ELSEVIER

Journal of Chromatography B, 769 (2002) 221–226

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Capillary gas chromatographic determination of 1,4-butanediol and γ-hydroxybutyrate in human plasma and urine

Benoît Blanchet, Karine Morand, Anne Hulin*, Alain Astier

Laboratory of Pharmacology and Toxicology, CHU Henri Mondor, 51 Avenue du mal de Lattre de Tassigny, 94010 Creteil Cédex, France

Received 4 May 2001; received in revised form 12 November 2001; accepted 21 November 2001

Abstract

This article describes two methods for the determination of 1,4-butanediol and γ-hydroxybutyrate in human plasma and urine using capillary gas chromatography. For 1,4-butanediol, plasma or urine samples (500 μl) were extracted by protein precipitation whereas for γ-hydroxybutyrate, plasma or urine samples (500 μl) were extracted and derivatised with BF_3–butanol. The compounds were separated on a Supelcowax-10 column and detection was achieved using a flame ionization detector. The methods are linear over the specific ranges investigated, accurate (with a percentage of the nominal concentration <109.8%) and showed intra-day and inter-day precision within the ranges of 5.0–12.0 and 7.0–10.1%, respectively. No interferences were observed in plasma and urine from hospitalized patients.

Keywords: 1,4-Butanediol; γ-Hydroxybutyrate

1. Introduction

1,4-Butanediol (1,4-BD) is a naturally occurring aliphatic alcohol and an industrial solvent. When ingested, 1,4-BD is rapidly converted by liver alcohol dehydrogenase to γ-hydroxybutyrate (GHB), a neuromodulator with depressant effects. 1,4-BD and its metabolite occur endogenously in humans in trace amounts [1]. This alcohol began to be marketed as a «natural and nontoxic» dietary supplement. It was declared a class I health hazard, with toxic effects including vomiting, respiratory depression, loss of consciousness, seizures and death [2,3]. Its metabolite, GHB, was marketed to bodybuilders for muscle building and fat loss [4,5]. Zvosec et al. reported toxic effects in eight patients including vomiting, urinary and faecal incontinence, agitation, combativeness, loss of consciousness, respiratory depression and death [6]. Poldrugo and Carter Snead showed that, in rats, effects of 1,4-butanediol are mediated through the brain and liver conversion of 1,4-BD to GHB [7].

To determine these two compounds in plasma and urine, some authors propose the use of gas chromatography (GC) or GC coupled with mass spectrometric (GC–MS) quantification [8–13]. GC–MS is a very sensitive technique. However, few laboratories in French hospitals could use this method which requires specific equipment and experienced technicians. Recently, some authors proposed high-per-

*Corresponding author. Tel.: +33-1-4981-2751; fax: +33-1-4981-2764.

E-mail address: anne.hulin@hmn.ap-hop-paris.fr (A. Hulin).

1570-0232/02/$ – see front matter
PII: S1570-0232(01)00565-7

formance liquid chromatography (HPLC) methods for GHB determination [14,15]. To define a rapid toxicological diagnosis in emergency situations, we have developed two simplified methods to quantify 1,4-BD and its metabolite in human plasma and urine using only one instrument, a capillary gas chromatograph coupled with a flame ionization detector. Capillary gas chromatography is usable in university or local hospitals and in less developed countries where chronic alcoholism can be observed.

2. Experimental

2.1. Chemicals

1,4-BD and GHB were purchased from Sigma (St. Louis, MO, USA). Octanol (OC) and DL-(α)-hydroxybutyric acid (HBA) were internal standards and were purchased from Sigma. All other chemicals were of analytical grade. Ethanol and acetonitrile were purchased from Merck Eurolab (Darmstadt, Germany).

2.2. Equipment

The equipment consisted of a capillary Autosystem gas chromatograph (Perkin-Elmer, Les Ulis, France). Data were recorded and analysed with a Model 1022 software system (Perkin-Elmer).

