**The Chemical Interconversion of GHB and GBL: Forensic Issues and Implications**

**ABSTRACT:** In this work, the interconversion of GHB and GBL in a variety of aqueous media was studied. The effects of solution pH and time were determined by spiking GHB or GBL into pure water and buffered aqueous solutions, and determining the GHB and GBL contents at various time intervals. The degree of GBL hydrolysis to GHB was determined for several commercial aqueous-based GBL products, and further studied as a function of time. The effects of temperature and time were also determined for five commercial beverages spiked with GHB or GBL. GHB and GBL contents were determined using high performance liquid chromatography (HPLC). GHB and/or GBL confirmations were made using gas chromatography-mass spectrometry (GC-MS) and/or infrared spectroscopy (IR).

Solution pH, time, and storage temperature were determined to be important factors affecting the rate and extent of GBL hydrolysis to GHB. Under strongly alkaline conditions (pH 12.0), GBL was completely converted to GHB within minutes. In pure water, GBL reacted to form an equilibrium mixture comprising ca. 2:1 GBL:GHB over a period of months. This same equilibrium mixture was established from either GHB or GBL in strongly acidic solution (pH 2.0) within days. A substantial portion of GBL (ca. 1/3) was hydrolyzed to GHB in aqueous-based GBL products, and in spiked commercial beverages, after ambient storage for a period ranging from several weeks to several months. Heat increased and refrigeration decreased the rate of GBL hydrolysis relative to ambient conditions. These studies show that hydrolysis of GBL to GHB does occur in aqueous-based solutions, with samples and time frames that are relevant to forensic testing. Implications for forensic testing and recommendations are discussed.

**KEYWORDS:** forensic science, GHB, GBL, gamma-hydroxybutyric acid, gamma-butyrolactone, gamma-hydroxybutyrate, interconversion, stability, high performance liquid chromatography

GHB (gamma-hydroxybutyric acid or gamma-hydroxybutyrate) is a hydroxylated short chain carboxylic acid, and GBL (gamma-butyrolactone) is the corresponding lactone (Fig. 1). GHB is currently being developed as a potential treatment for narcolepsy (1) and GBL has a variety of industrial uses (2,3). Although both GHB and GBL have potential or actual legal uses, both compounds are also drugs of abuse. GHB is typically manufactured either in clandestine laboratories or by end-users using GBL and sodium or potassium hydroxide in aqueous solution. The GHB product may be isolated as a powder, partially dried to a paste or wet mass, concentrated, or left as is in solution. However, at some point prior to consumption, the GHB product is typically redissolved and/or further diluted in aqueous-based media such as beverages. In addition to other illicit uses, GHB is commonly encountered in the “club drug” and “rave” scenes (4,5), and has frequently been detected in victims of drug-facilitated sexual assault or “date rape” (6–8).

GBL is frequently sold and consumed in aqueous solutions. Over the last several years, dozens of commercial GBL products have emerged under a variety of product names and labels (9). These products typically have label claims of 1 to 5 GBL per ounce and may also contain dyes, flavorings, nutritional supplements (e.g., vitamins), and other components. They have been marketed extensively over the Internet and in health food stores. Aqueous solutions of GBL are also sold in unlabeled containers from clandestine sources.

GHB and GBL are subject to interconversion in aqueous solution. GBL is converted to GHB via hydrolysis; GHB is converted to GBL via intramolecular esterification. In February 2000, GHB was added to the list of DEA Schedule 1 controlled substances (10). In this same legislation, GBL was made a List I chemical owing to its use in the manufacture of GHB (a “List I chemical” is defined in the Federal Controlled Substances Act as a chemical used in the manufacture of a controlled substance; the distribution of such chemicals is therefore monitored). Although GBL was not explicitly scheduled under the new law, the law does allow for GBL to be considered a scheduled analog of GHB depending on the specific circumstances (use and intent) of the case. At the state level, the scheduling status of GHB and GBL varies, with some states scheduling both substances and others carrying a legal distinction.

The legal distinctions between GHB and GBL, coupled with the potential for GBL to undergo interconversion with GHB, raises important issues for forensic scientists and law enforcement agencies. For example, the potential exists for aqueous-based GBL products to undergo conversion to GHB in the time between manufacture and consumption. In forensic analysis, the question of interconversion becomes paramount when developing or applying methods. Consequently, a thorough understanding of the interconversion process, especially with respect to the sample types and scenarios encountered in law enforcement, is needed.

In the current work, the stability and interconversion of GHB and GBL in aqueous solution were studied as a function of solution pH and time. In addition, commercial and clandestine GBL products were examined for the degree of conversion to GHB. Finally,
GHB and GBL were spiked into a variety of beverage matrices, and the interconversion was studied as a function of time and temperature. These studies provide a starting point for addressing some of the forensic issues related to GHB-GBL interconversion.

