DETERMINATION OF GAMMA-HYDROXYBUTYRATE (GHB) IN SERUM OR PLASMA WITH GC/MS

Alain G. VERSTRAETE[1], Eric VAN DE VELDE[1], Peter DE PAEPE[2], Marie Thérèse ROSSEEL[2]

[1]*Laboratory of Clinical Biology-Toxicology, University Hospital, Gent, Belgium*

[2]*Heymans Institute of Pharmacology, Gent, Belgium*

ABSTRACT: A method for the direct determination of GHB in serum or plasma, involving addition of deuterated internal standard, acetonitrile precipitation and derivatisation with BSTFA is presented.

KEY WORDS: GHB; GC/MS; Serum analysis.

Problems of Forensic Sciences, vol. XLII, 2000, 195–201
Received 9 September 1999; accepted 16 May 2000

INTRODUCTION

Gamma-hydroxybutyric acid (GHB, gamma-hydroxybutyrate, 4-hydroxybutanoic acid, 4-hydroxybutyrate; mol wt 104.11, $HO\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}COOH$; sodium salt = sodium oxybate, mol wt 126) is a naturally occurring metabolite of gamma-aminobutyric acid (GABA), thought to function as a neurotransmitter or neuromodulator. The highest concentration in humans is found in fetal cerebellum and adult hypothalamus.

It has been used as an intravenous anesthetic and in the treatment of narcolepsy; alcoholism and other drug abuse. Because of its side effects (nausea, vomiting, drowsiness, dizziness, respiratory depression and involuntary muscle spasms), its therapeutic use has been largely discontinued.

It is used illicitly as a "legal" drug, in date rape (it was found in 4% of the cases in the US [4] and in the body builder scene ("fat-burning" effects). It is scheduled in some countries, e.g. Belgium and France. It is usually sold in liquid form, in a dose of 1 or more grams. The purity is very variable, 40 ml (3–9 doses) may contain 3–20 g [19].

PHARMACOKINETICS AND TOXICOKINETICS

Both the oral absorption and the elimination of GHB are capacity-limited processes [17]. In humans, the peak plasma concentration is found 20–45 minutes after an oral

dose of 25 mg/kg [6]. Its effects last for 45–60 minutes. The half-life is dependent on the dose: after a dose of 14 µl/min/kg, it is about 20 min [11]. After an oral dose of 25 mg/kg, the mean peak concentration is 55 µg/ml (range 24–88) at 30 min, after 50 mg/kg, the peak concentration is 90 µg/ml (range 51–158) at 45 min [5]. After a single dose of 100 mg/kg, GHB is detectable for less than 8 h in blood and 12 h urine.

Concentrations > 260 µg/ml produce coma; at concentrations between 159 and 260 µg/ml, some spontaneous blinking occurs, between 52 and 156 µg/ml, light sleep with occasional eye opening is observed and at concentrations < 52 µg/ml patients awakened [12]. In one case of coma, plasma GHB was 125 µg/ml [14].

Less than 5% of an oral dose is excreted unchanged [9]. Peak urine concentrations are in the order of 1100 µg/ml, approximately 4 h after a 100 mg/kg oral dose. No drug is detected in urine after 12 h. In a case of driving under the influence, a urine concentration of 1975 µg/ml was measured [18]. Urine concentrations are 10 times higher than in blood [7].

Recently, de novo formation of GHB in blood samples (3–168 µg/ml) from deceased persons was reported. This phenomenon does not seem to occur in blood of living persons or in urine.

In overdose, one observes a profound, but short-lasting (< 5 h) decrease of consciousness, bradycardia, hypothermia, respiratory acidosis and emesis [3, 19].

There are some indications that GHB abuse is increasing [10]. As a consequence of the control of GHB in some countries, use of gamma-butyrolactone (GBL; which is not controlled), a precursor of GHB, can be expected, and it might be important to be able to distinguish GBL and GHB abuse.

ANALYTICAL METHODS

Analysis of GHB in body fluids is not straightforward: no immunoassays are available, it only absorbs ultraviolet light at very low wavelengths, and does not react with chromogenic field test kits. It is volatile and is transformed into gamma-butyrolactone (Figure 1) in acidic medium [13].

A colorimetric spot test was recently proposed [1]. A high pressure liquid chromatographic (HPLC) method with ultraviolet detection can only be used with seized materials, because of its high limit of detection (LOD, 50 ng) [16]. The method that is mostly used is gas chromatography (GC), with flame ionization (FID), electron capture (ECD) [2] or mass spectrometric (MS) detection [5]. Most frequently, GHB is first converted to GBL with acid [5]. GBL is then extracted with liquid-liquid extraction (e.g. with dichloromethane [20] or with headspace solid phase micro extraction (SPME)

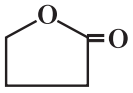

Fig. 1. Structure of gamma-butyrolactone.

