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γ-Hydroxybutyrate
Bridging the Clinical-Analytical Gap

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Contents

Abstract

Laboratory detection of γ-hydroxybutyrate (GHB) has been published as early as the 1960s. However, wide-scale use of GHB during the 1990s has led to the development of current analytic methods to test for GHB and related compounds. Detection of GHB and related compounds can be clinically useful in confirming the cause of coma in an overdose patient, determining its potential role in a postmortem victim, as well as evaluating its use in a drug-facilitated sexual assault victim. Analytical method sensitivity must be known in order to determine the usefulness and clinical application. Most laboratory cut-off levels are based on instrument sensitivity and will not establish endogenous versus exogenous GHB levels. Interpretation of GHB levels must include a knowledge base of endogenous GHB, metabolism of GHB and related compounds, as well as postmortem generation. Due to potential analytical limitations in various GHB methods, it is clinically relevant to specifically request for GHB as well as related GHB compounds if they are also in question. Various storage conditions (collection time, types of containers, use of preservatives, storage temperature) can also affect the analysis and interpretation of GHB and related compounds.

1. The History of γ-Hydroxybutyrate

During the past 12 years, illicit forms of γ-hydroxybutyrate (GHB), γ-butyrolactone (GBL) and 1,4-butanediol (1,4-BD) have been used recreationally by bodybuilders and club drug partiers, and to commit drug-facilitated sexual assaults. In the past, it was sold under such names as: Liquid Ecstasy, Liquid X, Somatomax PM and Georgia Home Boy. In the US, an approved form of GHB is federally listed as a schedule III controlled substance and indicated for the treatment of cataplexy in patients with narcolepsy (Xyrem® [1], Orphan Medical, Inc.); however, illicit forms of the drug are federally classified as schedule I. In Europe, it is approved for use as a general anaesthetic (Gamma-OH®, France; Somsanti®, Germany), and for the treatment of alcohol withdrawal (Alcover®, Italy).[1,2]

1 The use of trade names is for product identification purposes only and does not imply endorsement.

2. Physiochemical Properties

The chemical formula of GHB is $C_4H_8O_3$ and it has a molecular weight of 104.10. The sodium salt is a white/off-white powder with a molecular weight of 127.09, which forms a colourless solution in water.[1-3] In contrast, GBL ($C_4H_6O_2$) has a molecular weight of 86.10 and 1,4-BD ($C_4H_{10}O_2$) has a molecular weight of 90.1.[2] GHB is found endogenously in the mammalian brain where it is synthesised from the neurotransmitter γ-aminobutyric acid (GABA) via transamination to succinic semialdehyde and then reduction to GHB. Following ingestion, GHB is rapidly metabolised to succinic semialdehyde and oxidised to succinic acid, which enters the Krebs cycle where it is ultimately metabolised to carbon dioxide. The ingestion of GBL results in rapid conversion to GHB via plasma lactonase. After ingestion, 1,4-BD is initially converted to γ-hydroxybutyraldehyde (via alcohol dehydrogenase), followed by conversion to GHB (via aldehyde dehydrogenase).[2,4,5]

Despite GHB capacity-limited absorption and non-linear elimination kinetics, it is quickly absorbed and rapidly metabolised with an elimination half-life of 20–42 minutes with negligible amounts (1–5%) being excreted unchanged in the urine. After oral administration of therapeutic doses (12.5–50 mg/kg), GHB is almost completely eliminated from blood within 2–8 hours, with essentially complete urinary recovery within 8–12 hours of administration.[5-8]

To date, very few qualitative methods have been developed to detect GHB while many quantitative analytical methods have been developed. Although there exist approximately a dozen gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) methods cited between 1963–79 for the detection of GHB, this article will review basic analytical instrumentation terminology and apply it to recently developed analytical methods developed for the detection of GHB in blood and urine. A comprehensive list of cases involving the measurement of both endogenous and exogenous GHB levels, including analytical methods, can be found in table I and table II.

3. Review of Analytical Instrumentation and Detection Techniques

3.1 Gas Chromatography

Gas chromatography (GC) is an analytical process in which an organic solvent sample is injected into an injection port and volatilised into a stream of inert carrier gas, such as nitrogen, helium or hydrogen. The gas stream (mobile phase) is contained within a long column that is coated with a liquid layer (stationary phase) and the components of the sample partition between the gas phase and the liquid phase depending upon their physical and chemical properties; samples with greater affinity for the stationary phase are retained longer. Once the sample travels through the column, it passes through a detector. Currently, there are over 60 types of detectors used in GC, such as Fourier Transform infrared spectroscopy (FTIR), flame ionisation detection (FID) and mass spectrometry (MS).

Several types of capillary inlets are available for GC use (e.g. split or splitless injections, and 'on-column'). Sample methods for GC analysis typically include liquid-liquid and solid phase extraction. Special sampling methods also used for GC analysis include headspace sampling and solid phase microextraction (SPME). During headspace sampling, samples are volatilised before reaching GC injection port. Headspace sampling decreases instrument maintenance requirements since there is no direct liquid sample injection into the GC injection port or column; referred to as 'on-column injection'. SPME uses a fused silica fibre coated with a stationary phase. SPME fibre is placed in an aqueous solution-containing compound of interest where equilibrium is established and it is directly injected into the instrument. SPME is a quick extraction method that requires fewer extraction steps than conventional methods and generates less organic waste.

