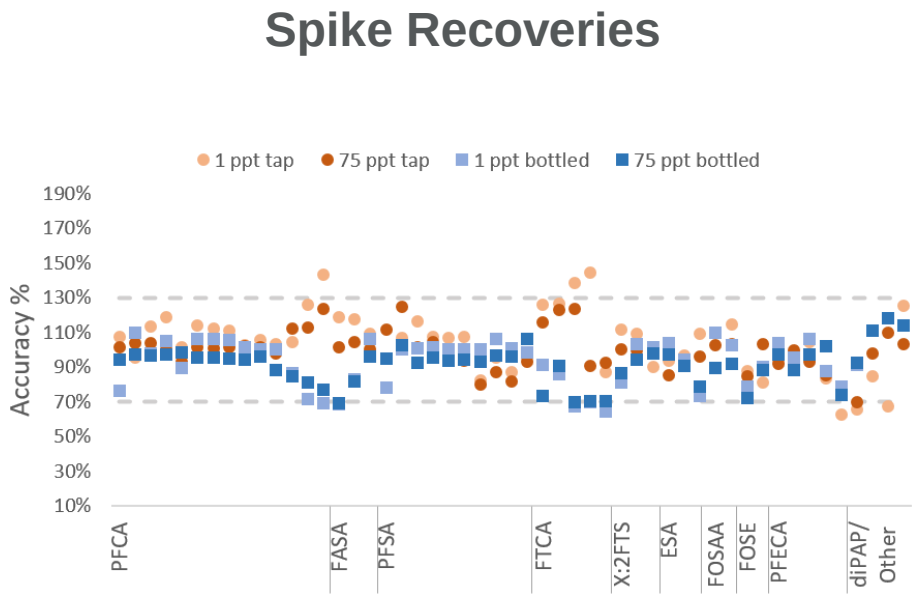
Water containing dispersion and extraction solvent

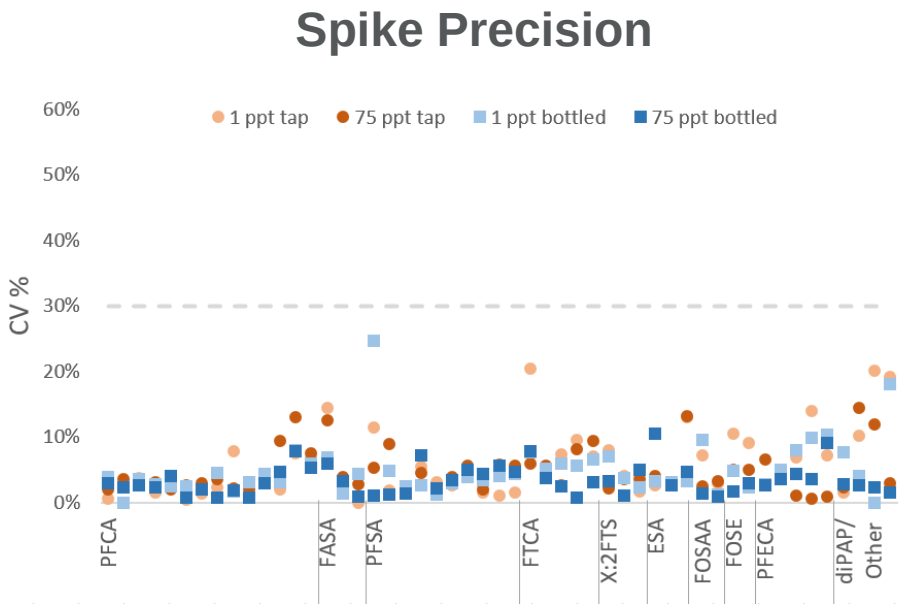
Injection from extract layer at the bottom of the vial

Targeted quantification results

Figure of merit for 56 target PFAS compounds

- The **mean accuracy and precision** of the workflow was evaluated for both spike levels (1 and 75 ng/L) in both bottled and tap water from 6 injections over 3 days.
- Majority of target PFAS compounds exhibited **spike recoveries** within 70–130% and **precision** (coefficient of variation, CV) within 20%.

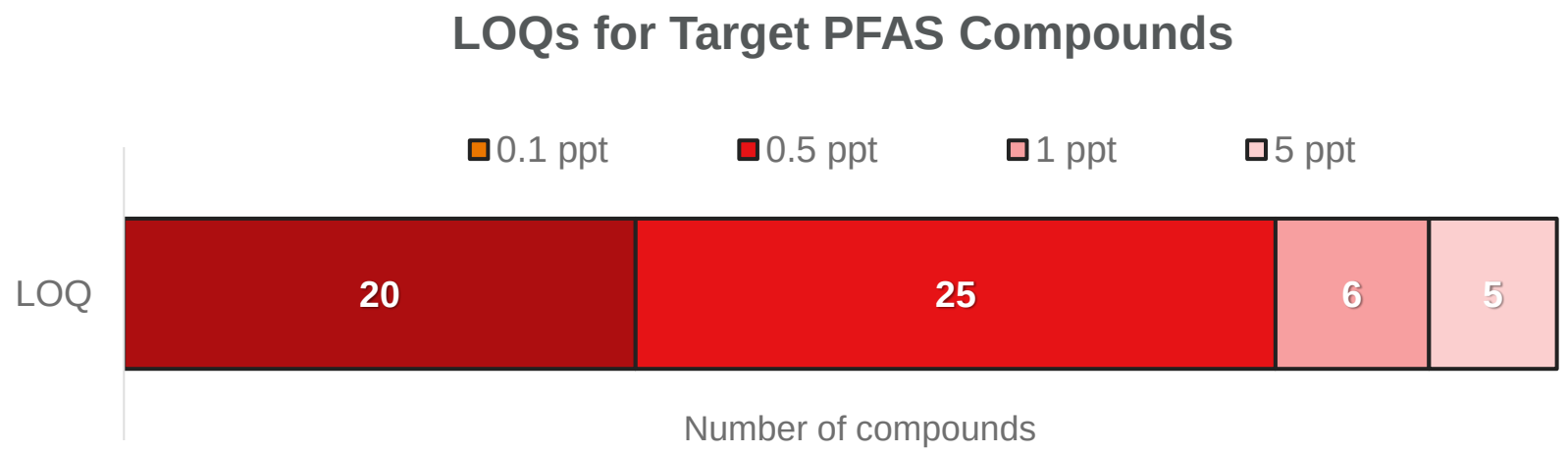




Targeted quantification results

Figure of merit for 56 target PFAS compounds

- **Limit of quantification (LOQ)** values were obtained based on the concentration level for which both reproducibility (<30%) and accuracy (70-130%) criteria were met on 6 injections over 3 days in neat solution.
- Majority of target PFAS compounds (~80%) exhibited LOQs of ≤0.5 ng/L, with the remaining ~20% exhibiting LOQs within the range of 1–5 ng/L.



Non-targeted analysis (NTA) of PFAS: The Wild West

Lots of great ideas and ambitions…….but no rules to follow

Goal of PFAS NTA workflows → **Detect unknown PFAS compounds and annotate them as confidently as possible using multiple layers of mass spectrometry evidence**

Picture obtained from LinkedIn (2024)

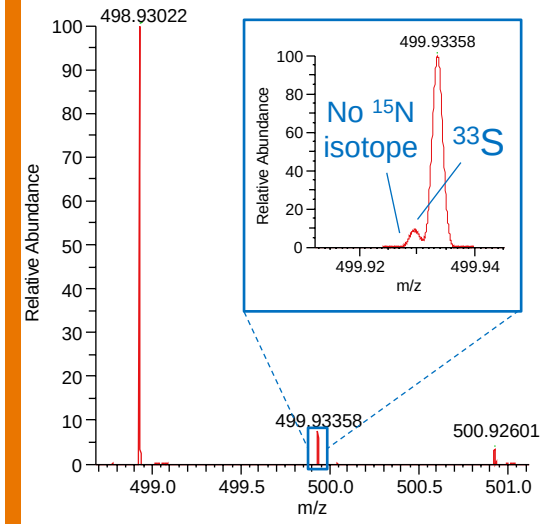
Increasing confidence in identifying PFAS compounds

Harnessing the Power of High Resolution Accurate Mass and MS^n Analysis

Increasing Confidence in Compound Identification

Level 5

MS^1 analysis at 240,000 mass resolution



Exact Mass
Using high resolution accurate mass (HRAM) MS

Level 4

Possible empirical formulas with $C_xH_yF_zO_wN_uS_v$ that are within 1 ppm mass error *AND* match the observed isotope pattern