[8]. Another alternative consists of extracting GHB and determining it directly with GC or GC-MS after silylation [15]. Recently, a GC-MS method involving a combination of solid-phase and liquid-liquid extraction and silyl derivatisation of GHB from urine has also been described [15].

We developed a GC/MS method for the quantitative determination of GHB in serum, plasma or urine, that requires a small amount of sample.

PROCEDURE

Twenty µl serum or plasma are pipetted in an Eppendorf tube, with 10 µl internal standard (GHB-D_6 (HO-CD_2-CD_2-CD_2-COONa), 100 µg/ml in methanol; Radian G-003) and 45 µl acetonitrile. After vortex-mixing and centrifugation at 12000 g for 5 minutes, 50 µl of the supernatant is pipetted into a glass evaporation vial and evaporated to dryness at room temperature under a gentle stream of nitrogen. Seventy-five microliters of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) are added. The tube is vortexed and incubated for 12 min at 90°C. One µl is injected (splitless, 250°C) into the GC/MS in SIM mode (m/z 147, 204, 206, 233, 239; the underlined ions are used for quantitation) on a Macherey Nagel Optima 1 column, 12 m x 0.2 mm, 0.35 µm film thickness with the following temperature program: 50°C for 0.6 min, then increased at 10°C/min to 100°C and further at 50°C/min to 250°C, hold for 1 minute (total duration: 9.6 minutes). The head pressure is 7.5 psi and the transfer line is heated to 290°C. The assay is calibrated with standards containing 0, 1.3, 2.6, 5.2, 10.3, 21, 41, 82, 165 µg/ml of GHB (Radian G-001, 1 mg/ml sodium GHB).

The method performs equally well in urine.

RESULTS

The "extraction" efficiency (the sample preparation only involves a precipitation step) is approximately 75–80%. An example of a calibration curve is given in Figure 2. The detection limit (LOD) is 1 µg/ml (S/N = 5) and the LOQ is 2.5 µg/ml, using 20 µl plasma. The within-run precision (coefficient of variation) at 5 and 100 µg/ml is 5.2 and 9.4% respectively (n = 6). The method has been used routinely for several months in the analysis of patient samples and also in some rat experiments. The between run precision at 10 and 100 µg/ml was 2.7 and 4.8% respectively (n = 8). The mass spectra of GHB, GHB-D_6, and a SIM chromatogram are shown in Figures 3–5.

CONCLUSION

We propose a simple and rapid method for the determination of GHB in serum or plasma. It has a good sensitivity and requires only a small sample volume. In combination with a method for GBL, it offers a possibility to differentiate GBL from GHB, which could be important in countries where GHB is scheduled, while GBL is not.

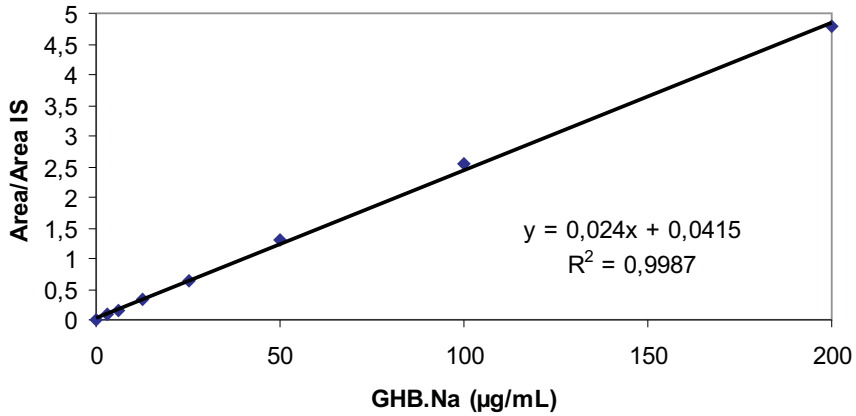

Fig. 2. Example of a calibration line.

