High-Resolution Data-Dependent Mass Spectrometry Guided by Real-Time Library Search for the Detection and Characterization of PFAS

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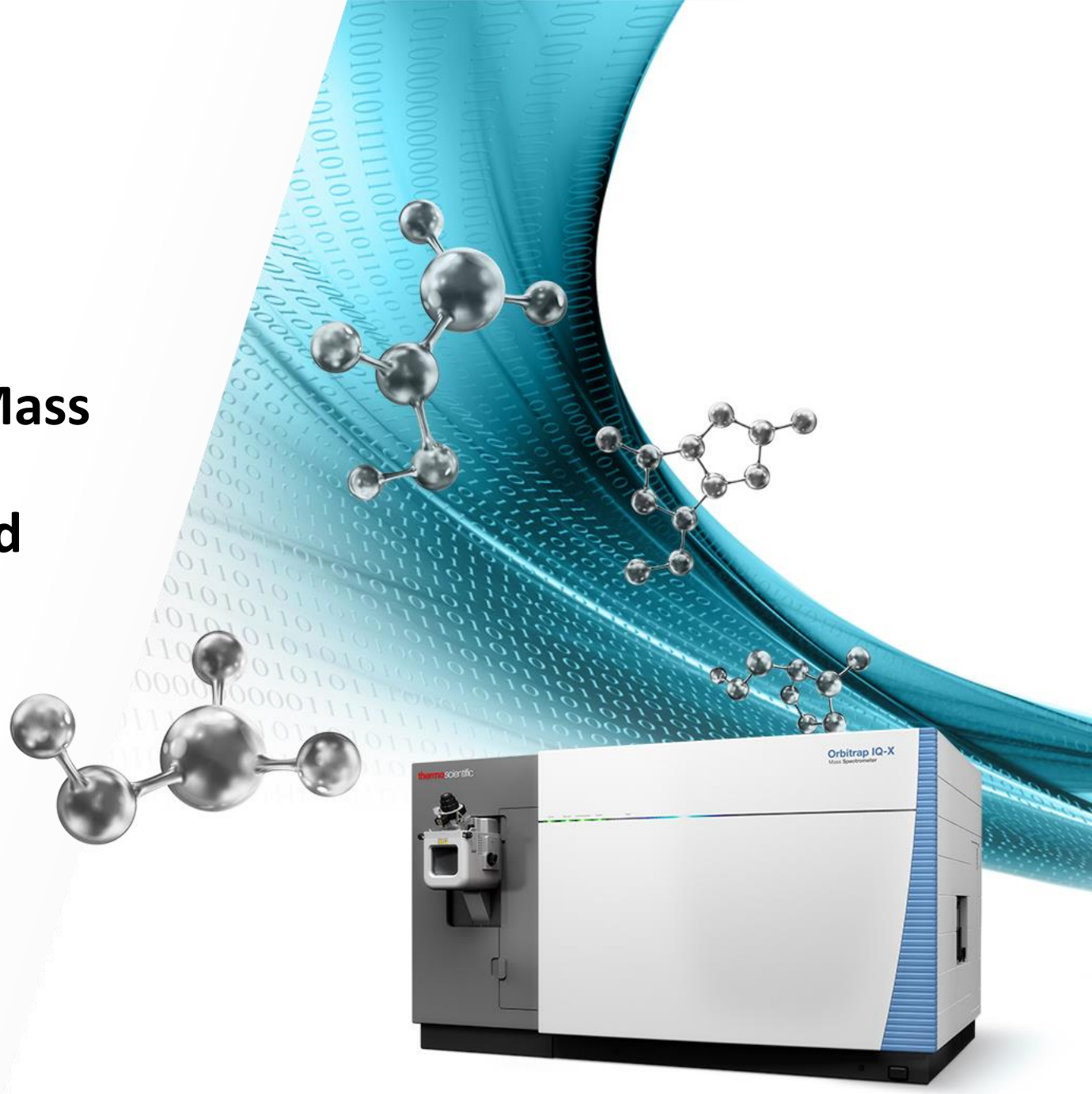
Product Manager

Thu 7
8/3/2023



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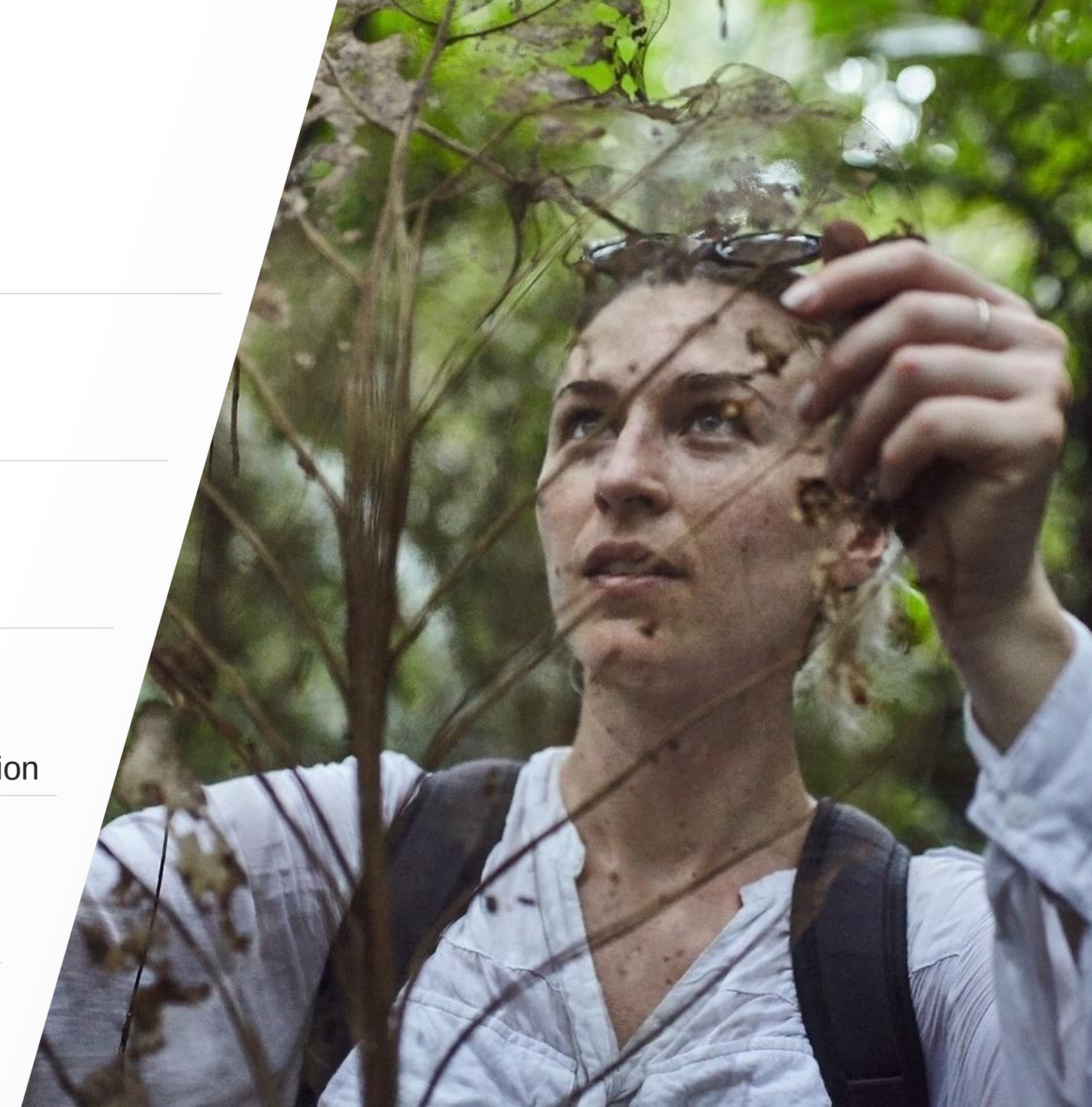
Conflict of interest disclosure

The presenter and fellow authors are employees of Thermo Fisher Scientific whose instrumentation and software were used to acquire and process the data.