Materials and Methods

Standards and Chemicals

Gamma-hydroxybutyric acid, sodium salt (minimum 99%) was obtained from Sigma Chemical (St. Louis, MO). Gamma-butyrolactone (reagent, minimum 98%) was obtained from Spectrum Chemical (New Brunswick, NJ). Potassium phosphate monobasic (reagent) was obtained from Fisher. Deionized water was obtained from Millipore Milli-Q filtration system. 99:1 BSTFA:TMCS (bis-(trimethylsilyl)trifluoroacetamide:trimethylchlorosilane) was obtained from Supelco. Pyridine (certified A.C.S.) was obtained from Fisher.

Buffered Solutions Study

Stock solutions of 1% w/w GHB or GBL were prepared in deionized water. Potassium phosphate monobasic solution (1 M) was prepared and then adjusted to pH 2.0, 4.0, 5.2, 6.4, 7.0, or 12.0 using aqueous phosphoric acid or sodium hydroxide. For preparation of GHB or GBL in the various phosphate buffers, equal volumes (1 mL) of the GHB or GBL stock solution and the phosphate buffer were added to 5 mL amber glass bottles and then vortexed for 10 s. For preparation of GHB or GBL in deionized water, water was substituted for the buffer portion. The final GHB or GBL concentrations were 0.5% w/w in 0.5 M buffer or deionized water. All pH's in the range 2.0 to 3.5 and using the molecular weight of the free acid (MW 104.1 g/mol) for test solutions or products with pH's in the range 2.0 to 3.5 and using the molecular weight of the anion (MW 103.1 g/mol) for test solutions or products with pH's at 7.0 or above. The average molecular weight of the free acid and anion (MWave 103.6 g/mol) was used for test solutions or products with intermediate pH's (pH range 4.0 to 6.5).

Infrared and GC-MS Confirmation of GHB and GBL in Buffered Solutions and Spiked Beverages

For each beverage matrix, a typical serving size was identified (see Table 1) and the beverage was spiked separately with GHB or GBL at a ratio of 3.0 g per serving (details concerning dose amount given in Results and Discussion section). Two portions were stored at room temperature (22°C), and one portion each was stored in the refrigerator (temperature 4°C) and in an oven (temperature 60°C).

HPLC Quantitative Analysis of GHB and GBL in Buffered Solutions and Spiked Beverages

After storage for the specified intervals, the test solutions were analyzed for GHB and GBL content using HPLC-UV. A Hewlett Packard 1100 Liquid Chromatograph equipped with a Hydrobond AQ column (4.6 mm by 15 cm, 5 μm) was 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 range pH 2.5 to 3.0. The injection volume was 10 or 15 μL; detection was at 215 nm. The column was thermostated at 30°C. The run time was 15 min. For the spiked beverages, a rinse step was added to the end of each LC run (stepped and held at 60:40 methanol:buffer for 10 min) in order to flush matrix components from the column between injections.

For analysis, the buffered solutions were diluted in mobile phase buffer to give concentrations in the range 150 to 1000 μg/mL. The commercial and clandestine GBL products and spiked beverages were diluted in water. For solutions that are highly alkaline or highly buffered at neutral or alkaline pH, dilution in the acidic mobile phase buffer may be required to prevent distortion of the GHB peak. GHB content was calculated using the molecular weight of the free acid (MW 104.1 g/mol) for test solutions or products with pH's in the range 2.0 to 3.5 and using the molecular weight of the anion (MW 103.1 g/mol) for test solutions or products with pH's at 7.0 or above. The average molecular weight of the free acid and anion (MWave 103.6 g/mol) was used for test solutions or products with intermediate pH's (pH range 4.0 to 6.5).

Spiked Beverages Study

Five commercial bottled beverages were purchased from a local grocery store for use in the study: (1) carbonated beverage (16.9 oz Sprite Lemon-Lime); (2) sports drink (32 oz. Gatorade Lemon-Lime); (3) alcohol cocktail (200 mL Jack Daniel’s Lynchburg Lemonade); (4) wine (750 mL Meier’s White Catawba Wine); (5) vodka (750 mL Popov Diluted Vodka). All of the beverage pH’s were acidic, ranging from pH 2.6 to 3.3 (see Table 1), and the alcohol contents of the latter three beverages were declared on the labels as 5.9, 11.5, and 21%, respectively.

TABLE 1—Summary of beverages for spiking study.

