Analysis of Perfluoroalkyl Substances (PFAS) using High Resolution Accurate Mass Data
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ABSTRACT
Purpose: Perfluoroalkyl substances are a district group of man-made compounds that have traditionally been analyzed with a targeted strategy using triple quadrupole mass spectrometry. In this work, we set out to address a growing need to identify novel compounds that may be similar in structures.

Methods: We developed a data-driven method for unknown perfluoroalkyl substances using a Thermo Scientific™ Q Exactive™ mass spectrometer. A standard compound mix and extracted samples were analyzed 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 reanalyzing the data set to acquire target novel compounds for the collection of MS2 spectra of the low abundant compounds of interest.

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

MATERIALS AND METHODS

Figure 1. Chemical Structures of Common PFAS

Table 1. LC Gradient for Sample Analysis

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% B</th>
<th>Flow Rate (μL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>0.5</td>
</tr>
<tr>
<td>15</td>
<td>75</td>
<td>0.5</td>
</tr>
<tr>
<td>20</td>
<td>100</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table 2. PFAS LOQ Obtained with Q Exactive

Concentration & Compound | PFOS | PFDA | PFHxS | PFHpA | PFDoA | PFPeA |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>LOQ (ng/L)</td>
<td>50</td>
<td>50</td>
<td>20</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

Results

We analyzed a standard mix of PFAS compounds in a method similar to previously published methods, using a PFAS adapted Van Deenen chromatogram and full scan/d_SLEEP characteristic of an untargeted workflow. Sensitivity of the optimized QGQ method was compared to the general Q instrument method (see Table 2). Many PFAS compounds were identified at the EPA relevant level for risk regulation; however, some compounds were not detected at the concentrations tested.

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

<table>
<thead>
<tr>
<th>Mass Spectrometer</th>
<th>PFAS List</th>
<th>LOQ (ng/L)</th>
<th>LC Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q Exactive</td>
<td>PFAS</td>
<td>50</td>
<td>LC Method</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
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</tr>
</tbody>
</table>

Figure 4. Identification of Targeted and Unknown Compounds

Figure 5. Untargeted strategy using Chromatogram View and Pattern Trace

PFAS software can be used to Cross Pattern Trace node. Pattern trace helps identify compounds with known chromatographic peaks. The high abudance unique compounds could be investigated via searching for the compound with the matching retention time and area.

Figure 6. Results Filter strategy

The unique tools available in Compound Discoverer software, a list of PFAS related compounds were identified. These lists included the Create Pattern Trace node and Pattern Scoring node. PFDDA and PFDDA formulas were entered in the Result Filter tool 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-hexyl) Sulfosuccinate which contains sulfur and carries similar structure to PFODA. Mass Defect is a complementary strategy to Pattern Trace, using the decimal information of the mix to predict related element compositions. PFDA and PFDo were also the reference compounds for the 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 theCloud library, and can be used in conjunction with FishR for identification after MS2 data is collected on the id-sample.

CONCLUSIONS

• Applying a Full MS Q Exactive method makes this workflow a versatile way to analyze complex PFAS datasets.

• The Known-Unknown strategy used in these experiments demonstrates the practicality of the features such as 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 PFRM 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.

REFERENCES

ACKNOWLEDGEMENTS
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TRADEMARKS/LICENSING
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