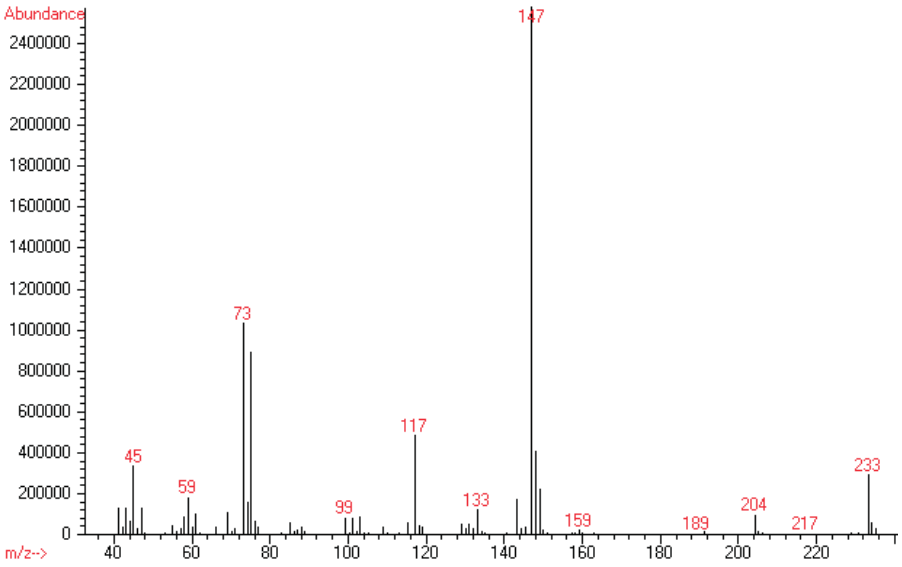

Fig. 3. GC/MS spectrum of GHB-bis-TMS.

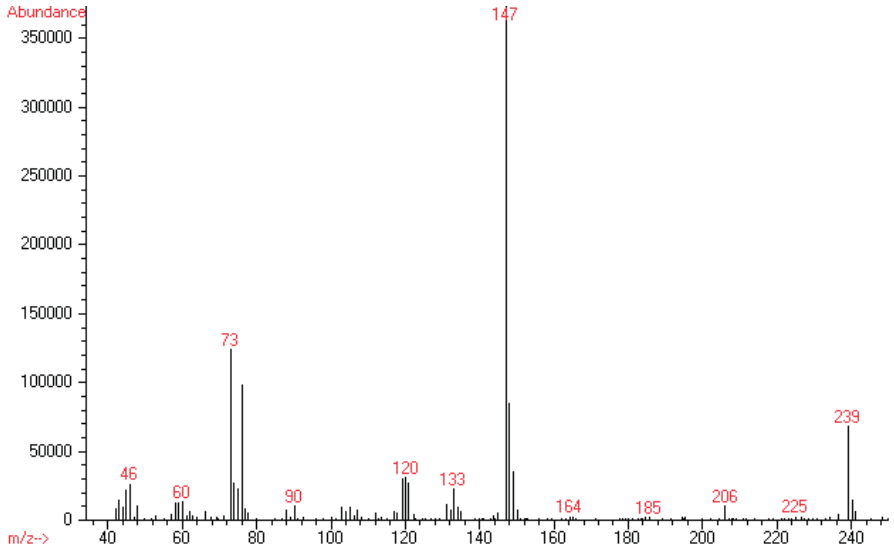

Fig. 4. GC/MS spectrum of GHB-D_6-bis-TMS.

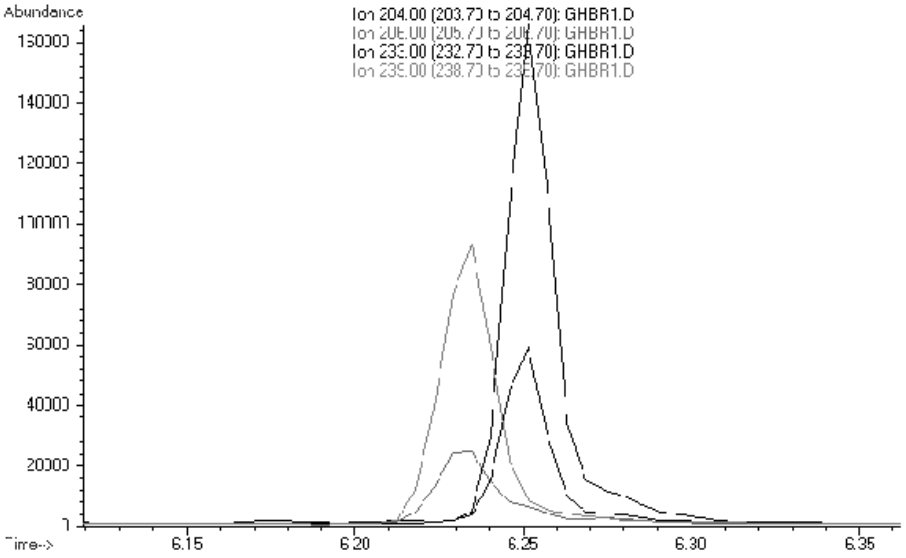

Fig. 5. SIM chromatogram of a serum sample containing 15 µg/ml of GHB.

References:

1. Badcock N. R., Zotti R., Rapid screening test for gamma-hydroxybutyric acid (GHB, Fantasy) in urine, *Therapeutic Drug Monitoring* 1999, vol. 21, p. 376.