<table>
<thead>
<tr>
<th>Beverage</th>
<th>pH</th>
<th>Alcohol Content Declared, %</th>
<th>Serving Size, oz.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemon lime carbonated soda</td>
<td>3.3</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Lemon sports drink</td>
<td>3.0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Lemon alcohol cocktail</td>
<td>2.6</td>
<td>5.9</td>
<td>7</td>
</tr>
<tr>
<td>White Catawba wine</td>
<td>2.9</td>
<td>11.5</td>
<td>6</td>
</tr>
<tr>
<td>Diluted vodka</td>
<td>3.2</td>
<td>21</td>
<td>1.5</td>
</tr>
</tbody>
</table>
trimethylchlorosilane) in the presence of pyridine with 30 min incubation at 70°C.

The infrared measurements were performed using a Nicolet Magna-IR 550 Fourier Transform Infrared (FT-IR) spectrometer interfaced with a NicPlan Infrared Microscope. For each sample, spectra were obtained for both the neat liquid and the residue resulting from air drying. For measurement of the neat liquid, a small droplet was pressed into a thin film between two BaF2 windows, and the spectrum was obtained using the main sample compartment. For measurement of the residue, approximately 1 µL of liquid was spotted on a BaF2 window, dried using a stream of air, and the measurement was obtained using the microscope.

**Results and Discussion**

The hydrolysis and reesterification of lactones is well known to chemists and has appeared as a subject in most basic organic chemistry texts over the last century (11–15). More advanced texts present mechanisms of hydrolysis (16,17). The hydrolysis is catalyzed under either acidic or basic conditions. Under acidic conditions, lactones are known to form an equilibrium with the corresponding open chain hydroxycarboxylic acids (13,14,17). Under basic conditions, the lactone/hydroxy- carboxylic acid equilibrium is driven to the hydroxy-carboxylate anion via dissociation of the acid (11,13,17). Hydrolysis may also be catalyzed by metal ions, as well as other chemical species (17). In “pure” water, the esterification of gamma-hydroxyacids to their corresponding lactones has been observed to occur readily, presumably due to the presence of trace quantities of acid (14,15,17). For this reason, isolation of the free acid form of GHB may not be possible, and free acid GHB has been reported to exist only in solution (12,15,17).

For the forensic chemist, specific information is needed concerning the hydrolysis of GHL to GHB and the reesterification of GHB to GBL. Under what time frames do the reactions occur? How will storage conditions and sample history affect the composition? How can analytical methods be designed to accurately represent the sample composition at the time of analysis? The current studies were designed to provide a scientific base of information to begin to address these and other questions related to the interconversion of GHB and GBL.

**Analytical Methodology: Addressing Questions of Interconversion and Method Capabilities**

Owing to the obvious concern with the analytical methods themselves, we conducted experiments to determine the potential for GHB-GBL interconversion with both the HPLC and GC-MS methods used in the current study. The HPLC method uses an acidic mobile phase buffer with a pH in the range 2.5 to 3.0. In daily practice, use of pure water for sample dilution is preferred. However, in cases where the sample is highly alkaline or highly buffered at pH 7.0 or above, it may become necessary to dilute the sample in the mobile phase buffer to prevent peak distortion resulting from mixed modes of retention for the GHB free acid and carboxylate forms. As shown in the subsequent studies, the course of GBL hydrolysis to GHB is slowest in pure water. Thus, for aqueous-based samples, it is highly unlikely that using water for sample dilution will further alter the relative proportions of GHB and GBL in the sample. There is also minimal concern when using the mobile phase buffer for sample dilution because the final pH typically falls in the weakly acidic range (pH 4.0 to 6.5), which also represents conditions under which the hydrolysis of GBL proceeds very slowly.

Nevertheless, experiments were conducted in order to determine the potential for interconversion resulting from three factors or circumstances which may occur during the HPLC analysis: (1) interconversion from reaction during the course of a single HPLC run after sample injection; (2) interconversion from reaction of a sample prepared in water prior to injection; and (3) interconversion from reaction of a sample prepared in mobile phase buffer prior to injection. Based on multiple studies and routine practice with GHB and GBL standards diluted in water over the last several years, we have never observed interconversion during the course of a single HPLC run (prior to elution of the peaks of interest, the injected sample resides for 10 min in mobile phase which comprises 95% buffer). In order to test the latter two factors, GHB and GBL standards were diluted in water or buffer (buffer pH 2.86), and then injected every 12 to 15 min to monitor for interconversion. No interconversion was observed for GHB or GBL diluted in water throughout the entire test period (17 h). Interconversion of ca. 0.5% on a relative basis was observed for both GHB and GBL standards diluted in the mobile phase buffer after 4 h. Therefore, when it becomes necessary to use the mobile phase buffer for sample dilution, it is prudent to conduct the HPLC analysis quickly, within a couple of hours to ensure no changes in sample composition. However, the experiments conducted using the mobile phase buffer for dilution represent a worst-case scenario, because dilution in the mobile phase buffer is used only for highly alkaline and/or highly buffered samples (the GHB and GBL standard solutions were not buffered and have pH’s of ca. 8 and 6, respectively).

