GHB IN URINE AND BLOOD WITHOUT CONVERSION TO GAMMA-BUTRYLACTONE (GBL) BY LC-MS/MS OR GC-MS CLEAN SCREEN® GHB EXTRACTION COLUMN

Part #

ZSGHB020 – CLEAN SCREEN® GHB 200 mg, 10 mL Tube

SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS

1. **PREPARE SAMPLE:**

   Blood: To 1 mL blood sample add internal standard and 0.5 mL of 100 mM phosphate buffer (pH 6.0).
   Mix/vortex.
   Rock for 10 minutes.
   Centrifuge for 10 minutes at 2700 rpm.

   Urine: To 200 µL of urine add internal standard(s) and 100 µL of 100 mM Phosphate buffer (pH 6.0)
   Mix/vortex

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**

   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH 6.0).
   NOTE: Aspirate at full vacuum or pressure

3. **APPLY SAMPLE:**

   Place test tubes into vacuum manifold for collection
   The sample loading and elute are both collected
   Load at 1 to 2 mL/minute
   Collect elute as it is applied to the column
   After the sample is off the columns apply full vacuum for about 15 seconds to remove any residual blood/urine.

4. **ELUTE GHB:**

   Remove test tubes and set aside
   Place clean tubes into vacuum manifold for collection
   1 x 2 mL Methano/ NH_4OH (99:1) onto SPE column and collect

5. **DRY ELUATE:**

   Evaporate to dryness at < 40°C.

6. **SAMPLE CLEAN-UP:**

   Add 200 µL 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. **DRY ELUATE:**

   Evaporate to dryness at < 40 °C.

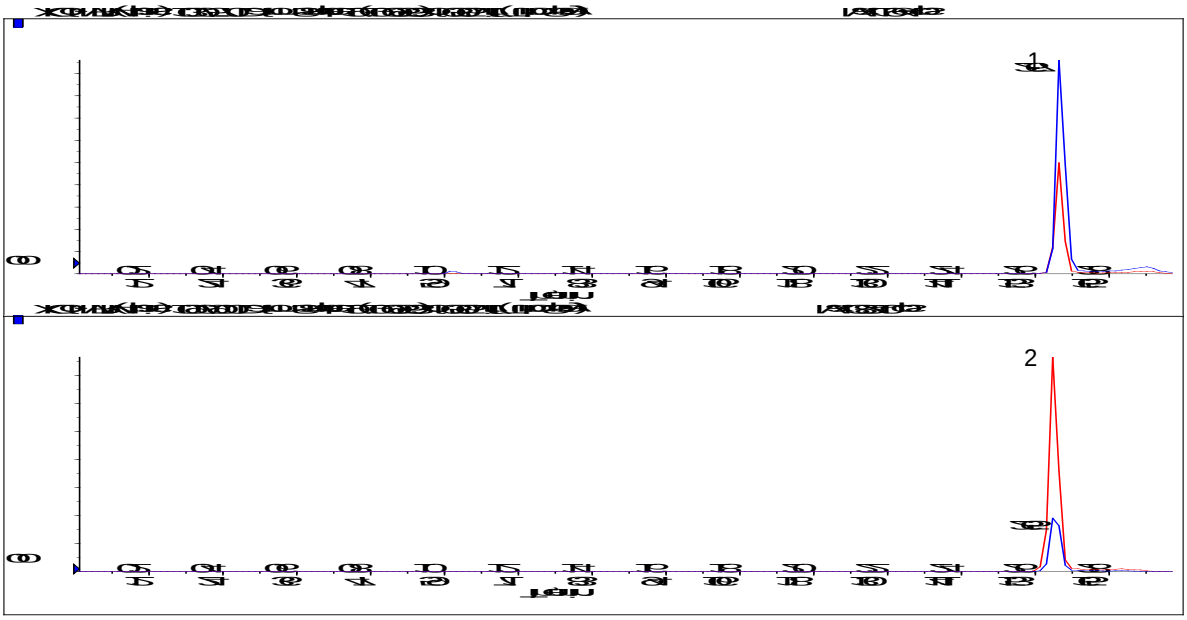
8. **RECONSTITUTE / DERIVATIZE**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 20 µL

