Tetracyclines in Serum

HPLC Method

Column: SymmetryShield™ RP8, 3.0 x 150 mm, 5 µm
Guard Column: Sentry™ guard column, 3.9 x 20 mm, 5µm
Part numbers: Column - WAT094243, Guard - WAT200675
Mobile phase: 0.1% TFA in Water, Methanol, Acetonitrile 91:2:7
Flow rate: 0.90 mL/min
Injection volume: 20 µL of reconstituted porcine serum extract
Detection: UV @ 350 nm

Compounds:
1: Minocycline
2: Tetracycline
3: Demeclocycline (I.S.)

Oasis® HLB Extraction Method

Oasis® HLB 1 cc/30 mg Extraction Cartridge
Part Number WAT094225

Condition
1 mL methanol/1 mL water

Load
1 mL spiked porcine serum with 20 µL phosphoric acid and 5.0 µg/mL demeclocycline (I.S.)

Wash
1 mL 5% methanol in water

Elute
1 mL methanol

Evaporate and Reconstitute
40 ºC under nitrogen stream
200 µL mobile phase

Chromatogram of Serum Extracts: A) Blank B) Spiked Sample

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration µg/mL</th>
<th>% Recovery</th>
<th>%RSD (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minocycline</td>
<td>2.50</td>
<td>96.1%</td>
<td>1.3%</td>
</tr>
<tr>
<td></td>
<td>0.500</td>
<td>88.0%</td>
<td>1.6%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>2.50</td>
<td>101.0%</td>
<td>0.5%</td>
</tr>
<tr>
<td></td>
<td>0.500</td>
<td>97.8%</td>
<td>1.4%</td>
</tr>
</tbody>
</table>

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