Qualitative Assessment of Extractables from Single-Use Systems Employed in the Storage or Manufacture of Biopharmaceuticals

Kevin Rowland
ASMS 2017
Leachables are:
Trace amounts of chemicals originating from packaging, containers, medical devices or process equipment that end up as contaminants in medicinal products or food resulting in exposure to patients or consumers.

Extractables are:
Substances that can be released from a medical device, pharmaceutical packaging or food contact surface using extraction solvents and/or extraction conditions that are expected to be at least as aggressive as the conditions of use.

From ISO-10993-12

Extractables & Leachables?
Model Single Use System

Bioprocess Bag
Pharma Grade Tubing
Hydrophilic Disk Filter

Use Condition:
37°C Saline
## Steps in an Extractables & Leachables Study

<table>
<thead>
<tr>
<th>Background Information</th>
<th>Study Design Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Device Composition</td>
<td>Analysis Techniques</td>
</tr>
<tr>
<td>Use Conditions</td>
<td>Sample Selection</td>
</tr>
<tr>
<td></td>
<td>Extraction Conditions</td>
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### Chemical Analysis

- **Sample and Control Preparation**
- **Extraction**
- **Identification**
- **Method Development**
- **Quantitation**
- **Toxicology**

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Steps in an Extractables & Leachables Study

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<td>Extraction Method Development</td>
</tr>
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<td></td>
<td>Toxicological Evaluation</td>
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Chemical Analysis
- Quantitation
- Method Development
- Toxicological Evaluation

Identification
Total Organic Carbon

Saline Extracts

- Tubing: 10.57
- Bag: 2.607
- Continuous Flow: 2.078
- Filter: 0.244
## Gravimetric Analysis

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Corrected Residue (mg/device)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bag</strong></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>0.005</td>
</tr>
<tr>
<td>50/50 H₂O/EtOH</td>
<td>3.650</td>
</tr>
<tr>
<td>Ethanol</td>
<td>29.245</td>
</tr>
<tr>
<td><strong>Tubing</strong></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>2.019</td>
</tr>
<tr>
<td>50/50 H₂O/EtOH</td>
<td>2.126</td>
</tr>
<tr>
<td>Ethanol</td>
<td>2575.819</td>
</tr>
<tr>
<td><strong>Filter</strong></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>0.106</td>
</tr>
<tr>
<td>50/50 H₂O/EtOH</td>
<td>0.349</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.158</td>
</tr>
</tbody>
</table>
Blue = Ethanol Tubing Extract
Red = Mineral Oil
UV-VIS Spectroscopy

UV Chromatograms of Saline Extracts
- Blank
- Bag
- Filter
- Tubing
- Flow Through Set

UV absorption (mAU)

Wavelength (nm)

UV Absorbance
Extraction

Scouting

Qualitative Analysis

QTOF-GCMS
ICP-MS
QTOF-LCMS
DHGCMS

Identification
Bioprocess Bag Extractables QTOF-LCMS

*Agilent 6545 QTOF LCMS*
*ESI positive ionization*

- Ethanol
- 50% Ethanol
- Water

Jordi Labs
Material Solutions. Uncompromising Integrity.
Advanced Qualitative Workflow

Find Compounds
- Recursive Feature Finding

Compare
- Statistical Analysis
- Fold Change Filtering
- Abundance Filtering

ID Compounds
- Formula Generation Databases
Advanced Qualitative Workflow

Find Compounds

Recursive Feature Finding

Compare

Statistical Analysis
Fold Change Filtering

ID Compounds

Abundance Filtering

Formula Generation Databases
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QTOF LCMS
Ethanol Extract of Bioprocess Bag

10⁷ Counts

Agilent 1290 UHPLC
Agilent 6545 QTOF LCMS
Dual ESI, Positive Ionization
QTOF LCMS
Ethanol Extract of Bioprocess Bag

Counts

Agilent 1290 UHPLC
Agilent 6545 QTOF LCMS
Dual ESI, Positive Ionization

10^6 Counts
QTOF LCMS

Ethanol Extract of Bioprocess Bag

$10^5$ Counts

Agilent 1290 UHPLC
Agilent 6545 QTOF LCMS
Dual ESI, Positive Ionization
Total Compounds Detected – QTOF LCMS

- **Tubing**
  - Ethanol: 175
  - 50% Ethanol: 151
  - Water: 288
  - Saline: 922