A typical chromatogram obtained using the HPLC method is given in Fig. 2; this chromatogram represents GBL spiked into the pH 2.0 buffer after a single day’s storage at ambient temperature. The detection wavelength used in the study (215 nm) is slightly above the λmax for both GHB (λmax 209 to 210 nm) and GBL (λmax 204 to 205 nm), resulting in ca. 20% less pure signal for both GHB and GBL. However, the 215 nm wavelength was chosen for detection for two reasons: (1) 215 nm is above the region where the phosphate buffer has a measurable absorbance (which reduces background absorbance); and (2) 215 nm provides for lower absorbance from unknown interferences, because the absorbance associated with a double bond (the simplest UV chromophore) increases dramatically in the spectral region below 210 nm. This latter concern is especially important for complex matrices such as flavored beverages. In the absence of chromatographic interferences, the HPLC approach was determined to be capable of detecting and measuring relative GHB contents of ≥0.4% in the presence of GBL, and relative GBL contents of ≥1% in the presence of GHB.

In addition to FTIR, GC-MS was used for confirmation of the presence of GHB in the test solutions and samples. For GC-MS analysis, GHB was identified as the di-TMS derivative. This approach has found routine use for the detection and identification of GHB in forensic samples (18,19) and biological specimens (20–22), and has been reported to be selective for GHB (20). It is generally well known that moisture will corrupt silylation reactions, and samples are taken to dryness to remove all traces of moisture using a stream of nitrogen (18,20,22) or dry air prior to derivatization. We conducted experiments to determine the potential for GBL to react to form the di-TMS derivative of GHB in both the absence and presence of moisture. No GHB was observed from a dry reaction mixture of neat GBL (1 µL), 200 µL of 99:1 BSTFA:TMCS (bis(trimethylsilyl)trifluoroacetamide:trimethylchlorosilane), and 200 µL pyridine with 30 min incubation at 70°C (experiment conducted in duplicate). In order to represent a fair
amount of moisture contamination, we repeated the experiment (triplicate trials) adding water back into the reaction vessel prior to derivatization (1 μL water, representing a 5 times molar excess of water with respect to GBL). Under these conditions, no conversion of GBL to GHB was observed. Finally, in order to represent copious water contamination, we repeated the experiment (duplicate trials) adding a large excess of water back into the reaction vessel prior to derivatization (20 μL water, representing a 100 times molar excess of water with respect to GBL). Under these conditions, a small amount of GHB was observed in one of the trials (ca. 2% on a relative basis) as the di-TMS derivative. In all of these experiments, the GBL was detected as unchanged GBL, indicating no reaction with the derivatizing agent. Although GHB formation was observed only when we overloaded the reaction with water, it is imperative to conduct thorough drying prior to derivatization in order to minimize the potential for interconversion and to maximize the efficiency of the derivatization reaction.

Buffered Solutions Studies: Hydrolysis and Reesterification of GBL Under Acidic, Neutral, and Basic Conditions

Solution pH is expected to be a critical factor affecting both the rate and extent of GBL hydrolysis to GHB, owing to both the catalytic effects of acids and bases and the direct influence of pH on the position of the GHB free acid/anion equilibrium. Although this may be understood in general terms, as our laboratory began receiving multiple and varied submissions of aqueous-based GBL products, we needed a direct knowledge of the actual rates of GBL hydrolysis that may occur in forensic samples, including the effects that solution pH has on GHB-GBL interconversion.

We conducted a series of pH studies, in which GHB or GBL were spiked at 0.5% w/w into solutions buffered at pH’s of 2.0, 7.0, and 12.0 (first series of experiments) and 4.0, 5.2, and 6.4 (second series). Phosphate (0.5 M) was chosen because it has a high buffering capacity at all three pH’s used in the first series of experiments (phosphate pKa’s of 2.2, 7.2, and 12.3) (23), and for consistency in the second series of experiments. The buffer strength was sufficient to maintain the buffer pH's throughout all the studies. The solutions were stored under ambient conditions, and then analyzed for GHB and GBL content using the HPLC method at various time intervals. For purposes of clarity, it is easiest to consider first the results for hydrolysis of GBL to GHB as a function of pH, and then later the results for esterification of GHB to GBL. The results for the hydrolysis of GBL to GHB from the pH studies are presented in Figs. 3 to 6. The data in these plots are represented as the relative proportion of GHB [(GHB content × 100%)/(GHB content + GBL content)], which was determined in the test solutions.
The hydrolysis of GBL in pure water (Fig. 3) proceeded slowly over a period of months, reaching a stable reaction mixture comprising ca. 2:1 GBL:GHB (67% GBL; 33% GHB) within 202 days. The solution pH was observed to decrease, reaching and maintaining a pH of ca. 3.3 after 108 days of storage. The decrease in pH is probably due to the partial dissociation of the GHB free acid upon forming; the pKₐ of GHB can be presumed to be near 5 based on comparison with butanoic acid (pKₐ 4.65) and 3-hydroxy-2-methyl-butanoic acid (pKₐ 4.82) (24). The results observed for the GBL–pure water solutions are consistent with the slow formation of an equilibrium mixture of GHB and GBL. The observed equilibrium composition is comparable to a textbook literature value cited as 72% GBL and 27% GHB (14); however, the exact conditions that produced the equilibrium mixture were not specified in the textbook and the primary reference was not provided.

