Review

Drug Testing and Analysis

Received: 6 September 2010 Revised: 5 December 2010 Accepted: 6 December 2010 Published online in Wiley Online Library: 4 March 2011

(www.drugtestinganalysis.com) DOI 10.1002/dta.254

An overview of gamma-hydroxybutyric acid: pharmacodynamics, pharmacokinetics, toxic effects, addiction, analytical methods, and interpretation of results

H. Andresen,[a]* B. E. Aydin,[b] A. Mueller[a] and S. Iwersen-Bergmann[a]

Abuse of gamma-hydroxybutyric acid (GHB) has been known since the early 1990's, but is not as widespread as the consumption of other illegal drugs. However, the number of severe intoxications with fatal outcomes is comparatively high; not the least of which is brought about by the consumption of the currently legal precursor substances gamma-butyrolactone (GBL) and 1,4-butanediol (1,4-BD). In regards to previous assumptions, addiction to GHB or its analogues can occur with severe symptoms of withdrawal. Moreover, GHB can be used for drug-facilitated sexual assaults. Its pharmacological effects are generated mainly by interaction with both $GABA_B$ and GHB receptors, as well as its influence on other transmitter systems in the human brain. Numerous analytical methods for determining GHB using chromatographic techniques were published in recent years, and an enzymatic screening method was established. However, the short window of GHB detection in blood or urine due to its rapid metabolism is a challenge. Furthermore, despite several studies addressing this problem, evaluation of analytical results can be difficult: GHB is a metabolite of GABA (gamma-aminobutyric acid); a differentiation between endogenous and exogenous concentrations has to be made. Apart from this, in samples with a longer storage interval and especially in postmortem specimens, higher levels can be measured due to GHB generation during this postmortem interval or storage time.

Keywords: gamma-hydroxybutyric acid; gamma-butyrolactone; 1,4-butanediol; gamma-valerolactone

History

Gamma-hydroxybutyric acid (GHB) was first synthesized in 1960 as a derivative of the endogenous neurotransmitter gamma-aminobutyric acid (GABA) to pass the blood-brain barrier. In some European countries, GHB is used as an anaesthetic drug, but it has insufficient analgesic effects that necessitates its combination with an analgesic; adverse effects, such as seizures or vomiting, frequently occur. Moreover, controlling dosage and duration of effects is difficult.[1] Later, GHB was also used in the treatment of alcohol and heroin withdrawal.[2] Since studies have shown that GHB has a positive effect on patients with narcolepsy and cataplexy, the substance was licensed for their use in the United States in 2002 and in Europe in 2005, despite the fact that these therapies have many side-effects.[3]

As early as the 1980s, GHB was offered in the USA as a dietary supplement. Bodybuilders, in particular, used GHB because of its suggested anabolic muscle-building effects through the increased release of growth hormones. It was also consumed for its supposed aphrodisiac and weight-loss effects, and as a sleeping aid.[1,4] After numerous cases of intoxication occurred over the following years, the US Food and Drug Administration (FDA) banned its sale to consumers in 1990.[5] However, this did not put a stop to misuse of the drug.[6] GHB gained in popularity; its users consumed 'liquid ecstasy' or 'soap' on the club scene as a party drug or at private events, as an alternative to, or in combination with, alcohol. From the USA, the new drug spread through the whole of Europe in the following years.

Legal regulations

Illicit forms of GHB have come under narcotics law in the USA since 2000 and are subject to the strictest categorization (Schedule I). An approved form of GHB is federally listed as a Schedule III controlled substance. In some parts of Europe (Germany, for example), GHB is still approved for treatment as an anaesthetic (Somsanit®; 4.0–6.0 g/70 kg BW (i.v.)) and for the treatment of narcolepsy (Xyrem®; 4.5–9.0 g/day (orally)).

In March 2001, GHB was added to Schedule IV of the 1971 UN Convention on Psychotropic Substances, thereby binding all EU member states to control it under their legislation addressing psychotropic substances. However, GHB is not yet fully under national control in all countries.[7]

Gamma-butyrolactone and 1,4-butanediol

Since GHB is subject to the Narcotics Act, legal substitutes such as gamma-butyrolactone (GBL) or 1,4-butanediol (BD),

* *Correspondence to: H. Andresen, Institute of Legal Medicine, Department of Toxicology, University Medical Centre, Hamburg-Eppendorf, Hamburg, Germany. E-mail: h.andresen@uke.uni-hamburg.de*

a *Institute of Legal Medicine, Department of Toxicology, University Medical Centre, Hamburg-Eppendorf, Hamburg, Germany*

b *Department for Addiction Treatment, Asklepios Clinic North/Ochsenzoll, Hamburg, Germany*

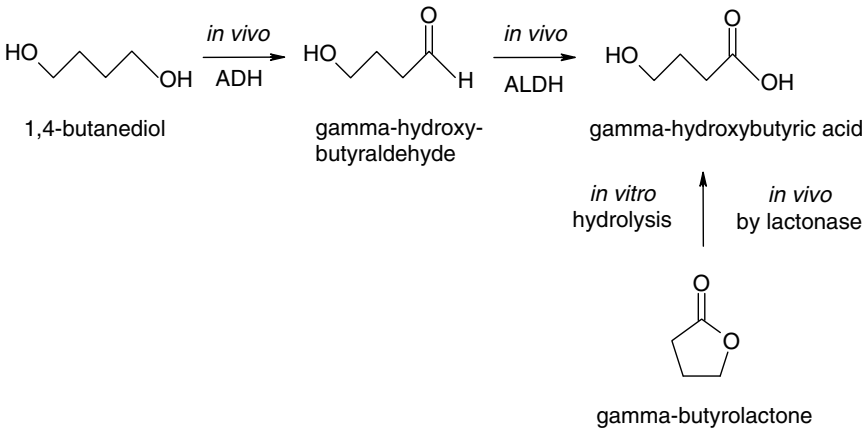

Figure 1. Conversion of GBL and 1,4-BD to GHB; ADH: Alcohol dehydrogenase ALDH: Aldehyde dehydrogenase.

the most commonly used GHB analogues, are currently being consumed. These substances are metabolized into GHB after oral administration (Figure 1) or they are transformed into GHB before ingestion by means of a simple chemical reaction, either by the dealers or the users themselves. Instructions on how to synthesize these substances, as well as complete 'chemistry kits', are available on the Internet. Since the actual effective substance is GHB, consumption of these precursors results in the same effects, side effects, and symptoms of intoxication.[8,9] For this reason, the FDA warned the public as early as 1999 not to consume GBL-containing products and called for a voluntary stop to the trade of the products. More seldom is the consumption of the substitutes gamma-valerolactone (GVL) or possibly gamma-hydroxybutyraldehyde.[10]

In most countries, the GHB precursors (GBL and 1,4-BD) are not controlled under current drug or medicine legislation owing to their widespread use as solvents and the associated consequences for the chemical industry. Although three EU member states (Sweden, Italy, and Latvia) have chosen to control them under the same, or similar legislation as that affecting GHB.[11] In some countries, however, voluntary monitoring systems are being established, such as the German Federal Criminal Office in 2002, in order to control the sale to private users. Since not all traders of the chemicals participate, however, it is still easy for potential consumers to buy GBL or 1,4-BD.

