J Forensic Sci, Jan. 2004, Vol. 49, No. 1
Paper ID JFS2003175_491
Available online at: www.astm.org

TECHNICAL NOTE

John S. Chappell,[1] *Ph.D.; Ashleigh W. Meyn,*[1] *B.A.; and Kenley K. Ngim,*[1] *Ph.D.*

The Extraction and Infrared Identification of *Gamma*-Hydroxybutyric Acid (GHB) from Aqueous Solutions

ABSTRACT: The chemical analysis of *gamma*-hydroxybutyric acid (GHB) in most forensic laboratories is complicated by the highly polar nature of the GHB molecule, which makes it unsuitable for direct analysis by gas chromatography (GC). Consequently, a popular analytical approach is to convert GHB into the corresponding lactone or a derivative compound that is then identified by mass spectrometry employed in conjunction with GC (GC/MS). An alternative approach is presented here where GHB may be isolated as a free acid specie from complex aqueous solutions employing a liquid–liquid extraction technique. This approach can yield a relatively pure residue of GHB that presents an infrared transmission spectrum that is sufficiently distinct for identification purposes. Infrared spectroscopy (IR) is a very popular technique that is available to most crime laboratories. The liquid–liquid extraction behavior of GHB is examined in detail and the uniqueness of the infrared spectrum is discussed.

KEYWORDS: forensic science, *gamma*-hydroxybutyric acid, *gamma*-hydroxybutyrate, *gamma*-butyrolactone, GHB, GBL, infrared spectroscopy, liquid–liquid extraction

Gamma-hydroxybutyric acid (GHB) has a history of abuse that dates back over 20 years. In the early1980s, GHB was first alleged to provide anabolic benefits following a study that showed GHB stimulated the release of plasma growth hormone (1). The ability of GHB to significantly increase muscle mass has since been disputed (2); however, the potential of GHB to produce euphoric effects in its users was discovered. An increase in GHB abuse followed, and by 1990 the Food and Drug Administration issued warnings of its use (3) coinciding with reports of medical emergencies involving GHB (4). Subsequently, GHB has become a part of the "rave" party culture, and its abuse has escalated (5). In the year 2000, GHB was federally controlled and designated a Schedule I depressant as an amendment to the Controlled Substances Act (6).

The chemical analysis of GHB is greatly influenced by its high polarity and thermal sensitivity, which prevents its direct detection by gas chromatographic (GC) instrumental techniques. Consequently, GHB analyses are largely restricted to approaches that rely on the chemical conversion of GHB into the corresponding lactone compound, *gamma*-butyrolactone (GBL) (7–9) or into derivative compounds (10–13) that are suitable for gas chromatographic analysis. The preparation of the trimethylsilyl (TMS) derivative of GHB is commonly employed for a GC-type analysis and specifically for identification by gas chromatography/mass spectrometry (GC/MS) (10–12). Derivatization methods are also desirable for toxicological analyses, where the low concentrations of GHB in complex biological fluids (blood, urine, etc.) require the selectivity and sensitivity of mass spectrometry detection. In contrast, GHB may be identified directly by a few other techniques, such as high-performance liquid chromatography with mass spectrometry (HPLC/MS) (14) or nuclear magnetic resonance (NMR) (15), but these instrumental techniques are not widely available to many forensic analysts.

An alternative approach is to employ infrared spectroscopy (IR), which is available in most crime laboratories. Infrared spectroscopy may be readily applied to relatively pure, solid samples of the salts of GHB (16,17). Unfortunately, this technique is not generally applicable to illicit solutions of GHB since isolation of the salts of GHB from aqueous matrices is problematic (18). However, relatively little attention has been given to the direct detection of the free acid specie, possibly due to the misconception that the free acid is not sufficiently stable to be isolated (or is too difficult to isolate) from aqueous solutions (19). In fact, the liquid–liquid extraction of the free acid specie has been exploited by one method for the analysis of biological fluids (12); however, this aspect to the solution chemistry of GHB has received little detailed attention in the forensic literature. The present study has examined the liquid–liquid extraction of GHB at the higher concentrations typically encountered in illicit samples and found that the free acid may be collected as a relatively pure substance from complex aqueous solutions. In addition, GHB presents an infrared transmission spectrum that is sufficiently distinct to serve as a means of identification. This approach allows for the direct identification of GHB recovered from illicit aqueous samples by instrumentation familiar to most crime laboratories without the need for derivatization. The infrared identification of the free acid of GHB and its liquid–liquid extraction from aqueous solutions is examined in greater detail in this paper.

Methods and Materials

Recovery studies examining the efficiency of the liquid–liquid extraction of GHB from aqueous solutions were conducted with

[1] Drug Enforcement Administration, Western Laboratory, San Francisco, CA.
Received 19 May 2003; and in revised form 24 August 2003; accepted 24 August 2003; published 17 Dec. 2003.

several water-immiscible organic solvents and as a function of aqueous solution pH. The organic liquids included methyl and ethyl acetate, diethyl ether, *n*-butanol, chloroform, and *n*-hexane. Aqueous solutions of GHB were prepared with the sodium salt of GHB (NaGHB) at a concentration of 0.08 *M* (10 mg of NaGHB per mL). The ionic strength of these solutions was established at approximately 1 and 5 *M* by the addition of an appropriate amount of NaCl. The solution pH was maintained with a dilute buffer system at a formal concentration of approximately 0.1 *M*. The buffers employed were either composed of phosphate (for pH values 1.0, 2.0, and 3.0) or bicarbonate (for pH values 4.0 and 5.0), where the pH was adjusted by the addition of HCl or NaOH. The organic extraction solvents, buffer agents, and other chemicals were all of reagent or higher grade. The sodium salt of GHB was acquired from Fluka Chemical Company. The pH value was measured with a Beckman model Φ32 pH meter.

