

Analysis of Perfluoroalkyl Substances (PFAS) using High Resolution Accurate Mass Data

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ABSTRACT

Purpose: Perfluoroalkyl substances are a distinct group of man-made compounds that have traditionally been analyzed with a targeted strategy using triple quadrupole mass spectrometry. In this work, we aim to address a growing need to identify novel compounds that may be similar in structures.

Methods: We developed a discovery method for unknown perfluoroalkyl substances using a Thermo Scientific™ Q Exactive™ mass spectrometer. A standard compound mix and extracted samples were searched using Compound Discoverer software.

Results: We have demonstrated a step by step method for unknown compound discovery at low abundance. The highlighted techniques include calculating a ratio with abundance of a standard sample to mark which compounds are unique to a sample, using the Result Filter tool, and resubmitting samples for data acquisition to target novel compounds for the collection of MS2 spectra of the low abundant compounds of interest.

INTRODUCTION

Perfluoroalkyl Substances (PFAS) are carcinogenic at even low concentrations¹. Chemical structure of two common PFAS are illustrated in Figure 1. These environmentally persistent toxic compounds continue to be synthesized for their film-forming properties for manufacturing processes and fire-retardants.² Many PFAS are known, and they have been monitored by the EPA since the late 1990's using targeted LCMS analysis.³ With a margin of protection from a lifetime of exposure to PFOA and PFOS from drinking water, EPA has established the health advisory levels at 70 parts per trillion⁴. To address the higher toxicity of the long-chain PFAS (e.g., Perfluorooctanoic acid), some companies have replaced known PFAS with alternative chemicals exhibiting similar properties². These new compounds may also be toxic although not yet studied. While known PFAS are typically analyzed in a targeted, highly selective QQQ workflow, an unknown screening workflow utilizing high-resolution accurate mass (HRAM) can be used to detect novel PFAS.

MATERIALS AND METHODS

Sample Preparation

24 PFAS standard solution and isotopically labeled PFAS standard were purchased from Wellington Labs to prepare calibration standards. The concentration of calibration standards ranged from 50 ppt - 50 ppb. Three unknown samples were tested for presence of PFAS: pizza box, carpet, and tap water. The solid samples were extracted with acetonitrile and drinking water was unaltered. All samples were diluted 1:1 with 200 ppt isotopic standards in 80% methanol.

LC/MS Method

The LC method was adapted from Thermo Scientific Application Note 65397⁵. Chromatographic separation was performed with a Thermo Scientific™ Vanquish™ Flex Binary U-HPLC system using a Thermo Scientific™ Accucore™ RP-MS column (2.6 µm, 2.1 × 100 mm). The LC was modified to reduce background PFAS, including the installation of Thermo Scientific™ PFC-free PEEK tubing and a delay column 3.0 x 50 mm, 1.9 µm Thermo Scientific™ Hypersil GOLD™. Mobile phase A was 5 mM ammonium Acetate and 0.1 % HAc in water. Mobile phase B was 5 mM ammonium acetate and 0.1 % HAc in MeOH. LC gradient is listed in Table 1.

Mass spectrometric analysis was performed on a Thermo Scientific™ Q Exactive Plus™ Orbitrap mass spectrometer operated in Full MS / ddMS2 mode. Analysis was performed in negative ion mode followed by top3 data-dependent MS/MS scans. Resolution for the Full MS scan was set at 70,000 and at 17,500 for the ddMS2 scans. The inclusion list contained PFAS standards and isotope standards with normalized collision energy. Analysis time, including column equilibration, was 21 min.