At pH 2.0, the hydrolysis of GBL (Fig. 4, open circles) proceeded much more rapidly relative to pure water, and produced a similar stable reaction mixture (68% GBL; 32% GHB) within only nine days of storage. Figure 4 also provides the results for the esterification of GHB at pH 2.0 (filled circles); the reaction mixture (67% GBL; 33% GHB) was again produced within 9 days of storage. The formation of the same stable reaction mixture starting from either GHB or GBL at pH 2.0 is evidence of a true equilibrium. The reaction mixture was monitored for 202 days and the composition remained constant.

The hydrolysis of GBL at pH 12.0 (Fig. 5) occurred rapidly, with greater than 90% conversion to GHB within 5 min, and complete conversion within 15 min. Under alkaline conditions, formation of GHB is driven via dissociation of the GHB free acid to the GHB anion or salt form, which is not subject to reesterification. In the current study, the reaction mixture was monitored for nearly seven months (202 days) and was stable. It is the rapid and complete conversion of GBL to GHB under alkaline conditions that makes this approach practical for the synthesis of GHB (17,25,26).

Figure 6 shows the results for hydrolysis of GBL at pH 7.0 (data from first series of experiments) and pH’s 6.4, 5.2, and 4.0 (data from second series of experiments). Although the pH 7.0 solution represents a neutral pH, the hydrolysis of GBL proceeded more rapidly than in pure water (see Fig. 3) and was also observed to proceed to near completion (97% conversion to GHB at the end of the study, 202 days, results not plotted). These results make sense because the solution pH was maintained at 7.0 in the buffer, and nearly all of the GHB that formed ultimately dissociated to the anion or salt form, driving the reaction to near completion. The rate and extent of GBL hydrolysis were lower for the lower pH’s relative to pH 7.0 (see Fig. 6, results for pH 4.0, 5.2, and 6.4 buffers).

However, hydrolysis continued to occur slowly, with the proportion of GHB obtained at the end of the study (220 days, results not plotted) increasing in the order pH 4.0 (39% GHB), pH 5.2 (61% GHB), and pH 6.4 (92% GHB). Thus, the degree of hydro-
ysis increased with increasing pH, consistent with an increasing degree of GHB dissociation.

The results for the esterification of GHB will now be considered. In strongly acidic solution, the esterification of GHB to GBL occurred readily, forming an equilibrium mixture comprising ca. 2:1 GBL:GHB (see Fig. 4 and previous discussion). In pure water, and in the pH 7.0 and 12.0 buffers, GHB was observed to be stable throughout the entire study (202 days, results not plotted). All GHB solutions were prepared using the sodium salt of GHB. The pH of the 0.5% GHB solutions prepared in pure water were measured as 7.8 at the beginning of the study and 7.5 at the end of the study. This pH range is sufficient to maintain GHB in its stable, dissociated anion form.

Relative to the pH 2.0 results, esterification of GHB to GBL was delayed over the pH range 4.0 to 6.4 (results not plotted). No esterification occurred for the pH 5.2 and 6.4 solutions throughout the first 17 days of the study; no esterification occurred for the pH 4.0 solutions throughout the first 10 days of the study. After these periods, esterification occurred gradually at all three pH's, with the degree of esterification increasing in the order pH 6.4, 5.2, and 4.0. The proportion of GHB remaining at the end of the study (220 days) at each of the pH's was as follows: 95% at pH 6.4; 85% at pH 5.2; and 72% at pH 4.0.

There was no evidence of side reactions occurring in any of the buffered solutions from the first or second study. The GBL that was lost due to hydrolysis was quantitatively converted to GHB, and the GHB that was lost due to esterification was quantitatively converted to GBL. Thus, although interconversion occurred, there was no loss in the total content of GHB and GBL observed as a function of time (results based on external calibration, no internal standard was used in the experiments).