Recently in Germany, several dealers of GBL were punished based on the pharmaceutical law. They were accused of selling so-called 'precarious pharmaceuticals' with a reasonable suspicion of harmful effects (on the basis of § 5 and § 95 of the German pharmaceutical act).

Prevalence/social implications

Quantitative data on the prevalence of GHB abuse is incomplete, but various qualitative measures indicate that a mini-epidemic of abuse began in the late 1980s and continues up to the present. Undoubtedly, the easy availability and low costs of GHB and its precursors have contributed to its popularity.[12] According to a study by the European Monitoring Centre for Drugs and Drug Addiction,[13] consumption of GHB or GBL is not as widespread as consumption of other illegal drugs. Studies have shown that the prevalence of young people's leisure use of GHB in the past month rarely exceeds 3%. However, there are also signs that consumption is becoming more common in private spheres, especially in certain population subgroups and certain social environments or geographical areas. Depending on the particular study and target group interviewed, the lifetime prevalence is 3% to 19% (six studies were included from the UK, the Netherlands, and Austria with data from years 1999 to 2007).[13]

According to various national reporting systems in the USA, there has been an emergence of a problem with GHB abuse beginning no later than 1994 or 1995. Quantitative data on the prevalence in the population is not available due to the fact that the use of GHB is not queried specifically, but is included under 'other drugs' in most surveys since 2001.[12] Since the final active agent in the body is GHB, even in cases of GBL or 1,4-BD consumption, an exact discrimination between these drugs is not possible in most cases.

In spite of the comparatively low popularity of GHB, cases of intoxication, some with a lethal result, have been increasing.[5,14,15] Exact numbers of deaths in Germany or other countries are currently not available.

Since GHB is not yet detected in routine toxicological screening, the prevalence of GHB abuse is thought to be underestimated.

Pharmacodynamics

Means of action

GHB is a naturally occurring hydroxy carboxylic acid in the human brain, blood, urine, and peripheral tissue.[16] It appears both as a metabolic product and as a precursor of GABA.[17] In spite of numerous studies, its function has not yet been clarified in detail, but there is evidence that GHB has different effects on the balance of neurotransmitters.[18] One possible mechanism of action in discussion is that GHB acts as a neuromodulator in the GABA system. Moreover, GHB causes a dose-response increase or decrease of dopamine levels in the brain and affects the cholinergic system (via $GABA_B$ receptors), as well as neurosteroids and, indirectly, opioids. Furthermore, GHB potentiates brain serotonin turnover and increases the secretion of growth hormones (Figure 2). In millimolar concentrations (above endogenous concentrations), GHB already activates $GABA_B$-receptors itself or by conversion to GABA and thereby inhibits the influx of Ca^{2+} and opens K^+-channels, as GABA does. In addition, the existence of a separate G-protein coupled GHB-receptor in the brain has been proven.[19] This receptor is

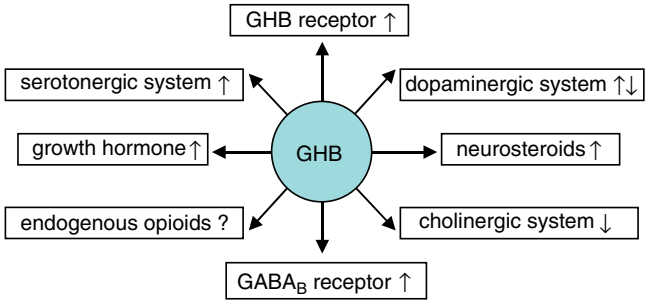

Figure 2. Postulated means of GHB action in the human brain.

thought to be stimulated already by endogenous concentrations of GHB (micromolar range). It is suggested that after exogenous admission, GHB will act both as a $GABA_B$- and GHB-receptor agonist.[18]

Dosage and symptoms

GHB is administered intravenously for the purpose of sedation, and orally for most other indications. Misuse occurs mostly by oral administration in liquid form, neat or diluted in drinks. Occasionally, capsules are ingested, which often contain GHB in the form of its sodium salt (sodium oxybate). GHB induces a biphasic time profile with an initial stimulant-like effect related to the simultaneous rise of plasma concentrations and a latter sedative effect not related to GHB kinetics.[20]

GHB has a narrow therapeutic index; the symptoms of poisoning are dose-dependent and partly similar to those of alcohol poisoning. Headache, nausea, vomiting, vertigo, and speech impairment may be present. Higher concentrations may lead to bradycardia, seizures, respiratory depression to the point of respiratory arrest, and impaired consciousness to the point of coma. In severe intoxication, myoclonus or seizures followed by loss of consciousness have often been described. A typical characteristic is a rapid onset and sudden clinical picture. Owing to the extremely fast metabolism of GHB, usually the effects last only 1–4 h, depending on the dose. This phenomenon is known as the 'fast-in, fast-out' effect[18] and causes a sudden awakening from (near) coma in most cases,[21,22] with patients usually recovering within 6–8 h after receiving symptom-oriented treatment.[4]

Patients who developed a coma (Glasgow Coma Scale (GCS) score of 3) had serum levels that ranged from 72 to 300 mg/L with a median of 193 mg/L and regained consciousness as levels decreased to the range of 75 to 150 mg/L.[23] In 15 patients with GCS <8, blood concentrations of 112 to 430 mg/L were determined (most of them co-ingested other drugs or ethanol).[22] One patient was in a state of coma (GCS 7) after ingestion of 1,4-BD, corresponding blood levels were 82 mg/L for 1,4-BD and 103 mg/L for GHB.[24] A woman who was admitted to an emergency department in a comatose state had 161 mg/L GHB in serum after ingestion of GBL from nail polish remover pads.[25]

Pharmacokinetics

Absorption and distribution

After oral administration, GHB is absorbed very rapidly; maximum plasma concentrations are reached after 25–45 min. The distribution throughout the body is fast and follows a two-compartment model.[14] The apparent distribution volume is reported with 0.19 to 0.4 L/kg[26–28] and was not significantly affected by gender, food, or liver cirrhosis.[28,29]

GBL has potentially a higher intestinal flux after oral administration and a shorter onset, therefore causing higher C_{max} of GHB.[30] 1,4-butanediol is also quickly absorbed and metabolized. It is extensively converted to GHB after oral administration, but significant inter-individual variability in the rate of metabolism, possibly related to genetic variants in the alcohol dehydrogenase, was observed.[27] Moreover, conversion of 1,4-BD is blocked competitively by ethanol.[31]

Biosynthesis, metabolism, and elimination

Endogenous GHB is formed from GABA by means of succinic semialdehyde reductase via the intermediate product succinic semialdehyde (SSA). Via the enzyme GHB dehydrogenase, GHB can be oxidated back into SSA. After a further oxidation step, SSA reaches the citric acid cycle, mainly as succinic acid[32] (Figure 3). Only a small proportion of GHB is excreted in an unchanged form through the kidneys (<2%). GHB is eliminated rapidly; the terminal elimination half-life in plasma averages 20–60 min after single oral doses, but there are indications that the elimination kinetics of GHB is not linear after administration of therapeutic doses.[14,33,34] Moreover, in some cases after ingestion of large doses, zero order kinetics seems to be more appropriate than first-order kinetics.[35]

