

Benzodiazepine Screen: Blood, Serum, Urine and Tissue For LC-MS/MS Using: 200 mg Clean Screen® BNZ 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 blood/ urine or 1 g of (1:4) tissue homogenate. Mix/ vortex. Add 3 mL of 100 mM phosphate buffer (pH= 6).
- Sample pH should be 6.0 ± 0.5 .
- Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate
- Vortex mix.
- Centrifuge as appropriate.

Procedure for Urine

- To 1 mL of acetate buffer (pH=5.0) containing 5000 F units/mL β -glucuronidase. Add internal standards*.
- To this solution add 1 mL of urine.
- Mix/Vortex.
- Hydrolyze for 3 hrs at 65 °C.
- Allow to cool.
- Centrifuge for 10 minutes at 2000 rpm and discard pellet.
- Add 3 mL of 100 mM phosphate buffer (pH 6.0) and mix.

2. Condition Clean Screen® CSBNZ Extraction Column

- 1 x 3 mL MeOH.
 - 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 (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 Benzodiazepines

- 1 x 3 mL ethyl acetate: ammonia (98:2 v/v).
- Collect eluate at 1-2 mL /minute.

6. Evaporation

- Evaporate eluate under a gentle stream of nitrogen < 40 °C.

7. Reconstitute

- Reconstitute sample in 50 μ L of 0.02% formic acid (aqueous)



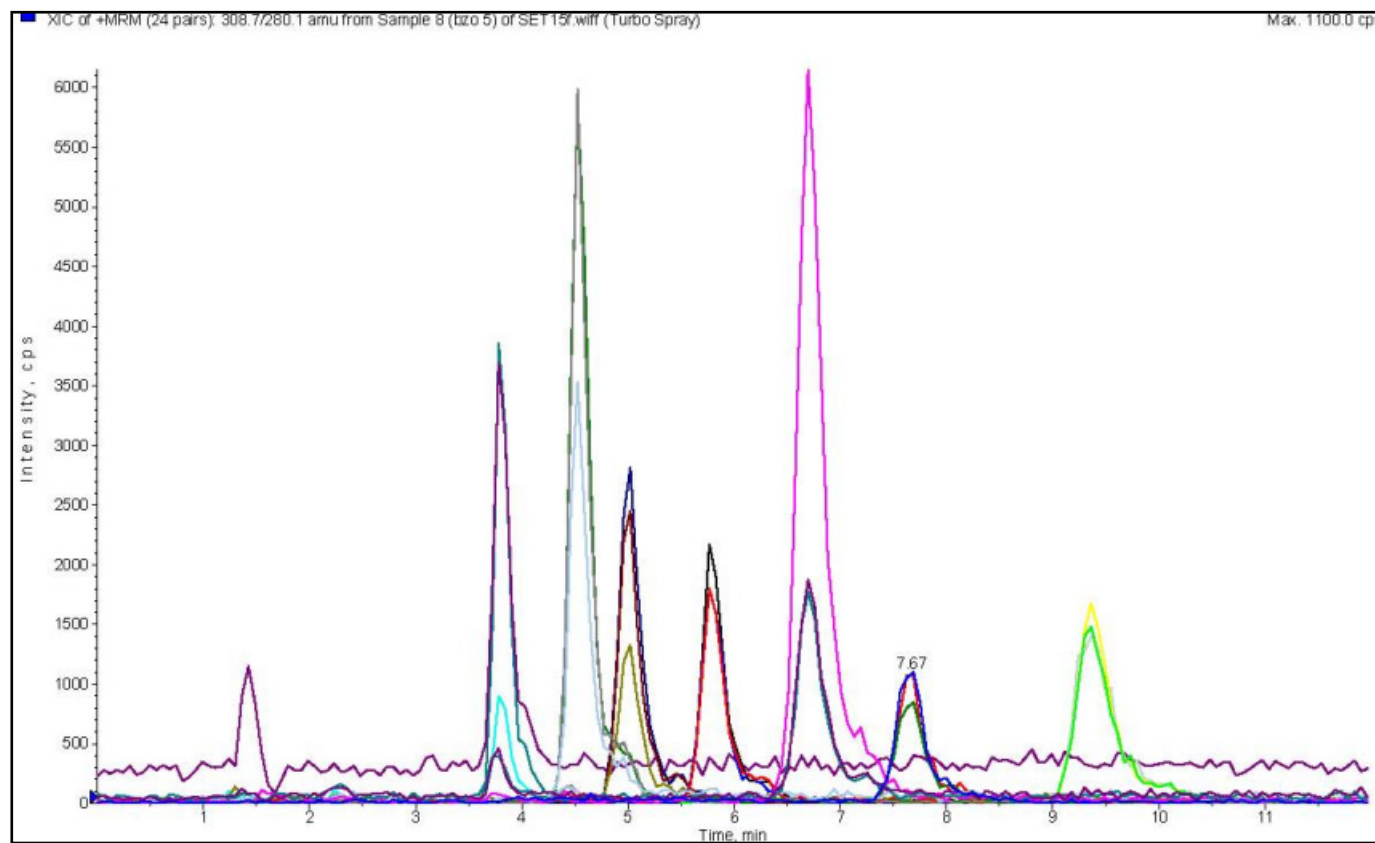
Instrumental Conditions: LC-MS/MS

Column	Thermo Fisher Gold PFP 150 x 2.1 mm, 3 µm
Mobile Phase	30: 70 (Acetonitrile: 0.02% aq. Formic acid)
Flow Rate	0.35 mL/ min
Column Temperature	Ambient
Injection Volume	5 µL
Mass Spectrometer	Applied Biosystem API2000

Compound	MRM Transition	Cerilliant #
Alprazolam	309.1/281.2	A-903
*Alprazolam-D5	314.1/286.2	A-910
Alpha-hydroxyalprazolam	325.1/242.9	A-907
*Alpha-hydroxyalprazolam-D5	330.1/302.2	A-902
Chlordiazepoxide	300.1/227.0	C-022
Diazepam	285.5/192.5	D-907
*Diazepam	292.2/198.2	D-902
Lorazepam	321.1/275.1	L-901
*Lorazepam-D4	325.1/279.0	L-902
Nordiazepam	271.1/140.1	N-905
*Nordiazepam-D5	275.6/140.1	N-903
Oxazepam	287.1/241.1	O-902
*Oxazepam-D5	290.2/198.2	O-901
Temazepam	301.1/255.1	T-907
*Temazepam-D5	306.1//260.1	T-902

*suggested internal standards



Chromatogram of 5 ng Benzodiazepines (without IS)

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