GC/FID is an extremely useful tool to identify substances by their retention time. In a FID, substances are burned in an oxyhydrogen flame, giving rise to a signal that is proportional to its carbon content. GC/FTIR is an alternative selective detector typically used for qualitative analysis.

3.2 Gas Chromatography/Mass Spectrometry

Using GC/MS, a substance travels through MS source (molecules are ionised via electron impact or chemical ionisation), into mass analyser (ions separated by mass-to-charge [m/z] via quadrapoles or ion traps), and to a detector (ions are counted and generate a mass spectrum plot – ion abundance vs m/z). The mass selective detector can be operated in either full-scan or selective ion monitoring mode.[39]

Identification is based on comparison of the peak retention times of GHB to the internal standard. Analysis is based on the quantitation and qualifier ions, their relative abundance, ratios between monitored ions and peak symmetry of ions (tailing factors). Quantitations are calculated based on the equation presented in figure 1.

Limit of detection (LOD) is the mean value at which the lowest concentration is measurable with all qualifying identity ratios being within ±20% of the corresponding ratios established by the calibrator. Limit of quantitation (LOQ) is established by the LOD parameters plus the quantitation must be within 20% of predetermined value. Limit of linearity (LOL) is the mean value at which the highest concentration is measurable with all qualifying identity ratios being within ±20% of the corresponding ratios established by the calibrator plus the quantitation must be within ±20% of the predetermined value.

Table I. Endogenous GHB studies

Study	Endogenous levels				Storage	Method
	live subjects	PM subjects[a]	urine (mg/L)	blood (mg/L)		
Fieler et al.[9]	20 NS		No	No		GC/MS (Ferrara method)
		25	No	0–168		GC/MS
		15 of 20		3.2–168		GC/MS and GC/FID Vanderpol method '95
Yeatman and Reid[10]	55 NS		<10		Unpreserved	GC/FID, as GBL
			0.9–3.5 (mean 1.65)		Unpreserved	GC/MS, as GBL (modified Stephen's method 1994)
Elliott[11]	119 (39 M, 80 F)		2 of 119 + (>2.5); (confirmed 3)		4°C, run within 3 days	GC/FID, as GBL GC/MS
	15 (3 M, 12 F)			0 of 15 + (>2.5); (confirmed <1)	4°C, run within 3 days	GC/FID, as GBL GC/MS
	1 M		0.22–2.33 (mean: 0.93)	−20°C		GC/MS
	1 F		0.31–1.51 (mean: 0.72)			
LeBeau et al.[12]	10 random			3.8–13.1 (mean 8.8 ± 3.1)	Citrate −20°C 6–36mo (mean 22 ± 10mo)	
LeBeau et al.[13]	5 M		0.00–6.63 (mean 1.59 ± 1.42)		Unpreserved at −20°C (mean 6–9mo)	Headspace GC/MS, as GBL
	3 F		0.00–1.70 (mean 0.31 ± 0.25)			
LeBeau et al.[14]	1 M		<0.19–1.65 (mean 0.63 ± 0.34) [42 samples over 1wk]			Headspace GC/MS, as GBL
	1 M		0.62–6.62 (mean 3.02 ± 1.52 [52 samples over 1wk])			
	1 M		<0.19–1.94 (mean 0.56 ± 1.55 [35 samples over 1wk])			
Elliot[15,16]		13 (mean 57)	0–197 (mean 56)	0–217	Unpreserved at 4°C × 4 days, then at −20°C	GC/MS and GC/FID (modified McCusker method)

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Table I. Contd

Study	Endogenous levels				Storage	Method
	live subjects	PM subjects[a]	urine (mg/L)	blood (mg/L)		
Stephens et al.[17]		3 of 17	5.1–9.5	19 (<5–76)	NaF	GC/MS Ferrara 1993
		6		20 (9–65)	NaF 60 days, 4°C	
		6		32 (<5–77)	NaF 60 days, 25°C	
		20		57 (9–433)	No, 40 days, 4°C	
		20			No, 40 days, 25°C	
LeBeau et al.[18]	1 M		0.25 (day 0); 1.04 (day 180)		Unpreserved at 25°C[b]	Headspace GC/MS, as GBL
			0.25 (day 0); 0.41 (day 180)		Unpreserved at 5°C[b]	
			0.25 (day 0); 0.39 (day 180)		Unpreserved at −10°C[b]	
	1 F		0.24 (day 0); 0.95 (day 180)		Unpreserved at 25°C[b]	Headspace GC/MS, as GBL
			0.24 (day 0); 0.74 (day 180)		Unpreserved at 5°C[b]	
			0.24 (day 0); 0.52 (day 180)		Unpreserved at −10°C[b]	
Kerrigan[19]	Pooled drug-free human urine[c]		1.3 (day 54); 4.8 (day 115–244)		21°C NaF + Na azide	GC/MS, PCI
			1.7 (day 54); 5.1 (day 244)		21°C NaF	
			2.8 (day 115); 3.0 (day 244)		4°C NaF + Na azide	
			4.0 (day 115); 3.1 (day 244)		4°C NaF	
			0.8 (day 165); 0.8 (day 244)		−20°C NaF + Na azide	
			0.7 (day 165); 0.6 (day 244)		−20°C NaF	
Elian[20]	670 urine samples		3080 (340–5750)			GC/MS (modified Elian 2001 methods)
	240 blood samples			740 (170–1510)		
Sakurada et al.[21]			0–2.6	0.4–7.3	−20°C	GC/MS (modified Couper method)
Kalasinsky et al.[22]		12		7.7 (3–14)		GC/MS (modified McCusker method)
	4 NS			1.4 (1–2)		

a PM subjects not specified.

b Means tested on days 0, 7, 21, 35, 90, 180.

c Levels are not detectable, <0.4 mg/L, until reported.

