

Sinead A. Hennessy,¹ B.Sc.; Siobhán M. Moane,¹ Ph.D.; and Seán D. McDermott,² Ph.D

The Reactivity of Gamma-hydroxybutyric acid (GHB) and Gamma-butyrolactone (GBL) in Alcoholic Solutions

ABSTRACT: In this work the stability of GBL (*gamma*-butyrolactone) and GHB (*gamma*-hydroxybutyric acid) in alcoholic media was studied. Under acidic conditions the GBL will react with ethanol or methanol to give the corresponding ethyl and methyl esters of GHB. It can be seen that ester formation is dependent on the type of alcohol, the alcohol content of the solution, and the pH of the solution. Under the same conditions it was shown that GHB does not give rise directly to the corresponding ester when merely in the presence of an alcohol; however the ester will be formed if the conditions are present for conversion of GHB to GBL followed by subsequent reaction with alcohol. In alcoholic beverage samples spiked with GBL the expected conversion to GHB occurred, and the formation of the ethyl ester of GHB was also seen in some samples. Wine samples were analyzed for the presence of the ethyl ester of GHB, and the effect of adding GHB/GBL to hot beverages was studied.

KEYWORDS: forensic science, *Gamma*-butyrolactone, GBL, *Gamma*-hydroxybutyric acid, GHB, stability, reactions with alcohols, ethyl-*gamma*-hydroxybutyrate, methyl-*gamma*-hydroxybutyrate

GHB (*gamma*-hydroxybutyric acid or *gamma*-hydroxybutyrate) is a naturally occurring short-chained fatty acid found in mammalian tissue (1,2). It was isolated and investigated originally by Laborit in 1960 in an effort to develop a *gamma*-aminobutyric acid (GABA) congener, which would readily cross the blood brain barrier. Earlier testing demonstrated that GHB could produce a dose-dependant sedation and anesthesia in laboratory animals and humans. GHB's action as a central nervous system (CNS) depressant was in some way similar to those of classical sedative/hypnotics, such as barbiturates and benzodiazepines (3).

GHB has a 30-year history of medicinal applications, particularly in Europe, and it was available for many years in the U.S. as a consumer product sold as a dietary supplement. It is known to promote the release of growth hormones and was used both for muscle growth and as a sleep aid among bodybuilders during the 1980s. Until the early 1990s, GHB had received little attention from researchers or from abusers. Increasing consumer use of GHB as a growth promoter or mild sedative brought about increased research interest (4,5). The intoxicating effects of GHB became more widely known, with increasing attention being paid to it in media particularly, the Internet. Reports also emerged that GHB had been used, often in combination with alcohol, to render women more vulnerable to sexual assault. The increase in abuse led to the U.S. FDA to declare GHB-containing products as unsafe and ban public sale in 1990 (6). In 2000 GHB was added to the list of DEA Schedule I controlled substances. However, due to its CNS suppressant ability, GHB is currently under study in some countries as a treatment for some sleep disorders (7) (i.e., narcolepsy) and alcoholism (8), and a medicinal preparation of GHB is currently a Schedule III controlled substance in the U.S.

Another related compound that has recently come to the forefront as a related drug of abuse is GBL (*gamma*-butyrolactone) (the corresponding lactone of GHB). However, while GHB is a controlled substance, GBL is not. GBL converts into GHB both *in-vivo* and in aqueous solutions, and it is therefore commonly substituted for GHB. Although GBL is included as a List I chemical in the U.S. (due to its use in the manufacture of GHB), it also has a variety of industrial uses, making it difficult to monitor or schedule.

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 in solution. However, at some point prior to consumption, the GHB product typically is re-dissolved or further diluted in aqueous-based media, such as beverages. In addition to other illicit drug uses, GHB is commonly encountered in the "club drug" and "rave" scenes (9) and has been detected frequently in victims of drug-facilitated sexual assault or "date rape" (10,11).

The physiological and neurochemical effects of GHB have been reviewed (12). That study also covered areas such as tolerance and dependence of GHB. The enhanced effect of GHB with alcohol and other drugs and the adverse reactions to GHB were also examined.

GHB and GBL are subject to interconversion in aqueous media. GBL is converted to GHB via hydrolysis, whereas GHB is converted to GBL via intramolecular esterification. Therefore, the potential exists for aqueous-based GBL products to undergo interconversion to GHB in the time between manufacture, consumption, and/or seizure. This interconversion is an important factor that must be considered when designing a method for the analysis of powders and/or liquids suspected of containing GHB. It is also a critical legal issue because GHB is a controlled substance, whereas GBL is not. The hydrolysis and re-esterification of lactones is a well-known chemical reaction. The conditions and mechanisms for the interconversion of the closed ring ester (lactone) to the corresponding open chain hydroxycarboxylic acid are widely published (13–16). GHB and GBL also have been the subject of stability studies (17,18).

¹ Department of Applied Sciences, Limerick Institute of Technology, Moylish, Limerick, Ireland.

² Forensic Science Laboratory Garda H.Q, Phoenix Park, Dublin 8, Ireland.

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One study examined the stability and interconversion of GHB and GBL in aqueous solutions over time at different pH values (19). The results show that, at a pH of 2.0, a stable mixture of 68:32 GBL:GHB will be established after 9 days, whether one starts with 100% GHB or 100% GBL. Under strongly alkaline conditions (pH 12.0), the conversion of GBL to GHB is 100% complete within minutes.

The analyses of GHB and GBL have been the subject of many analytical studies, including color tests, infra-red analysis, nuclear magnetic resonance (NMR), and mass-spectrometry (20–28). GHB and GBL also have been studied in many toxicology samples, examples of which include blood, urine, hair, and brain (29–34).

