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PAPER IN FOREFRONT

Source inference of exogenous gamma-hydroxybutyric acid (GHB) administered to humans by means of carbon isotopic ratio analysis: novel perspectives regarding forensic investigation and intelligence issues

François Marclay · Christophe Saudan · Julie Vienne · Mehdi Tafti · Martial Saugy

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Abstract γ-Hydroxybutyric acid (GHB) is an endogenous short-chain fatty acid popular as a recreational drug due to sedative and euphoric effects, but also often implicated in drug-facilitated sexual assaults owing to disinhibition and amnesic properties. Whilst discrimination between endogenous and exogenous GHB as required in intoxication cases may be achieved by the determination of the carbon isotope content, such information has not yet been exploited to answer source inference questions of forensic investigation and intelligence interests. However, potential isotopic fractionation effects occurring through the whole metabolism of GHB may be a major concern in this regard. Thus, urine specimens from six healthy male volunteers who ingested prescription GHB sodium salt, marketed as Xyrem®, were analysed by means of gas chromatography/combustion/isotope ratio mass spectrometry to assess this particular topic. A very narrow range of $\delta^{13}C$ values, spreading from −24.81‰ to −25.06‰, was observed, whilst mean $\delta^{13}C$ value of Xyrem® corresponded to −24.99‰. Since urine samples and prescription drug could not be distinguished by means of statistical analysis, carbon isotopic effects and subsequent influence on $\delta^{13}C$ values through GHB metabolism as a whole could be ruled out. Thus, a link between GHB as a raw matrix and found in a biological fluid may be established, bringing relevant information regarding source inference evaluation. Therefore, this study supports a diversified scope of exploitation for stable isotopes characterized in biological matrices from investigations on intoxication cases to drug intelligence programmes.

Keywords Gamma-hydroxybutyric acid · Gamma-butyrolactone · Sodium oxybate · Isotope ratio mass spectrometry · Urine · Source inference · Forensic

F. Marclay (✉) · C. Saudan · M. Saugy
Swiss Laboratory for Doping Analyses, University Center of Legal Medecine, Geneva and Lausanne, Centre Hospitalier Universitaire Vaudois and University of Lausanne,
Ch. des Croisettes 22,
1066 Epalinges, Switzerland
e-mail: francois.marclay@chuv.ch

J. Vienne · M. Tafti
Center for Integrative Genomics, University of Lausanne,
Génopode Building,
1015 Lausanne, Switzerland

Introduction

γ-Hydroxybutyric acid (GHB) is an endogenous short-chain fatty acid found in mammalian brain tissues as a metabolite of γ-aminobutyric acid (GABA), the primary inhibitory neurotransmitter of the central nervous system [1–3]. Biosynthesis also occurs through the peripheral lactonase of γ-butyrolactone (GBL) and the alcohol dehydrogenase of 1,4-butanediol (1,4-BD) into GHB upon direct oral consumption (Fig. 1) [4]. Binding to GHB-specific sites and $GABA_B$ receptors, this molecule exhibits pharmacological properties sought after for specific therapeutic purpose [5, 6]. Indeed, the sodium salt of GHB, referred to as sodium oxybate, is used in the treatment of narcolepsy, with cataplexy, and to help relieve alcohol and opiate withdrawal syndromes [2, 6–8]. Believed to increase the muscle mass due to a stimulatory effect on growth hormone production, GHB became popular amongst body builders as blended to nutritional supplements [9, 10]. Owing to its sedative and

