



# Analysis of Tricyclic Antidepressants by HPLC

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Tricyclic antidepressant drugs are tricyclic derivatives of diphenylamine or diphenylmethane. They have impetus or mood-enhancing effects. The cause of most depressive diseases is a decreasing level of biogenic amines (noradrenaline and serotonin) in the brain. Antidepressants inhibit the mechanism of inactivating these amines, which results in a higher concentration at the corresponding receptors. They have parasympatholytic side effects, which occur a few days after treatment. The desired antidepressant effects occur after two or three weeks.

Figure 1 shows the separation of the six tricyclic antidepressant drugs protriptyline, nortriptyline, doxepine, imipramine, amitriptyline and trimipramine using gradient analysis on a reversed phase column and UV detection. The autosampler temperature was set to 4 °C to avoid decomposition of the samples.

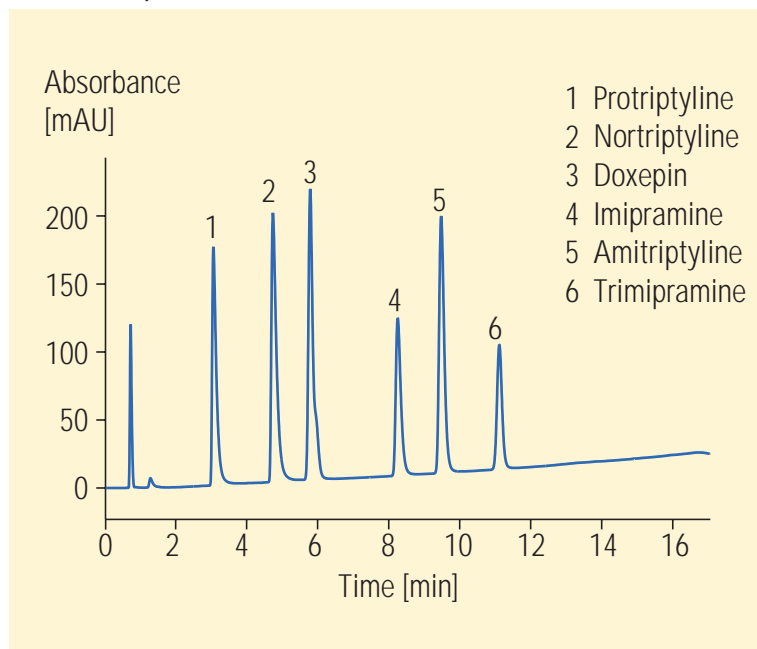


Figure 1  
Analysis of six tricyclic antidepressant drugs

## Conditions

### Column

4.6 x 75 mm Zorbax Eclipse XDB-C18, 3.5 µm

### Mobile phase

A = 0.025M  $\text{KH}_2\text{PO}_4$  in water (pH = 7),  
B = methanol

### Flow rate

1.0 ml/min

### Gradient

at 0 min 67 % B  
at 15 min 85 % B

### Column wash

at 17 min 67 % B

### UV detector

variable wavelength detector  
210 nm, standard cell

### Column compartment temperature

40 °C

### Stop time

17 min

### Post time

10 min

### Injection volume

5 µl



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## HPLC Performance

| Compound      | LOD for<br>S/N=2<br>(mg/l)* | Precision of RT<br>(RSD of 10 runs)<br>(100 mg/l)* | Precision of Area<br>(RSD of 10 runs)<br>(100 mg/l)* |
|---------------|-----------------------------|--|--|
| Protriptyline | 0.1                         | 0.32   | 0.54   |
| Nortriptyline | 0.1                         | 0.32   | 0.55   |
| Doxepine      | 0.5                         | 0.32   | 0.49   |
| Imipramine    | 1.0                         | 0.25   | 0.48   |
| Amitriptyline | 0.5                         | 0.22   | 0.48   |
| Trimipramine  | 1.0                         | 0.20   | 0.42   |

\* Injection volume: 5 µl

The performance of the HPLC method is shown in the table above.

The HPLC method presented here shows an easy but reliable and precise analysis of the tricyclic antidepressant drugs protriptyline, nortriptyline, doxepine, imipramine, amitriptyline and trimipramine. The values for LOD and precision of area show the good performance of the analysis.

## Equipment

### Agilent 1100 Series

- Quaternary pump (includes vacuum degasser)
- Thermostatted autosampler
- Thermostatted column compartment
- Variable wavelength detector, standard flow cell 10-mm path length, 13-µl cell volume

### Alternative:

- Binary pump
- Vacuum degasser
- Diode array detector standard flow cell 10-mm path length, 13-µl cell volume
- Agilent ChemStation + 3D software

### Columns

- Zorbax Eclipse XDB, 3.5 µm, 4.6 x 75 mm (Agilent part number 966967-902)
- Guard cartridges Zorbax Eclipse XDB, 5 µm, 4 x 4 mm (Agilent part number 7995118-504, 10/pk)

### Note:

Since the method was specifically developed on the Agilent 1100 Series system you might not be able to reproduce this analysis on an older system or even on a new system with lower performance. To avoid sample decomposition it is necessary to use a cooled autosampler, for example, the Agilent 1100 Series thermostatted autosampler.

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Released 12/98  
Publication Number 5968-2969E



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