Extraction entailed mixing an aliquot (3 to 5 mL) of the aqueous test solution of GHB with an equivalent volume of the organic solvent under study and vigorously shaking the two immiscible liquids together for 30 s. Once the two liquid phases separated, the organic extraction solution was physically removed (by Pasteur pipette) and transferred to a new container. The extraction efficiency was determined by a quantitative analysis of the organic extraction solution, whereby the organic solution was then mixed with an equal volume of an aqueous solution buffered to a pH of 6.5 and shaken for 30 s. This last step served to quantitatively extract GHB (greater than 99%) from the organic solution into an aqueous mobile phase suitable for quantitation by HPLC (20). The mobile phase consisted of 0.1 *M* sodium dihydrogen phosphate (NaH_2PO_4) and 0.005 *M* tetrabutylammonium hydrogen sulfate (TBAS), adjusted to pH 6.5 with NaOH. The quantitative measurements were made with a Hewlett-Packard model 1090 HPLC, employing a Phenomenex Aqua column (250 mm in length, 4.60 mm internal diameter, 5-μm particle size). The mobile phase was pumped at a flow of 1.2 mL per min at a temperature of 30°C. An ultraviolet diode array detector monitored the elution of GHB and GBL at a wavelength of 202 nm with a bandwidth of 6 nm. The quantitative method met a set of validation criteria that included linearity (linear response with correlation coefficient greater than 0.998) and precision (relative standard deviation less than 3%). The extraction studies were performed a minimum of three times, and their quantitative results exhibited a relative standard deviation of 10% or less.

Studies to isolate GHB and other similar hydroxy-carboxylic acids entailed conducting a liquid–liquid extraction of the acids from aqueous solutions with ethyl acetate. The acids were generated from the corresponding lactone compounds, since these compounds are commercially available from Sigma-Aldrich Company. Specifically, the compounds included *beta*-propiolactone, *beta*-butyrolactone, *gamma*-valerolactone, *delta*-valerolactone, and GBL. A sample (50 mg) of each of the lactones was hydrolyzed in aqueous solution (2 mL) by the addition of NaOH. Based on studies of GBL (21), hydrolysis is rapid in strongly basic aqueous solutions (pH > 12), occurring within a few minutes. After 15 min, the lactone is completely converted into the dissolved sodium salt of the corresponding hydroxy-carboxylic acid. The pH of the solution was then adjusted with HCl to a value between 2 and 3 to convert the carboxylate anion into the free acid specie (pH adjustment was simply monitored with pH test paper or strips). As a precaution, this solution was extracted with chloroform to remove any residual lactone that could be present. The aqueous phase was separated from the chloroform by pipette and placed in a new container with approximately 1 g of NaCl. The contents were thoroughly mixed until NaCl no longer dissolved into the aqueous solution. Approximately 10 mL of ethyl acetate was then introduced to the aqueous solution and shaken vigorously for 30 s. The ethyl acetate extract solution was isolated and passed over a column of anhydrous sodium sulfate to remove water that remained suspended or dissolved in the solution. This solution was then evaporated on a steam bath under a stream of air until ethyl acetate was no longer evident. A clear oily residue remained, which was examined by infrared spectroscopy. The residues were examined neat by placing a droplet between two potassium bromide disks (25 mm in diameter, 5 mm in thickness) and pressing the disks together to form a liquid film. The infrared transmission spectra were collected as an average of 16 scans with a Nicolet model Avatar 360 Fourier-transform spectrometer at 4 cm^{-1} resolution.

The free acid preparations of GHB and the other hydroxy-carboxylic acids were further characterized by the preparation of their TMS derivatives. A small sample (approximately 1 mg) of the pure liquid residue was dissolved into chloroform. Approximately 50 μL of bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was introduced to the chloroform solution to prepare the TMS derivative. The BSTFA reagent was acquired from Pierce Chemical. The solution was then examined by GC/MS to acquire the electron-impact mass spectrum for the derivative compound. The GC/MS instrument consisted of an Agilent model 6890 gas chromatograph fitted with an HP-1 capillary column (12 m length, 0.20 mm internal diameter) containing a cross-linked 1% phenyl methyl silicone stationary phase (0.33 μm phase thickness). Helium was employed as the carrier gas with a column flow of 1.0 mL per minute and a split vent flow of 50 mL per minute (split injection mode with split ratio of 50:1). Detection of the column eluant was made with an Agilent model 5973 mass selective detector set to scan mass fragments from 40 to 400 amu. The temperature of the GC inlet and the MS interface transfer line were maintained at 250 and 280°C, respectively. The GC oven temperature was initially held at 70°C for 1.2 min, increased to 280°C at 20°C/min, and then held for 2.3 min (total program time 14 min).

Studies were also conducted to assess the stability of GHB isolated as a pure substance, as well as in an ethyl acetate solution. The free acid of GHB was prepared from GBL as described previously for the infrared studies. The stability of the pure substance was monitored by sampling small portions (3 to 6 mg) of the oily liquid as the substance aged under ambient conditions (22°C, approximately 50% RH). The samples were examined qualitatively by both IR and HPLC to detect degradation of GHB. The stability of GHB in ethyl acetate solution was also considered by preparing a stock solution of GHB at a concentration of approximately 2 mg per mL. Aliquots of this solution were periodically withdrawn as the solution aged at room temperature, followed by evaporation of the sample to collect the oily liquid residue. An HPLC analysis of the residue then served to monitor GHB degradation, including GBL formation since the HPLC method resolves GBL from GHB (20).

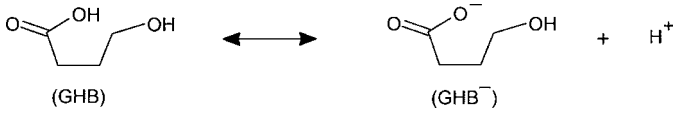
Lastly, the extraction of GHB from several familiar beverages was examined. Samples of the beverages were spiked with NaGHB at a concentration of 0.08 *M* [approximating the level commonly encountered in illicit samples (19,21)]. An extraction scheme was then applied to an aliquot (3 to 4 mL) of the spiked beverage after the pH was adjusted to a value between 2 and 3 with HCl. The aliquot was first extracted with approximately 5 mL of chloroform to remove any neutral organic compounds that could be present. This extraction step is also effective in removing any GBL that may be present in equilibrium with GHB. The aqueous solution was transferred to a new container, saturated with NaCl, and then extracted with ethyl acetate (10 mL) by vigorous shaking. Once the immiscible liquids separated, the ethyl acetate solution was isolated

and passed through a column of anhydrous sodium sulfate. The ethyl acetate solution was collected into a scintillation glass vial and the solvent evaporated to recover the residue for infrared examination.

