GHB and GBL from Biological Fluids by Headspace GC/MS(EI)

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1 INTRODUCTION

This procedure allows for the analysis of a specimen for GHB and GBL by headspace gas chromatography mass spectrometry (HS-GC/MS)

2 SCOPE

Analyses	☒ Screening ☒ Confirmation ☐ Quantitation
Matrices	Blood, urine, aqueous samples
Analytes	GHB, GBL
Personnel	This document applies to authorized personnel who perform the described tasks, singly or in combination.

3 PRINCIPLE

For the initial screen, an appropriate internal standard is added. The samples are treated with concentrated sulfuric acid and heat (for conversion of GHB to GBL). Methylene chloride is used to extract the GBL from the biological matrix. The organic extracts are concentrated and transferred to headspace autosampler vials. The vials are heated and the headspace is analyzed by gas chromatography/mass spectrometry (GC/MS).

4 SPECIMEN CRITERIA

This procedure can be performed on a biological fluid such as blood, serum, plasma, urine, or vitreous humor. 1.0 mL of specimen is required for analysis.

5 EQUIPMENT

5.1 Equipment

A. Centrifuge
B. Vortex mixer
C. Heating block
D. Evaporator with nitrogen
E. Balance
F. Rotator
G. Adjustable pipetters (0.01 - 1 mL) with appropriate tips
H. Routine laboratory supplies, including disposable glass pipets, autosampler vials with caps, spatulas, graduated cylinders, test tube racks, etc.

5.2 Consumables

A. 16 x 100 mm screw-top test tubes with caps
B. 10 mL conical-bottom screw-top centrifuge tubes with caps
C. 10 mL and 100 mL volumetric flasks

D. 20-mL headspace vials with magnetic caps

5.3 Instruments

5.3.1 GC/MS

Agilent Gas Chromatograph / Mass Spectrometer

5.3.2 Column

30 m x 0.25 mm x 1.4 µm film DB-624 (or equivalent) column

5.3.3 Headspace Autosampler

Gerstel CombiPal

5.4 Software

Component	Software	Version
Operating System	Microsoft Windows	7 Pro SP 1
GC/MS	Enhanced Chemstation	E.02.02.1431
Autosampler	Gerstel Maestro 1	1.5.3.3/3.5
Data Analysis	Xcalibur	2.0.7 SP1

5.5 Chemicals/Reagents

Storage and stability determined by manufacturer, unless otherwise indicated.

5.5.1 Purchased

A. Concentrated Sulfuric Acid	(Reagent Grade)
B. Methylene Chloride	(Optima Grade)
C. Methanol	(HPLC Grade)
D. Deionized water	

5.6 Standards/Controls

Storage/stability determined by manufacturer unless otherwise noted.

5.6.1 Purchased

A. GHB Na Stock Standard (1.0 mg/mL)
Purchased from Cerilliant Corporation or another approved supplier as the sodium salt.
B. GBL Stock Standard (1.0 mg/mL)
Purchased from Cerilliant Corporation or another approved supplier.
C. d_6-GHB Na Internal Standard (d_6-Gammahydroxybutyrate Sodium Salt; 100 µg/mL OR 1 mg/mL)
Purchased from Cerilliant Corporation or another approved supplier.

D. α Methylene-GBL:

Purchased from Sigma or another approved supplier.

E. Negative Control:

i. Urine samples:

a. Surine (artificial urine, Dyna-Tek, Inc.) or deionized water

b. Urine (UTAK, Cliniqa, or obtained in-house). Stored refrigerated or frozen.

ii. Blood samples:

a. Blood (UTAK, Cliniqa, or obtained in-house). Stored refrigerated or frozen.

Since GHB is endogenous, the most appropriate Negative Control is a deionized water sample or synthetic urine. However, a true matrix matched Negative Control (urine or blood) is also analyzed.

5.6.2 Prepared

A. GHB Na Working Standard (0.1 mg/ml)

Prepare by adding 1.0 mL of the GHB Na Stock Standard to a 10 mL volumetric flask. Bring to the mark with deionized water. Store refrigerated in glass. Stable for at least one year.

B. α Methylene-GBL Internal Standard (0.1 mg/mL)

To a 100 mL volumetric flask, add 10.0 mg of Alpha Methylene-Gammabutyrolactone. Bring volume to the mark with methanol. Store refrigerated in glass or plastic. Stable for at least 1 year.

C. Positive GHB Controls (5 and 10 µg/mL)

For urine screens, Positive Urine Controls at 5 and 10 µg/mL are analyzed. These are prepared fresh by adding 60 and 120 µL of the GHB Na Working Standard to 1 mL aliquots of Surine.

D. Positive GBL Control (10 µg/mL)

Prepared fresh by adding 10 µL of the GBL Stock Standard (1.0 mg/mL) to 1 mL of Surine.