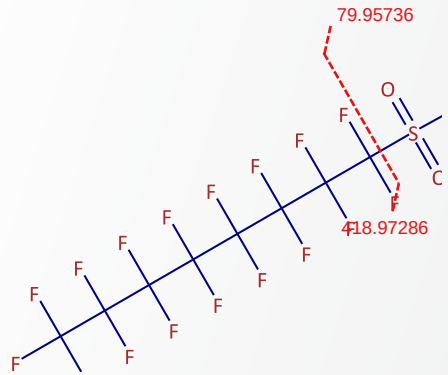
$C_8O_3F_{17}S$
$C_{13}O_4F_{13}S$
~~$C_{18}O_8N_2F_5S$~~
~~$C_{23}O_9N_2FS$~~
~~$C_2O_{10}N_8F_9S$~~

^{15}N isotope not observed in MS^1 spectrum

Elemental Formula
Combine measured MS^1 mass and isotope patterns

Using MS^1 Data

Level 3

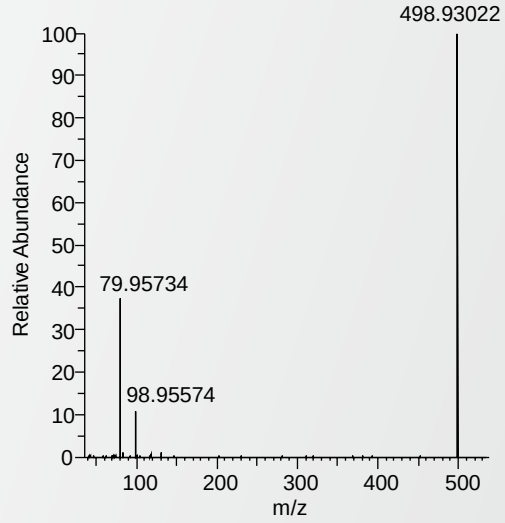


Perfluorooctanesulfonic acid
$C_8O_3F_{17}S$

Tentative Candidate
Compare MS^2 spectrum to in-silico fragmentation

Level 2

MS^2 analysis of m/z 498.93022 precursor ion



Probable Structure
Compare MS^2 spectrum to spectra library

Using MS^2 Data

See additional details on confidence levels in identifying small molecules in Schymanski et al. *Environ. Sci. Technol.* **2014**, 48, 2097–2098.

Workflow for non-targeted PFAS analysis

Compound Discoverer™ version 3.3 SP3

Each detected compound with a MS^2 spectrum is searched against the in-silico generated PFAS spectral library[1] from Duke University and 2023 NIST MS/MS library

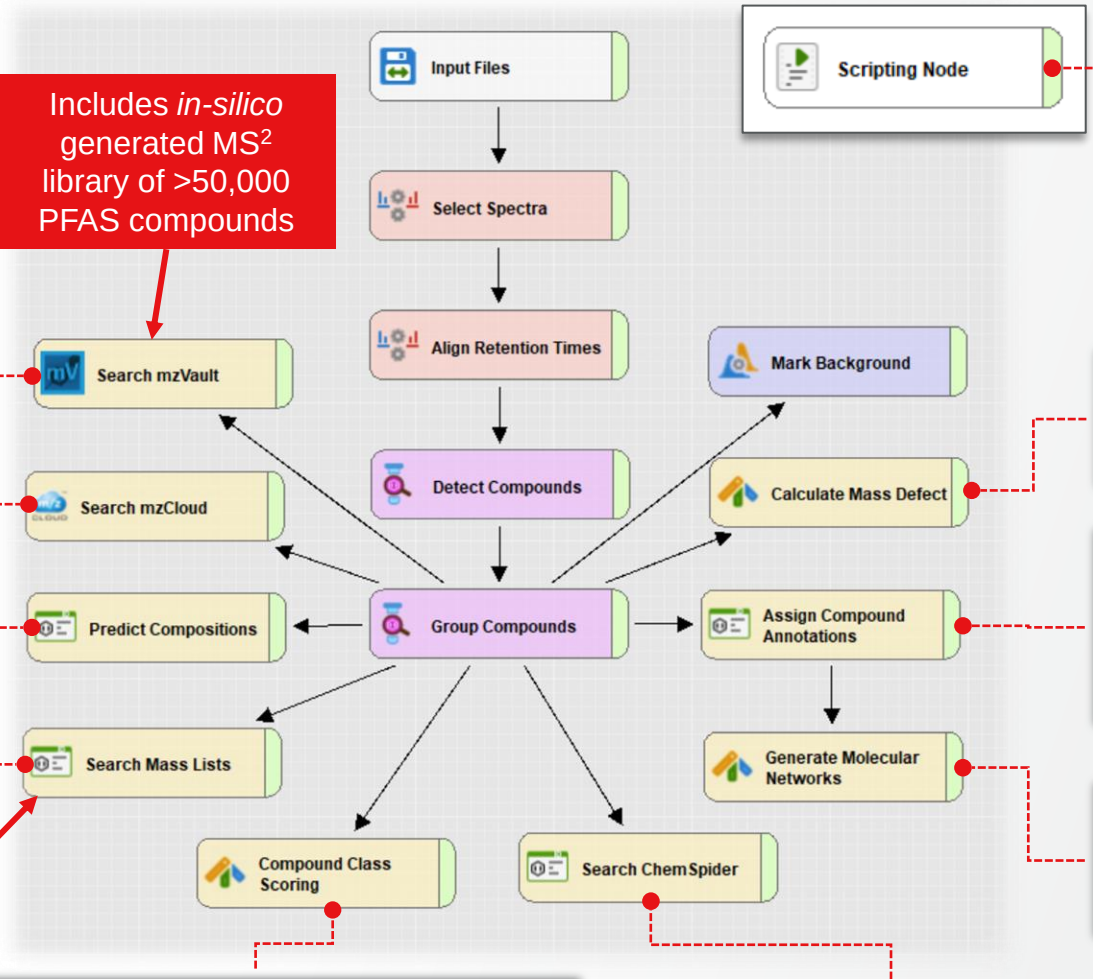
Includes *in-silico* generated MS^2 library of >50,000 PFAS compounds

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

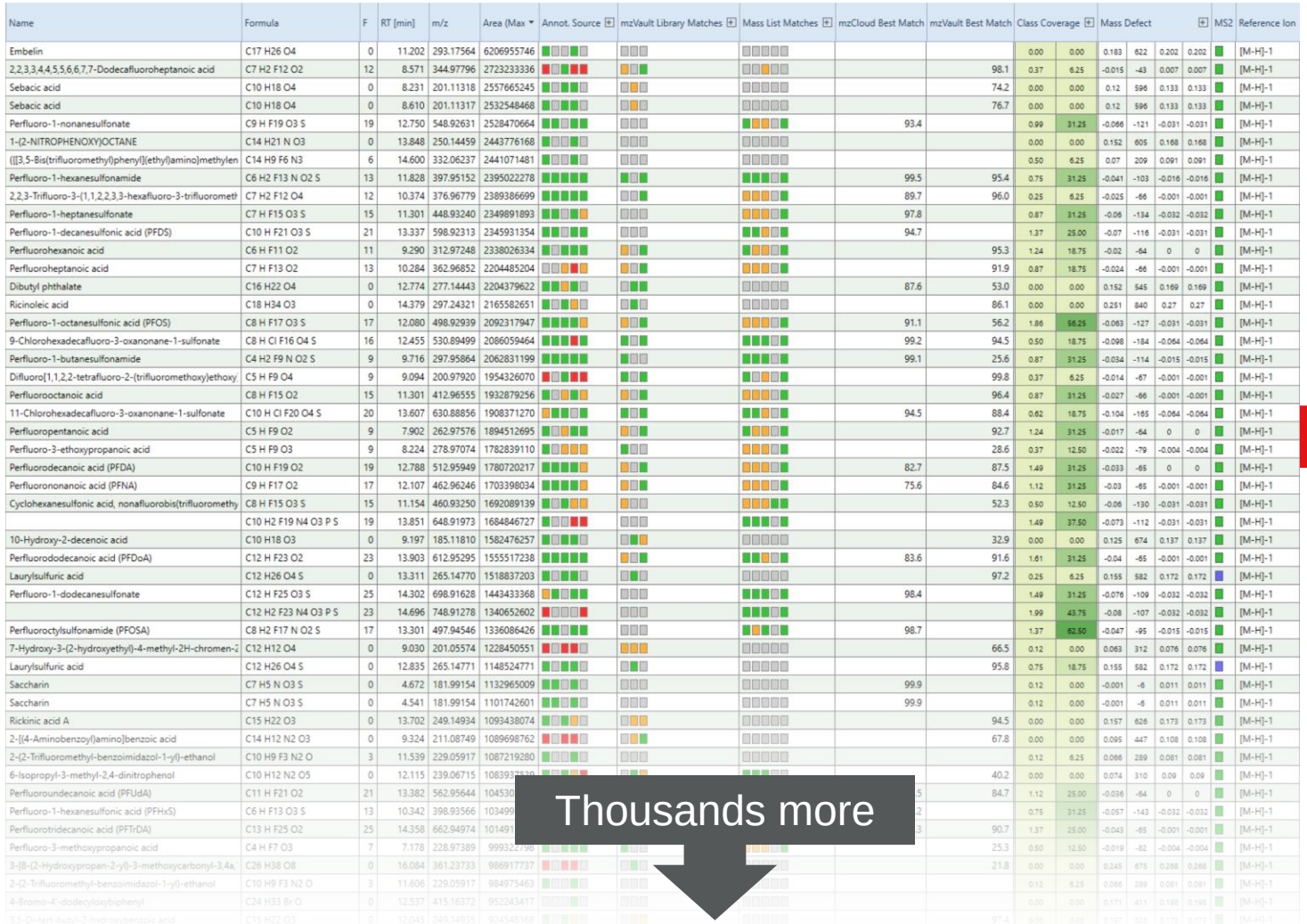
Compares MS^2 fragments of detected compounds to the FluoroMatch™ fragment library (InnovativeOmics) and PFAS fine structure fragment library

Each detected compound is searched against the ChemSpider chemical database

See additional details on the in-silico generated PFAS spectral library in Getzinger et al. *Anal. Chem.* **2021**, *93*, 2820–2827.