F = females; **GBL** = γ-butyrolactone; **GC/FID** = gas chromatography/flame ionisation detection; **GC/MS** = gas chromatography/mass spectrometry; **M** = males; **NS** = subjects not specified; **PCI** = positive chemical ionisation; **PM** = postmortem.

Table II. Exogenous γ-hydroxybutyrate (GHB) studies

Study	Patient status				Storage	Method
	live subjects	PM subjects	urine (mg/L)	blood (mg/L)		
Couper and Logan[23]	1 dfsa – 8h from incident			3.2	4°C NaF/K oxylate – blood only	GC/MS
	1 dui GBL		714	33		
	1 dui			34		
	2 OD		1600	130		
			2200	221 (serum 339)		
LeBeau et al.[12]	1 dfsa			14	Citrate	
	1 dfsa			31	Citrate	
Stillwell[24]	1 dfsa		26.9 at <12h			GC/MS, as GBL (modified Lettieri method 1978)
Pan et al.[25]	1 to a few h PI		800	16		GC/MS
	1 – 45 min PI		410	24		
	1			104		
		1 pt – 'short time' PM		174		
	1 dui			80		
	1 dui			350		
Stephens and Baselt[26]	1 dui – 2h PI		1975			GC/FID, as GBL
Duer et al.[27]		1 pt – 7h PM (1,4-BD ingestion)	870 1,4-BD	70 (peripheral) 78 (heart)	No preserv., 4°C	GC/MS, GHB GBL, as GHB
		1 pt – 10+h PM	320 GHB			
		(GBL + poly RX)	66 GBL			
		1 pt – 28h PM		67		
		(GHB + poly RX)				
LeBeau et al.[28]	1 dui			150		Headspace GC/FID, as GBL
				157		Headspace GC/MS, as GBL
	1 dfsa – 4h PI		308	47		
	1 dfsa – 9h PI		4.4			
Ferrara et al.[29]		1 pt – 2 days PM (mixed OD) 2 days PM	258.3	11.5		GC/MS
Elian[30]	1 dfsa		15			GC/MS
Louagie et al.[31]	1 OD			Serum: 125		GC/MS
Lora-Tamayo et al.[32]	Abuse		430 GHB 401 1,4-BD	103 GHB 82 1,4-BD		GC/MS

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Table II. Contd

Study	Patient status				Storage	Method
	live subjects	PM subjects	urine (mg/L)	blood (mg/L)		
Elian[33]	16 dfsa 2 dui			1.8–17.8 59–230		GC/MS
Karch et al.[34]		1 dfsa – 19h PM	1100	159 femoral	Both NaF preserved	GC, as GBL
		1 abuse – 17h PM	6000	B: 1400 femoral 1100 Rheart	All NaF preserved	GC, as GBL
Bosman and Lusthof[35]	2 dfsa		14–252		All possible citrate	GC/MS, as GBL (modified Ferrara method 1993)
	1 dfsa			18		
		3 dfsa		10–29		
	3 dui		100–2000			
	10 dui			51–194		
		2 GHB related?	23–35			
		10 GHB related?		9–40		
		3 GHB unrelated		6–15		
Deveaux et al.[36]	1 abuse 1 abuse 1 abuse		1450 436	165 132 114	4°C	GC/MS
Ferrara et al.[37]	50 pts/250 samples, 25 mg/kg, time 0 and 12h			Plasma: 55 (24–48)	−4°C × 1 day	
	50 pts/250 samples, 50 mg/kg, time 0 and 12h			Plasma: 90 (51–159)		
Kavanagh et al.[38]	1 pt – 1g (10 mg/kg)		0 (time 0) 7.7 (1h) 16.5 (1.5h) 4.0 (10h) 1.7 (16h)		−20°C	GC/MS (modified Elian method 2000)
	1 pt – 2g (20 mg/kg)		0 (time 0) 14.0 (1h) 29.1 (1.5h) 1.9 (10h) ND (16h)			
Kalasinsky et al.[22]		1 OD		330 femoral 648 heartblood		GC/MS

1,4-BD = 1,4-butanediol; **dfsa** = drug-facilitated sexual assault; **dui** = driving under the influence; **GBL** = γ-butyrolactone; **GC/FID** = gas chromatography/flame ionisation detection; **GC/MS** = gas chromatography/mass spectrometry; **ND** = none detected; **OD** = overdose; **PI** = post-injestion; **PM** = postmortem; **preserv.** = preservative; **pt** = patient; **Rheart** = right heart; **RX** = prescriptions/drug; **?** indicates uncertain if cause of death was GHB related.

$$\text{Test Sample: } \frac{\text{Select ion intensity (analyte)}}{\text{Select ion intensity (internal standard)}} \times \text{Standard concentration calibrator sample} = \text{Concentration test sample}$$

$$\text{Calibrator: } \frac{\text{Select ion intensity (analyte)}}{\text{Select ion intensity (internal standard)}}$$

Fig. 1. Sample quantitation using select ion mode single point calibration.