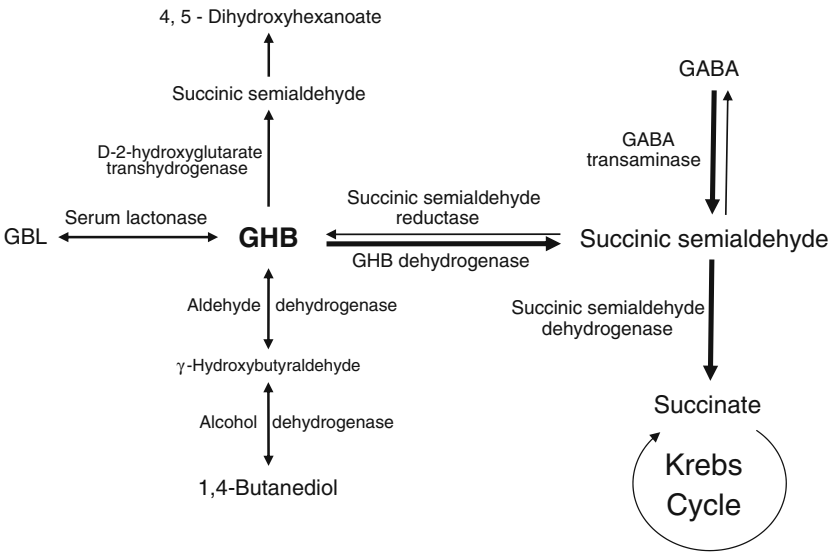

Fig. 1 Simplified metabolic pathway of GHB synthesis and metabolism [8]. The major pathways are shown by *thick arrows*

euphoric effects, this compound quickly gained reputation as a recreational drug due to its acknowledged use at nightclubs and raves [11, 12]. Subsequently, implication of GHB in drug-facilitated sexual assaults emerged, resulting from a combination of heightened sex drive, disinhibition and potential amnesia properties with an ease of spiking in beverages as a colourless and odourless liquid [13]. Whilst trends of both recreative and intoxicating use remain fairly stable, various international studies have reported severe intoxications and overdoses over the last decade, but also significant progression of the GBL consumption phenomenon [2, 14–19].

Therefore, discrimination between endogenous and exogenous GHB in biological fluids, including blood and urine, is often required in toxicological and forensic investigations. However, source identification of this compound is facing a number of challenges. One of the key issues is the short half-life of GHB, with a plasma half-life averaging <1 h, which translates into complete elimination from blood and urine within 6 and 12 h, respectively [20]. When considering the ordinary delay of several hours between drug ingestion and sample collection, along with the endogenous nature of GHB, origin assessment of low urinary or blood concentrations should be carefully interpreted. Accordingly, a general agreement on a cutoff limit for the distinction between endogenous and exogenous GHB is still prone to vast discussions [21–25]. However, this question may be addressed considering alternative biochemical markers. In particular, determination of the carbon isotope composition of this molecule has been proven promising to solve this issue [26, 27]. Indeed, incorporation of ^{13}C to the GHB molecule through biosynthesis appears to differ from synthesis through chemical precursors since the first reflects the C3 and C4 plant diet of an individual, whilst the latest corresponds to its chemical precursors, namely GBL and 1,4-BD, originating from petroleum extracts [28]. Thus, variations in the $^{13}C/^{12}C$ ratio ($\delta^{13}C$ values) allow discriminating between GHB of endogenous and exogenous origins.

Beyond this problem, investigations on intoxication cases and forensic drug intelligence could benefit from such information for the assessment of the linkage between GHB found in a biological sample and drug seizures. Actually, the potential of stable isotope analysis for drug profiling is valuable, as enlightened for cocaine, heroin, amphetamine-based molecules, marijuana and GBL [28–37]. However, this methodology has only been applied to the raw matrix at present, and linking the aforementioned molecules found in biological fluids and in seizure samples remains a promising topic yet to be explored.

Thus, this work proposes to evaluate the likelihood of isotopic fractionation due to metabolism in the body by comparing $\delta^{13}C$ values of prescription pharmaceutical sodium oxybate, marketed as Xyrem®, with urinary GHB of volunteers who ingested this specific prescription drug during a study on sleep. In that respect, measurements were achieved using an extensive sample cleanup procedure followed by the conversion of GHB into GBL prior to analysis by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) [26, 27].