2.3. Chromatographic conditions

The separation of 1,4-BD, GHB and their internal standards, OC and HBA, was carried out on a Supelcowax-10 30 m×0.53 mm column and a precolumn of 5 m (Alltech, Templeuve, France) using helium as carrier gas. Detection was achieved by flame ionisation detection (FID). Chromatographic analyses were performed at different temperature and gas flow programs. For all analyses, injector and detector temperatures were set at 240 and 280 °C, respectively. For 1,4-BD determinations, temperature programming of the column was from 120 to 240 °C at 8 °C/min for 3 min and 5 min at 240 °C. The helium carrier gas flow was 4.5 ml/min. For GHB, temperature programming was 110 to 135 °C at 2 °C/min, 135 to 150 °C at 6 °C/min, 150 to 260 °C at 16 °C/min and 10 min at 260 °C at a helium gas flow of 2.5 ml/min.

2.4. Preparation of standards

Stock solutions of 1,4 BD, GHB and the two internal standards, OC and HBA were prepared by dissolving the appropriate amount in distilled water (1,4-BD, 10 g/l), acetonitrile (OC, 6 g/l), and ethanol (GHB, 10 g/l; HBA, 13.2 g/l). These stock solutions were stable for at least 6 months at +4 °C. Working solutions were prepared by diluting these stock solutions and were used immediately for the preparation of quality control and calibration samples. Stock solutions were diluted in distilled water to obtain a concentration of 1 g/l for 1,4-BD and in ethanol to a concentration of 2 g/l for GHB. Working solutions of the internal standards were prepared by diluting the stock solutions in acetonitrile to 0.6 g/l for OC and in ethanol to 1.32 g/l for HBA.

2.5. Sample treatment

For 1,4-BD, 500 μl of the internal standard solution was added to 500 μl of plasma or urine (patient sample, quality control or calibration standard). The mixture was vortex-mixed for 30 s and centrifuged at 9000 *g* at room temperature. Aliquots of 0.3 μl of the supernatant were injected into the chromatographic system.

For GHB, 500 μl of plasma or urine (patient sample, quality control or calibration standard) was added to 25 μl of the internal standard solution. The mixture was vortex-mixed for 30 s and 500 μl of the mixture were taken. Precipitation of the proteins was carried out by the addition of 3 ml of cold ethanol. The mixture was vortex-mixed and centrifuged at 4 °C for 10 min at 1700 *g*. An aliquot of the supernatant (2.5 ml) was transferred to a glass tube and evaporated using a Speed Vac Plus SC110A Savant concentrator (Bioblock, Illkirch, Germany) at medium temperature for 30 min and then under nitrogen at 80 °C for 15 at 20 min. For better drying of the residues, 500 μl of ethanol was added and then evaporated. The derivatisation of GHB consisted of a butylation of the carboxylic group. For each extract, 200 μl of BF_3–butanol (Supelco,

Bellefonte, PA, USA) was added and and the tubes were vortex-mixed. Each tube was then well closed and kept at 100 °C for 15 min. To neutralize the resulting solution, 300 μl of 2 *M* hydrogencarbonate buffer, pH 10 (Merck, Darmstadt, Germany) was added to each sample. After vortexing, 1 ml of 5 *M* NaCl (Merck) was added to separate the organic phase. After mixing and centrifugation, at 1700 *g* for 5 min, 50 μl of the upper butanolic phase were taken, transferred into clean tubes and 0.3 μl aliquots were injected into the chromatographic system.

2.6. Specificity

Specificity was assessed by analysing plasma or urine of hospitalized patients. Samples were spiked with various aliphatic alcohols and diols which can provoke similar clinical signs such as ethylene or propylene glycol and several common drugs.

2.7. Linearity

Standard curves were obtained by spiking compound-free plasma or urine with 1,4-BD or GHB at the following concentration ranges: 1,4-BD (10 to 1000 mg/l) and GHB (25 to 2000 mg/l). Six concentrations were used to obtain each calibration curve. For the linearity estimation, five calibration curves were tested with the *F*-test for lack of fit.

