J Forensic Sci, Mar. 2003, Vol. 48, No. 2
Paper ID JFS2002186_482
Available online at: www.astm.org

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Identification and Quantitation of Gamma-Hydroxybutyrate (NaGHB) by Nuclear Magnetic Resonance Spectroscopy

ABSTRACT: The most common means of identification of gamma-hydroxybutyrate (NaGHB) involves using Fourier transform infrared spectroscopy (FTIR) or gas chromatography-mass spectrometry (GC-MS) of a suitable derivative. However, these methods may be complicated by possible shifts in chemical equilibrium between gamma-hydroxybutyric acid (GHB), GHB salts and the precursor lactone, gamma-butyrolactone (GBL). This paper addresses the technique of proton and carbon nuclear magnetic resonance spectroscopy (^{1}H and ^{13}C NMR) for the direct and accurate identification of GHB and GBL. The application of ^{1}H NMR for GHB quantitation is also discussed.

KEYWORDS: forensic science, nuclear magnetic resonance spectroscopy (NMR), gamma-hydroxybutyric acid (GHB), gamma-butyrolactone (GBL)

Gamma-hydroxybutyrate (NaGHB) is a central nervous system depressant originally used as an anesthetic adjunct in Europe more than 30 years ago (1) (Fig. 1). It is known to promote the release of growth hormones and was used both for muscle growth and as a sleep aid among bodybuilders during the 1980s (2,3). In 1989, NaGHB abuse began to increase due to its marketing as the replacement for L-tryptophan (4). In early 1990, hospital emergency rooms across the United States reported numerous cases of NaGHB overdose and related poisoning episodes (5), prompting several states to ban over-the-counter sales of NaGHB and NaGHB-containing "supplements" in 1990.

Following the ban, NaGHB was manufactured clandestinely from gamma-butyrolactone (GBL) (Fig. 2). NaGHB is currently abused for its hypnotic and euphoric effects and is commonly encountered at "rave parties" and in drug-aided sexual assault cases. There has been a widespread increase of NaGHB-related emergency room visits with 56 recorded visits in 1994 to 4969 visits in 2000 (6).

On February 18, 2000, the Hillory J. Farias and Samantha Reid Date-Rape Drug Prohibition Act of 1999 (Public Law 106-172) was signed and became Federal law. This law directed DEA to place GHB into Schedule I of the Controlled Substances Act (CSA). The final rule issued by DEA became effective on March 13, 2000 (the same day it was published in the *Federal Register*). It also placed GBL as a List I chemical. If, however, GBL is intended for human consumption and meets the definition of a Controlled Substance Analog in the CSA (21 USC 802(32), it could be treated as a Schedule I Controlled Substance. GHB-containing products manufactured, distributed or possessed in accordance with FDA authorized Investigational New Drug exemptions under the Federal Food, Drug and Cosmetic Act are placed into Schedule III, if or when they are approved.

The common means of identification of NaGHB involves using FTIR or derivatization followed by GC-MS. However, samples containing mixtures of NaGHB, GBL and/or other impurities require cleanup procedures such as acid/base extraction followed by evaporation of excess water prior to FTIR determination (7,8). Because NaGHB converts to GBL in heated injection ports, the identification of the original material by GC or GC-MS cannot be attempted without preliminary derivatization (9,10). In addition, sample preparation can affect the outcome of the analysis. The interconversion between NaGHB and GBL (Fig. 3) is very pH dependent, and any equilibrium shift during cleanup and derivatization can pose problems in the analysis (11). Although the lactone cannot be directly derivatized, in some cases, low levels of lactone may convert into GHB and then be derivatized. This has been detected in some derivatization experiments in the DEA Western laboratory using trimethylsilylating agents. High Pressure Liquid Chromatography/Ultraviolet-Visible Spectrophotometry (HPLC/UV-VIS), and HPLC/thermospray mass spectrometry have been used for separation and quantitation of GHB, and the latter method has also been used for confirmation (12). However, these techniques also have drawbacks since the mobile phase used may result in shifting the sample equilibria during analysis.