Data Analysis

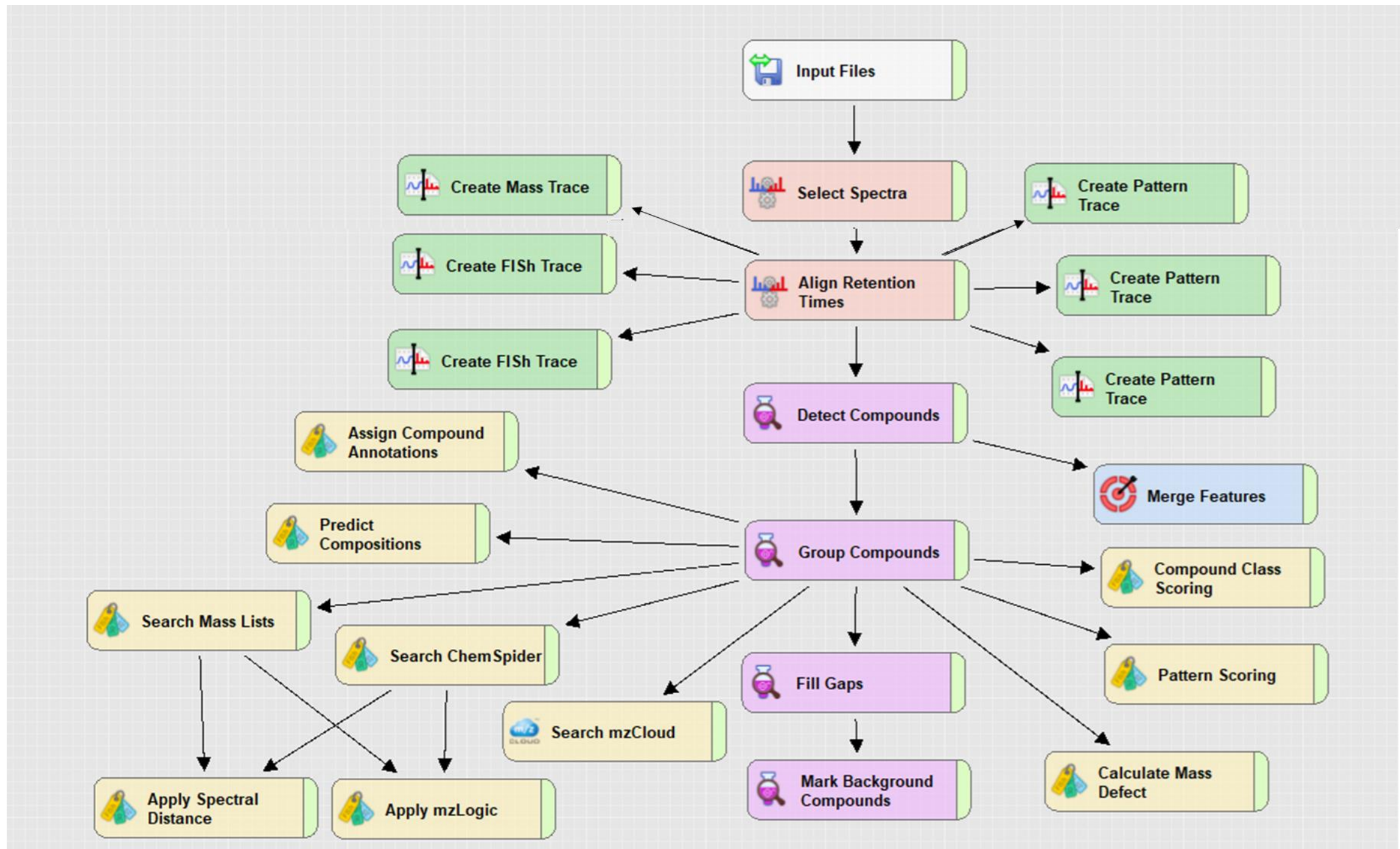
Standard compounds were quantified using Thermo Scientific™ TraceFinder™ 5.0 software. Standard and extracted samples were analyzed using Thermo Scientific™ Compound Discoverer™ 3.1 software. The processing workflow used for compound detection and compound identification is shown in Figure 2.