Initial results – All annotated compounds

Starting out with ~4,000 detected and annotated compounds, but how many PFAS?



Name	Formula	F	RT [min]	m/z	Area (Max	Annot. Source	mzVault Library Matches	Mass List Matches	mzCloud Best Match	mzVault Best Match	Class Coverage		Mass Defect				MS2	Reference Ion
Embelin	C17 H26 O4	0	11.202	293.17564	6206955746						0.00	0.00	0.183	622	0.202	0.202		[M-H]-1
2,2,3,3,4,4,5,5,6,6,7,7-Dodecafluoroheptanoic acid	C7 H2 F12 O2	12	8.571	344.97796	2723233336					98.1	0.37	6.25	-0.015	-43	0.007	0.007		[M-H]-1
Sebacic acid	C10 H18 O4	0	8.231	201.11318	2557665245					74.2	0.00	0.00	0.12	596	0.133	0.133		[M-H]-1
Sebacic acid	C10 H18 O4	0	8.610	201.11317	2532548468					76.7	0.00	0.00	0.12	596	0.133	0.133		[M-H]-1
Perfluoro-1-nonanesulfonate	C9 H F19 O3 S	19	12.750	548.92631	2528470664				93.4		0.99	31.25	-0.066	-121	-0.031	-0.031		[M-H]-1
1-(2-NITROPHENOXY)OCTANE	C14 H21 N O3	0	13.848	250.14459	2443776168						0.00	0.00	0.152	605	0.168	0.168		[M-H]-1
([3,5-Bis(trifluoromethyl)phenyl](ethyl)amino)methylen	C14 H9 F6 N3	6	14.600	332.06237	2441071481						0.50	6.25	0.07	209	0.091	0.091		[M-H]-1
Perfluoro-1-hexanesulfonamide	C6 H2 F13 N O2 S	13	11.828	397.95152	2395022278				99.5	95.4	0.75	31.25	-0.041	-103	-0.016	-0.016		[M-H]-1
2,2,3-Trifluoro-3-(1,1,2,2,3,3-hexafluoro-3-trifluorometh	C7 H2 F12 O4	12	10.374	376.96779	2389386699				89.7	96.0	0.25	6.25	-0.025	-66	-0.001	-0.001		[M-H]-1
Perfluoro-1-heptanesulfonate	C7 H F15 O3 S	15	11.301	448.93240	2349891893				97.8		0.87	31.25	-0.06	-134	-0.032	-0.032		[M-H]-1
Perfluoro-1-decanesulfonic acid (PFDS)	C10 H F21 O3 S	21	13.337	598.92313	2345931354				94.7		1.37	25.00	-0.07	-116	-0.031	-0.031		[M-H]-1
Perfluorohexanoic acid	C6 H F11 O2	11	9.290	312.97248	2338026334					95.3	1.24	18.75	-0.02	-64	0	0		[M-H]-1
Perfluoroheptanoic acid	C7 H F13 O2	13	10.284	362.96852	2204485204					91.9	0.87	18.75	-0.024	-66	-0.001	-0.001		[M-H]-1
Dibutyl phthalate	C16 H22 O4	0	12.774	277.14443	2204379622				87.6	53.0	0.00	0.00	0.152	545	0.169	0.169		[M-H]-1
Ricinoleic acid	C18 H34 O3	0	14.379	297.24321	2165582651					86.1	0.00	0.00	0.251	840	0.27	0.27		[M-H]-1
Perfluoro-1-octanesulfonic acid (PFOS)	C8 H F17 O3 S	17	12.080	498.92939	2092317947				91.1	56.2	1.86	56.25	-0.063	-127	-0.031	-0.031		[M-H]-1
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonate	C8 H Cl F16 O4 S	16	12.455	530.89499	2086059464				99.2	94.5	0.50	18.75	-0.098	-184	-0.064	-0.064		[M-H]-1
Perfluoro-1-butanesulfonamide	C4 H2 F9 N O2 S	9	9.716	297.95864	2062831199				99.1	25.6	0.87	31.25	-0.034	-114	-0.015	-0.015		[M-H]-1
Difluoro[1,1,2,2-tetrafluoro-2-(trifluoromethoxy)ethoxy	C5 H F9 O4	9	9.094	200.97920	1954326070					99.8	0.37	6.25	-0.014	-67	-0.001	-0.001		[M-H]-1
Perfluorooctanoic acid	C8 H F15 O2	15	11.301	412.96555	1932879256					96.4	0.87	31.25	-0.027	-66	-0.001	-0.001		[M-H]-1
11-Chlorohexadecafluoro-3-oxanonane-1-sulfonate	C10 H Cl F20 O4 S	20	13.607	630.88856	1908371270				94.5	88.4	0.62	18.75	-0.104	-165	-0.064	-0.064		[M-H]-1
Perfluoropentanoic acid	C5 H F9 O2	9	7.902	262.97576	1894512695					92.7	1.24	31.25	-0.017	-64	0	0		[M-H]-1
Perfluoro-3-ethoxypropanoic acid	C5 H F9 O3	9	8.224	278.97074	1782839110					28.6	0.37	12.50	-0.022	-79	-0.004	-0.004		[M-H]-1
Perfluorodecanoic acid (PFDA)	C10 H F19 O2	19	12.788	512.95949	1780720217				82.7	87.5	1.49	31.25	-0.033	-65	0	0		[M-H]-1
Perfluorononanoic acid (PFNA)	C9 H F17 O2	17	12.107	462.96246	1703398034				75.6	84.6	1.12	31.25	-0.03	-65	-0.001	-0.001		[M-H]-1
Cyclohexanesulfonic acid, nonafluorobis(trifluorometh	C8 H F15 O3 S	15	11.154	460.93250	1692089139					52.3	0.50	12.50	-0.06	-130	-0.031	-0.031		[M-H]-1
	C10 H2 F19 N4 O3 P S	19	13.851	648.91973	1684846727						1.49	37.50	-0.073	-112	-0.031	-0.031		[M-H]-1
10-Hydroxy-2-decenoic acid	C10 H18 O3	0	9.197	185.11810	1582476257					32.9	0.00	0.00	0.125	674	0.137	0.137		[M-H]-1
Perfluorododecanoic acid (PFDoA)	C12 H F23 O2	23	13.903	612.95295	1555517238				83.6	91.6	1.61	31.25	-0.04	-65	-0.001	-0.001		[M-H]-1
Laurylsulfuric acid	C12 H26 O4 S	0	13.311	265.14770	1518837203					97.2	0.25	6.25	0.155	582	0.172	0.172		[M-H]-1
Perfluoro-1-dodecanesulfonate	C12 H F25 O3 S	25	14.302	698.91628	1443433368				98.4		1.49	31.25	-0.076	-109	-0.032	-0.032		[M-H]-1
	C12 H2 F23 N4 O3 P S	23	14.696	748.91278	1340652602						1.99	43.75	-0.08	-107	-0.032	-0.032		[M-H]-1
Perfluorooctylsulfonamide (PFOSA)	C8 H2 F17 N O2 S	17	13.301	497.94546	1336086426				98.7		1.37	62.50	-0.047	-95	-0.015	-0.015		[M-H]-1
7-Hydroxy-3-(2-hydroxyethyl)-4-methyl-2H-chromen-2	C12 H12 O4	0	9.030	201.05574	1228450551					66.5	0.12	0.00	0.063	312	0.076	0.076		[M-H]-1
Laurylsulfuric acid	C12 H26 O4 S	0	12.835	265.14771	1148524771					95.8	0.75	18.75	0.155	582	0.172	0.172		[M-H]-1
Saccharin	C7 H5 N O3 S	0	4.672	181.99154	1132965009				99.9		0.12	0.00	-0.001	-6	0.011	0.011		[M-H]-1
Saccharin	C7 H5 N O3 S	0	4.541	181.99154	1101742601				99.9		0.12	0.00	-0.001	-6	0.011	0.011		[M-H]-1
Rickinic acid A	C15 H22 O3	0	13.702	249.14934	1093438074					94.5	0.00	0.00	0.157	626	0.173	0.173		[M-H]-1
2-[(4-Aminobenzoyl)amino]benzoic acid	C14 H12 N2 O3	0	9.324	211.08749	1089698762					67.8	0.00	0.00	0.095	447	0.108	0.108		[M-H]-1
2-(2-Trifluoromethyl-benzoimidazol-1-yl)-ethanol	C10 H9 F3 N2 O	3	11.539	229.05917	1087219280						0.12	6.25	0.066	289	0.081	0.081		[M-H]-1
6-Isopropyl-3-methyl-2,4-dinitrophenol	C10 H12 N2 O5	0	12.115	239.06715	1083937539					40.2	0.00	0.00	0.074	310	0.09	0.09		[M-H]-1
Perfluoroundecanoic acid (PFUdA)	C11 H F21 O2	21	13.382	562.95644	1045301				5	84.7	1.12	25.00	-0.036	-64	0	0		[M-H]-1
Perfluoro-1-hexanesulfonic acid (PFHxS)	C6 H F13 O3 S	13	10.342	398.93566	103499				2		0.75	31.25	-0.057	-143	-0.032	-0.032		[M-H]-1
Perfluorotridecanoic acid (PFTrDA)	C13 H F25 O2	25	14.358	662.94974	101491				3	90.7	1.37	25.00	-0.043	-65	-0.001	-0.001		[M-H]-1
Perfluoro-3-methoxypropanoic acid	C4 H F7 O3	7	7.178	228.97389	999322798					25.3	0.50	12.50	-0.019	-82	-0.004	-0.004		[M-H]-1
3-[8-(2-Hydroxypropan-2-yl)-3-methoxycarbonyl-3,4a	C26 H38 O8	0	16.084	361.23733	986917737					21.8	0.00	0.00	0.245	675	0.268	0.268		[M-H]-1
2-(2-Trifluoromethyl-benzoimidazol-1-yl)-ethanol	C10 H9 F3 N2 O	3	11.606	229.05917	984975463						0.12	6.25	0.066	289	0.081	0.081		[M-H]-1
4-Bromo-4'-dodecyloxybiphenyl	C24 H33 Br O	0	12.537	415.16372	952243417						0.00	0.00	0.171	411	0.198	0.198		[M-H]-1
3,5-Di-tert-butyl-2-hydroxybenzoic acid	C15 H22 O3	0	12.045	249.14935	924548168					97.4	0.00	0.00	0.157	626	0.173	0.173		[M-H]-1

