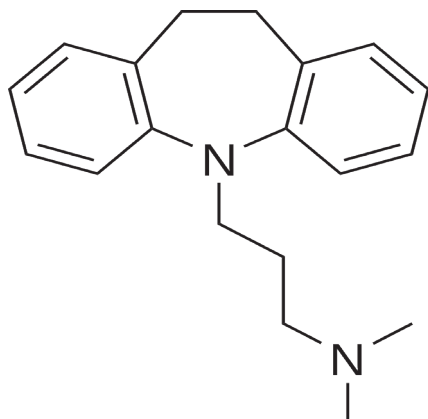


Tricyclic Antidepressants In Serum and Plasma For HPLC Using: 200 mg Clean Screen® Extraction Column



UCT Part Numbers

ZSDAU020
Clean Screen® DAU
10 mL, 200 mg sorbent
Without Tips

Or

ZCDAU020
Clean Screen® DAU
10 mL, 200 mg sorbent
With CLEAN-THRU® Tips

Procedure:

1. Prepare Sample

- To 2 mL of 100 mM phosphate buffer (pH= 6.0) add internal standard*. Add 1 mL of plasma/serum.
- Mix/vortex.
- Centrifuge for 10 minutes at 2000 rpm and discard pellet.
- Sample pH should be 6.0 ± 0.5 . Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

2. Condition Clean Screen® Extraction Column

- 1 x 3 mL CH₃OH.
 - 1 x 3 mL D.I. H₂O.
 - 1 x 1 mL 100 mM phosphate buffer (pH= 6.0).
- Note:** Aspirate at < 3 inches Hg to prevent sorbent drying.

3. Apply Sample

- Load at 1 mL/minute.

4. Wash Column

- 1 x 3 mL D.I. H₂O.
- 1 x 1 mL 100 mM acetic acid.
- 1 x 3 mL CH₃OH.
- Dry column (5 minutes at >10 inches Hg).

5. Elute

- 1 x 3 mL CH₂Cl₂/IPA/NH₄OH (78:20:2);
 - Collect eluate at mL/minute or use gravity flow.
- Note:** Prepare elution solvent fresh daily. Add IPA/NH₄OH, mix, then add CH₂Cl₂ (pH 11-12).

6. Dry Eluate

- Evaporate to dryness at < 40°C.

7. Quantitate

- Reconstitute with 200 µL ethyl acetate/D.I. H₂O (1:3).
- Mix/vortex vigorously for 30 seconds.
- Inject 100 µL onto HPLC.

HPLC Conditions

HPLC Column	Propylcyano, Endcapped 4.6 mm x 150 mm, 5 µm particle size
Column Temperature	30°C
Mobile Phase	Acetonitrile/ Buffer/ Methanol (60:25:15)
Buffer	0.01 M K ₂ HPO ₄ adjusted to pH 7.0 with H ₃ PO ₄
Flow Rate	1.75 mL/min



Analytes and Extraction Efficiency

Compound	Retention Time (min)	% Recovery	% RSD	Cerilliant #
Trimipramine ISTD*	2.048	100.0%	5.53%	T-904
Doxepin	3.048	96.5%	8.04%	D-905
Amitriptyline	3.433	98.9%	5.64%	A-923
Imipramine	3.865	97.2%	6.09%	I-902
Nortriptyline	5.349	88.9%	9.49%	N-907
Nordoxepin	5.788	85.0%	5.29%	D-007
Desipramine	6.067	85.3%	5.04%	D-906
Protriptyline ISTD*	6.476	86.3%	5.39%	P-903

* Internal Standards

Hints:

- [1] Silica Based HPLC columns are sensitive to pH. To prevent dissolution of the packing especially at the head of the column, it is best to place a silica column before the injector. This will saturate the mobile phase with silica.
- [2] Secondary Amines bind to glass and polyethylene. It is recommended to silylate all surfaces that come in contact with the sample. Immersion into 5% DMCS in toluene or vapor deposition will deactivate the surface by silylation.
- [3] To ensure the proper strength of elution solvent measure the apparent pH of the elution solvent. It should be pH 10 or higher. Add 1-2% of Ammonium Hydroxide and check again.

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