More recently, ^{1}H and ^{13}C NMR have been used successfully for GHB and GBL identification without altering the chemistry of the existing sample. ^{1}H and ^{13}C NMR spectra demonstrate sharp characteristic resonance peaks (8,13). In the DEA laboratories, at least 100 samples have been analyzed using NMR for identification.

NMR can also be used for quantitation of GHB and GBL either separately or in combination. This technique does not have the problems associated with other techniques, which include GC (14), free zone capillary electrophoresis (CE) (15,16) and HPLC (12,16,17). The GC quantitation method is relatively labor intensive and involves extraction of any free lactone present in the sam-

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Received 29 May 2002; and in revised form 6 Aug. and 15 Oct. 2002; accepted 19 Oct. 2002; published 3 Feb. 2003.

ple prior to the analysis. In addition, each of these methods relies on sample comparison with a known standard; this can be problematic since NaGHB is extremely hygroscopic and can absorb enough water from the air to convert it from a powder into a slush-like material, which will affect the quantitative results. Since NMR does not necessarily use GHB as an external standard, measurements are more reliable and consistent.

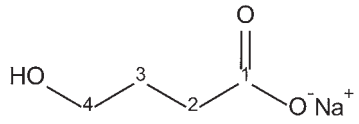

FIG. 1—*Structure of gamma-hydroxybutyric acid sodium salt.*

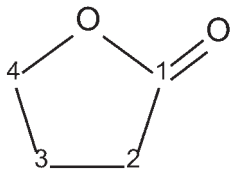

FIG. 2—*Structure of gamma-butyrolactone.*

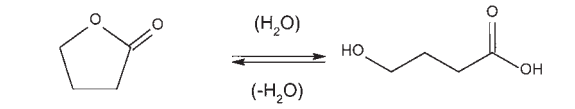

FIG. 3—*Reaction showing interconversion of GBL to GHB.*

Materials and Methods

Chemicals

Gamma-hydroxybutyric acid sodium salt was obtained from Fluka Chemical Corporation (Milwaukee, WI). Gamma-butyrolactone was obtained from Sigma Chemical Company (St. Louis, MO). Deuterium oxide (99.9 atm% D) and 1,4-dioxane (HPLC grade) were obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI).

NMR Spectrometry

Proton spectra were obtained on a Varian Gemini 2000 FT-NMR spectrometer using a 5 mm dual-channel room temperature probe and a proton observation frequency of 300 MHz. A SUN Ultra 5 computer running VNMRX software controlled the system. All samples were prepared in deuterium oxide containing sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as the internal reference. Wilmad 535 5 mm × 178 mm (0.19 by 7 in.) sample tubes containing 0.8 mL deuterated solvent were used for all data collection. A 30° pulse angle with 16 scans was applied on all qualitative experiments.

Carbon spectra were obtained using the above Varian NMR spectrometer at an observation frequency of 75 MHz. Sample concentrations were approximately 30 mg/mL. A 45° pulse angle was applied with 512 scans. All spectra were decoupled.

Homonuclear Correlation Spectra (COSY) (18) were obtained for both compounds. The parameters are as follows: 4 scans per increment with 64 increments, relaxation delay 1.000 s, WALTZ-16 decoupling, 0.3 Hz line broadening, FT size 512 × 512.

Heteronuclear Correlation Spectra (HETCOR) (19) were obtained for both compounds. The following parameters were used: 128 scans per increment with 256 increments, relaxation delay 1.000 s, WALTZ-16 decoupling, 1.0 Hz line broadening, FT size 2048 × 512.

