An integrated molecular networking based non-targeted PFAS analysis workflow by High-Resolution Mass Spectrometry (HRMS)

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ABSTRACT

Purpose: Develop an integrated non-targeted per- and polyfluoroalkyl substances (PFAS) analysis workflow coupling molecular networks and conventional techniques.

Methods: Negative mode data for landfill waste extracts was acquired in Full MS dd-MS2 on a Thermo Scientific™ Q Exactive™ Plus. Data was analyzed via the PFAS Unknown ID w Database Searches and Molecular Networks workflow in Thermo Scientific™ Compound Discoverer™ software.

Results: A highly curated molecular network of PFAS clustered by class was generated from data subjected to novel and conventional discrimination techniques.

INTRODUCTION

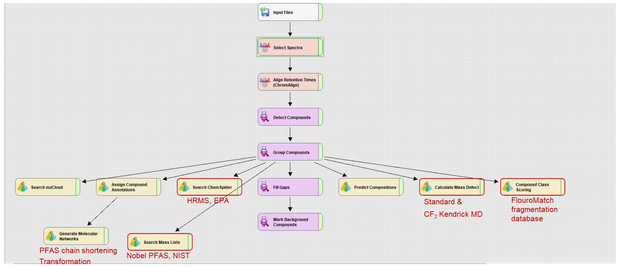
PFAS are a ubiquitous group of persistent toxic chemicals that have attracted the attention of regulatory agencies worldwide. Unlike traditional targets where reference standards are available, less than 200 PFAS standards exist for the more than 9,000 known PFAS, underscoring the need for a non-targeted workflow. Conventional non-targeted PFAS analysis workflows rely on signature fragments, homologous series with progressive retention times (RT) tied to chain length, a negative mass defect (MD), and Kendrick MD. These approaches are individually successful at identifying PFAS, but merging them requires expert knowledge. Here we describe the coupling of molecular networking with conventional and novel PFAS analysis techniques in an integrated workflow .

MATERIALS AND METHODS

The PFAS Unknown ID w Database Searches and Molecular Networks workflow (Figure 1) was applied to landfill waste extracts. Negative mode data was acquired in Full MS dd-MS2 on a Q Exactive Plus. Formula prediction was constrained to a maximum of 50 fluorine atoms. Sample spectra were searched via mzCloud™ and the manually curated Fluoromatch Suite database[1] of over 700 PFAS signature fragments.

Extensive mass lists of known and theoretical PFAS, background subtraction, and peak quality filters were used. MD filtering thresholds specific to fluorine-containing compounds, chemical transformations, and CF_2 Kendrick MD for identifying homologous series were applied. Onboard visualization encompassing Kendrick MD plots, molecular networks, and scripting node based orthogonal MS1 discrimination plots[2] were generated to assess the results.

Figure 1 Compound Discoverer PFAS workflow tree



This workflow illustrates the nodes used for the analysis of PFAS-containing samples.
(The optional scripting node is not shown.)

RESULTS

Figure 2. Result View

Results encompassing RT, MD, fragmentation library scores, areas, formulas, and other information pertinent to PFAS analysis via LC-MS/MS are displayed in the compounds table and its sub-tables. This view provides a way to interact with the analysis data, apply filters, and generate visualizations.

Figure 3. Result Filters

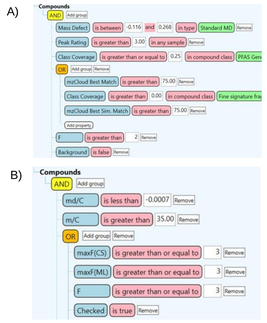
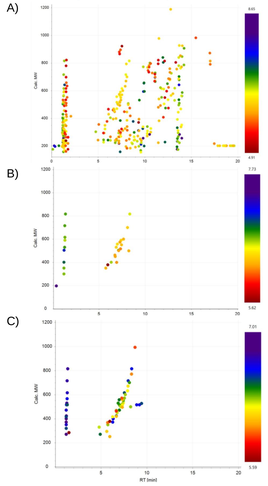


Figure 4. Multistep Filtering

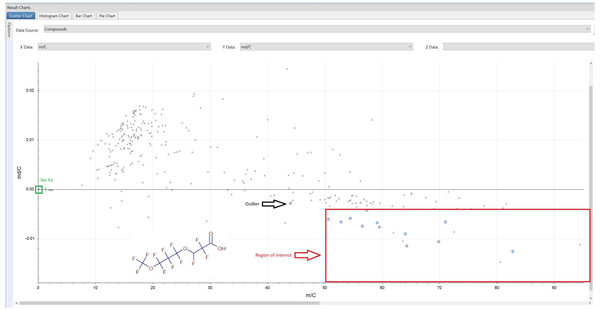


Filter based feature reduction. A) Fragment-based filtering approach. A MD filter was applied to retain PFAS based on literature values. The FluoroMatch database served as a coarse filter ensuring at least 3 matching fragments. After this, fine filters with lower thresholds were applied to retain only compounds matching the mzCloud library or fine signature fragment database. Formulas with less than 3 fluorine atoms and background compounds were excluded.

