



**BENZODIAZEPINES IN WHOLE BLOOD FOR GC OR GC/MS
CONFIRMATIONS USING:
300 mg CLEAN SCREEN[®] EXTRACTION COLUMN**
Part #: ZSBNZ030 without Tips or ZCBNZ030 with CLEAN-THRU[®] Tips

1. PREPARE SAMPLE

To 1 mL of pH 6 buffer add Internal standards*, add 2 mL of whole blood and vortex mix. Add 5 mL of pH 6 buffer Sonicate with a probe sonifier for ~10 seconds and centrifuge at ~2700rpm for 15 minutes.

2. CONDITION CLEAN SCREEN[®] EXTRACTION COLUMN

1 x 3 mL Ethyl Acetate.
1 x 3 mL MeOH.
1 x 3 mL DI H₂O.
1 x 3 mL 0.1M phosphate buffer (pH 6.0).

3. APPLY SAMPLE

Load sample by gravity.

4. WASH SAMPLE

1 x 3 mL DI H₂O.
1 x 3 mL 5% acetonitrile in 0.1M phosphate buffer (pH 6.0).
Dry columns 5 minutes at full vacuum or > 10 inches Hg.
1 x 3 mL Hexane.

5. ELUTE BENZODIAZEPINES

2 x 3 mL Ethyl Acetate.

6. DRY ELUATE

Evaporate to dryness under nitrogen at ~ 55° C.
Add external standards.*

7. DERIVATIZE

Add 100µL acetonitrile and 100µL MTBSTFA w/1% t-BDMCS.
Heat for 30 minutes at 70° C.
Remove from heat source to cool.
Inject 1µL into GC/MS-NCI.
Note: Do not evaporate MTBSTFA solution.

GC/MS Conditions:

Agilent 6890/5975 inert MSD
Inlet 260°, Split injection 2:1
Column: ZB-5MS, 15m, 0.25mm ID, 0.25µm film
Temperature program: 70° hold 0.25 min, ramp 20° per min to 300°, hold 4 min.
NCI parameters: Source 150°, Quad 106°, Methane as collision gas

Suggested Internal standards : Diazepam-D5 and Lorazepam-D4.