Drug facilitated sexual assaults/chemical submission

GHB and its precursors are colourless liquids, transparent, and almost entirely without taste. They can thus be mixed with drinks to administer to unsuspecting victims without their noticing, and are used with criminal intent to weaken the resistance of individuals, for example to exploit their property or body without their consent and without them having the slightest recollection afterwards of what happened (anterograde amnesia). Criminals tend to use substances such as GHB to facilitate the commitment of their offences. They also tend to use substances currently not under international control, such as ketamine, 1,4-BD and GBL, since they are easily available through legitimate channels.[7]

GHB can result in impaired consciousness, including coma with anterograde amnesia. Studies have shown that GHB is one of the substances most often used for the purpose of drug facilitated sexual assault (DFSA) in the USA.[5,36,37] Similar cases have also been reported in Germany and other European countries.[11,38] For a detailed summary of the use of GHB in DFSA, see Marinetti and LeBeau.[37]

Generally, the true incidence of these cases will be higher because of the non-reporting or delayed reporting by the victim, or as a result of the failure to collect samples in time and as a consequence of the very short detection window of GHB (approx. 5–8 h in blood and up to 12 h in urine samples).[36]

Therefore, in Resolution 52/8, the Commission on Narcotic Drugs urged member states to adopt measures to address the emerging problem of the use of substances to facilitate sexual assault. The substances covered by that resolution include, for example, controlled narcotic drugs such as cannabis, benzodiazepines, GHB, and zolpidem, and substances that are not under international control, such as alcohol, 1,4-BD, GBL, chloral hydrate, ketamine,

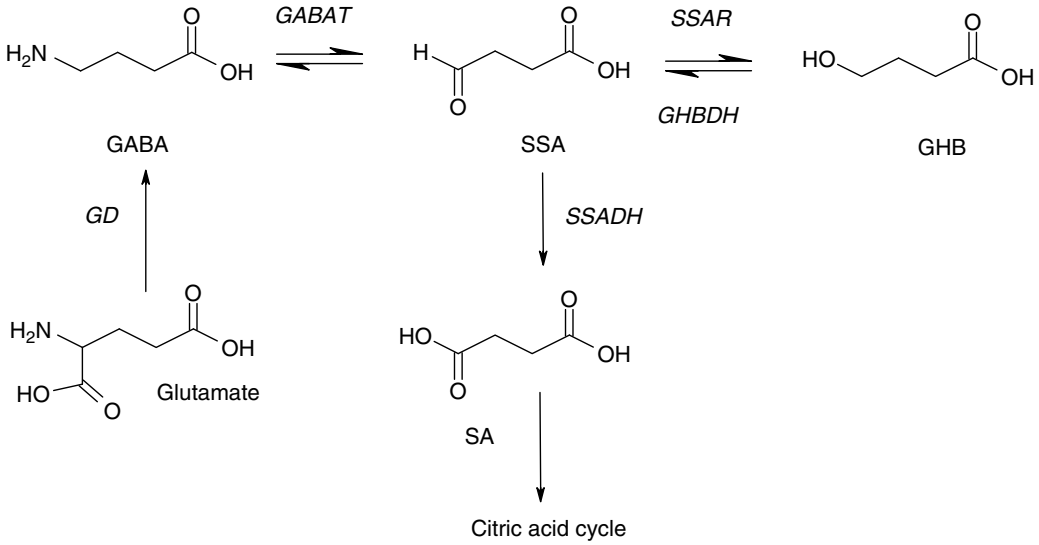

Figure 3. Main metabolic pathways for GABA and GHB; GABAT: GABA-Transaminase; GD: Glutamate decarboxylase; GHBDH: GHB-Dehydrogenase; SA: succinic acid; SSA: succinic semialdehyde; SSAR: SSA-Reductase; SSADH: SSA-Dehydrogenase.

and scopolamine. Furthermore, the Commission urged member states to enhance public awareness of the problem and to consider imposing stricter controls or take other measures aimed at discouraging the use of such substances for the commission of DFSA, including those substances not under international control. Member states were also encouraged to share, through bilateral, regional, and international channels, information on emerging trends in the use of drugs to commit such offences.[7]

Driving under the influence

Because of its depressant effect on the central nervous system, the use of GHB and its analogues is also important in cases of driving under the influence of drugs (DUID). For example, Jones *et al*. identified in their database 548 DUID cases with GHB (alone or together with other recreational drugs) for the years 1997 to 2007 in Sweden.[39] Bosman and Lusthof found 13 DUID cases with high concentrations of GHB within two years.[40] Couper and Logan reported the case of a 38-year-old male who was arrested for driving under the influence of GHB/GBL seven times over an 8-month period.[41]

Fatalities

Fatalities have been reported with accidental overdoses of GHB,[9,15,42,43] suicidal intent, or trauma as a result of impaired driving.[44,45] Fatal overdoses occur also with the precursors GBL[25] or 1,4-BD.[44,45]

Especially post-mortem analytical GHB results should be interpreted with caution. This problem will be discussed later. Furthermore, it should be recognized that abrupt GHB withdrawal can cause life-threatening clinical courses which can even lead to death.

Addiction

Initial studies after clinical administration of GHB in patients with narcolepsy have not shown any cases of misuse or of a developing tolerance. More recent studies, however, indicate the potential for the drug as mentally and physically addictive and suggest a high abuse liability.[20] GHB abuse can lead to addiction with severe withdrawal complications; the same is valid for its precursors. GHB addiction is characterized by round-the-clock dosing, typically every 1–3 h. According to our experience, typical doses are 20 to 70 millilitres per day; reported doses of patients with GBL/1,4-BD withdrawal in the literature ranged from 'two sips every night' up to 900 mL/day during five weeks to four years.[10] Addiction can develop within a few weeks. Withdrawal symptoms after abrupt GHB discontinuation manifested very quickly within 2–6 h.[46] Withdrawal complications of dependence can be associated with a range of neurological phenomena including tremor, anxiety attacks, confusion, epileptic fits, and memory loss. These initial symptoms may progress to severe delirium with auditory and visual hallucinations and autonomic instability. Cardiovascular effects included severe tachycardia and hypertension. Life-threatening clinical courses with admission to the intensive care unit have been documented.[47] Most patients typically requested medical assistance for continued withdrawal symptoms from three days to up to 14 days or more after the last dose.[10,47,48] Benzodiazepines, barbiturates, and antipsychotic medication are the most used drugs in the treatment of GHB withdrawal symptoms.[49] However, in many cases, high doses of benzodiazepines and barbiturates proved ineffective. A sufficient medical treatment of the GHB withdrawal syndrome comparable to the treatment of alcohol or benzodiazepine addiction is not yet clearly defined.[50]

Analytic

Sampling

One general problem in GHB analysis is the very small window of detection, due to the rapid elimination of this acid.[36] In urine, GHB was detectable for approximately 12 h and probably less than 6 h in blood.[33] It is therefore important that the specimens are obtained as early as possible. Given that some authors observed time- and temperature-dependent *in vitro* changes

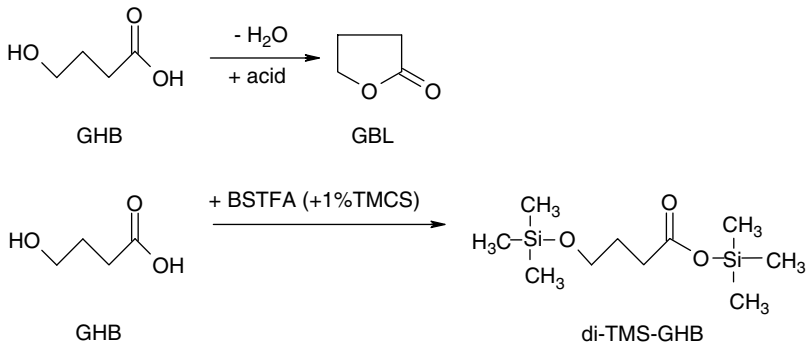

Figure 4. Two methods of formation derivatives prior to GHB analysis.