2.8. Precision and accuracy

Six samples of two quality controls at low and high concentrations were analysed to calculate the relative standard deviation (RSD) and thus were used for intra-day validation. Inter-day validation was studied over 3 days using the two quality controls with six samples at D1, three at D2 and three at D3. Accuracy was calculated as the percentage of the nominal concentration.

2.9. Recovery

The recovery after deproteinisation for the 1,4-BD determination was estimated by the ratio of the peak areas of five samples, at two concentrations, to the peak areas of standard aqueous solutions at the same concentrations. The recovery for GHB determination was not studied because esterification was necessary to detect the compound. It was not possible to define absolute recovery in comparison with aqueous solutions without esterification.

2.10. Limit of quantification

Plasma samples were spiked with decreasing concentrations of the studied compounds and analysed. The limit of quantification was considered to be the lowest concentration that could be measured with an RSD less than 25%.

2.11. Calculation procedures for determination of quality controls and patient samples

Daily calibration curves were constructed using the ratio of the observed peak area of each compound to this of its internal standard. For quality controls and patient samples, the unknown concentrations of 1,4-BD and GHB were obtained from the linear regression equation of the peak area ratio against concentration of the respective calibration curve. Plasma and urine samples from healthy volunteers ($n=4$) were treated under the above conditions. Plasma and urine samples from two patients were assayed and showed.

3. Results

A complete validation was performed on plasma samples. Fig. 1 shows a chromatogram of a plasma sample spiked with 1,4-BD and OC, its internal standard. The approximate retention times were 5.0 and 10.5 min, respectively, for OC and 1,4-BD. Fig. 2 shows a chromatogram of a plasma sample spiked with GHB and its internal standard, HBA. Their retention times were 14.7 and 10.8 min, respectively.

The 1,4-BD determination method showed good intra-day and inter-day precision with RSDs ranging from 5.8 to 11% and from 7 to 10.1% for the 1,4-BD determination (Table 1). For GHB determination, intra-day and inter-day precisions were good with RSDs ranging from 5.0 to 12% and from 8.6 to 8.7%, respectively (Table 1).

Both methods proved to be accurate with average percentages of less than 109.8% for 1,4-BD and

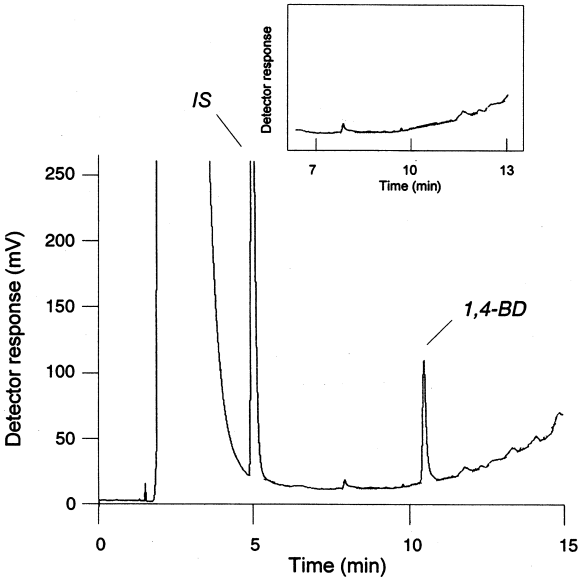

Fig. 1. Chromatogram of a plasma spiked with 250 mg/l 1,4-butanediol and 500 μl of a 0.6 g/l solution of internal standard (I.S.). In front of figure, chromatogram of a blank plasma.