Experimental

Reagents and chemicals

Methanol (≥99.9%), dichloromethane (≥99.9%) and acetic acid (glacial, 100%) were purchased from Merck (Darmstadt, Germany). Acetonitrile (≥99.7%) was obtained from Biosolve B.V. (Chemie Brunschwig, Basel, Switzerland) and hydro-

chloric acid fuming (37%) and sodium chloride (>99.5%) from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland). ε-Caprolactone (≥99.0%, Lot no. 087K3521) was supplied by Fluka (Buchs, Switzerland). Ultrapure water was produced by a Milli-Q Gradient A10 water purification system with a Q-Gard® 2 and a QuantumTM EX Ultrapure organex cartridge purchased by Millipore Corp. (Billerica, MA, USA). Helium (Quality 60, >99.9999%) and carbon dioxide gas (quality 40, >99.99%) were obtained from Carbagas (Domdidier, Switzerland). Oasis® MCX cartridge 30 μm (6 cc, 150 mg) were supplied by Waters (Milford, MA, USA) and Bond Elute SAX SPE cartridges 40 μm (3 mL, 500 mg) by Varian Inc. (Palo Alto, CA, USA). GHB enzymatic assay kits were purchased from Bühlmann Laboratories AG (Basel, Switzerland). Sodium oxybate, trademarked as Xyrem® (Lot no. A13870), was obtained from UCB-Pharma SA (Bulle, Switzerland).

GHB urine specimens and prescription medication

Urine specimens were collected from 13 healthy male volunteers, aged 20–26 years (mean age, 23.5±1.6 years), participating in a study on effects on sleep of a sodium oxybate oral preparation, trademarked as Xyrem®. Actually, each subject ingested a sodium oxybate dose equivalent to 30 mg/kg body weight dissolved in 60 mL water twice during the course of this study: once prior to sleep at 11 P.M. and once prior to a 2-h nap at 3 P.M., with a minimum of 1-week interval between both intakes. Urine samples were collected shortly after waking up in the morning and between 9 and 10 P.M. after the nap, resulting in a 6- to 8-h delay after oral consumption, and stored at −20 °C since collection until GC-MS and GC/C/IRMS analyses. Endogenous levels of GHB were assessed by the collection of urine specimens at six different times over the treatment period and storage under equal conditions. Xyrem® from the batch used for oral administration was analysed as well.

Evidence on in vitro production of GHB in antemortem urine samples has been highlighted as a process that is dependent on storage conditions. Indeed, storage at ambient temperature or refrigerated at 5 °C over a long period of time can result in a significant increase of endogenous GHB concentration [38, 39]. However, keeping urine samples frozen at −20 °C has been reported as significantly reducing this phenomenon to a minimum, even after up to 8 months [40]. Thus, these were the preferred storage conditions for this study.

Quantitative determination of GHB by enzymatic assay

Direct and quantitative determination of GHB in urine has been performed by an enzymatic assay kit on the Dimension® Xpand™ Plus Integrated Chemistry System (Siemens Healthcare Diagnostics SA, Düdingen, Switzerland), an automated chemistry and immunoassay analyser for central laboratories. Reagents, calibrator and validator vials were reconstituted and kept refrigerated at 4 °C prior to analysis. Calibration was performed over the 10- to 100-μg/mL range, with calibration standards at low, medium and high concentration levels (k=3) and validator standards (QC) at two concentration levels (k=2) analysed in triplicate (n=3) each time. A calibration curve was built using a linear regression whose suitability for quantification purpose was verified by comparing concentration measurements of QCs to confidence limits specified in the kit. Eventually, a volume of 12 μL urine was used for each enzymatic assay.

Conversion of prescription pharmaceutical GHB into GBL

In a 10-mL glass tube, 1 mL of 6 M hydrochloric acid was added to 10 μg of Xyrem® prior to vortex mixing for ~30 s. Then, liquid–liquid extraction (LLE) was performed by adding 1 mL dichloromethane and shaking the mixture by inversion for 10 min using a rotator unit. After centrifugation at 2,500 rpm for 5 min, the aqueous layer was transferred and extraction was repeated once with 1 mL dichloromethane. Eventually, the combined organic layer was evaporated in a conical glass tube to ~100 μL under a gentle stream of nitrogen (20 psi) at 25 °C after the addition of 5 μL of 1 mg/mL internal standard (IS) solution (ε-caprolactone in dichloromethane).