Table 1. Selected published analytical methods for GHB analysis by GC/MS with silylization. Mode of extraction, internal standard (IS), derivatization reagent, detection technique and limit of detection (LOD) and/or limit of quantification (LOQ) (if available) are displayed

Extraction/ separation	IS	Derivatisation reagent	Detection	LOD	LOQ	Author(s)
liquid/liquid (ACN)	GHB-d6	BSTFA (+1%TMCS)	GC/MS		1 mg/L, serum, urine	Villain *et al.*[55]
liquid/liquid (EtAc)	GHB-d6	BSTFA (+1%TMCS)	GC/MS		n.a.	Elian[56]
liquid/liquid (EtAc)	GHB-d6	BSTFA (+1%TMCS)	GC/MS	0.2 mg/L, urine, plasma		Elliott[57]
liquid/liquid (EtAc)	GHB-d6	BSTFA (+1%TMCS)	GC/MS	0.2 mg/L, urine	0.4 mg/L, urine	Kerrigan[51]
liquid/liquid (EtAc)	diethylene glycol	BSTFA (+1%TMCS)	GC/MS		1 mg/L, serum	Couper and Logan[1]
liquid/liquid (TBME)	Gamma-caprolactone (4-hydroxy-caproic acid)	BSTFA (+1%TMCS)	GC/MS	<2 mg/L, urine		Kavanagh *et al.*[58]
liquid/liquid (ACN)	valproic acid	MSTFA	GC/MS	2 mg/L, serum		Louagi *et al.*[21]
precipitation (MeOH)	2-hydroxy-caproic acid	BSTFA (+1%TMCS)	GC/MS		0.01 mg/L, urine	Shima *et al.*[59]
SPE	GHB-d6	BSTFA (+1%TMCS)	GC/MS		>5 mg/L, urine	Mc Cusker *et al.*[60]
SPE	GHB-d6	BSTFA (+1%TMCS)	GC/MS	1 mg/L, blood		Kalasinsky *et al.*[42]

n.a. not available.
ACN: acetonitrile; BSTFA: bis-trimethyl-silyl-trifluoroacetamide, EtAc: ethyl acetate; GC/MS: gas chromatography/mass spectrometry; MeOH: methanol; MSTFA: *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide; SPE: solid phase extraction; TBME: *tert*-butylmethylether; TMCS: trimethyl-chlorosilane.

of GHB concentrations,[51,52] storage in refrigerated or frozen conditions is important. Some investigators described a possible influence of additives: LeBeau *et al*. supposed an *in vitro* increase of GHB in citrate-buffered blood after long-term storage.[53] Therefore, sodium fluoride or no preservatives should be used in samples for GHB analysis.

Chromatographic methods

Currently, there are numerous chromatographic methods that have been published for determining GHB in blood, urine, and other specimens. Basic principles of these methods (Figure 4) are either cyclization of the carbonic acid to lactone structure (GBL) (first described by Doherty *et al*.)[54] and detection with gas chromatography/flame ionization detector (GC/FID) or gas chromatography/mass spectrometry (GC/MS), or turning into silyl derivatives and detection via GC/MS. Most of these methods differ only in the choice of the internal standards and extraction procedures. Moreover, other techniques such as liquid chromatography/mass spectrometry (LC/MS), ionic or thin layer chromatography, and capillary electrophoresis were used. In addition, the procedures show different limits of detection (LOD) and limits of quantification (LOQ) (Tables 1–3).

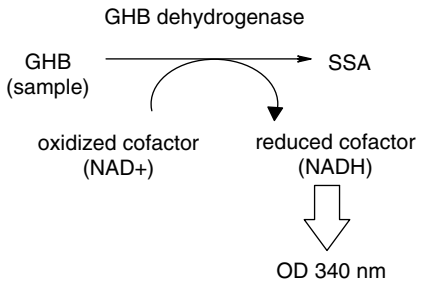

Figure 5. Principle of the enzymatic assay of GHB OD: optical density.

Rapid screening method

Until now, analysis of GHB was only possible by laborious chromatographic methods. Due to its non-characteristic chemical structure, an immunological test could not be developed.

Recently, however, an enzymatic GHB test was developed and evaluated for clinical chemistry analyzers (Bühlmann Laboratories, Schönenbuch, CH) using the degradation step of GHB to succinic semialdehyde (SSA) by GHB-dehydrogenase. GHB is converted to its metabolite by the action of GHB-specific recombinant enzyme and oxidized nicotinamide adenine dinucleotide (NAD^+). The

Table 2. Selected published analytical methods for GHB analysis by GC/MS with lactone conversion. Mode of extraction, internal standard (IS), derivatization reagent, detection technique and limit of detection (LOD) and/or limit of quantification (LOQ) (if available) are displayed

Extraction/ separation	IS	Derivatisation reagent	Detection	LOD	LOQ	Author(s)
liquid/liquid (chloroform)	GHB-d6	+ perchloric acid: GHB → GBL	GC/MS		n.a.	Bosman and Lusthof[40]
liquid/liquid (MeCl)	GHB-d6,	+ H_2SO_4: GHB → GBL	HS-GC/MS	0.5 mg/L, urine, blood		LeBeau *et al*.[61]
liquid/liquid (MeOH)	GVA	+ NaOH: GBL → GHB MSTFA	GC/MS	1.5 mg/L, urine, blood, brain tissue, ocular fluid	4.9 mg/L urine, blood, brain tissue, ocular fluid	Duer *et al*.[9]
liquid/liquid (chloroform)	GVL	+ trifluoroacetic acid: GHB → GBL	GC/MS		0.5 mg/L plasma; 0.2 mg/L urine	Brenneisen *et al*.[14]
liquid/liquid (benzene)	delta-valerolactone	+ hydrochloric acid (urine)/perchloric acid (plasma): GHB → GBL	GC/MS	0.2 mg/L plasma, 0.1 mg/L urine		Ferrara *et al*.[62]
HS-SPME	GHB-d6,	+ H_2SO_4: GHB → GBL	GC/MS	0.6 mg/L serum, 5.0 mg/L urine		Merckel *et al*.[63]
SPDE	GBL-d6	+ H_2SO_4: GHB → GBL	GC/MS	0.5 mg/L serum, urine	2 mg/L serum, 4 mg/L urine	Lenz *et al*.[25]

n.a.: not available.
GC/MS: Gas chromatography/mass spectrometry; GVA: Gamma-valeric acid; HS: head space; MeCl: methylene chloride; MeOH: methanol; MSTFA: *N*-methyl-*N*-trimethylsilyltrifluoroacetamide; SPDE: solid-phase dynamic extraction; SPME: solid phase microextraction.

