J Forensic Sci, March 2006, Vol. 51, No. 2
doi:10.1111/j.1556-4029.2006.00073.x
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GHB Free Acid: I. Solution Formation Studies and Spectroscopic Characterization by ^{1}HNMR and FT-IR

ABSTRACT: In forensic evidence, γ-hydroxybutyric acid (GHB) has frequently been encountered in one of its salt forms (γ-hydroxybutyrate), but has also been encountered in its free acid form (GHB). Owing to the physical properties, encounters of the free acid have been largely restricted to forensic exhibits comprising aqueous solutions, such as acidic beverages that have been "spiked" or formulated with GHB salts or γ-butyrolactone (GBL). The analysis of GHB free acid presents particular difficulties including the potential for altering the original proportions of GHB free acid, GHB carboxylate, and GBL in the course of analysis, and discrimination between GHB free acid and carboxylate forms. In this work, the formation of GHB free acid in aqueous solutions (water and/or D_2O) was studied as a function of solution pH. Proton nuclear magnetic resonance (^{1}HNMR) and Fourier-transform infrared spectrometry (FT-IR) measurements were obtained on freshly prepared mixtures of NaGHB and HCl stock solutions representing a series of points along the GHB titration curve. Both ^{1}HNMR and FT-IR were shown to track the changing proportions of GHB free acid and carboxylate forms as a function of pH, while simultaneously monitoring for the formation of the lactone (GBL). The results were consistent with acid–base conversion behavior for a carboxylic acid. ^{1}HNMR was shown to provide an ideal means for analysis of aqueous-based GHB/GBL forensic exhibits based on simple dilution of the neat liquid exhibit, without altering the original proportions of GHB free acid, carboxylate, and GBL in the samples.

KEYWORDS: forensic science, GHB free acid, γ-hydroxybutyric acid, GHB, γ-hydroxybutyrate, solution spectroscopic analysis, FT-IR, NMR, ^{1}HNMR, GBL, interconversion, sodium oxybate

Clandestine manufacture of γ-hydroxybutyric acid (GHB) typically produces GHB in a salt form (1). The salt most commonly found is the sodium salt, although potassium and lithium salts and salt mixtures have also been reported (1–5). Salt forms of GHB may occur as relatively pure solids, wet pastes, or in solution. Although the manufactured form of GHB has typically been a salt form, the presence of GHB as the free acid in forensic samples has occurred in aqueous beverages adulterated with either GHB salts or γ-butyrolactone (GBL) (6,7). Depending on the age and storage conditions of GBL formulations, the amount of GHB free acid formed may vary from a trace amount to approximately one third of the original GBL content of the product (6). To date, GHB free acid has not been reported in an isolated, "neat," or pure form in forensic samples.

Some of the forensic issues associated with interconversion of GHB and GBL have been addressed previously (6,7). The distinction between GHB free acid and carboxylate forms from a forensic analysis perspective has also been discussed (7). In the United States, the legal distinction between GHB and GBL at the federal level was clearly defined in the year 2000: GHB, in all of its forms (acid or salts), was declared to be a schedule I controlled substance, except for a pharmaceutical GHB preparation that was relegated to a schedule III status (8). At the same time, GBL was classified as a list 1 chemical that could be considered a schedule I analog of GHB under the analog provisions (8,9). However, the broader legal distinctions between GHB and GBL still exist at the international level (10,11). In March 2001, the United Nations Office on Drugs and Crime, Commission on Narcotic Drugs (ODCCP) adopted a World Health Organization (WHO) proposal to place GHB in schedule IV under the 1971 Convention on Psychotropic Substances (10). However, no formal action regarding GBL was announced by ODCCP. In the European Union, the legal status and control mechanisms for GHB vary across its member states, and GBL is subject to voluntary monitoring by the chemical industry owing to its role as a GHB precursor (11).

Analytical methods that have been reported for the identification of GHB in forensic analysis include gas chromatographic–mass spectrometric (GC–MS) (2,12–15), infrared (IR) (2–4,13,16–18), liquid chromatographic–mass spectrometric (LC–MS) (19,20), and both ^{1}H and ^{13}C nuclear magnetic resonance (NMR) (3,21). However, except for a recent report using NMR (21), these analytical methods or approaches have not been used to discriminate between GHB free acid and carboxylate forms. In GC–MS analysis, GHB is typically derivatized with a silylating agent such as BSTFA and detected as the di-trimethylsilyl (TMS) derivative. Discrimination of the free acid from the carboxylate is lost with the addition of pyridine or other organic bases in the derivatization process. In LC–MS analysis, speciation of the two is also lost with the use of acidic buffers in the mobile phase, and to the facile interconversion of GHB free acid and carboxylate forms in the ion source.

Previous reports of GHB forensic analysis using IR have involved one or more GHB salts (2–4,13,16,17). The earliest reported isolation of the free acid with characterization by NMR was from a 1989 study into the biological activity of GHB derivatives of D-glucosamine (22). Even though GHB free acid has been encountered in large proportions in acidic, aqueous GBL

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Presented at the 55th Annual Meeting of the American Academy of Forensic Sciences, Chicago, IL, 2003.

Received 26 April 2005; and in revised form 21 Aug. 2005; accepted 28 Aug. 2005; published 14 Feb. 2006.

products, its IR spectrum is largely masked in measurements of the neat products because of interference from the GBL carbonyl and water bands (23). This scenario may lead to discrepancies in the detection of the free acid in forensic samples analyzed by IR vs. other analytical techniques.

The overall goal of the current study was to address issues associated with the forensic analysis of GHB free acid including the development of methods and approaches that discriminate between the free acid and carboxylate forms without altering the original proportions of GHB free acid, GHB carboxylate, and GBL. A specific objective was to develop a means to detect the presence of GHB free acid before any substantial sample manipulation. This would minimize the possibility of generating GHB by conversion from GBL in the course of analysis. Another objective was to develop a simple procedure for preparation of a GHB free acid reference standard.

In Part I, we report on studies of GHB free acid formation in aqueous solutions (H_2O and D_2O) as a function of solution pH. Simultaneous measurements were acquired on freshly prepared mixtures of NaGHB and HCl stock solutions using FT-IR, proton nuclear magnetic resonance (^{1}HNMR), and high-performance liquid chromatography (HPLC)–UV. IR and ^{1}HNMR spectroscopy were used to discriminate between the GHB free acid and carboxylate forms, and to detect formation of GBL. In Part II (23), we investigated the isolation of small amounts (<10 mg) of GHB free acid for use as a reference standard, as well as the isolation and spectroscopic analysis of GHB from forensic exhibits.