Our study of the stability of GHB and GBL in alcoholic media was prompted by the presence of a second peak in the GC trace of a sample of GBL in methanol. This was identified as the methyl ester of GHB. The above product formation, coupled with the knowledge that GHB and GBL are frequently encountered in alcoholic media, prompted us to examine the stability of the GHB and GBL with reference to (a) alcohol type, (b) variations of pH, and (c) alcoholic strength. We also examined spiked beverages with specific reference to the presence of the ethyl ester of GHB, which is produced when GBL reacts with ethanol.

Materials and Methods

Reagents and Standards

GHB (*gamma*-hydroxybutyric acid) sodium salt and GBL (*gamma*-butyrolactone) were obtained from Fluka Chemicals and Sigma-Aldrich, respectively. Phosphoric acid and triethylamine were obtained from BDH. Ethanol and isopropanol were obtained from Merck. Methanol and chloroform were obtained from Lab Scan Analytical. All dilutions were made with distilled water.

Sample Preparation

Solutions of GHB and GBL were prepared at a concentration of 10 mg/mL (in the various alcohol types and alcohol concentrations) and stored at room temperature throughout the study. Stock solutions (50 mL) of the various alcohol concentrations were prepared by dilution with distilled water. The pH was adjusted by the dropwise addition of concentrated hydrochloric acid.

All pH measurements were taken using a Delta Ohm microprocessor multi-use pH meter (HD 8705).

HPLC Conditions

An HP 1100 series HPLC was employed. This system consisted of a quaternary pump, 100-vial auto-sampler, on-line degasser, and photodiode array detector. The components were separated using a Spherisorb 5 ODS1 column of 150 × 4.6 mm × 5 μm maintained at 30°C. The mobile phase consisted of 70% phosphate buffer and 30% methanol at a flow rate of 1.0 mL/min. UV detection was performed at 215 nm with a reference wavelength of 360 nm. A sample volume of 2 μL was injected onto the column.

Phosphate Buffer Preparation

The phosphate buffer was prepared as a concentrate composed of 1.0 M phosphoric acid (68 mL of 85% phosphoric acid diluted to 1 L with distilled water) titrated to pH 2.5 with triethylamine (approximately 10 mL per 100 mL of 1.0 M phosphoric acid).

A working solution was then prepared by the dilution of 10 mL of the concentrate solution to 1 L with distilled water giving a 10 mM triethylamine-phosphate (TEAP) working buffer (pH 2.75).

Working solutions were made fresh when required.

GC Conditions

An HP 6890 GC equipped with a 5973 MS was used.

The following column was used: HP Ultra-1 (Crosslinked Methyl Siloxane 12 m, internal diameter 0.2 mm, film thickness 0.33 μm), Helium flow 1 mL/min, 50:1 split ratio.

Temperature program was as follows: Initial temp: 60°C for 2 min; then 60–180°C at 15°C/min with no hold time; then 180–290°C at 25°C/min, with a final hold time of 3 min. The total run time was 17.4 min.

MS Conditions

Low mass was 40, high mass 550, with a solvent delay of 1.5 min.

Spiked Beverages

Alcoholic beverages were prepared to simulate the approximate alcohol concentration that may be found in real cases. Therefore, some of the alcoholic beverages were diluted 1:3 in a suitable mixer (e.g., cola, orange juice, or tonic). GBL was added to give a concentration of 10 mg/mL.

Wine Samples

For GC analysis GBL was extracted from the wine sample by addition of 1 mL of chloroform to 5 mL of wine. The resulting extractions were then concentrated, in a stream of dry air, to 0.2 mL and injected onto the GC/MS system.

For HPLC analysis the wine samples were injected directly onto the HPLC column without dilution or extraction.

Tea/Coffee

Tea and coffee were prepared fresh (temperature = 95°C) and added to vials containing GBL or GHB.

Quantitative Calculations

The % composition in each case was calculated based on peak area where the sum of the three components was taken to be 100% (peak area normalization, no internal standard used) as follows:

$$\% \text{ Ester} = [\text{Area of Ester}] / [\text{Area of Ester}] + [\text{Area of GHB}] + [\text{Area of GBL}]$$

Results and Discussion

GBL Stability

Lactones are cyclic esters and react similarly to other esters. One of these reactions is alcoholysis (cleavage by an alcohol). The alcoholysis of an ester is more commonly called transesterification. The alcohol acts as a nucleophilic reagent, and the reaction is acid or base catalyzed. The basic reaction is demonstrated in Fig. 1.



FIG. 1—*Transesterification reaction of an ester with an alcohol.*

When GBL was added to methanol and the sample analyzed by GC/MS after two days, a second product was seen in the mixture. This product was the methyl ester of *gamma*-hydroxybutyric acid (methyl-*gamma*-hydroxybutyrate or GHB methyl ester).

The GC/MS trace for this sample is shown in Fig. 2.

The reaction of GBL with ethanol does not lead to any initial product formation. However, the addition of a few drops of concentrated hydrochloric acid to the GBL/ethanol mix gives rise to a peak at 3.71 min (Fig. 3), which is ethyl ester of *gamma*-hydroxybutyric acid (ethyl-*gamma*-hydroxybutyrate or GHB ethyl ester).

The interconversion of GBL and GHB in aqueous media is a widely known reaction, however when alcohol is added to the mix there are two competitive reactions in progress (Fig. 4), namely the hydrolysis reaction resulting in the formation of GHB and the alcoholysis (transesterification) reaction resulting in the formation of the corresponding GHB-ester. The basic mechanism of the alcoholysis reaction is protonation of the carbonyl oxygen of GBL followed by nucleophilic attack by the alcohol. Therefore, when GBL reacts with methanol or ethanol, the resulting products are the GHB methyl ester (I) and GHB ethyl ester (II), respectively.

These esters have been synthesized previously by a variety of routes (35–37).

The interpretation of the main peaks in the mass-spectra of the methyl ester (I), and the ethyl ester (II) is given in Table 1.

