



DETECTABUSE™ "NO VACUUM" GRAVITY SERIES GVSA METHOD FOR THE ANALYSIS OF GAMMA-HYDROXYBUTYRATE (GHB) IN URINE BY GC-MS

Revised: May, 2003

This method is a preliminary procedure for investigational use only. Although it has performed well in our laboratory it must be validated by your laboratory before it is used to report patient values. We would appreciate your comments on it's performance and welcome Your suggestions for improvements or enhancements.

General - Allow all solutions to pass through the column by gravity flow. All steps are done without column drying (i.e. vacuum or positive pressure).

1. Column Conditioning:

- Add 3 ml 0.2M Acetic Acid in Acetone.
- Add 3 mL 10% HCl in H₂O.
- Add 6 mL Deionized H₂O.

2. Sample Preparation:

- Add 1 ml 0.025M **Hepes Buffer**, pH 8.2 to 50 uL of urine or serum.
- Add 1 ug GHB-d6 as internal standard.
- Vortex mix for 10 seconds.

Note: Prepare Hepes Buffer, 0.025 as follows:

- Weigh 6.0 Gm Hepes (N-Hydroxyethyl Piperazine N2 Ethanesulfonic Acid).
- Dissolve in H₂O and bring it up to one liter.
- Adjust the pH to 8.2 with 2N Sodium Hydroxide.

2. Sample addition:

- Pour prepared sample onto the column.
- Add 6.0 mL of Deionized Water as a wash solution to remove excess Hepes buffer.
- Add 2.0 mL of Acetone to remove non-ionic contaminants.

3. Elution:

- Elute with 2.0 mL of 0.05M Acetic Acid in Methanol.
- Dry eluates at less than 45.°C

4. Derivatization:

- Add 100 uL Ethyl Acetate and 50 uL BSTFA with 1% TMCS. Incubate for 20 minutes @ 65°C.
- Allow to cool.
- Inject 1 uL of derivatized sample.

GC/MS:

GC/MS was performed using a Hewlett Packard Series 5890 GC coupled to a HP 5970 series MSD. A 15 M cross linked capillary column was used (Alltech EC-5), I.D. 0.25 mm, film thickness 0.25 uM). The MSD is run in SIM mode.

The following ions are monitored at a dwell time of 20 ms:

- GHB-di-TMS 233, 234, 235 Retention = 4.80 minutes
- GHB-D6-di-TMS 239, 240, 241, Retention = 4.74 minutes

Temperatures:

- Injector Temp: 265°C
- Transfer Line: 290°C

Oven Temp. Program:

- 60°C - 130°C @ 10°/min.
- 130°C - 285°C @ 40°/min

Go to:

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