HPLC Application
ID No.: 20032

Tetracyclines in Meat by LC/MS/MS

Column: Gemini® 5 µm C18 110 Å, LC Column 50 x 4.6 mm, Ea
Dimensions: 50 x 4.6 mm ID
Order No: 008-4435-E0
Elution Type: Gradient
Eluent A: 0.1% Formic Acid in Water
Eluent B: Acetonitrile

Gradent Profile:

<table>
<thead>
<tr>
<th>Step No.</th>
<th>Time (min)</th>
<th>Pct A</th>
<th>Pct B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>2.1</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>6.5</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>7.1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>8.1</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>98</td>
<td>2</td>
</tr>
</tbody>
</table>

Flow Rate: 0.8 mL/min
Col. Temp.: 40 °C
Detection: Tandem Mass Spec (MS-MS) @ (600 °C)
Detector Info: 3200 QTRAP LC/MS/MS

Analyst Note:
SecurityGuard™ Guard Cartridge System extends column lifetime.
- SecurityGuard Cartridges, Gemini C18 4 x 3.0mm ID, 10/Pk Part No.: AJ0-7597
- Holder Part No.: KJ0-4282

ANALYTES:

1. Oxytetracycline (461.1 to 426.1/201.2)
   Retention Time: 3.64 min

2. Tetracycline (445.2 to 410.1/427)
   Retention Time: 3.71 min

3. Chlortetracycline (479.1 to 444.1/462.2)
   Retention Time: 4.26 min

4. Methacycline (443.1 to 426.1/201.2)
   Retention Time: 4.3 min

5. Doxycycline (445.2 to 428.3/98.1)
   Retention Time: 4.42 min
**Sample Preparation Details**

for HPLC  Application ID No.: 20032

**Tetracyclines in Meat by LC/MS/MS**

**PRODUCT DESCRIPTION:**
Strata™-X 33 µm Polymeric Reversed Phase, 60 mg / 3 mL, Tubes, 50/Pk

Order No.: 8B-S100-UBJ

**SOLID PHASE EXTRACTION (SPE) PROCEDURE:**

**Note:** The solvent volumes shown below are for a 60 mg bed mass.

The solvent volumes will need to be adjusted for a smaller or larger bed mass.

**Condition:**

**Load:**
Measure 5 g of sample into an 85 mL centrifuge tube. Add 25 mL of acidified methanol/1M HCl mixture (0.833 mL of 1M HCl in a total volume of 200 mL methanol). Mix for 1 min using an Ultra-Turrax mixer at a velocity setting of 2. Agitate further for 5 min.

**Wash:**

**Dry:**
Evaporate to dryness using an eppendorf concentrator set at 45°C or blow dry with nitrogen.

**Elute:**

**Final Prep and Analysis:**
Inject: 50 µL on HPLC Tandem Mass Spec (MS-MS) @ (600°C)

**ANALYTES:**

<table>
<thead>
<tr>
<th></th>
<th>Spiked Conc. (ng/mL)</th>
<th>Log P</th>
<th>pKa</th>
<th>% Rec</th>
<th>%RSC (n=0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oxytetracycline</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Tetracycline</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Chlortetracycline</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Methacycline</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Doxycycline</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
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</tbody>
</table>

**Note:** This method is designed as a convenient starting point for further investigation and can be tailored to meet your extraction goals. Call your local Phenomenex Representative for assistance in method development and optimization techniques.

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