**Commercial and Clandestine Aqueous-Based GBL Products**

Dozens of aqueous-based commercial products with labels claiming to contain GBL in the range 1 to 5 g per ounce have been received and analyzed by our laboratory. In addition to GBL, GHB was readily detected in most of these products. Based on the pH studies, we began examining these products for the amount of conversion and obtained product pH measurements at the time of analysis. Results are given in Table 2 for four such products, representing three different “brand names,” designated “A,” “B,” or “C” in the table.

The total content (both GHB and GBL) for each product ranged from 2 to 5 g per ounce. Product A, with a pH of 5.23, had the lowest proportion of GHB, less than 10%. The GHB proportion for Product B, with a more acidic pH of 2.58, was 27%. The pH of both products labeled as C was 2.9, and the GHB proportion for both products was about 30%. The proportions of GHB observed for Products B and C are similar to the equilibrium proportion of GHB (about 1/3) observed in the pH 2.0 studies. Since Products B and C also had fairly acidic pH’s, and assuming that the products were formulated with GBL in water, both products had undergone extensive hydrolysis prior to analysis. Products A and B were reanalyzed after a long period of storage (≥10 months) under ambient conditions. The pH for Product A dropped over 1 full unit, to 4.10, and the proportion of GHB increased substantially (28%). The pH for Product B did not change significantly, and the proportion of GHB increased slightly (31%). These results are consistent with continuing GBL hydrolysis for Product A and probably indicate an established equilibrium for Product B.

The stability of an aqueous-based “clandestine” GBL was examined as a function of time (Table 3). This “clandestine” product was sold on the street as a clear liquid in an unlabeled container and probably represented GBL obtained from an industrial source and diluted in water. When the product was first received, the pH was about 5, with little to no GHB, and with a GBL content of 3.3 g per ounce. The product was stored under ambient conditions and reanalyzed several times over a ten-month period. The pH of the product decreased with time while the proportion of GHB increased. The pH and composition of the product was not observed to change significantly between the last two analyses, conducted after approximately three months and ten months of storage, respectively. The final GHB proportion was 32% with a pH of 2.9. The results for this product are consistent with a freshly manufactured aqueous-based GBL product at the time of the initial analysis and the establishment of a GHB/GBL equilibrium during storage.

The results for both the commercial and clandestine GBL products are consistent with aqueous-based GBL products that have little to no buffering capacity. (Although no buffering capacity measurements were made, the pH of these products was observed to decrease with time.) These results are consistent with the ongoing hydrolysis of GBL resulting in the establishment of an equilibrium GHB/GBL mixture. The results for the commercial and clandestine products are also consistent with the pH-hydrolysis studies presented. Note that the pH-hydrolysis studies were conducted using GBL concentrations (0.5% w/w) which were 10 to 40 times less concentrated than the commercial and clandestine GBL products. Thus, with respect to the equilibrium proportions of GHB and GBL established in aqueous solution, there was no major impact observed due to concentration over this range.

**TABLE 2—Commercial aqueous-based GBL products.**

<table>
<thead>
<tr>
<th>Product</th>
<th>Total Content (g/oz)</th>
<th>First Analysis</th>
<th>Second Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Product pH</td>
<td>Relative Proportion GHB (%)</td>
</tr>
<tr>
<td>A*</td>
<td>2.4</td>
<td>5.23</td>
<td>7.6</td>
</tr>
<tr>
<td>B††</td>
<td>4.8</td>
<td>2.58†</td>
<td>27</td>
</tr>
<tr>
<td>C-1</td>
<td>2.9</td>
<td>2.93</td>
<td>31</td>
</tr>
<tr>
<td>C-2</td>
<td>4.1</td>
<td>2.87</td>
<td>30</td>
</tr>
</tbody>
</table>

* Products stored under ambient conditions between first and second analysis; elapsed time between analyses 10 months for Product A and 14½ months for Product B.
† pH measurement not obtained for Product B in conjunction with first analysis; first pH measurement obtained 3½ months after first analysis.

**TABLE 3—Clandestine aqueous-based GBL product.**

<table>
<thead>
<tr>
<th>Analysis Date (Time Elapsed)</th>
<th>Product pH</th>
<th>Relative Proportion GHB (%)</th>
<th>Relative Proportion GBL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-3-99†</td>
<td>4.94</td>
<td>trace</td>
<td>99+</td>
</tr>
<tr>
<td>9-2-99 (2 days)</td>
<td>...</td>
<td>trace</td>
<td>99+</td>
</tr>
<tr>
<td>10-5-99 (35 days)</td>
<td>3.35</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>12-8-99 (99 days)</td>
<td>2.92</td>
<td>31</td>
<td>69</td>
</tr>
<tr>
<td>6-30-00 (304 days)</td>
<td>2.87</td>
<td>32</td>
<td>68</td>
</tr>
</tbody>
</table>

* The total content for this product was 3.3 g/oz (initially all GBL).
† Date of initial analysis (day 0).
Spiked Beverages

There are various scenarios by which GHB or GBL may be consumed after mixing into a beverage. Among these, these include the preference of the end-user and intentional adulteration of another person’s beverage with the intent to commit sexual assault (6–8). In our laboratory, we have encountered cases involving both alcoholic and nonalcoholic beverages. Most commercial beverages contain water or will be mixed into an aqueous base prior to consumption and have acidic pH’s. The question of how the beverage matrix will effect the GHB/GBL interconversion becomes an issue at the moment the beverage is spiked.