4. Laboratory Analysis of of γ-Hydroxybutyrate, γ-Butyrolactone and 1,4-Butanediol

4.1 Screening Methods

Qualitative tests are designed to provide a rapid indication that the substance of interest is either present or not. The sensitivity of the method must be known in order to determine its usefulness and clinical application. When possible, it is useful to know if there are any substances that can potentially cause false positive results. As with any preliminary positive qualitative test, results must be confirmed by an alternative chemical method, typically via GC/MS.

Recently, a number of rapid colorimetric assays have been developed. A rapid screening test for GHB has been reported that can produce qualitative results with 0.25mL of urine within 10 minutes. This test involves the conversion of GHB to GBL and therefore cannot be used to distinguish these two compounds. Positive samples immediately turn a blue/olive green colour upon mixing the prepared layers. The sensitivity of the assay is reported to be 0.1 mg/mL.[40]

An alternative rapid screening test for GHB has been developed that can produce qualitative results with 0.3–1.0mL of urine within 5 minutes. This assay also involves the conversion of GHB to GBL; positive samples turn purple. The sensitivity of the assay is reported be 0.5 mg/mL with 0.3mL of urine and 0.1 mg/mL with 1mL of urine.[41] A few other rapid screen tests for GHB have been reported that can produce qualitative results:[42]

- colour test 1 will detect positive GHB as orange-red to dark red and GBL as yellow colour;
- colour test 2 will detect positive GHB as purple and GBL as yellow colour;
- colour test 3 will detect positive GHB as dark green and GBL as yellow-orange colour; and
- colour test 4 will detect positive GHB as pink-to-violet colour.

The availability of two other colorimetric tests has been published; however, they will not be discussed since they are only available to law enforcement personnel.[43,44] In addition to colorimetric methods, a microcrystal test has been developed that is specific for GHB at concentrations of approximately 4 mg/mL, as rectangular crystal formations in about 5 minutes. This procedure, while specific for GHB, is not very sensitive but could be used for product identification.[45]

Other, more sophisticated qualitative analysis procedures for GHB include GC-FID (see table III) and another involves the acid conversion of GHB to its cyclic lactonic acid, GBL followed by extraction with methylene chloride put onto a 7694 Headspace autoinjector. This procedure tests for GHB and GBL concurrently by having one sample mixed with acid to convert GHB to GBL and one sample without acid to detect existing GBL. Blood LOD is 0.5 mg/L with linearity from 5–1000 mg/L. Urine LOD is 0.5 mg/L with linearity from 5–1000 mg/L.[28] Another similar method utilises a modification of the Stephens 1994 method (see section

Table III. Gas chromatography/mass spectrometry analysis of γ-hydroxybutyrate (GHB) to γ-butyrolactone (GBL)

Study	GBL ions	ISTD ions	LOD (mg/L)	LOQ (mg/L)	LIN (mg/L)
Ferrara et al.[37]	GBL: 41, 42, 56, 86[a]	d-Valerolactone: 41, 42, 56, 100[a]	0.2 (urine) 0.1 (plasma)		0–150 (urine) 0–200 (plasma)
LeBeau et al.[28]	GBL: 86[a]	GHB-d6: 92	0.5		5–1000
LeBeau et al.[13,18]	GBL: 86[a]	GHB-d6: 92	0.06	0.19	Calibration curve: 0.2–10
Stillwell[24]	GBL: 86[a], 56, 42, 28	GBL-d6: 92[a], 60, 48, 32			
Bosman and Lusthof[35]	GBL: 42, 56, 86[a]	GBL-d6: 46, 48, 60, 92[a]			

a Quantification ion.

GBL-d6 = γ-butyrolactone-deuterated 6; **GHB-d6** = γ-hydroxybutyrate-deuterated 6; **ISTD** = internal standard; **LIN** = linearity; **LOD** = limit of detection; **LOQ** = limit of quantitation.

4.2), which also involves conversion of GHB to GBL followed by extraction with chloroform and analysis on a GC/FID and GC-MS in the full-scan mode. This method used a positive cut-off concentration of 10 mg/L.[10,11]

The literature contains mention of GHB screened via nitrogen phosphorus detection (GC-NPD); however, no details are available for discussion.[32] Other screening and potential confirmation methods are used infrequently as they lack sensitivity and are difficult to quantitate at low levels. These include: ^{1}H nuclear magnetic resonance spectroscopy (LOD: serum 2.3 mmol/L; urine 25.4 mmol/L),[46] micellar electrokinetic chromatography (LOD: GHB 5.1 mg/L; GBL 0.34 g/L; 1,4-BD 0.25 g/L; 1,4-BD comigrates with ethanol, and is difficult to quantitate at low levels),[47] capillary zone electrophoresis with indirect UV absorption detection (LOD and LOQ vary with urine concentration),[48] SPME analysed via high performance liquid chromatography (HPLC)/UV (no details available),[49] and HPLC/UV-visible spectrophotometry.[50]