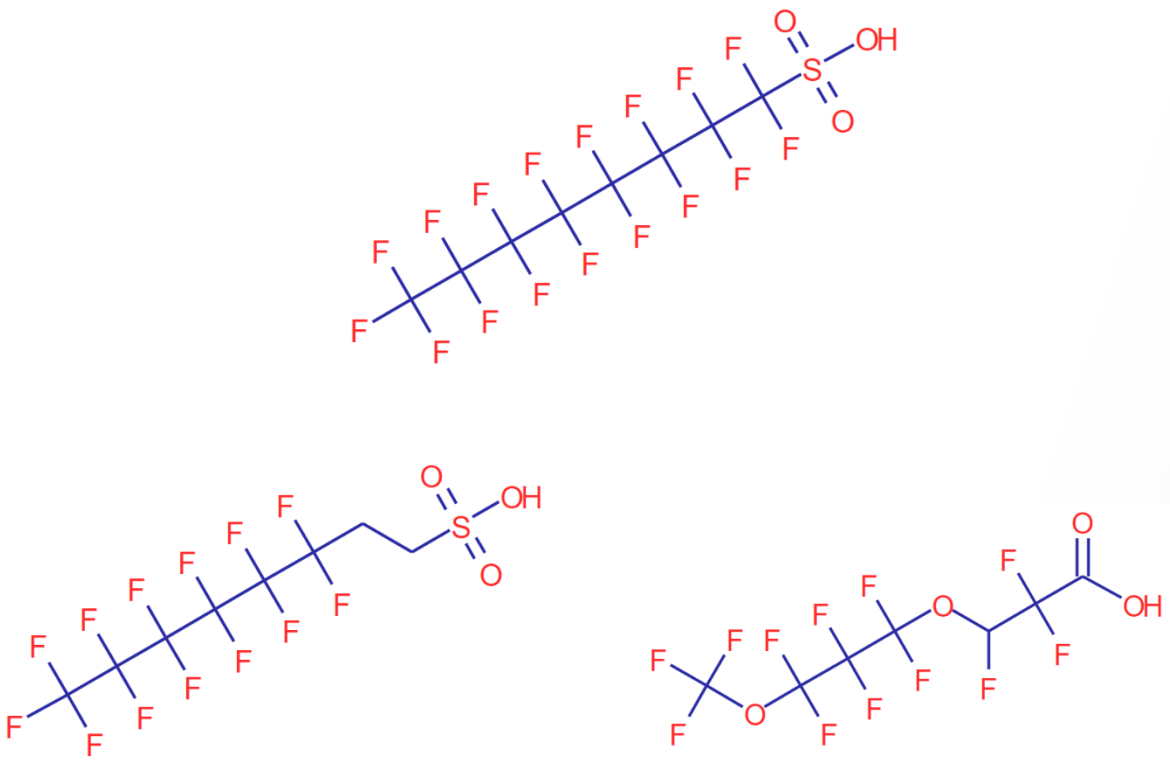
Outline

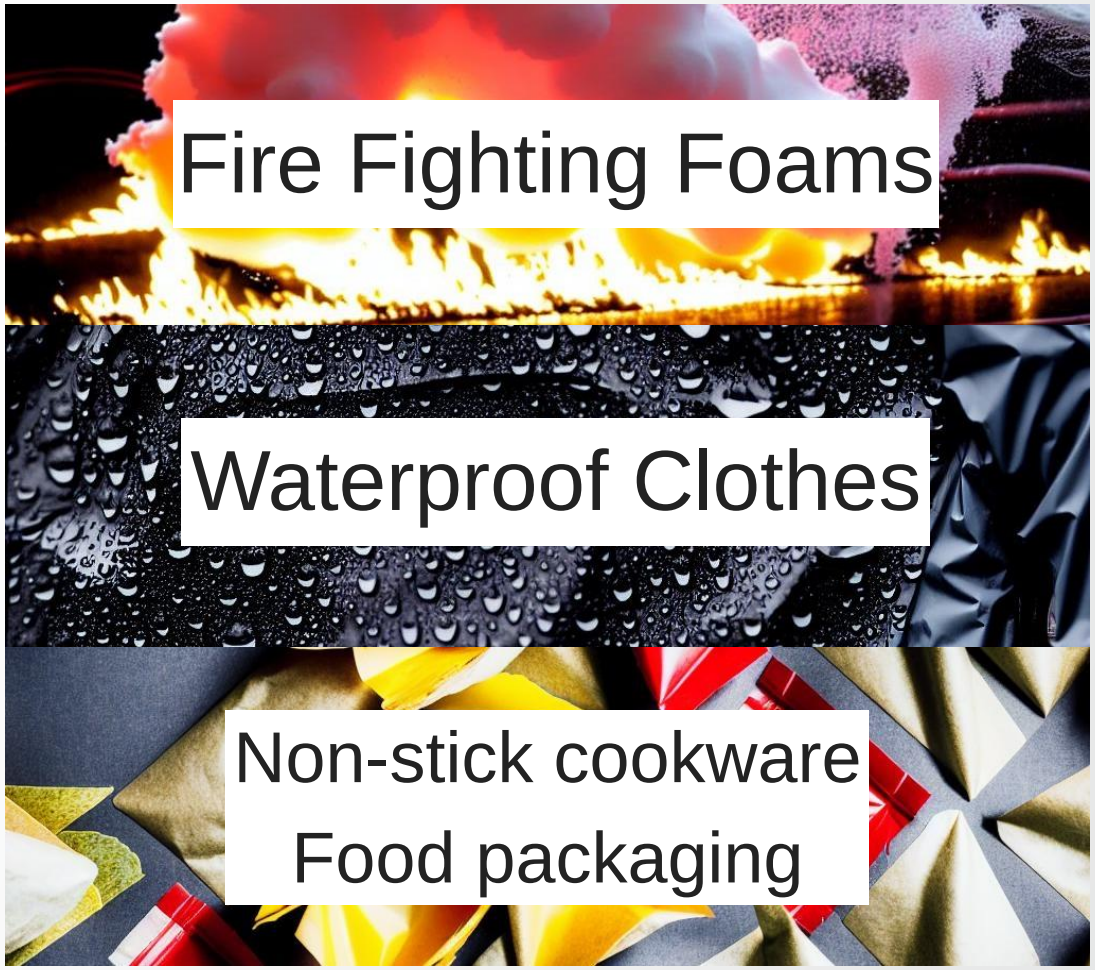
1. PFAS and why are they important
2. Analytical challenges for unknown PFAS
3. Mass filtering to focus analysis
4. Real-Time Library Search for additional information
5. Data processing



PFAS

Per- and PolyFluoroAlkyl Substances contain fully fluorinated methyl or methylene carbon atoms





Problem overview

- **Ubiquitous** – PFAS have been found in water, soil, air, food, and biological samples

- **Long Lasting** – PFAS can take years to degrade in the body

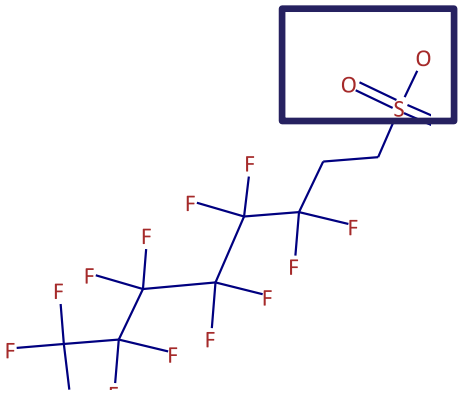
- [1]**Health Impacts** – PFAS has been found to have links to:
  - Endocrine disruptions of the thyroid
  - Reduced immune system functionality
  - Low birth weights
  - Liver Toxicity

- [2]**Emerging Regulations** – March 2023 the EPA proposed a new regulation on six PFAS in drinking water

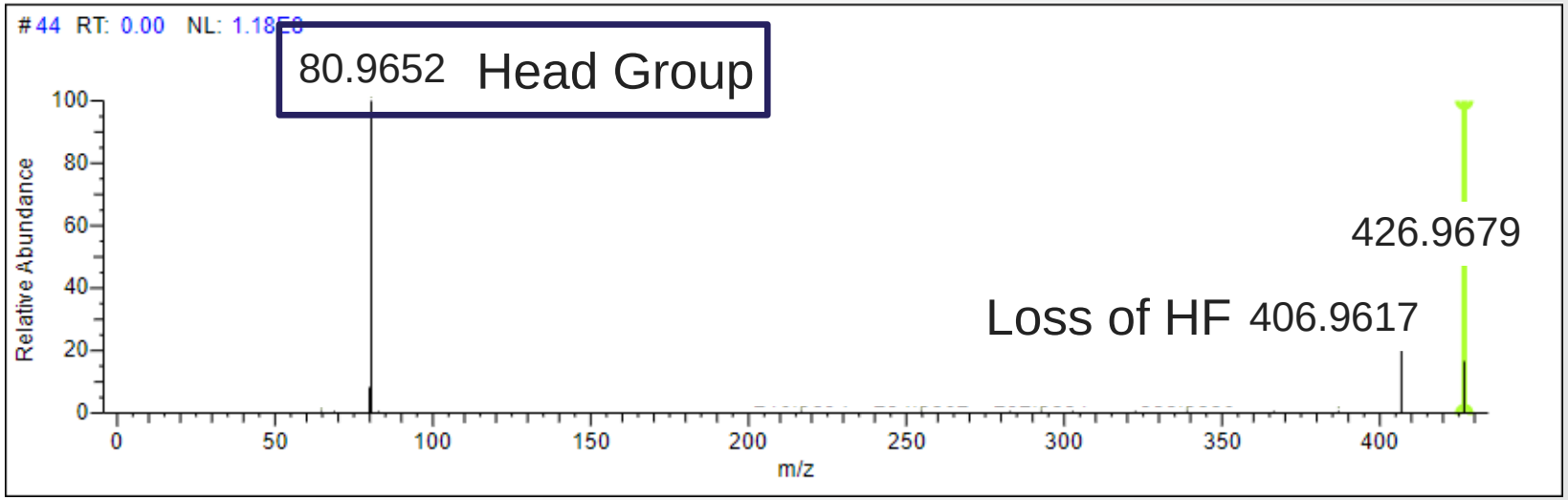
1. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Perfluoroalkyls. May **2021**
2. EPA. Per- and Polyfluoroalkyl Substances (PFAS) Proposed PFAS National Primary Drinking Water Regulation. March **2023**

Analytical Challenges

- Often present at lower concentrations in complex matrices
- Over 12,000 PFAS compounds known
- Minimal commercial standards available
- Collision based MS^2 doesn't always give enough information to piece together the structure