- **Filter**
  - Ethanol: 128
  - 50% Ethanol: 125
  - Water: 154
  - Saline: 296

- **Bag**
  - Ethanol: 117
  - 50% Ethanol: 125
  - Water: 204
  - Saline: 726

Agilent 1290 UHPLC
Agilent 6545 QTOF LCMS
Dual ESI
+ Ionization
With this many **Compounds** which are **Important**?
Advanced Qualitative Workflow

Find Compounds  Compare  ID Compounds

Recursive Feature Finding  Statistical Analysis  Fold Change Filtering

Fold Change Filtering  Abundance Filtering

Formula Generation Databases
Bioprocess Bag Ethanol Extract

Recursive Feature Finding
Agilent Profinder

726 Compounds

Agilent Mass Profiler Professional

Significance and Fold Change Filtering

575 Extractable Compounds

288 Compounds from Control

Agilent 1290 UHPLC
Agilent 6545 QTOF LCMS
Dual ESI, + Ionization
Tubing Extractables by Solvent

50% Ethanol

Ethanol

Water

Agilent 1290 UHPLC
Agilent 6545 QTOF LCMS
Dual ESI, Positive Ionization
Tubing Extractables by Solvent

- 50% Ethanol: 96 items
- Ethanol: 723 items
- Water: 18 items

Agilent 1290 UHPLC
Agilent 6545 QTOF LCMS
Dual ESI, Positive Ionization
Tubing Extractables vs. Saline

Extractables

Saline Extract

Agilent 1290 UHPLC
Agilent 6545 QTOF LCMS
Dual ESI, Positive Ionization
Tubing Extractables vs. Saline

Extractables

Saline Extract

875

32

17

Agilent 1290 UHPLC
Agilent 6545 QTOF LCMS
Dual ESI, Positive Ionization
Advanced Qualitative Workflow

Find Compounds

Recursive Feature Finding

Compare

Statistical Analysis
Fold Change Filtering

Abundance Filtering

ID Compounds

Formula Generation Databases
Value Based on
Safety Concern Threshold (SCT)
Product Configuration
Analytical Method Used

$$AET = \left( \frac{SCT}{\text{doses/day}} \right) \times \left( \frac{\text{labeled doses}}{\text{Container}} \right)$$
Analytical Evaluation Threshold

SCT = 0.15 µg/day

Safety Concern Threshold

Each system provides 500 doses
Each patient takes 2 doses/day

\[
\frac{0.15 \, \mu g}{\text{day}} \times \frac{1 \, \text{day}}{2 \, \text{doses}} \times \frac{500 \, \text{doses}}{\text{System}}
\]

AET = 37.5 µg/Device

Estimated Analytical Evaluation Threshold
Estimated AET = 37.5 µg/Device
Value Based on
Safety Concern Threshold (SCT)
Product Configuration
Analytical method used

\[ AET = \left( \frac{SCT}{\text{doses/day}} \right) \times \left( \frac{\text{labeled doses}}{\text{Container}} \right) \]
Analytical Evaluation Threshold

AET

Concentration

Instrument Response
Analytical Evaluation Threshold

AET
Concentration
Analytical Standard
Instrument Response
Relative Response Factor based on **Erucamide**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative Response Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irganox 259</td>
<td>2.157</td>
</tr>
<tr>
<td>Didecyl phthalate</td>
<td>1.857</td>
</tr>
<tr>
<td>Dioctyl phthalate</td>
<td>1.595</td>
</tr>
<tr>
<td>Benzyl butyl phthalate</td>
<td>1.113</td>
</tr>
<tr>
<td>Oleamide</td>
<td>0.974</td>
</tr>
<tr>
<td>Stearamide</td>
<td>0.786</td>
</tr>
<tr>
<td>Irganox 1010</td>
<td>0.643</td>
</tr>
<tr>
<td>Caprolactam</td>
<td>0.598</td>
</tr>
<tr>
<td>Irganox 3114</td>
<td>0.555</td>
</tr>
<tr>
<td>Irganox 1135</td>
<td>0.545</td>
</tr>
<tr>
<td>Monobutyl phthalate</td>
<td>0.097</td>
</tr>
<tr>
<td>Trially trazine trione</td>
<td>0.006</td>
</tr>
<tr>
<td>Irganox 1141</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Relative Response Factor based on Irganox 1010
Adjusting AET