Sample preparation

Urine sample cleanup is based on a method previously published and shortened to our particular needs [26]. Considering the concentration of GHB, the volume of urine aliquots was determined as follows:

$$V_{\text{urine}} = \frac{10}{\text{GHB}_{\text{concentration}}} \times \mu\text{g}, \quad (1)$$

with V_{urine} in millilitres and $\text{GHB}_{\text{concentration}}$ in micrograms per millilitre.

Then, urine aliquots were diluted up to a volume of 2 mL with water prior to centrifugation for 5 min at 2,500 rpm. Solid phase extraction (SPE) was performed on an Oasis® MCX cartridge 30 μm (6 cc, 150 mg) previously conditioned by successive addition of 2 mL methanol and 2 mL water. Elution of GHB was carried out with 2.5 mL of methanol/0.1% formic acid in water (10:90, v/v). After evaporation of the eluate to ~0.5 mL under an air stream (20 psi) at 50 °C, further purification was performed on a Bond Elute SAX SPE cartridge 40 μm (3 mL, 500 mg). Conditioning was achieved by successive addition of 2 mL

methanol, 8 mL of 10% acetic acid and 2 mL water at a flow rate of 0.5 mL/min. After loading of the urine extract, interaction with the solid phase occurred for 15 min prior to washing with 1 mL water, 1 mL water/methanol (50:50, *v*/*v*) and 0.5 mL methanol at a flow rate of 0.5 mL/min. Elution of the analyte was carried out with 3 mL of 10% acetic acid in acetonitrile at a flow rate of 0.5 mL/min. After evaporation of the eluate to dryness under a gentle air stream (5 psi) at 50 °C, the residue was dissolved in 1 mL of 6 M hydrochloric acid prior to vortex mixing for ~30 s. Then, LLE was performed with 1 mL dichloromethane for 10 min using a rotator unit. After centrifugation at 2,500 rpm for 5 min, the aqueous layer was transferred and extraction was repeated once with 1 mL dichloromethane. Eventually, the combined organic layer was evaporated to ~100 μL in a conical glass tube under a gentle stream of nitrogen (20 psi) at 25 °C after the addition of 5 μL of 1 mg/mL IS solution.

GC/C/IRMS analysis

The carbon isotope measurements were performed on a Delta^{Plus} XL IRMS system (ThermoFinnigan MAT, Bremen, Germany) coupled to an Agilent 6890A Gas Chromatograph (HP Analytical Division) via a Finnigan GC Combustion III interface (ThermoFinnigan MAT). The samples were injected using a CombiPal autosampler (CTC Analytics AG, Zwingen, Switzerland). The mass spectrometer consisted of an electron impact source held at a 3.0-kV acceleration voltage for CO_2 gas, a magnet and three Faraday collectors for the measurement of the ions at *m/z* 44, 45 and 46. Chromatographic separation was achieved on a DB-17MS capillary column (30 m × 0.25 mm i.d., 0.25-μm film thickness) from J&W Scientific (Folsom, CA, USA). Helium was used as carrier gas with a constant flow of 1.3 mL/min. The GC injection port, combustion oven and reduction oven temperatures were set to 280, 940 and 600 °C, respectively. Standard on–off tests (reference carbon dioxide gas pulses of 20-s duration) were introduced six times during the chromatographic separation. Regarding the analysis of the samples containing GBL and the IS, the oven temperature was increased from 80 °C (5 min) to 240 °C at 20 °C/min, then to 300 °C at 30 °C/min, and maintained at the final temperature for 2 min. The volume of injection was 1 μL and the samples were injected in the splitless mode (1.50 min). Oxidation of the combustion reactor was performed over 1 h after every batch of 20 samples.

The symbol δ is the standard notation for expressing carbon isotope ratios. It is defined as the parts per thousand deviation of isotopic compositions versus that of Vienna Pee Dee Belemnite and is calculated according to [41]:

$$\delta^{13}\mathrm{C}\,/‰ = \frac{(^{13}\mathrm{C}/^{12}\mathrm{C})_{\mathrm{sample}} - (^{13}\mathrm{C}/^{12}\mathrm{C})_{\mathrm{standard}}}{(^{13}\mathrm{C}/^{12}\mathrm{C})_{\mathrm{standard}}} \times 1000 \quad (2)$$

Calibration of the reference gas was previously performed using a mixture of three alkanes (Chiron AS, Trondheim, Norway), C_{15} (*n*-pentadecane), C_{20} (*n*-eicosane) and C_{25} (*n*-pentacosane), with $\delta^{13}C$ values of −30.22‰, −33.06‰ and −28.21‰, respectively.