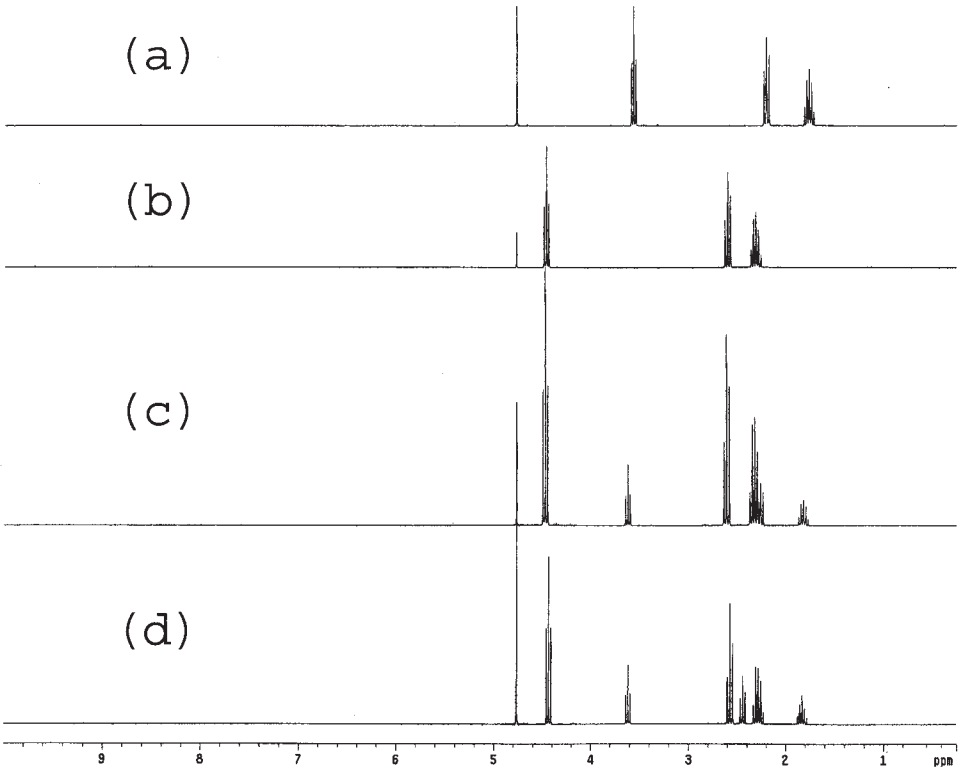

FIG. 4—(a) *^{1}H NMR spectrum of NaGHB,* (b) *^{1}H NMR spectrum of GBL,* (c) *^{1}H NMR spectrum of a mixture of NaGHB and GBL,* (d) *^{1}H NMR spectrum of GBL and GHB free acid interconversion.*

TABLE 1—*Proton and carbon chemical shift assignments for GHB and GBL individually in D_2O, a mixture of GHB and GBL in D_2O, and GHB free acid and GBL in D_20 with DSS as the reference. Proton-proton coupling constants in Hertz are in parentheses. Peak multiplicity code: t = triplet, m = multiplet (five line pattern).*

	Individually		Mixture		GHB Free Acid and GBL	
	GHB	GBL	GHB	GBL	GHB	GBL
H-2	2.20 t (7.7)	2.60 t (8.1)	2.26 t (7.7)	2.62 t (8.1)	2.43 t (7.7)	2.57 t (8.1)
H-3	1.76 m (7.1)	2.31 m (7.6)	1.82 m (7.1)	2.32 m (7.6)	1.82 m (7.1)	2.28 m (7.6)
H-4	3.56 t (6.7)	4.46 t (7.3)	3.62 t (6.7)	4.45 t (7.3)	3.61 t (6.7)	4.43 t (7.3)
C-2	36.67	30.57	36.78	30.59	32.96	30.52
C-3	31.05	24.21	31.18	24.24	29.41	24.15
C-4	64.15	73.21	64.25	73.21	63.34	73.17
C-1	185.77	185.59	185.53	185.47	180.92	185.58