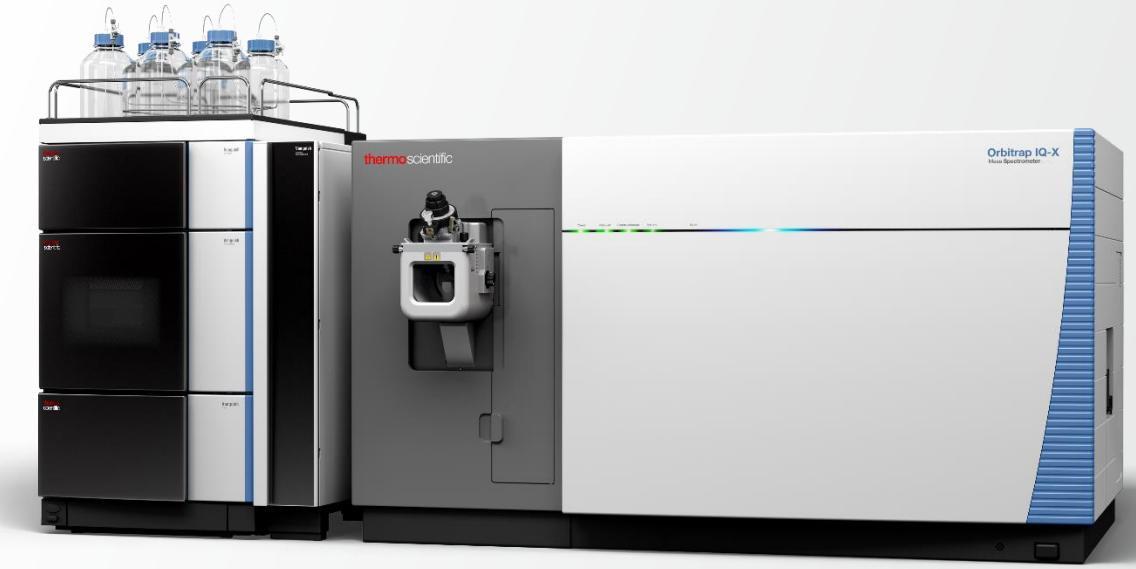


6:2 Fluorinated telomer sulfonate

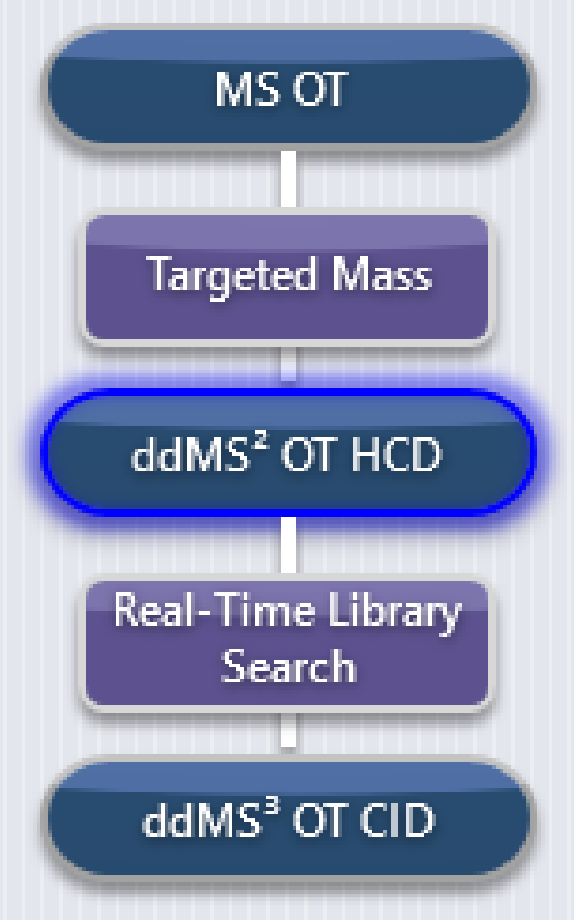


Thermo Scientific™ Orbitrap™ IQ-X™ Tribrid™ mass spectrometer

- Orbitrap resolution up to 500K standard or 1M with upgrade
- Ion Trap for on-the-fly CE optimization as well as CID and MS^n fragmentation
- Highly customizable method editor to tailor the method to the analytes of interest
- Real-Time Library Search to selectively target compounds in real time using a spectral library



Customizable methods focuses analysis on PFAS



HRAM data helps differentiate isobars

A mass filter to prioritize compounds with a negative mass defect

Assisted CE optimizes settings on the fly

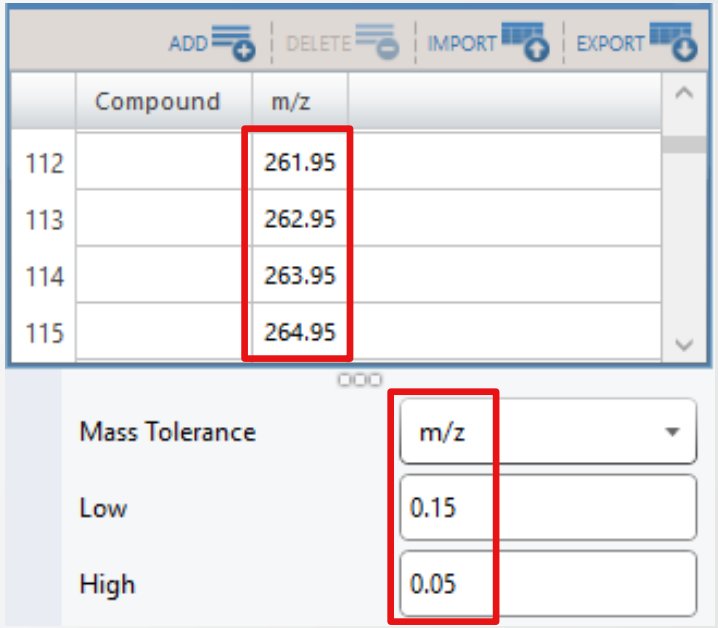
RTLS checks against a library in real time then triggers scans on likely PFAS

ddMS^3 and CID fragmentation gets additional structural information on unknown PFAS compounds

A mass filter to prioritize a negative mass defect

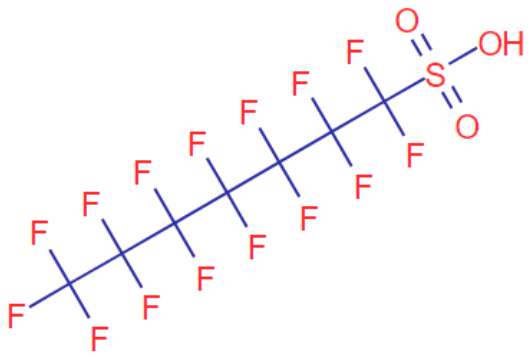
- Highly fluorinated compounds tend to have m/z below the integer values
- An inclusion list focuses ddMS^2 on those mass ranges during the analysis

PFAS compound	m/z
Perfluorohexanoic acid	312.9728
Perfluoro-1-butanesulfonamide	297.9590
HFPO-DA	328.9677
6:2 Fluorinated telomer sulfonate	426.9679
N-MePFOSAA	569.9673