Acquisition and evaluation of the GC/C/IRMS data were performed with the ISODAT 2.5 software (ThermoFisher Scientific, Bremen, Germany).

GC-MS analysis

Prior to GC/C/IRMS analysis, identification of the substance was ensured by GC-MS chromatographic retention time and by measurement of the full EI-MS spectrum between *m/z* 40 and 300. The diagnostic ions selected for the identification of each compound were the following: GBL (*m/z* 56, 86 and 42) and ε-caprolactone (*m/z* 55, 75, 84 and 114).

GC-MS analysis was performed on a Hewlett-Packard 5890 Serie II Plus chromatograph (HP Analytical Division, Waldbronn, Germany) equipped with a HP 7673 autosampler and coupled with a HP 5971 mass selective detector. GC separation was achieved on a DB-17MS capillary column (30 m×0.25 mm i.d., 0.25-μm film thickness) from J&W Scientific. Helium was used as carrier gas with a constant flow of 0.8 mL/min and at the initial column head pressure of 15 psi. For a robust identification of the target compounds, the GC operating conditions were identical to GC/C/IRMS analysis.

Identification criteria

Identification criteria were defined according to a technical document addressing this particular topic [42]. The chromatographic retention time (t_R) tolerance window must be within ±1% of the retention time of the reference material analysed in the same batch. Concerning full EI-MS experiments, at least two diagnostic ions are required, with the relative intensity of any of the ions not differing by more than 20% from that of the quality control material. A signal-to-noise ratio >3 must also be considered.

Data treatment and analysis

The $\delta^{13}C$ values were analysed statistically using S-PLUS® 7.0 for Windows. For distribution testing, a Kolmogorov–Smirnov test of normality has been employed. Equality of the

variances was assessed using a Levene test. Then, statistical differences among samples were tested using the two-sample t test, with $p<0.05$ considered statistically significant.

Results and discussion

Quantitative determination of GHB in urine by enzymatic assay

Quantification by enzymatic assay has been favoured for the reason that virtually no sample cleanup was required, providing a high throughput particularly suitable for use as a screening procedure. Indeed, such method allowed targeting samples of interest in a rapid and straightforward fashion. Noteworthy is that endogenous levels of GHB in urine specimens collected at six different times over the treatment period and reliability of storage conditions could be assessed simultaneously along with this single batch of analyses. Also, storage condition effects were limited as this procedure allowed avoiding an additional freeze and thaw cycle before urine extraction for compound identification and isotopic measurements. Actually, this simple and fast quantification method could be followed by a sample preparation for GC/MS and GC/C/IRMS analyses within ~1 h after thawing of the urine specimens.

A concentration range comprising expected urinary levels of both endogenous and exogenous GHB was initially determined, with consideration of the performance characteristics described in technical notes supplied with the enzymatic assay kit. Thus, calibration was performed over the 10- to 100-μg/mL range using calibrators at the 10-, 50- and 100-μg/mL concentration levels ($k=3$) analysed in triplicate ($n=3$). Due to the linear response, unweighted linear least-squares regression was chosen for quantification purpose, with R^2 corresponding to the calibration curve being >0.99. Also, the suitability of direct quantification of GHB in urine over the assay range was established as the concentration values obtained for calibration standards and standard deviations (SD) met the guidelines specified in the aforementioned technical notes. Also, samples exceeding the upper limit of quantification were diluted 1:10 (v/v) with 0.9% sodium chloride solution, as indicated by the manufacturer, and concentration values were multiplied by 10.

