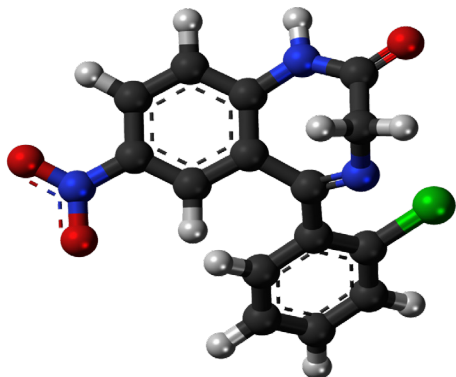


Clonazepam/Flunitrazepam & Metabolites In Blood, Plasma/Serum and Urine For LC-MS/MS Confirmations Using: 200 mg Clean Screen® CSBNZ Extraction Column



UCT Part Numbers

CSBNZ203
Clean Screen BNZ
200 mg 3 mL

Procedure:

1. Prepare Sample

- To 1 mL of 100 mM phosphate buffer (pH 6) add internal standards*.
- Add 1 mL whole blood, Serum/Plasma or Urine. Add 3 mL of 100 mM phosphate buffer (pH 6).
- Vortex and centrifuge as appropriate.

2. Condition Column

- 1 x 3 mL CH₃OH.
 - 1 x 3 mL 100 mM phosphate buffer (pH 6).
- Note:** Aspirate at < 3 inches Hg to prevent sorbent drying.

3. Apply Sample

- Load at 1 to 2 mL/minute.

4. Wash Column

- 1 x 3 mL of 5 % (v/v) acetonitrile in 100 mM phosphate buffer (pH= 6).
- Dry column (5 minutes at > 10 inches Hg).
- 1 x 3 mL of hexane.
- Dry column (5 minutes at > 10 inches Hg).

5. Elute Clonazepam / 7-Aminoclonazepam - Flunitrazepam/7 - Aminoflunitrazepam

- 2 x 3 mL ethyl acetate / ammonium hydroxide (96:4).
- Collect eluate at 1-2 mL /minute.

6. Evaporation

- Evaporate eluates under a gentle stream of nitrogen < 40°C
- Dissolve residue in 50 µL CH₃OH.

Instrument Conditions

Column	150 x 2.1 mm (3 µm) Gold PFP (ThermoFisher)
Mobile Phase	Acetonitrile: 0.1% Formic acid (33:67)
Flowrate	0.35 mL/ minute
Injection Volume	5 µL
Column Temperature	ambient
Detector	API 2000 MS/MS

Compound	MRM Transition	Cerilliant #
7-Aminoclonazepam	286.1/121.1	A-915
*7-Aminoclonazepam-D4	290.2/121.1	A-917
Clonazepam	316.0/270.2	C-907
*Clonazepam-D4	320.1/270.4	C-905
7-Aminoflunitrazepam	284.2/135.0	A-912
*7-Aminoflunitrazepam-D7	291.3/138.2	A-917
Flunitrazepam	314.1/268.2	F-907
*Flunitrazepam-D7	321.1/275.2	F-915



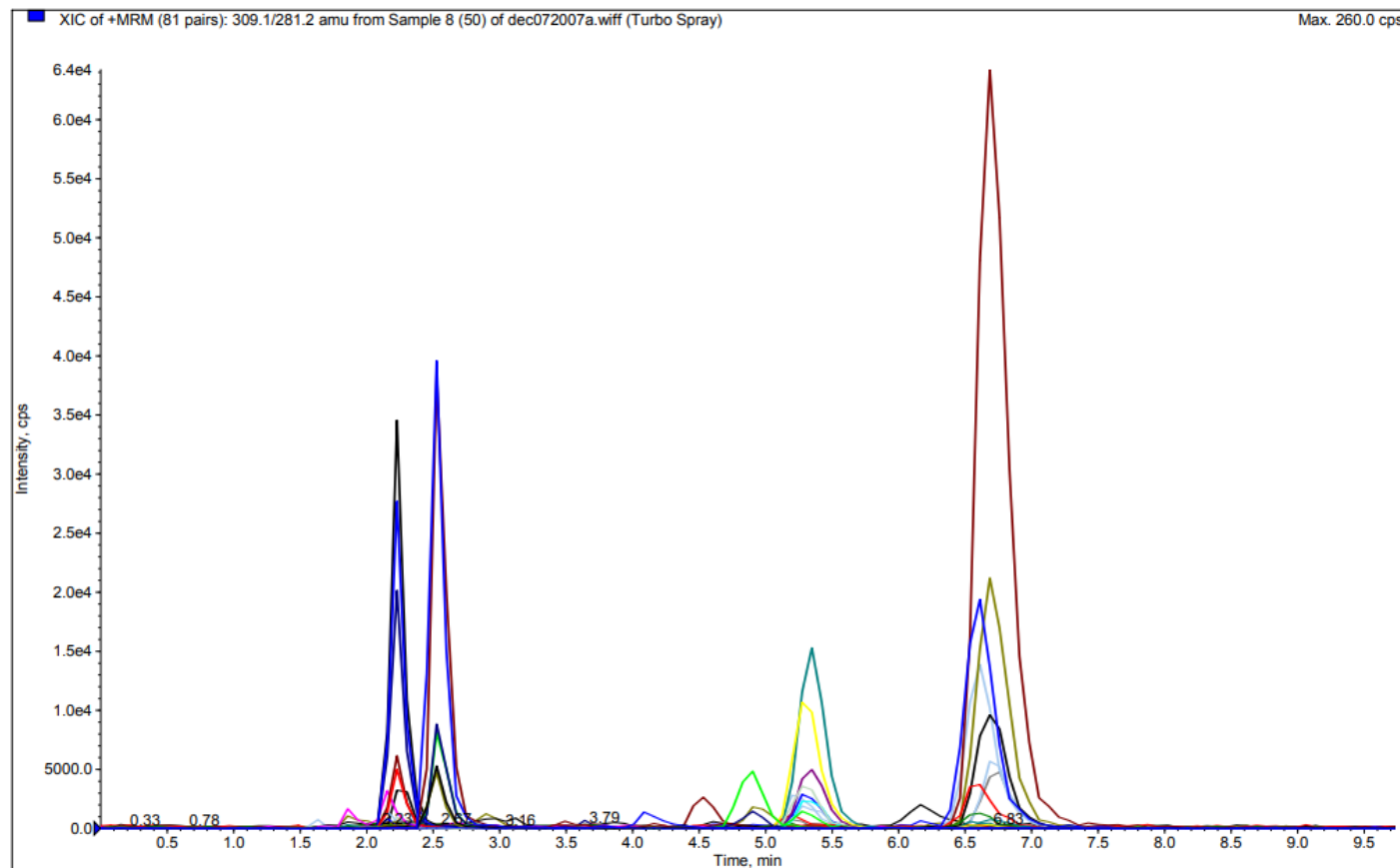
Chromatogram of:

7- aminoclonazepam/ 7-aminoclonazepam-D4

7-aminoflunitrazepam/ 7-aminoflunitrazepam-D7

Clonazepam/ Clonazepam-D4

Flunitrazepam/ Flunitrazepam-D7

**Recovery:** > 90% (N= 20)**LOD:** 1 ng/mL

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