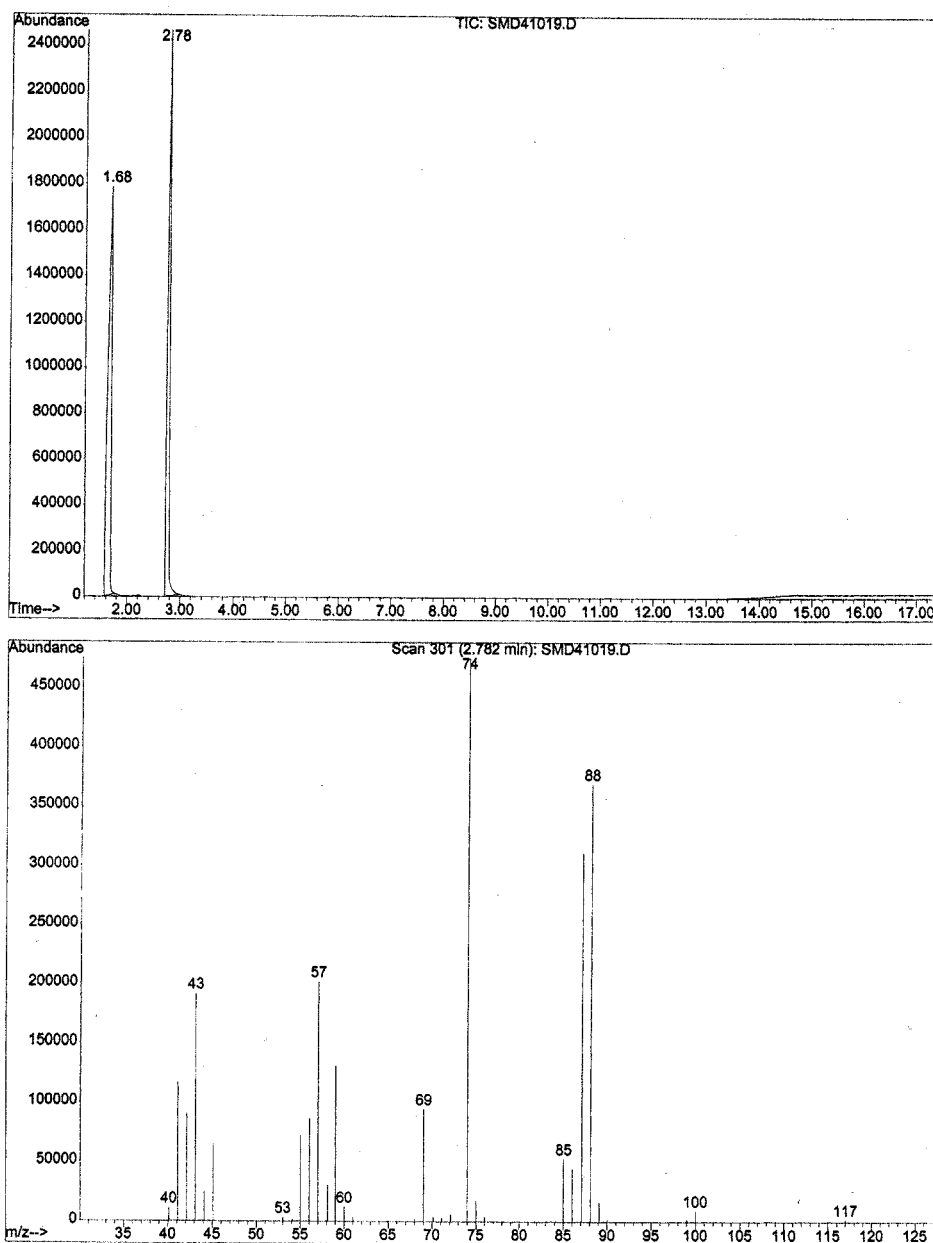


FIG. 2—*GC trace of the product of the reaction of GBL with methanol. GBL retention time = 1.68 min. Methyl ester of GHB, retention time = 2.78 min. Mass-spectrum of ester also shown.*

