



**CLOZAPINE AND METABOLITES IN WHOLE BLOOD,
SERUM/PLASMA AND URINE USING 200 mg CLEAN-UP[®]
EXTRACTION COLUMN AND LC-MS/MS or HPLC-UV ANALYSIS**

PART #:

CECNP123 – CLEAN-UP[®] CYANOPROPYL 200 mg, 3 mL Tube

1. PREPARE SAMPLE:

To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standard.*

Add 1 mL of blood, serum/plasma or urine. Add 2 mL of 100 mM phosphate buffer (pH 6.0).

Mix/vortex Centrifuge as appropriate.

2. CONDITION CLEAN-UP[®] CECNP123 EXTRACTION COLUMN:

1 x 1 mL Methanol.

1 x 1 mL H₂O.

1 x 0.5 mL 100 mM phosphate buffer (pH 6.0).

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. H₂O

1 x 0.5 mL 1% NH₄OH in D.I. H₂O.

Dry column (5 minutes at > 10 inches Hg).

5. ELUTE CLOZAPINE:

1 x 0.2 mL 1% NH₄OH in Methanol.

Collect eluate at 1-2 mL /minute.

Inject 5 µL (LC-MS/MS).

Inject 20 µL (HPLC-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/min.

Column Temperature: ambient.

Detector: API 2000 MS/MS.

HP1100 Diode-Array (230 nm).

<u>Compound</u>	<u>MRM Transition</u>
Clozapine	327.1/270.1
Desmethylozapine	313.1/192.1
Clozapine-N-oxide	343.1/243.1
*Flurazepam	388.1/315.2

