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**\*\* “*BENCH METHOD*” FOR DETECTABUSE® “NO VACUUM” GRAVITY GV-65 / GV-65C METHOD FOR THE ANALYSIS OF HYDROLYZED OR “FREE” EXTENDED OPIATES IN URINE, SERUM OR ORAL FLUIDS BY GC/MS**

**SAMPLE SIZE**

1 mL

**INTERNAL STANDARD**

Appropriate Amount

**OXIME FORMATION for TMS:**

Oxime or methoxy Hydroxylamine or Methoxyamine (10%)

**HYDROLYSIS** – Enzymatic or Acid

0.5 mL 1.0 M Acetate Buffer

5000 units beta-glucuronidase per mL sample

2 hr at 55 – 60° C

**pH**

pH samples to 2-3

10% HCL for enzyme hydrolysis

Phosphate Buffer and 20% KOH for acid hydrolysis

**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**

3.0 mL 0.01% HCl

2.0 mL MeOH

1.0 mL Ethyl Acetate

**ELUTION**

1.5 mL n-butyl chloride:ethyl acetate:TEA (76:20:4)

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

**DERIVATIZE**

MBTFA:Ethyl Acetate (50:50)

BSTFA:Ethyl Acetate (50:50)

Propionic Anhydride:Pyridine (50:50)

Acetic Anhydride: Pyridine (50:50)

Heat, evaporate or transfer to vials and cap

**\*\* SEE COMPLETE METHODS FOR DETAILS**