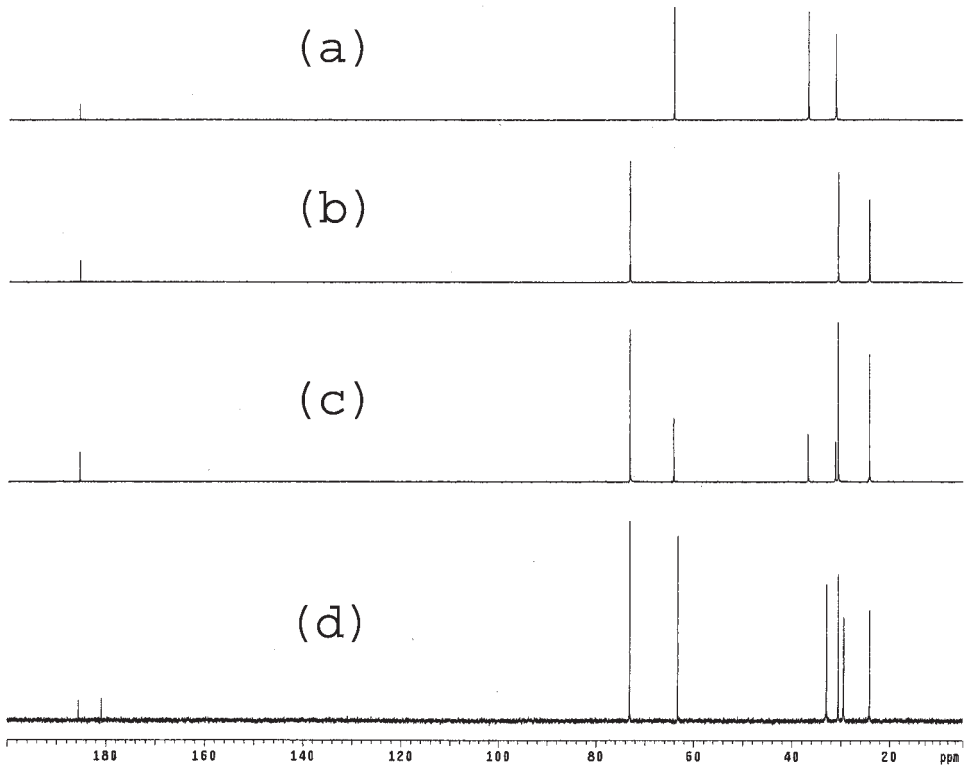

FIG. 5—(a) *^{13}C NMR spectrum of NaGHB,* (b) *^{13}C NMR spectrum of GBL,* (c) *^{13}C NMR spectrum of a mixture of NaGHB and GBL,* (d) *^{13}C NMR spectrum of GBL and GHB free acid interconversion.*

Quantitation of NaGHB was accomplished using ^1H NMR with a 90° pulse angle, a 30 s delay between pulses, and 16 scans. Concentrations of NaGHB ranged from 5–100 mg/mL with 1,4-dioxane (2 mg/mL) as the internal standard. Figure 7 is the ^1H spectrum for the quantitation of GHB. The 30 s delay was used to allow the resonances to return to equilibrium. This exceeds the $5\times T_{1max}$ for the compounds.

Results and Discussion

The ^1H spectra of GHB and GBL each exhibit three principal resonance signals with very different chemical shifts. The ^{13}C NMR of GHB and GBL may also be used for spectroscopic identification. ^{13}C Spectra of GHB and GBL display four carbon resonance peaks, which are easily distinguishable and specific. Even if co-mingled in a sample, the chemical shifts of either compound are not changed significantly and, except for the carbonyl carbon, do not coincide.

The interpretation of ^1H one-dimensional (1D) spectra of NaGHB and GBL is straightforward with three signals: two triplets and one five-line multiplet. The triplets are a result of spin-spin couplings with two protons on the adjacent carbons and not complicated by other adjacent protons. The five-line multiplet is the result of rapid conformational averaging by spin-spin couplings from protons on the adjacent carbons (20–24). The chemical shift of the H-2 is consistent with a methylene group adjacent to a carboxylic acid. The H-4 chemical shift agrees with a methylene group adjacent to an alcohol. Figure 4 is the ^1H spectra of GHB (a) and GBL (b), a mixture of NaGHB and GBL (c) and an equilibrium mixture of GBL and NaGHB showing the partial hydrolysis of GBL into GHB after a six-month period (d) (11).

