



TRICYCLIC ANTIDEPRESSANTS IN SERUM AND PLASMA FOR HPLC USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU® Tips

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 1 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 K₂HPO₄ adjusted to pH 7.0 with H₃PO₄

FLOW RATE = 1.75 mL/min.

ANALYTES AND EXTRACTION EFFICIENCY

<u>COMPOUND</u>	<u>Retention Time (min)</u>	<u>% Recovery</u>	<u>%RSD</u>	<u>Cerilliant #</u>
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.