Thousands more

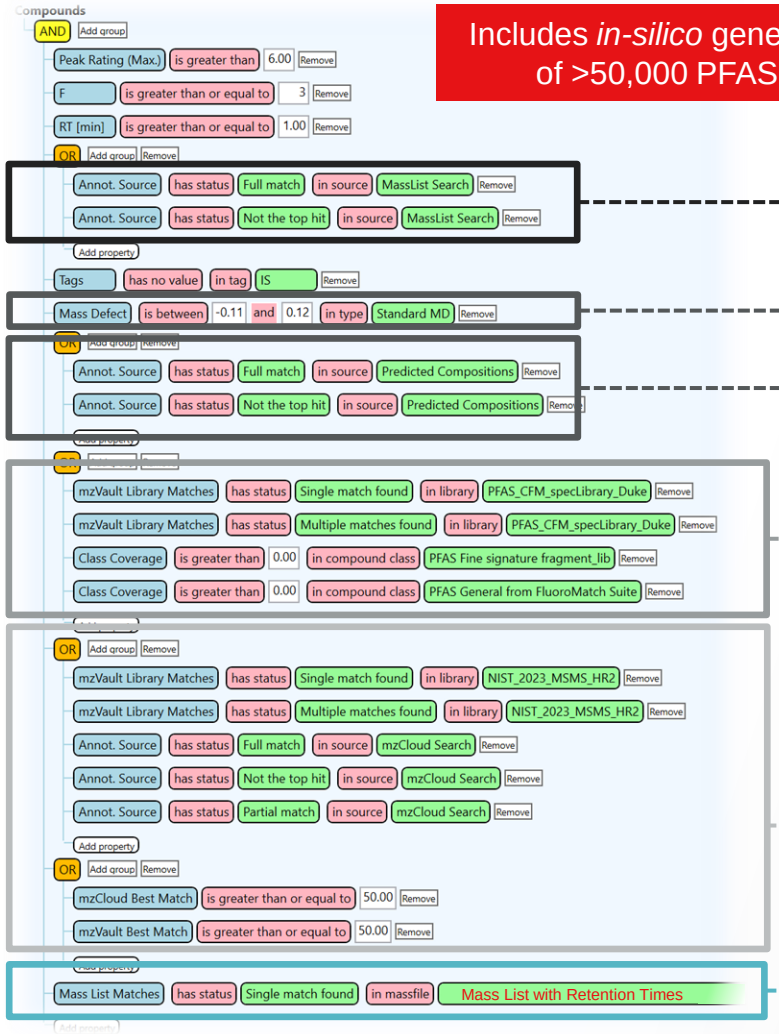
We need to narrow this list down to only PFAS, and then determine the confidence levels of each annotation

Data reduction approach

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

Includes *in-silico* generated MS² library of >50,000 PFAS compounds

- Search Mass Lists
- Search ChemSpider
- Predict Compositions
- Compound Class Scoring
- Search mzVault in-silico library
- Search mzVault 2023 NIST MS/MS
- Search mzCloud
- Search Mass Lists with Retention Times



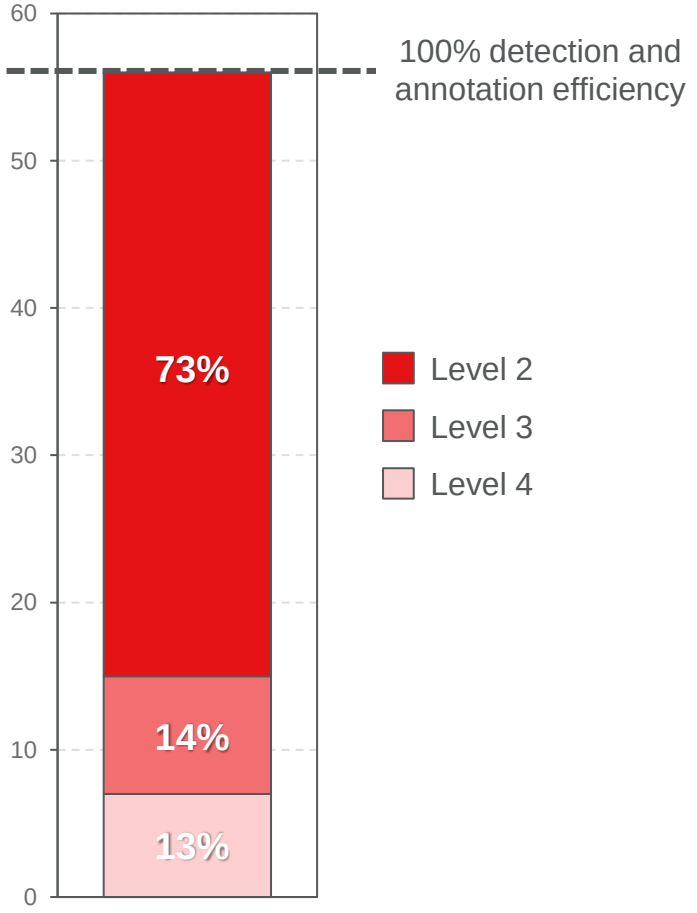
Annotation Criteria	Annotation Confidence Level 5	4	3	2	1
Measured mass ±2 ppm of at least one PFAS Mass List	✓	✓	✓	✓	✓
Standard mass defect is between -0.11–0.12		✓	✓	✓	✓
Isotopic pattern match		✓	✓	✓	✓
≥1 MS^2 fragment with match to FluoroMatch database and/or >50% similarity match to in-silico PFAS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

Annotation of PFAS in DLLME extracts of spiked water samples

Evaluating the detection and annotation efficiency of 56 spiked PFAS compounds

Annotation Criteria	Annotation Confidence Level 5	4	3	2
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 and/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				

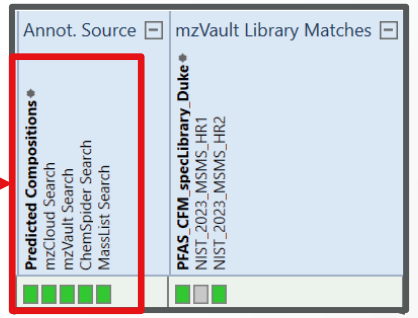


Finalized list of annotated PFAS compounds

Easy-to-evaluate source contributions to each final compound annotation

Tagging feature to mark each identified compound with their annotation confidence level

Percentage of fragments within the FluoroMatch database that match to fragments within the measures MS2 spectrum



