LC/MS/MS Analysis of Methadone from urine using Strata-X-Drug B on a Kinetex C18 2.6µm, 50x2.1mm

Column: Kinetex® 2.6 µm C18 100 Å, LC Column 50 x 2.1 mm, Ea

Dimensions: 50 x 2.1 mm ID

Order No: 008-4462-AN

Elution Type: Gradient

Eluent A: 0.1% Formic acid

Eluent B: 0.1% Formic acid in acetonitrile

Gradient

<table>
<thead>
<tr>
<th>Profile</th>
<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>60</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.5</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.51</td>
<td>60</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6.5</td>
<td>60</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

Flow Rate: 400 µL/min

Col. Temp.: ambient

Detection: Mass Spectrometer (MS) @ amu (ambient)

Detector Info: SCIEX®

Source: Cur-11, CAD-12, IS-4500, Tem-450, NEB-12

Dwell: 25ms

Polarity: Positive

Analyst Note: SecurityGuard™ ULTRA Guard Cartridge System extends column lifetime.

- SecurityGuard ULTRA Cartridges, UHPLC C18 for 2.1mm ID Columns, 3/Pk Part No.: AJ0-8782
- Holder Part No.: AJ0-9000

19703

Analytes:

1. Methadone-d9 (319.4>268.4)
   Retention Time: 2.55 min

2. Methadone (310.2>265.3, 310.2>105.1)
   Retention Time: 2.55 min

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PRODUCT DESCRIPTION:
Strata™-X-Drug B 33 µm Polymeric Strong Cation, 60 mg / 6 mL, Tubes , 30/Pk

Order No.: 8B-S128-UCH

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:
To 2mL urine, add 2 mLs of 100mM sodium acetate buffer with I.S. (300ng/mL). Make sure pH is ~4.0-6.0. Load pre-treated sample directly onto SPE cartridge. NO CONDITIONING REQUIRED

Wash:

Dry:
10 minutes @ full vacuum

Elute:

Final Prep and Analysis:
Evaporate to dryness under a stream of nitrogen at 50 C. Reconstitute in 1 mL of 40% acetonitrile.
Inject: 2.5 µL on HPLC Mass Spectrometer (MS) @ amu (ambient)

<table>
<thead>
<tr>
<th>ANALYTES</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 Methadone-d9</td>
<td>300</td>
<td></td>
<td>100</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>2 Methadone</td>
<td>300</td>
<td></td>
<td>97.59</td>
<td>2.48</td>
<td></td>
</tr>
</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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