



**BENZODIAZEPINE SCREEN: BLOOD, SERUM, URINE AND TISSUE
FOR LC-MSMS USING: 300 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 blood/ Urine or 1g 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 (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 BENZODIAZEPINES

1 x 3 mL ethyl acetate; ammonia (98:2 v/v).

Collect eluate at 1-2 mL /minute.

6. EVAPORATION

Evaporate eluates under a gentle stream of nitrogen < 40°.

7. Reconstitute sample in 50 µL of 0.02% formic acid (aqueous).

INSTRUMENTAL CONDITIONS: LC-MSMS

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

<u>Compound</u>	<u>MRM Transition</u>	<u>Cerilliant#</u>
Alprazolam	309.1/281.2	A-903
*Alprazolam-D5	314.1/286.2	A-910
Alphahydroxyalprazolam	325.1/242.9	A-907
*Alphahydroxyalprazolam-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

Chromatogram of 5 ng Benzodiazepines (without IS)