4.2 Confirmation Methods

Quantitative methods are the most sensitive and reliable methods. Typically, the preferred analysis is performed via GC/MS. A review of the literature mentions GHB confirmed via GC-FID, and GC-FID with nitrogen; however, no details are available for discussion.[26,51]

Several GC/MS methods have been developed for the analysis of GHB. Some methods involve acid conversion of GHB to its cyclic lactonic acid, GBL, followed by extraction with benzene,[37] chloroform[24,35] or methylene chloride.[13,18,28] Monitored ions, LOD, LOQ and LOL can be found in table III. Many other GHB methods involve liquid-liquid extraction with acetonitrile,[25,31,32,52] ethyl acetate,[11,19-21,23,30,33] methanol[27] or tert-butylmethylether,[38] followed by derivatisation (deriv) with bis-trimethyl-silyl-trifluroacetamide/trimethyl-chlorosilane,[10,11,19-23,30,32,33,52,53] *N*-methyl-*N*-trimethylsilyltrifluoroacetamide,[31] *N*-(tert-butyldimethylsilyl)-*N*-methyltrifluroacetamide,[25] or (tert-butyldimethylsilyl), acetonitrile, tert-butyldimethylsilyltrifluoroacetamide.[38] Monitored ions, LOD, LOQ and LOL can be found in table IV.

Additional GC/MS extraction procedures include solid phase extraction[10,53] and solid phase microextraction, with[54] and without[22,55] conversion of GHB to GBL. A few GC/MS methods use headspace GC/MS.[13,14,18,28] Another GC/MS method that is becoming more popular is positive chemical ionisation (PCI)-GC/MS.[19]

Table IV. Gas chromatography/mass spectrometry analysis of γ-hydroxybutyrate (GHB) derivatives

Study	Deriv. GHB ions	Deriv. ISTD ions	LOD (mg/L)	LOQ (mg/L)	LIN (mg/L)
McCusker et al.[53]	GHB: 233[a], 234, 235	GHB-d6: 239[a], 240, 241			5–500 (urine)
Elian[20,30,33]	GHB: 233[a], 234, 235	GHB-d6: 239[a], 240, 241			2–50 (urine)
			1		1–200 (blood)
Couper and Logan[23]	GHB: 233, 204, 117	ISTD DEG: 235, 103, 117		0.5	1–200 (urine)
			0.5	1	1–1000 (whole blood)
Duer et al.[27]	GHB: 233, 234, 235	GHB-d6: 239, 240, 241	2.0	6.5	600
	GHB: 233, 234, 235	ISTD GVA: 230, 231, 232, 233	1.5	4.9	700
Kavanagh et al.[38]	GHB: 275	ISTD GCL: 303	set at 2.0		2–100
Kalasinsky et al.[22]	GHB: 233, 234, 235	GHB-d6: 239, 240, 241	0.001		0.1–10
Deveaux et al.[36]			1–2	2.5–5	2.5–200
Kerrigan[19]	GHB: 277, 249, 233, 159		0.2	0.4	
Elliott[11]	GHB: 233	GHB-d6: 239		0.2	
Lora-Tamayo et al.[32]	GHB: 233, 117, 204	GHB-d6: 239, 120, 206			2.5–85
	1,4-BD: 177, 116, 219				4.8–160
Villain et al.[52]	GHB: 233, 204, 147	GHB-d6: 239	0.2	1	1–200 (urine)
			0.1	1	1–200 (blood)
Yeatman and Reid[10]	GHB: 233, 234, 235		0.5	0.75	1–100
Louagie et al.[31]	GHB: 233	ISTD VAL: 201	2		200
Sakurada et al.[21]	GHB: 73, 117, 147[a], 204, 233	ISTD DEG: 73[a], 103, 117, 147, 235			0–200
	1,4-BD: 73, 101, 116, 147, 171, 219				

a Quantification ion.

1,4-BD = 1,4-butanediol; **DEG** = diethylene glycol; **Deriv.** = derivatised; **GCL** = γ-caprolactone; **GHB-d6** = γ-butyrolactone-deuterated 6; **GVA** = γ-valerolactone; **ISTD** = internal standard; **LIN** = linearity; **LOD** = limit of detection; **LOQ** = limit of quantitation; **VAL** = valproic acid.

Other confirmation methods that have been infrequently used include: SPME analysed via HPLC/MS (no details available),[49] negative electrospray ionisation ion-trap tandem MS (LOD: 5 mg/L),[48] and HPLC/thermospray mass spectrometry.[50]

5. Potential Pitfalls in the Interpretation of Results

5.1 Storage Conditions

Timely sample collection, proper containers and appropriate storage conditions are essential when interpreting GHB levels (see table I). Whole blood samples collected and stored simultaneously in yellow-top anticoagulant-citrate buffer tubes (trisodium citrate, citric acid, dextrose) versus purple-top anticoagulant edetate disodium acetate (EDTA) tubes have demonstrated that GHB can be artificially produced in citrate buffered blood specimens that were negative in EDTA blood specimens. This has been confirmed experimentally.[12]