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

	Tags	Name	Formula	F	RT [min]	m/z	Area (Max.)	Annot. Sou	mzVault Library Matches	Mass List Matches	mzCloud Best Match	mzVault Best Match	Class Coverage	
1		10:2 Fluorinated telomer sulfonate	C12 H5 F21 O3 S	21	13.949	626.95429	515738822				96.0	39.5	0.75	12.50
2		Perfluoropentanoic acid	C5 H F9 O2	9	8.682	262.97596	51082095					79.1	1.12	25.00
3		Perfluoro-1-octanesulfonic acid (PFOS)	C8 H F17 O3 S	17	11.545	498.92966	14782802				89.8	61.1	1.86	37.50
4		N-Methylperfluoro-1-octanesulfonamidoacetic acid (N	C11 H6 F17 N O4 S	17	13.163	569.96704	29234176				70.5	71.7	1.49	37.50
5		Heptafluoropropan-2-ol	C3 H F7 O	7	12.144	184.98428	31191121					36.1	0.37	12.50
6		N-Ethylperfluoro-1-octanesulfonamidoacetic acid (N-E	C12 H8 F17 N O4 S	17	13.619	583.98270	45973262				76.7	62.8	1.12	50.00
7		Potassium perfluoro-1-(perfluoroethyl)cyclohexanesulf	C8 H F15 O3 S	15	11.006	460.93285	17252539					64.5	1.12	25.00
8		Perfluorohexadecanoic acid	C16 H F31 O2	31	15.391	812.93968	532195883					43.6	1.49	25.00
9		Perfluoro-1-dodecanesulfonate	C12 H F25 O3 S	25	14.302	698.91628	1443433368				98.4		1.49	31.25
10		Glycine, N-[(1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecaflu	C11 H6 F17 N O4 S	17	13.905	569.96686	10117450					39.4	1.24	37.50
11		11-Chlorohexadecafluoro-3-oxanonane-1-sulfonate	C10 H Cl F20 O4 S	20	13.607	630.88856	1908371270				94.5	88.4	0.62	18.75
12		Perfluoropentanoic acid	C5 H F9 O2	9	7.902	262.97576	1894512695					92.7	1.24	31.25
13		Heptadecafluoro-N-methylisooctanesulphonamide	C9 H4 F17 N O2 S	17	14.132	511.96128	202111808					31.4	1.99	43.75
14		N-Methylperfluoro-1-octanesulfonamidoacetic acid (N-	C11 H6 F17 N O4 S	17	13.437	569.96704	214202729				93.7	79.1	1.37	31.25
15		N-Methylperfluoro-1-octanesulfonamidoacetic acid (N-	C11 H6 F17 N O4 S	17	13.248	569.96694	140100161				82.2	69.5	1.12	31.25
16		Methyl Perfluorononanoate	C10 H3 F17 O2	17	12.898	476.97834	170603032						0.87	12.50
17		Perfluoro-1-octanesulfonic acid (PFOS)	C8 H F17 O3 S	17	11.789	498.92954	173684522				99.4	20.3	0.99	18.75
18		1-Butanesulfonamide, 1,1,2,2,3,3,4,4,4-nonafluoro-N-m	C5 H4 F9 N O2 S	9	10.864	311.97443	319551437						0.99	31.25
19		Ammonium bis[2-(perfluorohexyl)ethyl] phosphate	C16 H9 F26 O4 P	26	14.562	788.97397	129368813					26.8	0.37	0.00
20		Sulfluramide	C10 H6 F17 N O2 S	17	14.486	525.97702	112749995				98.5	78.5	0.99	37.50
21		3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptadecafluoro-2-m	C11 H5 F17 O2	17	14.095	490.99402	109642967						1.99	12.50
22		6:2/8:2 Fluorotelomer phosphate diester	C18 H9 F30 O4 P	30	15.128	888.96744	80731951					49.2	0.50	0.00
23		Perfluoro-1-hexanesulfonic acid (PFHxS)	C6 H F13 O3 S	13	10.117	398.93586	139586578				98.1		0.99	31.25
24		2-(2-hydroxyethyl)ammonium bis[(perfluorooctyl)ethyl	C20 H9 F34 O4 P	34	15.568	988.96100	56893102					31.5	0.37	0.00

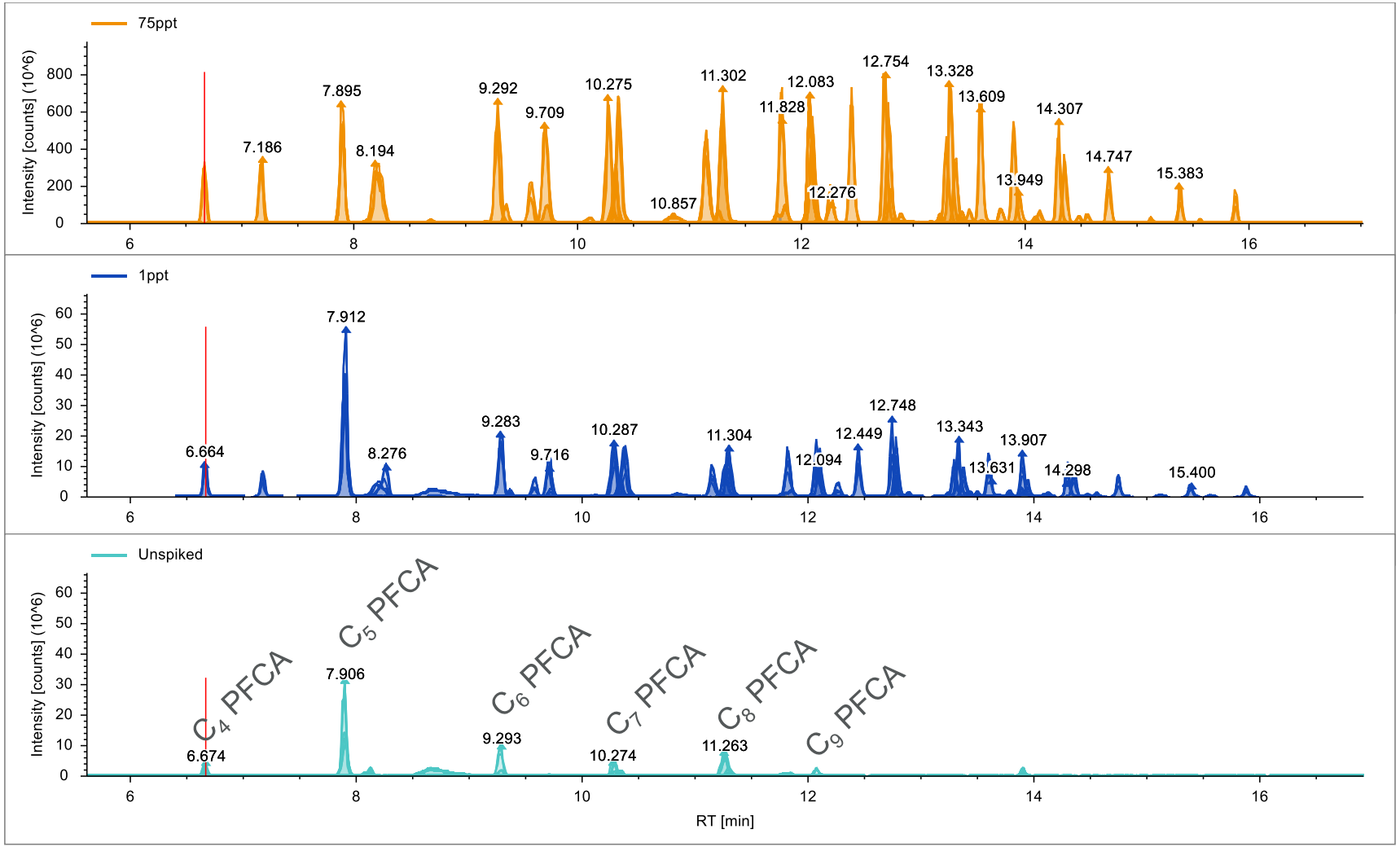
Overlaid Extracted Ion Chromatograms of annotated PFAS