Table 1. LC Gradient for Sample Analysis

Time (min)	B%	Flow Rate ml/min
0	0	0.3
1	30	0.3
6	45	0.3
13	80	0.3
14	100	0.3
17	100	0.3
18	0	0.3
21	0	0.3

Figure 2. Compound Discoverer Known-Unknown Workflow

The Known-Unknown Workflow emphasizes using known compounds for identification of similar compounds. Similarities in isotope pattern are identified through Create Pattern Trace and Pattern Scoring nodes. Similarities in elemental composition are identified through Calculate Mass Defect node. Structural similarities are identified using MS2 spectra, including Compound Class Scoring and Create FISH Trace nodes.



RESULTS

We analyzed a standard mix of PFAS compounds in a method similar to previously published methods, using a PFAS adapted Vanquish liquid chromatograph and full scan / ddMS2 characteristic of an untargeted workflow. Sensitivity of the optimized QQQ method was compared to the generic QE instrument method (see Table 2). Many PFAS compounds were identified at the EPA-relevant level for required regulation, however, some compounds were not detected at the concentrations tested.

The strategy for identifying PFAS standard compounds with a Q Exactive mass spectrometer and Compound Discoverer Software is depicted in Figure 3. To compensate for the lack of sensitivity in the ddMS2 experiment, we used a high concentration 24-PAR polyfluoroalkyl standard along with experimental samples to mark the retention time of expected compounds. Slight differences in retention time between nearly identical known PFAS compounds were distinguished in the pizza box, carpet, and water samples via this targeted strategy. (see Figure 4).

Through the unique tools available in Compound Discoverer software, a list of PFAS related compounds were identified. These tools included the Create Pattern Trace node and Pattern Scoring node. PFOA and PFOS formula were entered in the node parameters for matching similar isotope patterns of unknown compounds (see Figure 5). For example, compound eluted at retention time 14.701 min was identified as 1,2-Bis (2-ethylhexyl) Sulfosuccinate which contains sulfur and shares similar structure to PFOA. Mass Defect is a complementary strategy to Pattern Trace, using the decimal information of the m/z to predict related element compositions. PFOA and PFOS were also the reference compounds for this node. We used Mass Defect in a filter to strategically refine results (see Figure 6). Compound Classes were developed in conjunction with unique fragments identified in mzCloud library, and can be used in conjunction with FISH for identification after MS2 data is collected on the dilute sample.

Pattern Trace, Compound Class Scoring, Mass Defect to can be used independently to identify the unknown PFAS related compounds (see Figure 6). And user can filter out compounds that do not meet the criteria using the Filter tool. A ratio of the abundance of each compound was calculated against the standard sample, to help identify if any compounds were unique to the unknown samples. This was set up in the Grouping and Ratios before processing the dataset. Compounds that did not meet our subjective criteria for the chromatogram peak and mass spectrum isotope/adduct pattern were removed from the Compound Table by manually editing the name to include the word "discard" then filter as shown. MS2 data for confirmation can be acquired in the follow up experiment (Figure 7A and 7B).

In summary, 52 unknown compounds were identified using the filter for further investigation. Mass Defect filter identified 34 compounds, one was identified using Class Coverage, 35 were identified by Pattern Trace and Formula, and four contained mzCloud™ and 16 contained mzLogic™ data. Of the targeted compounds from the Mass List, 20 PFAS standard and internal standard compounds out of 52 were detected, and 19 of them found matches from mzCloud.

Figure 3. Strategy to identify PFAS Standard Compounds via Q Exactive Mass Spectrometer and Compound Discoverer Software

PFAS standards can be identified using a single source mass list using both accurate mass and retention time and confirmed by spectral libraries via untargeted search. Customized PFAS Mass List was built via importing inclusion list from Q Exactive method editor to Compound Discoverer software Mass Lists panel.