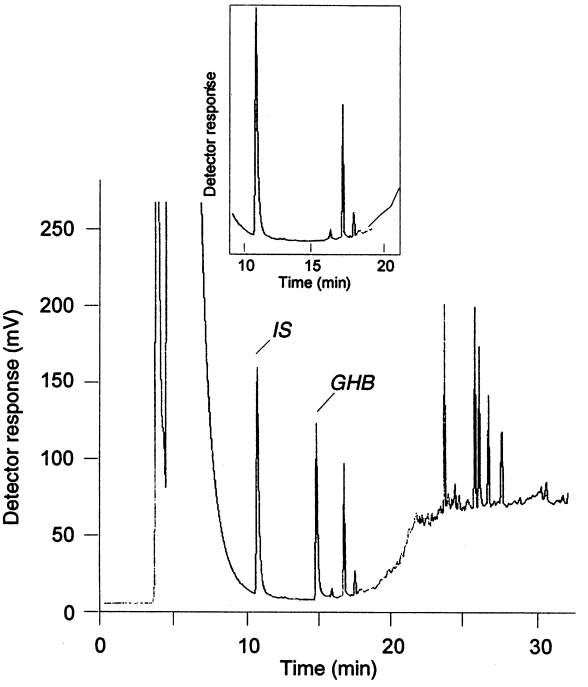

Fig. 2. Chromatogram of a plasma spiked with 1000 mg/l of γ-hydroxybutyrate and 25 μl of a 1 m*M* solution of internal standard (I.S.). In front of figure, chromatogram of a blank plasma containing the same internal standard concentration.

107.3% for GHB (Table 1). The mean extraction recoveries were 92.3±2.5% for 1,4-DB and 102.2±5.2% for its internal standard. Limits of quantification were, respectively, 10 mg/l for 1,4-BD (RSD=21.6%, $n=4$) and 25 mg/l (RSD=9.7%, $n=6$) for GHB.

The linearity of both methods were evaluated between 10 and 1000 mg/l for 1,4-BD and between 25 and 2000 mg/l for GHB. The linear regression equations of the calibrations curves were $y=-5.9$ (± 4.5)+1129 (± 84)x ($n=5$) with a mean correlation coefficient of 0.989 for 1,4-BD determination and $y=0.045$ (± 0.40)+3.54 (± 0.29)x ($n=5$) with a mean correlation coefficient of 0.991 for GHB determination.

In order to determine GHB in several samples, it was critical to assess the stability of the butylated extracts. Thus, we studied the conservation of the derivatized mixtures. Six independent pretreatment procedures were performed the same day on two blood samples containing 100 and 500 mg/l GHB. Three derivatized extracts were immediately analysed and three other samples were injected after being kept at 4 °C for 12 h in air-tight tubes, screened from light (data not shown). No significant difference was observed between the ratios of GHB to its internal standard. Thus, for practical reasons, we decided to extract and derivatise all samples (calibrators, controls and patient) the same day and to inject them the next day.

No interference was observed with methanol, ethanol, *n*-propanol, isopropanol, *n*-butanol, *n*-pentanol, isopentanol, acetone, chloroform, ether, acetonitrile, acetaldehyde, ethylmethylceton, methylisobutylceton, ethylene glycol, and propylene glycol. Concerning drugs, no interference was obtained with digoxin, salicylate, vancomycin, amikacin, gentamicin, cefotaxime, cyclosporin, tacrolimus, mycophenolate mofetil, phenobarbital, carbamazepin, valproate ($n=15$).

Comparable results were obtained with urine samples spiked with 1,4-BD and GHB. Retention times were the same than those observed for plasma. Intra-day and inter-day precisions were acceptable

Table 1
Intra-day, inter-day precision and inaccuracy for the determination of 1,4-butanediol and γ-hydroxybutyrate in human plasma

	Intra-day validation			Inter-day validation			Accuracy (%, range) (n=12)
	Spiked plasma concentration (mg/l)	Mean determined concentration (mg/l)	RSD (%) (n=6)	Spiked plasma concentration (mg/l)	Mean determined concentration (mg/l)	RSD (%) (n=12)	
1,4-Butanediol							
High	375	378	11.0	375	364.5	10.1	100.7 (92–119)
Low	75	82.5	5.8	75	81.0	7.0	109.8 (96–120)
GHB							
High	1500	1290	5.0	1500	1370	8.6	102.3 (95–117)
Low	250	275.4	12.0	250	268.2	8.7	107.3 (94–112)

with RSDs ranging from 6.1 to 12.7% for 1,4-BD and from 5.4 to 11.3% for GHB. The mean urinary extraction recovery was 93.2±3.5% for 1,4-BD.

