

TECHNICAL NOTE

Criminalistics

Smartphone-based colorimetric determination of gamma-butyrolactone and gamma-hydroxybutyrate in alcoholic beverage samples

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Abstract

Gamma-hydroxybutyrate (GBH) is a popular recreational drug. Its strong sedative and amnesic effects have led to drug-facilitated sexual assaults, poisonings, overdose, and death. As a result, legislation has restricted its availability leading to GHB, consumers switching to its pro-drug, gamma-butyrolactone (GBL). Consequently, there is a growing need for methods capable of their determination in complex samples such as beverages. Previous studies have shown the possibility to colorimetrically qualitatively determine both GBH and GBL by the formation of the lactone and its reaction with hydroxylamine and ferric chloride to give a purple-colored complex. In this present investigation, we have shown the possibility of using this approach to both quantify GBL and GHB using both UV/Vis spectrometry and by the application of the camera of a smartphone to record images of the purple color developed. Via subsequent use of a downloadable free App, to extract the numerical values of the Red, Green, and Blue (RGB) color components, it was shown possible to construct a calibration curve and to quantitatively determine the concentration of the drugs present in fortified alcoholic beverage samples. It was found that by simple mathematical normalization of the RGB values the effects of camera distance and elimination could be readily overcome. Using the smartphone approach, GBL determinations on a sample of lager beer gave a mean recovery of 103% (%CV = 0.70%, $n = 5$) at a concentration of 0.56 mg/ml indicating the method holds promise for the determination of GBL and GHB in such samples.

KEYWORDS

beverages, colorimetric, gamma-butyrolactone, gamma-hydroxybutyrate, Red, Green, and Blue, smartphone

Highlights

- Colorimetric method to determine gamma-hydroxybutyrate and gamma-butyrolactone in beverages.
- Quantified by RGB color component using a free, downloadable app on a smartphone.
- Quantitative determination in a beverage sample is reported based on the RGB values.
- Can be applied without the need for a laboratory, at the point of need in criminal investigations.

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1 | INTRODUCTION

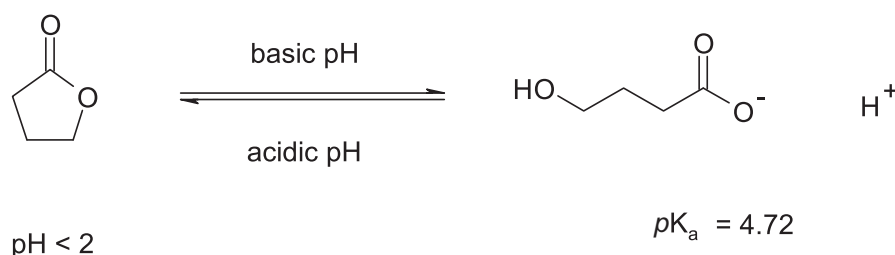
Gamma-hydroxybutyrate (GHB) is a popular recreational drug and due to its strong sedative and amnesic effects it has been connected to drug-facilitated sexual assault cases, poisoning, overdose, date rape, and death [1–5]. The National Drug Intelligence Centre has affirmed that GHB has surpassed Rohypnol as the most common drug implicated in sexual assault cases [6] and as a result, it is now designated as a Class C drug in the UK [7] and is prohibited for sale as a supplement in the USA by the FDA [8]. As a result of these restrictions on the purchase of GHB, consumers have switched to its pro-drug, gamma-butyrolactone (GBL) [9,10]. As GBL products are commercially available, being commonly utilized as an industrial solvent and as a precursor for the synthesis of other chemicals [11] it is easier to obtain than GHB, and justifications for its possession can be made. However, GBL can be readily converted to GHB, either by relatively simple chemistry such as pH adjustment, with GHB being the predominant species at pH values >12 [12] (Scheme 1) or enzymatically in vivo by peripheral lactonases with a half-life of less than 1 min [13]. Under acidic conditions, the particularly stable lactone, GBL is formed, playing a significant role in the solution chemistry of GHB [12,14]. Gamma-lactones reportedly [15] form from the corresponding hydroxy acid so readily that is often not necessary to add acid to catalyze the intramolecular esterification. Mere traces of acid in the solvent or on the glassware can be sufficient to bring about lactonization (Scheme 1). Chappell et al. [16] have made investigations of the pH behavior of GHB and GBL and showed an increase in the speed of the rate of conversion of GHB to GBL with decreasing pH; and found an equilibrium of 2:1 GBL to GHB achieved at pH 2, similar to that seen in pure water [12]. Significant deviation from this gradual change in the GBL:GHB ratio at pH 1.0 has also been reported [16]. LeBeau et al. [17] showed that the addition of 0.15 ml of concentrated sulfuric acid added to 1.0 ml of the sample was sufficient to convert GHB to GBL to allow for its extraction in the nonpolar solvent, dichloromethane. A recovery of 96.5% of GHB as GBL was reported, demonstrating the high conversion rate of GHB to GBL under these conditions.