Following quantification, urine specimens of interest for further extraction and isotopic measurement were reduced down to nine samples originating from seven subjects. Indeed, in order to avoid more extensive purification steps prior to IRMS measurements and to support the exogenous origin of GHB found in urine, only samples with a concentration above 20 μg/mL were selected (Table 1). Noteworthy is that a few volunteers presented levels of GHB lower than 10 μg/mL even after treatment with Xyrem®, which may be explained by the short half-life of this substance joint to a longer delay before urine collection due to a prolonged sleep. Additionally, endogenous GHB in urine specimens collected over the treatment period was not detected. This ensured that subjects involved in this study did not suffer from 4-hydroxybutyric aciduria, which would result in naturally elevated endogenous levels of GHB [43, 44], and also excluded potential in vitro production issues.

Identification of GBL by GC/MS analysis

GC/MS analysis of Xyrem® and urine specimens allowed the identification of target compounds and suitability assessment for subsequent isotopic measurements. Satisfactory sample cleanup was achieved for eight of the nine samples, corresponding to six of the seven volunteers. Indeed, identification criteria were met for GBL and ε-caprolactone, with adequate chromatographic resolution and complete conversion of GHB into GBL (Fig. 2), except for one sample due to a co-eluting compound. Whilst the nature of that molecule could not be clearly identified, an exogenous origin may be hypothesized as there was no previous observation of this compound in urine extracts, even in trace amount. Thus, the latter was not submitted to

Table 1 Quantification of GHB by enzymatic assay and carbon isotopes determination in urine specimens of interest by GC/C/IRMS ($n=8$)

Type	Subject	Concentration (μg/mL)	$\delta^{13}C$ (‰)	SD (‰)
Urine specimen	1	86.9	−24.97	0.06
		76.5	−24.81	0.23
	2	168.6	−24.92	0.08
		97.4	−24.92	0.13
	3	33.3	−25.02	0.20
	4	22.6	−25.05	0.12
	5	39.6	−25.06	0.25
	6	35.5	−24.89	0.23
Standard	Xyrem®		−24.99	0.02

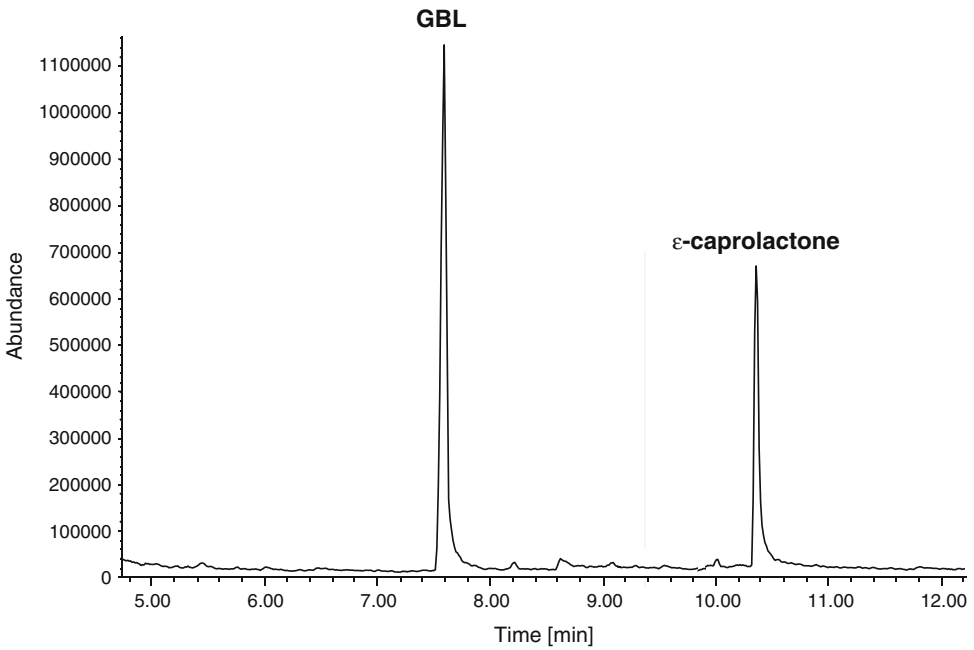

Fig. 2 GC-MS chromatogram of a urine specimen containing GHB at a concentration of 100 μg/mL and ε-caprolactone at 50 μg/mL

IRMS analysis. Also, Xyrem® was found to be of excellent purity.

