Ultrapure water: LC-MS suitability tests

Application details
• Contaminations such as ions and organic traces present in ultrapure water affect LC-MS analyses and decrease performance of the analytical instrumentation. The use of high purity solvents in LC-MS analyses is critical as it allows to avoid ghost peaks, high background, signal suppression and formation of adducts.
• Specifications for LC-MS grade bottled water vary from one manufacture to another. Standard tests for LC-MS system performance were adapted to demonstrate the suitability and the benefits of using fresh ultrapure water for LC-MS analyses.
• Ultrapure water quality can degrade significantly during storage. Flow injection analyses of stored ultrapure water show the effect of storage on ultrapure water quality.
• Laboratory environment can strongly degrade ultrapure water quality resulting in ghost peaks and noisy MS background. The benefits of obtaining freshly produced ultrapure water by discarding several liters prior to ultrapure water prior to collection are described.

Ultrapure water from Merck Millipore water purification systems
• Fresh ultrapure water used in all the experiments was produced from a Milli-Q® water purification system fed by an Elix system. The Milli-Q® system was equipped with online resistivity and TOC monitors. Unless otherwise specified, a 0.22 µm membrane was used at the point-of-delivery purifier (Millipak).
Reserpine test of different water samples

Suitability test using reserpine allows the evaluation of potential organic contaminants that can affect MS analyses especially when the analytes are in the high molecular weight range. Reserpine was dissolved in different high purity water samples to verify their suitability for LC-MS analyses.

**MS Conditions**

- **System**: Applied Biosystems API2000
- **Injection rate**: 10 µL/min
- **Detection**: pos. ESI-MS (m/z 200 - 1000)
- **Sample diluent (v/v)**: Acetonitrile/water 50:50 (v:v)
- **Samples**: Four different water samples spiked with 50 ppb reserpine

**Reserpine**

\[
\text{Reserpine } C_{33}H_{40}N_2O_9 \quad (M = 608 \text{ g/mol})
\]

**High purity water sample**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Reserpine signal intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshly produced Milli-Q® water</td>
<td>8.86 \times 10^4</td>
</tr>
<tr>
<td>Bottled water competitor B</td>
<td>7.97 \times 10^4</td>
</tr>
<tr>
<td>Bottled water competitor S</td>
<td>6.40 \times 10^4</td>
</tr>
<tr>
<td>Bottled water competitor F</td>
<td>4.69 \times 10^4</td>
</tr>
</tbody>
</table>
Caffeine test of different water samples

Suitability test using caffeine allows the evaluation of potential organic contaminants that can affect MS analyses especially when the analytes are in the high molecular weight range. Reserpine was dissolved in different high purity water samples to verify their suitability for LC-MS analyses.

**High purity water sample** | **[M+H]^+** intensity | **[M+Na]^+** intensity
---|---|---
Freshly produced Milli-Q® water | 2.61 x 10^4 | 1.68 x 10^4
Bottled water competitor B | 2.46 x 10^4 | 2.36 x 10^4
Bottled water competitor S | 3.15 x 10^4 | 1.87 x 10^4
Bottled water competitor F | 0.63 x 10^4 | 3.99 x 10^4

**MS Conditions**
- **System**: Applied Biosystems API2000
- **Injection rate**: 10 µL/min
- **Detection**: pos. ESI-MS (m/z 50 - 400)
- **Sample diluent (v/v)**: Water
- **Samples**: Four different water samples spiked with 110 ppb caffeine

Caffeine (M = 194 g/mol)
Dinitrophenol test of different water samples

Suitability test using dinitrophenol allows the evaluation of potential organic contaminants that can affect MS analyses especially when the analytes are in the high molecular weight range. Reserpine was dissolved in different high purity water samples to verify their suitability for LC-MS analyses.

![Graphs showing MS intensity for different water samples](image)

### MS Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>System</strong></td>
<td>Applied Biosystems API2000</td>
</tr>
<tr>
<td><strong>Injection rate</strong></td>
<td>10 µL/min</td>
</tr>
<tr>
<td><strong>Detection</strong></td>
<td>pos. ESI-MS (m/z 100 - 300)</td>
</tr>
<tr>
<td><strong>Sample diluent (v/v)</strong></td>
<td>Acetonitrile hypergrade for chromatography LiChrosolv®/water 80:20 (v:v)</td>
</tr>
<tr>
<td><strong>Samples</strong></td>
<td>Four different water samples spiked with 100 ppb 2,4-dinitrophenol</td>
</tr>
</tbody>
</table>

### High purity water sample Dinitrophenol signal intensity

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dinitrophenol signal intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshly produced Milli-Q® water</td>
<td>3.65 x 10⁶</td>
</tr>
<tr>
<td>Bottled water competitor B</td>
<td>2.14 x 10⁶</td>
</tr>
<tr>
<td>Bottled water competitor S</td>
<td>1.31 x 10⁶</td>
</tr>
<tr>
<td>Bottled water competitor F</td>
<td>1.98 x 10⁶</td>
</tr>
</tbody>
</table>

2,4-Dinitrophenol (M = 184 g/mol)
Effect of storage on ultrapure water quality

As the demand of high purity solvents becomes nowadays more critical, especially with the advances of MS, it is expected that solvents used in MS produce very low background signals. The purity of ultrapure water stored in poor quality containers or in contaminated lab environment can decrease with time resulting in high background which complicates data interpretation.

Chromatographic Conditions

<table>
<thead>
<tr>
<th>Systems</th>
<th>Waters Acquity UPLC and Xevo® TQ MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection volume</td>
<td>10 µL</td>
</tr>
<tr>
<td>Detection</td>
<td>pos. ESI-MS (m/z 50 - 700)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Acetonitrile hypergrade for chromatography LiChrosolv®/water 2:98 (v:v) + 0.1% formic acid</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.45 mL/min</td>
</tr>
<tr>
<td>Samples</td>
<td>Two different water samples</td>
</tr>
</tbody>
</table>
Maintaining quality of freshly produced ultrapure water (1)

Laboratory environment can strongly decrease ultrapure water quality resulting in ghost peaks, signal suppression and noisy MS background. In particular, contaminants present in the laboratory environment can adhere to the final pollisher membrane, become washed out during water tapping and elute as ghost peaks in subsequent LC runs. This issue can be easily solved by simply discarding the first few liters a water purification delivers every morning before collecting freshly produced ultrapure water for the daily use.

Chromatographic Conditions

Column 2.1x150 mm C18 (3 µm)
Systems Waters HPLC Alliance 2695, Waters ZQ 2000 single quadrupole
Detection pos. ESI-M
Mobile phase A: Freshly produced Milli-Q® water
B: Acetonitrile hypergrade from LC-MS LiChrosolv®
Gradient 0' 0% B, 30' 100% B
Flow rate 0.25 mL/min
Samples Water samples taken after different amounts of water were tapped from a water purification system.
Maintaining quality of freshly produced ultrapure water (2)

MS Conditions
- **System**: Bruker Esquire 3000plus
- **Injection rate**: 200 µL/min
- **Detection**: pos. ESI-MS (m/z 100 - 2000)
- **Samples**: Water samples taken after different amounts of water were tapped from a water purification system.