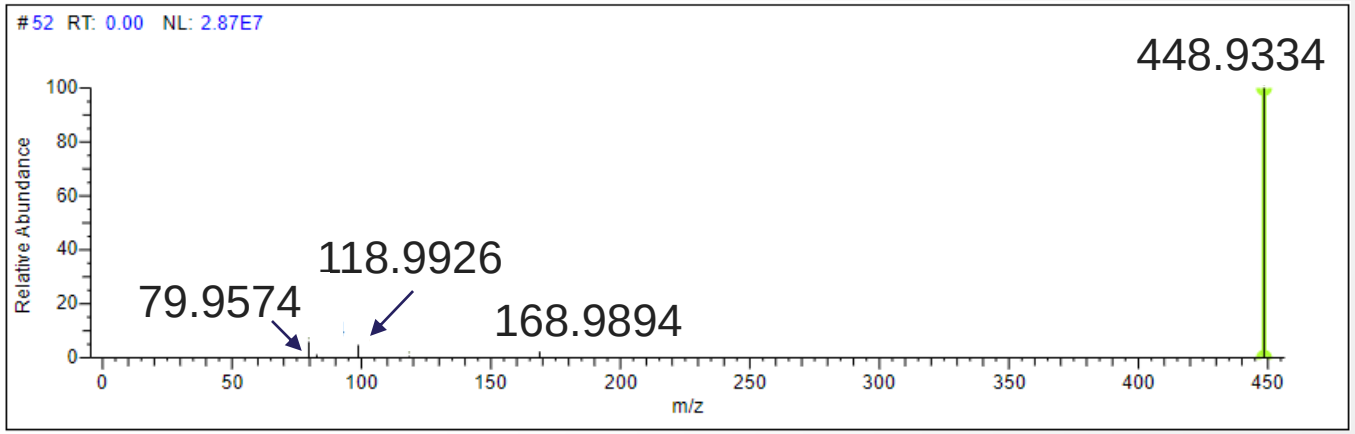


Assisted CE

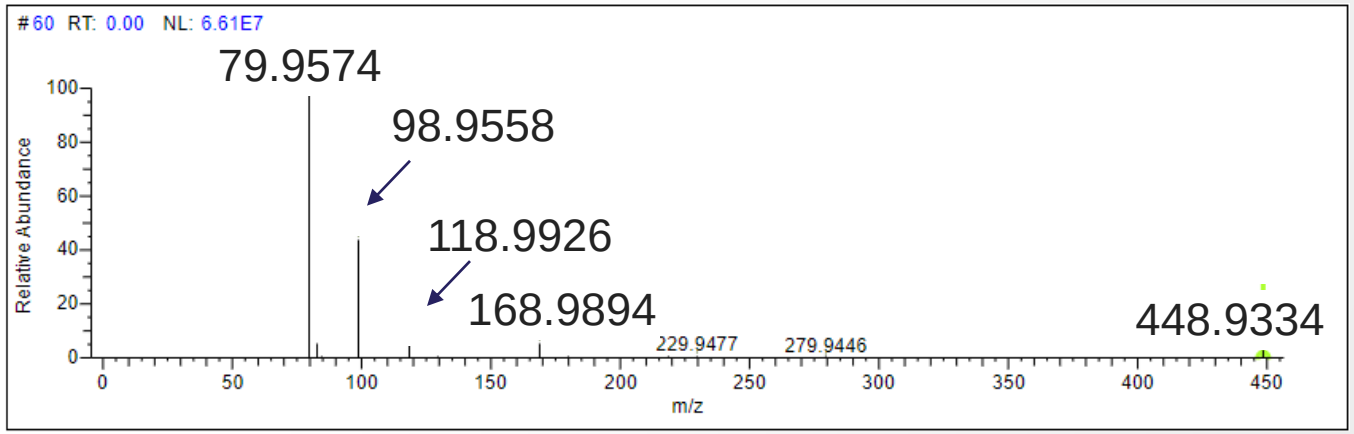
- The ion trap can check up to 5 collision energies in parallel with Orbitrap acquisition
- The CE that fragments at least 80% of the precursor is used for the ddMS^2 acquisition



Perfluoro-1-heptanesulfonate



Collision Energy 30

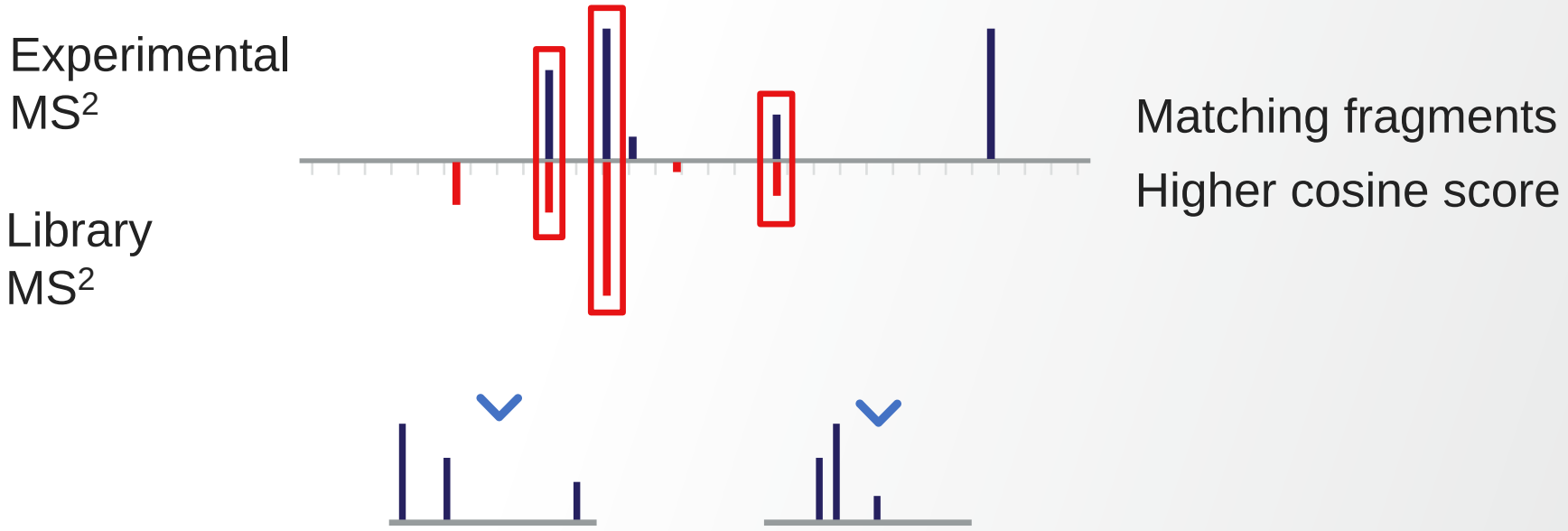


Collision Energy 50

Real-Time Library Search

- Real-Time Library Search

  - ddMS^2 data is compared to a library in real time to find similar spectral features and generate a cosine score



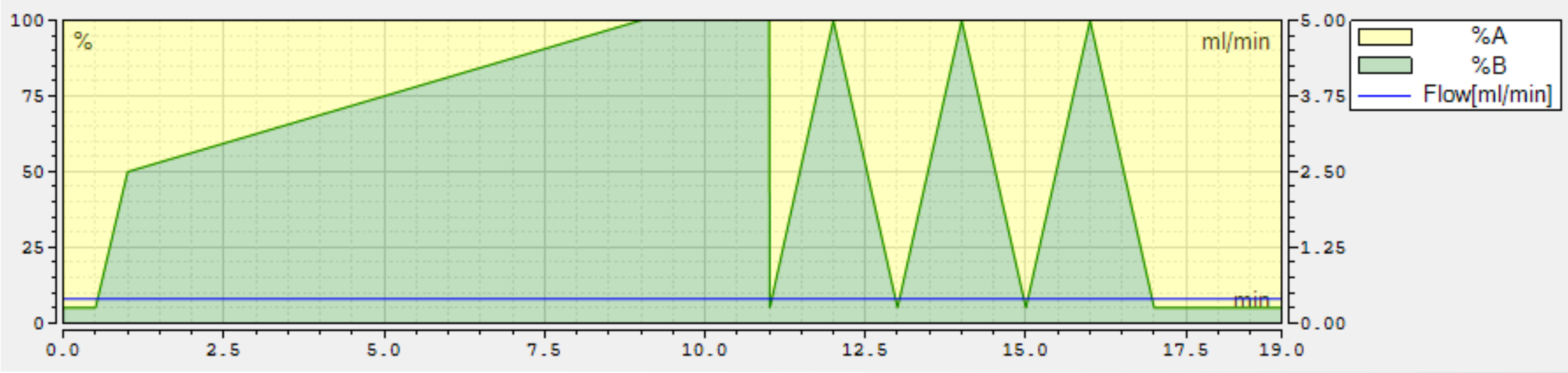
  - Likely PFAS trigger additional experiments like ddMS^3 or CID

Bills *et al. Anal. Chem.* **2022,** 94, 9, 3749–3755

Experimental details

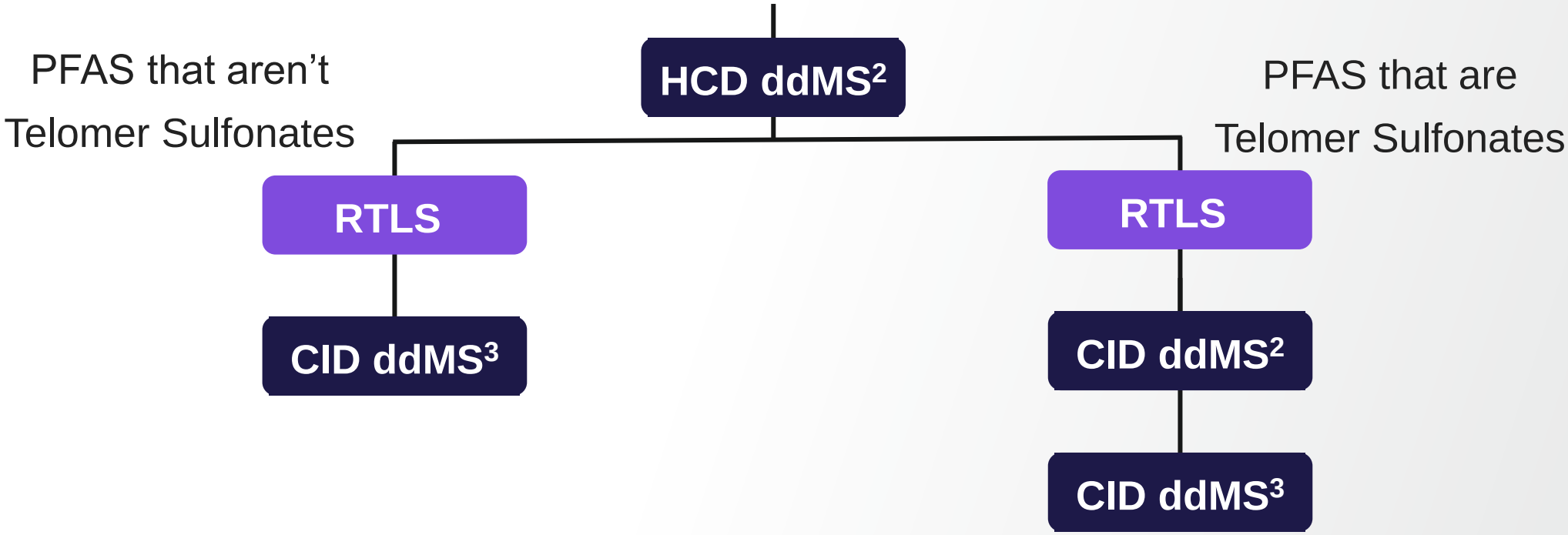
- A mixture of 30 PFAS compounds (PFAC30PAR from Wellington Laboratories) were run both diluted in solvent at 10 ppb and spiked into plasma extract at 1 ppb

