Fast, Reproducible LC-MS/MS Analysis of Dextromethorphan and Dextrorphan

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Introduction

Dextromethorphan (Figure 1) is used as a cough suppressant and can be found in many over-the-counter cough and cold remedies. Dextromethorphan is quickly metabolized to dextrorphan (Figure 2) in the liver. This process can be used to differentiate fast and slow metabolizers, which can be useful information when starting metabolism drug research. The extraction from plasma using SOLA solid phase extraction (SPE) products and LC analysis of dextromethorphan and dextrorphan are demonstrated in this application.

SOLA SPE products introduce next-generation, innovative technological advancements, which give unparalleled performance characteristics compared to conventional SPE, phospholipid, and protein precipitation products.

- Higher levels of reproducibility
- Higher levels of extract cleanliness
- Reduced solvent requirements
- Increased sensitivity

SOLA SPE plates or cartridges have significant advantages when analyzing compounds in complex matrices, particularly in high-throughput bioanalytical and clinical research laboratories where reduced failure rates, higher analysis speed, and lower solvent requirements are critical. SOLA products’ superior performance gives higher confidence in analytical results and lowers cost without compromising ease-of-use or requiring complex method development.

Key Words

Accucore C18, dextromethorphan, dextrorphan, SOLA CX

Abstract

A liquid chromatography–tandem mass spectrometry method for the analysis of dextromethorphan and dextrorphan from rat plasma has been developed. A limit of quantification of 0.1 ng/mL is readily achieved. When using Thermo Scientific™ SOLA™ CX cartridges or plates, sample preparation is fast and reproducible giving excellent precision, low matrix suppression, and good recovery. The analysis was carried out on a Thermo Scientific™ Accucore™ C18 50 × 2.1 mm HPLC column for a fast separation with a cycle time of 2 minutes and good peak shape.
Experimental Details

Sample Handling

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fisher Scientific™ LC-MS grade water</td>
<td>W/011217</td>
</tr>
<tr>
<td>Fisher Scientific™ LC-MS grade methanol</td>
<td>M/4062/17</td>
</tr>
<tr>
<td>Fisher Scientific™ LC-MS grade acetonitrile</td>
<td>A/0626/17</td>
</tr>
<tr>
<td>Fisher Scientific™ Analytical grade formic acid</td>
<td>F/1900/PB08</td>
</tr>
<tr>
<td>Fisher Scientific™ HPLC grade ammonia solution</td>
<td>A/3295/PB05</td>
</tr>
<tr>
<td>Thermo Scientific™ National™ Mass Spec Target DP Certified 2 mL clear vial with ID patch, blue DP cap with bonded PTFE/silicone septum</td>
<td>MSCERT4000-34W</td>
</tr>
</tbody>
</table>

Sample Handling Equipment

<table>
<thead>
<tr>
<th>Equipment Description</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermo Scientific™ 96 well plate vacuum manifold</td>
<td>60103-351</td>
</tr>
<tr>
<td>Thermo Scientific™ UltraVap™</td>
<td>CLS-229070</td>
</tr>
</tbody>
</table>

Sample Pretreatment

Standard spiking solutions of dextromethorphan and dextrorphan were prepared in acetonitrile. A working internal standard solution (dextromethorphan-d$_3$) was prepared in acetonitrile. Spiking solutions for each level of calibrant were made in acetonitrile. 180 μL of blank plasma or sample was taken and added to a clean vial. For standards and quality control (QC) samples, 10 μL of standard spiking solution was added, with 10 μL of acetonitrile added for blanks. For standards and QCs, 10 μL of working internal standard solution was added. For blanks, 10 μL of acetonitrile was added. All samples were vortexed for 30 seconds and 200 μL of 0.1% formic acid in water was added. All samples were vortexed for 30 seconds and centrifuged at 14,000 rpm for 5 min.