Unpreserved urine specimens from non-GHB users detected endogenous GHB levels with approximately a 2- to 2.5-fold difference when stored at 25°C versus 5°C; no real detection difference demonstrated between 5°C and –10°C.[18]. Urine specimens, preserved with sodium fluoride (NaF) ± Na azide, from pooled drug-free human urine detected no endogenous GHB levels (<0.4 mg/L) at day 0. Endogenous GHB levels in this study demonstrated that *in vitro* GHB production might be capacity limited. Upon reanalysis over 244 days, specimens stored at –20°C had comparable GHB levels (0.8 vs 0.6 mg/L) regardless of which preservative was used. Specimens stored at 4°C had comparable GHB levels (3.0 vs 3.1 mg/L) regardless of which preservative; however, they were three times higher than those stored at –20°C. Likewise, specimens stored at 21°C had comparable levels (4.8 vs 5.1 mg/L) regardless of which preservative; however, they were more than five times higher than those stored at –20°C.[19]

The stability and interconversion of GHB and GBL in various aqueous solutions (1% weight-in-weight in deionised water, 3.0g per serving in lemon-lime soda, lemon-lime sports drink, lynchburg lemonade, white wine, vodka) was studied under various physical and chemical conditions (solution pH 2–12; temperature 4°C, 22°C, 60°C). In water, conversion of GBL to GHB at a ratio of 2 : 1 occurs within 202 days in pure water, within 9 days in acidic solution (pH 2.0), and within 15 minutes in alkaline solution (pH 12.0). Conversion of GHB to GBL did not occur in pure water or in alkaline solution (pH 12.0) but occurred readily at a ratio of 1 : 2 in acidic solution (pH 2.0). Analysis of GBL and GHB in the other aqueous solutions demonstrated greater stability for GHB than GBL. Results demonstrate the potential factors that must be considered during product identification.[56]

Postmortem blood preserved with sodium fluoride 10 mg/mL, can be stored at either room temperature (25°C) or refrigerator (4°C) with little to no effect on endogenous GHB formation. However, when postmortem blood was not preserved, samples demonstrated 50% higher endogenous GHB formation when stored under refrigeration, and when stored at room temperature exhibited nearly double the value of those stored under refrigeration.[17]

Although most reports do not show elevation of GHB in postmortem urine, one study has reported GHB elevations in both postmortem blood and urine.[15,16]

5.2 Clinical Considerations

Most studies (see table I) report endogenous GHB urine and blood levels somewhere below 10 mg/L. GHB levels between the LOQ of the analytical instrument and reported endogenous levels ('grey zone') are troublesome and difficult to interpret. In patients with GHB levels within the grey zone (due to GHB's short half-life and potential for level diurnal variation), it might be clinically relevant for the clinician to check an additional blood/urine sample on the following day at the exact same time of day in which the original sample was collected, to serve as a control. If collection occurs, they should also collect several other samples throughout the day to serve as a patient's baseline GHB level. However, levels must be interpreted within the context of other symptoms and findings. Remember, some individuals may have a tendency toward diurnal variation in urine GHB levels (creatinine-normalised).[13] GHB has demonstrated intra- and inter-individual variation in urinary excretion.[11,14]

Qualitative procedures can be useful for product identification and rapid screening of an overdose patient. Due to the typical time delay in taking samples from a drug-facilitated sexual assault victim, qualitative tests are usually not sensitive enough for detection of GHB in biological fluids. There are many similar quantitative methods for detection of GHB and its congeners (GBL, 1,4-BD). Some analytical methods are not capable of distinguishing GHB from GBL. It is important for clinicians to know which method of detection will be used to analyse GHB in order to properly assess its advantages and limitations. One must keep in mind that laboratory cut-off levels are typically based upon the detection limits of the instrument and will not necessarily reflect endogenous versus exogenous GHB levels.

It is of the utmost importance to have an analytical chemist/toxicologist review the chromatographs to ensure that proper quantification conditions (retention time, calibrator ratios, identity ratios, tailing factors) were carried out and that there was no sign of either carry-over or co-eluting substances. Any known information regarding the patient's medication history (licit and illicit) should be conveyed to the analytical laboratory. Future analytical methods should include analysis of potentially interfering substances, e.g. urea.[19,25]

It is apparent that there are ongoing needs to further evaluate endogenous GHB levels in biological fluids. Storage conditions, preservatives and time from storage to analysis must also be taken into account when interpreting GHB levels. Statistically significant population-based endogenous GHB levels need to be determined to further assist clinicians with their ability to evaluate theoretical exogenous cut-off levels.

6. Conclusion

The dramatic increase in GHB abuse during the 1990s has lead to rapid advances in laboratory detection of GHB. Questions that remain to be answered are: which GHB levels are truly endogenous and which are exogenous, did storage conditions either increase or decrease the original results, and what was the original substance anyway?

