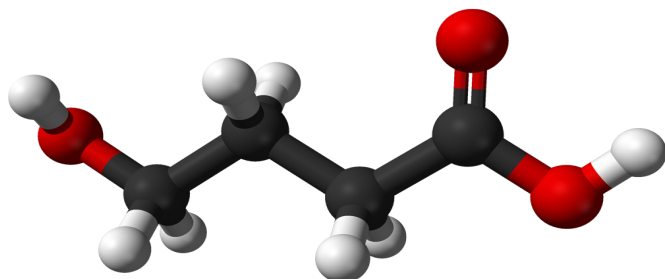


A Solid Phase Method for Gamma-Hydroxybutyrate (GHB) In Blood, Urine, Vitreous, or Tissue Without Conversion to Gamma-Butyrolactone (GBL)



UCT Part Numbers

ZSGHB020
Clean Screen® GHB
200 mg, 10 mL

SBSTFA-1-1
Selectra-Sil BSTFA
with 1% TMCS 1g vial

GHB working standard; 200 µg/mL in H₂O; prepared from Cerilliant stock 1 mg/mL.

GHB -D₆ working internal standard; 100 µg/mL; use as supplied Cerilliant stock (0.1 mg/mL).

Working Standard	Whole Blood	Concentration
10 µL	200 µL	10 µg/mL
25 µL	200 µL	25 µg/mL
50 µL	200 µL	50 µg/mL
100 µL	200 µL	100 µg/mL



Sample Pretreatment:

- Make calibration standards and Pipette 200 μ L of sample into appropriately labeled 1.5 mL plastic centrifuge tubes
Note: Samples include urine, vitreous humor or homogenized tissue (1:4)
- Add 25 μ L of internal standard.
- Add 1 mL of acetone; Vortex 15 seconds.
- Centrifuge; Transfer acetone layer to culture tubes.
- Evaporate extracts @ 70°C w/nitrogen.
- Reconstitute the dried extracts with 200 μ L of 100 mM Phosphate Buffer (pH 6.0); Vortex 15 seconds.

SPE Procedure:

1. Condition Clean Screen® GHB Extraction Column:

- a) 1 x 3 mL of CH₃OH
- b) 1 x 3 mL of D.I. H₂O
- c) 1 x 1 mL of 100 mM Phosphate Buffer (pH 6.0)

Note: Aspirate at 3 inches of Hg or less to prevent sorbent drying.

2. Apply Sample

- a) Add sample with Eppendorf pipette.
- b) Aspirate at ~1 inch Hg.

3. Elute GHB

- a) Place clean test tubes into vacuum manifold.
- b) Add 1 mL of CH₃OH/NH₄OH (99:1) to original sample test tube; Vortex.
- c) Decant onto column and collect extract.
- d) Aspirate ~1 inch Hg.

4. Concentrate

- a) Remove test tube from Vacuum Manifold.
- b) Evaporate to dryness at 70 °C using a stream of nitrogen or air.

5. Derivatize

- a) Add 100 μ L of ethyl acetate and 100 μ L of BSTFA with 1% TCMS. Mix/Vortex.
- b) Heat at 70 °C for 30 minutes.

6. Quantitate

- a) Inject 1 to 2 μ L onto gas chromatograph.



Quantitation Ion:

Compound	Primary Ion	Secondary	Tertiary	Cerilliant #
GHB-D ₆ -di-TMS	239	240	241	G-006
GHB-di-TMS	233	234	235	G-001

Quality Control NOTE: Quality control samples were prepared using drug free blood and 1 mg/mL in house stock standard prepared using GHB stock from Sigma (#H-3635). A negative, low and high QC sample was prepared and stored frozen in 0.5-mL aliquots until use.

References:

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