The ^{13}C 1D spectra of NaGHB and GBL are straightforward with four signals. The signal at approximately 185 ppm is the car-

bonyl (25). The signal in the 65–75 ppm region correlates with a signal for an alcoholic carbon (C-OH). Table 1 lists the ^{1}H and ^{13}C chemical shifts and J-couplings for both compounds. Figure 5 is the ^{13}C spectra of NaGHB (a) and GBL (b), a mixture of GHB and GBL (c) and an equilibrium mixture of GBL and GHB free acid interconversion after six months (d) (11).

The symmetrized COSY spectra show cross correlations between H2–H3 and H3–H4, but no correlation between H2–H4. This is consistent with the structure of GHB and GBL (26). Figures 6 and 7 are the COSY spectra of NaGHB and GBL, respectively.

The HETCOR spectra show the carbon-hydrogen correlations and are straightforward and unambiguous (27). Figures 8 and 9 are the HETCOR spectra of NaGHB and GBL, respectively.

No significant chemical shift changes were observed during proton and carbon NMR experiments on mixtures of GHB sodium salt and GBL in D_2O (see Table 1).

The NMR experiments showed no observable conversion of GBL to GHB in D_2O solvent for the first 21 days. However, conversion of GBL into GHB-free acid in D_2O did occur after a longer period of time. GBL/GHB interconversion is shown in Figs. 4(d) and 5(d) as the ^{1}H and ^{13}C spectra, respectively. The conversion of GBL to GHB-free acid eventually reached an equilibrium ratio of 2/1, as suggested in some previous studies [e.g., see 11]. This was confirmed in our NMR experiments. No further difference in the proportion between the lactone and free acid was observed in the same sample tested at six months as compared to three months. GHB-free acid can be easily identified using its chemical shifts (21), which are markedly different from its salt form, especially on the H-2 protons and C-1, C-2 and C-3 carbons (see Table 1). The protons of the hydroxyl and the carboxyl group exchange quite rapidly with the deuterium in the solvent, and give rise to a large HDO peak at 4.8 ppm.