Results and Discussion

Solution Chemistry of GHB

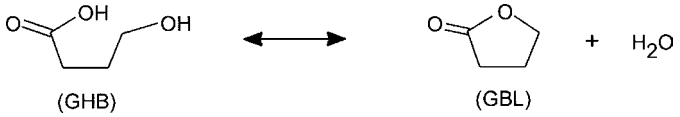
The chemistry of GHB in aqueous solution is essentially described by two equilibria. Since GHB is an acidic compound, the free acid may dissociate into an anionic specie (GHB^-) and a solvated proton (H^+):



(1)

The acid dissociation constant K_a for this process is estimated to be near 2.5×10^{-5} L/mol^{-1} (or a pK_a value of 4.6) (21). This value implies that the free acid specie predominates over the anion specie in acidic aqueous solutions of pH less than 4.0. At higher pH, dissociation is favored and the anion becomes established to an extent that the concentration of the free acid specie is negligible for pH values above 7.0.

The free acid of GHB may also undergo further reaction and convert into the corresponding lactone compound, GBL,



(2)

where GHB self condenses to form a cyclic ester. GBL is particularly stable among the family of lactone compounds and therefore plays an important role in the solution chemistry of GHB (19,21). The rate of this reaction, however, is relatively slow in mildly acidic solutions, and significant conversion of GHB into GBL requires a time scale of several hours (or longer) for solutions of pH 2.0 or greater. Consequently, the free acid specie of GHB is sufficiently stable in moderately acidic solutions ($2.0 \leq \text{pH} \leq 4.0$) to allow its separation from aqueous solutions by an extraction procedure.

The liquid–liquid extraction of GHB from aqueous solution may be treated as a simple equilibrium of the free acid specie between two immiscible liquid phases. This equilibrium is classically described by a partition coefficient P_{org},

$$P_{org} = [GHB]_{org} \div [GHB]_{aq} \quad (3)$$

where the concentration of GHB in the organic liquid $[GHB]_{org}$ is proportional to that in the aqueous solution $[GHB]_{aq}$ at equilibrium. Since the anion GHB^- does not partition to any significant extent into most water-immiscible organic solvents, any appreciable partition of the free acid into the organic solvent may provide a basis for a clean extraction of GHB. The efficiency of the extraction is then determined by the magnitude of P_{org} for a given organic solvent.

Extraction of the Free Acid

To assess the effectiveness of a liquid–liquid extraction procedure, a variety of water-immiscible organic solvents were employed in the extraction of aqueous solutions of GHB at a pH of 2.0 and at either a 1.0 or 5.0 *M* (saturated) concentration of NaCl. The results of these tests are displayed in Table 1. The partition coefficient of the GHB extraction varies widely among the solvents, ranging from negligible to a value of nearly 1. The effectiveness of the solvents to extract GHB is dictated by several chemical factors, although the relative affinity of GHB to dissolve into a particular solvent is a primary issue. For GHB, the solvating interactions are strongly influenced by the potential of the solvent to hydrogen bond to GHB, which largely favors solvation into aqueous solutions, and therefore the extraction efficiencies of most of the organic solvents are relatively low. However, some organic solvents perform significantly better than others, which likely bears some relation to their respective functional groups. As shown in Table 1, the solvents with an oxygen functionality demonstrated a higher capacity to extract GHB from an aqueous solution. Specifically, *n*-butanol and the acetate esters exhibited the greatest extraction of GHB among the solvents considered. This is likely attributable to the hydrogen-bonding potential of the hydroxyl or the carbonyl groups of these respective solvents. In contrast, the purely hydrocarbon solvent of *n*-hexane exhibited negligible extraction of GHB, as may be expected due to its nonpolar character. Interestingly, chloroform also demonstrated little affinity to extract GHB despite its significant polarity. This finding suggests that the nature of the hydrogen bond formed with oxygen-containing functional groups is more significant to the solvation of GHB than the generic polar interaction presented by a chlorinated hydrocarbon.

TABLE 1—*GHB free acid extraction by several common organic solvents. partition coefficient.**

Solvent	1 *M* NaCl	Saturated NaCl
Ethyl acetate	0.17	0.26
Ethyl acetate	~0.3†	~0.5†
n-butanol	0.78	0.96
Diethyl ether	0.065	0.11
Chloroform	<0.005	<0.005
n-hexane	<0.005	<0.005

* The partition coefficient P_{org} (defined by Eq 3) was measured following extraction of an aqueous solution of GHB (initial concentration 80 m*M*) at pH 2.0.

† Value of P_{org} is approximate due to significant water solubility within the methyl acetate extract solution.

An appreciable enhancement of GHB extraction is observed upon saturation of the aqueous solution with sodium chloride. This effect, due to an increase in the ionic strength of the aqueous solution, derives from an increase in the activity coefficient of GHB. As a result, the partition coefficient (as defined by Eq 3) only approximates the equilibrium constant and becomes more a function of the solution ionic strength.

The magnitude of the partition coefficient observed for three of the organic test solvents is sufficient to enable an effective extraction of GHB for a qualitative analysis. However, some of the solvents are not suitable for this methodology since a relatively volatile solvent is necessary. Specifically, *n*-butanol has a normal boiling point above 100°C and is not readily evaporated on a steam bath. Another concern is the limited solubility of water in the extraction solvent. Methyl acetate dissolves a detectable amount of water and therefore leaves a particularly wet residue of GHB following evaporation of the solvent. Consequently, ethyl acetate was selected as the most appropriate extraction solvent to recover GHB as a pure residue.