Material	Details
LC	Thermo Scientific™ Vanquish™ Flex UHPLC system with PFAS HPLC Kit
Column	Thermo Scientific™ Accucore™ C18 HPLC Column
Solvent A	100% water + 10 mM ammonium acetate
Solvent B	75:20:5 MeOH : ACN : Water + 10mM Ammonium acetate
Sample Vials	Polypropylene Vials and caps



RTLS Method

- A library was curated in mzVault without 7 of the compounds
- A method was created using a mass defect filter and Real-Time Library Search in similarity mode

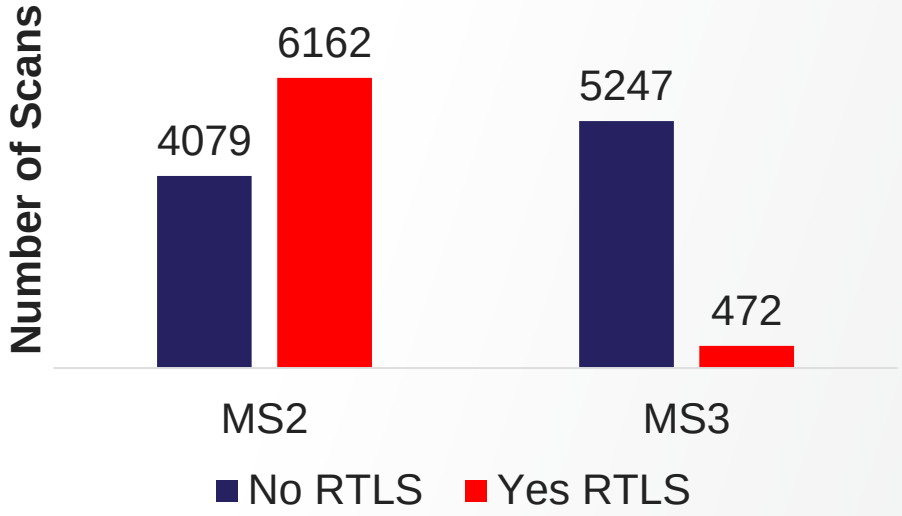


More relevant scans with RTLS

- Without guidance, ddMS^3 is collected on every compound regardless of relevance
- By only triggering compounds on likely PFAS, ddMS^3 is limited to relevant compounds
- For example, look at how the instrument spends its time when analyzing a single injection of plasma spiked with a mixture of 30 PFAS compounds

Because time isn't wasted on irrelevant scans

MS^2 is collected on more compounds



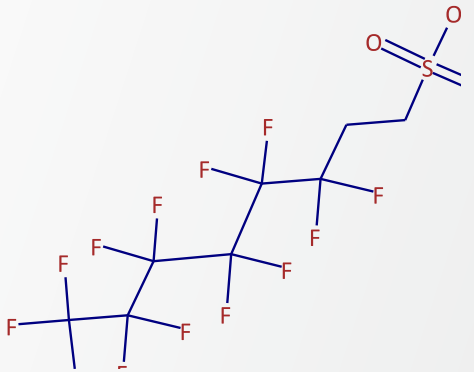
Instead of thousands of MS^3 collected on non-PFAS

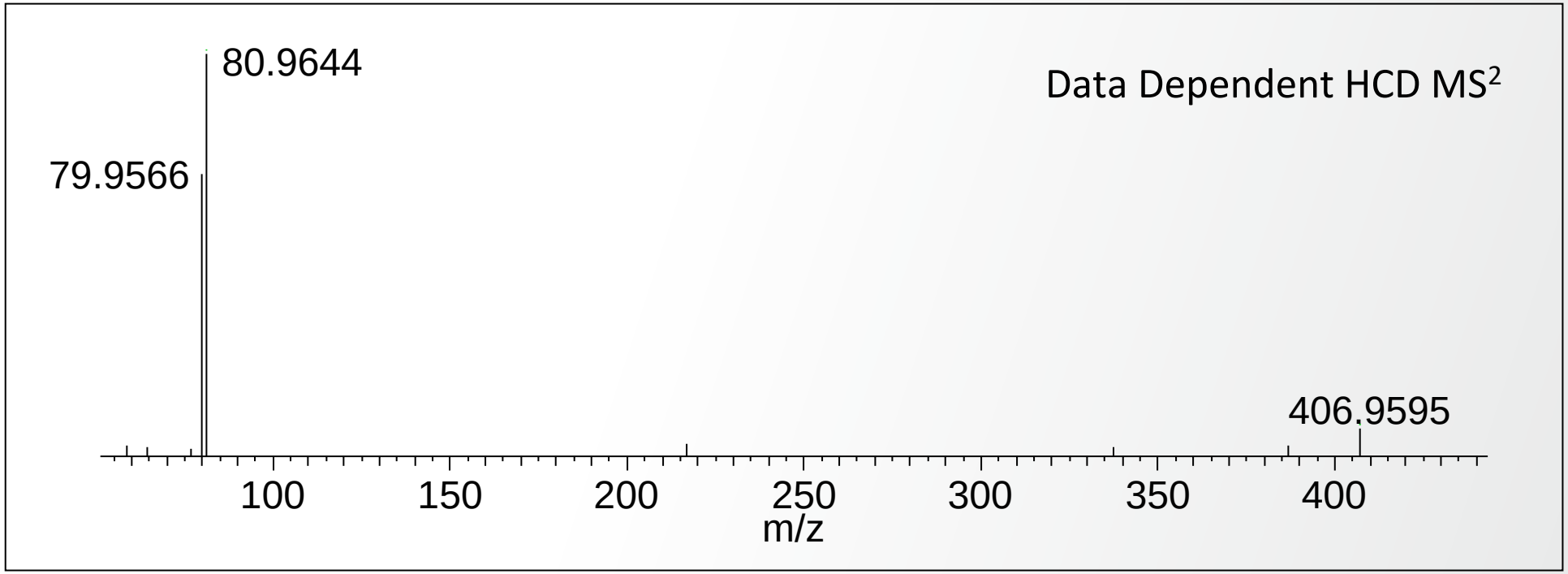
there is an easier to review data set with MS^3 collected only on likely PFAS

Data collected on Standards

- All 30 standards in neat solvent were detectable and triggered ddMS^2 and 29 triggered ddMS^3 data

- CID MS^2/MS^3 was collected on 6:2 Fluorinated telomer sulfonate despite not being in the library





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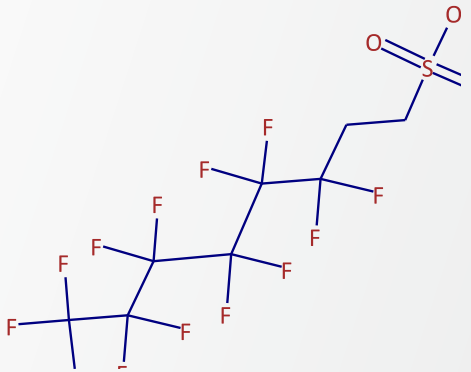