Sample Preparation

<table>
<thead>
<tr>
<th>Compound(s):</th>
<th>Dextromethorphan, dextromethorphan-d$_3$, and dextrorphan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrix:</td>
<td>Plasma</td>
</tr>
<tr>
<td>Plate type:</td>
<td>Thermo Scientific SOLA CX</td>
</tr>
<tr>
<td>Conditioning stage:</td>
<td>Apply 500 μL of methanol, then 500 μL 0.1% formic acid in water to the SPE plate</td>
</tr>
<tr>
<td>Application stage:</td>
<td>Apply all supernatant to the SPE plate at a flow rate of 0.5 mL/min</td>
</tr>
<tr>
<td>Washing stage:</td>
<td>Apply 500 μL of methanol / water (40:60 v/v) to the SPE plate</td>
</tr>
<tr>
<td>Elution stage:</td>
<td>Apply 4 × 250 μL 5% ammonia in methanol to the SPE plate and dry well</td>
</tr>
<tr>
<td>Additional stage:</td>
<td>Dry down under nitrogen and reconstitute in 200 μL acetonitrile / water (50:50 v/v). Mix well.</td>
</tr>
</tbody>
</table>

Accucore HPLC columns use Core Enhanced Technology™ to facilitate fast and highly efficient separations. The 2.6 μm diameter particles are not totally porous, but instead have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. The carbon loading of Accucore C18 columns provides high retention of non-polar analytes via a predominantly hydrophobic interaction mechanism. The tightly controlled 2.6 μm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 μm materials.
Separation Conditions

<table>
<thead>
<tr>
<th>Instrumentation:</th>
<th>Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC HPLC System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column:</td>
<td>Accucore C18 2.6 μm, 50 x 2.1 mm 17126-052130</td>
</tr>
<tr>
<td>Mobile phase A:</td>
<td>Water + 0.1% formic acid</td>
</tr>
<tr>
<td>Mobile phase B:</td>
<td>Acetonitrile + 0.1% formic acid</td>
</tr>
<tr>
<td>Gradient:</td>
<td>Time (min) %B</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>95</td>
</tr>
<tr>
<td>1.01</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Flow rate:</td>
<td>1.4 mL/min</td>
</tr>
<tr>
<td>Column temperature:</td>
<td>40 °C</td>
</tr>
<tr>
<td>Pressure:</td>
<td>360 Bar</td>
</tr>
<tr>
<td>Injection details:</td>
<td>2 μL</td>
</tr>
</tbody>
</table>

MS Conditions

<table>
<thead>
<tr>
<th>Instrumentation:</th>
<th>Thermo Scientific™ TSQ Vantage™ MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionization conditions:</td>
<td>HESI</td>
</tr>
<tr>
<td>Polarity:</td>
<td>Positive</td>
</tr>
<tr>
<td>Spray voltage (V):</td>
<td>5000</td>
</tr>
<tr>
<td>Vaporizer temperature (°C):</td>
<td>450</td>
</tr>
<tr>
<td>Sheath gas pressure (Arb):</td>
<td>60</td>
</tr>
<tr>
<td>Aux gas pressure (Arb):</td>
<td>40</td>
</tr>
<tr>
<td>Capillary temp (°C):</td>
<td>300</td>
</tr>
<tr>
<td>Collision pressure (m Torr):</td>
<td>1.5</td>
</tr>
<tr>
<td>Q1 (FWHM):</td>
<td>0.7</td>
</tr>
<tr>
<td>Q3 (FWHM):</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Compound transition details are provided in Table 1.

<table>
<thead>
<tr>
<th>Compound Transition</th>
<th>Dextromethorphan (m/z)</th>
<th>Dextrophan (m/z)</th>
<th>Dextromethorphan-d₃ (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parent</strong></td>
<td>272.2</td>
<td>258.2</td>
<td>275.2</td>
</tr>
<tr>
<td><strong>Products</strong></td>
<td>215.2</td>
<td>157.1</td>
<td>215.1</td>
</tr>
<tr>
<td><strong>Collision energy</strong></td>
<td>22</td>
<td>37</td>
<td>22</td>
</tr>
<tr>
<td><strong>S Lens (Arb)</strong></td>
<td>76</td>
<td>84</td>
<td>77</td>
</tr>
</tbody>
</table>

Table 1. Compound transition details

Data Processing

| Software: | Thermo Scientific™ LC QUAN™ 2.6 |
Results

Chromatography
The Accucore C18 HPLC column gave excellent peak shape. The chromatography of the QCM at 2.5 ng/mL is shown in Figure 3.

