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

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INTRODUCTION

PFAS (Per- and Polyfluoroalkyl Substances) is a group of compounds made by human for a variety of applications. The group contains many different compounds, and the NIH (National Institute of Environmental Health Sciences) states 9,000 have been identified so far. Some of the studies have linked exposure of some of these PFAS compounds to adverse health effects for human and animals. For LC-MS analysis the focus on the development of a sensitive PFAS assay will be a quantitation method for limited number of compounds. However, with such large number of PFAS compounds the interest in Identifying and confirming presence of possible other in matrices is of interest. In this publication workflow of acquisition and data processing using Compound Discoverer will be shown.

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 database1 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 CF2 Kendrick MD for identifying homologous series were applied. Onboard visualization encompassing Kendrick MD plots, molecular networks, and scripting node based orthogonal MS1 discrimination plots2 were generated to assess the results.

Figure 1 Method Example PFAS FS-ddMS2 acquisition method

Below an example method Full Scan and data dependant MS2 on the Exploris. Full Scan can show all compounds detected and the MS2 is to obtain more information about these compounds or to verify suspected compounds in a target list. The MS2 spectrum is obtained by Isolating the precursor m/z of the adduct this will be fragmented by application of collision energy and subsequent fragments are detected in the orbitrap.

Figure 2 Vanquish and Exploris instrument

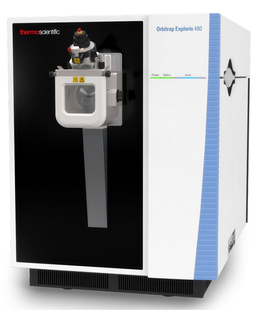


Figure 3 integrated Compound Discoverer PFAS workflow tree

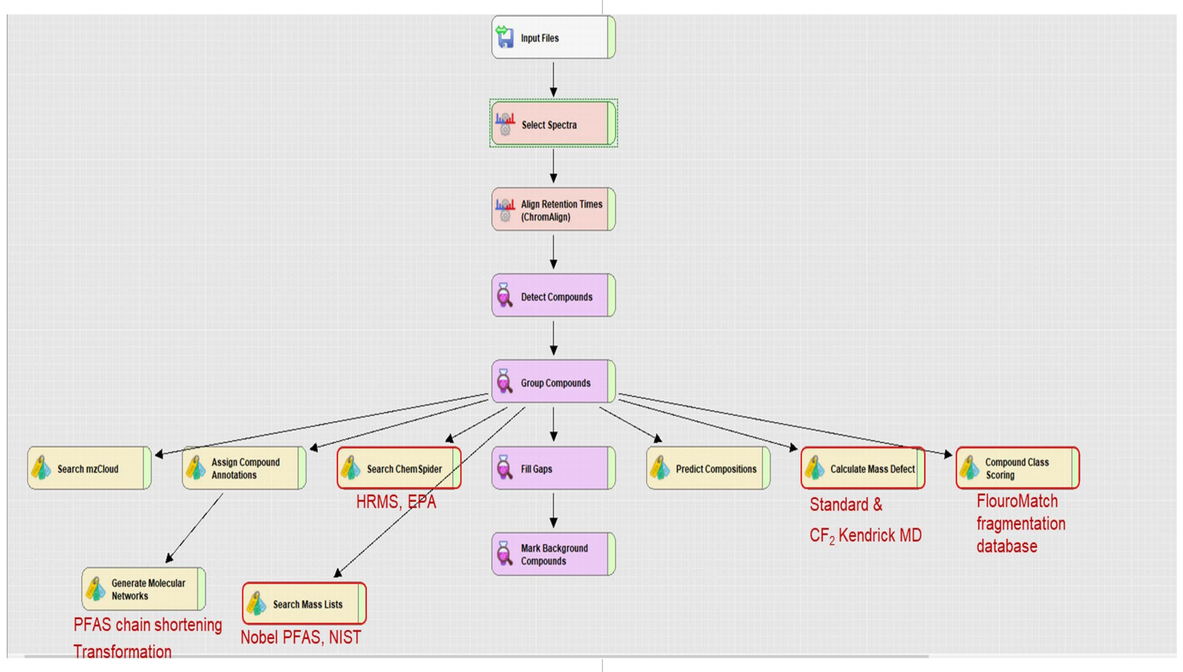
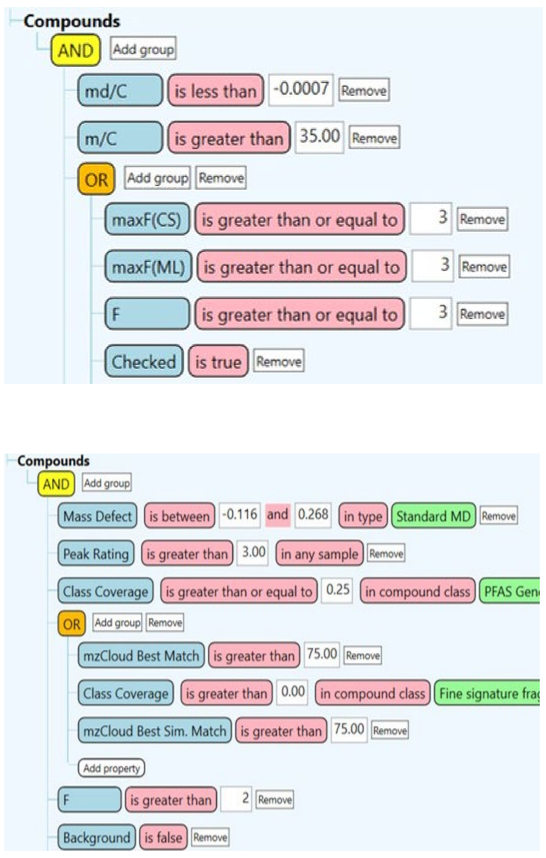