Isotope measurements of GBL in urine by GC/C/IRMS

Conversion of GHB into GBL was favoured over conventional derivatization with di-TMS in order to avoid the addition of carbon atoms to the molecule [26, 45]. Indeed, subsequent calculation of a correction factor accounting for this phenomenon is necessary, resulting in $\delta^{13}C$ values with a larger SD [27]. Alternatively, conversion into GBL leads to the loss of a molecule of water, with no influence on carbon atoms attached to the original molecule of GHB. Thus, translation of isotopic measurements of GBL into $\delta^{13}C$ values for GHB is straightforward.

Each sample preparation was spiked with ε-caprolactone, a molecule very close in structure to GBL and displaying a slightly different chromatographic retention. Potential mass discrimination during the course of GC/C/IRMS analysis was tested with ε-caprolactone serving as internal standard [28]. The reproducibility of isotopic measurements was assessed accordingly, relying on the $\delta^{13}C$ value of the IS calibrated previously to this study (mean $\delta^{13}C$ value=−23.40‰, SD=0.21‰, n=30), using a 95% confidence interval as a run acceptance criteria. Also, the stability of the system was evaluated introducing reference carbon dioxide gas pulses (20-s width) six times during the chromatographic separation, and pulses at 420 and 690 s were used to normalize $\delta^{13}C$ values (Fig. 3).

Prior to the isotopic measurements of urine specimens, the linear response of the IRMS was defined by injecting different amounts of GHB converted into GBL from Xyrem®. Indeed, the accuracy of the carbon isotopic ratio determination may be significantly affected when the signal intensity is outside the linearity range [46]. Thus, increasing quantities of GBL from 10 to 100 ng were injected, resulting in signal intensities ranging from 389 to 4,432 mV, respectively. A linear response was observed, as demonstrated by the stable $\delta^{13}C$ values (0.03‰ per millivolt) obtained.

Each urine sample was extracted in triplicate, and the isotopic measurements of GHB are summarized in Table 1. The $\delta^{13}C$ values determined in this study range from −24.81‰ to −25.06‰, with relatively small deviations (SD<0.26‰). The stability of IRMS measurements during the chromatographic separation proved satisfying, as verified by the standard on–off tests (mean $\delta^{13}C$ value=−28.50‰, SD=0.10‰, n=162 for six reference carbon dioxide gas pulses). Likewise, reproducible $\delta^{13}C$ values were collected for the IS (mean $\delta^{13}C$ value=−23.38‰, SD=0.19‰, n=27), with every carbon isotope ratio comprised in the confidence interval previously established (two-sample t test).

Noteworthy is that the distribution of $\delta^{13}C$ values did not show significant deviation from the isotopic values and related SD of Xyrem® (mean $\delta^{13}C$ value −24.99‰, SD=0.02‰), as revealed by statistical tests. Indeed, equality of the variances was verified using a Levene test and statistical differences among urine specimens were ruled out by the two-sample t test, with p<0.05 considered statistically significant. Thus, metabolism of GHB did not induce a significant carbon isotopic fractionation, as reported for each of the six subjects.

Several pharmacokinetic studies described the conversion of GHB to succinic semialdehyde and further to succinate prior to entering into the Krebs cycle as the major