The extraction of GHB was also examined as a function of solution pH. The recovery of GHB from an ethyl acetate extraction of buffered aqueous solutions is shown in Fig. 1. Two sets of data are presented, where the solution ionic strength was adjusted to 1 *M*

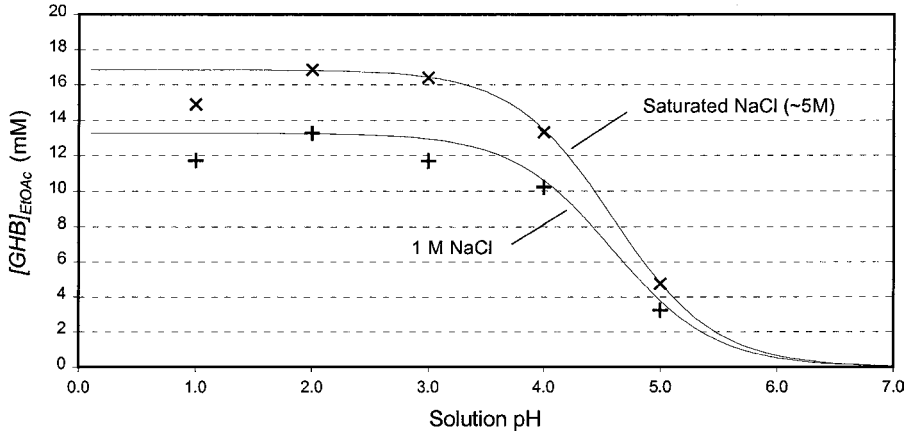


FIG. 1—*The GHB concentration of an ethyl acetate extract solution $[GHB]_{EtOAc}$ following from the equal volume extraction of an aqueous solution of GHB (initial concentration 80 mM) as a function of the aqueous solution pH. Two aqueous solutions are considered: one solution composed of 1 M NaCl (data points represented by +), the other solution saturated with NaCl (data points represented by ×). The GHB concentration in the extract solution exhibits a pH dependence that correlates with the behavior predicted for the free acid specie (solid lines).*

(1 *M* NaCl) and approximately 5 *M* (saturated NaCl). Both sets of data exhibit a similar dependence on the aqueous solution pH, where the greatest recovery of GHB is observed below a pH value of 3. Above this pH value, the extracted GHB concentration steadily decreases to the point where above pH 5 the recovery of GHB is minimal. This functional dependence on solution pH appears to be proportional to the concentration of the free acid specie as predicted from the acid dissociation constant for GHB (21). This relationship is illustrated by the solid lines plotted in Fig. 1, which are derived from the calculated concentration of the free acid specie based on the acid dissociation constant (2.5×10^{-5} L/mol^{-1}) and partition coefficients in Table 1. The one significant deviation from the calculated recovery occurs at a solution pH of 1.0. This effect is likely due to lactonization of GHB (Eq 2), which is sufficiently fast at pH 1.0 that detectable reaction may occur over the time period of the extraction procedure (i.e., several minutes) (21).

The free acid specie of GHB may be collected as a relatively pure residue by the evaporation of the extraction solvent under a stream of air and with gentle heating over a steam bath. A colorless, viscous liquid remains once ethyl acetate is removed. The stability of this form of GHB under ambient conditions was considered by examining preparations of the free acid as a function of time. Studies by IR indicated that the free acid slowly degrades to GBL, as evident by the appearance of several weak spectral features (absorption peaks near 1770, 1170, 1036, and 992 cm^{-1}) attributable to the formation of the lactone. The lactone features were not initially detected for several hours following evaporation of ethyl acetate, although these peaks intensified over a period of several weeks as the liquid aged at room temperature. HPLC analysis of samples taken from the free acid preparation confirmed the slow formation of GBL, where approximately 3% of the GHB converted into GBL after the first day. This conversion is not observed in solutions of the free acid in ethyl acetate for over several weeks at room temperature. Upon evaporation of the solvent, however, equilibrium with an ambient atmosphere apparently allows for the release of water and thereby favors the condensation of the free acid into GBL (this conversion may be additionally driven by the affinity of pure GHB to absorb water and generate a dehydrating environment in the pure liquid). Heating the free acid also activates this reaction, and so excessive heating of the free acid residue was avoided following solvent evaporation. IR studies detected degradation of the pure liquid after heating on a steam bath for approximately 30 min.

Infrared Identification of the Free Acid

The infrared transmission spectrum of the GHB residue collected from the evaporation of ethyl acetate extraction solutions is displayed in Fig. 2 along with the spectra of GBL and NaGHB. In contrast to the spectra of the lactone and the sodium salt, relatively broad absorption peaks characterize the spectrum of GHB. This general appearance is consistent with that expected for GHB, where strong intermolecular (and likely intramolecular) hydrogen bonds occur among the carboxylic and hydroxyl groups of the molecule in a condensed state (22,23). Several of these features overlap with the absorption bands of ethyl acetate, although the neat infrared spectrum of ethyl acetate also exhibits a strong, sharp peak (1372 cm^{-1}) and several weaker peaks (845, 632, and 606 cm^{-1}) that are absent in the spectrum of GHB, indicating any residual ethyl acetate is minimal in this manner of preparation. One prominent feature of the spectrum is the strong absorption peak at 1709 cm^{-1} due to the carbonyl-stretching mode. The position of this peak occurs at a lower wave number than the corresponding peak in the lactone spectrum (1770 cm^{-1}), a general effect observed among carboxylic acids due to a weakening of the carbonyl bond from hydrogen bonds in the condensed state (22). This effect is amplified in the crystalline state of the sodium salt due to the formation of the carboxylate anion, where delocalization of the carbonyl *pi*-bond further lowers the stretching frequency of the carbon–oxygen bond (1560 cm^{-1}).

Another strong feature of the spectrum of GHB is the broad absorption envelope between 3600 and 2400 cm^{-1}. This absorption band primarily arises from the hydrogen-stretching modes of the hydroxyl and carboxyl groups, which is extremely broadened by hydrogen bond formation (23). Typically, a carboxylic acid presents a broad band centered near 3000 cm^{-1} due to a dimer arrangement in the condensed state, where the carboxylic groups of two molecules form a symmetric pair of hydrogen bonds. In GHB, a hydroxyl group also contributes a broad band near 3400 cm^{-1}, which together with the carboxyl mode accounts for the strong and broad absorption over this region. The smaller absorption feature at 2956 cm^{-1} is due to the carbon-hydrogen stretching motions.