Efficient detection and annotation from unspiked to 75 ppt

Spiked at 75 ppt

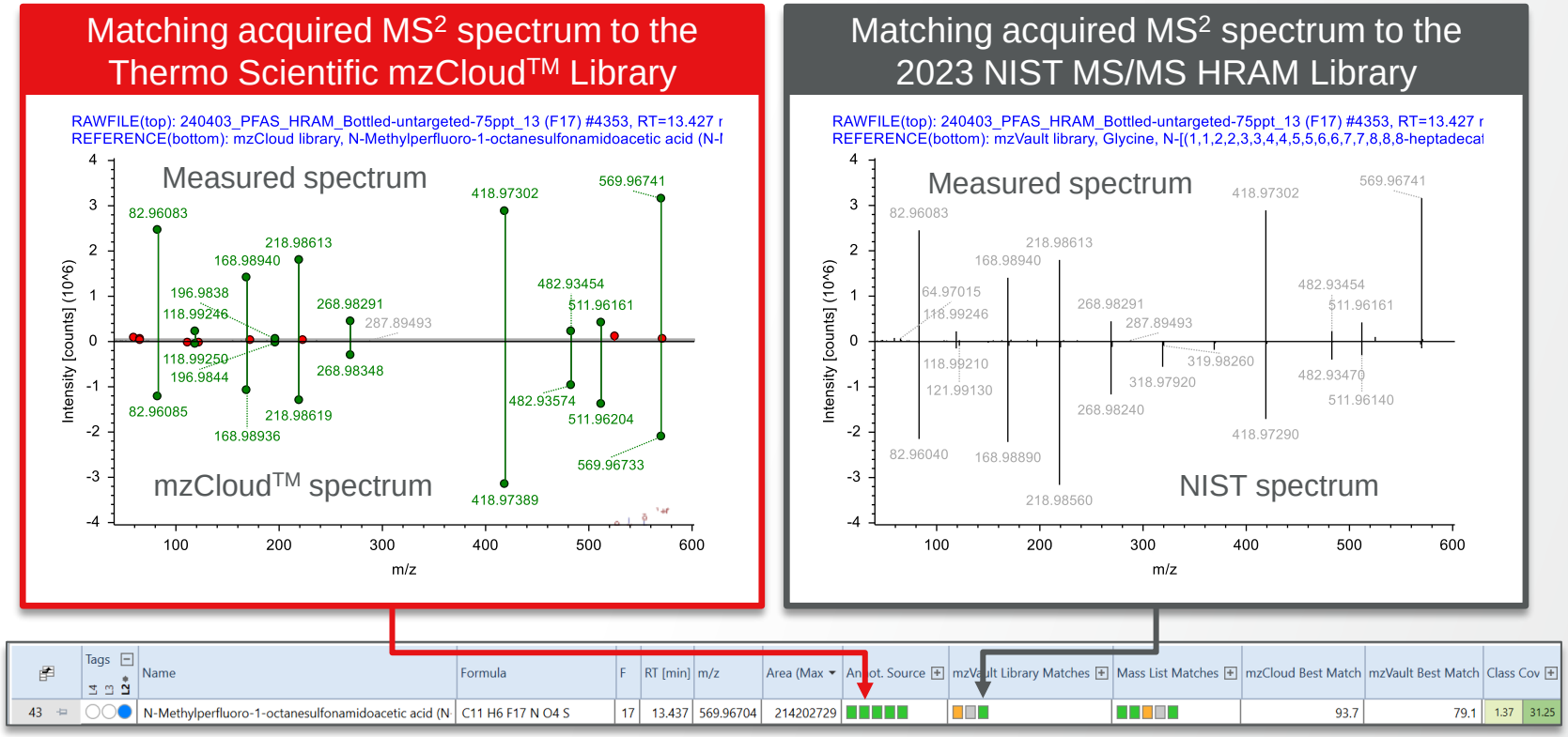
Spiked at 1 ppt

Unspiked



Confidently annotating unknown PFAS compounds

Bringing together multiple resources to annotate at Level 2 confidence

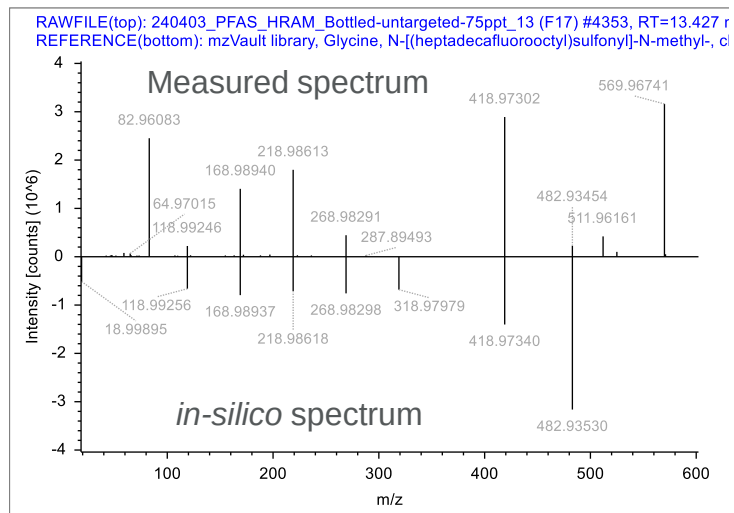


Confidently annotating unknown PFAS compounds

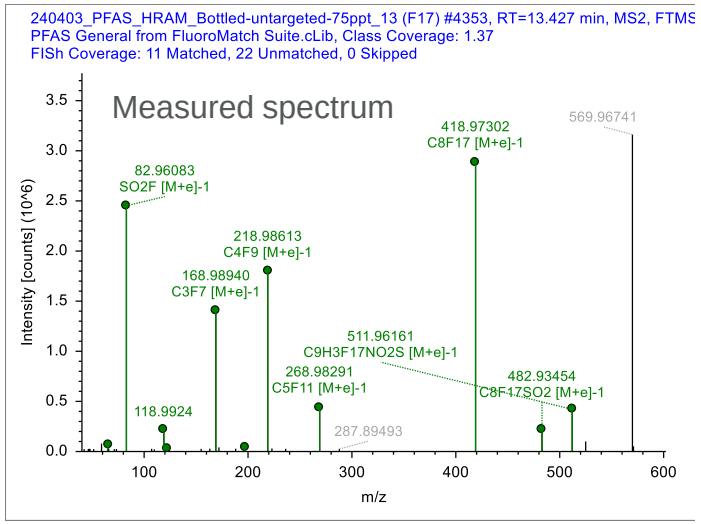
Bringing together multiple resources to annotate at Level 3 confidence

	Tags	Name	Formula	F	RT [min]	m/z	Area (Max	Annot. Source	mzVault Library Matches	Mass List Matches	mzCloud Best Match	mzVault Best Match	Class Cov	
43		N-Methylperfluoro-1-octanesulfonamidoacetic acid (N	C11 H6 F17 N O4 S	17	13.437	569.96704	214202729				93.7	79.1	1.37	31.25

Matching acquired MS^2 spectrum to the Duke University *in-silico* generated PFAS library

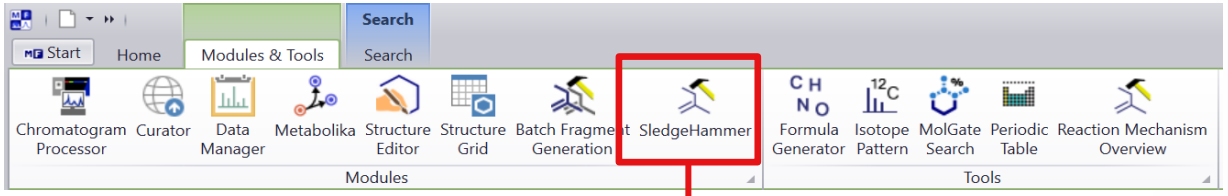


Matching acquired MS^2 spectrum to the FluoroMatch PFAS fragment database

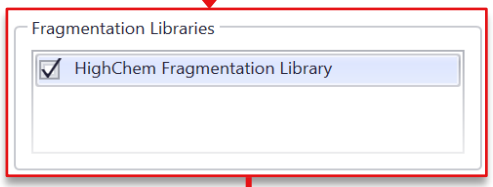


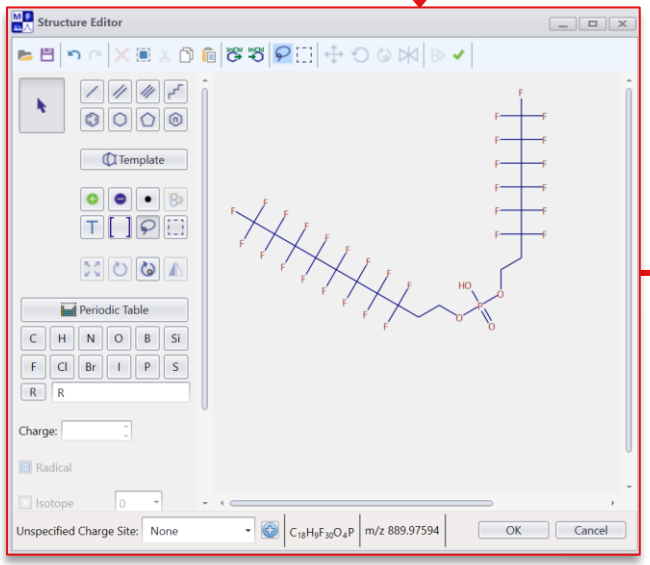
Diving deeper in the interpretation of MS2 spectra

Utilizing the SledgeHammer tool in Mass Frontier to better understand MS^2 fragmentation

