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**** “BENCH METHOD” FOR DETECTABUSE® GRAVITY SERIES
GV-65 / GV-65C METHOD FOR THE ANALYSIS OF
URINARY BENZODIAZEPINES**

SAMPLE SIZE

1 mL

INTERNAL STANDARD

Appropriate Amount

HYDROLYSIS

0.5 mL 0.25 M Acetate Buffer pH 5

5,000 units beta-glucuronidase per mL sample

2 hrs at 55-60°C

pH

1.0 mL 0.25 M Phosphate Buffer pH 10

Adjust pH to 10 with 2N KOH

COLUMN CONDITIONING – GV-65C

1 mL MeOH – Extract within 20 min

COLUMN CONDITIONING – GV-65

1 mL MeOH – Extract within 20 min

1 mL Bisulfite Solution

1 mL Deionized Water

EXTRACTION

Decant samples onto columns

RINSE

2.0 mL MeOH:water (20:80)

DRY

>7 mm Hg for 10 minutes

ELUTION

1.5 mL Ethyl Acetate:Isopropyl Alcohol:TEA (93:5:2)

Evaporate at 40°C - to dryness (over-drying may cause losses)

DERIVATIZE

MTBSTFA:Ethyl Acetate (50:50)

Heat at 70°C for 20 min

Transfer to vials and cap

**** SEE COMPLETE METHODS FOR DETAILS**