The remaining features of the GHB spectrum are largely attributable to other modes of the carboxyl and hydroxyl groups. This includes the broad absorption feature between 1450 and 1150 cm^{-1} (assignable to both the in-plane deformation of the carboxyl hydrogen and stretching of the carbon–oxygen bond in the carboxyl

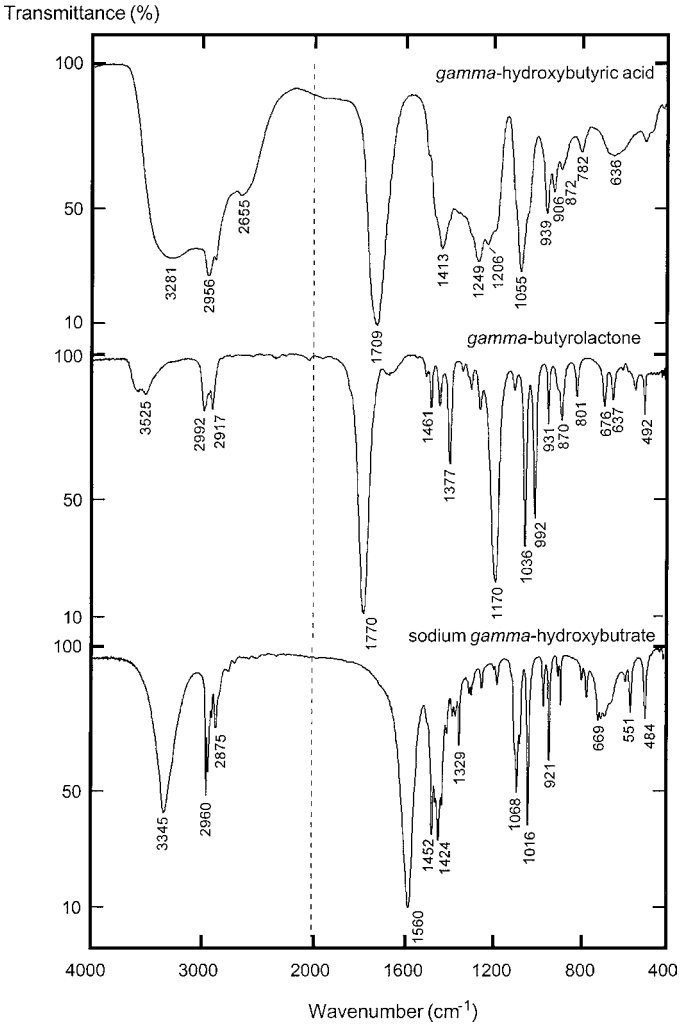


FIG. 2—*Infrared transmission spectra (4 cm^{-1} resolution) for gamma-hydroxybutyric acid (neat between KBr disks), gamma-butyrolactone (neat between KBr disks), and sodium gamma-hydroxybutyrate (powder dispersed in a KBr matrix).*

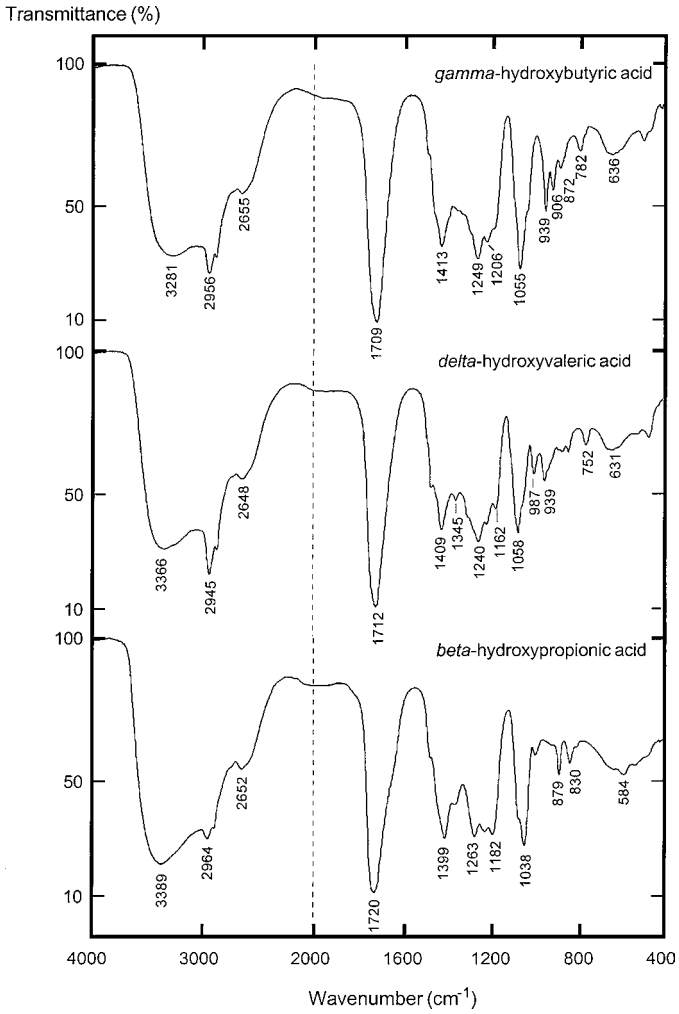


FIG. 3—*Infrared transmission spectra (4 cm^{-1} resolution) for gamma-hydroxybutyric acid, delta-hydroxyvaleric acid, and beta-hydroxypropionic acid, each examined neat between KBr disks.*

group) and the single strong peak at 1055 cm^{-1} (due to the stretching mode of the carbon–oxygen bond of the terminal hydroxyl group). The series of weaker peaks below 1000 cm^{-1} derive from out-of-plane deformation modes of both the carboxyl and hydroxyl hydrogen atoms, since these bending motions become viable vibrations in a hydrogen-bonded condensed state (24). These bending motions are expected to absorb between 950 and 900 cm^{-1} for the carboxyl group and near 650 cm^{-1} for the hydroxyl group.

