

# Tricyclic Antidepressants in Plasma

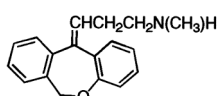
## HPLC Method

**Column:** SymmetryShield™ RP<sub>8</sub>, 4.6 x 75 mm, 3.5 μm  
**Part number:** WAT094263  
**Mobile phase:** 50 mM phosphate, pH 7/methanol 26:74  
**Flow rate:** 1.4 mL/min  
**Injection volume:** 1 mL spiked plasma plus 20 μL concentrated phosphoric acid, 100 μL injection  
**Temperature:** 29° C  
**Detection:** UV @ 254 nm

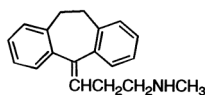
### Compounds:

Spiked at 500 ng/mL versus Blank:

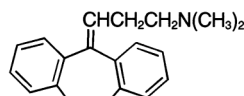
1. Nordoxepin (I.S.)
2. Nortriptyline
3. Doxepin
4. Imipramine
5. Amitriptyline
6. Trimipramine



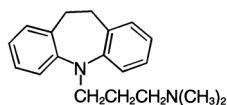
1. Nordoxepin



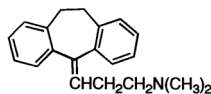
2. Nortriptyline



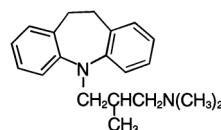
3. Doxepin



4. Imipramine



5. Amitriptyline



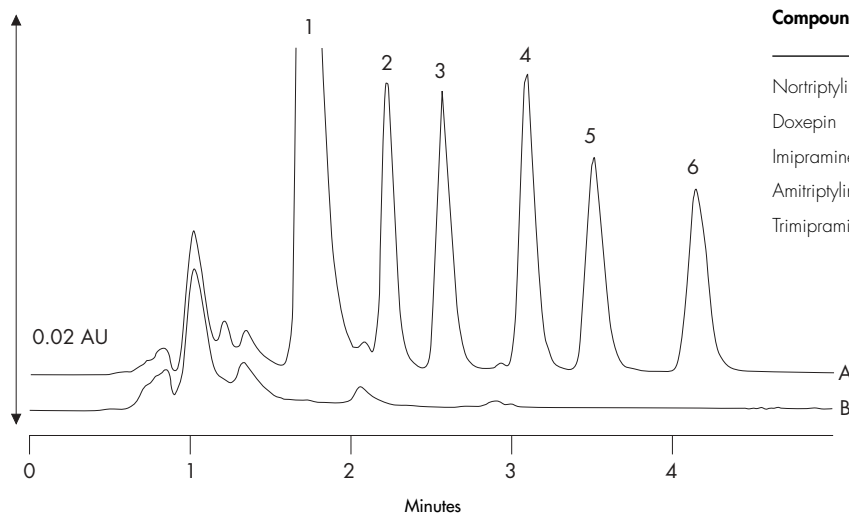
6. Trimipramine

## Oasis® HLB Extraction Method

Oasis® HLB Extraction Plate, 30 mg/96-well  
 Part Number WAT058951

|   |
|---|
| <b>Condition</b><br>1 mL methanol/1 mL water  |
| <b>Load</b><br>1 mL spiked plasma plus<br>20 μL concentrated phosphoric acid  |
| <b>Wash</b><br>A. 1 mL 2% ammonium<br>hydroxide in 5% methanol<br>B. 1 mL 2% ammonium<br>hydroxide in 65% methanol<br>C. 1 mL 2% acetic acid in 5% methanol |
| <b>Elute</b><br>600 μL 65% methanol   |
| Add internal standard (I.S.)<br>60 μL 36 μg/mL nordoxepin in<br>10% ammonium hydroxide  |

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



| Compound      | 500 ng/mL |            | 100 ng/mL |            |
|---------------|-----------|------------|-----------|------------|
|               | Recovery  | RSD (n=96) | Recovery  | RSD (n=95) |
| Nortriptyline | 92.3%     | 1.4%       | 90.8%     | 5.7%       |
| Doxepin       | 90.6%     | 1.4%       | 90.4%     | 4.7%       |
| Imipramine    | 92.2%     | 1.7%       | 86.4%     | 5.3%       |
| Amitriptyline | 90.2%     | 1.6%       | 85.3%     | 5.8%       |
| Trimipramine  | 90.3%     | 1.9%       | 89.8%     | 6.1%       |