Name	Formula	RT [min]	Annotation	Mass List Matches	mzCloud	MS2
Perfluoro-1-heptanesulfonate	C7 H F15 O3 S	11.656				91.7
8:2 Fluorinated telomer sulfonate	C10 H5 F17 O3 S	13.320				88.8
Perfluoro-1-decanesulfonic acid (PFDS)	C10 H F21 O3 S	14.006				88.3
Perfluoro-1-pentanesulfonate	C5 H F11 O3 S	8.910				87.9
Perfluoro-1-nonanesulfonate	C9 H F19 O3 S	13.347				87.8
N-Ethylperfluoro-1-octanesulfonamidoacetic acid (N-EtPFOSAA)	C12 H8 F17 N O4 S	14.468				87.2
Perfluorooctylsulfonamide (PFOSA)	C8 H2 F17 N O2 S	14.313				86.4
Perfluoro-1-hexanesulfonic acid (PFHxS)	C6 H F13 O3 S	10.507				86.0
4:2 Fluorinated telomer sulfonate	C6 H5 F9 O3 S	8.198				83.3
Perfluorotridecanoic acid (PFTriDA)	C13 H F25 O2	14.933				81.9
Perfluorotetradecanoic acid (PFTeDA)	C14 H F27 O2	15.118				81.5
Perfluoro-1-octanesulfonic acid (PFOS)	C8 H F17 O3 S	12.330				81.0
6:2 Fluorinated telomer sulfonate	C8 H5 F13 O3 S	11.489				80.2
Perfluoro-1-hexanesulfonic acid (PFHxS)	C6 H F13 O3 S	10.205				71.0
Perfluorododecanoic acid (PFDoA)	C12 H F23 O2	14.599				60.9

Figure 4. Identification of Targeted and Unknown Compounds

Isobaric and unknown compounds can be differentiated based on information such as mzCloud match, FISH analysis, Mass List retention time match, and sample comparison to a standard mixture using concentration-dependent chromatographic peak area.

