Tricyclic antidepressants
Determination of tricyclic antidepressants in plasma

Application Note

BioPharma

Authors
Agilent Technologies, Inc.

Introduction
Gas chromatography using an Agilent CP-Sil 5 CB column separates 13 tricyclic antidepressants in plasma in 14 minutes.
Conditions

Technique: GC-capillary

Column: Agilent CP-Sil 5 CB, 0.22 mm x 25 m fused silica
    WCOT CP-Sil 5 CB (0.12 µm)  (Part no. CP7710)

Temperature: 100 °C → 175 °C, 25 °C/min; → 250 °C, 10 °C/min;
    → 290 °C, 25 °C/min; 290 °C (2 min)

Carrier Gas: N₂, 1 mL/min

Injector: Splitless
    T = 250 °C

Detector: NPD
    T = 300 °C

Sample Size: 1-2 µL

Concentration range: 100 ng/mL

Courtesy: Stichting Ziekenhuisapotheek, Laboratory of
    Pharmacy, Venray, The Netherlands

Peak identification

Ret. time:
1. amitriptyline  8.82
2. nortriptyline  8.91
3. clomipramine  10.52
4. desmethylclomipramine  10.67
5. thioridazine  15.60
6. mesoridazine  18.47
7. sulforidazine  18.99
8. ring-sulfoxide  20.64
9. mianserine  8.79
10. imipramine  9.02
    desipramine  9.16
    doxepine  9.06
    trimipramine  9.05
    desmethylmianserine  9.07
11. maprotyline  9.88
12. levomepromazine  11.39
13. leponex  13.45
Sample preparation

2.5 mL plasma
0.5 mL NaOH, 0.25 molar
1.0 mL molar aqueous NaHCO$_3$ solution, pH 9.0
50 μL standard solution
6.0 mL heptane/iso-amylalcohol (98.5/1.5 v/v)

Shake 10 minutes vigorously on Vortex.
Centrifuge. Transfer organic phase to another tube.
Add 1.5 mL 0.1 molar aqueous HCl solution. Shake 10 minutes vigorously.
Centrifuge.

Remove the organic phase and transfer aqueous phase to another tube.
Add 1.5 mL 1.0 molar aqueous NaHCO$_3$ buffer pH 9.0 and add 100 μL toluene - isoamylalcohol 85:15. Shake 10 minutes vigorously and remove aqueous layer.
Centrifuge.

Transfer organic phase to sample vial.

Standard solutions

Stock: Dissolve 5 mg of the base in 10 mL methanol.
Dissolve 50 μL of the stock solution in 5 mL water immediately before the analysis. Add 50 μL of this solution to 2.5 mL plasma. Concentration is 100 ng/mL plasma.

Carbonate buffer 1 mol pH 9: Dissolve 8.4 g NaHCO$_3$ in 100 mL freshly distilled aqua dest. and adjust to pH 9 with NaOH. Brown coated glassware with ground glass stoppers should be used for the analysis.

Peak identification

<table>
<thead>
<tr>
<th>Ret. time</th>
<th>Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.82</td>
<td>amitriptyline</td>
</tr>
<tr>
<td>8.91</td>
<td>nortriptyline</td>
</tr>
<tr>
<td>10.52</td>
<td>clomipramine</td>
</tr>
<tr>
<td>10.67</td>
<td>desmethylocipramine</td>
</tr>
<tr>
<td>15.60</td>
<td>thioridazine</td>
</tr>
<tr>
<td>18.47</td>
<td>mesoridazine</td>
</tr>
<tr>
<td>18.99</td>
<td>sulfonidazine</td>
</tr>
<tr>
<td>20.64</td>
<td>ring-sulfoxide</td>
</tr>
<tr>
<td>8.79</td>
<td>mianserine</td>
</tr>
<tr>
<td>9.02</td>
<td>imipramine</td>
</tr>
<tr>
<td>9.16</td>
<td>desipramine</td>
</tr>
<tr>
<td>9.06</td>
<td>doxepine</td>
</tr>
<tr>
<td>9.05</td>
<td>trimipramine</td>
</tr>
<tr>
<td>9.07</td>
<td>desmethylocipramine</td>
</tr>
<tr>
<td>9.88</td>
<td>maprotyline</td>
</tr>
<tr>
<td>11.39</td>
<td>levomepromazine</td>
</tr>
<tr>
<td>13.45</td>
<td>leponex</td>
</tr>
</tbody>
</table>