Determination of Gamma-Hydroxy-Butyrate (GHB) in Biological Samples

Application Note

Forensic Toxicology

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Abstract

A method has been developed on the Agilent 220 Quadrupole Ion Trap using EI-MS for the identification and quantification of GHB in biological samples. A working range of 10–200 µg/mL shows linearity for GHB. In the analysis of GHB, the benefits of using GC Quadrupole Ion Trap MS cannot be underestimated, in terms of reducing sample matrix interference, improving signal to noise and coupling its high selectivity and sensitivity.

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Introduction

Gamma-Hydroxy-butyrate (GHB) is an endogenous metabolite found in most mammalian tissues at nanomolar concentrations. The sodium salt has been promoted as a steroid alternative as well as a tryptophan replacement. GHB often involves oral doses of the salt dissolved in water. GHB is a metabolic by-product of 1,4-Butanediol, a solvent used in the production of certain plastics and fibers, 1,4-Butanediol has also been used as a recreational substance.

This application note describes a method for the analysis of GHB in serum, whole blood, vitreous fluid, urine, and tissue homogenates. A minimum of 0.5 mL of sample is required for the analysis.

GHB is extracted from the biological samples using protein precipitation. The dried extracts are then derivitized with BSTFA prior to GCMS analysis. The MS data is acquired in a narrow scan range encompassing the masses of the specific derivative ions and qualifiers for GHB and GHB d-6 the internal standard.

Experimental

Standards and reagents

Reagents
Acetonitrile, Methanol – Reagent Grade. BSTFA, (United Chemical Technologies).

Standards
GHB (G-001) and GHB d-6 (G-006) internal standard were purchased from Cerilliant.

4-Hydroxybutyric acid, sodium salt was purchased from Sigma Chemical (H-3635).

Working standards were then made or used as supplied, GHB-1mg/mL, GHB d-6 -25 µg/mL (1 mL of Stock standard to 40 mL acetonitrile), GHB QC -1 mg/mL (12.2 mg of GHB sodium salt (Sigma) to methanol (10 mL total volume).

Store at 2–8 °C, stable for 2 years.

Controls

Negative control - Drug free packed red blood cells was obtained from American Red Cross; dilute 1:1 with normal saline (0.9%) store at –20 °C, stable for 1 year.

Low control - (20 µg/mL) 20 µL of working GHB QC Standard to 980 µL blank blood in a 16 × 100 mm culture tube.

High control 1 - (80 µg/mL) 80 µL of working GHB QC Standard to 920 µL blank blood in a 16 × 100 mm culture tube.

High control 2 - (150 µg/mL) 150 µL of working GHB QC Standard to 850 µL blank blood in a 16 × 100 mm culture tube.

Sample preparation and calibration standards

Prepare a calibration curve using the calibration standard and drug free blood in 16 × 100 mm culture tubes as follows:

- 10 µg/mL - 10 µL std. and 990 µL drug free blood
- 25 µg/mL - 25 µL std. and 975 µL drug free blood
- 50 µg/mL - 50 µL std. and 950 µL drug free blood
- 100 µg/mL - 100 µL std. and 900 µL drug free blood
- 200 µg/mL - 200 µL std. and 800 µL drug free blood

Pipet 20 µL of standards, samples, negative, and positive controls into labeled 1.5-mL centrifuge tubes. Add 100 µL of working internal standard to each tube. Cap and vortex (approximately 15 seconds). Centrifuge at 3,000 rpm for a minimum of 10 minutes in the microcentrifuge. Transfer the upper layer into clean 16 × 100 mm disposable culture tubes, dry with nitrogen at 70 °C. Add 100 µL BSTFA with 1% TMS to each dried extract. Cap and vortex mix. Incubate for 20 minutes at 70 °C. Transfer the BSTFA mix to autosampler vials with inserts, cap and transfer to GCMS for analysis.