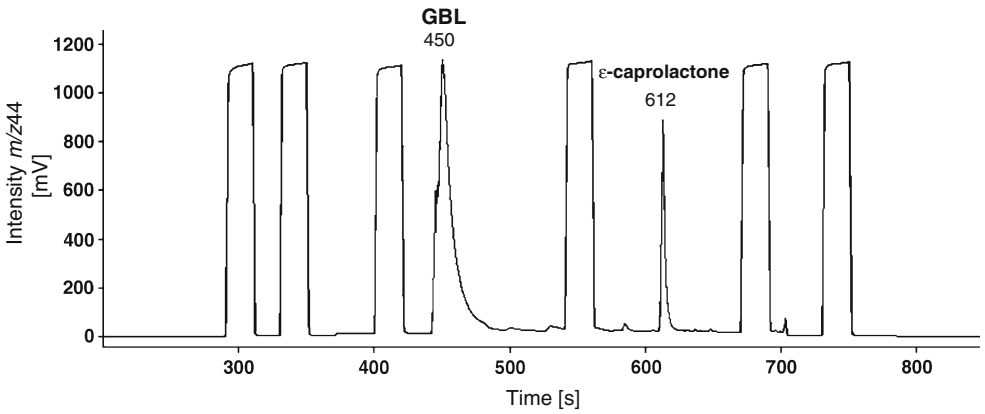

Fig. 3 GC/C/IRMS chromatogram (*m/z* 44) of a urine specimen containing GHB at a concentration of 100 μg/mL and ε-caprolactone at 50 μg/mL. The *square-topped peaks* represent pulses of CO_2 reference gas

metabolic pathway, along with other minor metabolic routes (Fig. 1) [8, 47, 48]. Due to this extensive hepatic metabolism, <1% of the dose is excreted unchanged in urine. Thus, verifying whether the carbon isotopic composition of this small amount of GHB recovered in urine displayed variations with respect to the original pharmaceutical preparation was a crucial point. Indeed, reduction of GHB into succinic semialdehyde by GHB dehydrogenase may lead to carbon isotopic fractionation due to the kinetic isotope effect. Considering such effect, the reaction rate of ^{13}C-enriched isotopologues is known to be slower in bond making or breaking chemical processes [49, 50]. Therefore, the small fraction of GHB found unchanged in urine may potentially exhibit ^{13}C enrichment compared to the isotopic signature of the original dose administered to the subjects. Also, renal reabsorption and metabolic clearance of GHB could be hypothesized as an additional source of carbon isotopic fractionation. However, determination of the carbon isotope ratio for each of the six subjects did not highlight variations in the isotopic composition through GHB metabolism as a whole. Accordingly, the potential sources of carbon isotopic fractionation previously mentioned should not significantly affect the $\delta^{13}C$ values.

Our findings tend to demonstrate the possibility of connecting GHB in biological samples and this substance as a prescription or illicit drug by means of carbon isotope determination. Noteworthy is that Xyrem® quantity administered to the volunteers corresponded to an average dose for recreational use, which is significantly less than both therapeutic and intoxicating doses. Therefore, the methodology applied in this study could be used at ease with higher urinary concentrations of GHB. Such findings would be very valuable when investigating intoxication cases and in support of drug intelligence as they may fill the gap between the raw matrix and biological fluids with valuable information. Indeed, corresponding carbon isotope contents would allow linking drug seizures to urine specimens, and by extension drug traffickers to individuals charged with substance abuse felony or suspicious individuals to drug poisoning victims. This would bring an additional level of evidence to tackle the aforementioned issues of forensic interest. In addition, considering the increasing popularity of GBL consumption, such studies could be followed up by the assessment of metabolism influence on the carbon isotopic profile of GBL excreted as GHB in biological fluids.

Conclusion

Determination of the carbon isotope content of GHB by GC/C/IRMS, performed with a previously published method [26], was applied to eight urine specimens of six healthy male volunteers who ingested pharmaceutical GHB sodium salt, known as sodium oxybate and trademarked as Xyrem®, as part of a study on sleep. A very limited range of $\delta^{13}C$ values, from −24.81‰ to −25.06‰, was observed, corresponding to the carbon isotopic values of Xyrem® (mean $\delta^{13}C$ value = −24.99‰) used for treatment. Since urine samples and prescription drug could not be discriminated by means of statistical analysis, metabolism of GHB demonstrated no significant influence on $\delta^{13}C$ values.

This study provides a baseline for further studies and for the exploitation of stable isotopes characterized in biological matrices in both intoxication cases brought to court and drug intelligence programmes. Indeed, source inference subsequent to carbon isotope determination appears achievable as highlighted by the link established between GHB as a raw matrix or found in a biological fluid. In addition, such studies may be extended to GBL, a chemical precursor of increasing interest amongst substance users, to assess potential isotopic fractionation related to its metabolism prior to excretion as GHB in biological fluids.

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