Plants, Food, Environment and Microbes

Library Matching in Real-Time for Guided Data-Dependent HRAM Analysis of Unknown PFAS

Brandon Bills[1], Sunandini Yedla[1], Juan Sanchez[1], Cynthia Grim[1], Ed George[1], Tim Stratton[1], Ralf Tautenhahn[1], Vlad Zabrouskov[1]
[1]Thermo Fisher Scientific, 355 River Oaks Pkwy, San Jose, CA, U.S.A. , 95134

Abstract

Purpose: PFAS are a prolific class of environmental contaminants characterized by a carbon backbone with near or complete saturation with fluorine. Characterizing these chemicals can be difficult due to the thousands of variations of the synthesized chemicals, isomeric structures, and the limited availability of reference standards. This work evaluates on the fly techniques to focus data dependent analysis on likely PFAS to generate more relevant data.

Methods: A Tribrid mass spectrometer using Real-Time Library Searching was used to acquire data. A mixture of 30 PFAS compounds, both neat and spiked into plasma extract, were analyzed using a mass filter to target compounds with a negative mass defect and Real-Time Library Search to collect additional information on compounds similar to those in a library of PFAS. Data was processed using the PFAS workflow in Thermo Scientific™ Compound Discoverer™ software.

Results: 29 of the 30 standards triggered additional data collection when run neat. 7 additional PFAS standards were detected in a single run of the plasma spiked with PFAS.

Introduction

PFAS are synthetic chemicals characterized by a carbon backbone with near or complete saturation with fluorine. Valued for their hydrophobic and oleophobic traits, PFAS have been used for decades in non-stick cookware, waterproof clothing, and fire-fighting foams. These compounds resist degradation and can bioaccumulate in plants and animals and have emerged as a prolific environmental contaminant. Characterizing these chemicals can be difficult due to the thousands of variations of the synthesized chemicals, isomeric structures, and the limited availability of reference standards. In our work we use the Real-Time Library Search feature available on the Thermo Scientific™ Orbitrap IQ-X™ Tribrid™ mass spectrometer paired with the new PFAS data processing workflow in Compound discoverer to simplify detecting and characterizing PFAS compounds.

Materials and methods

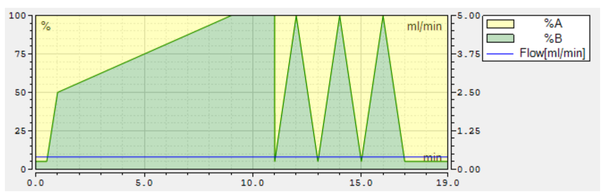
Sample Preparation

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

Test Method

Chromatography

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



Mass Spectrometer

Thermo Scientific Orbitrap IQ-X mass spectrometer

Data Analysis

Chromatograms and mass spectra were processed using Thermo Scientific Compound Discoverer software.

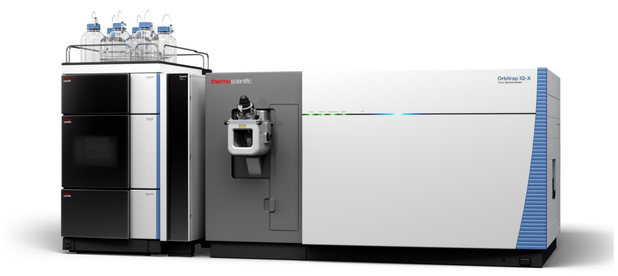


Figure 1. Thermo Scientific Horizon Vanquish LC and Orbitrap IQ-X mass spectrometer

Results

Focusing on PFAS for ddMS²

PFAS are often found in low abundance in complex matrices. This means data dependent methods will often target higher intensity background peaks. However, likely PFAS compounds can be singled out at the Full MS level by looking at their mass defect. Fluorinated compounds, such as those in table 1, often have a negative mass defect (mass values just below the integer value).

Table 1. Example PFAS compounds and their m/z values

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

If an inclusion list is set up to target masses in that range, see figure 2, organic compounds low in halogens can be screened out so the instrument can focus on likely PFAS.