6 PROCEDURE

	Step	Note	Reference/Lot
	A. Samples		
	1. To labeled 16 x 100 mm screw-top tubes:		
☐	i. Add 1.0 mL of biological fluid		
	B. Controls		
	1. Prepare Negative Control(s)		
☐	i. Synthetic		
☐	ii. Natural		
	2. Prepare Positive Control(s), as required *Prepare one extra positive control for use as a GC/MS performance standard.*		
	i. GHB (GHB Working Standard, 0.1 mg/mL)		
☐	a. Low Control (5 µg/mL): Add 60 µL		
☐	b. High Control (10 µg/mL): Add 120 µL		
	ii. GBL (GBL Stock Standard, 1.0 mg/mL)		
☐	a. GBL Control (10 µg/mL): Add 10 µL		
	C. Internal Standard(s)(One option is selected)		
	1. GHB Analysis		
☐	i. Add 10 µL of 1.0 mg/mL d_6-GHB Internal Standard -OR-		
☐	ii. Add 100 µL of 0.1 mg/mL d_6-GHB Internal Standard		
	2. GBL Analysis		
☐	i. Add 20 µL of 0.1 mg/ mL α-methylene-GBL Internal Standard, and skip to Step E		
	D. Adjust pH		
☐	1. Add 0.150 mL of concentrated sulfuric acid to all tubes and cap		
☐	2. Vortex		
☐	3. Heat at ~70°C for 5 minutes		
☐	4. Let cool to ~room temperature		
	E. Extract		
☐	1. Add 4 mL methylene chloride to each tube and cap		
☐	2. Rotate for 5 minutes		

☐	3. Centrifuge 5 minutes at ~3000 rpm		
☐	4. Remove top (aqueous) layer to waste		
☐	5. Transfer bottom (organic) layer to a labeled conical tube		
	F. Concentrate		
☐	1. Evaporate to ~0.100 mL at ~35°C (do not dry fully)		
☐	**G. Transfer concentrated solvent to 20 mL headspace vial and cap**		
☐	**H. Instrumental Analysis** 1. GC/MS: analyze 1 mL i. Analyze GC/MS performance standard prior to batch analysis		
☐	ii. Analyze sample extracts		

7 ANALYTICAL PARAMETERS

7.1 Agilent Gas Chromatograph

7.1.1 Oven – Standard Conditions

Step	Temperature (°C)	Hold (min)	Ramp (°C/min)
1	50	3	20
2	150	7	

Total Run Time (min): 15.00

7.1.2 Inlet/Carrier/Column

Inlet		**Carrier**		**Column**	
Temperature (°C)	150	**Gas**	ultrapure helium	**Type**	DB-624
Injection Mode	Split	**Mode**	constant flow	**Length (m)**	30
Split Ratio	10:1	**Flow (mL/min)**	0.87	**Internal Diameter (mm)**	0.25
				Film Thickness (µm)	1.40

7.2 Agilent Mass Spectrometer

	Standard Conditions
Ionization Mode	Electron Impact (+)
Scan Mode	Full Scan
Scan Range (m/z)	35-200
Multiplier Offset (V)	200
Solvent Delay (min)	5.0
Temperatures (°C)	
Source	230
Quadrupole	150
Transfer Line	260

7.3 Autosampler

Mode	Headspace	Syringe	
Incubation		Temperature (°C)	110
Temperature (°C)	100	Sample Fill Volume (mL)	1.0
Time (min)	15	Sample Fill Rate (mL/sec)	0.5
Agitator Speed (rpm)	300	Sample Fill Strokes	4
Agitator Timing (s) On/Off	10/1	Sample Injection Speed (mL/sec)	1.0
Cycle Time (min)	20	Sample Flush Time (min)	4.0

8 DATA ANALYSIS

8.1 Decision Criteria

Matrix	Target/IS Ratio	Interpretation
Urine	≥ ratio of 10 ug/mL control	Requires confirmation/confirmed
Blood	≥ ratio of 2 ug/mL control	Requires confirmation/confirmed

8.1.1 Chromatography

The peak of interest will show good chromatographic fidelity, with reasonable peak shape, width, and resolution. In order to be determined acceptable, a chromatographic peak in an unknown sample will compare favorably to a chromatographic peak of the same analyte in a known sample analyzed on the same system in the same or subsequent analytical runs. Additionally, the following two criteria should be met.

8.1.1.1 Retention Time

The retention time of the peak will be within ±2% of the retention time (relative or absolute, as appropriate) obtained from injection of a reference standard, an extracted Positive Control, or an appropriate deuterated analog.

8.1.1.2 Signal-to-Noise

To justify the existence of a peak, its baseline signal to peak-to-peak noise ratio will exceed 3. Further, the baseline signal for the peak of interest will be at least 10 fold greater than that for any observed peak at similar retention time in a Negative Control or solvent blank injected just prior to the sample.

8.1.2 Mass Spectrometry

The mass spectrum of the analyte of interest will compare favorably to a reference standard or an extracted Positive Control. See the Guidelines for Comparison of Mass Spectra (TOX-104) for further guidance.

8.2 Calculations

The Target/IS Ratio is calculated by dividing the GHB/GBL area by the corresponding internal standard: m/z 86 (GHB) ÷ m/z 92 (d6-GHB).

9 REPORTING

Care should be taken in interpretation of GHB levels. GHB is a naturally occurring product in the body. Further, studies have shown that GHB is elevated in blood collection tubes containing citrate. Exercise care in reporting and interpreting low values of GHB.

When analyzing specimens from living persons, in most cases, amounts of GHB in blood below 2 µg/mL, and/or amounts of GHB in urine below 10 µg/mL should not be reported as positive.

10 Corrective Measures

See TOX-101 for guidance.

11 Performance Characteristics

11.1 Limit of Detection

Urine	5 µg/mL (administratively set)
Blood	2 µg/mL

11.2 Carryover

None detected during validation.

12 Limitations

12.1 Postmortem/Sample Stability

GHB levels may be elevated in postmortem blood samples and/or unpreserved blood samples. Therefore, a positive GHB finding in a postmortem blood sample should always be confirmed in a second specimen such as urine or vitreous humor.

12.2 Interferences

None known. Grossly decomposed or putrefied samples may affect both detection and quantitation limits. Yellow-top (citrate) tubes will interfere with the analysis of GHB.

13 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

14 Revision History

Revision	Issued	Changes
06	02/11/2022	Document reformat.