GC/MS ion trap analysis

GC Conditions

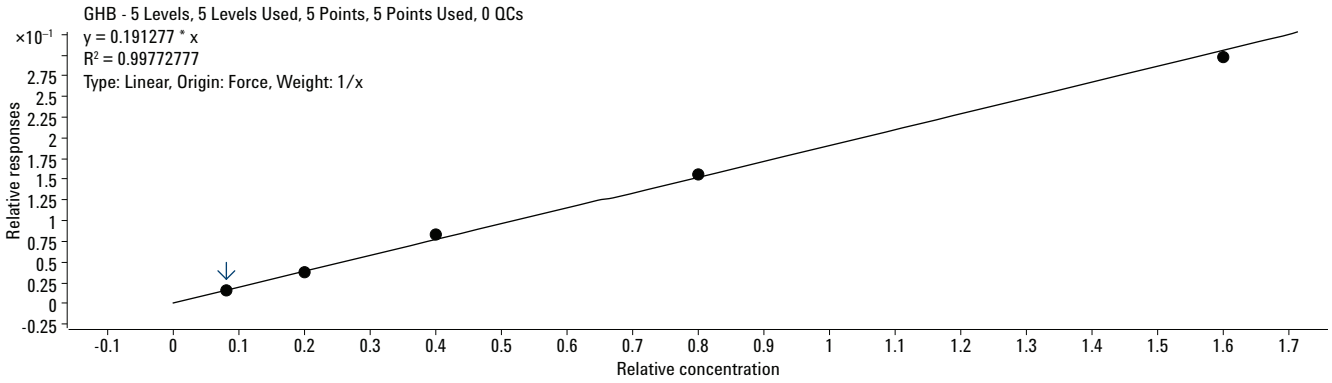
Column	DB-5MS or equivalent 25 m × 200 mm, 0.33 µm
Injection volume	1 µL
Injection mode	Splitless
Inlet temperature	250 °C
Carrier gas	Helium
Column flow	1.2 mL/min.
Oven program	60 °C; 1 minute hold
	10 °C/min to 200 °C;
	200 °C/min to 280 °C; 1 minute hold

Quadrupole ion trap MS conditions

Tune	Auto-tune
Acquisition	EI-Scan 200–250 da
Solvent delay	7.0 minutes
MS temperatures	Trap 210 °C, Manifold 50 °C, Transfer line 310 °C
Target	5,000
Filament current	5 µA

Compound	Rt(min)	Quant ion	Qualifiers
GHB	9.38	233	234/204
GHB d-6	9.33	239	240/206

GHB calibration



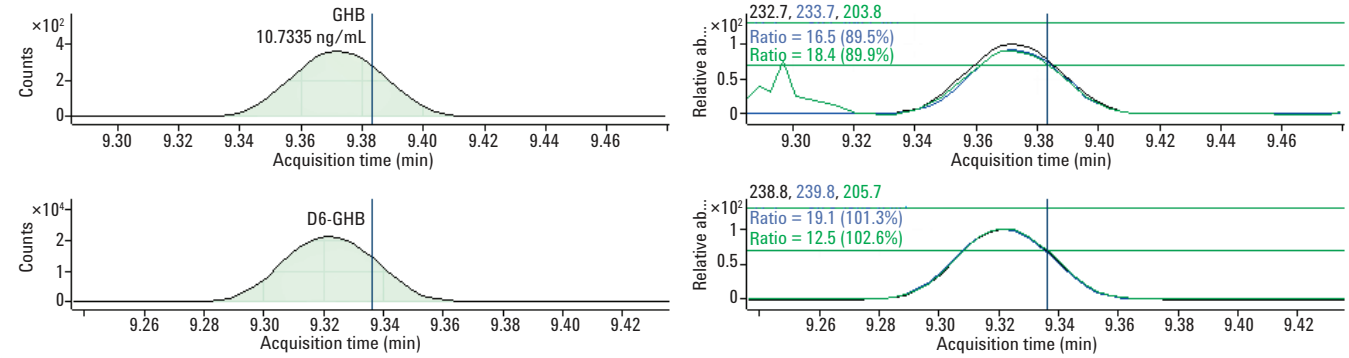
Results and Discussion

The following criteria are used to determine the presence and amount of GHB:

- The chromatography must be acceptable (separation of peaks, peak symmetry, and absence of carryover). The selected ions for quantitation and qualification are present. Ion ratios are within 20% of the target values determined from the calibration. The retention times of the presumed GHB from the test specimen is within ± 2% of the retention times for the latest calibration.
- The area of the GHB and the internal standard quantitative ions are used for quantitative analysis. Quantitation is accomplished by comparison of the relative response of unknowns and controls against a calibration curve produced from the relative responses for each calibrator concentration. The positive controls must be within their target ranges and GHB must be absent in the negative control.

Method limits

Linearity	10–200 µg/mL
Limit of Detection (LOD)	5 µg/mL
Limit of Quantitation (LOQ)	10 µg/mL
Carryover	No carryover noted after measured concentrations of 200 µg/mL.
Interferences	None were noted when blood and urine from random samples of known negative cases were analyzed.



