



## DETERMINATION OF GAMMA HYDROXYBUTYRATE (GHB) IN HAIR USING SOLID PHASE EXTRACTION AND LC-MS/MS OR GC-MS CLEAN-UP<sup>®</sup> QAX EXTRACTION COLUMN

Part #

CUQAX156 – CLEAN-UP<sup>®</sup> QAX 500 mg, 6 mL Tube

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

### 1. PREPARE SAMPLE:

To a clean glass tube add 100 mg of decontaminated hair sample.

Add 1 mL of CH<sub>3</sub>OH and internal standard\*, vortex mix

Incubate at 40 °C for approx. 12 hours

Centrifuge sample at 3000 rpm for 10 minutes

Transfer organic phase to a clean glass tube

Evaporate to dryness < 40 °C

Dissolve residue in 3 mL of D.I. H<sub>2</sub>O (pH 7)

Vortex Mix

### 2. CONDITION CLEAN-UP<sup>®</sup> EXTRACTION COLUMN:

1 x 3 mL CH<sub>3</sub>OH.

1 x 3 mL D.I. H<sub>2</sub>O.

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. H<sub>2</sub>O.

1 x 3 mL CH<sub>3</sub>OH.

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

### 5. ELUTE GHB:

2 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 µL of mobile phase  
Inject 20 µL

- **GC-MS: DERIVATIZE with TMS**

Add 50 µL Ethyl Acetate and 50 µL BSTFA (with 1% TMCS)

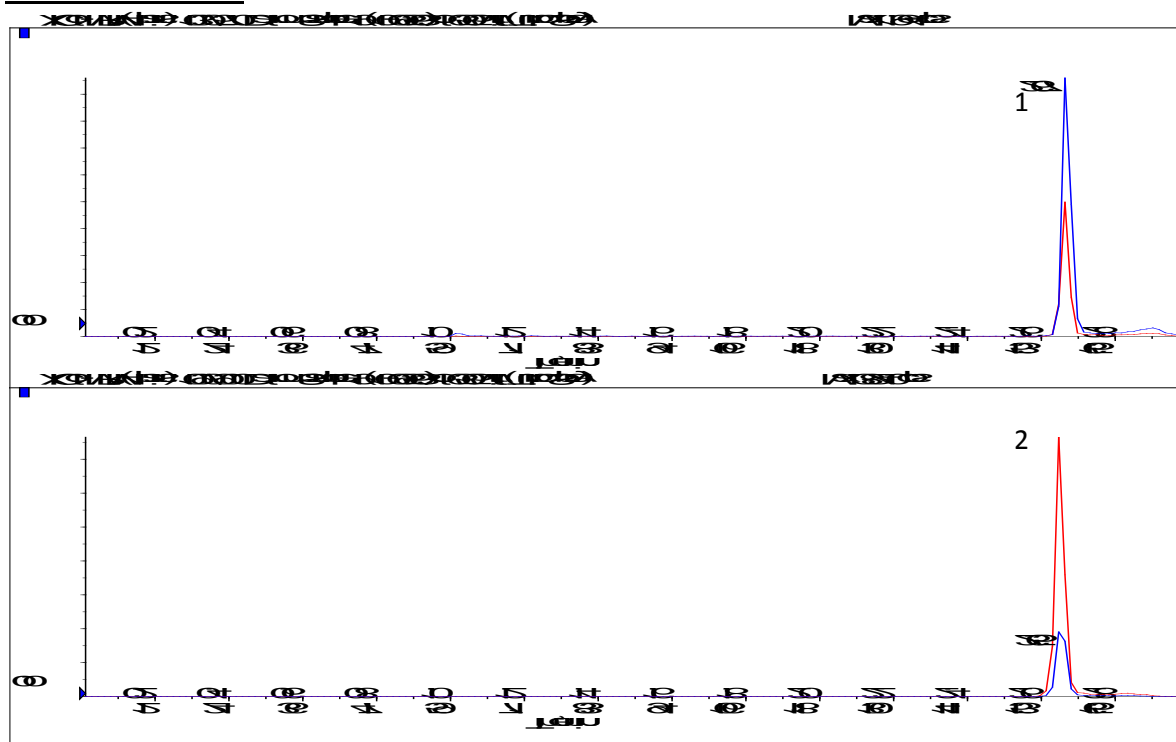
Overlay with N<sub>2</sub> and cap. Mix/vortex.

