Biochemical Diagnostics, Inc.
A Kova International Company

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DETECTABUSE® GRAVITY SERIES GVSA-200 METHOD FOR THE ANALYSIS OF GAMMA-HYDROXYBUTYRATE (GHB) IN URINE OR SERUM BY GC/MS

SAMPLE PREPARATION - (Please see Notes and Supplemental Information before proceeding)
1. Add 1mL of 0.025M Hepes Buffer pH 8.2 to a 16 x 100 mm disposable glass culture tube.
2. Add 50 µL of sample.
3. Add 1 µg of GHB-D6 .
4. *Vortex mix for 10 seconds.*

Note: When adding an internal standard dissolved in an organic solvent to a urine or blood sample, the solvent volume must not exceed 3% of the buffered sample volume. Higher solvent concentrations may produce extraction losses.

HARDWARE SETUP - (Please refer to the Detectabuse Hardware Setup Instructions)

COLUMN CONDITIONING
1.Add 3 mL of 0.2M Acetic Acid in Acetone.
2. Add 3 mL 10% HCL in DI water.
3. Add 6 mL DI water
4.Proceed to Sample Extraction within 20 minutes of column conditioning.

SAMPLE EXTRACTION - (Please see Notes at end of this section before proceeding)
1. Pour samples onto preconditioned column.
2. Wash column with 6.0 mL of deionized water to remove excess Hepes Buffer.
3. Wash with 2.0 mL of Acetone to remove non-ionic contaminants.

Note*: If liquids do not elute freely by gravity flow, there is probably air trapped within the column bed or frits. Tapping the column mounting plate onto the vacuum box should initiate flow. Any columns that have not emptied within 5 or 6 min. may be induced with a low vacuum from a small vacuum pump.*

SAMPLE ELUTION
1. Place the column mounting plate on the elution rack loaded with corresponding labeled 12 x 75 mm or 16 x 100 mm tubes. Make sure that the hole pattern on the plate matches the hole pattern on the rack.
2. Add 2.0 mL of of 0.05M Acetic Acid in Methanol.
3. Dry under N2 or argon at less than 45°C.

Note: *If a sample does not elute freely by gravity flow, there is either air trapped within the column bed or frits or aqueous phase remaining on the column because of weak vacuum during the column drying step. In most cases, tapping the column will initiate flow. If this does not do the job, use a rubber bulb to gently push a few drops of elution solvent and trapped air into the collection tube. Allow the remainder of solvent to flow by gravity.*

DERIVATIZATION
1. To each dried extract add 100 µL ethyl acetate and 50 µL BSTFA with 1% TMCS.
2. Incubate for 20 minutes at 65°C
3. Allow to cool.
4. Transfer to vials with inserts and cap.

SUPPLEMENT - When using an automated robotic system all liquids may be allowed to flow unassisted through the column or may be pulled through the column with vacuum or pushed through with positive pressure.

Assisted flow parameters may be set as follows:

Column Conditioning - Pass through column in approximately 20 seconds (± 20%).

Sample, Sample Washes and Elution Solvent - Pass through column in approximately 60 seconds (± 20%).

Column Drying Steps – Use 12 – 15 PSI positive pressure for 40 seconds or vacuum set at 15” Hg for 30 seconds (These drying parameters are for individual columns).

GC/MS ANALYSIS

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.

MSD SIM PROGRAM Ions
Drug Monitored
GHB-di-TMS 233, 234, 235
GHB-D6-di-TMS 239, 240, 241

NOTES:

1. HEPES BUFFER: to prepare 0.025M Hepes Buffer Dissolve 6 gms of HEPES (N-Hydroxyethyl Piperazine N2 Ethanesulfonic Acid) in DI water and QS to 1 liter. Adjust pH to 8.2 with 2N Sodium Hydroxide.
2. SAMPLES AND WASHES – Allow all samples and washes to gravity flow completely through the resin bed before adding the next liquid.
3. INTERNAL STANDARDS – When preparing the Internal Standard the quantity added per mL of sample should approximate the cutoff value of the compound(s) being tested for. The Internal Standard can almost always be prepared in an aqueous matrix. If prepared in an organic solvent the solvent must not exceed 3% of the final prepared sample.
4. TURBID SAMPLES may need to be centrifuged
5. RINSE SOLVENTS should be delivered to the top part of the column to better remove the aqueous.
6. POLAR SOLVENTS used (e.g. acetonitrile and ethyl acetate) may absorb moisture. Flush bottles with nitrogen, keep stock bottles full or use sodium sulfate to minimize moisture.
7. AIR TRAPPED within the column bed or frits may prevent the liquids from eluting freely by gravity flow. Tapping the column mounting plate onto the vacuum box should initiate flow.
8. IDEAL FRAGMENTS should be determined by full scans

180 Heartland Blvd, Edgewood, NY 11717● Phone (800) 223-4835
Fax (631) 595-9204 ● www.biochemicaldiagnostics.com