Materials and Methods

Note that two separate series of GHB free acid solution and isolation experiments were conducted in two separate laboratories: the FDA Forensic Chemistry Center in Cincinnati, OH, and the DEA North Central Regional Laboratory in Chicago, IL. As a result, materials were obtained from multiple sources, and both the IR measurements and HPLC experiments were conducted on multiple systems.

Standards and Chemicals

GHB, sodium salt (NaGHB, minimum 99%) was obtained from Sigma (St. Louis, MO). GBL (reagent grade, 99+%) was obtained from Aldrich (Milwaukee, WI) and Sigma. Hydrochloric acid (Baker Analyzed) was obtained from JT Baker (Phillipsburg, NJ). Deionized water was obtained from a Millipore Milli-Q filtration system, or EM Science (Omnisolv water, Gibbstown, NJ). Deuterium oxide (99.9 atom %D) was obtained from Aldrich.

Free Acid Formation Solution Experiments: Water and D_2O

Aqueous stock solutions of NaGHB (approximately 1 M, 13–14% w/v) and HCl (3 M nominal) were prepared. In initial experiments, small volume aliquots of the NaGHB (30–100 μL) and HCl solutions (10–50 μL) were mixed in varying molar ratios representing a series of points along the titration curve. Larger volume aliquots of the NaGHB stock (5.0 mL) and HCl stock (100 μL–3.0 mL) solutions were used in subsequent experiments for pH measurement with a conventional electrode. Sample preparation involved first placing an aliquot of NaGHB solution in a narrow glass test tube or glass scintillation vial. Next, immediately upon addition of the aqueous HCl aliquot, the container was vortexed, and separate aliquots of the reaction mixture were removed for analysis by ^{1}HNMR, FT-IR, HPLC, and pH measurement. The pH measurements were made using pH paper (pHydrion range 1–12) for the small volume aliquots, and a pH meter (Denver or Orion Model 420A, Orion Research Inc, Boston, MA) for the larger volume reaction mixtures.

Analogous experiments were conducted substituting D_2O for water, thus eliminating the interference from the water OH bend that occurs in the IR at 1650 cm^{-1} (see Fig. 1, aqueous solution spectra for GHB sodium salt shown in water and D_2O). The use of

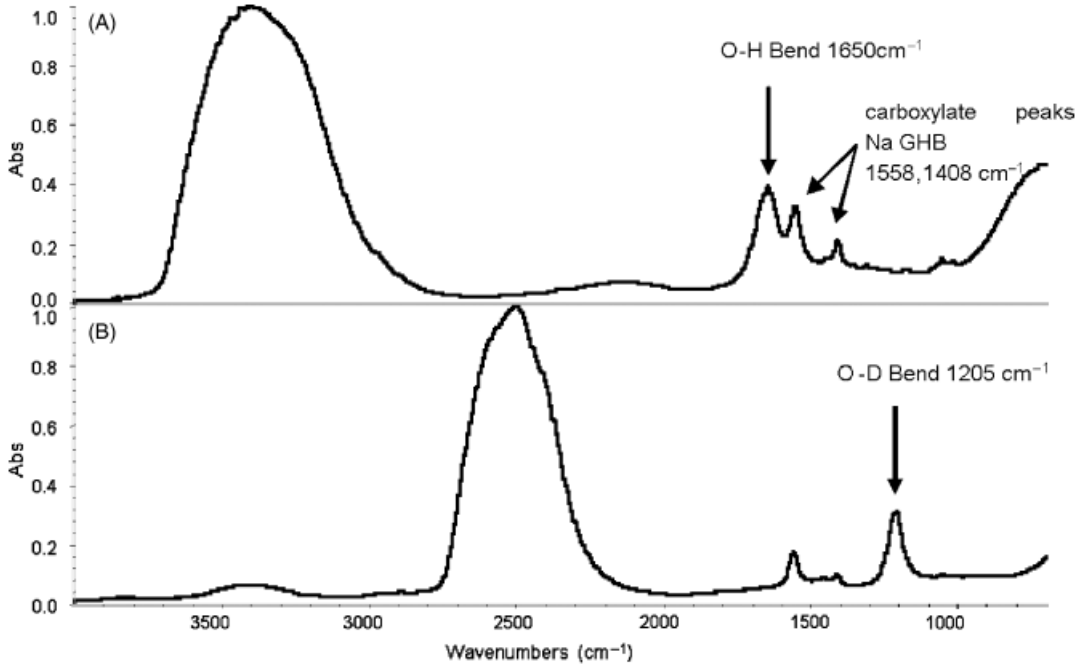

FIG. 1—*Comparison of the Fourier-transform infrared spectrometry spectra of NaGHB in H_2O (A) and D_2O (B). In D_2O, the O–D bend shifts to a much lower wavelength and out of the region of interest.*

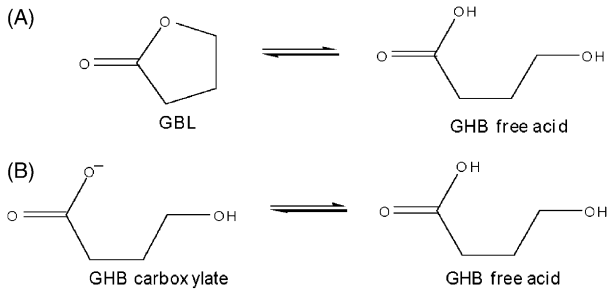

FIG. 2—*Formation of γ-hydroxybutyric acid (GHB) free acid from hydrolysis of γ-butyrolactone (GBL) (A) or protonation of γ-hydroxybutyrate (B).*

D_2O allowed for simultaneous observation of the free acid C = O band at 1700 cm^{-1} and the C = O band associated with GBL at 1741 cm^{-1} in later experiments. The shift in the peak maxima for the free acid C = O and the C = O associated with GBL is due to deuterium bonding (analogous to hydrogen bonding in water). Two separate series of experiments were conducted using NaGHB concentrations of 1.7 M (21% w/v) with ca. 3 M HCl, or 0.4 M NaGHB (5% w/v) with ca. 1 M HCl.