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References

1. Sweetman SC. Martindale. 33rd ed. Chicago (IL): Pharmaceutical Press, 2002: 1268
2. Ferrara SD, Frison G, Tedeschi L, et al. Gamma-hydroxybutyrate (GHB) and related products. In: LeBeau M, Mozayani A, editors. Drug-facilitated sexual assault. San Diego (CA): Academic Press, 2001: 107-26
3. O'Neil MJ, Smith A, Heckelman PE, et al. Merck index. 13th ed. Whitehouse Station (NJ): Merck & Co., 2001; 4840: 862
4. Tunnicliff G. Sites of action of gamma-hydroxybutyrate (GHB): a neuroactive drug with abuse potential. J Toxicol Clin Toxicol 1997; 35 (6): 581-90
5. Scharf MB, Lai AA, Branigan B, et al. Pharmacokinetics of gammahydoxybutyrate (GHB) in narcoleptic patients. Sleep 1998; 21 (5): 507-14
6. Palatine P, Tedeschi L, Frison G, et al. Dose-dependant absorption and elimination of gamma-hydroxybutyric acid in healthy volunteers. Eur J Clin Pharmacol 1993; 45: 353-6
7. Ferrara SD, Zotti S, Tedeschi L, et al. Pharmacokinetics of γ-hydroxybutyric acid in alcohol dependant patients after single and repeated oral doses. Br J Clin Pharmacol 1992; 34: 231-5
8. Hoes MJAJM, Vree TB, Guelen PJM. Gamma-hydroxybutyric acid (*) as hypnotic. L'encephale 1980; 6: 93-9
9. Fieler EL, Coleman DE, Baselt RC. γ-hydroxybutyrate concentrations in pre- and postmortem blood and urine [letter]. Clin Chem 1998; 44 (3): 692
10. Yeatman DT, Reid K. A study of urinary endogenous gamma-hydroxybutyrate (GHB) levels. J Anal Toxicol 2003; 27 (1): 40-2
11. Elliott SP. Gamma hydroxybutyric acid (GHB) concentrations in humans and factors affecting endogenous production. Forensic Sci Int 2003; 133 (1-2): 9-16
12. LeBeau M, Montgomery MA, Jufer RA, et al. Elevated GHB in citrate-buffered blood [letter]. J Anal Toxicol 2000; 24: 383-4
13. LeBeau MA, Christenson RH, Levine B, et al. Intra- and interindividual variations in urinary concentrations of endogenous gamma-hydroxybutyrate. J Anal Toxicol 2002; 26: 340-6
14. LeBeau M, Darwin WD, Huestis MA. Intra- and interindividual variations in urinary levels of endogenous GHB [abstract]. SOFT Meeting 1999; San Juan
15. Elliot S. The presence of gamma-hydroxybutyric acid (GHB) in postmortem biological fluids [letter]. J Anal Toxicol 2001; 25: 152
16. Elliot S. Response to the presence of gamma-hydroxybutyric acid (GHB) in postmortem biological fluids [letter]. J Anal Toxicol 2001; 25: 357
17. Stephens BG, Coleman DE, Baselt RC. In vitro stability of endogenous gamma-hydroxybutyrate in postmortem blood [letter]. J Forensic Sci 1999; 44 (1): 231-2
18. LeBeau MA, Miller ML, Levine B. Effect of storage temperature on endogenous GHB levels in urine. Forensic Sci Int 2001; 119 (2): 161-7
19. Kerrigan S. In vitro production of gamma-hydroxybutyrate in antemortem urine samples. J Anal Toxicol 2002; 26 (8): 571-4
20. Elian AA. Determination of endogenous gamma-hydroxybutyric acid (GHB) levels in antemortem urine and blood. Forensic Sci Int 2002; 128: 120-2
21. Sakurada K, Kobayashi M, Iwase H, et al. Production of γ-hydroxybutyric acid in postmortem liver increases with time of death. Toxicol Lett 2002; 129: 207-17
22. Kalasinsky KS, Dixon MM, Schmunk GA, et al. Blood, brain, and hair GHB concentrations following a fatal ingestion. J Forensic Sci 2001; 46 (3): 728-30
23. Couper FJ, Logan BK. Determination of γ-hydroxybutyrate (GHB) in biological specimens by gas chromatography: mass spectrometry. J Anal Toxicol 2000; 24 (1): 1-7
24. Stillwell ME. Drug-facilitated sexual assault involving gamma-hydroxybutyric acid. J Forensic Sci 2002; 47 (5): 1133-4
25. Pan YM, Gill GN, Tilson CS, et al. Improved procedure for the analysis of gamma-hydroxybutyrate and ethylene glycol in whole blood. J Anal Toxicol 2001; 25 (5): 328-32
26. Stephens BG, Baselt RG. Driving under the influence of GHB? J Anal Toxicol 1994; 18: 357-8
27. Duer WC, Byers KL, Martin JV. Application of a convenient extraction procedure to analyze gamma-hydroxybutyric acid in fatalities involving gamma-hydroxybutyric acid, gamma-butyrolactone, and 1,4-butanediol. J Anal Toxicol 2001; 25 (7): 576-82
28. LeBeau MA, Montgomery MA, Miller ML, et al. Analysis of biofluids for gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL) by headspace GC-FID and GC-MS. J Anal Toxicol 2000; 24: 421-8
29. Ferrara SD, Tedeschi L, Frison G, et al. Fatality due to gamma-hydroxybutyric acid (GHB) and heroin intoxication. J Forensic Sci 1995; 40 (3): 501-4
30. Elian AA. A novel method for GHB detection in urine and its application in drug-facilitated sexual assaults. Forensic Sci Int 2000; 109 (3): 183-7
31. Louagie HK, Verstraete AG, DeSoete CJ, et al. A sudden awakening from near coma after combined intake of gamma-hydroxybutyric acid (GHB) and ethanol. J Toxicol Clin Toxicol 1997; 35 (6): 591-8
32. Lora-Tamayo C, Tena T, Rodriguez A, et al. Intoxication due to 1,4-butanediol. Forensic Sci Int 2003; 133: 256-9
33. Elian AA. GC-MS determination of gamma-hydroxybutyric acid (GHB) in blood. Forensic Sci Int 2001; 122 (1): 43-7
34. Karch SB, Stephens BG, Nazareno GV. GHB: club drug or confusing artifact? Am J Forensic Med Pathol 2001; 22 (3): 266-9
35. Bosman IJ, Lusthof KJ. Forensic cases involving the use of GHB in The Netherlands. Forensic Sci Int 2003; 133 (1-2): 17-21
36. Deveaux M, Renet S, Renet V, et al. Is gamma-hydroxybutyric acid (GHB) really used in rave-parties and as a date-rape drug in France? Acta Clin Belg 2002; 57 (1 Suppl. 1): 37-40
37. Ferrara SD, Tedeschi L, Frison G, et al. Therapeutic gamma-hydroxybutyric acid monitoring in plasma and urine by gas chromatography-mass spectrometry. J Pharm Biomed Anal 1993; 11 (6): 483-7
38. Kavanagh PV, Kenny P, Feely J. The urinary excretion of γ-hydroxybutyric acid in man. J Pharm Pharmacol 2001; 53 (3): 399-402
39. McNair HM, Miller JM. Basic gas chromatography. New York: Wiley Interscience, 1998
40. Badcock N, Zotti R. Rapid screening test for gamma-hydroxybutyric acid (GHB, fantasy) in urine [letter]. Ther Drug Monit 1999; 21 (3): 376-7
41. Alston II WC, Ng K. Rapid colorimetric screening test for γ-hydroxybutyric acid (liquid X) in human urine. Forensic Sci Int 2002; 126: 114-7
42. Moffat AC, Osselton MD, Widdop B, et al. Clarke's analysis of drugs and poisons. 3rd ed. Chicago (IL): Pharmaceutical Press, 2004: 300
43. Morris JA. Extraction of GHB for ftir analysis and a new color test for gamma butyrolactone (GBL). Microgram 1999; 32: 215-21
44. Koppenhaven DJ. GHB color test. Microgram 1997; 30: 130
45. Andera KM, Hiram BS, Evans HK, et al. Microchemical identification of gamma-hydroxybutyrate (GHB). J Forensic Sci 2000; 45: 665-8