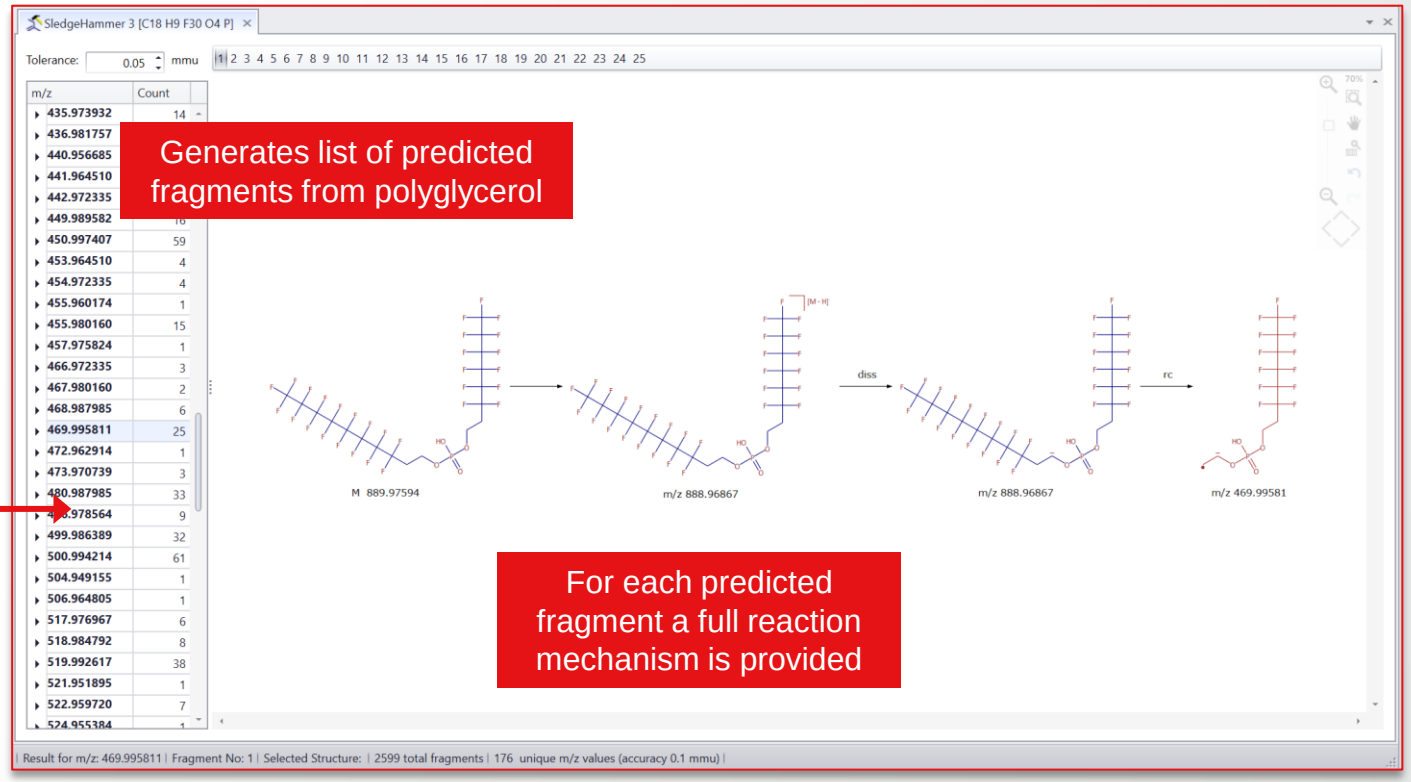


Leverages >54,000 fragmentation schemes that cover mechanisms published in major journals





Generates list of predicted fragments from polyglycerol



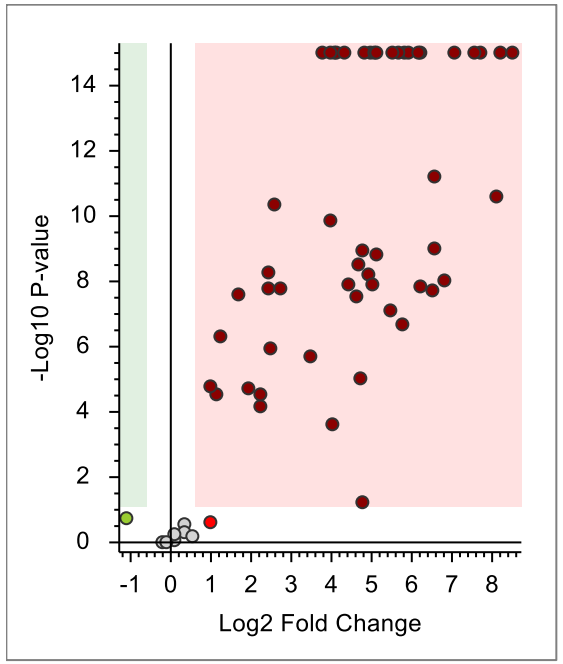
For each predicted fragment a full reaction mechanism is provided

Evaluating overall PFAS composition across samples

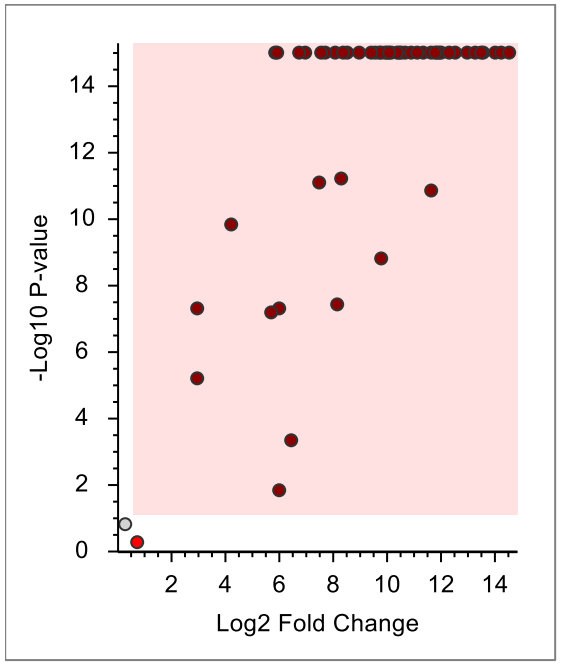
Built-in plotting tools to visually investigate difference in PFAS composition