^{1}HNMR Measurements

Nearly all ^{1}HNMR measurements were acquired using a Varian Gemini 300 FT-NMR Spectrometer (Varian Inc., Palo Alto, CA) (300 MHz, see Fig. 4), except for the spectrum shown in Fig. 7*B*, which was acquired using a Varian Mercury 400 FT-NMR Spectrometer (Varian Inc.) (400 MHz). All chemical shifts reported are downfield relative to the TMS proton signal (sharp singlet at 0.0 ppm) from the internal reference compound sodium 3-trimethylsilyl-propanesulfonate (DSS). Note that the broad singlet at ca. 4.8 ppm arises from the resonance of hydroxyl protons in both H_2O and D_2O (through rapid exchange between O–H and O–D), as well as both of the O–H functions of GHB. As these protons are rapidly exchanged among the various hydroxyl-containing moieties, this signal provided no significant interpretive value in this work. For NaGHB, the methylene signals appear at the following frequencies: 1.780 ppm (a, HO–CH_2–$C\mathbf{H_2}$–CH_2–CO_2Na, quintet, j = 6.9 and 7.3 Hz), 2.218 ppm (b, HO–CH_2–CH_2–$C\mathbf{H_2}$–CO_2Na, triplet, j = 7.8 Hz), and 3.584 ppm (c, HO–$C\mathbf{H_2}$–CH_2–CH_2–CO_2Na, triplet, j = 6.7 Hz). For GHB free acid, the methylene signals appear at the following frequencies: 1.834 ppm (a, HO–CH_2–$C\mathbf{H_2}$–CH_2–CO_2H, quintet, j = 7.0 & 6.9 Hz), 2.448 ppm (b, HO–CH_2–CH_2–$C\mathbf{H_2}$–CO_2H, triplet, j = 7.6 Hz), and 3.620 ppm (c, HO–$C\mathbf{H_2}$–CH_2–CH_2–CO_2H, triplet, j = 6.5 Hz). For GBL, the methylene signals appear at the following frequencies: 2.280 ppm (a, –O–CH_2–$C\mathbf{H_2}$–CH_2-C = O–, quintet, j = 7.5 & 7.8 Hz), 2.574 ppm (b, –O–CH_2–CH_2–$C\mathbf{H_2}$-C = O–, triplet, j = 8.2 Hz), and 4.436 ppm (c, –O–$C\mathbf{H_2}$–CH_2–CH_2- C = O–, triplet, j = 7.0 Hz).

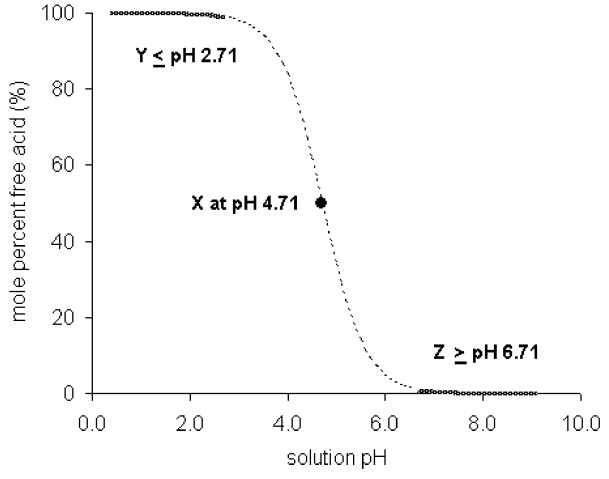

FIG. 3—*Change in mol% γ-hydroxybutyric acid free acid as a function of pH for a pK_a value of 4.71. See text for discussion.*

IR Spectroscopic Measurements

Initial FT-IR measurements for the solution reaction mixtures were made between BaF_2 windows using a Perkin Elmer Spectrum GX FT-IR (Perkin Elmer, Wellesley, MA) with an AutoImage System Microscope. All spectra were collected using the main bench sample compartment. The data collection parameters used were the following: resolution 4 cm^{-1}, 64 scans, a strong apodization function, a DTGS detector with a spectral range of 4000–370 cm^{-1}, and a detector gain = 1. For each sample 3 μL of liquid was placed between clean BaF_2 windows and an IR spectrum was obtained. Clean BaF_2 windows were used to ratio against the sample spectra. All IR spectra were treated after data collection first by applying a smoothing function (Savitsky–Golay 3°polynomial with nine-point smoothing) and then baseline corrected using a two-point correction in the region 1800–1400 cm^{-1}. While these data exhibited the trends discussed in this paper, variability in the intensities of the peak absorbance bands was observed because of the lack of a fixed pathlength when making liquid measurements between windows.

The entire experiment was repeated by making duplicate measurements for each solution using a Nicolet Magna 550 FT-IR with a SensIR Durascope single reflectance attenuated total reflectance (ATR) accessory. The use of the Durascope ATR accessory provided for the fixed pathlength that was absent in the initial experiments, resulting in excellent precision for the duplicate measurements. The data collection parameters used were the following: resolution 4 cm^{-1}, 64 scans, a Happ–Genzel apodization function, a TE-cooled DTGS detector with a spectral range of 4000–650 cm^{-1}, and a detector gain = 1. For each sample, 10 μL of liquid was placed directly onto the ATR diamond-coated zinc selenide (ZnSe) internal reflectance element (IRE) and an IR spectrum was obtained. A clean ATR IRE was used to ratio against the sample spectra. No postdata treatment of the FT-IR ATR measurements was performed, and the peak heights were measured directly from the FT-IR ATR data.

HPLC Quantitative Analysis of GHB and GBL

HPLC–UV analysis was conducted according to the previously reported method (6). Two Hewlett Packard 1100 Liquid Chromatograph Systems (Agilent Technologies, Palo Alto, CA) equipped with a Hydrobond AQ column (4.6 mm × 15 cm, 5 μm) were used for all analyses. The mobile phase comprised 95:5 buffer: methanol at a flow rate of 0.5 mL/min. The buffer was 50 mm phosphate, in the pH range 2.5–3.0. The injection volume was 10 or 15 μL; detection was at 215 nm. The column was thermostatted at 30°C. The run time was 15 min. Standard solutions for GHB were each prepared from the sodium salt.