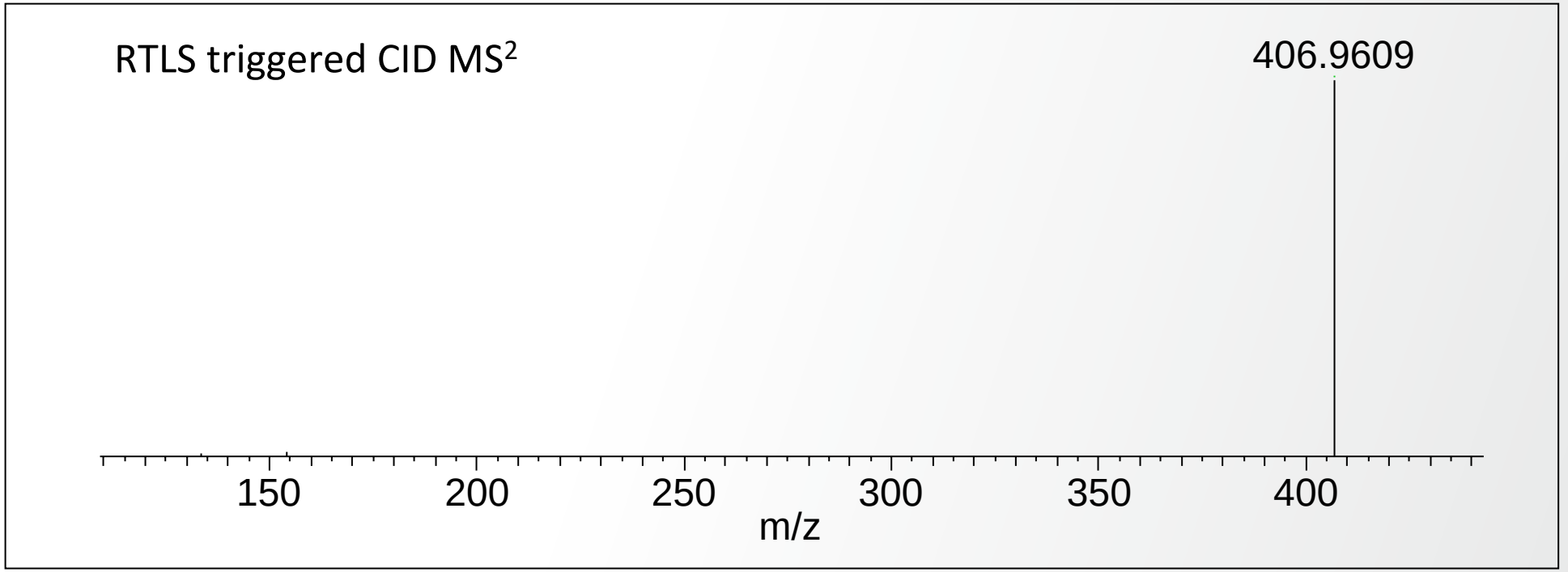

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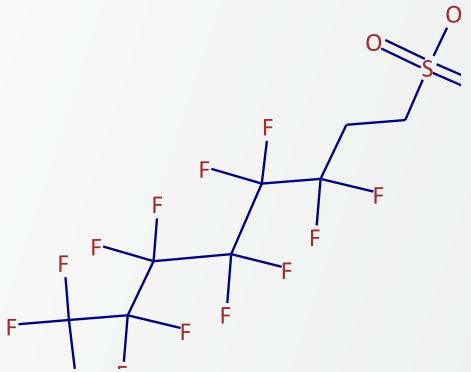
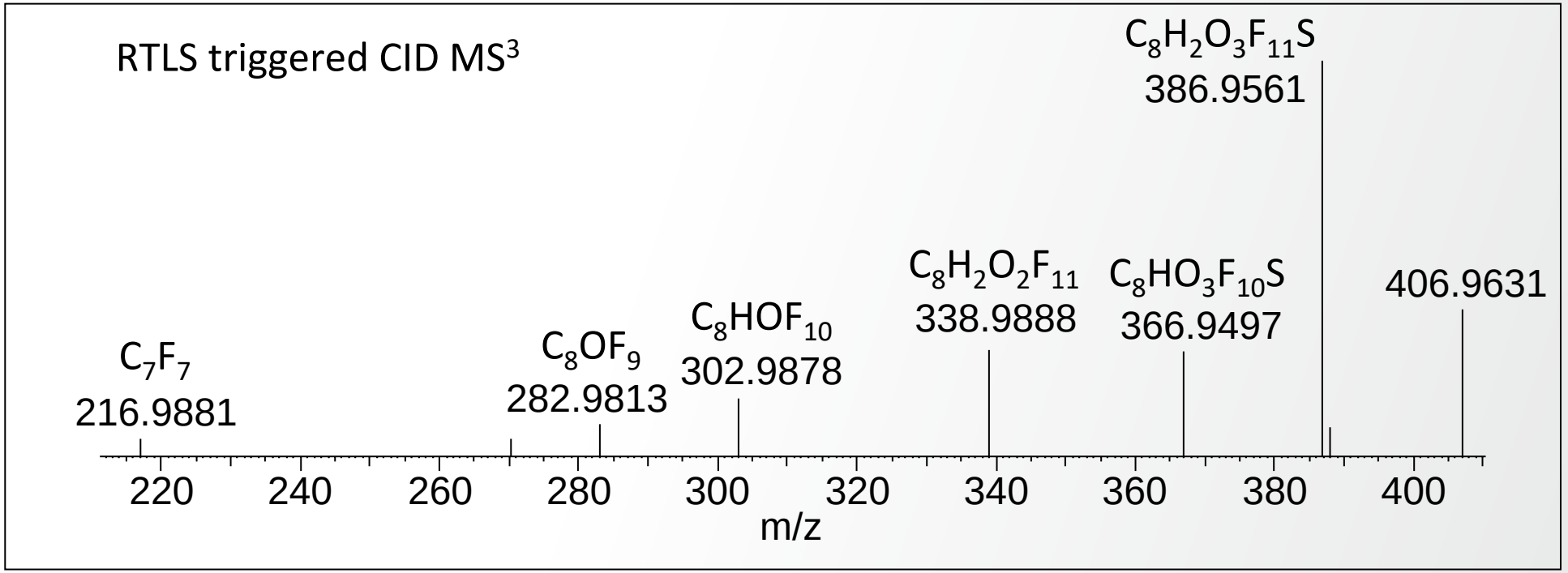
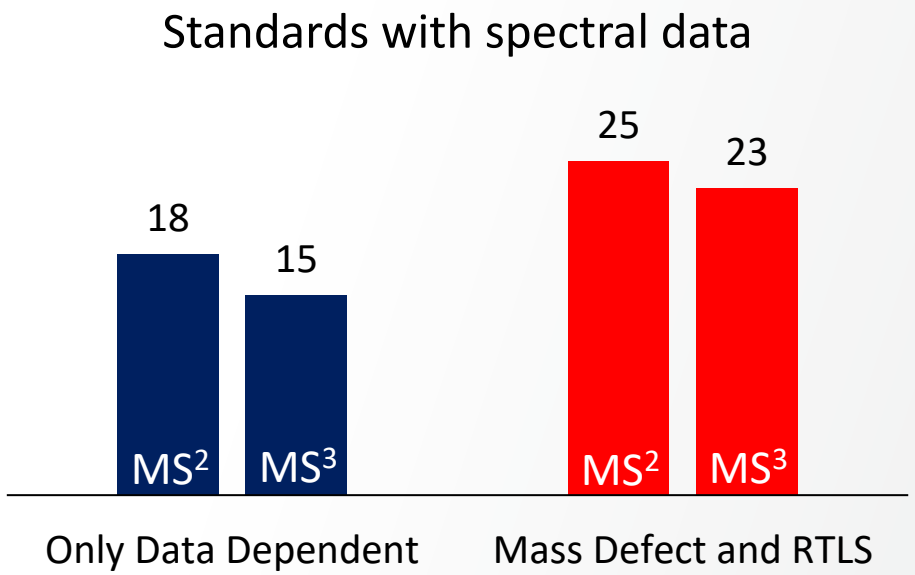