Table 3. Selected published analytical methods for GHB analysis with other detection techniques. Mode of extraction, internal standard (IS), derivatization reagent, detection technique and limit of detection (LOD) and/or limit of quantification (LOQ) (if available) are displayed

Extraction/separation	IS	Derivatisation reagent	Detection	LOD	LOQ	Author(s)
liquid/liquid (EtAc o. MTBE)	GHB-d6		LC/MS		0.6 mg/L serum, 2.4 mg/L urine	Kaufmann *et al*.[64]
liquid/liquid (MeCl)	AMGBL	+ H_2SO_4: GHB → GBL	GC/FID	0.5 mg/L serum, urine		LeBeau *et al*.[61]
liquid/liquid (chloroform)	hexanoic acid	+ H_2SO_4: GHB → GBL	GC/FID	2.5 mg/L		Elliott[57]
capillary electrophoresis	AHA		CE-C^4D		2.4 mg/L urine	Gong *et al*.[65]
capillary electrophoresis	AHA	+ NaOH: GBL → GHB	indirect UV	approx. 3 mg/L, urine, serum		Bortolotti *et al*.[66]
capillary electrophoresis	maleic acid		MS		20 mg/L urine	Gottardo *et al*.[67]
		+ Br-DMEQ → fluorescent derivative	TLC		100 mg/L urine	Matsuda *et al*.[68]

AHA: Alpha-hydroxybutyric acid; AMGBL: alpha-methyl-gamma-butyrolactone; Br-DMEQ: 3-bromomethyl-6,7-dimethoxy-1-methyl-1,2-dihydroquinoxaline-2-one; CE-C^4D: capacitively coupled conductivity detection; EtAc: ethyl acetate; GC/FID: Gas chromatography/flame ionization detection; HS: head space; MeCl: methylene chloride; LC/MS: Liquid chromatography/mass spectrometry; MS: Mass spectrometry; MTBE: methyl-tert-butyl-ether; TLC: Thin layer chromatography.

increase in absorbance at 340 nm resulting from the reduction of NAD^+ into NADH is proportional to the amount of GHB in the sample (Figure 5). Pursuant to the assay information of the manufacturer, the functional sensitivity is given with 7 mg/L and 3 mg/L for serum and urine respectively. Thereby, a fast and economic testing method for urine and serum for clinical purposes or abstinence controls is possible and less equipped laboratories will also be able to measure GHB. Of course, particularly in forensic cases, confirmation tests are indispensable.

Colour tests

For qualitative screening, some methods were published for rapid colourimetric tests. They are suitable for urine or liquids with higher GHB concentrations. Some of them involve the conversion of GHB to GBL; therefore they are not suitable to distinguish these two compounds.[69]

Detection of GBL, 1,4-BD, GVL

Generally, GHB is the effective agent even in cases of GBL or 1,4-BD use. The metabolism of GBL is very fast; therefore a short time after ingestion of this substance, GHB will be solely, or at least mainly, present in blood. In cases of 1,4-BD toxicity, 1,4-BD can be detected alongside GHB in some cases, but typically in considerably smaller amounts.[9,25]

Given that GHB is the active agent, a determination of GBL or 1,4-BD will not be necessary, especially in persons who

show symptoms of GHB action. In some forensic cases, particularly in secured liquids, it could be important to differentiate between GHB and other drugs. In cases of liquids, the conversion from GHB to GBL in acidic solutions has to be considered.[70]

Methods which use the conversion from GHB to GBL[62] could also detect native GBL, but cannot distinguish these two compounds. A test of GBL is possible by having one sample mixed with acid to convert GHB to GBL and one sample without acid to detect existing GBL.[61]

Lora-Tamayo *et al*. published a method to detect 1,4-BD and GHB in parallel by GC/MS (extraction with ethyl acetate, derivatization with BSTFA + 1% TMCS, IS: GHB-d6). In one case of intoxication, they found 103 mg/L GHB and still 82 mg/L 1,4-BD in blood.[24] Duer *et al*. determined 1,4-BD by HPLC-DAD after derivatization with 3,5-dinitrobenzoyl chloride.[9]

Gamma-valerolactone is reported to also be an alternative to illegal GHB. Its active form, built *in vivo* via lactonase, is the carboxylic acid (gamma-hydroxyvaleric acid/gamma-methyl gamma-hydroxybutyrate (4-Me-GHB)), which is not yet available for commercial purchase. Therefore, synthesis of the active agent (as described by Duer *et al*.,[9] for example) and detection via GC/MS as di-TMS derivative,[8] or analysis after cyclization to GVL and detection of the lactone (as described for GVL as internal standard, for example by Brenneisen *et al*.),[14] is possible.

Cut-Offs

Because GHB is an endogenous substance, generally traces or smaller amounts can be detected in human tissue and specimens. Therefore, discrimination between endogenous and exogenous GHB is complicated, particularly in those samples with low concentrations. Numerous authors recommend cut-offs for ante-mortem specimens (urine, plasma/serum) to facilitate such a decision. However, a standard procedure has not yet been specified.

Ante-mortem samples

For blood (serum/plasma) samples, 4 mg/L[57] and 5 mg/L[40,71] as threshold concentrations between exogenous and endogenous GHB were suggested. For urine, cut-offs between 2 mg/L[58] and 10 mg/L[40,57,71] were proposed.

Brenneisen *et al*. argue that a recommended 10 mg/L cut-off in urine may be too high to be optimally sensitive for forensic purposes.[14] Mari *et al*. present new data from volunteers with GHB therapy and conclude that one must act with caution when applying the currently used cut-off of 10 mg/L for urine.[72] Recently, we recommended 4 mg/L and 6 mg/L as cut-offs for ante-mortem serum and urine samples, respectively.[73] Hopefully, new methods such as carbon isotopic ratio analysis will help to discriminate exogenous from endogenous GHB levels in human specimens in the future.[74]

Post-mortem specimen

In post-mortem peripheral blood samples, high concentrations of GHB from zero to 197 mg/L could be measured, even in cases without GHB ingestion (Table 4). These levels overlap with the range of reported fatal GHB intoxications with concentrations of 27 to 2937 mg/L (Table 5) and could possibly lead to misinterpretation as intoxication. The origin of these high amounts of GHB is not yet clarified, but it could be slightly reduced by the use of preservatives (sodium fluoride) and/or freezing specimens immediately after sampling.[45,52,75]

Table 4. GHB concentrations in post-mortem peripheral blood samples without GHB uptake

GHB concentration (mg/L)	authors
0–197	Elliott[81]
0–29	Elliott[79]
3.2–168	Fieler *et al*.[80]
17–44 (HB: 0.4–409)	Kintz *et al*.[78]
11–97	Marinetti *et al*.[8]
0–43.0	Moriya and Hashimoto[82]
0–11.6	Moriya and Hashimoto[76]
5–77	Stephens *et al*.[83]

HB: heart blood samples.