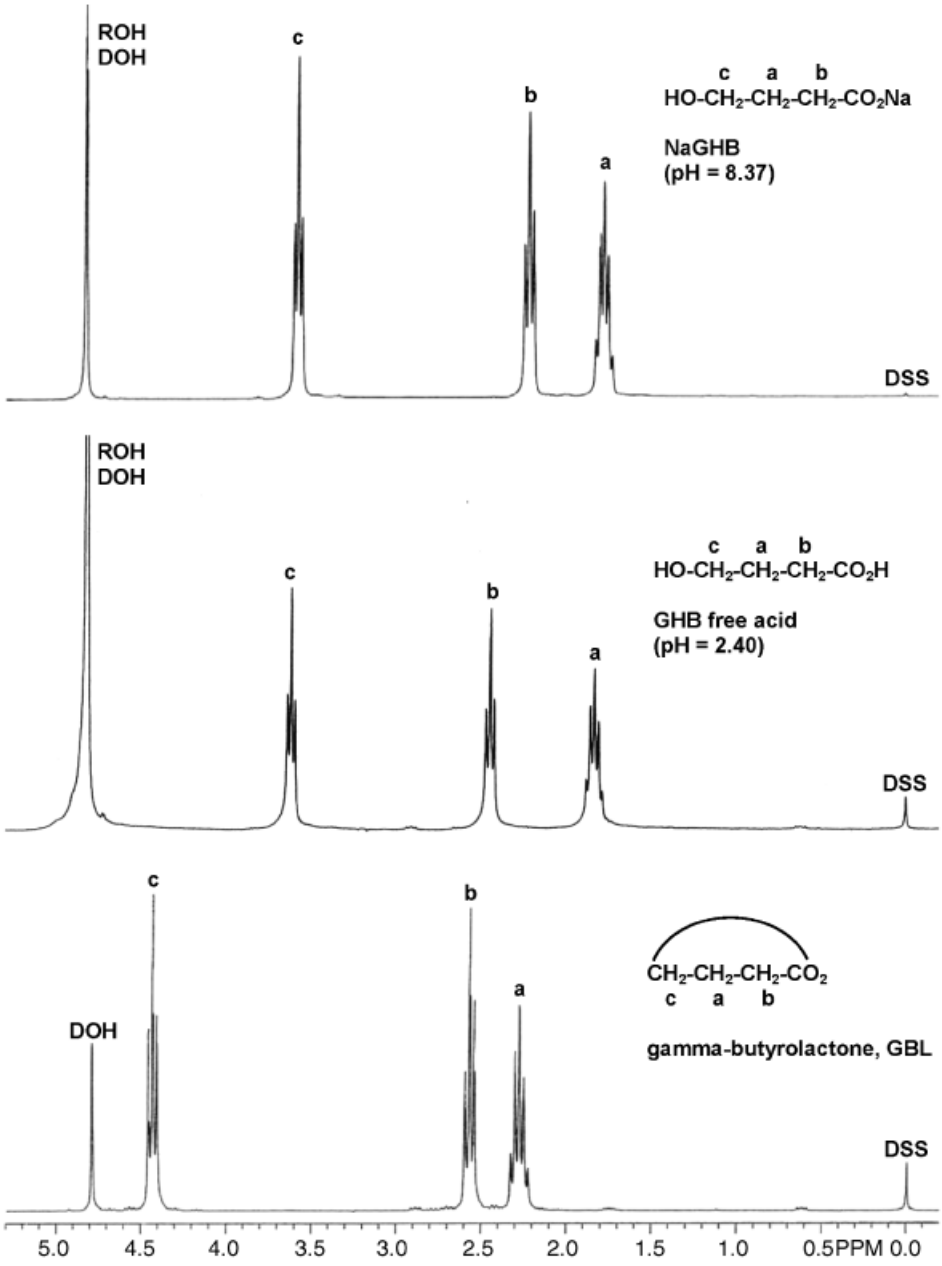

FIG. 4—*Proton nuclear magnetic resonance (^{1}HNMR) spectra of NaGHB, γ-hydroxybutyric acid (GHB) free acid, and γ-butyrolactone (GBL).*

Results and Discussion

The main evidence type containing the GHB free acid is neutral to moderately acidic aqueous solutions, in which a portion of the GBL has undergone hydrolysis to GHB free acid (6,7). Although the form of GHB (free acid vs. carboxylate) is irrelevant subject to its scheduling status (in the United States), the ability to discriminate between GHB free acid and carboxylate forms nonetheless provides valuable intelligence. Methods that can directly analyze for the presence of GHB free acid in forensic evidence are needed to avoid the unintended generation of GHB free acid in the course of analysis. Furthermore, establishing the source of GHB in a given exhibit may be important in an investigation and subsequent prosecution (e.g., to determine whether a beverage was adulterated with GBL or a GHB salt).

The analysis of GHB free acid in forensic evidence is problematic for several reasons. First, there is no commercially available standard or reference material for GHB free acid. Second, the most commonly used analytical approaches do not discriminate between GHB free acid and salt forms. Third, in aqueous-based GBL solutions containing GHB free acid, the IR spectrum of the free acid is largely masked in measurements performed on the neat sample liquid (23). FT-IR is frequently used for the analysis of GHB-related evidence (2–4,13,16–18). As such, this scenario may lead to discrepancies in the detection of GHB free acid in forensic samples analyzed by IR vs. other analytical techniques.

Formation of GHB Free Acid and Free Acid pH Dependence

An understanding of the role of solution pH in GHB/GBL aqueous solution chemistry is critical for designing valid analyt-