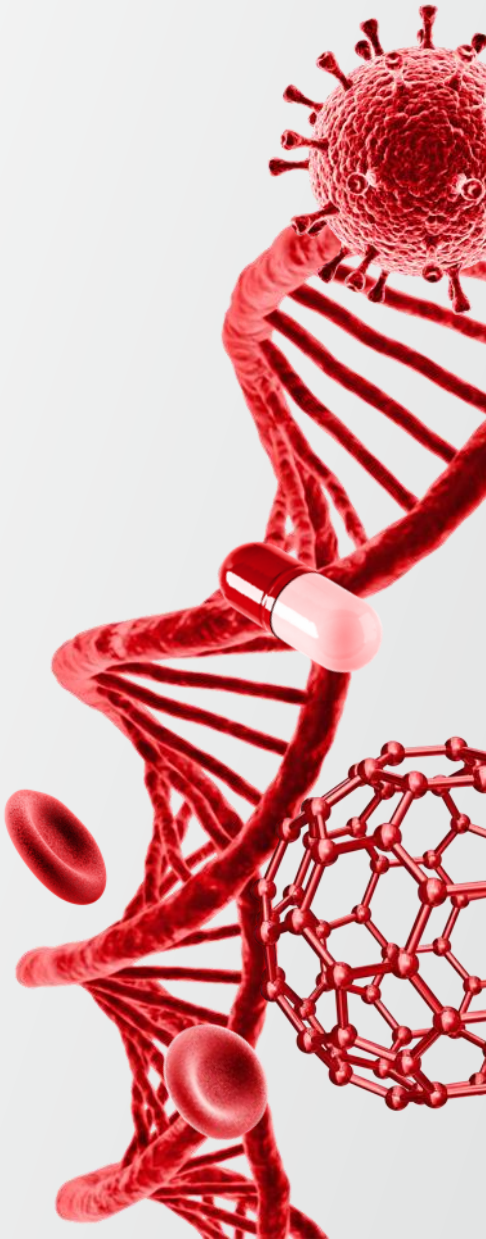


Advantages of Mass Defect Filter and RTLS

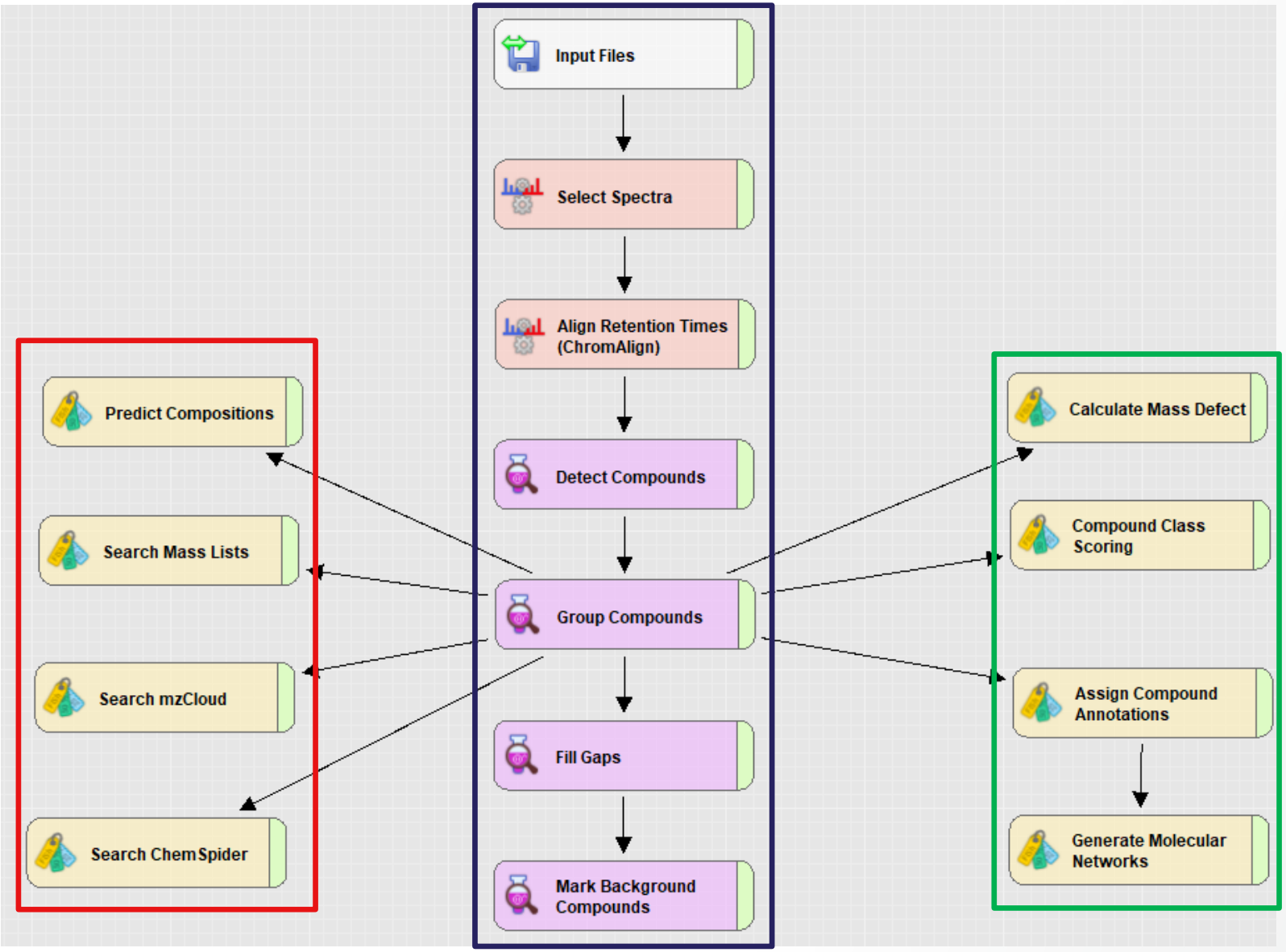
- In the plasma sample, unguided ddMS^3 often targets higher mass matrix compounds
- With a filter for mass defect, ddMS^2 is limited to compounds that are potential PFAS
- RTLS limited ddMS^3 data collection to compounds similar to PFAS in the given library



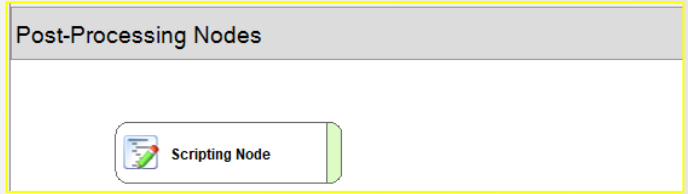
Data Processing in Compound Discoverer



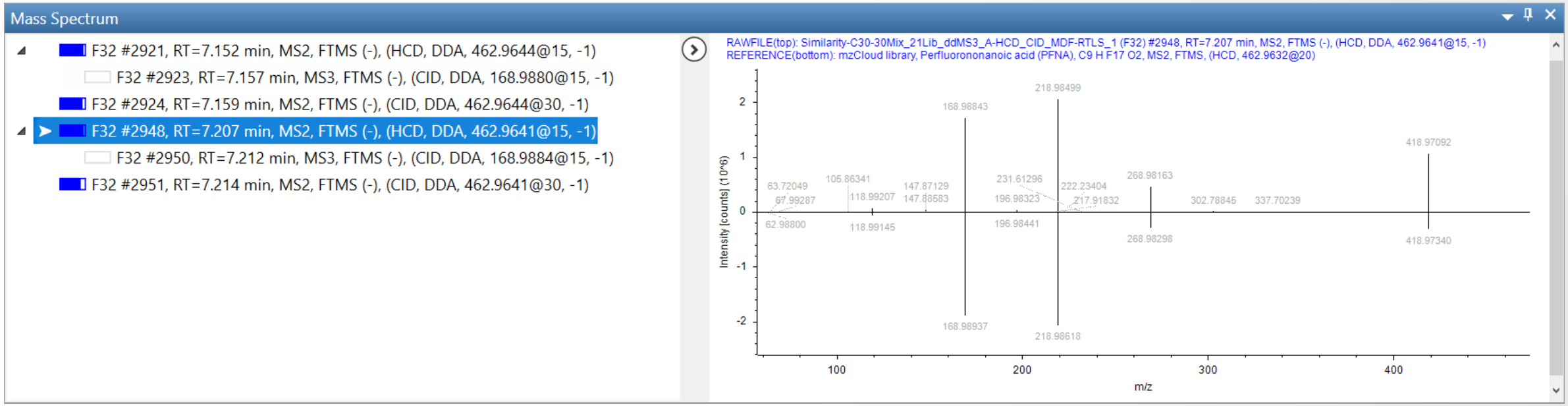
Thermo Scientific™ Compound Discoverer™



- Takes in files, detects compounds, aligns between files, marks background
- Predicts the composition and searches for annotations from multiple sources
- Calculates mass defect, scores their similarity to PFAS, and generates molecular networks



Compound Discoverer results



- Annotations from mzCloud can be directly compared against experimental results

	Name	Formula	Annot. Source	Annot. ΔMass [ppm]	Calc. MW	m/z	RT [min]	Area (Max.)	mzCloud Best Match	Mass Defect			Class Coverage		MS Depth
1	Perfluorobutanoic acid	C4 H F7 O2		0.42	213.98657	212.97928	3.652	34884364	86.3	-0.013	-63	0	0.25	6.25	3
2	Perfluoropentanoic acid	C5 H F9 O2		0.54	263.98343	262.97613	4.234	46425737	87.9	-0.017	-63	0	0.25	12.50	3
3	Perfluoro-1-butanesulfonamide	C4 H2 F9 N O2 S		-0.02	298.96625	297.95897	5.474	131675699	90.8	-0.034	-113	-0.015	0.25	12.50	3
4	Perfluoro-1-butanesulfonic acid (PFBS)	C4 H F9 O3 S		-0.20	299.95021	298.94293	4.446	123178856	88.3	-0.05	-166	-0.031	0.62	25.00	3
5	Perfluorohexanoic acid	C6 H F11 O2		1.19	313.98046	312.97317	4.896	51681327	88.9	-0.02	-62	0.001	0.25	0.00	3

- Results are displayed in a table including annotation, formula, mass defect, and PFAS class coverage

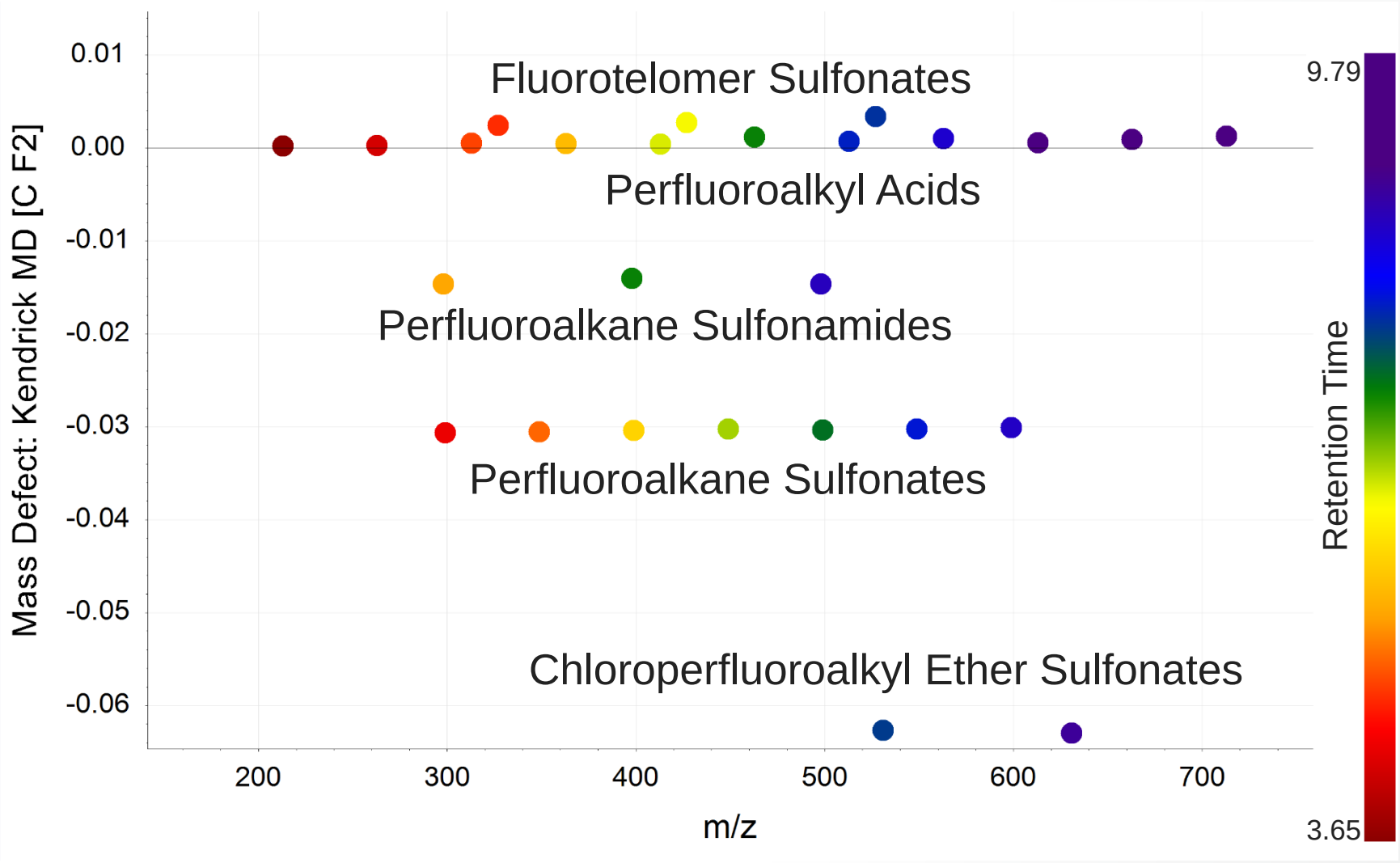
Kendrick Mass Defect

- Kendrick mass normalizes the mass based on mass of a repeating subunit (CF_2 in this case)
- Plotting compounds by Kendrick mass defect and molecular weight shows homologous series

