APPLICATION

Per- and Polyfluorinated Alkyl Substances (PFAS) from Milk, Eggs, Butter, Cheese, and Fish using QuEChERS, SPE, and LC-MS/MS

Agustin Pierri, PhD and Scott Krepich

1 Weck Laboratories, City of Industry, CA 91745, USA
2 Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Introduction

Per- and polyfluorinated alkyl substances (PFAS), are a group of synthetic fluorinated carbon polymers used on various commercial products, such as fire-fighting foams, non-stick surfaces, and food packaging. These have been detected throughout the global environment, food products, even human plasma. PFAS are associated with various adverse health effects, they are bio-accumulative, ubiquitous, and their analysis level requirements are very low, due to an expected lifetime of exposure.

In the following method we explore sample preparation techniques using a QuEChERS extraction for dairy and fish samples to achieve low ppb sensitivities, and a subsequent solid phase extraction (SPE) for further sample clean-up and ppt sensitivities. In addition, a range of buffers were explored to maximize ionization efficiency, mass spec sensitivity, and maintaining selectivity throughout the wide aliphatic range, including branched isomers.

The method described is a fast (4-minute gradient) and effective LC-MS/MS methodology. Using a Luna® Omega PS C18, a novel C18 column chemistry with a positive surface charge for the determination of PFAS at a 0.05-1000 ppb range in dairy and fish samples, a QuEChERS extraction for accurate 1 ppb recoveries in matrix, and secondary solid phase extraction clean-up for further accurate 0.1 ppb recoveries in matrix.

Experimental Conditions

Sample Preparation

Step 1. QuEChERS Extraction and dSPE Protocol

roQ Extraction Kit (Part No. KS0-8910)

• Take 1 g homogenized sample and add into 50 mL centrifuge tube
• Spike samples with IS/surrogate and/or analytical spike as appropriate
• Add 10 mL Acetonitrile and 10 mL Water
• Add salts in salt packets (4 g MgSO₄ + 1 g NaCl salts)

Caution: Exothermic Reaction

Note: Salt pack will clump (vortexing is not violent enough to break up clumps), specifically shake the sample violently until homogenous and then vortex for 30 seconds.

• Centrifuge until there is distinct separation between Acetonitrile, Water, and Solids layers
• Transfer 200 µL to autosampler vial for LC-MS/MS analysis, or remove 1 mL clean Acetonitrile and proceed to dSPE Protocol

roQ QuEChERS dSPE Kit (Part No. KS0-8920)

• Transfer 1 mL of Acetonitrile and add to dSPE Kit Tube (150 mg MgSO₄ and 50 mg PSA)
• Vortex for 30 seconds and centrifuge
• Remove 1 mL clean Acetonitrile and analyze, or proceed to Step 2 SPE Protocol

Step 2. Solid Phase Extraction (SPE) Protocol

Pre-treatment:

Transfer 500 µL of clean acetonitrile from QuEChERS Protocol and dilute to ~15 mL with water

Solid Phase Extraction

Cartridge: Strata®-X-AW 200 mg, 3 mL
Part No.: 8B-5038-FBJ
Condition: Add 3 aliquots of 2 mL 0.3 % NH₄OH/ACN
Equilibrate: 3 mL Water
Load: ~15 mL of diluted QuEChERS Extract
Wash: 5 mL Water
Elute: 4 mL 0.3 % NH₄OH/ACN
Dry: Evaporate to near dryness and reconstitute to 500 µL

LC-MS/MS Conditions

Column: Luna Omega 1.6 µm PS C18
Dimensions: 100 x 2.1 mm
Part No.: 00D-4752-AN
Mobile Phase: A: 5 mM Ammonium Acetate in Water
B: Acetonitrile
Gradient: Time (min) % B
0 40
0.5 40
3 90
3.1 100
4 100
Flow Rate: 0.55 mL/min
Injection: 20 µL
Temperature: 40 °C
UHPLC System: Agilent® 1290
Detection: Agilent 6460 QQQ
Analytes:
1. PFBA
2. PFPeA
3. PFBS
4. PFHxS
5. PFHxA
6. PFHxS
8. PFOS
9. PFHpS
10. PFOS
11. PFNA
12. FOSA
13. Me-FOSE
14. 8:2 FTS
15. Me-FOSA
16. PFDA
17. Et-FOSE
18. Et-FOSA
19. PFDS
20. PFDoA
21. PFOA
22. PFTrDA
23. PFTeDA
24. PFUdA

For additional technical notes, visit www.phenomenex.com
Results and Discussion
Optimized LC-MS/MS conditions were explored, where ammonium fluoride is often a nice negative ion-mode enhancing modifier. Ammonium acetate, acetic acid, and ammonium formate were also screened (Figure 1), with ammonium formate yielding the best ionization efficiency and sensitivity for these particular compounds, and branched isomer resolution was maintained (Figure 2).

The system was calibrated from 0.05 ppb – 1000 ppb (Figure 3), with a 0.05 ppb LLOQ (Figure 4) and 0.5 ppb mid level (Figure 5).

Butter, cheese, egg, milk, and fish samples were screened using various QuEChERS-like extractions, including C18 dSPE, C18 dSPE with a hexane, PSA d-SPE with a hexane, C18+GCB dSPE with hexane, and PSA dSPE (Figure 6). The PSA dSPE with the traditional water+acetonitrile extraction solvents gave the best recoveries for all of the samples with a 1 ng/g sensitivity in matrix (Figures 7-11), and then further optional clean-up using a Strata®-X-AW (mixed-mode reversed phase – weak anion-exchange) solid phase extraction yielded sensitivity in matrix down to 0.1 ng/g (Figure 12).