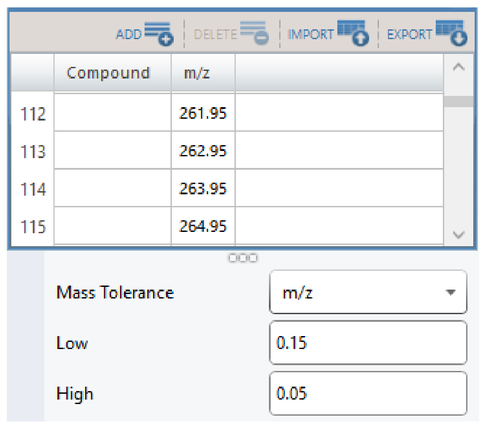


Figure 2. Inclusion list targeting compounds with a negative mass defect

Optimizing collision energy on the fly

Different classes of PFAS fragment optimally at different collision energies. The ion trap can check up to 5 collision energies in parallel with Orbitrap acquisition to pick the CE that fragments at least 80% of the precursor is used for the ddMS² acquisition. For example, in figure 3, perfluoro-1-heptanesulfonate yields far more fragment ions if a collision energy of 50 is used.

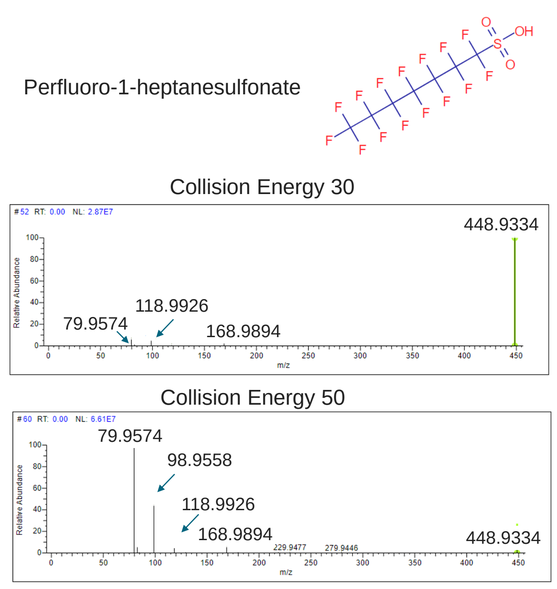


Figure 3. HCD fragmentation spectra for perfluoro-1-heptanesulfonate at collision energy 30 and 50. Assisted collision energy lets the instrument choose CE 50 in real time.

Additional information with Real-Time Library Search (RTLS)

The Orbitrap IQ-X has multiple tools to collect additional spectral information. This could include data dependent MSⁿ scans or alternate fragmentation like CID. However, every additional scan takes additional time. In order to avoid spending instrument time collecting additional information on irrelevant compounds, the Real-Time Library Search (RTLS) filter, shown in figure 4, can compare experimental spectra against a given library in real time and trigger additional scans only on likely PFAS compounds. As shown in figure 5, by limiting additional scans to only likely PFAS compounds, additional time can be spent collecting MS² and the list of compounds with MS³ ends up as a concise list of compounds that met the triggering criteria.

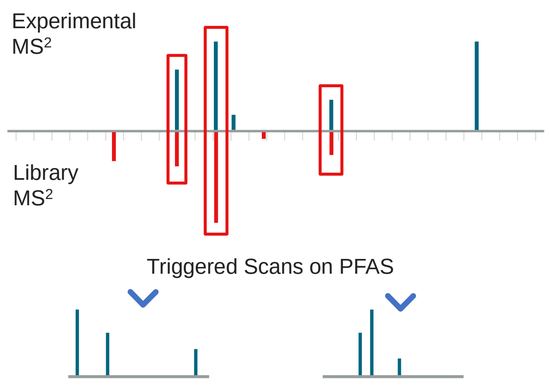


Figure 4. RTLS triggering additional scans based on matching fragments

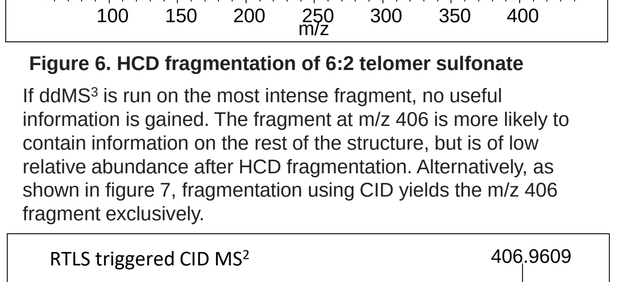


Figure 5. Number of ddMS² and ddMS³ scans collected with and without RTLS

RTLS triggering of specific scans for a class of PFAS

Not every class of PFAS fragments to give useful structural information using the same fragmentation method. For example, in figure 6 the HCD fragmentation of 6:2 Fluorotelomer sulfonate converts most of the precursor to the low mass headgroup.