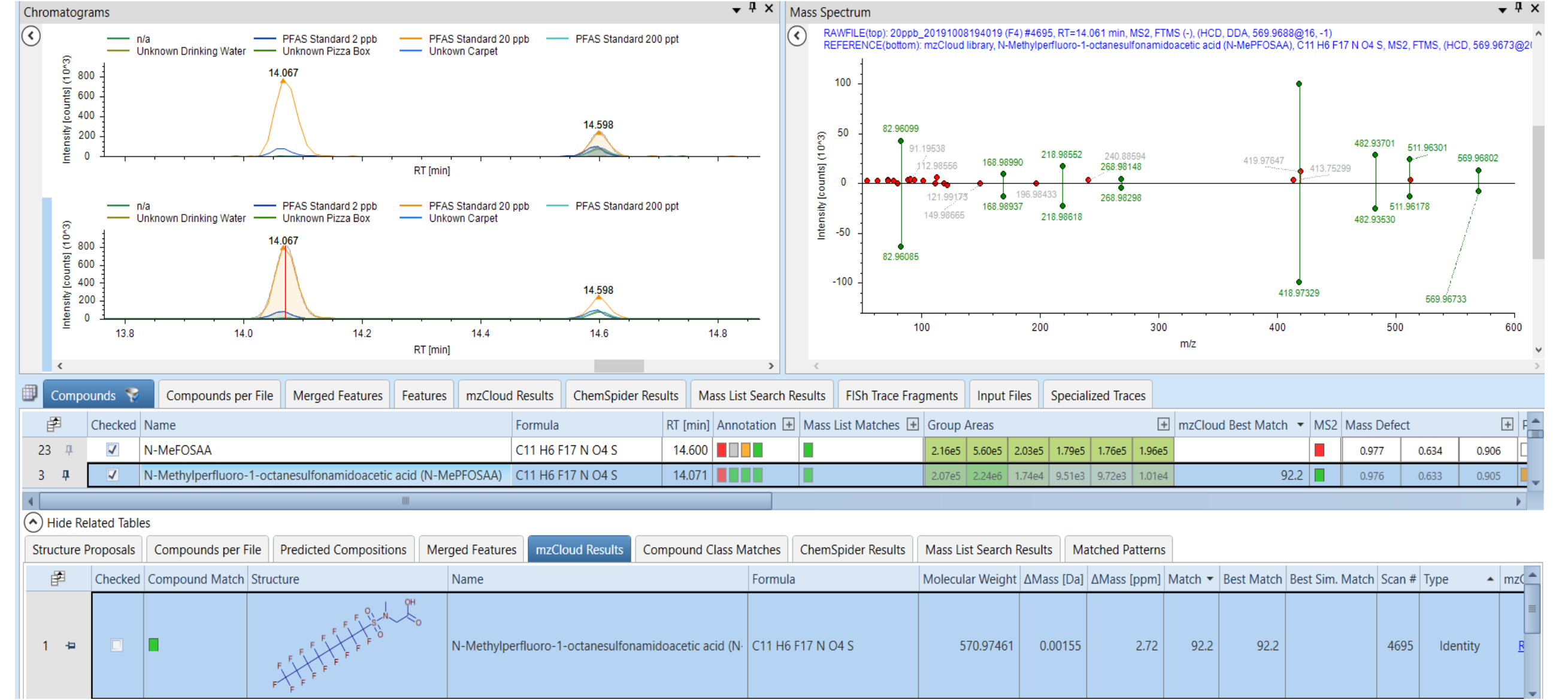


Figure 5. Untargeted strategy using Chromatogram View and Pattern Trace.

PFOS Related Compound Search via Create Pattern Trace Node, and known characteristic pattern trace can be extracted, such as S isotope pattern, PFOS and PFOA isotope patterns. The high abundance unique compounds could be investigated via searching the compounds table for a matching retention time and area.