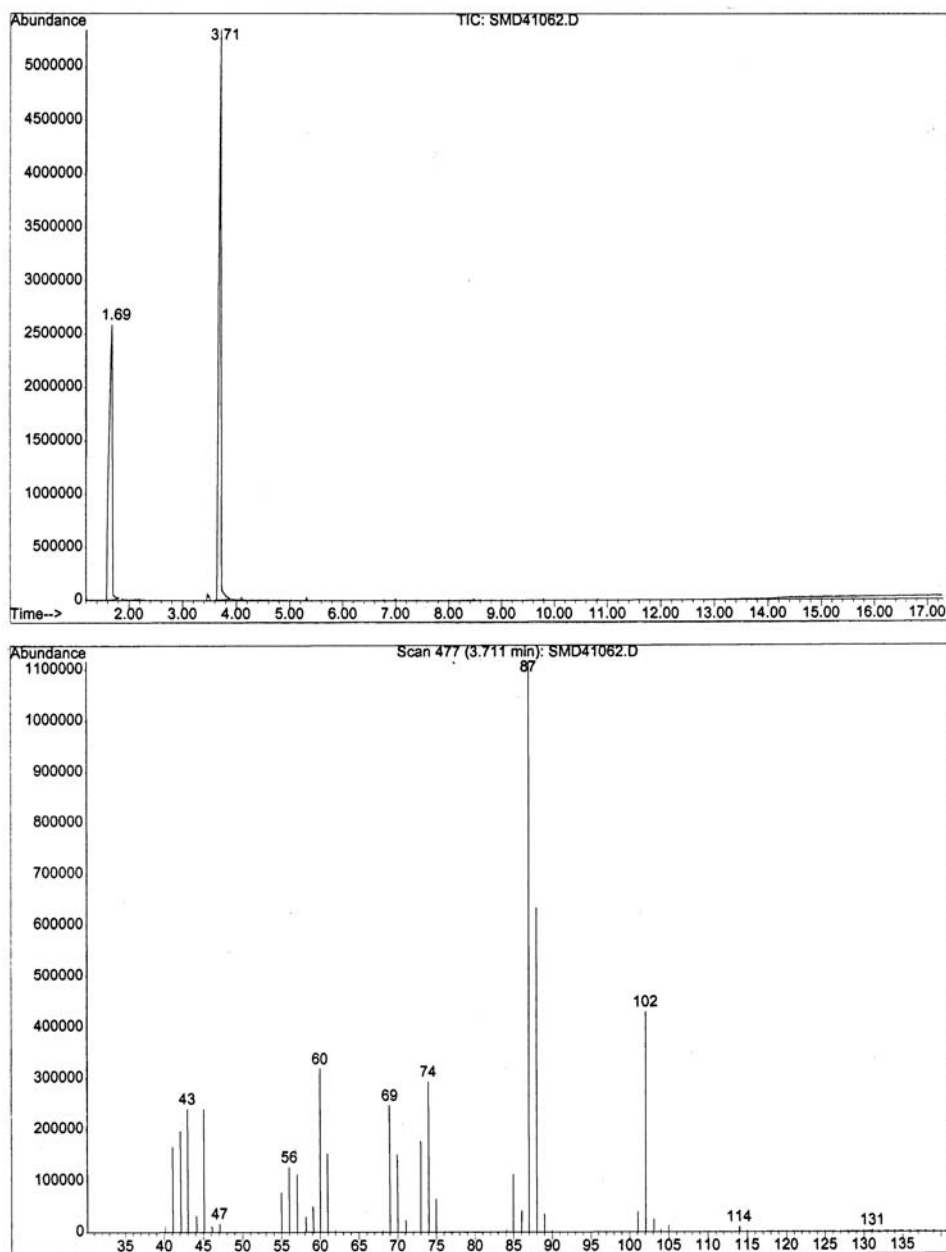


FIG. 3—GC trace of the product of the reaction of GBL with ethanol. GBL retention time = 1.69 min. Ethyl ester of GHB, retention time = 3.71 min. Mass-spectrum of ester also shown.

TABLE 1—Interpretation of main peaks in mass-spectra of methyl ester of GHB (I) and ethyl ester of GHB (II).

Methyl ester (I) (mw = 118)	Ethyl ester (II) (mw = 132)	Interpretation
m/z = 117	m/z = 131	Loss of H
m/z = 100	m/z = 114	Loss of H ₂ O
m/z = 88	m/z = 102	α -cleavage of alcohol
m/z = 87	m/z = 87	α -cleavage of ester
m/z = 74	m/z = 87	McLafferty rearrangement product
m/z = 69	m/z = 69	Loss of H ₂ O from m/z = 87
m/z = 43	m/z = 43	CH ₃ C = O

We examined the reactions in Fig. 4 in more detail. Initially a 50% acidified ethanol/water mix (pH 2.0) was examined with

GBL. It can be seen in Fig. 5 that at first the rate of formation of GHB is more rapid than the ethyl ester; however, after 7 days the ester formation has overtaken the GHB formation. The reaction progress was monitored by HPLC as the GHB, GBL, and ester give three separate peaks (Fig. 6). Because of the conversion of GHB to GBL in the heated injection port, a direct analysis by GC cannot be used to distinguish between GHB and GBL.

After 14 days a stable reaction mix is established of GBL: Ester: GHB (46:31:23).

In a similar reaction with an acidified 50% methanol/water solution (pH 2.0), we see the ester formation overtake the GHB formation very early (i.e., on day 2) (Fig. 7). Analysis of the product mix was by HPLC (Fig. 8), and the final equilibrium was GBL:Ester:GHB (37.5:47:15.5).

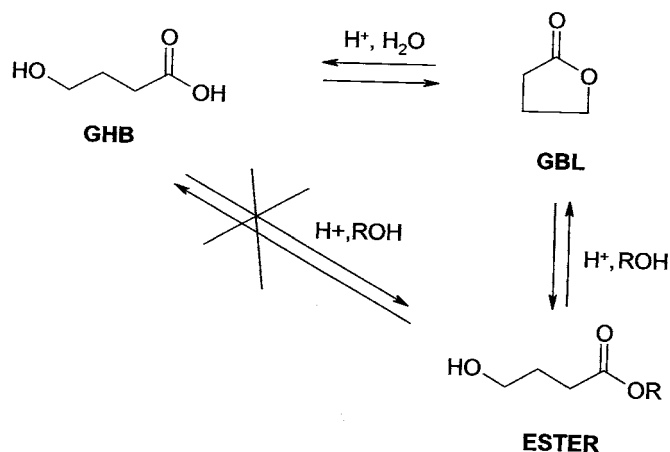


FIG. 4—Interconversion of GHB and GBL and the conversion of GBL to an open chain ester.

Effect of Alcohol Type

In a series of experiments, we examined the effect of varying the type of alcohol while keeping the pH constant (pH 2.0). The solution in each case was 50% alcohol at pH 2.0. When an alcohol acts as a nucleophile in acid-catalyzed transesterification reactions, the dissociation constant (pKa) of the alcohol is a measure of the nucleophilic strength. The nucleophilic strength of methanol (pKa = 15.5), ethanol (pKa = 15.9), and isopropanol (pKa = 17.1) can be clearly seen in Fig. 9, where the rates of ester formation for each alcohol are shown.

Effect of Alcohol Concentration

In a series of experiments (using ethanol), the alcohol concentration of the solution was varied, and the pH was kept constant (pH 2.0). The rates of ester and GHB formation from GBL were examined.