Table 5. GHB concentrations in post-mortem peripheral blood samples with known GHB uptake

GHB concentration (mg/L)	authors
27–1030	Baselt[26]
330	Kalasinsky *et al*.[42]
2937	Kintz *et al*.[43]
170–2200#	Knudsen *et al*.[77]
280	Kraner *et al*.[84]
165, 957	Lenz *et al*.[25]
461	Mazarr-Proo and Kerrigan[15]
18–4400*	Zvosec *et al*.[44]

* In this study, peripheral and heart blood samples were analyzed.
# GHB-poisoning with no or minor influence of other drugs.

Some authors recommended cut-offs for post-mortem specimens, such as Moriya and Hashimoto, who proposed 10 mg/L for urine and 30 mg/L for blood in decedents showing little or no putrefaction (post-mortem interval $<$48 h).[76] This cut-off was supported for femoral vein blood samples by two recently published retrospective studies from Sweden.[45,77] Kintz *et al*. proposed 50 mg/L for post-mortem blood samples, where below this, it is not possible to determine whether any GHB detected is of endogenous synthesis or exogenous intake.[78] Some authors recommend analysis of urine in addition to blood samples because of typically (but not generally)[79] lower GHB concentrations due to less post-mortem generation.[80] Other authors suggested vitreous humour as the specimen of choice, in addition to femoral vein blood.[8,78]

Regarding the concentrations determined by several authors in post-mortem samples with known GHB uptake and in samples of deceased persons who definitely did not consume GHB, determining a cut-off does not seem possible at present. Therefore, the appraisal of post-mortem GHB results remains a challenge.

Summary

Gamma-hydroxybutyric acid is a substance which is used as an abused drug in the USA and Europe. Besides GHB, the legal precursors GBL and 1,4-BD (and possibly GVL) are used because

these solvents are not included as scheduled substances yet. Severe intoxications and also fatal casualties occur. In addition, GHB is one of the substances which are used in cases of chemical submission (e.g. date rape) because of the short elimination half-life and thus a limited detection window. In the last years, cases of addiction have also been reported; withdrawal symptoms are difficult to treat.

There exist numerous analytical methods to determine GHB in human specimens, mainly using two basic principles (cyclization to GBL or silylization) combined with chromatographic techniques. Apart from this, an enzymatic test and other methods are possible. However, analysis of GHB cases is complex because of the small detection window, post-mortem generation of GHB, and the necessity to discriminate between endogenous and exogenous GHB.

References

[1] F. J. Couper, B. K. Logan. Determination of gamma-hydroxybutyrate (GHB) in biological specimens by gas chromatography-mass spectrometry. *J. Anal. Toxicol.* **2000**, *24*, 1.
[2] L. Gallimberti, M. Spella, C. Soncini, G. Gessa. Gamma-hydroxybutyric acid in the treatment of alcohol and heroin dependence. *Alcohol* **2000**, *20*, 257.
[3] D. Fuller, C. Hornfeldt. From club drug to orphan drug: sodium oxybate (Xyrem) for the treatment of cataplexy. *Pharmacotherapy* **2003**, *23*, 1205.
[4] A. Templeton, H-J. Vonesch. Intoxikation mit GHB ('liquid ecstasy'). *Schweiz. Med. Forum* **2005**, *5*, 115.
[5] M. A. El Sohly, S. J. Salamone. Prevalence of drug used in cases of alleged sexual assault. *J. Anal. Toxicol.* **1999**, *23*, 141.
[6] Centers of Disease Control. Multistate outbreak of poisonings associated with illicit use of gamma hydroxy butyrate. *JAMA* **1991**, *265*, 447.
[7] INCB. Available at: http://www.incb.org/incb/en/annual-report-2009.html [24 February 2010].
[8] L. J. Marinetti, D. S. Isenschmid, B. R. Hepler, S. Kanluen. Analysis of GHB and 4-methyl-GHB in postmortem matrices after long-term storage. *J. Anal. Toxicol.* **2005**, *29*, 41.
[9] W. C. Duer, K. L. Byers, J. V. Martin. 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*, 576.
[10] R. Palmer. Gamma-butyrolactone and 1,4-butanediol; abused analogues of gamma-hydroxybutyrate. *Toxicol. Rev.* **2004**, *23*, 21.
[11] EMCDDA. Sexual assaults facilitated by drugs or alcohol. Available at: http://www.emcdda.europa.eu/html.cfm/index7291EN.html [March 2008].
[12] K. L. Nicholson, R. L. Balster. GHB: a new and novel drug of abuse. *Drug Alcohol Depen.* **2001**, *63*, 1.
[13] EMCDDA. GHB and its precursor GBL: an emerging trend case study. Available at: http://www.emcdda.europa.eu/html.cfm/index7079EN.html [March 2008].
[14] R. Brenneisen, M. A. ElSohly, T. P. Murphy, J. Passarelli, S. Russmann, S. J. Salamone, D. E. Watson. Pharmacokinetics and excretion of gamma-hydroxybutyrate (GHB) in healthy subjects. *J. Anal. Toxicol.* **2004**, *28*, 625.
[15] S. Mazarr-Proo, S. Kerrigan. Distribution of GHB in tissues and fluids following a fatal overdose. *J. Anal. Toxicol.* **2005**, *29*, 398.
[16] R. H. Roth, N. J. Giarman. Natural occurrence of gamma-hydroxybutyrate in mammalian brain. *Biochem. Pharmacol.* **1970**, *19*, 1087.