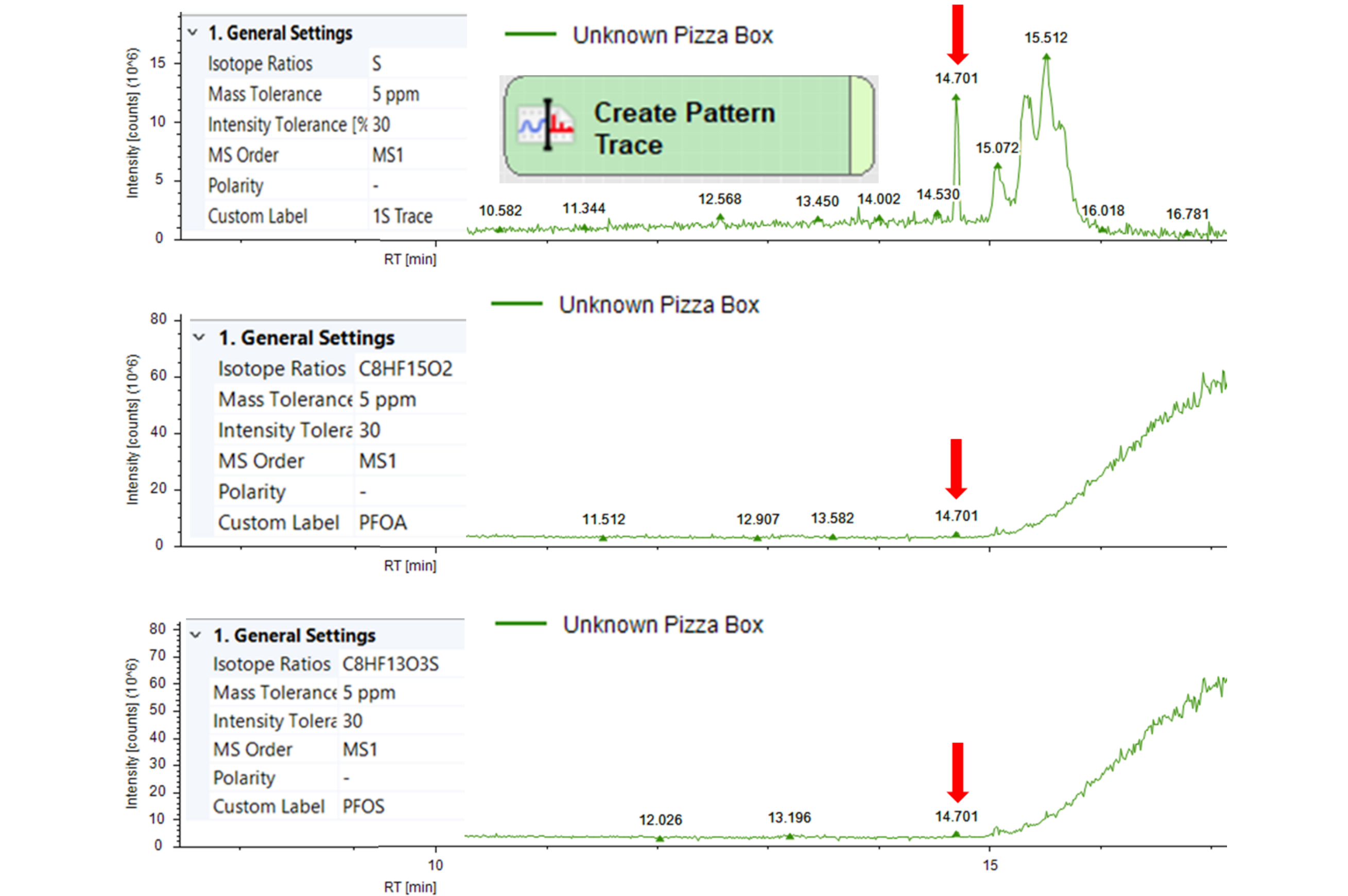


Figure 6. Results Filter strategy

We require compounds to meet Background, Retention Time, Area, and Formula criteria to be filtered out. Remaining compounds must use at least one of the unique features such as Mass Defect, Compound Class, Fluorine in the Formula, and Pattern Trace. Compounds must also meet Area Ratio criteria (compared to the 24PAR standard mix) to highlight PFAS related compounds. Compounds manually labeled as Discard can be removed.