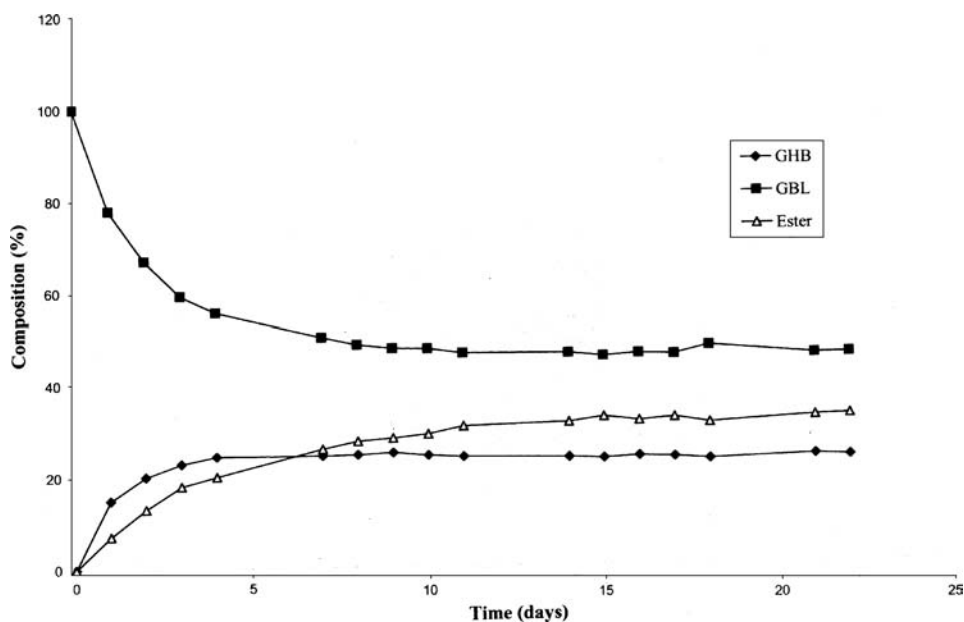


FIG. 5—Plot of product formation when GBL is dissolved in 50% ethanol/water at pH 2.0.

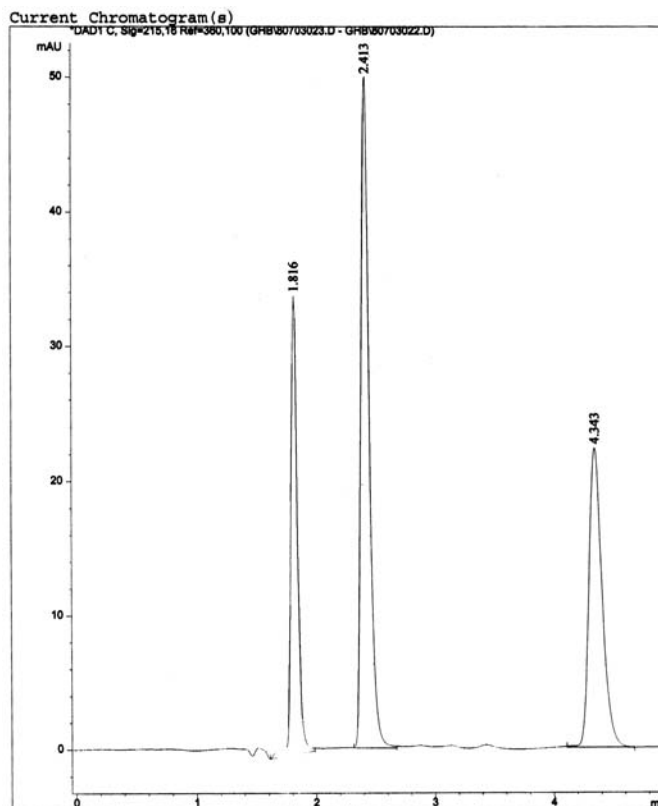


FIG. 6—HPLC trace showing GHB (retention time 1.816 min), GBL (retention time 2.413 min), Ethyl ester of GHB (retention time 4.343 min).

In each case the reaction came to equilibrium. The rates of ester formation are seen in Fig. 10. It can be seen that both the rate of formation and the final concentration of the ester formed were increased greatly by the strength of the alcohol in each mix. For a mixture with 100% ethanol, the final ester content is 70%, but for a 50% ethanol solution, the final ester content is 31%, and for a 5% solution, the final ester content is only 2%.

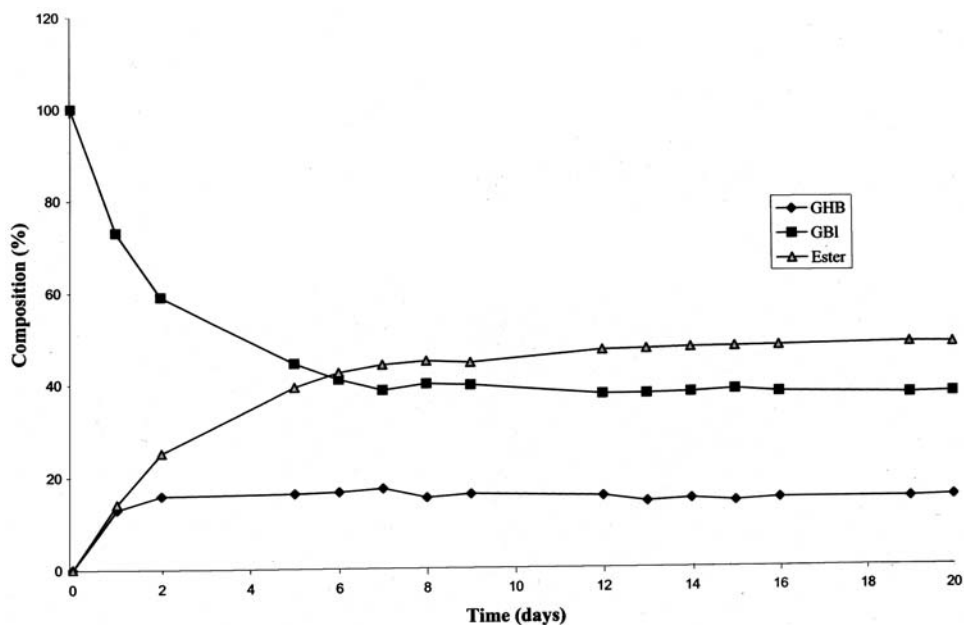


FIG. 7—Plot of product formation when GBL is dissolved in 50% methanol/water at pH 2.0.

