



**CLONAZEPAM / FLUNITRAZEPAM & METABOLITES IN BLOOD,
PLASMA/ SERUM AND URINE FOR LC-MSMS CONFIRMATIONS
USING: 200 mg CLEAN SCREEN[®] CSBNZ EXTRACTION COLUMN**
Part #: CSBNZ203

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 out.

3. APPLY SAMPLE:

Load sample at 1-2 mL / minute.

4. WASH COLUMN:

1 x 3 mL of 5 % (v/v) acetonitrile in 100 mM phosphate buffer (pH6).
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°.
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.

<u>Compound</u>	<u>MRM Transition</u>	<u>Cerilliant #</u>
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

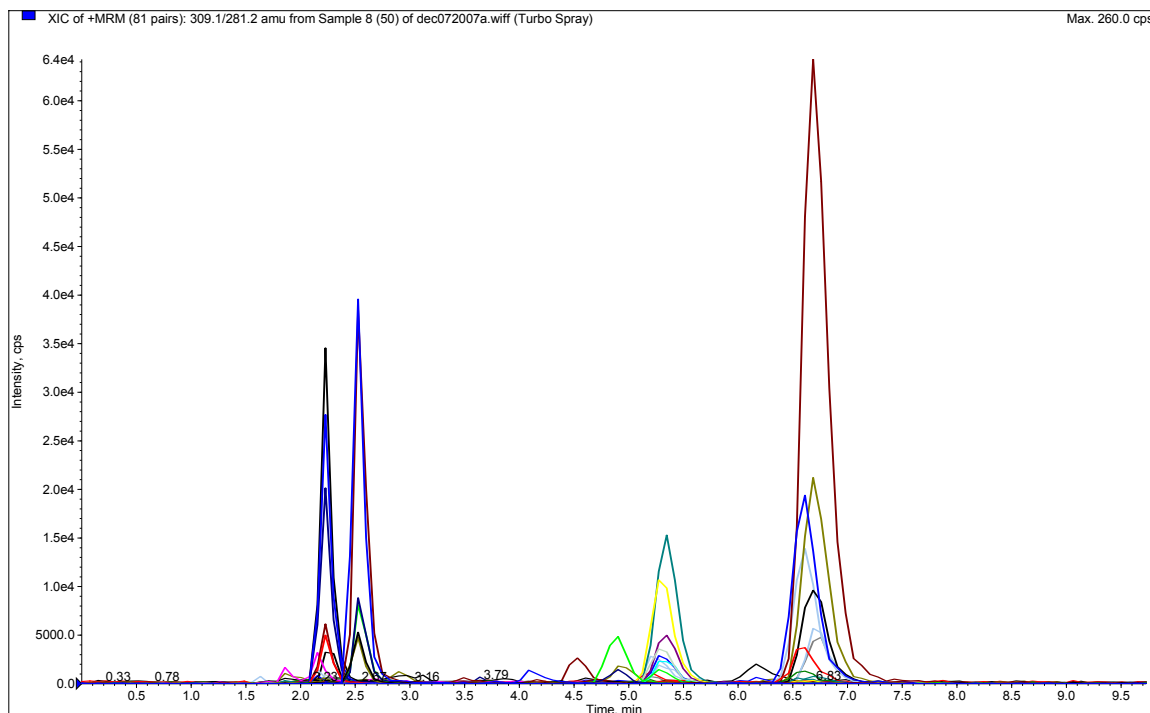
Chromatogram of:

7- aminoclonazepam/ 7-aminoclonazepam-D4

7-aminoflunitrazepam/ 7-aminoflunitrazepam-D7

Clonazepam/ Clonazepam-D4

Flunitrazepam/ Flunitrazepam-D7



Recovery: > 90% (N=20)

LOD: 1 ng/ mL