**AET = 37.5 µg/Device**

*Estimated Analytical Evaluation Threshold*

Mean Relative Response Factor = 0.78  
Standard Deviation = 0.646  
%RSD = 83%

\[
37.5 \text{ µg/Device} - \left(37.5 \text{ µg/Device} \times 0.83\right)
\]

**AET = 6.4 µg/Device**

*Final Analytical Evaluation Threshold*
Estimated AET = 37.5 µg/Device

Final AET = 6.4 µg/Device
Abundance Filtering at AET
Identification

Jordi Proprietary Additives and Polymer Database

Agilent E&L Database Molecular Formula Generation
Confirmation

High Resolution MSMS Fragmentation Spectra

Analysis of Reference Standards Confirmation of Molecular Weight

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Low Energy EI GC/QTOF

7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione

Monoisotopic Mass = 276.1725

Low energy EI (10 eV)
Formula: $C_{17}H_{24}O_3$
Mass diff: -0.4 ppm
Extraction

Scouting

Qualitative Analysis

QTOF-GCMS ICP-MS

QTOF-LCMS DHGCMS

Identification
## Daily Limit

for Sb, by Inhalation **20 µg/day**

<table>
<thead>
<tr>
<th>Class (ICH Guidelines)</th>
<th>As</th>
<th>Cd</th>
<th>Hg</th>
<th>Pb</th>
<th>Co</th>
<th>Ni</th>
<th>Cr</th>
<th>Mo</th>
<th>Sb</th>
<th>Ba</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filters</td>
<td>0.0004</td>
<td>&lt;DL</td>
<td>0.0003</td>
<td>&lt;DL</td>
<td>&lt;DL</td>
<td>&lt;DL</td>
<td>0.0005</td>
<td>0.0006</td>
<td>1.4267</td>
<td>0.0001</td>
</tr>
<tr>
<td>Tube</td>
<td>&lt;DL</td>
<td>0.0048</td>
<td>0.0070</td>
<td>0.0043</td>
<td>0.1018</td>
<td>&lt;DL</td>
<td>0.0054</td>
<td>&lt;DL</td>
<td>0.0161</td>
<td>0.0048</td>
</tr>
<tr>
<td>Bag</td>
<td>0.0012</td>
<td>0.0025</td>
<td>0.0056</td>
<td>0.0062</td>
<td>&lt;DL</td>
<td>&lt;DL</td>
<td>0.0025</td>
<td>&lt;DL</td>
<td>0.6346</td>
<td>0.1785</td>
</tr>
<tr>
<td>Flow Through</td>
<td>0.0180</td>
<td>0.0024</td>
<td>0.0228</td>
<td>0.0840</td>
<td>0.0984</td>
<td>&lt;DL</td>
<td>0.0468</td>
<td>0.0312</td>
<td>0.3096</td>
<td>&lt;DL</td>
</tr>
<tr>
<td>Filters</td>
<td>0.0010</td>
<td>&lt;DL</td>
<td>0.0001</td>
<td>&lt;DL</td>
<td>&lt;DL</td>
<td>&lt;DL</td>
<td>0.0004</td>
<td>&lt;DL</td>
<td>0.2724</td>
<td>0.0027</td>
</tr>
<tr>
<td>Bag</td>
<td>&lt;DL</td>
<td>0.0054</td>
<td>0.0027</td>
<td>0.0145</td>
<td>&lt;DL</td>
<td>&lt;DL</td>
<td>&lt;DL</td>
<td>&lt;DL</td>
<td>0.0547</td>
<td></td>
</tr>
<tr>
<td>Tube</td>
<td>&lt;DL</td>
<td>0.0106</td>
<td>0.0037</td>
<td>0.0081</td>
<td>0.2016</td>
<td>0.106</td>
<td>&lt;DL</td>
<td>0.0025</td>
<td>&lt;DL</td>
<td>0.0356</td>
</tr>
</tbody>
</table>

**Results in ng/Day**

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Bioprocess System

Extract Preparation
Scouting Analyses by FTIR, TOC, Gravimetric and UV-VIS
Qualitative Screening (DHS, ICP-MS, QTOF-MS)

Unknown Identification
Feature Finding, Statistical Analysis and Abundance Filtering using Agilent Profinder and MPP
Unknown Identification by Database and MFG