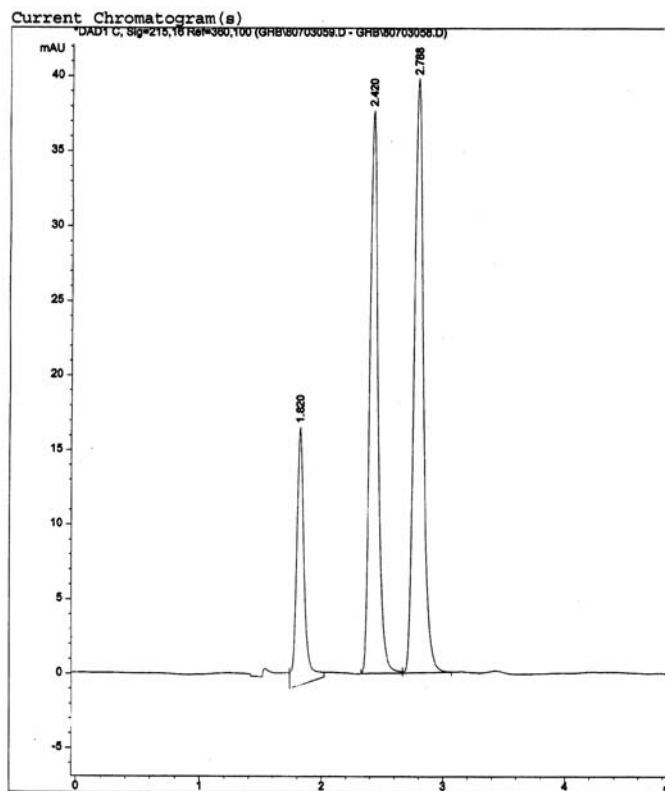


FIG. 8—HPLC trace showing GHB (retention time 1.820 min), GBL (retention time 2.420 min), Methyl ester of GHB (retention time 2.788 min).

Effect of pH

In a further series of experiments, starting with GBL and using a 50% ethanol solution, the pH was varied from 1.0–4.0. At pH 1.0, the reaction came to equilibrium after only 20 h. At pH 2.0, the reaction was much slower, taking approximately 12 days to reach equilibrium. However, the final ratio of GBL:Ester:GHB (48:31:21)

is almost identical to that of pH 1.0 (Fig. 11). At pH 3.0, the reaction is slower again where the amount of ester formed at 22 days is 14% (experiment stopped after 22 days, equilibrium not yet established). At pH 4.0, there is no change, i.e., no formation of GHB or ester (not plotted).

GHB Stability

We also studied the stability of GHB in various alcoholic solutions. A possible alternative reaction mechanism to that in Fig. 4 is the direct reaction of an alcohol with GHB to form the corresponding ester. In order to test this we examined GHB in some alcoholic solutions. The results are summarized in Table 2. It can be seen that in 100% methanol or 100% ethanol (isopropanol not used) no ester is formed after 30 days, even under acidic conditions (pH 2.0). With no water in the mixture the conditions are not favorable for GBL formation at room temperature.

Ester formation is observed only when GBL is formed by the interconversion of the GHB and subsequent reaction of the alcohol with the GBL (50% methanol or 50% ethanol). This can be seen if we examine the plot of the reaction of GHB in 50% methanol at

TABLE 2—Summary of results of reaction of GHB in various alcoholic solutions.

Solvent	pH	Product at Day 30		
		GHB (%)	GBL (%)	Ester (%)
100% EtOH	6.0	100	0	0
100% MeOH	6.0	100	0	0
100% EtOH/HCl	2.0	100	0	0
100% MeOH/HCl	2.0	100	0	0
50% EtOH/HCl	4.0	100	0	0
50% MeOH/HCl	4.0	100	0	0
50% EtOH/HCl*	2.0	40	44	15
50% MeOH/HCl*	2.0	16	37	47
50% EtOH/HCl*	1.0	22	48	30
50% MeOH/HCl*	1.0	14	40	46

* Products have not reached equilibrium.

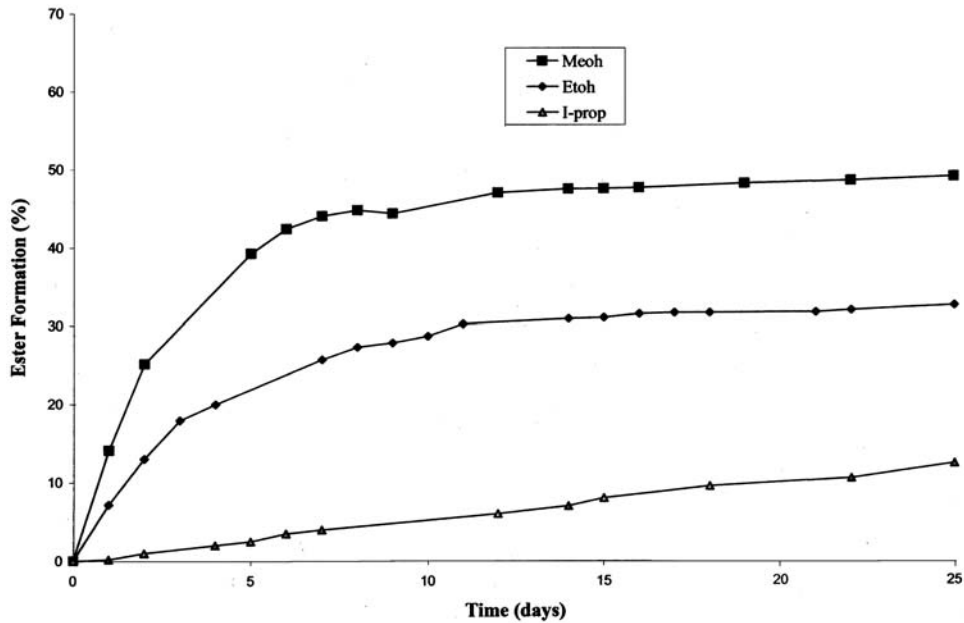


FIG. 9—Rate of ester formation when GBL is dissolved in 50% methanol/water, 50% ethanol/water, and 50% isopropanol/water. All solutions were at pH 2.0.