Conclusion
Even with decreased use of PFAS on commercial products, PFAS contamination will persist throughout the environment due to their bio-accumulative properties. Thus, demand for PFAS analysis is not limited only to environmental samples, but also at low levels in food matrices for human consumption. Presented is a fast and sensitive LC-MS/MS method developed to meet PFAS analysis needs in diverse food samples, including dairy and fish, down to low ppb levels. The use of the QuEChERS technique along with further clean up using solid phase extraction is an effective extraction and clean up procedure to ensure the optimum effectiveness of LC-MS/MS analysis.

Acknowledgement
We would like to provide special thanks to Weck Laboratories for contributing this application.
Figure 2: Branched isomer resolution using Ammonium Formate Buffer

Figure 3: Dynamic Range (0.05-1000 ppb)
Figure 4:
Chromatogram of a 0.05 ppb LLOQ

Figure 5:
Chromatogram of 0.5 ppb mid-level standard
Figure 6: Water + Acetonitrile with PSA dSPE
From left to right: Blank, Butter, Cheese, Egg, Milk, and Fish
Figure 7: QuEChERS Recoveries 1 ng/g (n=4)

Milk recoveries

Figure 8: QuEChERS Recoveries 1 ng/g (n=4)

Butter recoveries

Figure 9: QuEChERS Recoveries 1 ng/g (n=4)

Tuna recoveries
Figure 10:
QuEChERS Recoveries 1 ng/g (n=4)

Figure 11:
QuEChERS Recoveries 1 ng/g (n=4)

Figure 12:
QuEChERS + SPE Recoveries 0.1 ng/g (n=4)
APPLICATION

Luna® Omega Ordering Information

<table>
<thead>
<tr>
<th>Part No.</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>00D-4753-AN</td>
<td>0.8 µm Minibore Columns (mm)</td>
<td>50/pk</td>
</tr>
<tr>
<td>00D-4753-EO</td>
<td>5.0 µm Minibore Columns (mm)</td>
<td>50/pk</td>
</tr>
<tr>
<td>00B-4753-AN</td>
<td>2.0 - 3.0 mm</td>
<td>50/pk</td>
</tr>
<tr>
<td>00B-4753-Y0</td>
<td>3.0 x 100 mm</td>
<td>50/pk</td>
</tr>
</tbody>
</table>

Strata®-X-AW Ordering Information

<table>
<thead>
<tr>
<th>Format</th>
<th>Sorbent Mass</th>
<th>Part Number</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube</td>
<td>100 mg</td>
<td>BB-S038-EBJ</td>
<td>3 mL (50/box)</td>
</tr>
<tr>
<td>100 mg</td>
<td>BB-S038-ECH</td>
<td>6 mL (30/box)</td>
<td></td>
</tr>
<tr>
<td>200 mg</td>
<td>BB-S038-FBJ</td>
<td>6 mL (30/box)</td>
<td></td>
</tr>
<tr>
<td>200 mg</td>
<td>BB-S038-FCH</td>
<td>6 mL (30/box)</td>
<td></td>
</tr>
<tr>
<td>500 mg</td>
<td>BB-S038-HBJ</td>
<td>6 mL (30/box)</td>
<td></td>
</tr>
<tr>
<td>500 mg</td>
<td>BB-S038-HCH</td>
<td>6 mL (30/box)</td>
<td></td>
</tr>
</tbody>
</table>

roQ® Extraction Kits

Extraction kits contain fifty easy-pour salt packets and fifty 50 mL stand-alone centrifuge tubes

<table>
<thead>
<tr>
<th>Description</th>
<th>Unit</th>
<th>Part No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOAC 2007.01 Method Extraction Kits</td>
<td>50/pk</td>
<td>KSO-8911*</td>
</tr>
<tr>
<td>EN 15662 Method Extraction Kits</td>
<td>50/pk</td>
<td>KSO-8909*</td>
</tr>
</tbody>
</table>

Original Non-buffered Method Extraction Kits

<table>
<thead>
<tr>
<th>Description</th>
<th>Unit</th>
<th>Part No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0 g MgSO₄, 1 g NaCl</td>
<td>50/pk</td>
<td>KSO-89810</td>
</tr>
<tr>
<td>6.0 g MgSO₄, 1.5 g NaCl</td>
<td>50/pk</td>
<td>KSO-89812</td>
</tr>
</tbody>
</table>

roQ® dSPE Kits

dSPE Kits contain pre-weighed sorbents/salts inside 2 mL or 15 mL centrifuge tubes

<table>
<thead>
<tr>
<th>Description</th>
<th>Unit</th>
<th>Part No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 mL dSPE Kits</td>
<td>100/µL</td>
<td>KSO-8921</td>
</tr>
<tr>
<td>15 mL dSPE Kits</td>
<td>100/µL</td>
<td>KSO-8921</td>
</tr>
</tbody>
</table>

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

Terms and Conditions
Subject to Phenomenex Standard Terms and Conditions which may be viewed at www.phenomenex.com/TermsAndConditions

Trademarks
Luna and Strata are registered trademarks and roQ, MidBore, and SecurityGuard are trademarks of Phenomenex. Agilent is a registered trademark of Agilent Technologies, Inc.

Disclaimer
Phenomenex is not affiliated with Agilent Technologies.