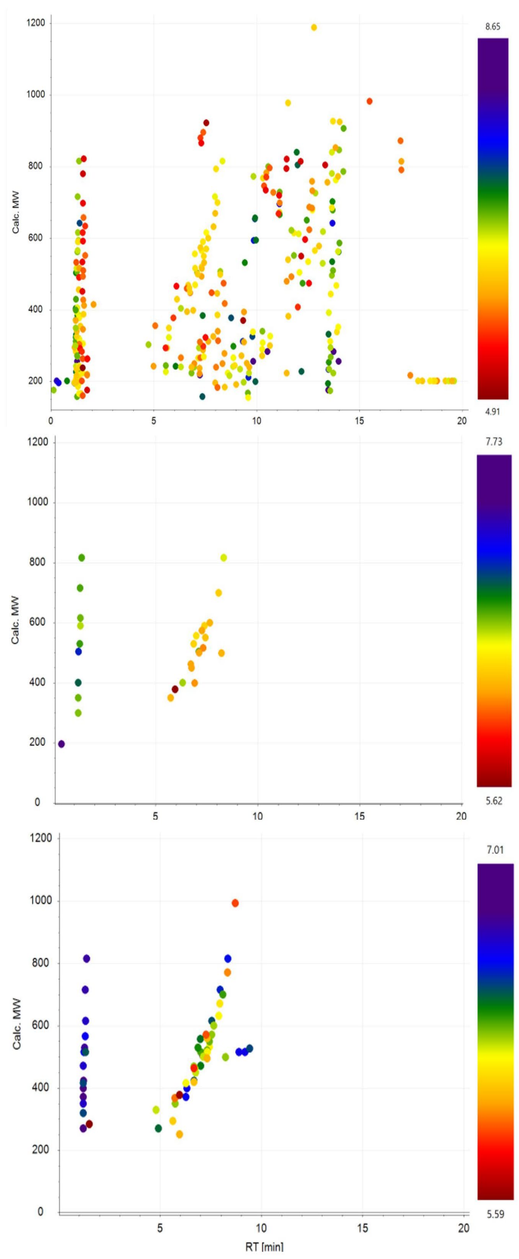
Figure 3. Result Filters used with PFAS script



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.

Figure 4. Multistep Filtering



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.

RESULTS

Figure 6 Result View

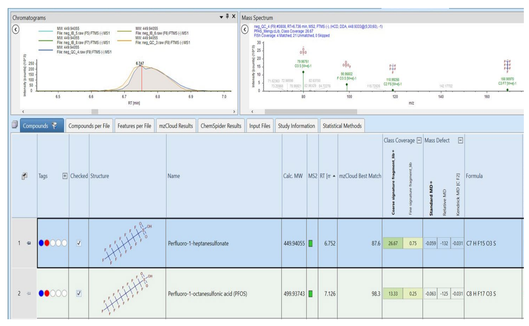
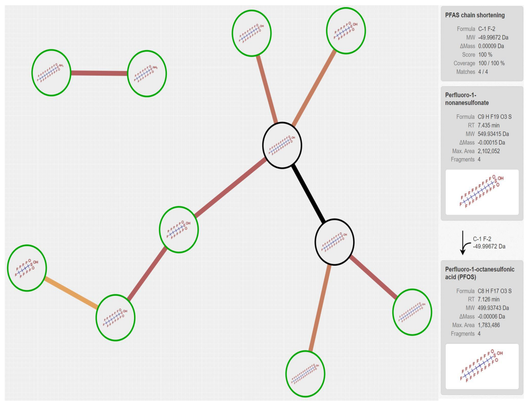


Figure 7 Molecular networking



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