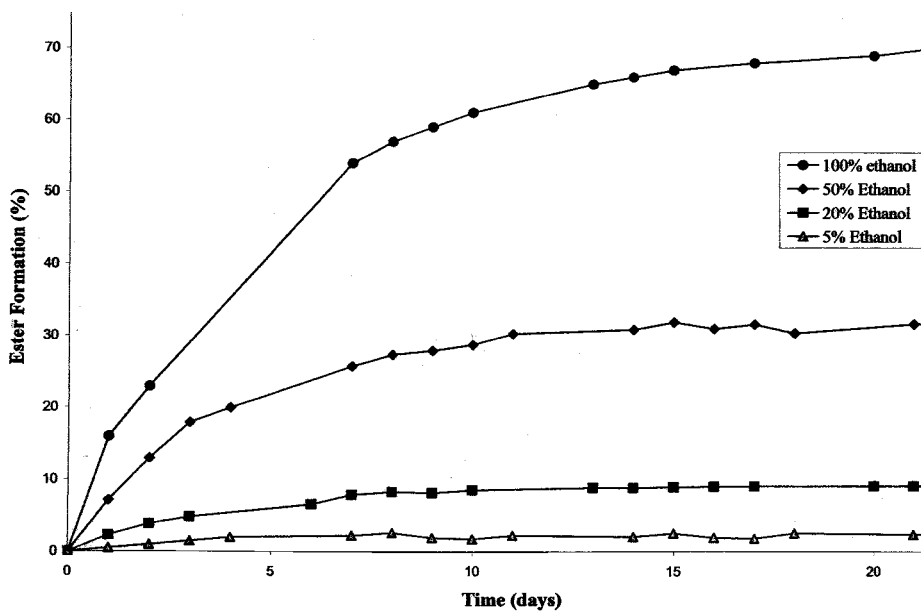


FIG. 10—Plots showing the rate of ester formation from GBL as the ethanol strength of the solution is varied.

pH 1.0 (Fig. 12). We see the initial rapid formation (time for this plot is in hours) of GBL, which is being converted simultaneously to the ester. The GBL level reaches a peak after 2 h and then decreases, as the ester is further formed.

We can conclude that when GHB is added to alcohol, unless the conditions are favorable for conversion to GBL, at room temperature GHB will not give rise directly to the ester.

Spiked Beverages

The results of our studies with beverages spiked with GBL (GHB not used) are listed in Table 3.

After 30 days, the ester formation was observed in only two instances (i.e., in Bacardi/Cola and Vodka/Cola). The pH of these

beverages is less than 3.0, and the alcohol content was 9.4%, conditions that favor ester formation.

These results indicate that the presence of the ester in samples suspected of being spiked in “date-rape” scenarios could be used to indicate that GBL was added to the drink some time earlier.

In the case of WKD (a vodka based “alcopop”), the pH is less than 3.0, but the alcohol content of 5.5% is too low for ester formation.

Wine Samples

We examined a number of wine samples for the presence of GBL and the ethyl ester of GHB. As previously reported (38), GBL is present as a natural product of certain wines. We wished

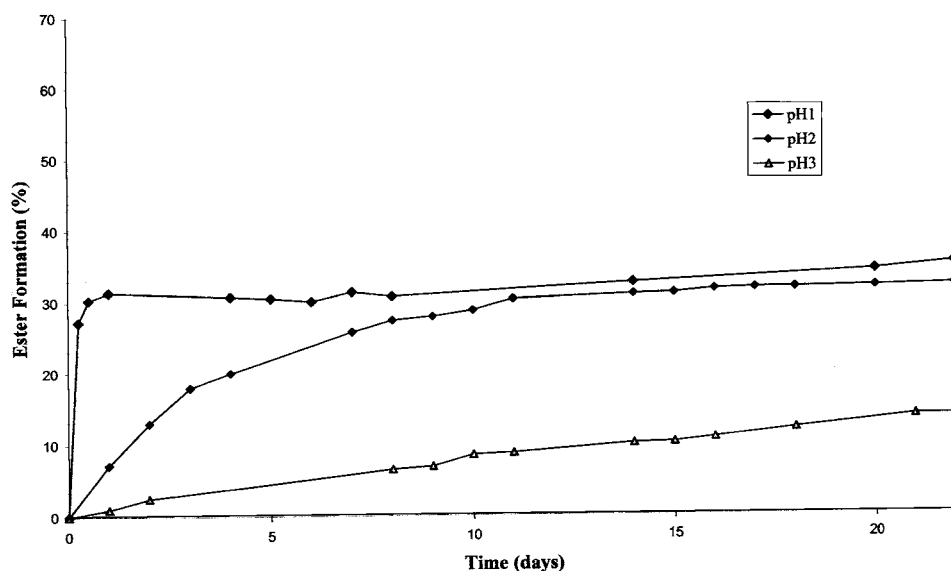


FIG. 11—Plots showing the rate of ester formation from GBL in 50% ethanol/water, for varying pH values.