Differential analysis plot

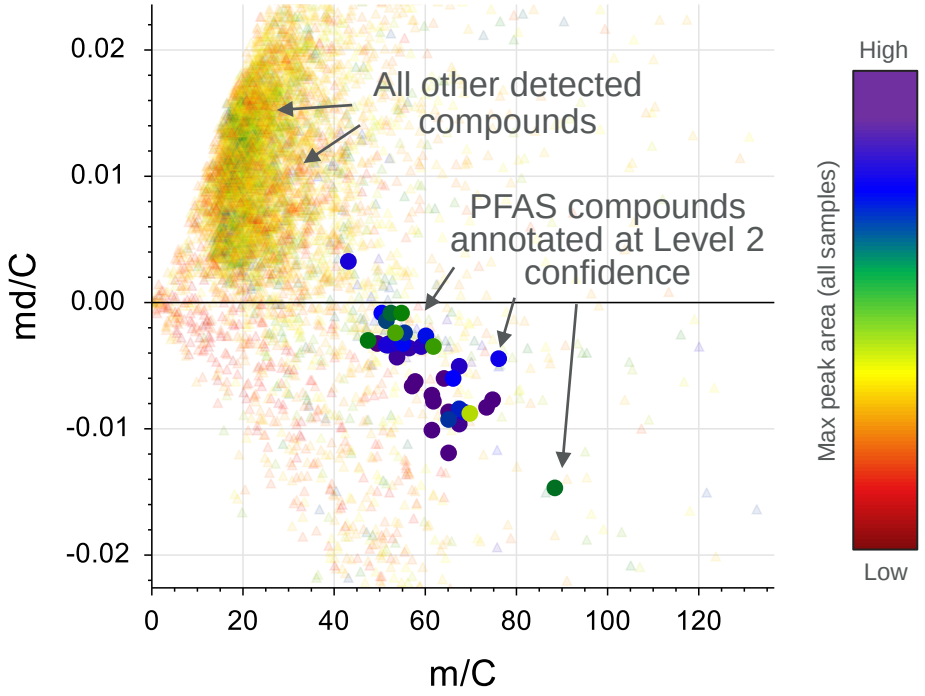
Spiked (1 ppt) vs. Unspiked



Spiked (75 ppt) vs. Unspiked



Orthogonal MS1 plot*



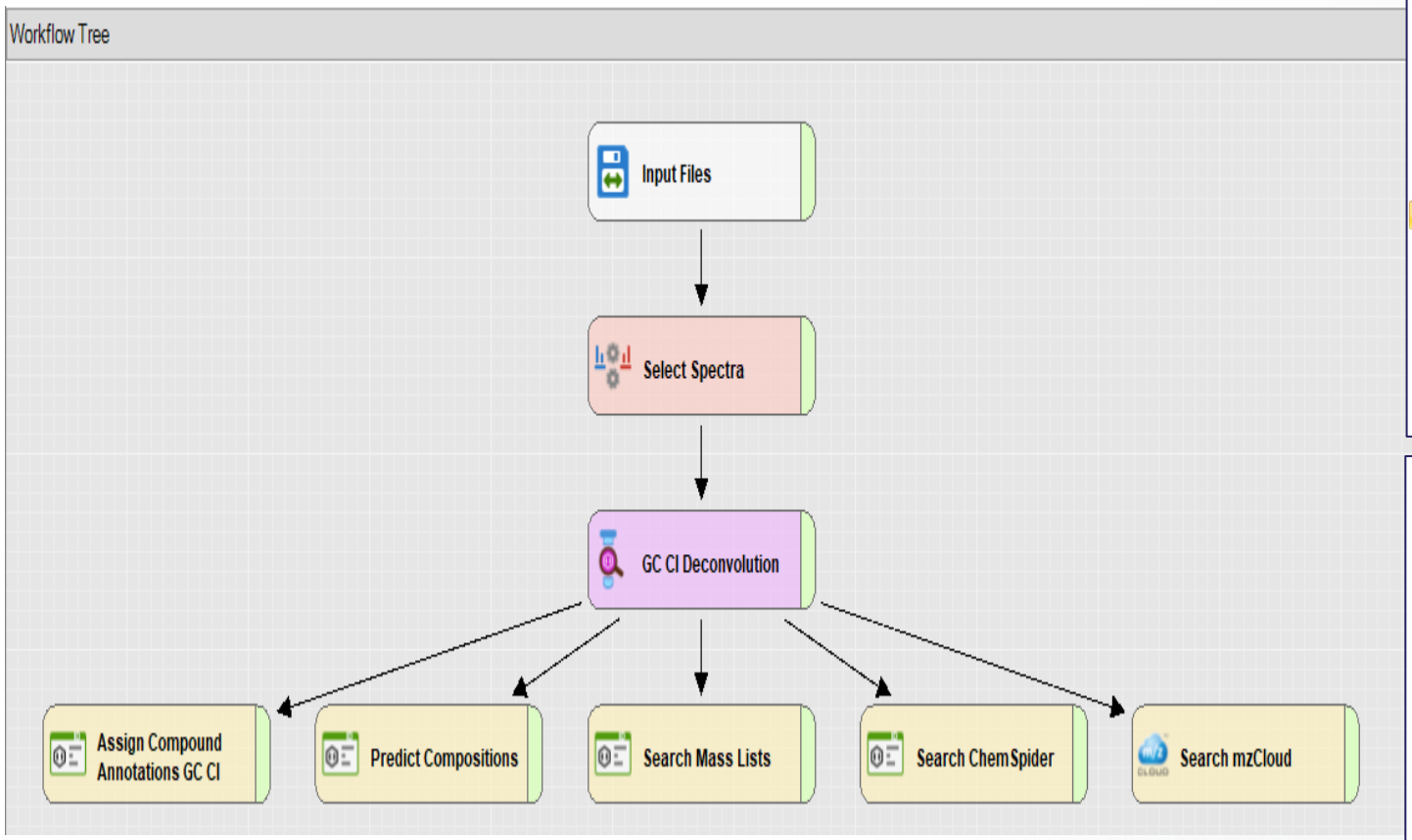
*Orthogonal MS1 plots were developed by Kaufman et al. **2022**, 105 (5), *Journal of AOAC International*

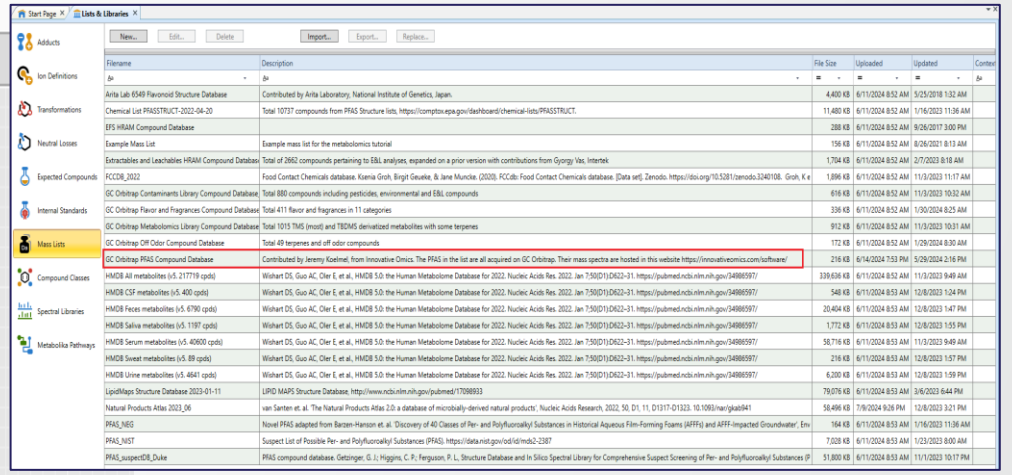
Why PFAS by GCMS ?

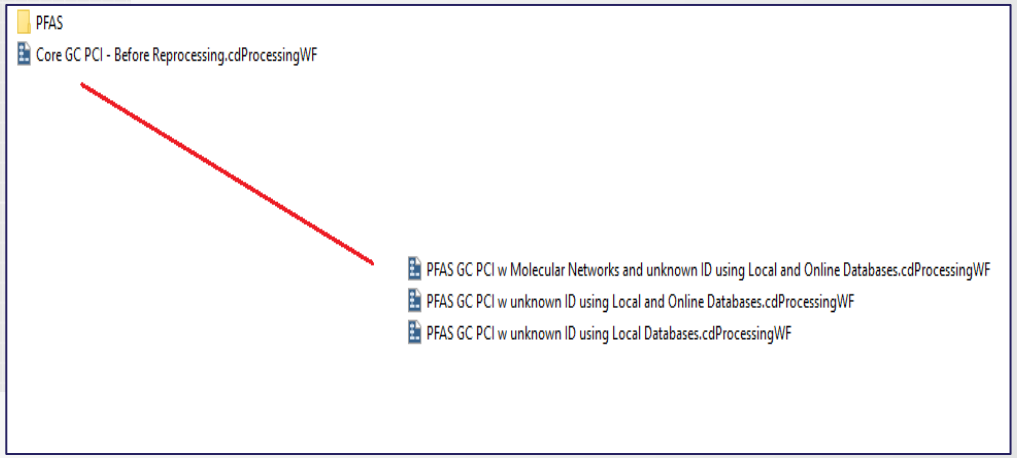
- Added value to LCMS
- Complementary results, alternative for LC difficult compounds
- Cross confirmation of results
- Target quantitation, screening, and non-targeted (unexpected) analyses all available
- High throughput
- System always ready- EI, PCI modes and easy change between them
- Obtain Solvent Quality information in mobile phase or sample prep

Tools added for GC-Orbitrap NTA analysis- CD version 3.4

- Dedicated GC-Orbitrap PFAS workflow template
- Updated PFAS compound database
- Adding more chemicals to GC Orbitrap Contaminants library
- CSC and collaborator labs completing full methodology







Some recent publications PFAS using GC-HRMS

Journal of Chromatography A 1693 (2023) 463884

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

The use of gas chromatography – high resolution mass spectrometry for suspect screening and non-targeted analysis of per- and polyfluoroalkyl substances

Jonathan S Casey[a], Stephen R Jackson[b], Jeff Ryan[b], Seth R Newton[b,*]

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Environmental Science & Technology

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Article

Characterization of Per- and Polyfluorinated Alkyl Substances Present in Commercial Anti-fog Products and Their *In Vitro* Adipogenic Activity

Nicholas J. Herkert, Christopher D. Kassotis, Sharon Zhang, Yuling Han, Vivek Francis Pulikkal, Mei Sun, P. Lee Ferguson, and Heather M. Stapleton*

Cite This: *Environ. Sci. Technol.* 2022, 56, 1162–1173

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Conclusions

- Automated dispersive liquid-liquid microextraction (DLLME) is a promising technique to extract and concentrate PFAS from drinking water samples. The resulting extract from a single sample preparation can then be measured by both quantitative targeted and non-targeted analysis in separate runs, as well as further exploration by GC-Orbitrap analysis.
- Quantitative targeted analysis showed good accuracies at low (1 ng/L) and high (75 ng/L) spiking level, as well as reproducibility (<30%, n=7) over several days. The use of internal standards for selected PFAS classes can additionally improve obtained results in terms of accuracy.
- The non-targeted analysis workflow in Compound Discoverer 3.3 SP3 provides a comprehensive package enabling confident annotation of unknown PFAS compounds through the use of multiple spectral libraries, fragmentation databases and as well as understanding differences in PFAS composition across samples.
- CD version 3.4 will greatly expand the workflow capabilities for NTA using GC-Orbitrap

richard.cochran@thermofisher.com

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  - Aristide Ganci
  - Bénédicte Gauriat
  - Jean-François Garnier