![Figure 3: Representative chromatogram of dextromethorphan and dextrorphan SRM, extracted from plasma at 2.5 ng/mL](image)

Linearity
Standards of dextromethorphan and dextrorphan extracted from spiked rat plasma gave a linear calibration curve over the dynamic range of 0.1 to 100 ng/mL with an \( r^2 \) of 0.998 and 0.999, respectively (Figures 4 and 5 and Table 2).

![Figure 4: Dextromethorphan linearity over the dynamic range 0.1 to 100 ng/mL](image)

![Figure 5: Dextrorphan linearity over the dynamic range 0.1 to 100 ng/mL](image)
Accuracy

Eight standards were run over the linear range 0.1 to 100 ng/mL. The accuracy of the back calculated values was ≤14% in all cases.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Specified Concentration (ng/mL)</th>
<th>Dextromethorphan Calculated Concentration (ng/mL)</th>
<th>Dextromethorphan % Diff</th>
<th>Dextrophopan Calculated Concentration (ng/mL)</th>
<th>Dextrorphan % Diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0.1</td>
<td>0.101</td>
<td>1</td>
<td>0.090</td>
<td>-10</td>
</tr>
<tr>
<td>S2</td>
<td>0.2</td>
<td>0.204</td>
<td>2</td>
<td>0.228</td>
<td>14</td>
</tr>
<tr>
<td>S3</td>
<td>0.5</td>
<td>0.480</td>
<td>-4</td>
<td>0.503</td>
<td>1</td>
</tr>
<tr>
<td>S4</td>
<td>1</td>
<td>1.10</td>
<td>10</td>
<td>0.904</td>
<td>-10</td>
</tr>
<tr>
<td>S5</td>
<td>5</td>
<td>1.73</td>
<td>-5</td>
<td>5.06</td>
<td>1</td>
</tr>
<tr>
<td>S6</td>
<td>10</td>
<td>9.86</td>
<td>-1</td>
<td>10.3</td>
<td>3</td>
</tr>
<tr>
<td>S7</td>
<td>50</td>
<td>47.2</td>
<td>-6</td>
<td>50.4</td>
<td>1</td>
</tr>
<tr>
<td>S8</td>
<td>100</td>
<td>103</td>
<td>3</td>
<td>99.2</td>
<td>-1</td>
</tr>
</tbody>
</table>

Table 2: Accuracy data for eight extracted dextromethorphan and dextrorphan standards over the linear range 0.1 to 100 ng/mL

Precision

QC samples were run in replicates of six at concentrations of 0.25, 3, and 25 ng/mL. The precision of the QC level was ≤ 8% CV in all cases (Table 3).

<table>
<thead>
<tr>
<th>Standard</th>
<th>Concentration (ng/mL)</th>
<th>Dextromethorphan Average Calculated Concentration (n=6)</th>
<th>Dextromethorphan % CV</th>
<th>Dextrophopan Average Calculated Concentration (n=6)</th>
<th>Dextrorphan % CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>QCL</td>
<td>0.25</td>
<td>0.236</td>
<td>7</td>
<td>0.236</td>
<td>8</td>
</tr>
<tr>
<td>QCM</td>
<td>3</td>
<td>2.67</td>
<td>3</td>
<td>3.08</td>
<td>7</td>
</tr>
<tr>
<td>QCH</td>
<td>25</td>
<td>24.5</td>
<td>8</td>
<td>27.4</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 3: Average precision data for six replicate QCs at three levels for dextromethorphan and dextrorphan