We chose five commercial beverages for study (Table 1). These beverages comprised both alcoholic and nonalcoholic beverages. The alcohol content of the latter three beverages varied from 5.9 to 21% as declared on the respective labels. The pH’s of all five beverages were acidic, ranging from 2.6 to 3.3. For purposes of the study, we wanted to represent a scenario in which a dose of GHB or GBL, which was likely to cause serious effects or health consequences, was spiked into the beverage. Thus, each beverage was spiked at a ratio of 3.0 g per serving using freshly prepared GHB or GBL aqueous stock solutions. Oral consumption of 3.0 g of GHB or GBL represents a common dose encountered in illicit use (5,27,28,31) and is likely to induce sleep or unconsciousness in the average adult who has not developed a tolerance (27–31). More serious effects such as anesthesia or coma may also result (27,28,30,31), depending on the individual’s body weight, consumption with alcohol or other drugs, and other factors. The serving sizes used for each beverage are given in Table 1.

In order to determine the effect of storage temperature, the spiked beverage portions were subsequently stored under ambient conditions (two duplicate portions), in a refrigerator (one portion), and in an oven at 60°C (one portion). Under ambient conditions, detectable levels of GHB were observed after a single day’s storage for four of the five beverage matrices spiked with GBL (ranging from 2 to 6% GHB on a relative basis), and for all five beverage matrices after three days storage (ranging from 3 to 13% GHB on a relative basis). Some differences in the actual rate of hydrolysis of GBL to GHB were observed among the five beverages. Results for the hydrolysis of GBL in two of the commercial beverages under all three storage temperatures are given in Fig. 7A (alcohol lemon cocktail) and Fig. 7B (diluted vodka), respectively. The results for these two beverages represent the matrices that were observed to have the least (Fig. 7A) and greatest (Fig. 7B) overall stability for GBL.

The same pattern was observed for all five beverages with respect to the effect of temperature (see Fig. 7). Hydrolysis occurred more rapidly in the oven, and more slowly in the refrigerator, relative to ambient conditions. GHB proportions (27 to 34%), close to the equilibrium proportion observed at pH 2.0, were observed for all five beverages within three days storage in the oven. Under ambient conditions, similar GHB proportions (27 to 34%) were observed after two weeks to two months storage. Hydrolysis of GBL was retarded by storing the solutions in the refrigerator (4°C). Analyses for GHB and GBL content were conducted after 1, 3, 8, 15, and 66 days storage in the refrigerator. No hydrolysis was observed (no GHB detected) for four of the beverage matrices after a full day of storage. GBL remained stable for at least 8 days in two of the beverages. Although hydrolysis was significantly retarded under refrigerated conditions, GHB was readily detected in all five beverage matrices within 1 to 15 days storage in the refrigerator. The GBL stability data for the five beverages including the relative GHB proportion and time interval when first detected are summarized in Table 4.

Overall, GHB was more stable in the five beverage matrices relative to GBL (results not plotted). Under either ambient or refrigerated conditions, no esterification was observed (no GBL detected) for four of the beverage matrices throughout the entire study (66 days). Under ambient or refrigerated conditions, no esterification was observed for the fifth beverage matrix (wine) throughout the first three days of the study; in this matrix, the proportion of GHB lost due to esterification under either of these conditions was less than 10% at the end of the study. No esterification...
of GHB was observed for the five beverage matrices after one day's oven storage, and no esterification was observed for the vodka matrix throughout the entire storage period in the oven. The proportion of GHB lost due to esterification after 15 days oven storage ranged from 10 to 30% for the other four beverage matrices. Analyses conducted on these beverages after 15 days oven storage could not be used because degradation occurred, producing analytical interferences.

Summary, Implications, and Recommendations

Solution pH, time, and storage temperature were determined to be important factors that affect the rate and extent of GBL hydrolysis and conversion to GHB. The pH-hydrolysis studies showed that GBL hydrolysis occurs under acidic, basic, and neutral conditions with the rate and extent of conversion to GHB varying widely according to the solution pH. At ambient temperature, and under strongly alkaline conditions (pH 12.0), the conversion time frame was within minutes, with 100% conversion to GHB. Under strongly acidic conditions (pH 2.0), the conversion time frame was days with the formation of an equilibrium mixture comprising ca. 2:1 GBL:GHB. In pure water, the same ca. 2:1 GBL:GHB equilibrium mixture was ultimately formed with gradual conversion occurring over more than six months storage (202 days) at ambient temperature. At intermediate pH's (pH 4.0 to 7.0), conversion to GHB occurred continually over several months, with the extent of conversion ranging from 72 to 97% after ca. seven months of storage.