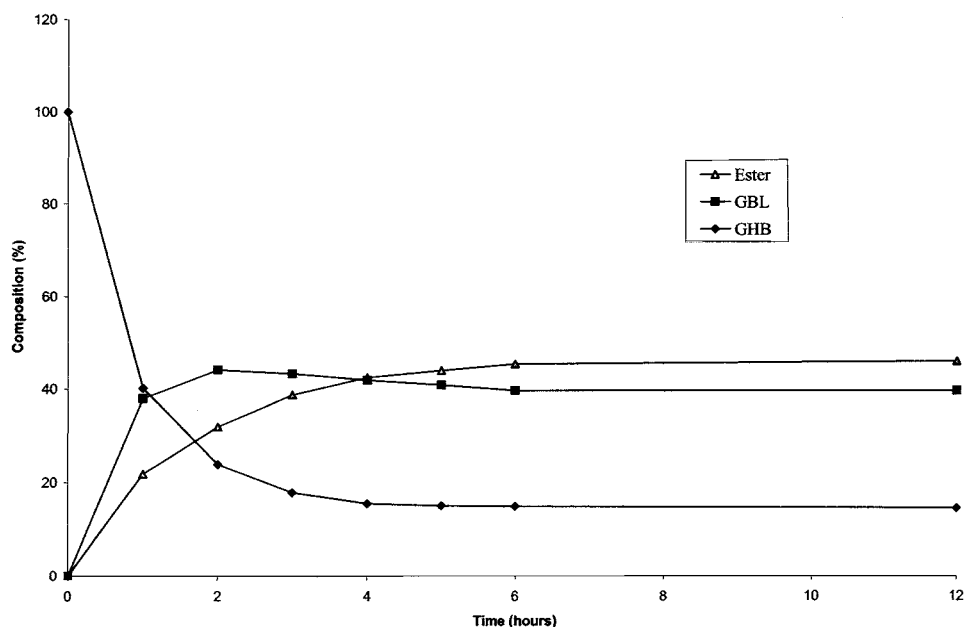


FIG. 12—Plot showing the rate of product formation from GHB in a 50% methanol/water solution at pH 1.0.

to establish if the ethyl ester also was present in the same wine samples. Analysis of the five wine samples (Table 4) confirmed the presence of GBL, but there was no evidence of ester formation. The pH of these wines would not be low enough for ester formation, although at an alcohol content of approximately 13%, we would expect ester formation if the conditions were acidic enough (i.e., less than pH 3.0).

We did not measure the percentage GBL content of the wines, but the absence of the ethyl ester shows that any GBL present had not been converted to the ester over time.

In addition, we examined the wine samples for the presence of GHB using HPLC. This was not previously considered, as the earlier examination was by GC, which would convert GHB to GBL. We found no evidence of GHB in the wine samples; however, the wine samples had some early eluting com-

pounds that had retention times close to the retention time of GHB.

Hot Beverages

Another possible method of spiking beverages in “date-rape” situations is the addition of GHB or GBL to a hot beverage such as tea or coffee.

When GHB or GBL was added to tea/coffee at 95°C, no inter-conversion was observed.

Conclusion

When GBL is added to aqueous solutions containing alcohol, under acidic conditions both GHB and the corresponding ester of

TABLE 3—Summary of results after 30 days using beverages spiked with GBL.

Beverage	Dilution	pH	Alcohol Content (% Vol)	% GHB	% Ester
Bacardi (& Cola)	1:3	2.65	9.4	30	3
Bacardi (& Orange Juice)	1:3	3.91	9.4	0	0
Bacardi Breezer*	Neat	3.26	5.4	19	0
Brandy	Neat	3.17	40	8	0
Cider	Neat	3.12	6.0	22	0
Gin (& Tonic)	1:3	3.22	9.4	23	0
Lager	Neat	4.19	4.3	0	0
Vodka (& Cola)	1:3	2.63	9.4	30	4
W.K.D.**	Neat	2.74	5.5	29	0

*A bacardi based "alcopop" available in 250 mL bottles.

**A vodka based "alcopop" available in 250 mL bottles.

TABLE 4—Details of wine samples examined.

Wine/Grape	Region of Origin	Vintage	pH	Alcohol Content	GBL Detected
Rioja	Spain	1994	3.5	13%	Yes
Cabernet Sauvignon	California	2001	3.6	13.5%	Yes
Sauvignon Blanc	Chile	2002	3.2	12.5%	Yes
Chardonnay	New Zealand	2000	3.4	12.5%	Yes
Zinfandel	California	2001	3.1	10.5%	Yes

the GHB will be formed. The extent of ester formation depends on alcohol type, i.e., the lower the pKa, the greater the ester formation. Ester formation is also dependent on the acidity of the solution and the percent alcohol present. In the case of a 50% ethanol solution, ester formation will occur at pH 3.0 but not at pH 4.0. A 5% solution of ethanol at pH 2.0 will only give rise to a small amount (2%) of ester.

Alcoholic beverages spiked with GBL gave rise to GHB and the ethyl ester of GHB, depending on the acidity and alcohol content of the beverage. Ester formation was only seen in beverages with a pH below 3.0 and an alcohol content above 9.0%. The presence of the ester in samples suspected of being spiked in "date-rape" cases could be used to indicate that GBL was added to the mix some time earlier. The analysis of samples such as these brought to forensic science laboratories must be carried out immediately to avoid any confusion about composition at time of seizure.

Wine samples, which contain GBL as a natural component in very low concentrations, did not display any evidence of ester formation.

When GBL or GHB was added to hot tea or coffee, no interconversion was observed.

The results of this study, coupled with earlier work, will help analysts to understand the product mix encountered when suspect GHB/GBL samples are submitted to forensic science laboratories.

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Additional information and reprint requests:

Seán McDermott, Ph.D.
Forensic Science Laboratory
Garda H.Q
Phoenix Park
Dublin 8
Ireland
E-mail: Sdmcdermott@fsl.gov.ie