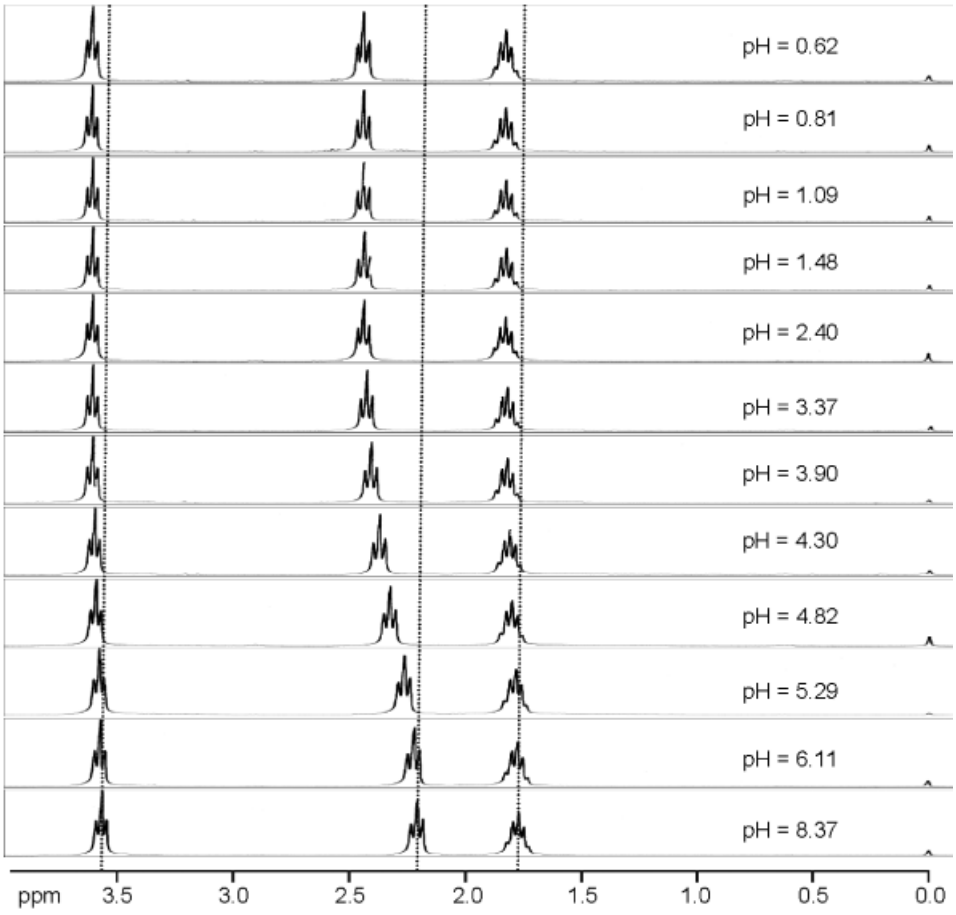

FIG. 5—*Stacked plot of proton nuclear magnetic resonance spectra of NaGHB/HCl reaction mixtures in D_2O as a function of solution pH after reaction.*

ical approaches and for preparation of a suitable GHB free acid reference material. GHB free acid may be formed in aqueous solution by either acid-catalyzed hydrolysis of GBL or protonation of the carboxylate form (see Fig. 2). Both of these routes are reversible to the extent that the free acid may revert to either the lactone via esterification (see Fig. 2*A*, back reaction) or to the carboxylate form via acid dissociation (see Fig. 2*B*, back reaction). Although both reactions are pH dependent and thus affected by the hydrogen ion concentration, the role and effect of the hydrogen ion are different for the two reactions. For hydrolysis/esterification, the effect is mainly catalytic, with the rate of reaction decreasing exponentially as $[H^+]$ decreases. Thus, the reaction rate decreases by several orders of magnitude as the pH increases from strongly acidic to weakly acidic (i.e., pH $\sim$ 0–4) (6,7,24,25). Further increases in solution pH (>4) result in a change in the mechanism of hydrolysis to neutral, and eventually basic mechanisms (24,26). The rate of hydrolysis is slowest at intermediate pH (pH 4–7), and then increases with increasing $[OH^-]$ by several orders of magnitude above pH 7 (6,7,27). For the carboxylate protonation route, the hydrogen ion is a reagent in the reaction, affecting mainly the extent of reaction. The equilibrium constant that governs proton exchange is the acid dissociation constant (K_a). The reaction can be expressed as follows:

$$HA \rightleftarrows A^- + H^+ \qquad (1)$$

where HA represents the GHB free acid and A^- represents the carboxylate. The dissociation constant is related to the molar concentrations of the free acid ([HA]), carboxylate ion ($[A^-]$), and hydrogen ion $[H^+]$ as follows:

$$K_a = \frac{[A^-][H^+]}{[HA]} \qquad (2)$$

The literature pK_a value (where p$K_a = -\log K_a$) for GHB is 4.71 (28), which is typical for a carboxylic acid. For the purposes of this work, the literature pK_a value is considered to be a good estimate of the actual pK_a values in the solutions studied. Strictly speaking, the actual pK_a value changes as a function of temperature and ionic strength (7,25); however, the pK_a value will be a constant for a given solution set. As such, changes in the equilibrium molar ratio of the GHB free acid and carboxylate forms in any aqueous system are controlled by changes in the solution pH.

Figure 3 depicts the changing molar ratio of the free acid and carboxylate forms as a function of solution pH (molar ratio is expressed as mol % free acid). This plot has several distinct features that are analogous to a titration curve. At pH 4.71, designated as point "*X*" in the figure, there exists an equimolar amount of GHB free acid (HA) and the carboxylate form (A^-). At this point, the solution pH is equal to the pK_a (p$K_a = -\log K_a$). At pH $<$ 2.71, designated as region "*Y*" in the figure, the free acid form (HA) dominates, and the proportion of carboxylate (A^-) is negligible (<1 mol%). This represents a pH region at least two units below the pK_a of GHB. Conversely, at pH $>$ 6.71, designated as region "*Z*" in the figure, the carboxylate form (A^-) dominates and the proportion of free acid (HA) is negligible (<1 mol%). This rep-

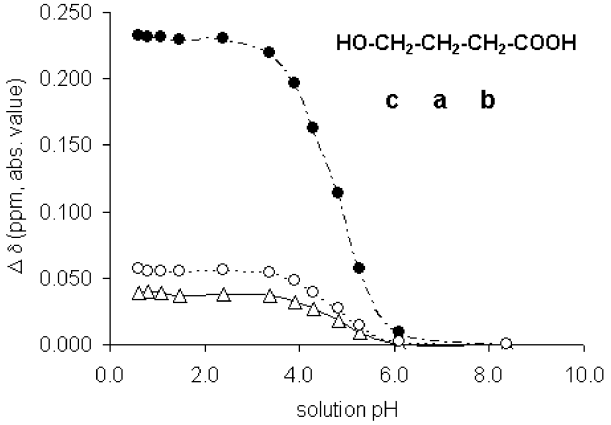

FIG. 6—*Change in proton nuclear magnetic resonance chemical shift value for the three sets of methylene protons in γ-hydroxybutyric acid as a function of solution pH: (●) "b" methylene protons adjacent to carboxylic acid carbon; (○) "a" central methylene protons; and (△) "c" methylene protons adjacent to hydroxyl carbon.*

resents a pH region that is at least two units above the pK_a of GHB. Finally, there exists a region of steep change that spans from pH 2.7 to 6.7, in which the mol% of free acid (HA) decreases as solution pH increases.