2. Doherty J., Snead O., Roth R., A sensitive method for quantitation of gamma-hydroxybutyric acid and gamma-butyrolactone in brain by electron capture gas chromatography, *Analytical Biochemistry* 1975, vol. 69, pp. 268–277.
3. Dyer J. E., Gamma-hydroxybutyrate: a health-food product producing coma and seizure-like activity, *American Journal of Emergency Medicine* 1991, vol. 9, pp. 321–324.
4. ElSohly M. A., Salamone S. J., Prevalence of drugs used in cases of alleged sexual assault, *Journal of Analytical Toxicology* 1999, vol. 23, pp. 141–146.
5. Ferrara S. D., Tedeschi L., Frison G., Castagna F., Gallimberti L., Giorgetti R., Gessa G. L., Palatini P., Therapeutic gamma-hydroxybutyric acid [4-hydroxybutyric acid] monitoring in plasma and urine by gas chromatography-mass spectrometry, *Journal of Pharmaceutical and Biomedical Analysis* 1993, vol. 11, pp. 483–487.
6. Ferrara S. D., Zotti S., Tedeschi L., Frison G., Castagna F., Gallimberti L., Gessa G. L., Palatini P., Pharmacokinetics of gamma-hydroxybutyric acid in alcohol dependent patients after single and repeated oral doses, *British Journal of Clinical Pharmacology* 1992, vol. 34, pp. 231–235.
7. Fieler E. L., Coleman D. E., Baselt R. C., Gamma-hydroxybutyrate concentrations in pre- and postmortem blood and urine, *Clinical Chemistry* 1998, vol. 44, pp. 692–692.
8. Frison G., Tedeschi L., Maietti S., Ferrara S. D., Determination of gamma-hydroxybutyrate (GHB) in plasma and urine by headspace solid-phase microextraction (SPME) and gas chromatography-positive ion chemical ionization-mass spectrometry, Proceedings of the joint 1998 SOFT/TIAFT International Meeting (TIAFT), Spiehler Verlag, Newport Beach 1999, pp. 394–404.
9. Frommhold S., Gamma-hydroxybutyrate (GHB): what is "the scoop"?, *Toxi-News* 1997, vol. 16, pp. 3–8.
10. Gamma Hydroxybutyrate use – New York and Texas, 1995–1996, *Morbidity and Mortality Weekly Report* 1997, vol. 46, pp. 281–283.
11. Ghysel M., Le GHB: l'acide gamma hydroxy butyrique. Revue de la litérature, *Toxicorama* 1999, vol. 11, pp. 1–16.
12. Helrich M., McAslan T., Skolnik S. [et al.], Correlation of blood levels of 4-hydroxybutyrate with state of consciousness, *Anesthesiology* 1964, vol. 25, pp. 771–775.
13. Letteri J., Fung H., Evaluation and development of gas chromatographic procedures for the determination of gamma-hydroxybutyric acid and gamma-butyrolactone in plasma, *Biochemical Medicine* 1978, vol. 20, pp. 70–80.
14. Louagie H. K., Verstraete A. G., De Soete C. J., Baetens D. G., Calle P. A., A sudden awakening from a near coma after combined intake of gamma-hydroxybutyric acid (GHB) and ethanol, *Journal of Toxicology: Clinical Toxicology* 1997, vol. 35, pp. 591–594.
15. McCusker R. R., Paget W. H., Chronister C. W., Goldberger B. A., ElSohly M. A., Analysis of gamma-hydroxybutyrate (GHB) in urine by gas chromatography-mass spectrometry, *Journal of Analytical Toxicology* 1999, vol. 23, pp. 301–305.
16. Mesmer M. Z., Satzger R. D., Determination of gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL) by HPLC/UV-VIS spectrophotometry and HPLC/thermospray mass spectrometry, *Journal of Forensic Sciences*, vol. 43, pp. 489, 1259–1260.
17. Palatini P., Tedeschi L., Frison G., Padrini R., Zordan R., Orlando R., Gallimberti L., Gessa G. L., Ferrara S. D., Dose-dependent absorption and elimi-

nation of gamma-hydroxybutyric acid in healthy volunteers, *European Journal of Clinical Pharmacology* 1993, vol. 45, pp. 353–356.

18. Stephens B. G., Baselt R. C., Driving under the influence of GHB?, *Journal of Analytical Toxicology* 1994, vol. 18, pp. 357–358.
19. Thomas G., Bonner S., Gascoigne A., Coma induced by abuse of gamma-hydroxybutyrate (GBH or liquid ecstasy): a case report, *British Medical Journal* 1997, vol. 314, pp. 35–36.
20. Thomas B., Schöntube E., Gaschromatographische Bestimmung von Gamma-Hydroxybüttersäure in Human Plasma, *Pharmazie* 1993, Bd. 48, S. 623–624.