[17] M. Maitre. The gamma-hydroxybutyrate signalling system in brain: organization and functional implications. *Prog. Neurobiol.* **1997**, *51*, 337.
[18] K. Drasbek, J. Christensen, K. Jensen. Gamma-hydroxybutyrate – a drug of abuse. *Acta Neurol. Scand.* **2006**, *114*, 145.
[19] O. Snead. Evidence for a G protein-coupled gamma-hydroxybutyric acid receptor. *J Neurochem* **2000**, *75*, 1986.
[20] S. Abanades, M. Farré, D. Barral, M. Torrens, N. Closas, K. Langohr, A. Pastor, R. De la Torre. Relative abuse liability of gamma-hydroxybutyric acid, flunitrazepam, and ethanol in club drug users. *J. Clin. Psychopharm.* **2007**, *17*, 625.
[21] H. K. Louagie, A. G. Verstraete, C. J. De Soete, D. G. Baetens, P. A. Calle. A sudden awakening from a near coma after combined intake of gamma-hydroxybutyric acid (GHB) and ethanol. *Clin. Toxicol.* **1997**, *35*, 591.
[22] D. van Sassenbroeck, N. De Neve, P. De Paepe, F. Belpaire, A. Verstraete, P. Calle, W. Buylaert. Abrupt awakening phenomenon associated with gamma-hydroxybutyrate use: a case series. *Clin Toxicol* **2007**, *45*, 533.
[23] K. Sporer, R. Chin, J. Dyer, R. Lamb. Gamma-hydroxybutyrate serum levels and clinical syndrome after severe overdose. *Ann. Emerg. Med.* **2003**, *42*, 3.
[24] C. Lora-Tamayo, T. Tena, A. Rodríguez, J. Sancho, E. Molina. Intoxication due to 1,4-butanediol. *Forensic Sci. Int.* **2003**, *133*, 256.
[25] D. Lenz, M. Rothschild, L. Kroener. Intoxications due to ingestion of gamma-butyrolactone: organ distribution of gamma-hydroxybutyric acid and gamma-butyrolactone. *Ther. Drug Monit.* **2008**, *30*, 755.
[26] R. C. Baselt, *Gamma-Hydroxybutyrate*, 8th ed. Biomedical Publications: Foster City, **2008**.
[27] D. Thai, J. Dyer, P. Jacob, C. Haller. Clinical pharmacology of 1,4-butanediol and gamma-hydroxybutyrate after oral 1,4-butanediol administration to healthy volunteers. *Clin. Pharmacol. Ther.* **2007**, *81*, 178.
[28] L. Borgen, R. Okerholm, D. Morrison, A. Lai. The influence of gender and food on the pharmacokinetics of sodium oxybate oral solution in healthy subjects. *J. Clin. Pharmacol.* **2003**, *43*, 59.
[29] S. Ferrara, L. Tedeschi, G. Frison, R. Orlando, M. Mazzo, R. Zordan, R. Padrini, P. Palatini. Effect of moderate or severe liver dysfunction on the pharmacokinetics of gamma-hydroxybutyric acid. *Eur. J. Clin. Pharmacol.* **1996**, *50*, 305.
[30] A. Guidotti, P. Ballotti. Relationship between pharmacological effects and blood and brain levels of gamma-butyrolactone and gamma-hydroxybutyrate. *Biochem. Pharmacol.* **1969**, *19*, 883.
[31] D. K. Van Sassenbroeck, P. De Paepe, F. M. Belpaire, W. A. Buylaert. Characterization of the pharmacokinetic and pharmacodynamic interaction between gamma-hydroxybutyrate and ethanol in the rat. *Toxicol. Sci.* **2003**, *73*, 270.
[32] E. Kaufman, T. Nelson. Evidence for the participation of a cytosolic NADP+-dependent oxidoreductase in the catabolism of gamma-hydroxybutyrate in vivo. *J. Neurochem.* **1987**, *48*, 1935.
[33] P. Palatini, L. Tedeschi, G. Frison, R. Padrini, R. Zordan, R. Orlando, L. Gallimberti, G. L. Gessa, S. D. Ferrara. Dose-dependent absorption and elimination of gamma-hydroxybutyric acid in healthy volunteers. *Eur. J. Clin. Pharmacol.* **1993**, *45*, 353.
[34] S. Ferrara, S. Zotti, L. Tedeschi, G. Frison, F. Castagna, L. Gallimberti, G. Gessa, P. Palatini. Pharmacokinetics of gamma-hydroxybutyric acid in alcohol dependent patients after single and repeated doses. *Brit. J. Clin. Pharmaco.* **1992**, *34*, 231.
[35] A. Jones, A. Eklund, R. Kronstrand. Concentration-time profiles of gamma-hydroxybutyrate in blood after recreational doses are best described by zero-order rather than first-order kinetics. *J. Anal. Toxicol.* **2009**, *33*, 332.
[36] M. A. LeBeau, W. Andollo, W. Hearn, R. Baselt, E. Cone, B. Finkle, D. Fraser, A. Jenkins, J. Mayer, A. Negrusz, A. Poklis, H. Walls, L. Raymon, M. Robertson, J. Saady. Recommendation for toxicological investigations of drug-facilitated sexual assaults. *J. Forensic Sci.* **1999**, *44*, 227.
[37] L. Marinetti, M. LeBeau. The use of GHB and analogs to facilitate sexual assault. *Forensic Sci. Rev.* **2010**, *22*, 41.
[38] M. Scott-Ham, F. C. Burton. Toxicological findings in cases of alleged drug-facilitated sexual assault in the United Kingdom over a 3-year period. *J. Clin. Forensic Med.* **2005**, *12*, 175.
[39] A. Jones, A. Holmgren, F. Kugelberg. Driving under the influence of gamma-hydroxybutyrate (GHB). *Forensic Sci. Med. Pathol.* **2008**, *4*, 205.
[40] I. J. Bosman, K. J. Lusthof. Forensic cases involving the use of GHB in the Netherlands. *Forensic Sci. Int.* **2003**, *133*, 17.
[41] F. J. Couper, B. K. Logan. Addicted to driving under the influence – a GHB/GBL case report. *J. Anal. Toxicol.* **2004**, *28*, 512.
[42] K. S. Kalasinsky, M. M. Dixon, G. A. Schmunk, S. J. Kish. Blood, brain and hair GHB concentrations following fatal ingestion. *J. Forensic Sci.* **2001**, *46*, 728.