46. Imbenotte M, Azaroual N, Cartigny B, et al. Identification and quantitation of xenobiotics by ^{1}H NMR spectroscopy in poisoning cases. Forensic Sci Int 2003; 133 (1-2): 132-5
47. Dahlen J, Vriesman T. Simulataeous analysis of γ-butyrolactone, and 1,4-butanediol by micellar electrokinetic chromatography. Forensic Sci Int 2002; 25 (2-3): 113-9
48. Baldacci A, Theurillat R, Caslavska J, et al. Determination of γ-hydroxybutyric acid in human urine by capillary electrophoresis with indirect UV detection and confirmation with electrospray ionization ion-trap mass spectrometry. J Chromatog A 2003; 990: 99-110
49. Benson A, Sabucedo A, Almirall J, et al. Detection and identification of illicit drugs including GHB, rohypnol, ketamine, and psilocybin by refocused solid phase microextraction high performance liquid chromatography mass spectrometry, (SPME/HPLC/MS). Abstracts from the 223rd ACS National Meeting; 2002 Apr 7-11; Orlando (FL). Am Chem Soc 2002; 223 (1-2): #ANYL 54
50. Mesmer NZ, Satzger RD. Determination of gamma-hydroxybutyrate (GHB) and gamma-butyrolacton (GBL) by HPLC/UV/VIS spectrophotometry and HPLC thermospray mass spectrometry. J Forensic Sci 1998; 43 (3): 489-92
51. LoVecchio F, Curry S, Bagnasco T. Butyrolactone-induced central nervous system depression after ingestion of RenewTrient, a 'dietary supplement' [letter]. N Engl J Med 1998; 339 (12): 847-8
52. Villain M, Cirimele V, Ludes B, et al. Ultra-rapid procedure to test for γ-hydroxybutyric acid in blood and urine by gas chromatography-mass spectrometry. J Chromatogr B 2003; 792 (1): 83-7
53. McCusker RR, Paget-Wilkes H, Chronister CW, et al. Analysis of gamma-hydroxybutyrate (GHB) in urine by gas chromatography-mass spectrometry. J Anal Toxicol 1999; 23: 301-5
54. Frison G, Tedeschi L, Maietti S, et al. Determination of γ-hydroxybutyric acid (GHB) in plasma and urine by headspace solid-phase microextraction and gas chromatography/positive ion chemical ionization mass spectrometry. Rapid Commun Mass Spectrom 2000; 14: 2401-7
55. Blair S, Song M, Hall B, et al. Determination of gamma-hydroxybutyrate in water and human urine by solid phase microextraction-gas chromatography/quadrupole ion trap spectrometry. J Forensic Sci 2001; 46 (3): 688-93
56. Ciolino LA, Mesmer MZ, Satzger D, et al. The chemical interconversion of GHB and GBL: forensic issues and implications. J Forensic Sci 2001; 46 (6): 1315-23

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