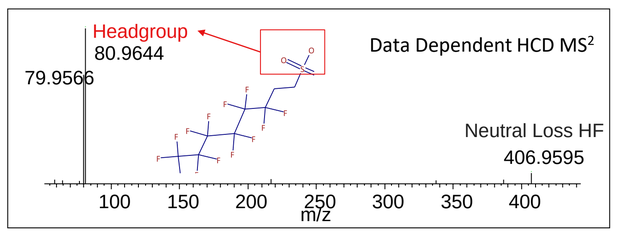


Figure 6. HCD fragmentation of 6:2 telomer sulfonate

If ddMS³ is run on the most intense fragment, no useful information is gained. The fragment at m/z 406 is more likely to contain information on the rest of the structure, but is of low relative abundance after HCD fragmentation. Alternatively, as shown in figure 7, fragmentation using CID yields the m/z 406 fragment exclusively.

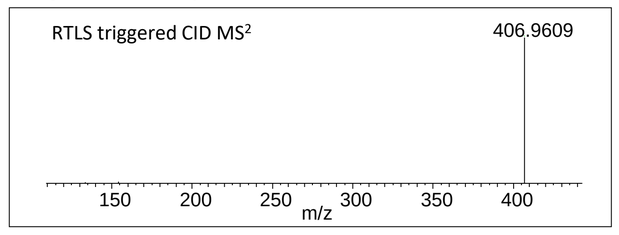


Figure 7. CID fragmentation of 6:2 telomer sulfonate

RTLS can also be configured to select compounds of a known class for specific experiments. As shown in figure 8, the method has an additional branch for telomer sulfonates. The library used does not contain 6:2 telomer sulfonates, but other telomer sulfonates have similar HCD fragments as those shown in figure 6. So if the top result from the library is a telomer sulfonate, the instrument will trigger ddMS² followed by ddMS³ to give more useful spectra like what is shown in figure 9.

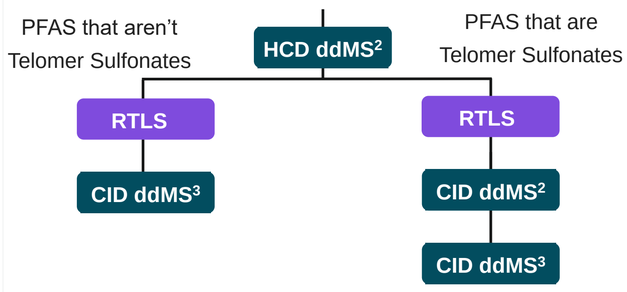


Figure 8. Structure of a ddMS³ experiment that will trigger a specific experiment when a telomer sulfonate is detected

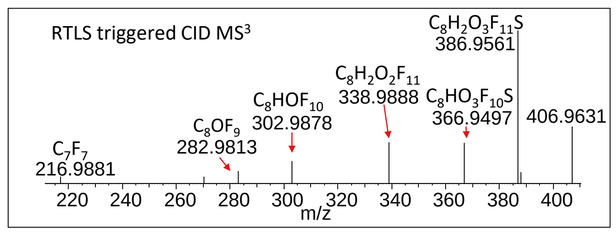


Figure 9. MS³ CID fragmentation of 6:2 telomer sulfonate

Combining RTLS with an inclusion filter targeting negative mass defects both guides the instrument towards collecting the most useful spectral information while maximizing the number of detected PFAS compounds. For example, when a sample of extracted plasma was spiked a mixture of 30 PFAS standards and run using only a ddMS3 method, only 18 standards were able to trigger ddMS² and only 15 of those triggered ddMS³ (See figure 10). However, with added inclusion list and RTLS filters, the number of PFAS standards detected in a single run increased to 25 with 23 triggering additional scans.