The general appearance observed for the infrared transmission spectrum of GHB is quite similar to that observed for some other short-chain hydroxy-carboxylic acids that may be regarded as analogues of GHB. The spectra for *delta*-hydroxyvaleric (5-hydroxypentanoic) acid and *beta*-hydroxypropionic (3-hydroxypropanoic) acid are shown in Fig. 3 alongside GHB (4-hydroxybutanoic acid). All three compounds possess a terminal hydroxyl group and only differ by the length of the carbon chain in their molecular structure. The three spectra display the prominent carbonyl-stretching band (approximately 1710 cm^{-1}) and broad absorption envelope (between 3600 and 2400 cm^{-1}) that are characteristic of a hydrogen-bonded carboxylic acid. Similar absorption features are also present throughout the remaining spectrum, although some distinguishable differences are evident. Interestingly, these differences are most apparent with the pattern of bands within the region between 1000 and 800 cm^{-1}, which are attributable to the out-of-plane deformation of the carboxyl hydrogen. Hydrogen-bonding interactions in the condensed state may produce these spectral differences since the geometry of the interactions may be influenced by the skeletal structure of these molecules (including the length of the carbon chain) (24). In theory, these effects may apply to any of the vibrational modes of the carboxyl or hydroxyl hydrogen atoms, although, depending upon the relative strength of the interactions, some vibrational modes may be more sensitive than others. The out-of-plane deformations of the carboxyl hydrogen may also appear the most distinctive since their absorption in the spectral region between 1000 to 800 cm^{-1} does not coincide with other stronger absorption bands, which is an issue with many of the other modes.

As a further comparison, the spectrum of GHB is shown with those of *gamma*-hydroxyvaleric (4-hydroxypentanoic) acid and *beta*-hydroxybutyric (3-hydroxybutanoic) acid in Fig. 4. Unlike GHB, the hydroxyl group for both of these compounds is attached to the carbon chain at the site adjacent to the terminal carbon. As a result, a secondary alcohol with a terminal methyl group characterizes their molecular structures. The infrared spectra reflect this difference in structure with some additional features to the generic pattern of bands observed in Fig. 3. Specifically, a pair of peaks

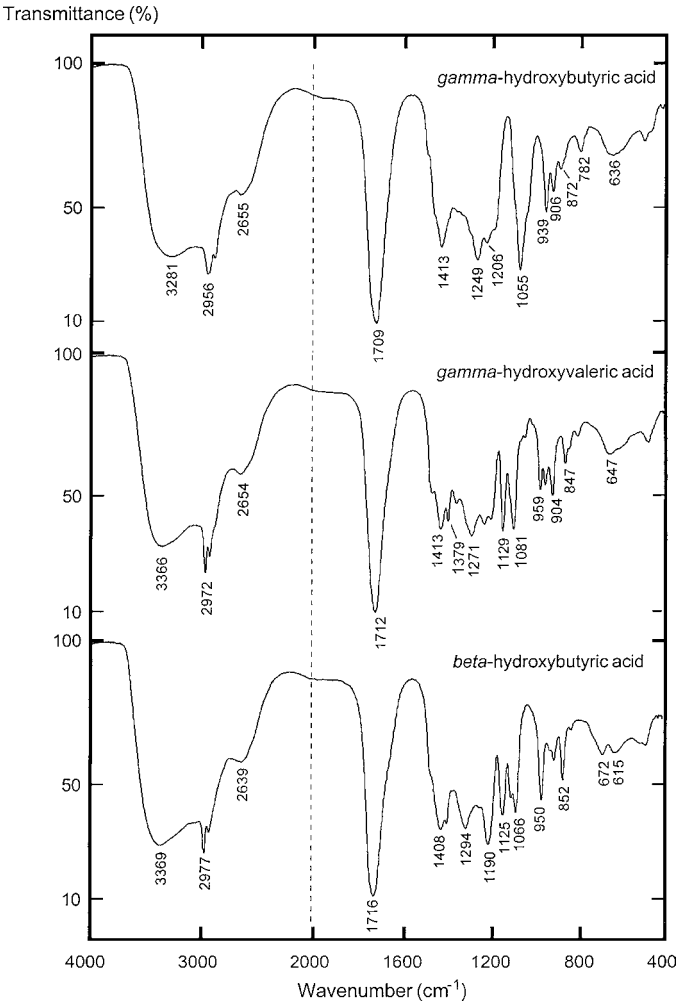


FIG. 4—*Infrared transmission spectra (4 cm^{-1} resolution) for gamma-hydroxybutyric acid, gamma-hydroxyvaleric acid, and beta-hydroxybutyric acid, each examined neat between KBr disks.*

occurs between 1150 and 1000 cm^{-1}, where before a single peak was observed. The appearance of a band at a higher wave number is consistent with the carbon–oxygen stretching mode of a secondary alcohol (22). Small but significant changes to the shape of the broad absorption envelope between 1450 and 1150 cm^{-1} are also apparent, likely due to the carbon–hydrogen deformations of the terminal methyl group. Significant differences in the pattern of peaks between 1000 and 800 cm^{-1} are also observed. All five spectra in Figs. 3 and 4 are therefore distinguishable in spite of a general similarity over much of the mid-infrared region. Given these observations, the infrared spectra of the free acid of GHB and other potential analog compounds appear suitably distinct for the purpose of identification.

The identity of the free acid preparations of GHB and the other hydroxy-carboxylic acids was further confirmed by the preparation of their TMS derivatives. The GC/MS analysis of the derivative preparations showed the presence of a compound consistent with the di-substituted TMS derivative for each of the hydroxy-carboxylic acids. The mass spectrum observed for the free acid preparation of GHB exhibited the same prominent mass fragments reported previously for GHB (10). The mass spectra for all five of the derivative compounds displayed a base peak with a mass-to-charge ratio (m/z) of 147 amu, commonly observed for di-substituted TMS derivatives. Each of the spectra also exhibited a prominent mass fragment with an m/z value consistent with M-15, where M is the molecular mass of the di-substituted TMS derivative. Specifically, this mass fragment observed in the respective spectra was 219 amu for the *beta*-hydroxypropionic acid derivative (M = 234 amu), 233 amu for the *beta*- and *gamma*-hydroxybutyric acid derivatives (M = 248 amu), and 247 amu for the *gamma*- and *delta*-hydroxyvaleric acid derivatives (M = 262 amu). The M-15 mass fragment (due to the loss of a methyl group) is recognized as a prominent feature of TMS derivative mass spectra, since the molecular ion is often too weak for detection (10). Notably, the mass spectra of all five TMS derivative compounds exhibited mass fragmentation patterns that are readily distinguishable from one another.