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 <sub>6</sub>	109.13	90.0	2.65

### PARAMETERS

**Mobile Phase A:** 0.1% Formic Acid in D.I. H<sub>2</sub>O

**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

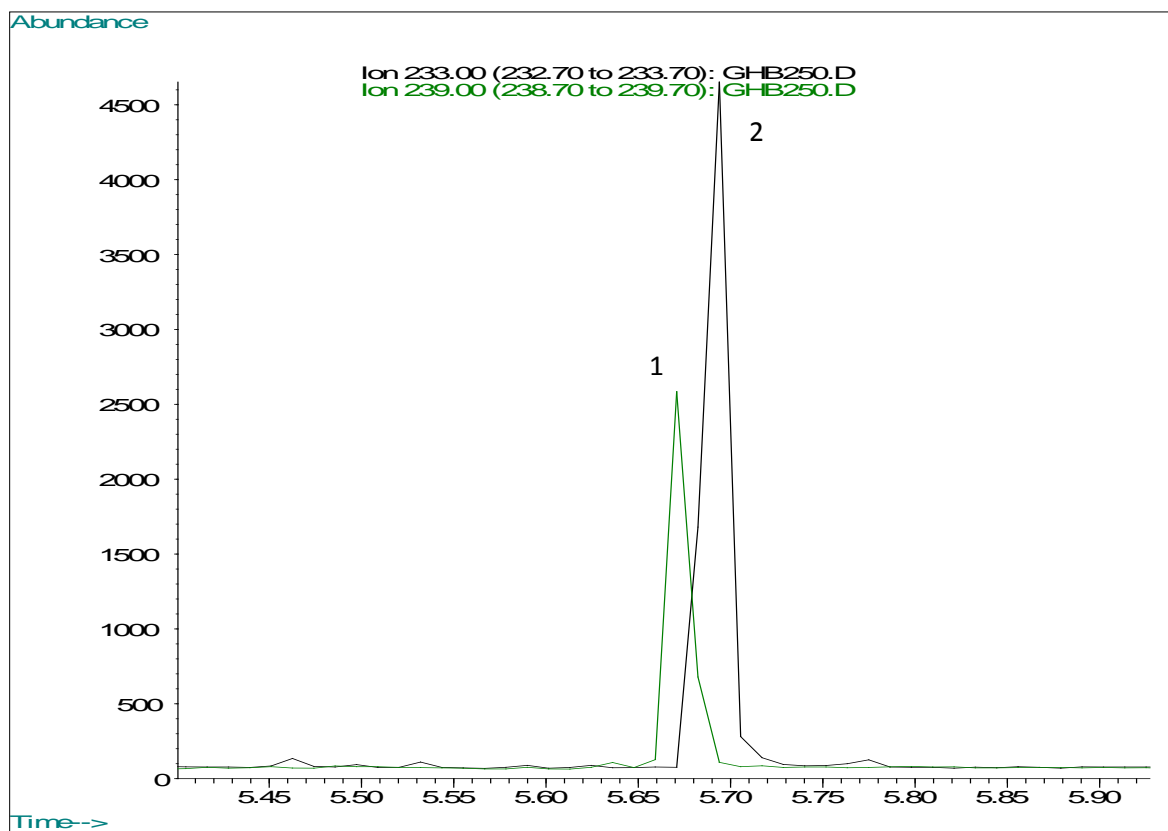
**Instrument:** API 3200 Qtrap MS/MS with Agilent 1200 Binary Pump SL

### 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 <sub>6</sub>	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 x0.25 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