Recovery

Overspikes (post extracted fortified blanks) were run in triplicate at concentrations of 0.25, 3, and 25 ng/mL and used to calculate the percentage recovery level for dextromethorphan and dextrorphan (Table 4). The recovery of dextromethorphan was 95.4% and that of dextrorphan was 101.6%. The recovery proved to be consistent throughout the batch for all compounds.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Response Ratio</th>
<th>% Recovery</th>
<th>Average % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextromethorphan</td>
<td>Average QCL response ratio</td>
<td>0.596</td>
<td>98.8</td>
</tr>
<tr>
<td></td>
<td>Average QCL overspike response ratio</td>
<td>0.594</td>
<td>95.6</td>
</tr>
<tr>
<td></td>
<td>Average QCM response ratio</td>
<td>5.45</td>
<td>91.9</td>
</tr>
<tr>
<td></td>
<td>Average QCM overspike response ratio</td>
<td>5.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average QCH response ratio</td>
<td>49.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average QCH overspike response ratio</td>
<td>54.2</td>
<td></td>
</tr>
<tr>
<td>Dextrorphan</td>
<td>Average QCL response ratio</td>
<td>1.18</td>
<td>102.9</td>
</tr>
<tr>
<td></td>
<td>Average QCL overspike response ratio</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average QCM response ratio</td>
<td>15.1</td>
<td>102.0</td>
</tr>
<tr>
<td></td>
<td>Average QCM overspike response ratio</td>
<td>14.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average QCH response ratio</td>
<td>134</td>
<td>99.8</td>
</tr>
<tr>
<td></td>
<td>Average QCH overspike response ratio</td>
<td>134</td>
<td>101.6</td>
</tr>
</tbody>
</table>

Table 4: Recovery data for dextromethorphan, dextrorphan and dextromethorphan-d₃
Matrix Suppression

Overspikes and solution standards were run in triplicate at concentrations of 0.25, 3, and 2.5 ng/mL and used to calculate the percentage matrix suppression for dextromethorphan and dextrorphan (Table 5). There was very little matrix suppression: -0.5% and 7.4% for dextromethorphan and dextrorphan, respectively. The matrix effects were consistently low throughout the batch for all compounds.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Response Ratio</th>
<th>% Matrix Suppression</th>
<th>Average % Matrix Suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextromethorphan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average QCL overspike response ratio</td>
<td>0.594</td>
<td>-5.9</td>
<td>-0.5</td>
</tr>
<tr>
<td>Average QCL solution response ratio</td>
<td>0.560</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average QCM overspike response ratio</td>
<td>5.71</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Average QCM solution response ratio</td>
<td>5.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average QCH overspike response ratio</td>
<td>54.2</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Average QCH solution response ratio</td>
<td>56.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrorphan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average QCL overspike response ratio</td>
<td>1.15</td>
<td>14.6</td>
<td></td>
</tr>
<tr>
<td>Average QCL solution response ratio</td>
<td>1.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average QCM overspike response ratio</td>
<td>14.8</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Average QCM solution response ratio</td>
<td>15.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average QCH overspike response ratio</td>
<td>134</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>Average QCH solution response ratio</td>
<td>142</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Matrix suppression data for dextromethorphan, dextrorphan and dextromethorphan-d₃.

Conclusion

• SOLA CX SPE plates and Accucore C18 HPLC columns allow for simple extraction and rapid quantification of dextromethorphan and dextrorphan from plasma.

• Accucore C18 HPLC columns give a fast runtime for dextromethorphan and dextrorphan of 2 min.

• Accucore C18 HPLC columns give excellent peak shape for dextromethorphan and dextrorphan.

• An LLOQ of 0.1 ng/mL was achieved.

• Extraction recovery was 95.4% and 101.6% for dextromethorphan and dextrorphan, respectively.

• SOLA CX SPE plates achieved excellent precision on the extraction of dextromethorphan and dextrorphan from plasma samples at low concentrations with % CV (n=6) ≤8%

• SOLA CX SPE plates achieved low matrix recovery. This gave excellent matrix suppression effects at 7% and 12% for dextromethorphan and dextrorphan, respectively.

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