Plasma and urine samples from four healthy volunteers treated under the described conditions showed no peak for 1,4-BD and GHB. A poisoning patient has been sampled (blood and urine). Determination of 1,4-BD showed no 1,4-BD peak while determination of GHB showed 470 and 520 mg/l GHB concentrations, respectively, in plasma and in urine (Figs. 3 and 4). A GHB determination was performed in plasma and urine of a patient treated with γ-hydroxybutyrate (3000 mg dose). Concentrations obtained in this patient were 1100 mg/l in plasma and 24 400 mg/l in urine.

4. Discussion and conclusion

We have developed two methods using a single analytical instrument for the determination of 1,4-BD and GHB in human plasma and urine. These methods do not require the expensive and complex equipment used by the previously described methods such as GC–MS. Mainly for this reason, some hospital laboratories are currently unable to determine 1,4-BD and its metabolite in emergency situa-

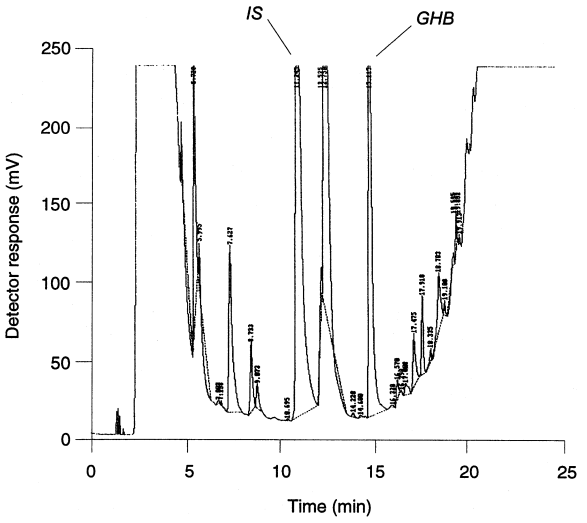

Fig. 3. Chromatogram of plasma from a poisoning patient containing 470 mg/l GHB and 25 μl of a 1 m*M* solution of internal standard (I.S.).

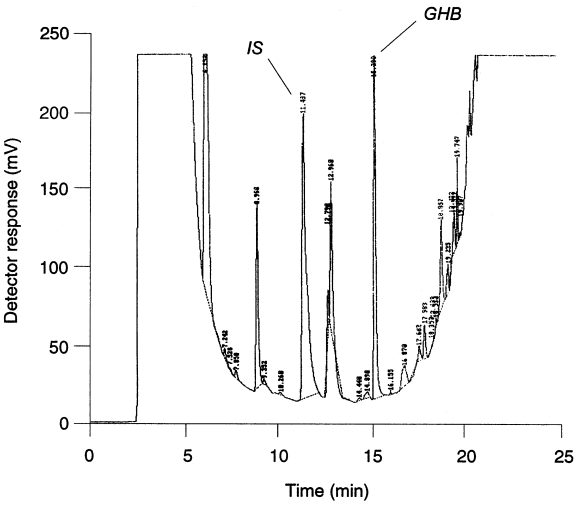

Fig. 4. Chromatogram of urine from a poisoning patient containing 520 mg/l GHB and 25 μl of a 1 m*M* solution of internal standard (I.S.).

tions. Since it is essential to furnish to the clinicians a rapid and specific toxicological confirmation of a suspected 1,4-BD poisoning, the use of an analytical method, which can be easily performed night and day by any technicians, is obviously required. In comparison with GC–MS, our technique presents a non-specific detection. However, our objective was not a forensic analysis which requires the use of specific methods. In our clinical practice, the aim is to define a medical diagnosis and begin a treatment. We work in accordance with clinical signs like metabolic acidosis. Concurrently, we observed no interference to our methods by other commonly detected volatiles and industrial solvents. We think that is unlikely to obtain an unknown interference which induces metabolic acidosis. Moreover, the determination of ethanol, methanol or ethylene glycol is currently performed in most hospital laboratories by capillary GC–FID methods using a polar stationary phase such as Carbowax-20M. Therefore, a similar chromatographic system could permit the determination of several alcohols and diols, including 1,4-BD, and their metabolites.