$$Kendrick\ Mass = Observed\ Mass \times \frac{50.0000}{49.9968}$$

$$Kendrick\ Mass\ Defect = Nominal\ Mass - Kendrick\ Mass$$

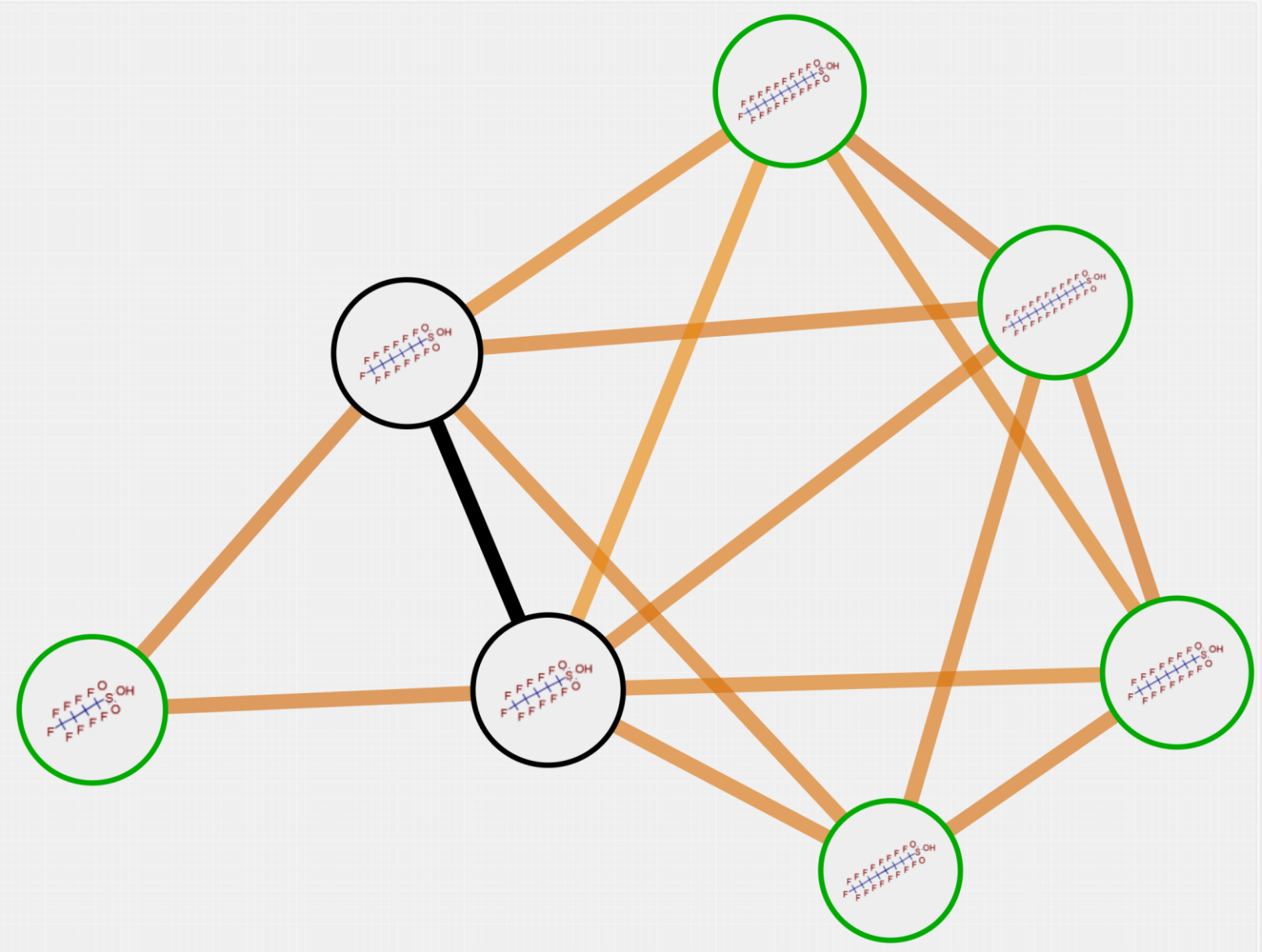
Homologous Series



- 5 homologous series PFAS were graphed
- Compounds in a series have the same Kendrick mass defect and are spaced in increments of 50 amu
- The RT is color coded as a third dimension to provide a simple verification of increasing RT based on PFAS chain length.

Molecular networks for homologous series

- Compounds are linked based on their relations to other compounds
- Clicking on a link displays information about both compounds and how they are related



PFAS Chain Shortening

Formula	C-1 F-2
MW	-49.99696 Da
ΔMass	-0.00016 Da
Score	81 %
Coverage	77 / 85 %
Matches	36 / 23

Perfluoro-1-hexanesulfonic acid (PFHxS)

Formula	C6 H F13 O3 S
RT	5.864 min
MW	399.94406 Da
ΔMass	0.00018 Da
Max. Area	125,235,137
Fragments	47

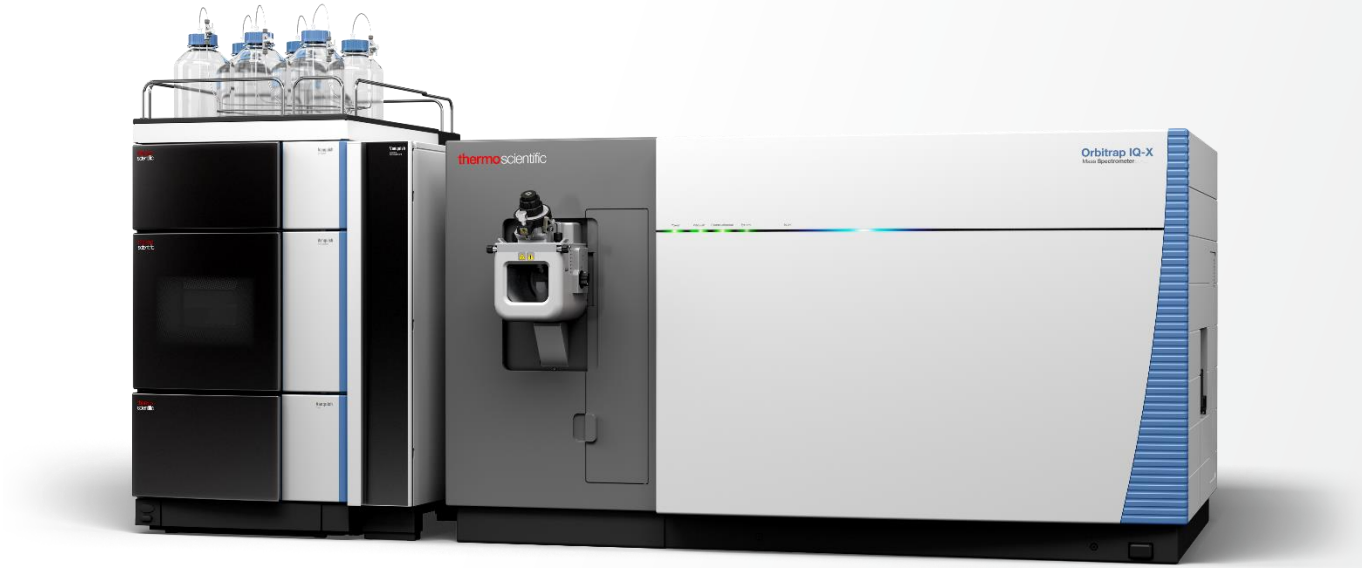
C-1 F-2
-49.99696 Da

Perfluoro-1-pentanesulfonate

Formula	C5 H F11 O3 S
RT	5.117 min
MW	349.9471 Da
ΔMass	0.00002 Da
Max. Area	136,287,615
Fragments	27

Conclusions

- PFAS is a complex compound class that poses numerous analytical challenges
- The Orbitrap IQ-X offers tools such as Real-Time Library Search to focus data acquisition on relevant compounds
- $ddMS^3$ and CID provides unique information to help identify unknown structures
- Compound Discoverer helps process and visualize PFAS results



Acknowledgments

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Ed George
Cynthia Grim
Vlad Zabrouskov

Thank you

I would be happy to answer questions