Drug substances and products may become contaminated by chemical compounds from primary and secondary packaging materials. Compounds which can be extracted directly from the container closure systems are called “extractables” while compounds, which are found within the formulation are called “leachables” which are often a subset of extractables. The US FDA has issued guidance on container closure systems for packaging human drugs and biologics due to the potential risk impurities pose to consumer health. The guidance document includes protection, safety and compatibility guidelines.

In general, profiling extractables and leachables (E&L) is a complex analytical challenge due to the following factors:

- The wide range of materials used in making of primary and secondary containers.
- The diversity of physicochemical properties of the extracted and leached impurities.
- Varying concentration levels in samples (ranging from pg/mL to µg/mL).
- Detection of these compounds in a wide range of different matrices.

To overcome these challenges, multiple and often complementary analytical techniques such as LC/MS, GC/MS, and ICP/MS are required. Here we present LC/MS technique.

During screening for impurities, it is likely that E&Ls are present in the blank solvent originating from its container. Typically, a single background subtraction during the data processing will remove potential E&Ls also from the samples. Therefore, it is important to perform sample-to-sample comparison to retain compounds based on their intensity differences. This is performed by Mass Profiler (MP) software. MP is a statistical program that helps to compare two individual samples, replicates of the single sample, or replicates of two samples groups.

The methodology shown in Figure 1 was used for analysis of E&Ls in an ophthalmic drug product (ODP). This methodology enables the rapid and accurate identification of extractables and leachables.

### Sample Preparation

**Extractable sample**

An ophthalmic medicine bottle was purchased from a local store. It was washed with water and filled with extraction solvent (1:1 methanol:water) and incubated in an oven at 55 °C for 72 hours. The extract was used for a direct injection into the LC-MS/MS system. A second sample was analyzed as a blank, which contained the pure extraction solvent.

**Leachable samples**

A first leachable sample, designated as the “stressed sample” was obtained by heating the ophthalmic drug formulation and its container at 55 °C for 24 hours. The stressed temperature was injected directly into the LC-MS/MS system. A second leachable sample designated as the “non-stressed sample” was the ophthalmic drug formulation stored at recommended conditions and was directly injected into the system.

**Instrument**

Agilent 1298 Infinity Binary LC System and an Agilent G-TOF G6450A System with a Dual Agilent Jet Stream source were used for LC/MS/MS analysis. The Agilent 1298 Infinity UHPLC System comprised of a binary pump (p/n G4220A), autosampler (p/n G4220A), ALS Thermostat (p/n G1339B), and TCC (p/n G1316A). The Mobile Phase was A: 0.1% formic acid and Mobile Phase B: methanol.

**Parameter**

- Gradient: 0-40 min
- Flow rate: 1 mL/min
- Injection volume: 5 µL
- Source temperature: 350°C
- Source pressure: 1300 psi
- ESI voltage: 4000 V
- Mass range: 50-5000
- Autosampler temperature: 6 °C

### Experimental

**Data Analysis:**

**PCDL (Personal Compound Data Library) Manager Software:** An user-generated, custom database, containing E&Ls reported in the literature, was created in this software using molecular formulae and structure.

**Mass Profiler Software:** The control group was either the extractable or leachable stressed sample while the experimental group was either solvent blank or leachable non-stressed sample. A statistical analysis and fold change was performed on the replicate groups. Compound occurrence frequency with > 50% in at least one group and 2.0 fold change, was considered. The differential features obtained from the fold change analysis were matched against the custom database with a mass accuracy criteria < 5 ppm.

**Targeted Leachable Analysis:** In addition to untargeted analysis, targeted analysis was performed on the leachable samples to identify “known” leachable impurities. The formulae in E&L custom database were used for “Batch Target Feature Extraction” analysis by Mass Profiler Software and exported to MP software.

**Results**

**Data Comparison and Identification using MassHunter Mass Profiler Software:**

Extracts from the empty ophthalmic bottle analyzed in positive ion mode revealed 200 compounds of which the abundance of 45 compounds were significantly higher compared to the solvent blank. Due to the criteria of a fold change of greater than 2.5, the presence of dinonyl phthalate has been confirmed and not eliminated from the sample. Figure 2 shows a logarithmic abundance plot of compounds found in the extractables study with one, two, and four fold change intensity cut-offs indicated.

By combining the positive and negative ionization analysis results 54 compounds have been detected in the leachable study. The negative ionization mode contributed with 38% to this number of detected compounds.

**Identification**

The identification functionality within MP software was used to identify the compounds based on their accurate mass. Figure 3 shows the identity, isotopic distribution and structure of dinonyl phthalate, which was identified in the empty ophthalmic bottle extract. 11 extractable compounds were identified with the use of the user generated database. Table 2 shows the list of identified extractable and leachable compounds from positive and negative ionization modes.

**Table 1: LC and MS method parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase A: B</td>
<td>Measured</td>
</tr>
<tr>
<td>Injection volume</td>
<td>5 µL</td>
</tr>
<tr>
<td>Source temperature</td>
<td>350°C</td>
</tr>
<tr>
<td>Source pressure</td>
<td>1300 psi</td>
</tr>
<tr>
<td>ESI voltage</td>
<td>4000 V</td>
</tr>
<tr>
<td>Mass range</td>
<td>50-5000</td>
</tr>
<tr>
<td>Autosampler temperature</td>
<td>6 °C</td>
</tr>
<tr>
<td>Mobile Phase A: B</td>
<td>Measured</td>
</tr>
</tbody>
</table>

**Table 2: Compound mass (user created) database assisted identification of E&Ls**

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Mass Error (ppm)</th>
<th>CAS Number</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diisononyl phthalate</td>
<td>4.01</td>
<td>331-03-8</td>
<td>leachable</td>
</tr>
<tr>
<td>Diisooctyl phthalate</td>
<td>4.89</td>
<td>120-70-4</td>
<td>leachable</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>1.17</td>
<td>108-91-8</td>
<td>leachable</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>1.17</td>
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</tbody>
</table>

**Results and Discussion**

**Molecular Structure Correlator (MSC) analysis:** MSC analysis has been performed to identify unknown compounds which were not included in the PCDL. The auto MS/MS results were exported to the MSC software. Here, “ChemSpider” was selected as the structure source for unknown analysis.

**Figure 4. Mass Profiler results showing plot of logarithmic abundance of leachables and extractables**

**Figure 5. Molecular Structure Correlator Software for confirmation of diisooctyl phthalate**

**Conclusions**

- In this work, an Agilent 1298 Infinity UHPLC System coupled to an Agilent ESI G-TOF was used to analyze E&L compounds from ophthalmic drug products and their container.
- Statistical comparison to determine E&L compounds from control samples was performed using Agilent Mass Profiler Software.
- The compounds that differed significantly between samples were identified using an accurate mass method.
- The analysis of MS/MS data using MSC software facilitated the structural elucidation of unknown compounds.
- Agilent E&L compounds were found to be present in each E&L sample.
- The results of this study identified several compounds that could pose a potential health risk. Semi-quantification results (data not shown) of leachables study show that 3 compounds - diisononyl phthalate, and camphor, could lead to stressed drug product and were found in excess of 1 µg/mL concentration and therefore are to be reported to regulatory authorities.

For more information please see the app note.**

**References**


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