LC/MS/MS Analysis with on-line cartridge for removal of phospholipids from protein precipitation biological fluid samples

Candace Price, David Bell, Emily Barrey, Anders Fridstrom, Jason Wrigley
MilliporeSigma®, Bellefonte, PA

Introduction
Phospholipids are abundantly present in biological fluids such as blood, plasma, serum, cerebrospinal fluid, among others. They often co-extracted with a broad range of analytes of interest during sample preparation. The phospholipids present in a sample are notorious in producing various issues in LC/MS-based bioanalysis. They may cause ion suppression or, in rarer cases, ion enhancement, in MS detection. They also tend to build up on a reverse-phase LC column (e.g. C8 and C18 column), fouling the chromatographic separation and ultimately shortening the column lifetime. Consequently, the reproducibility, and sensitivity of the LC/MS bioanalysis may be greatly compromised if the phospholipids are not removed.

We have developed a HybridSPE®-Phospholipid technology for selective and rapid depletion of phospholipids from biological samples prior to LC/MS analysis of small molecules. The technology utilizes the affinity of zirconia particles for selective binding and removal of phospholipids. The technology was introduced a few years ago in two product formats: 96-well filter plates for high throughput sample preparation and cartridges for low sample volume, respectively. Here we introduce a new product format, on-line cartridge, as an alternative option of phospholipid removal and sample preparation. The setup of the on-line cartridges with an LC/MS column is devised and efficiency in phospholipid removal from protein precipitated plasma samples is evaluated. Their applicability was demonstrated with two sets of compounds of different chemical properties.

Experimental
Material:
Rat Plasma 2-EDTA (Lot Number T304407): Protein precipitation solvent: acetonitrile with 1% formic acid or methanol with 1% (v/v) ammonium formate.

HybridSPE® on-line cartridge (2 cm x 4 mm I.D.). About 100 mg of material was packed in each cartridge.

Sample Preparation:
The rat plasma spiked with analytes was protein precipitated by vortex mixing the rat plasma with the precipitation solvent at 1/2 ratio. Then the mixture was centrifuged at 10000 rpm x 3 min and the resulting supernatant was collected for LC/MS analysis.

Results

Figure 2A. Phospholipids in Plasma Sample without HybridSPE® Cartridge

The phospholipids in plasma are separated in two broad peaks with high intensities:
- 1.25 million counts of peak height of 1-chain phospholipids
- 4.0 million counts of peak height of 2-chain phospholipids

Figure 2B. #120° Injection with a HybridSPE® Cartridges

No phospholipid peaks were detected at 120° injection with HybridSPE cartridge.

Applications with Different Types of Analytes
The analyte recovery and reproducibility from the on-line phospholipid removal LC/MS methods were evaluated. Two sets of analytes representing basic and neutral compounds were tested with cartridges packed with different lots of HybridSPE® material.

Figure 3. Representative LC/MS Chromatogram of Basic Analytes

Table 1. Analyte’s Recovery and Reproducibility

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Retention Time (min)</th>
<th>LC/MS Quantifier Recovery (Avg. n=2)</th>
<th>Recovery Reproducibility RSD (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diginon</td>
<td>6.4</td>
<td>276.5 / 451.6</td>
<td>4.9</td>
</tr>
<tr>
<td>Digonon</td>
<td>8.6</td>
<td>276.5 / 451.6</td>
<td>4.9</td>
</tr>
<tr>
<td>Ipradone</td>
<td>6.4</td>
<td>411.3 / 191.3</td>
<td>2.2</td>
</tr>
<tr>
<td>Ipradone</td>
<td>8.6</td>
<td>411.3 / 191.3</td>
<td>2.2</td>
</tr>
<tr>
<td>Comprad</td>
<td>6.4</td>
<td>315.2 / 90.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Comprad</td>
<td>8.6</td>
<td>315.2 / 90.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Tammouine</td>
<td>6.4</td>
<td>372.3 / 72.2</td>
<td>9.9</td>
</tr>
<tr>
<td>Tammouine</td>
<td>8.6</td>
<td>372.3 / 72.2</td>
<td>9.9</td>
</tr>
</tbody>
</table>

For all of the analytes, including the basic, polar, and non-polar analytes:
- A recovery of 94%-120% was obtained
- A reproducibility of 1%-5% was achieved

Conclusion
An on-line cartridge packed with zirconia-coated silica particles has been successfully developed for the on-line phospholipid removal during LC/MS analysis of biological samples.

- Performance testing shows the on-line cartridges are capable of removing >95% of phospholipids from a 1 μL of plasma samples even after 120 consecutive injections.
- Two applications have been established using on-line HybridSPE® with LC/MS detection. For all of the analytes:
  - A recovery of 94%-120% was obtained
  - A reproducibility of 1%-5% was achieved
  - Narrow and symmetric peaks were observed: peak width at half height is <6s and tailing factors 0.9-1.3, respectively.

www.merckgroup.com/life-science
The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.
© 2019 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.
MilliporeSigma, Supelco, HybridSPE, Ascentis and the vibrant M are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources. Lit. No. T419003