[43] P. Kintz, M. Villain, A.-L. Pélisser, V. Cirimele, G. Leonetti. Unusually high concentrations in a fatal GHB case. *J. Anal. Toxicol.* **2005**, *29*, 582.
[44] D. Zvosec, S. Smith, T. Porrata, A. Strobl, J. Dyer. Case series of 226 gamma-hydroxybutyrate-associated deaths: lethal toxicity and trauma. *Am. J. Emerg. Med.* **2010**, Apr 26 [Epub ahaed of print].
[45] F. Kugelberg, A. Holmgren, A. Eklund, A. Jones. Forensic toxicology findings in deaths involving gamma-hydroxybutyrate. *Int. J. Legal Med.* **2010**, *124*, 1.
[46] J. Wojtowicz, M. Yarema, P. Wax. Withdrawal from gamma-hydroxybutyrate, 1,4-butanediol and gamma-butyrolactone: a case report and systematic review. *CJEM* **2008**, *10*, 69.
[47] J. E. Dyer, B. Roth, B. Hyma. Gamma-hydroxybutyrate withdrawal syndrome. *Ann. Emerg. Med.* **2001**, *37*, 147.
[48] C. Mc Daniel, K. Miotto. Gamma hydroxybutyrate (GHB) and gamma butyrolactone (GBL) withdrawal: five case studies. *J. Psychoactive Drugs* **2001**, *33*, 143.
[49] M. van Noorden, L. van Dongen, F. Zitman, T. Vergouwen. Gamma-hydroxybutyrate withdrawal syndrome: dangerous but not well-known. *Gen. Hosp. Psychiat.* **2009**, *31*, 394.
[50] M. Mc Donough, N. Kennedy, A. Glasper, J. Beam. Clinical features and management of gamma-hydroxybutyrate (GHB) withdrawal: a review. *Drug Alcohol Depend.* **2004**, *15*, 3.
[51] S. Kerrigan. In vitro production of gamma-hydroxybutyrate in ante-mortem urine samples. *J. Anal. Toxicol.* **2002**, *26*, 571.
[52] M. A. LeBeau, M. A. Montgomery, C. Morris-Kukoski, J. E. Schaff, A. Deakin. Further evidence of in vitro production of gamma-hydroxybutyrate (GHB) in urine samples. *Forensic Sci. Int.* **2007**, *169*, 152.
[53] M. A. LeBeau, M. A. Montgomery, R. A. Jufer, M. L. Miller. Elevated GHB in citrate-buffered blood. *J. Anal. Toxicol.* **2000**, *24*, 383.
[54] J. Doherty, S. Hattox, O. Snead, R. Roth. Identification of endogenous gamma-hydroxybutyrate in human and bovine brain and its regional distribution in human, guinea pig and rhesus monkey brain. *J. Pharmacol. Exp. Ther.* **1978**, *207*, 130.
[55] M. Villain, V. Cirimele, B. Ludes, P. Kintz. Ultra-rapid procedure to test for gamma-hydroxybutyric acid in blood and urine by gas chromatography-mass spectrometry. *J. Chromatogr.* **2003**, *792*, 83.
[56] A. A. Elian. A novel method for GHB detection in urine and its application in drug-facilitated sexual assaults. *Forensic Sci. Int.* **2000**, *109*, 183.
[57] S. Elliott. Gamma hydroxybutyric acid (GHB) concentrations in humans and factors affecting endogenous production. *Forensic Sci. Int.* **2003**, *133*, 9.
[58] P. Kavanagh, P. Kenny, J. Feely. The urinary excretion of gamma-hydroxybutyric acid in man. *J. Pharm. Pharmacol.* **2001**, *53*, 399.
[59] N. Shima, A. Miki, T. Kamata, M. Katagi, H. Tsuchihashi. Endogenous level and in vitro production of GHB in blood from healthy humans, and the interpretation of GHB levels detected in ante-mortem blood samples. *J. Health Sci.* **2005**, *51*, 147.
[60] R. R. Mc Cusker, H. Paget-Wilkes, C. W. Chronister, B. A. Goldberger, M. A. ElSohly. Analysis of gamma-hydroxybutyrate (GHB) in urine by gas chromatography-mass spectrometry. *J. Anal. Toxicol.* **1999**, *23*, 301.
[61] M. A. LeBeau, M. A. Montgomery, M. L. Miller, S. G. Burmeister. Analysis of biofluids for gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL) by headspace GC-FID and GC-MS. *J. Anal. Toxicol.* **2000**, *24*, 421.
[62] S. Ferrara, L. Tedeschi, G. Frison, F. Castagna, L. Gallimberti, R. Giorgetti, G. Gessa, P. Palatini. Therapeutic gamma-hydroxybutyric acid monitoring in plasma and urine by gas chromatography-mass spectrometry. *J. Pharm. Biomed. Anal.* **1993**, *11*, 483.
[63] C. Merckel, V. Auwärter, D. Simmert, F. Pragst. Gamma-hydroxybuttersäure (GHB) in notfallmedizinischen Proben: Bestimmung durch HS-SPME/GC-MS und Fallbeispiele. *Toxichem Krimtech* **2003**, *70*, 93.
[64] E. Kaufmann, A. Alt. Determination of GHB in urine and serum by LC/MS using a simple one-step derivative. *Forensic Sci. Int.* **2007**, *168*, 133.
[65] X. Gong, P. Kubán, A. Scholer, P. Hauser. Determination of gamma-hydroxybutyric acid in clinical samples using capillary electrophoresis with contactless conductivity detection. *J. Chromatogr. A* **2008**, *1231*, 100.
[66] F. Bortolotti, G. De Paoli, R. Gottardo, M. Trattene, F. Tagliaro. Determination of gamma-hydroxybutyric acid in biological fluids by using capillary electrophoresis with indirect detection. *J. Chromatogr. B* **2004**, *800*, 239.
[67] R. Gottardo, F. Bortolotti, M. Trettene, G. De Paoli, F. Tagliaro. Rapid and direct analysis of gamma-hydroxybutyric acid in urine by capillary electrophoresis-electrospray ionization ion-trap mass spectrometry. *J. Chromatogr. A* **2004**, *1051*, 207.
[68] K. Matsuda, N. Asakawa, M. Iwanaga, A. Gohda, S. Fukushima, Y. Ishii, H. Yamada. Conversion of gamma-hydroxybutyric acid to a fluorescent derivative: a method for screening. *Forensic Toxicol.* **2006**, *24*, 41.
[69] C. Morris-Kukoski. Gamma-hydroxybutyrate; bridging the clinical-analytical gap. *Toxicol. Rev.* **2004**, *23*, 33.
[70] L. Ciolino, M. Mesmer, R. Satzger, A. Machal, H. McCauley, A. Mohrhaus. The chemical interconversion of GHB and GBL: forensic issues and implications. *J. Forensic Sci.* **2001**, *46*, 1315.
[71] A. A. Elian. Determination of endogenous gamma-hydroxybutyric acid (GHB) levels in ante-mortem urine and blood. *Forensic Sci. Int.* **2002**, *128*, 120.
[72] F. Mari, L. Politi, C. Trignano, M. Di Milia, M. Di Padua, E. Bertol. What constitutes a normal ante-mortem urine GHB concentration? *J. Forensic Legal Med.* **2009**, *16*, 148.
[73] H. Andresen, N. Sprys, A. Schmoldt, A. Mueller, S. Iwersen-Bergmann. Gamma-hydroxybutyrate in urine and serum: additional data supporting current cut-off recommendations. *Forensic Sci. Int.* **2010**, *200*, 93.
[74] C. Saudan, M. Augsburger, P. Mangin, M. Saugy. Carbon isotopic ratio analysis by gas chromatography/combustion/isotope ratio mass spectrometry for the detection of gamma-hydroxybutyric acid/GHB) administrations to humans. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 3956.
[75] K. Beránková, K. Mutnanská, M. Balíková. Gamma-hydroxybutyric acid stability and formation in blood and urine. *Forensic Sci. Int.* **2006**, *161*, 158.
[76] F. Moriya, Y. Hashimoto. Site-dependent production of gamma-hydroxybutyric acid in the early post-mortem period. *Forensic Sci. Int.* **2005**, *148*, 139.
[77] K. Knudsen, U. Jonsson, J. Abrahamsson. Twenty-three deaths with γ-hydroxybutyrate overdose in western Sweden between 2000 and 2007. *Acta Anaesth. Scand.* **2010**, *54*, 987.
[78] P. Kintz, M. Villain, V. Cirimele, B. Ludes. GHB in post-mortem toxicology. Discrimination between endogenous production from exposure using multiple specimens. *Forensic Sci. Int.* **2004**, *143*, 177.
[79] S. Elliott. Further evidence for the presence of GHB in post-mortem biological fluid: implications for the interpretation of findings. *J. Anal. Toxicol.* **2004**, *28*, 20.
[80] E. L. Fieler, D. E. Coleman, R. C. Baselt. Gamma-hydroxybutyrate concentrations in pre- and postmortem blood and urine. *Clin. Chem.* **1998**, *44*, 692.
[81] S. Elliott. The presence of gamma-hydroxybutyric acid (GHB) in post-mortem biological fluids. *J. Anal. Toxicol.* **2001**, *25*, 152.
[82] F. Moriya, Y. Hashimoto. Endogenous gamma-hydroxybutyric acid levels in post-mortem blood. *Leg. Med.* **2004**, *6*, 47.
[83] B. Stephens, D. Coleman, R. Baselt. In vitro stability of endogenous gamma-hydroxybutyrate in postmortem blood. *J. Forensic Sci.* **1999**, *44*, 231.
[84] J. Kraner, J. Plassard, D. McCoy, J. Rorabeck, M. Witeck, M. Evans. Fatal overdose from ingestion of 1,4-butanediol, a GHB precursor. *Clin. Toxicol.* **2000**, *38*, 534.