^{1}HNMR Measurement of Aqueous GHB Solutions as a Function of pH

The ^{1}HNMR spectra of NaGHB, GHB free acid, and GBL are displayed in Fig. 4. The multiplicity of each signal follows simple first-order principles, and the signal pattern is the same in each of the three compounds (two triplets and a quintet). The only differences in the spectra are the positions (frequencies) of the three signals. In GBL, the signal positions are governed by the ester functionality. When comparing the carboxylate with the free acid form, the structural change is quite subtle, differing only in the absence or presence of a proton (carboxylate and free acid, respectively). Despite this seemingly subtle structural difference, a relatively large shift is observed in the signal frequencies, especially for the methylene protons adjacent to the carbonyl carbon. The magnitude of the shift is greatest for the methylene protons adjacent to the carbonyl and diminishes as the methylene protons become more distant from the carbonyl. This behavior is due to the fact that protonation causes an electronic change in the molecule and the frequency of resonance signals is very sensitive to the electronic environment. Thus, the influence of the electronic perturbation is most profound in the methylene protons closest to the site of protonation and diminishes rapidly as a function of distance.

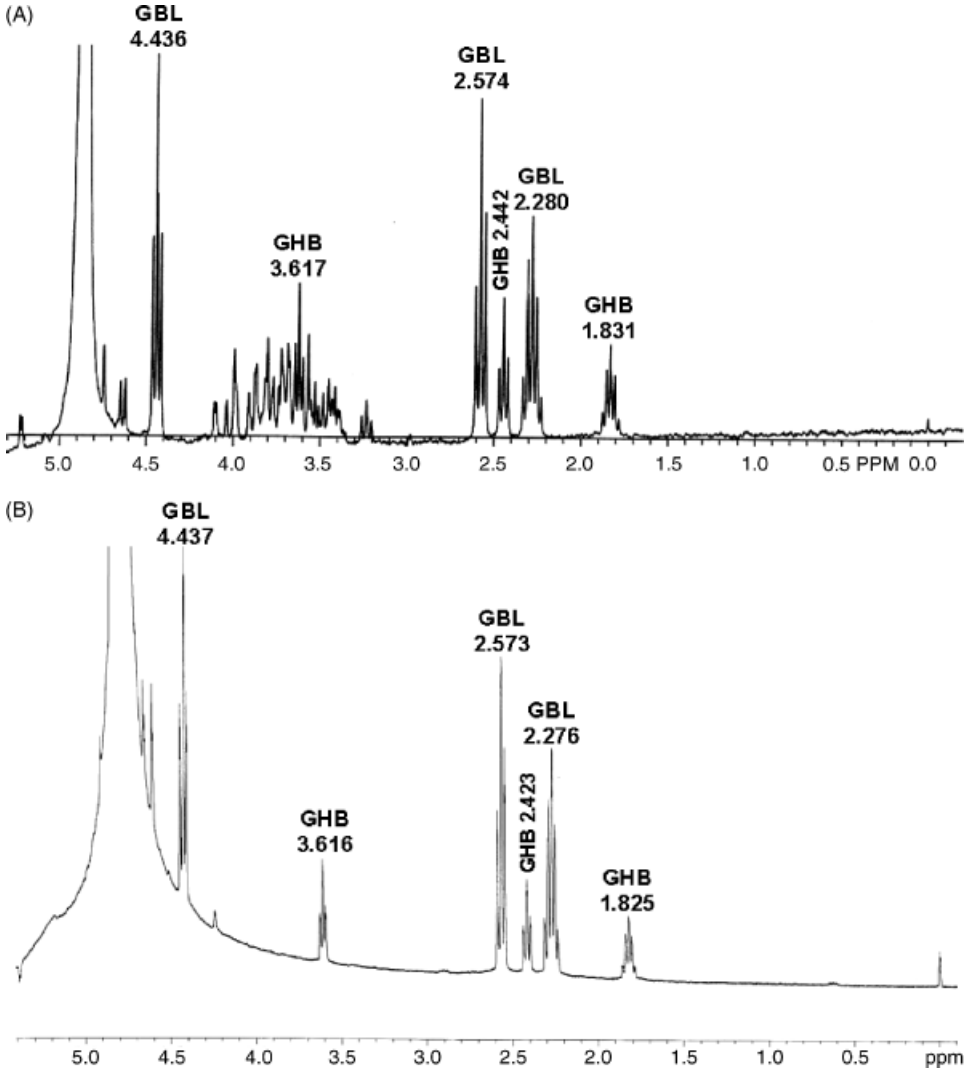

FIG. 7—*Proton nuclear magnetic resonance spectra of aqueous-based GBL forensic exhibits containing γ-hydroxybutyric acid (GHB) free acid: a commercially formulated γ-butyrolactone (GBL) "beverage" (A); residue from a soft drink bottle used for storage of GBL and rinsed with water (B).*

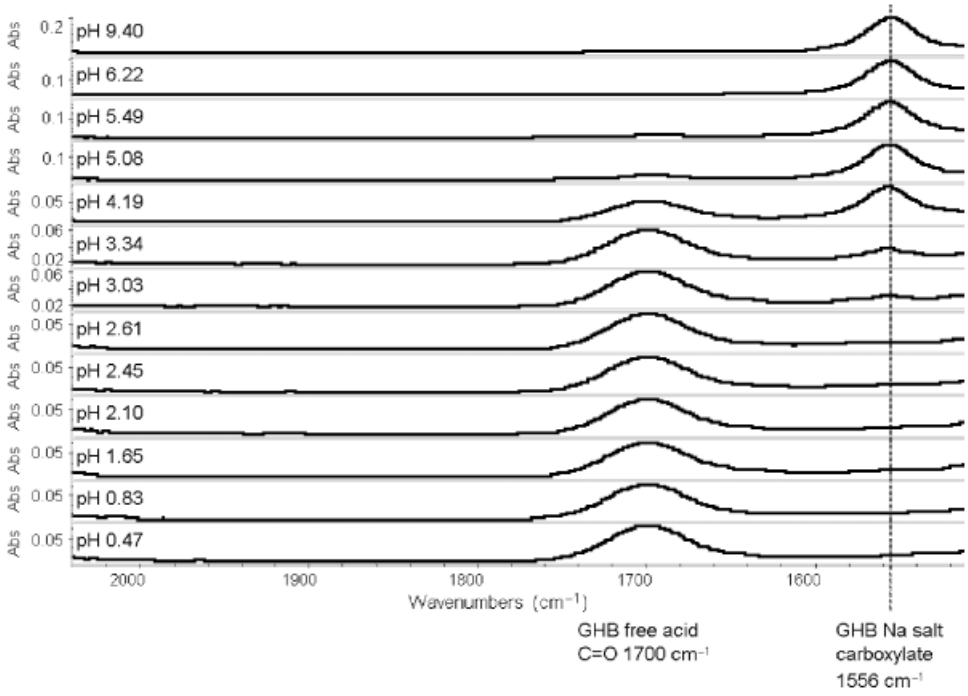

FIG. 8—*Stacked plot of expanded region infrared spectra of NaGHB/HCl reaction mixtures in D_2O as a function of solution pH after reaction.*

