A SOLID PHASE METHOD FOR GAMMA-HYDROXYBUTYRATE (GHB) IN BLOOD, URINE, VITREOUS OR TISSUE WITHOUT CONVERSION TO GAMMA-BUTRYLACTONE (GBL)

Part #:
ZSGHB020 – CLEAN SCREEN® GHB 200 mg, 10mL Tube
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TCMS

GHB working standard; 200 µg/mL in D.I. H_2O; prepared from Cerilliant stock 1 mg/mL.

GHB –D6working 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

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

   NOTE: *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 @ 70°C w/nitrogen.

6. Reconstitute the dried extracts with 200 µL of 100 mM Phosphate Buffer (pH 6.0); Vortex 15 seconds.

7. **CONDITION CLEAN SCREEN® GHB EXTRACTION COLUMN:**
   1 x 3 mL of CH_3OH.
   1 x 3 mL of D.I. H_2O.
   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.

8. **APPLY SAMPLE:**
   Add sample with Eppendorf pipette.
   Aspirate at ~1 inch Hg.

9. ELUTE GHB:

Place clean test tubes into vacuum manifold

Add 1 mL of CH_3OH/ NH_4OH (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.

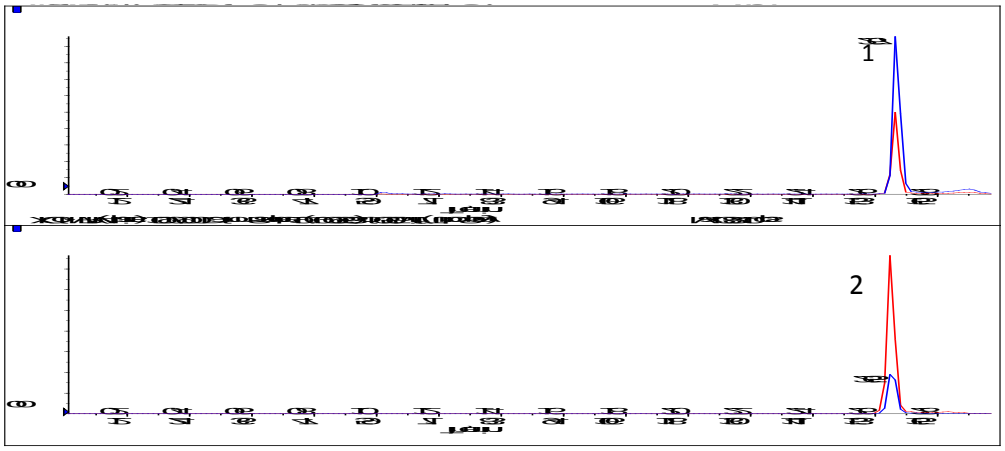
Evaporate to dryness at 70 °C using a steam of nitrogen or air.

11. RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 20µL.
- **GC-MS**: Dissolve residue in 100 µL of Ethyl Acetate and 100 µL of BSTFA with 1% TMCS. Mix/Vortex; Heat at 70°C for 30 minutes.

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. GHB	103.02	84.9	2.67
2. GHB-D_6	109.13	90.0	2.65

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O

Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Flow Rate: 1.25mL/minute

Polarity: Negative

Reconstitute: 100µl

Injection Volume: 20µl

LC Column: Biphenyl HPLC Column 150 x 4.6mm 5µm

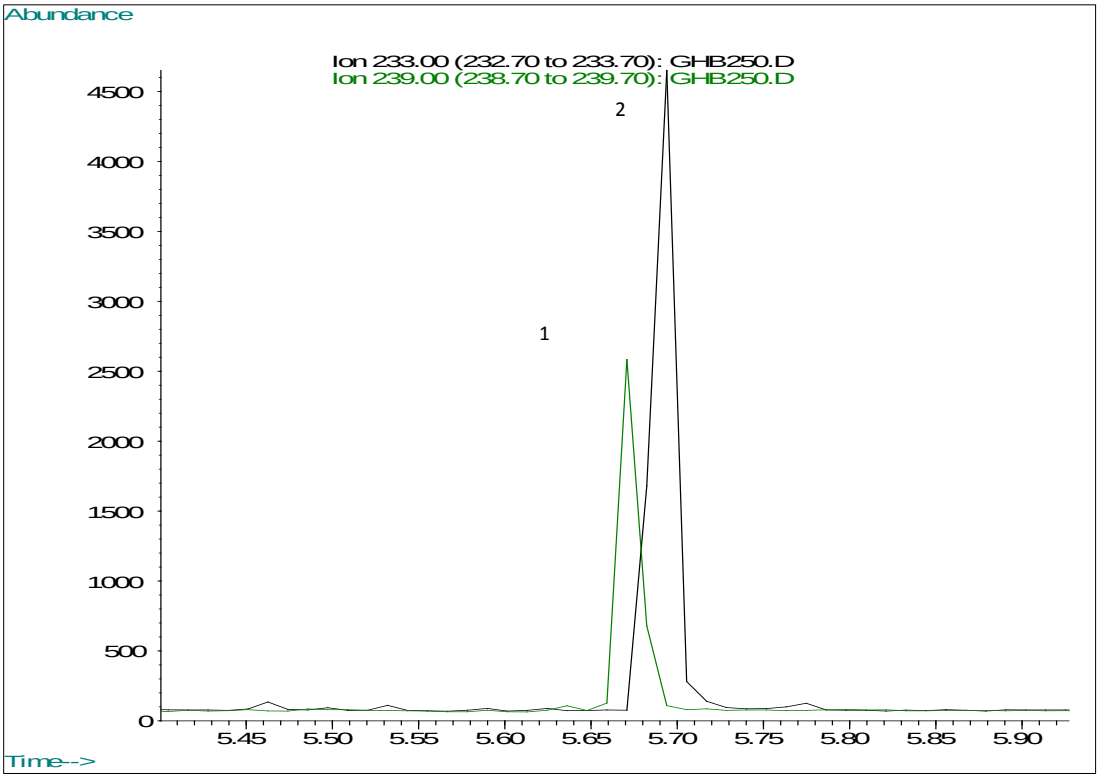
Instrument: API 3200 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Gradient:

Time	%A	%B
0.0	95	5
1.5	95	5
2.5	50	50
3.1	95	5
4.1	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



BSTFA-OXIME DERIVATIVES

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (minutes)
1.GHB-D_6	239	240		5.67
2.GHB	233	234	235	5.69

PARAMETERS

GC/MS: HP 5890 5972MSD GC/MS System with 7673 ALS System

GC capillary column: 30m x0.25mm (0.25um) RTX-5MS

Injector: 1µL Splitless 250°C

Oven temperature program: 70°C for 1 min; 15°C/min to 130°C, then to 300°C 50°C/min. Hold for 0.1min

Carrier gas: Helium

MSD condition: Aux temperature: 280 ºC, MS Source: 250 ºC, MS Quad: 150 ºC

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