Studies with a series of commercial and clandestine aqueous-based GBL products with GBL contents between 2 to 5 g per ounce showed that conversion of a substantial portion of GBL to GHB does occur in these products. GHB contents close to the equilibrium proportion (28 to 31% relative) were already established in many of the products upon receipt in our laboratory, or formed upon ambient storage in our laboratory over a period of 3 to 15 months. For the clandestine GBL product, detectable levels of GHB formed within two weeks of ambient storage. Spiking studies with a series of commercial beverages showed that conversion of a substantial portion of GBL to GHB also occurs in these beverages. For these latter products, the effects of storage temperature were also determined. The effect of heat was to significantly increase the rate of hydrolysis relative to ambient temperature, with GHB contents close to the equilibrium proportion (27 to 34%) established within three days of oven storage (60°C) and two weeks to two months of ambient storage. Refrigeration (4°C) significantly retarded GBL hydrolysis relative to ambient storage; however, the presence of GHB was readily detected in these spiked beverages within 1 to 15 days storage in the refrigerator.

The current studies show that the hydrolysis of GBL to GHB occurs in aqueous-based forensic samples, and that the rate and extent of conversion to GHB will vary according to the specific solution composition. Readily detectable levels of GHB can be expected to form within one or more days of ambient storage in many aqueous-based GBL solutions, and within hours for other solutions, especially if a catalytic substance is present. For commercial products, the time frames and storage conditions between manufacture, distribution, sale, and consumption can be expected to affect the relative proportions of GHB and GBL.

The time frame and storage conditions between evidence collection and sample analysis can also be expected to affect the relative amounts of GHB and GBL. The potential for hydrolysis can be minimized by storing the evidence in a cooler or refrigerator during transit and at the analyzing laboratory. Freezing sample evidence may be a possibility to retard hydrolysis, but more studies would be needed to determine if freezing introduces other problems, such as poor freeze/thaw stability. The addition of stabilizing agents could also be investigated.

For the forensic chemist, GHB-GBL interconversion must also be considered when developing or applying analytical methods. For any method, the potential for interconversion from sample preparation throughout detection should be understood. Approaches that rely on the intentional conversion of GHB to GBL provide no information concerning the source of GHB in the sample evidence. Many forensic labs test only for the presence of GHB. This approach alone does not address the issue of interconversion for aqueous-based sample evidence. In addition, as presented in detail earlier, care should be taken to ensure thorough drying when applying derivatization methods for GC-MS analysis.

Quantitative analysis for both GHB and GBL (i.e., HPLC analysis) may indicate whether the evidence represents a GBL product that is undergoing natural hydrolysis, or a GHB product intentionally manufactured from GBL. In order to characterize the sample evidence further, a pH measurement should be obtained at the time of analysis. If practical in a given case for samples with pH's in the range 4.0 to 7.0, the evidence could be reanalyzed as a function of time providing information as to whether an equilibrium is being established. For samples in this pH range, this may further differentiate between aqueous GBL products and intentionally manufactured GHB batches that were subsequently neutralized with slight excesses of acid.

If warranted, sample evidence may be also be analyzed for sodium and/or potassium content to indicate whether an attempt to synthesize GHB was made using sodium or potassium hydroxide. In our laboratory, the analysis for sodium and/or potassium content has been especially useful for cases involving GHB kits (i.e., GBL, sodium or potassium hydroxide, and batches of GHB made from the kits). In cases where a beverage has been spiked, a comparative analysis for the sodium and/or potassium contents of the spiked beverage and a control beverage sample (representing a matched manufacturer's lot) may indicate whether the beverage was spiked with a GHB salt vs. GBL. Our laboratory typically uses ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy) to conduct such analyses.

The hydrolysis of GBL has legal implications for aqueous-based GBL sample evidence. Since GBL will hydrolyze to GHB within relatively short time frames, there is a high potential for all aqueous-based GBL solutions to contain GHB, a DEA Schedule I controlled substance. Under the law, the presence of any detectable GHB content defines the evidence as a Schedule 1 controlled substance. From a practical standpoint, the likelihood for formation of readily detectable levels of GHB in commercial aqueous-based GBL products prior to sale and consumption is high. From a legal standpoint, this may provide a basis to define these commercial products as Schedule 1 controlled substances.

References


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