Figure 5 is a stacked plot illustrating the gradual shift in the three signal positions for GHB as the solution pH (experiment conducted in D_2O) is incrementally adjusted downward from pH 8.37 (99.99% carboxylate form) to pH 0.62 (99.99% free acid form). As aqueous HCl is added to the NaGHB solution, protonation of the carboxylate occurs almost instantaneously. The resultant ^{1}HNMR spectrum at any given pH represents a composite spectrum of the GHB free acid and carboxylate forms, with the two forms rapidly interconverting. Proton exchange for acid–base pairs in water are known to occur on time scales measured in pico or femtoseconds (29). The proton exchange between the two forms occurs on a much faster time scale than the ^{1}HNMR data acquisition. If the proton exchange occurred at a slower rate than the data acquisition, separate signals would be observed for the free acid and carboxylate forms, resulting in a total of six signals when both species are present at detectable levels: three from the free acid and three from the carboxylate. Note that in all cases, the spectra were obtained within minutes of preparing the solutions and examined for the presence of GBL. No evidence of GBL formation was observed in any of the spectra, except for the experiments at pH 0.62 and 0.81, for which low amounts were detected (at pH 0.62 [GBL] $\sim$ 2% at 2 min, 5.6% at 7 min, 9.3% at 16 min, 13% at 28 min, 15% at 36 min, and 17% at 41 min; at pH 0.81 [GBL] $<$ 1% at 2 min and 3.5% at 12 min). Figure 6 graphically depicts the change in signal frequency for each of the three sets of protons in GHB as a function of solution pH. These plots mimic an acid–base titration curve, indicating that ^{1}HNMR tracks the changing proportions of the free acid and carboxylate forms of GHB as solution pH changes (compare Figs. 3 and 6).

Upon closer analysis of the NMR curves, it is evident that the frequencies of the three signals reach plateaus at about pH 3.3 and 6.1. The lower limit at pH 3.3 represents the point at which the free acid (HA) predominates, whereas the upper limit at pH 6.1 represents the point at which the carboxylate (A^-) predominates. Given the aforementioned assumption, the midpoint of this range at pH 4.7, which represents an equimolar distribution of HA and A^- (i.e., [HA] = [A^-]), is in agreement with the literature pK_a of 4.71. It is interesting to note that measurable movement in the frequency of the "b" protons signal as a function of pH can be detected over a wider pH range than movement in the "a" or "c" protons. This increased sensitivity is consistent with the fact that the "b" protons are closest to the point of protonation/deprotonation, and thus most accurately indicative of the free acid/carboxylate ratio.

^{1}HNMR Measurement of GHB Free Acid in Forensic Samples and Spiked Beverages

At the DEA laboratory in Chicago, ^{1}HNMR has been routinely applied for direct identification of both GHB and GBL in aqueous-

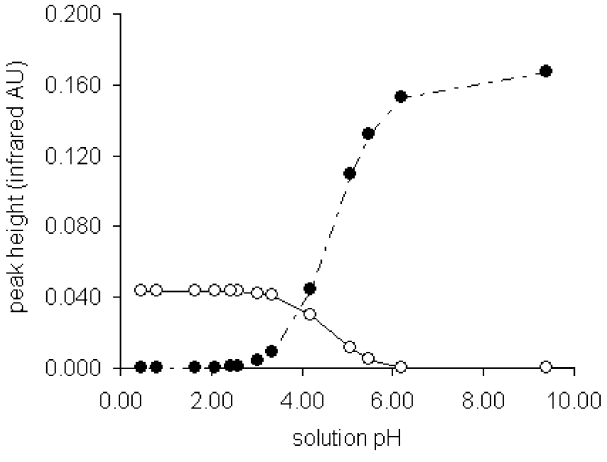

FIG. 9—*Change in Fourier-transform infrared spectrometry absorbance intensity as a function of solution pH in D_2O for the γ-hydroxybutyric acid (GHB) free acid carbonyl (○, 1700 cm^{-1}) and GHB carboxylate asymmetric stretch (●, 1556 cm^{-1}).*

based GBL formulations (see Figs. 7*A* and *B*). Sample manipulation is minimal, comprising of simple dilution of the neat sample in D_2O and addition of an internal reference (i.e., DSS). The relative proportions of the GHB free acid and GBL were readily estimated from the ^{1}HNMR signal intensities.

FT-IR Measurement of Aqueous GHB Solutions as a Function of pH

Figure 8 shows a stacked plot of the IR spectra collected for the GHB in D_2O experiment as a function of solution pH. At a pH of 9.4, the predominant solution species is the sodium salt that is evident by the presence of the carboxylate asymmetric stretch observed at 1556 cm^{-1}. At a pH of 0.47, the predominant solution species is the free acid that is evident by the presence of the free acid C = O observed at 1700 cm^{-1} (the position of the carbonyl band is shifted to a lower wavenumber in deuterated water). As the pH is varied, the intensity of the carboxylate peak at 1556 cm^{-1} decreases and the intensity of the free acid C = O band at 1700 cm^{-1} increases. No peak associated with the free acid was observed above pH of 6.2, and no peak associated with the carboxylate was observed at/below a pH of 2.1.

A simple plot of the peak intensities (IR absorbance peak heights) for the free acid C = O (1700 cm^{-1}) and carboxylate (1556 cm^{-1}) signals as a function of solution pH is given in Fig. 9. As was observed for the ^{1}HNMR experiments, the IR absorbance plots for the free acid and carboxylate signals closely resemble acid–base titration curves. The change in the free acid carbonyl signal intensity tracks the theoretical change in mol% free acid as a function of pH (see Fig. 3). However, in the case of the FT-IR experiments, the signals from the free acid and carboxylate forms are independently measured and observed. Note that the IR molar absorptivity for the carboxylate stretch (1556 cm^{-1}) is greater than the IR molar absorptivity for the free acid carbonyl band (1700 cm^{-1}), resulting in a steeper "titration" curve for the carboxylate plot. Thus, despite the larger signal for the carboxylate observed at a pH of 4.71, the molar amounts of free acid and carboxylate forms in solution are equal at this pH (pH = pK_a).