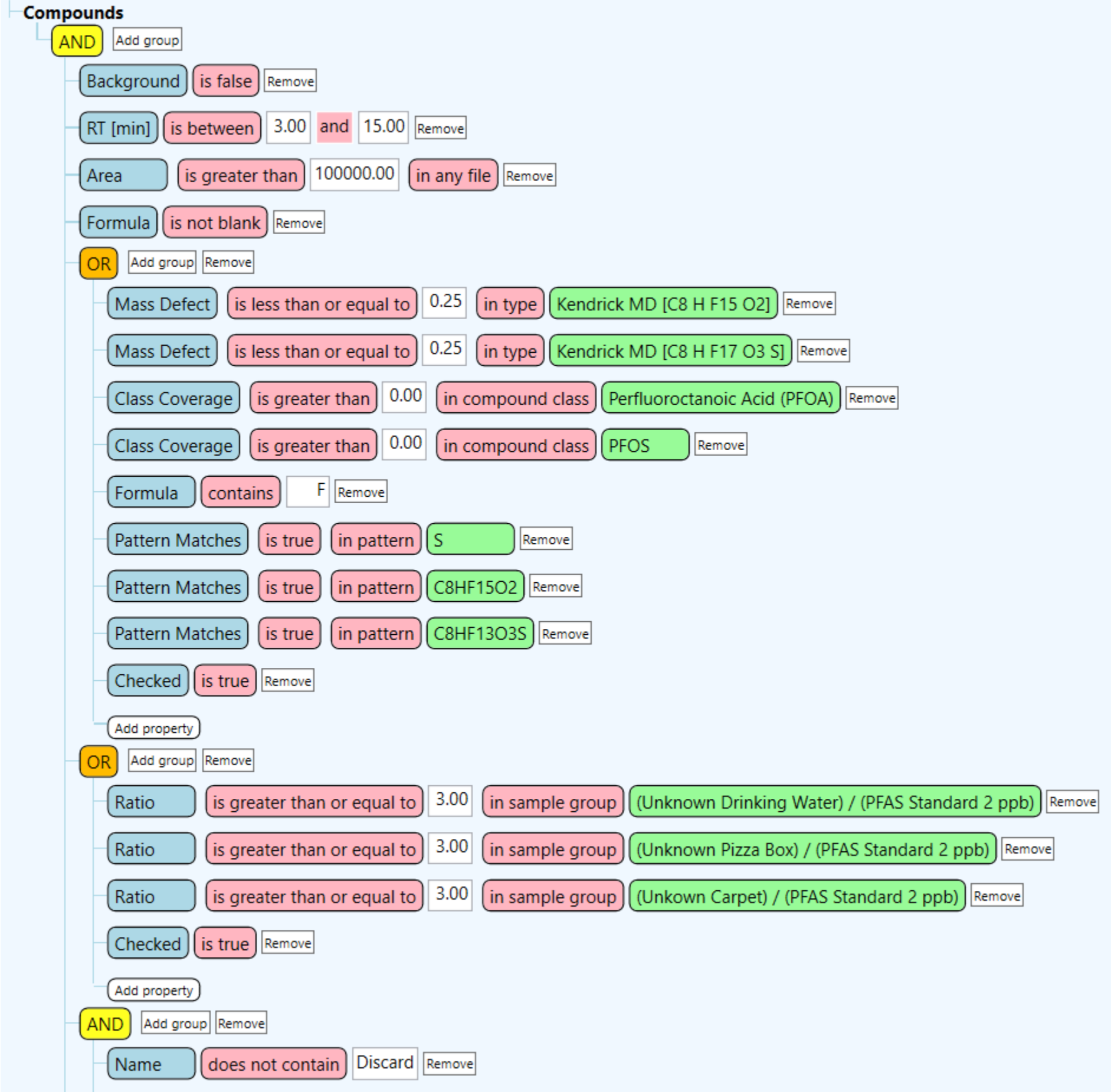
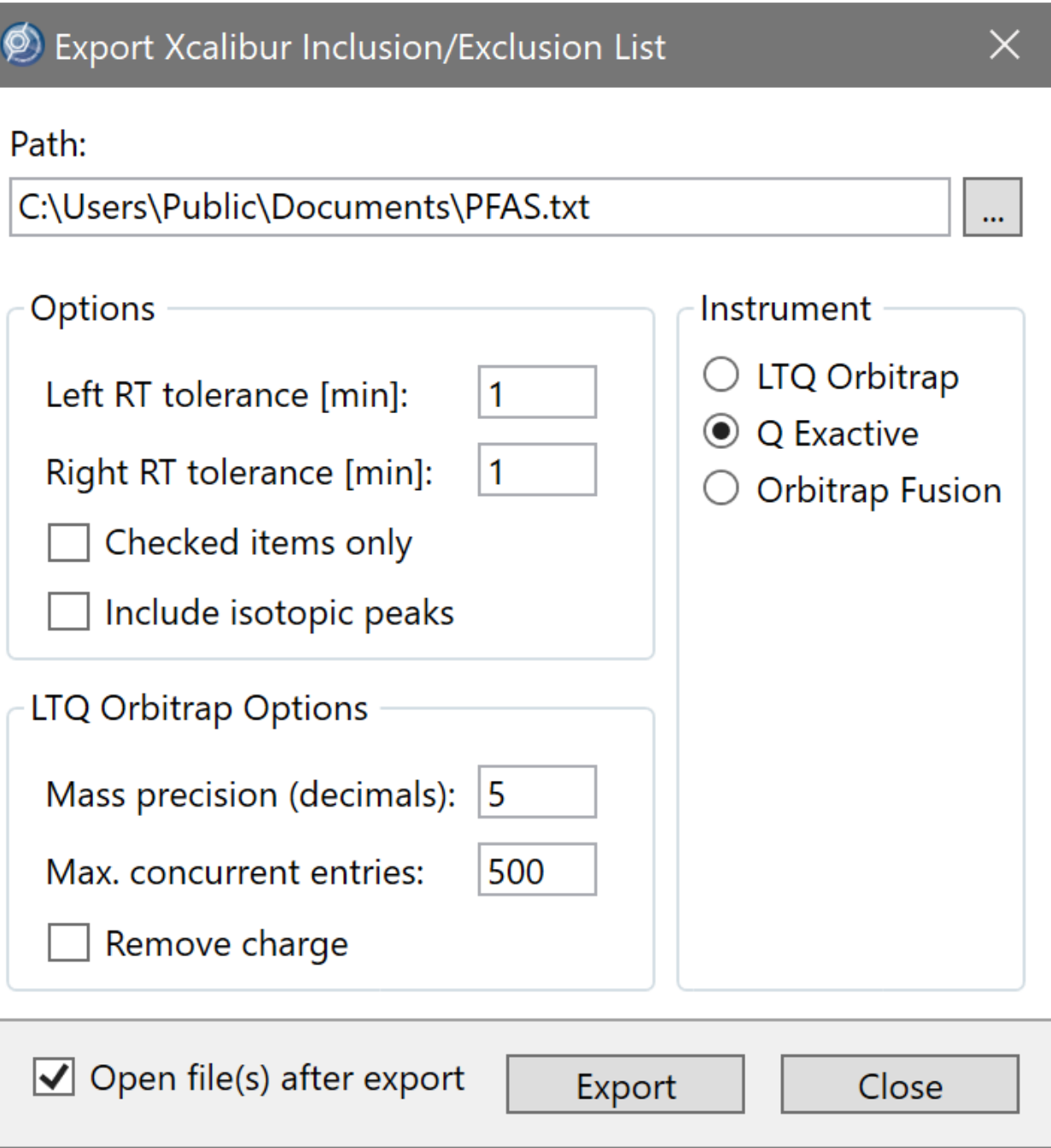


