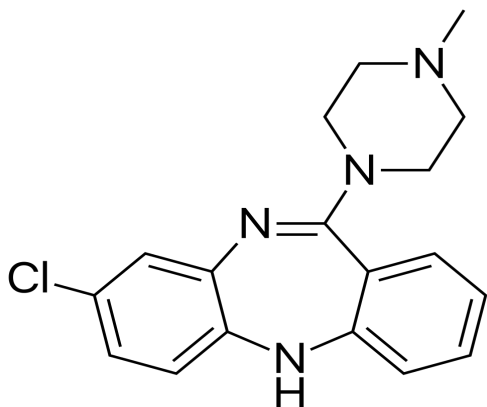


Clozapine and Metabolites In Whole Blood, Serum/Plasma, and Urine Using: 300 mg Clean-Up® Extraction Column



UCT Part Numbers

CECN4123
Clean-Up®
Cn4 200 mg 3 mL

Procedure:

1. Prepare Sample

- To 1 mL of 100 mM phosphate buffer (pH= 6) add internal standard.*
- Add 1 mL of blood, serum/plasma or urine. Add 2 mL of 100 mM phosphate buffer (pH=6).
- Mix/vortex Centrifuge as appropriate.

2. Condition Clean Screen® CECN41L# Extraction Column

- 1 x 1 mL MeOH.
 - 1 x 1 mL H₂O.
 - 1 x 0.5 mL 100 mM phosphate buffer (pH= 6).
- Note:** Aspirate at < 3 inches Hg to prevent sorbent drying.

3. Apply Sample

- Load sample at 1-2 mL/minute.

4. Wash Column

- 1 x 1 mL D.I. Water
- 1 x 0.5 mL 1% ammonium hydroxide in D.I. water.
- Dry column (5 minutes at > 10 inches Hg).

5. Elute Clozapine

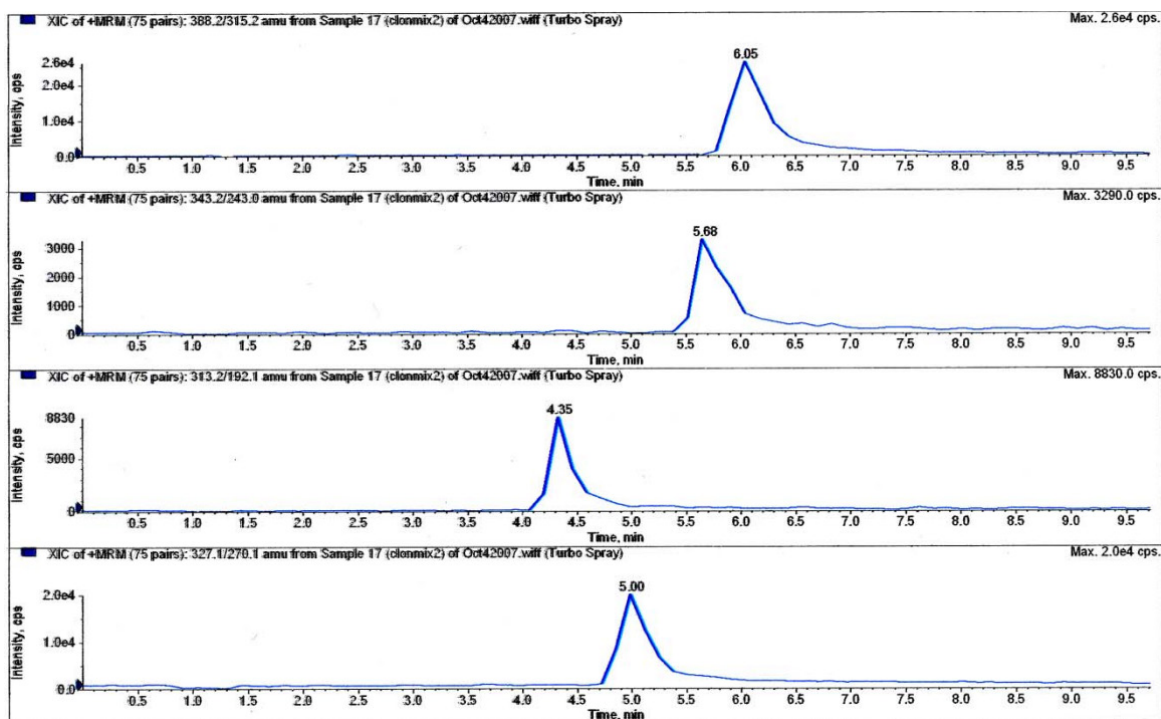
- 1 x 0.2 mL 1% ammonium hydroxide in methanol.
- Collect eluate at 1-2 mL /minute.
- Inject 5 µL (LCMSMS).
- Inject 20 µL (LC-UV).



Instrument Conditions

Column	150 x 2.1 mm (3 µm) Zorbax: Agilent Technologies
Mobile Phase	Acetonitrile: 0.1% Formic acid (33:67)
Flow Rate	0.35 mL/minute
Column Temperature	ambient
Detector	API 2000 MS/MS. HP1100 Diode-Array (230 nm).

Compound	MRM Transition	Cerilliant #
Clozapine	327.1/270.1	C-059
Desmethyloclozapine	313.1/192.1	
Clozapine-N-oxide	343.1/243.1	
*Flurazepam	388.1/315.2	F-003



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