

A Solid Phase method for Gamma-Hydroxybutyrate (GHB) In Urine without conversion to Gamma-Butyrolactone (GBL)

(Part # ZSGHB020 without Tips or ZCGHB020 with CLEAN-THRU® Tips)

Developed by:
United Chemical Technologies, Inc.

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1. PREPARE SAMPLE

To 200 μ L of urine add internal standard (GHB-D6) and 100 μ L of 0.1 M phosphate buffer (pH 6.0).
Mix/vortex.

2. CONDITION CLEAN SCREEN® GHB EXTRACTION COLUMN

1 x 3 mL CH₃OH; aspirate.
1 x 3 mL DI H₂O; aspirate.
1 x 0.5 mL 0.1 M phosphate buffer (pH6.0); aspirate.
Note: Aspirate at < 3 inches of Hg to prevent sorbent drying.

3. APPLY SAMPLE

Place test tubes into vacuum manifold for collection.
The sample loading and wash are both collected.
Decant sample onto column. Aspirate at ~1 inch Hg.

4. WASH COLUMN

Add 1 mL of CH₃OH /NH₄OH (99/1) to original sample test tube; vortex.
Decant wash onto column.
Note: Aspirate at ~1 inch of Hg.

5. CONCENTRATE

Remove test tubes from vacuum manifold.
Evaporate to dryness at 60° C using a stream of air or N₂.

6. SAMPLE CLEAN UP

Add 200 μ L of dimethylformamide.
Add 1 mL of hexane saturated with dimethylformamide.
Mix by inversion for 5 minutes.
Centrifuge at 3000 rpm for 5 minutes.
Transfer lower dimethylformamide layer to a clean test tube.

7. CONCENTRATE

Evaporate to dryness at 50° C using a stream of air or N₂.

8. DERIVATIZE

Add 100 μ L ethyl acetate and 100 μ L BSTFA (with 1% TMCS).
Mix/vortex.

9. QUANTITATE

Inject 1 to 2 μ L onto gas chromatograph.
For MSD monitor the following ions:

GHB-diTMS	233*, 234, 235
GHB-D6-diTMS	239*, 240, 241

* Quantitation ion

Blood GHB Extraction Procedure
Using:
(United Chemical Technologies, Inc. Worldwide Monitoring® part number ZSGHB020)

by: Mr. Jim Oeldrich
Wisconsin State Crime Lab Milwaukee, WI

- 1. PREPARE SAMPLE**
To 1 mL blood sample add internal standard and 0.5 mL of 0.1 M phosphate buffer (pH 6.0).
Mix/vortex.
Rock for 10 minutes
Centrifuge for 10 minutes at 2700 rpm.
- 2. CONDITION CLEAN SCREEN® GHB EXTRACTION COLUMN**
1 x 3 mL Methanol; aspirate.
1 x 3 mL DI Water; aspirate.
1 x 0.5 mL 0.1 M phosphate buffer (pH 6.0); aspirate.
Note: Aspirate at less than 3 inches of Hg to prevent sorbent drying.
- 3. APPLY SAMPLE**
Place centrifuge tubes into vacuum manifold for collection.
The sample loading is collected.
Decant sample onto column. Aspirate at about 1 inch Hg.
After the sample is off the columns apply full vacuum for about 15 seconds to remove any residual blood.
- 4. ELUTE GHB COLUMN**
Replace the centrifuge with clean centrifuge tubes.
Add 2 mL of CH₃OH /NH₄OH (99/1) to the column. Aspirate at about 1 inch of Hg.
- 5. CONCENTRATE**
Remove test tubes from vacuum manifold.
Vortex the sample prior to concentrating.
Evaporate to dryness at 60° C using a stream of nitrogen.
- 6. SAMPLE CLEAN UP**
Add 200 µL of dimethylformamide.
Add 1 mL of hexane saturated with dimethylformamide.
Rock for 5 minutes.
Centrifuge at 5 minutes at 2700 rpm.
Transfer lower dimethylformamide layer to a clean test tube.
(If necessary transfer all liquid to a clean tube and allow to separate, then proceed to extract the lower layer)
- 7. CONCENTRATE**
Evaporate to dryness at 50° C using a stream of air or Nitrogen.
- 8. DERIVATIZE**
Add 25 µL ethyl acetate and 25 µL BSTFA w/1% TMCS
Mix/vortex.
Heat at 70° C for 30 minutes.
- 9. QUANTITATE**
Inject a 1 to 2 µL of the sample onto GC/MS.

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In Blood, Urine, Vitreous or Tissue
without conversion to Gamma-Butyrolactone (GBL)
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GHB working standard; 200 µg/mL in H₂O; prepared from Radian stock 1 mg/mL.

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

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

- 1) Make calibration standards and pipet 200 µL of QC and unknown bloods* into appropriately labeled 1.5 mL plastic centrifuge tubes.

***ALL SAMPLES INCLUDING URINE, VITREOUS OR HOMOGENIZED TISSUES (1:4)**

- 2) Add 25 µL of internal standard.
- 3) Add 1 mL of acetone; vortex 15 seconds
- 4) Centrifuge; transfer acetone layer to culture tubes.
- 5) Evaporate extracts @ 80°C w/Nitrogen
- 6) Reconstitute the dried extracts with 200 µL of 0.1 Phosphate Buffer pH 6.0 buffer; vortex 15 seconds.
- 7) **Prepare CLEAN SCREEN® GHB** (200 mg in a 10 mL tube)
Extraction columns as follows:

- 1 x 3 mL of CH₃OH; aspirate.
- 1 x 3 mL of DIH₂O; aspirate.
- 1 x 3 mL of 0.1 M Phosphate Buffer (pH 6.0) aspirate.

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

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8) APPLY SAMPLE

Place test tubes into vacuum manifold for collection.

The sample loading and wash are both collected.

Decant sample onto column. Aspirate at ~1 inch Hg.

9) ELUTE GHB

Add 1 mL of CH₃OH/NH₄OH (99/1) to original sample test tube; vortex

Decant onto column and collect extract.

Aspirate ~1 inch Hg.

10) Concentrate

Remove test tube from Vacuum Manifold.

Evaporate to dryness at 70°C using a stream of Nitrogen or air.

11) Derivatize

Add 100 µL of ethyl acetate and 100 µL of BSTFA with 1% TCMS. Mix/Vortex.

No heating is required.

12) QUANTITATE

Inject 1 to 2 µL onto gas chromatograph.

For MSD monitor the following ions:

GHB-diTMS 233*, 234, 235

GHB-D6-diTMS 239*, 240, 241

*Quantitation ion

Quality Control NOTE:

Quality control samples were prepared using drug free blood and 1mg/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.



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