Figure 7A. Export compounds for Inclusion List

The unknown compounds were exported to an Xcalibur™ Inclusion list through the right-click menu in the Compounds Results Table

Figure 7B. Follow-up analysis for structural elucidation

The unknown compounds inclusion list was imported to the FullMS/ddMS2 method through inclusion list drop down menu



CONCLUSIONS

- Applying a Full MS / ddMS2 method makes this workflow a versatile way to analyze complex PFAS datasets.
- The Known-Unknown strategy used in these experiments demonstrates the practicality of features such as the Pattern Trace, Mass Defect, and Compound Class Scoring in Compound Discoverer software.
- Because of the lower sensitivity of the Q Exactive full scan compared to triple stage quadrupole methods, a targeted approach is recommended to mark known PFAS compounds in Compound Discoverer results. For higher sensitivity using the Q Exactive, other targeted scan types such as PRM scan could be used.
- A second injection of the sample using an inclusion list from Compound Discoverer can provide the MS2 data required for structural elucidation of the low abundant unknown compounds.

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ACKNOWLEDGEMENTS

We would like to thank Kevin McHale for his help to adapt the Triple Stage Quadrupole LC methods for use in this study. Also we would like to thank Ken Matuszak for sharing his expertise with the targeted approach to PFAS analysis using the Q Exactive mass spectrometer. Cristina Jacob and Amanda Souza established the standard methods used as a foundation for this research, with EPA 8327 PFAS analysis and unknown Compound Discoverer workflows, respectively.

TRADEMARKS/LICENSING

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