Extraction of the Free Acid from Familiar Beverages

The liquid–liquid extraction of GHB was examined for a variety of beverages using ethyl acetate as the extraction solvent. The beverages included familiar carbonated soft drinks (Coca-Cola®, Sprite®), sport drinks (Gatorade®), fruit juices (cranberry, orange) and alcoholic drinks (vodka, rum, beer, wine). In spite of the complexity of many of these aqueous matrices, the extraction scheme (see Materials and Methods) proved effective at isolating the free acid in a relatively pure form from all the beverages. This observation is illustrated in Fig. 5, where the infrared spectra for two

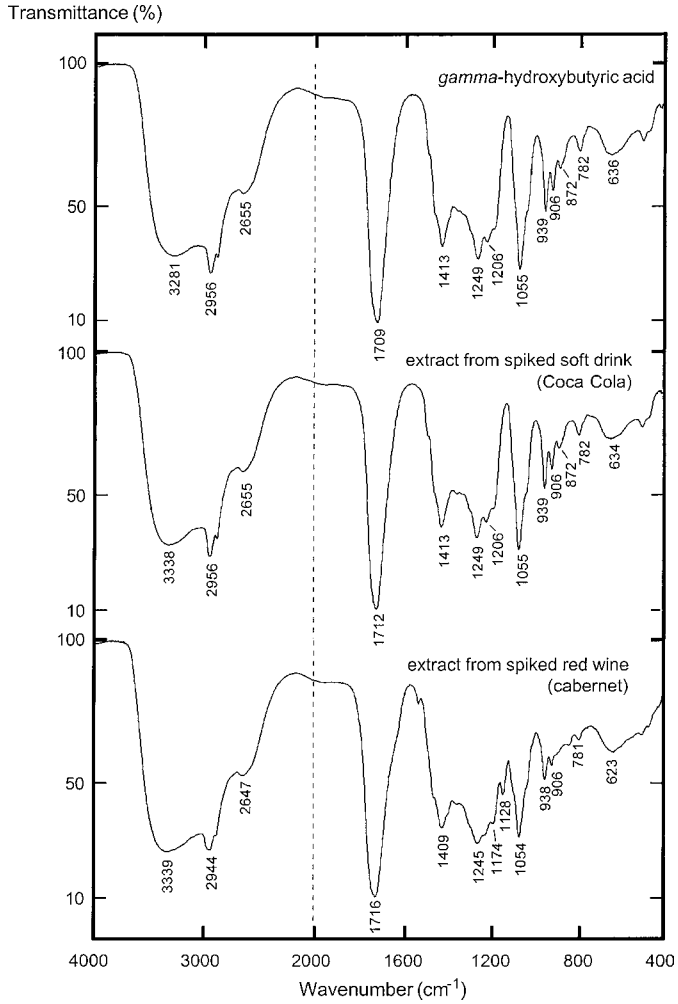


FIG. 5—*Infrared transmission spectra (4 cm^{-1} resolution) for gamma-hydroxybutyric acid and residues extracted from two spiked beverages (Coca Cola® and red wine), each examined neat between KBr disks.*

examples are shown with the spectrum of GHB. For several of the beverages (soft drinks, cranberry juice, vodka, rum, beer), the spectra exhibited good agreement with that of the free acid (by an overlay comparison) as illustrated by the case of a soft drink (Coca-Cola®) in Fig. 5. The extraction from some other beverages (some sport drinks, orange juice, red wine) gave spectra that were readily recognizable as composed of the free acid but displayed some weak spectral features that deviated from that of the free acid. The most significant differences were observed with red wine, as shown in Fig. 5. The deviations are presumably due to the extraction of one or more ingredients that compose the beverages, albeit at a low concentration relative to the amount of GHB present.

The infrared identification of GHB via the extraction scheme is dependent upon the appreciable concentration of GHB (0.08 *M*) introduced into the beverages. The extraction is applicable to solutions of much lower concentrations, as has been demonstrated with biological fluids with GHB concentrations below 0.1 m*M* (12). However, at GHB levels less than 0.01 *M*, the actual quantity of GHB collected by this extraction scheme (less than 1 mg) may become impractical for an infrared analysis. For these cases where the extract residue is of insufficient quantity or not suitably pure to identify by infrared spectroscopy the extraction still serves to separate GHB from the original aqueous solution for an alternative analysis. This manner of preparation is particularly suited for derivatization techniques that require a solvent that is nonprotic. As discussed earlier, the TMS derivative may be readily prepared from the free acid preparation and identified by GC/MS. The liquid–liquid extraction of the free acid and subsequent TMS derivatization is commonly practiced in this laboratory as a complementary test for the identification of GHB.

It should be noted that an essential step to this extraction scheme is the preliminary extraction of the aqueous sample with chloroform (or methylene chloride). This step may not only remove some extraneous organic components of the beverage, but also effectively removes any GBL that may be present in equilibrium with GHB. The level of GBL may be highly variable in illicit preparations, depending upon a variety of factors, including whether the solution was initially spiked with GBL or a GHB salt, the solution pH, and age of the solution. GBL may also convert to GHB following adjustment of the sample to an acidic pH, although the rate of conversion is very slow for a solution pH between 2 and 3 (conversion of 1% GBL into GHB is estimated to require an excess of 1 h (21)). Therefore, a preliminary extraction of the sample with chloroform is recommended to eliminate the possibility of a false positive detection of GHB.