GHB Free Acid Solution IR Spectra

The solution IR spectrum for the stoichiometric reaction mixture of GHB sodium salt and HCl in water is given in Fig. 10*A*. Despite the absence of GBL, the emergence of the free acid carbonyl absorbance (1710 cm^{-1}) for GHB is still difficult to discern because of interference from the water OH bend (1640 cm^{-1}). However, when this same experiment is conducted in D_2O, the OD bend is shifted to a lower frequency, and thus the free acid carbonyl absorbance is readily observed (1700 cm^{-1}, Fig. 10*B*). There is also no evidence of either GBL formation (no carbonyl absorbance at 1741 cm^{-1}) or residual carboxylate (no absorbance at 1556 cm^{-1}). The stoichiometric reaction mixtures were stable for a period of time before any measurable reversion to the lactone (no evidence of GBL formation was observed via ^{1}HNMR data over a 12-h period). To our knowledge, these spectra represent the first reported aqueous solution infrared spectra for GHB free acid.

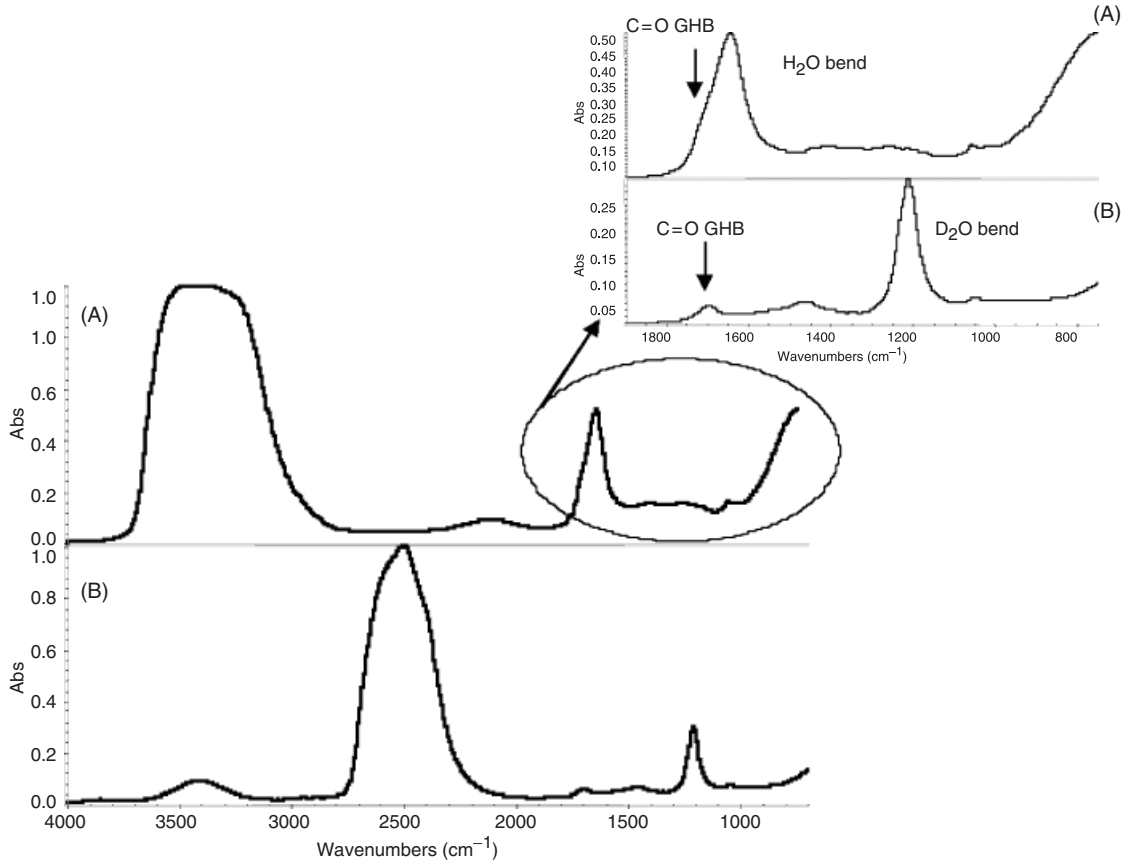

FIG. 10—*Aqueous solution Fourier-transform infrared spectrometry spectra for γ-hydroxybutyric acid (GHB) free acid in water, H_2O (A), and deuterated water, D_2O (B). Inset: Interference to free acid carbonyl band from water bend (A), and interference resolved in deuterated water (B).*

Conclusion

At a solution pH below 7, forensic evidence suspected of containing GHB may actually contain a mixture of GHB free acid and carboxylate forms, as well as GBL. Legal implications related to the interconversion of GHB and GBL still exist at both the domestic and international levels. Analytical strategies are desirable that can affect the analysis of GHB-related evidence while minimizing any concerns of altering the original proportions of GHB free acid, GHB carboxylate, and GBL. To this end, a detailed investigation of the role of solution pH in aqueous GHB chemistry was conducted in this work. Simultaneous measurements were obtained on freshly prepared solutions of GHB using FT-IR, ^{1}HNMR, and HPLC–UV. Both the FT-IR and ^{1}HNMR spectra demonstrated the ability to track the changing proportions of GHB free acid and carboxylate forms that occur as a function of pH, while also detecting the presence of lactone (GBL) that rapidly forms in GHB solutions at low pH. Results were consistent with the expected acid–base conversion behavior of a carboxylic acid as a function of pH. An aqueous solution spectrum for GHB free acid was presented for the first time in the absence of either GHB carboxylate or GBL.

^{1}HNMR provides a rapid, convenient means for both direct identification and quantitation of GHB and GBL in aqueous samples. Sample preparation was shown to be minimal, comprising of simple dilution in D_2O. As the sample pH was not adjusted, the original proportions of GHB free acid, GHB carboxylate, and GBL in the evidence were preserved. This approach minimizes concerns regarding GHB/GBL interconversion and may provide additional information about the sample relevant to the evidence history.

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