Low calibration standard-10 µg/mL

Sample							GHB Method	GHB Results						Qualifier...		Qualifier...		D6-GHB (ISTD)...		Qualifier...		Qualifier...	
		Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	Ratio	MI	RT	Resp.	Ratio	MI	Ratio	MI
▶		10STD	10STD 3-28-2012 11-58-19 AM.SMS.D	Cal	1	3/28/2012 9:58 AM	10.0000	9.372	796		10.7335	10.7335	107.3	16.5		18.4		9.321	48482	19.1		12.5	
		25STD	25STD 3-28-2012 12-25-23 PM.SMS.D	Cal	2	3/28/2012 10:25 AM	25.0000	9.377	1337		24.1522	24.1522	96.6	17.8		18.0		9.325	36165	18.1		12.7	
		50STD	50STD 3-28-2012 12-52-31 PM.SMS.D	Cal	3	3/28/2012 10:52 AM	50.0000	9.379	3205		53.8446	53.8446	107.7	18.4		19.8		9.330	38895	18.7		12.6	
		100STD	100STD 3-28-2012 1-19-37 PM.SMS.D	Cal	4	3/28/2012 11:19 AM	100.0000	9.382	7580		101.5021	101.5021	101.5	18.5		20.5		9.329	48802	18.9		12.2	
		200STD	200STD 3-28-2012 1-46-44 PM.SMS.D	Cal	5	3/28/2012 11:46 AM	200.0000	9.376	10603		194.7676	194.7676	97.4	18.5		18.3		9.329	35577	18.7		12.6	
		NEG	NEG 3-28-2012 2-13-52 PM.SMS.D	Sample		3/28/2012 12:13 PM												9.330	38058	19.6		13.2	
		LOW	LOW 3-28-2012 2-41-05 PM.SMS.D	Sample		3/28/2012 12:41 PM		9.375	1384		21.0558	21.0558		20.7		22.6		9.327	42959	20.1		13.1	
		HIGH1	HIGH1 3-28-2012 3-08-10 PM.SMS.D	Sample		3/28/2012 1:08 PM		9.379	6791		97.2311	97.2311		18.9		19.2		9.331	45646	19.6		12.8	
		HIGH2	HIGH2 3-28-2012 3-35-21 PM.SMS.D	Sample		3/28/2012 1:35 PM		9.378	9480		166.8828	166.8828		19.1		20.6		9.330	37125	20.2		12.6	
		BLANK	BLANK 3-28-2012 4-02-33 PM.SMS.D	Sample		3/28/2012 2:02 PM																	
		1972B	1972B 3-28-2012 4-29-48 PM.SMS.D	Sample		3/28/2012 2:29 PM		9.376	113		1.9509	1.9509						9.324	37765	20.5		13.0	
		2051B	2051B 3-28-2012 4-56-59 PM.SMS.D	Sample		3/28/2012 2:57 PM		9.380	224		3.2764	3.2764						9.328	44747	20.8		13.3	
		2132B	2132B 3-28-2012 5-24-07 PM.SMS.D	Sample		3/28/2012 3:24 PM		9.379	2289		80.0402	80.0402		21.3		21.3		9.331	18690	20.2		14.9	
		2132UR	2132UR 3-28-2012 5-51-14 PM.SMS.D	Sample		3/28/2012 3:51 PM		9.374	938		16.9244	16.9244		18.4		19.4		9.326	36205	20.7		13.2	

Note tags for outliers and below calibration level.

Conclusion

This application note presents a sensitive, selective, and robust analytical method to determine GHB in biological samples using GHB d-6 as an internal standard. For the analysis of GHB, the benefits of GC Quadrupole Ion Trap MS cannot be underestimated. In terms of reducing sample matrix interference, improving signal to noise and coupling its high selectivity and sensitivity the GC Quadrupole Ion Trap MS provides a more confidence driven solution for the analysis of GHB. GC Quadrupole Ion Trap MS analysis has the potential to reduce false positive and negatives as well as providing an additional degree of confidence in the results obtained. Using the optimized method listed above, a fast, targeted GC/MS analytical method can be used to solve the current GHB analysis problem facing forensic laboratories today. Three levels of positive controls were used in conjunction with a negative control to assure accurate quantification and rule out false negatives in the unknown biological samples. Low µg/mL detection limits were observed for GHB in the various sample matrices.

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