Conclusions

In summary, GHB may be readily extracted from aqueous solutions as the free acid specie, which offers a reasonably rapid and simple means of detecting GHB that does not require conversion to GBL. The liquid–liquid extraction is not a quantitative separation because the partition coefficient observed for some appropriate organic solvents ranges from 0.1 to 0.5. The extraction, however, is sufficient for most qualitative analyses since the concentration of most illicit solutions is adequate to acquire a suitable quantity of GHB.

A comparison of the infrared transmission spectrum of GHB with that of other similar hydroxy-carboxylic acids reveals spectral differences that are suitably distinct to allow an unambiguous identification of GHB. When applied to several familiar beverages spiked with GHB, the liquid–liquid extraction technique was effective at isolating GHB as a relative pure substance. The infrared spectrum of this residue was suitable for infrared identification in several examples, but not for all aqueous matrices. In these latter cases, the extraction is still an effective means to separate GHB from the aqueous matrix for analysis by other techniques. The liquid–liquid extraction is also applicable to analog compounds of GHB (other short-chain, hydroxy-carboxylic acids), which may also be subject to abuse (25).

References

1. Takahara J, Yunoki S, Yakushiji W, Yamane Y, Ofuji T. Stimulatory effects of *gamma*-hydroxybutyric acid on growth hormone and prolactin release in humans. J Clin Endocrinol Metab 1977;44:1014–7. [PubMed]
2. Addolorato G, Capristo E, Gessa GL, Caputo F, Stefanini GF, Gasbarrini G. Long-term administration of GHB does not affect muscular mass in alcoholics. Life Sci 1999;65:191–6.
3. Food and Drug Administration. *Gamma*-hydroxybutyric acid (press release). November 1990.
4. Centers for Disease Control. Multistate outbreak of poisonings associated with illicit use of *gamma*-hydroxybutyrate. JAMA 1991;256:447–8.
5. Galloway GP, Frederick SL, Staggers FE, Gonzales M, Stalcup SA, Smith DE. *Gamma*-hydroxybutyrate: an emerging drug of abuse that causes physical dependence. Addiction 1997;92:89–92. [PubMed]
6. Addition of *gamma*-hydroxybutyric acid to Schedule I. Federal Register 2000;65:13235–8.
7. LeBeau MA, Montgomery MA, Miller ML, Burmeister SG. Analysis of biofluids for *gamma*-hydroxybutyrate (GHB) and *gamma*-butyrolactone GBL) by headspace GC/FID and GC/MS. J Anal Toxicol 24;2000: 421–8. [PubMed]
8. Vree TB, Van Der Kleijn E, Knop HJ. Rapid determination of 4-hydroxybutyric acid (*Gamma* OH) and 2-propyl pentanoate (Depakine) in human plasma by means of gas-liquid chromatography. J Chromatgr 1976;121:150–2.
9. Ferrara SD, Tedeschi L, Frison G, Castagna F, Gallimberti L, Giorgetta R, et al. Therapeutic *gamma*-hydroxybutyric acid monitoring in plasma and urine by gas chromatography–mass spectrometry. J Pharm Biomed Anal 1993;11:483–7. [PubMed]
10. Blackledge RD, Miller MD. The identification of GHB. Microgram 1991;24:172–9.
11. McCusker RR, Paget-Wilkes H, Chronister CW, Goldberger BA, Elsohly MA. Analysis of *gamma*-hydroxybutyrate (GHB) in urine by gas chromatography–mass spectrometry. J Anal Toxicol 1999;23:301–5. [PubMed]
12. Couper FJ, Logan BK. Determination of *gamma*-hydroxybutyrate (GHB) in biological specimens by gas chromatography–mass spectrometry. J Anal Toxicol 2000;24:1–7. [PubMed]
13. Blair S, Song M, Hall B, Brodbelt J. Determination of *gamma*-hydroxybutyrate in water and human urine by solid phase microextraction-gas chromatography/quadrupole ion trap spectrometry. J Forensic Sci 2001;46:688–93. [PubMed]
14. Mesmer MZ, Satzger RD. Determination of *gamma*-hydroxybutyrate (GHB) and *gamma*-butyrolactone (GBL) by HPLC/UV-VIS spectrophotometry and HPLC/thermospray mass spectrometry. J Forensic Sci 1998;43:489–92.
15. Chew SL, Meyers JA. Identification and quantitation of *gamma*-hydroxybutyrate (NaGHB) by nuclear magnetic resonance spectroscopy. J Forensic Sci 2003;48:292–8. [PubMed]
16. Bommarito C. Analytical profile of *gamma*-hydroxybutyric acid (GHB). JCLIC 1993;3:10–2.
17. Walker L. Identification of the potassium salt of *gamma*-hydroxybutyrate (GHB-K^+). JCLIC 1999;9:17–8.
18. Morris JA. Extraction of GHB for FTIR analyses and a new color test for *gamma*-butyrolactone (GBL). Microgram 1999;32:215–21.
19. Ciolino LA, Mesmer MZ, Satzger RD, Machal AC, McCauley HA, Mohrhaus AS. The chemical interconversion of GHB and GBL: forensic issues and implications. J Forensic Sci 2001;46:1315–23. [PubMed]
20. Rees DK, Wasem SE, Patierno ER. Identification and quantitation of *gamma*-hydroxybutyrate in illicit drug samples. Microgram 2001;34: 329–39.

21. Chappell JS. The non-equilibrium aqueous solution chemistry of *gamma*-hydroxybutyric acid. JCLIC 2002;12:20–7.
22. Colthup NB, Daly LH, Wiberly SE. Introduction to infrared and Raman spectroscopy. New York: Academic Press, 1964.
23. Cannon CG. The nature of hydrogen bonding. Spectrochim. Acta 1958; 10:341–8.
24. Hadzi D, Sheppard N. Infra-red absorption bands associated with the COOH and COOD groups in dimeric carboxylic acids. I. The region from 1500 to 500 cm^{-1}. Proc Roy Soc Ser A 1953;216,247–66.
25. Morris JA. Analogs of GHB; Part 2: Theoretical perspective. JCLIC 2001;10,14–6.

Additional information and reprint requests:
John S. Chappell
Drug Enforcement Administration
Western Laboratory
390 Main Street, Room 700
San Francisco, CA 94105