Determination of 1,4-BD in the poisoning patient showed no peak. This patient had ingested a small dose and sampled were taken 10 h after ingestion. Thus, its conversion to GHB was soon observed in urine and plasma. The intoxication of our patient was not fatal and he was hospitalized in medical care unit for 3 days. Determination of GHB in treated patients (continuous infusion) showed high concentrations. Literature reports that 1,4-BD levels from patients who died ranged from 220 to 1750 mg/l. The lowest lethal dose in humans is listed by the National Toxicology Program as 0.3 g/kg, although no source is given. However, it has been noticed that GHB blood levels ranged from 300 to 900 mg/l and from 400 to 5500 mg/l in urine of all poisoned patients [6]. Thus, the determination of GBH may be very important to monitor severe 1,4-BD poisoning, especially to follow-up the efficiency of haemodialysis or of antidotal treatment by blockade of alcohol deshydrogenase by ethanol or fomepizole. Since the pretreatment of samples containing 1,4-BD includes only a deproteinisation step, no particular analytical problems were expected, as observed. Moreover, the recovery experiments showed that the precipitation of proteins by acetonitrile gave an excellent extraction of the hydrophilic diol. Since octanol poisoning has never been described, it was unlikely to be found in samples and thus was chosen as internal standard.

However, for GHB determination, the entire process was more complex and longer, including an esterification step, but was found to be well adapted for toxicological purposes. Esterification by BF_3–butanol is a well known method to obtain volatile derivatives of aliphatic acids, including hydroxy-acids, under moderate conditions [16,17]. Since the butyl esters are less soluble in water than the methyl esters and since the addition of NaCl to the reacting mixture induces a salting out effect, this procedure permits thus a better separation of the butanolic phase and an optimal recovery.

Our simplified method permits the obtention of sufficient sensitivity and specificity to achieve the clinical goal. Thus, these validation parameters were comparable to those obtained with the previously described GC–MS method [8] and our limits of quantification were acceptable considering the levels observed in case of acute poisoning.

References

[1] P. Vayer, P. Mandel, M. Maitre, Life Sci. 41 (1987) 1547.
[2] MMWR Morb. Mortal Wkly. Rep. 39 (1990) 861.
[3] MMWR Morb. Mortal Wkly. Rep. 46 (1997) 281.
[4] FDA talk paper T99-5, 21 January 1999, p. 511.
[5] FDA Talk Paper T99-21, 11 May 1999, p. 1852.
[6] D.L. Zvosec, W.S. Smith, J.R. McCutcheon, J. Spillane, B.J. Hall, E.A. Peacock, New Engl. J. Med. 344 (2001) 87.
[7] F. Poldrugo, O. Carter Snead, Neuropharmacology 23 (1984) 109.
[8] S.D. Ferrara, L. Tedeschi, G. Frison, J. Pharm. Biomed. Anal. 11 (1993) 483.
[9] A.A. Elian, Forensic Sci. Int. 122 (2001) 43.
[10] G. Frison, L. Tedeschi, S. Maietti, S.D. Ferrara, Rapid Commun. Mass Spectrom. 14 (2000) 2401.
[11] O.C. Snead, R. Furner, C.C. Liu, Biochem. Pharmacol. 15 (1989) 4375.
[12] T.B. Vree, E. van der Kleijn, H.J. Knop, J. Chromatogr. B 121 (1976) 150.
[13] F.J. Couper, B.K. Logan, J. Anal. Toxicol. 24 (2000) 1.
[14] C.A. de Vriendt, D.K. van Sassenbroeck, M.T. Rosseel, E.J. van de Velbe, A.G. Verstraete, Y. Vander Heyden, F.M. Belpaire, J. Chromatogr. B 752 (2001) 85.
[15] I. Yanagisawa, M. Yamane, J. Chromatogr. B 345 (1985) 229.
[16] Alltech Chromatography: Catalog 400, Alltech, 1997, p. 452.
[17] K. Eder, J. Chromatogr. B 671 (1995) 113.