   - **GC-MS**: **DERIVATIZE with TMS**
     Add 50 µL Ethyl Acetate and 50 µL BSTFA w/ 1% TMCS
     Overlay with N_2 and cap. Mix/vortex.
     React 30 minutes at 70 °C. Remove from heat source to cool.
     NOTE: Do not evaporate BSTFA solution

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	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: 1.25 mL/minute

Polarity: Negative

Reconstitute: 100 µL

Injection Volume: 20 µL

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

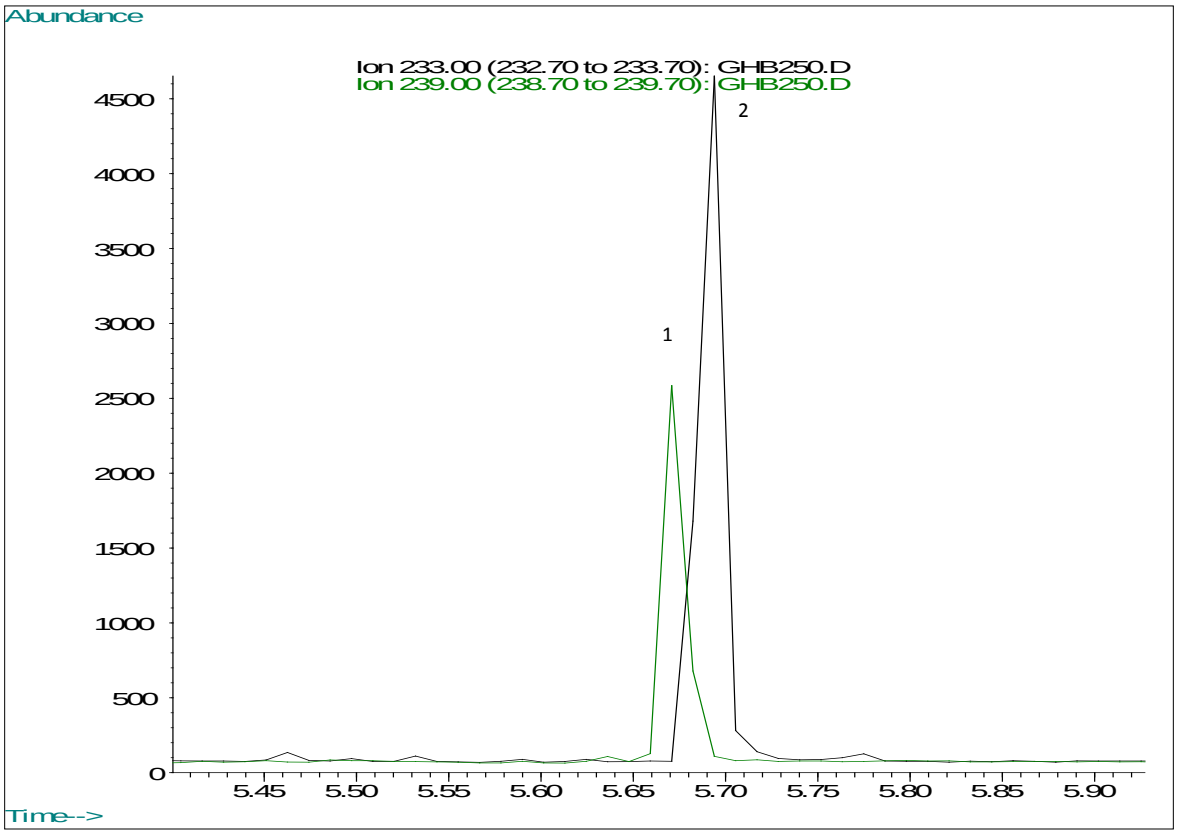
Instrument: API 3200 Qtrap MS/MS with Agilent 1200 Binary Pump SL

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 (min)
1. GHB-D_6	239	240	241	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: 30 m x0.25 mm (0.25 µm) 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 0.1 min

Carrier gas: Helium

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