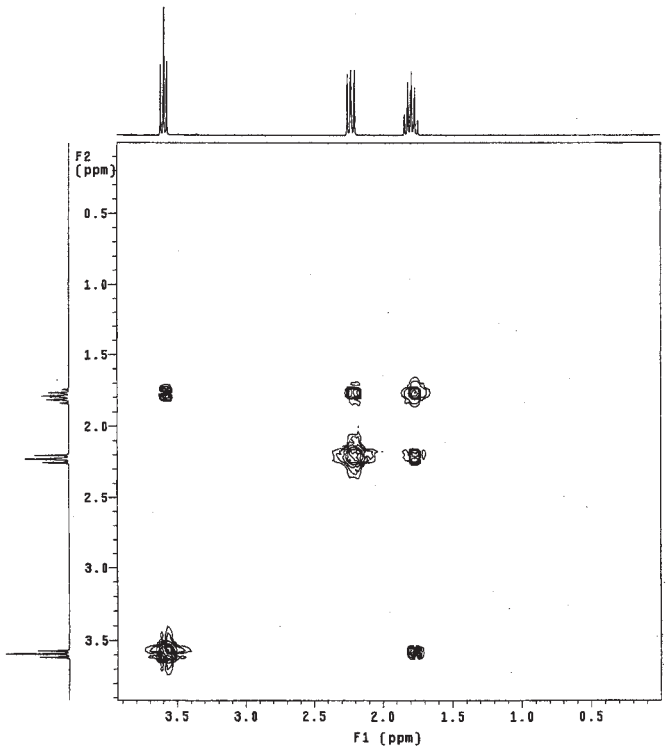

FIG. 6—*COSY spectrum of NaGHB.*

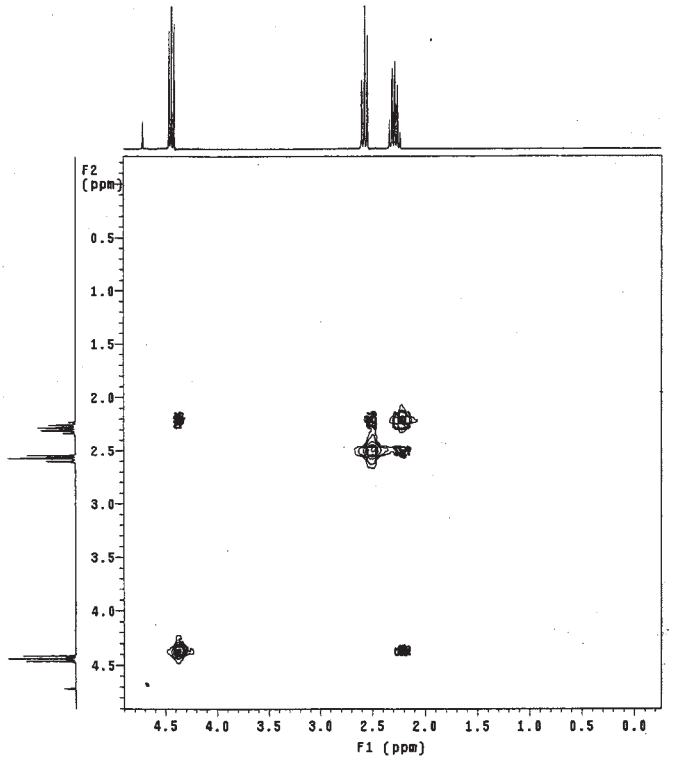

FIG. 7—*COSY spectrum of GBL.*

Quantitation

Figure 10 shows a ^{1}H NMR spectrum of the NaGHB in D_2O acquired with the internal standard 1,4-dioxane represented by eight protons with a single resonance signal at 3.75 ppm. Quantitation of GHB in the sample (Fig. 11) is determined by comparing integrated signal intensities of the internal standard and the NaGHB/H-4 protons (Table 2). The calibration curve is linear from 5–100 mg/mL of NaGHB with a correlation coefficient of 0.99999 for a five-point calibration. The measured values at each concentration represent the mean value of the three separate trials.

Precision was determined at each concentration by comparing the experimental results of three trials. The relative standard deviation (RSD) of the three trials at each concentration ranged from 0.14 to 0.88%. Accuracy, expressed in percent error, was measured by comparing the mean experimental value at each concentration to the weighed concentration. The percent error ranged from 0.40 to 1.60. The experiments demonstrated a high level of reproducibility as well as accurate quantitative measurements of the NaGHB sample based on NMR theory (28). The accurate proportionality between the signal intensities and the number of hydrogen nuclei resonating can be used to accurately determine the concentration of a sample, even though a standard for that compound is not present (29). In this particular instance, both NaGHB and GBL can be quantitated simultaneously.

