



**CLOZAPINE AND METABOLITES IN WHOLE BLOOD,
SERUM/PLASMA AND URINE USING: 300 mg CLEAN-UP®
EXTRACTION COLUMN**

PART #: CECN41L3

LC-MSMS / LC-UV

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 out.

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).

Flowrate: 0.35 mL/minute.

Column Temperature: ambient.

Detector: API 2000 MS/MS.

HP1100 Diode-Array (230 nm).

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