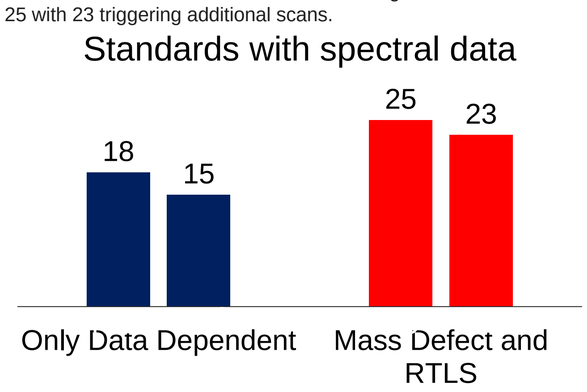


Figure 10. Number of PFAS standards (out of 30) detected in a single run of extracted plasma with and without Mass Defect inclusion list and RTLS

Visualizing results in Compound Discoverer

Compound Discoverer has a dedicated PFAS processing workflow that can help annotate unknown PFAS. In addition, there are useful tools to help visualize how found PFAS are related. For example in figure 11 compounds are graphed as a function of m/z and Kendrick mass defect (the mass defect normalized based on a CF_2 subunit, see equations below). Compounds that are part of a homologous series (compounds with the same head group and an increasing number of CF_2 units) will have similar Kendrick Mass defects and be spaced at increments of 50 m/z. In addition, the compounds can be color coded based on retention time (RT) so that is easy to see as the number of CF_2 units increases and the RT likewise increases.

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

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

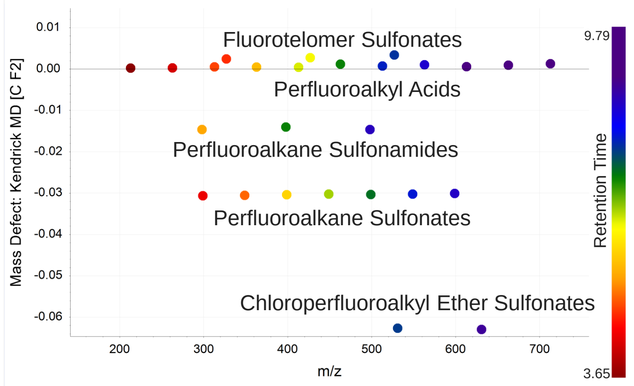


Figure 11. Homologous PFAS series graphed as a function of Kendrick mass defect, m/z, and retention time

Further relationships between compounds with fragmentation data can be visualized by looking at the molecular network. As shown in figure 12, related compounds cluster together with links that when selected will show the relationship.

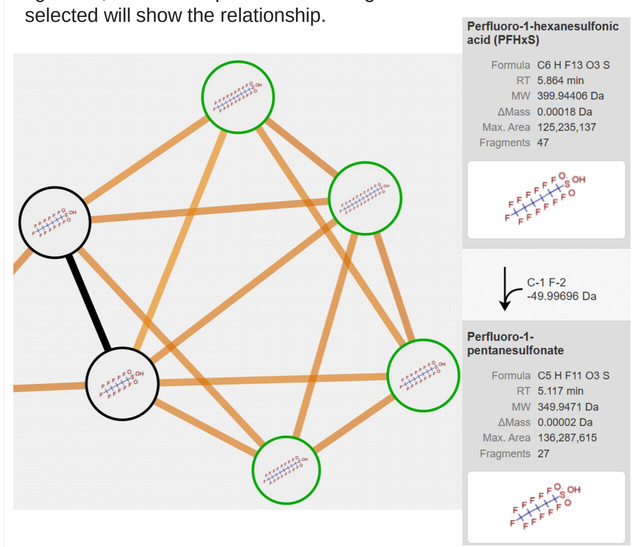


Figure 12. Molecular network of perfluoroalkane sulfonates with sample of compound relationship information for linked compounds

Conclusions

The goal of this work was to evaluate the usefulness of the tools available on the Orbitrap IQ-X for targeting and acquiring useful data on PFAS compounds and processing the resulting data using Compound Discoverer.

- An inclusion list to helps limit ddMS² to compounds with a negative mass defect.
- Assisted collision energy uses the ion trap to test energies quickly to select the optimal setting for generating useful fragments before running an Orbitrap scan.
- Real-Time Library Searching helps limit additional scan acquisitions to relevant compounds, even if a compound isn't in the library.
- The compound class filter in the RTLS node allows the instrument to perform specific scans when the top library hit matches a relevant compound class.
- Combining these tools to focus instrument time on likely PFAS compounds increased the number of found standards by 7.
- Compound Discoverer has multiple tools for visualizing results.

Trademarks/licensing

PN: PO66162-EN0422S

Science at a scan
Scan the QR code on the right with your mobile device to download this and many more scientific posters.

Learn more at thermofisher.com/