



GHB IN URINE WITHOUT CONVERSION TO GAMMA-BUTYRLACTONE (GBL) BY LC-MS/MS OR GC-MS CLEAN-UP[®] QAX EXTRACTION COLUMN

Part #

CUQAX156 – CLEAN-UP[®] QAX 500 mg, 6 mL Tube

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

1. PREPARE SAMPLE:

Urine: To 50 μ L of urine add internal standard(s) and 5 mL of D.I. H₂O
Mix/vortex

2. CONDITION CLEAN-UP[®] EXTRACTION COLUMN:

1 x 3 mL CH₃OH.

1 x 3 mL D.I. H₂O.

NOTE: Aspirate at full vacuum or pressure

3. APPLY SAMPLE:

Load at 1 to 2 mL/minute

4. WASH COLUMN:

1 x 3 mL D.I. H₂O.

1 x 3 mL CH₃OH.

Dry column (10 minutes at >10 inches Hg).

5. ELUTE GHB:

1 x 3 mL 6% Glacial Acetic Acid/ 94% Methanol

7. DRY ELUATE:

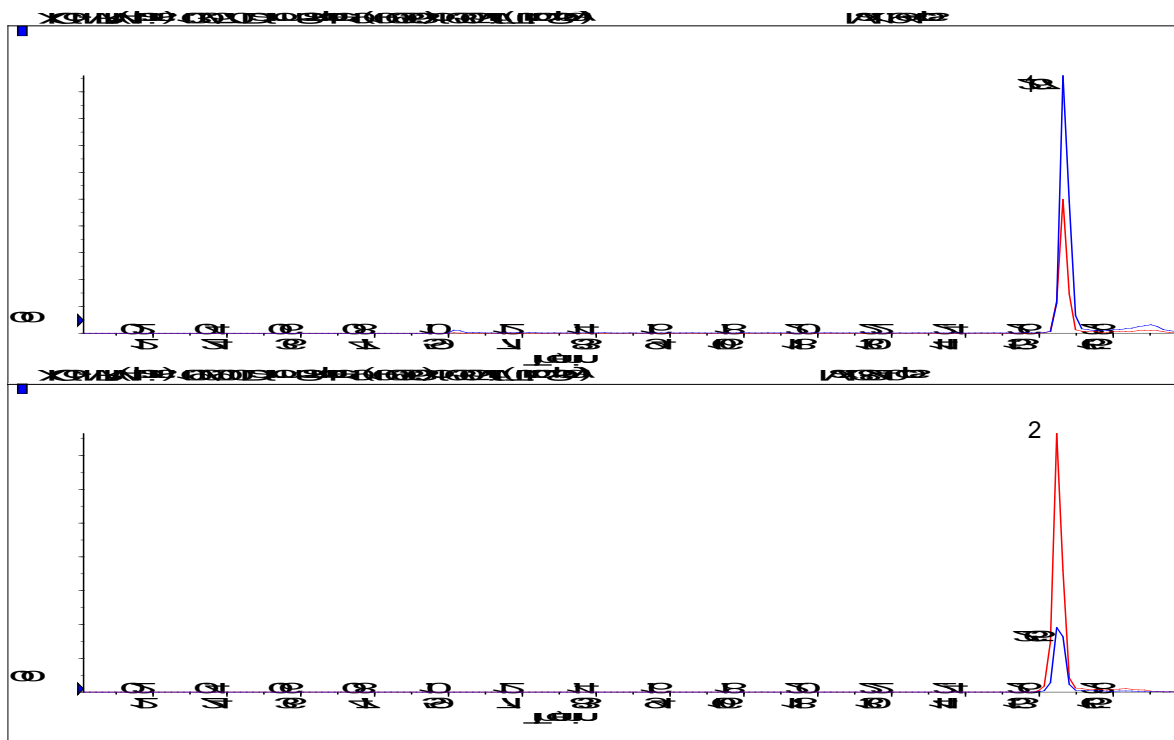
Evaporate to dryness at < 40°C.

8. RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase
Inject 20 μ L
- **GC-MS: DERIVATIZE with TMS**
Add 50 μ L Ethyl Acetate and 50 μ L BSTFA (with 1% TMCS)
Overlay with N₂ 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. H₂O

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

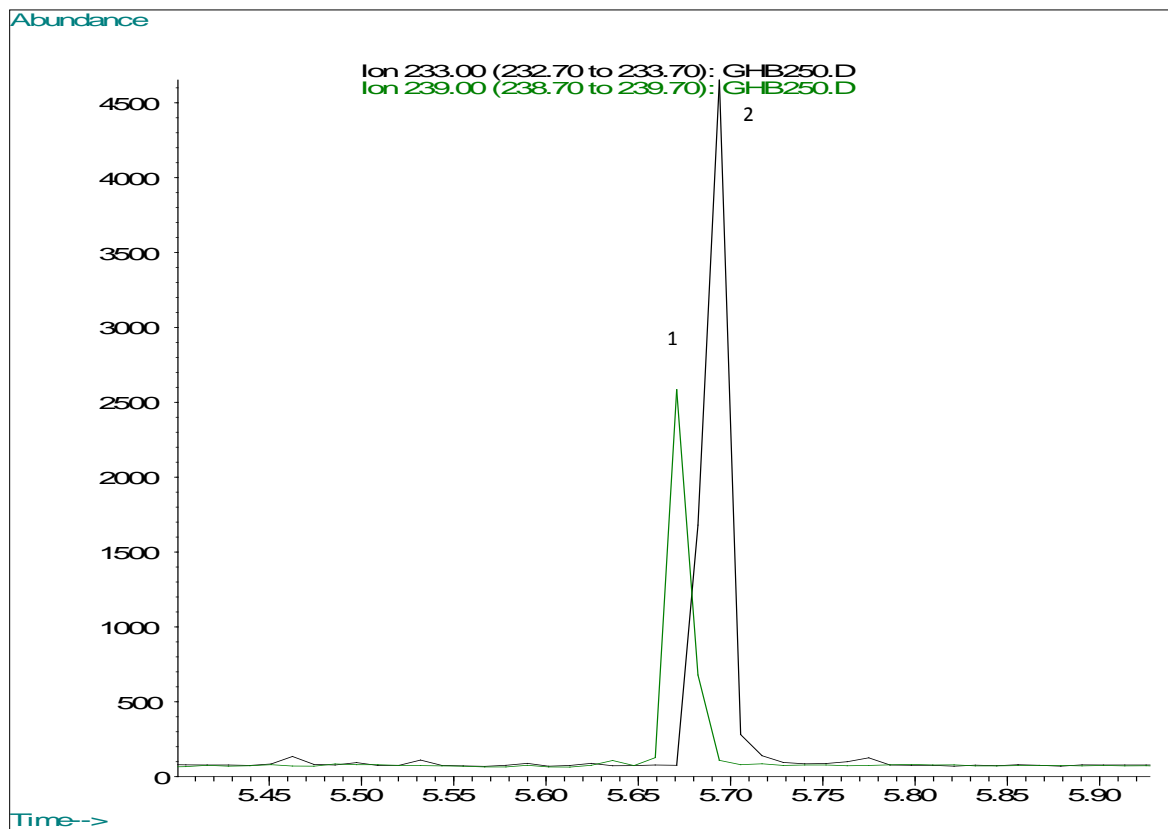
Instrument: API 3200 Qtrap MS/MS with Agilent 1200 Binary Pump SL

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	Qualifer Ion 1	Qualifier Ion 2	Relative Retention Time (min)
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: 30 m x 0.25 mm (0.25 µm) RTX-5MS

Injector: 1 µL 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 °C, MS Source: 250 °C, MS Quad: 150 °C