B) Filtering approach amenable to fragmentation independent orthogonal discrimination. Compounds falling within the experimentally determined region of interest are preserved. Max F values indicating the formula with the maximum number of fluorine atoms within all the annotation sources are leveraged to elucidate previously missed targets.

Filter based feature reduction. A) No filters applied, 373 compounds displayed. B) fragment-based filtering approach, 28 compounds retained. C) shows fragmentation independent orthogonal discrimination filter applied, 60 compounds retained.

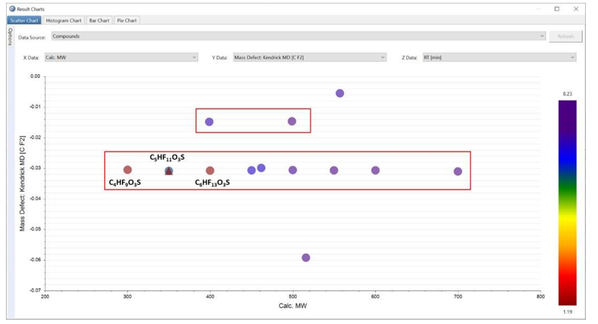
Figure 5. Orthogonal MS1 PFAS discrimination plots



The orthogonal discrimination tool relies on a scripting node to estimate the number of carbons independently from Compound Discoverer software's formula assignment. The measured A0 (first isotopic peak) and A1 (corresponding monoisotopic peak) distribution are used as inputs to approximate the number of carbons enabling the creation of new m/C (molecular mass divided by number of carbon atoms) and md/C (MD divided by number of carbon atoms) ratios.

When plotted, PFAS containing molecules cluster on the bottom region of interest outlined by a red rectangle. Compounds incompatible with this approach, which lack an A1, are outlined by a green square at the origin. A black arrow denotes an outlier with multiple carbon atom substitutions by oxygen.

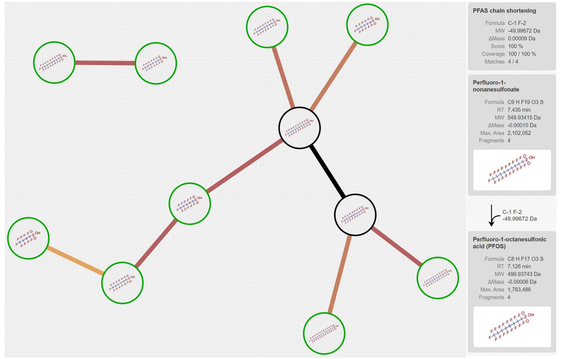
Figure 6. Kendrick mass defect



The CF_2 Kendrick MD is visualized. Data from the Compounds table are plotted in three dimensions using the Kendrick MD of approximately 50 Da or exactly one CF2 to elucidate the presence of homologous PFAS series. The RT is color coded as a third dimension to provide a simple verification of increasing RT based on PFAS chain length.

Two homologous series are identified at Kendrick MD [CF_2] of -0.03 and -0.015. Part of a homologous series is labeled, from left to right: perfluorobutanesulfonic acid, $C_4HF_9O_3S$; perfluoropentanesulfonic acid, $C_5HF_{11}O_3S$; and perfluorohexanesulfonic acid, $C_6HF_{13}O_3S$.

Figure 7. Molecular networking



Molecular networks exhibiting class-based clustering of perfluorosulfonic acid and perflurosulfonamide homologous series. RT trends, MS2 spectra match scores, and the PFAS chain shortening transformation are showcased by the link between perfluorononane sulfonate and perfluroctane sulfonate in the perfluoro sulfonic acid cluster.

CONCLUSIONS

Compound Discoverer provides a comprehensive turnkey solution for the untargeted analysis of PFAS in complex matrices. The incorporation of analysis techniques, best practices from the literature, compilation of PFAS databases, orthogonal discrimination tool, and molecular networks enables a simplified approach for analyzing PFAS.

REFERENCES

1. Koelmel, J.P. et al. Fluoromatch 2.0—Making Automated and Comprehensive Non-Targeted PFAS Annotation a Reality. Analytical and Bioanalytical Chemistry 2021, 414(3), 1201–15.

2. Kaufmann, A. et al. Simplifying Nontargeted Analysis of PFAS in Complex Food Matrixes. Journal of AOAC International 2022, 105(5), 1280–87.

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TRADEMARKS/LICENSING

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