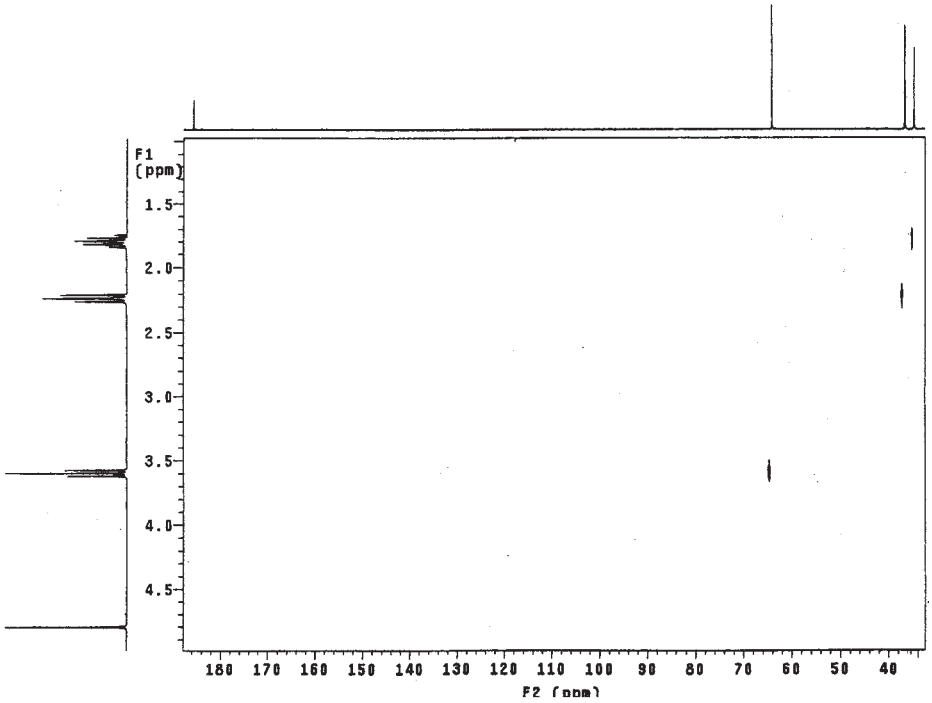

FIG. 8—*HETCOR spectrum of NaGHB.*

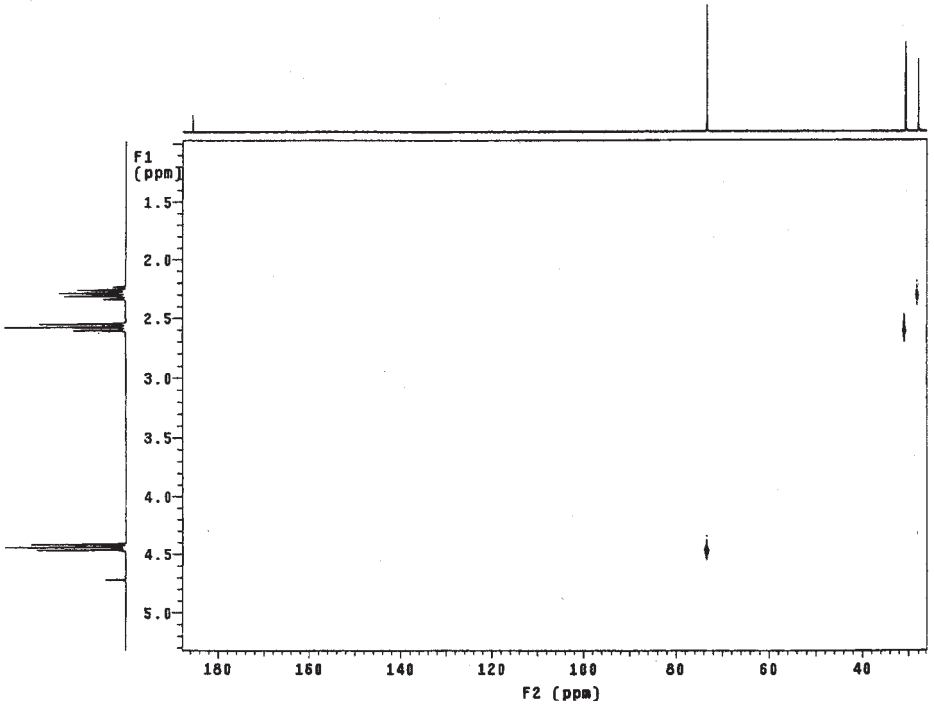

FIG. 9—*HETCOR spectrum of GBL.*

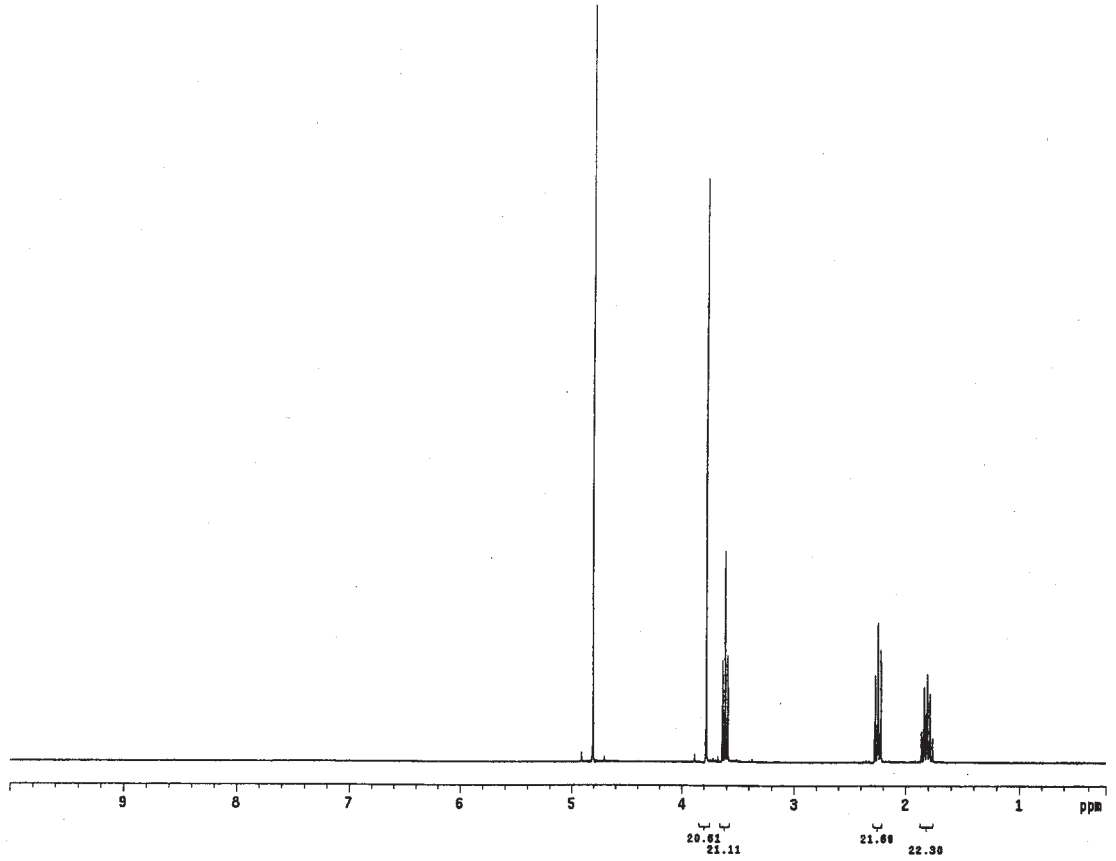

FIG. 10—*^{1}H NMR spectrum of NaGHB in D_2O; internal standard 1,4-dioxane.*

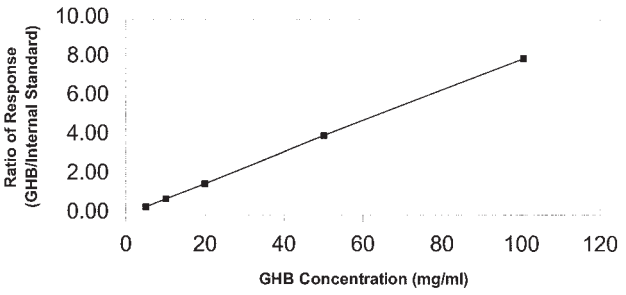

FIG. 11—*Linearity chart R = 0.999986 for GHB quantitation.*

TABLE 2—*GHB quantitation using 1,4-dioxane as internal standard in D_20 on three trials.*

GHB Conc. (mg/mL)	*Measured (mg/mL)	RSD (%)	% Error
5.04	5.06	0.88	0.40
10.01	10.16	0.73	1.55
20.01	20.33	0.24	1.60
50.12	50.92	0.14	1.60
100.40	101.00	0.69	0.60

*Mean experimental value.

Conclusion

NMR provides a simple, rapid and sensitive analysis to identify and quantify NaGHB, GBL, and mixtures of NaGHB and GBL. The quantitation does not require prior extractions, derivatizations or manipulations, and therefore does not change the equilibrium of the sample during analysis. This method also does not require the use of a NaGHB standard, the use of which can be problematic due to its hygroscopic nature.

Acknowledgments

The authors wish to acknowledge Kathleen M. Andrews, Drug Enforcement Administration forensic chemist at the Western Laboratory, San Francisco, California, for her information and helpful insights, as well as her exceptional contributions during discussions throughout this research project.

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