

**Benzodiazepines in Whole Blood for GC or GC/MS Confirmations Using:  
300mg Clean Screen® Extraction Column**

(Part # ZSBNZ030 without tips or  
ZCBNZ030 with tips)

**1. PREPARE SAMPLE**

Standards were prepared to the following concentrations in 2mL blood:

Lorazepam: 25 ng/mL

Oxazepam, Temazepam, Midazolam: 50 ng/mL each

Nordiazepam: 200 ng/mL

Diazepam, Alprazolam, 7-aminoclonazepam,

Triazolam, Clonazepam: 100 ng/mL each.

Blood preparation: 6mL of pH6 buffer is added to a 15-mL polypropylene tube.

Standards as above are added, then 2mL of hemolyzed postmortem blood. Samples are sonified using a probe sonifier for ~10 seconds and centrifuged at ~2700rpm for 15 minutes.

**2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN**

1 x 3 ml Ethyl Acetate

1x 3 ml MeOH

1 x 3 ml DI H<sub>2</sub>O

1 x 3ml 0.1M phosphate buffer (pH 6.0)

**3. APPLY SAMPLE**

Load sample by gravity

**4. WASH SAMPLE**

1 x 3 ml DI H<sub>2</sub>O

1 x 3mL 5% acetonitrile in 0.1M phosphate buffer (pH 6.0)

Dry columns 5 minutes at full vacuum or > 10 inches Hg)

1 x 3ml Hexane

**5. ELUTE BENZODIAZEPINES**

2 x 3ml 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

\*External standards: 200 ng/mL d5-diazepam and 50 ng/mL d4-lorazepam

**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