Gamma-hydroxybutyrate overdose is relatively common, due to its narrow therapeutic index and synergistic effects reported with common drugs such as ethanol [18]. A study undertaken by Carter et al. [19] showed that accidental overdose was more likely when using GHB than other common sedative drugs, such as triazolam, pentobarbital, partly due to the steep dose-response curve seen with GHB [20].

Nevertheless, illicit recreational users of GHB reportedly take relatively high concentrations of GHB. One or more “capfuls” of liquid, equating to ca. 5 g of GHB, or an approximate concentration of 1 g/ml of pure GHB solution 1–6 times per week, 1–3 times per day [21]. In a recent toxicology study [22] into the metabolism of GHB in humans, doses between 33.1 and 60.1 mg/kg body weight were given in a series of 250 ml orange drinks, and levels as high as 80 mg/ml GHB [23] have been reported in a near-fatal incident after the consumption of a so-called “health drink” in the UK. Recent studies of electronic cigarette vaping fluids showed some to have GBL levels as high as 0.37 mg/ml [24].

A number of different methods for the determination of GHB and its analogs have been achieved for both clinical and forensic analyses [25]. However, these generally require a well-equipped laboratory and trained staff for their implementation. In an attempt to meet the demands by members of the public to be able to check their own drinks a number of different commercially available colorimetric spot test devices have been developed. However, these are mostly qualitative. Notably, both Alston and Ng [26] and Zhang and Huang [27] have reported on the utilization of a qualitative colorimetric method for GHB and GBL based on the formation of the purple-colored ferric-hydroxamate complex [28]. This approach is based on a two-part chemical reaction. First, the addition of acid to the sample is undertaken to convert the GHB present to its lactone, GBL (Scheme 1). Following this, the solution is made basic by the addition of sodium hydroxide. Under these conditions [29], in the presence of hydroxylamine, the lactone can then be converted to the corresponding hydroxamate. The addition of ferric chloride results in the formation of a purple-colored complex allowing for its qualitative detection.

Smartphones have become increasingly more commonplace, and awareness of the large number of functions and various applications (Apps) that can be downloaded is widely understood [30]. Smartphones have a range of sensors allowing for numerous applications, including: cameras, barometers, accelerometers, gyroscopes, luxometers, magnetometers, among others, that can be used to take a variety of measurements with good precision [31,32]. Previously, it has been shown possible to colorimetrically determine the number of different drugs [33–39] and either qualitatively or quantitatively determine these using the camera on a smartphone. These previous investigations have utilized subsequent processing of the images obtained, with a readily available, downloadable free App, to examine the red, green, blue (RGB) color balance of the colorimetric reaction.



SCHEME 1 Solution pH behaviour of GHB and GBL

Different formats for photographs taken with smartphones are available; however, most cameras give an 8-bit jpeg image. The 8-bit picture provides 256 variations (2^8) for each color channel (red, green, and blue) expressed as a numerical value between 0 and 255. These recorded RGB numerical values can be plotted against known concentrations of the analyte to construct a calibration curve. Analyte concentrations of unknown samples can then be found following simple interpolation or rearrangement of the equation of a straight-line plot ($y = mx + c$). Such an approach offers a number of advantages including the possibility of point-of-need or care use [40]. The application uses technology available to virtually everybody, outside of a laboratory setting and is operable without professional training or complex laboratory instrumentation.

This present investigation represents the first example of this approach being applied to the possibility of determining GBL and GHB in a beverage sample. Initial studies were made to ascertain the possibility of quantitatively determining GBL via the formation of hydroxamate derivative by UV/Vis spectroscopy. The possibility of developing a simple quantitative smartphone-based sensor utilizing the RGB color model was then explored for the determination of GBL in an alcoholic beverage. To our knowledge, this is the first example of the application of this colorimetric procedure for the quantitative determination of GBL and GHB, and also the first employing smartphone technology.

2 | MATERIALS AND METHODS

2.1 | Materials

All chemicals were obtained from Fischer Scientific Ltd. unless otherwise stated. Deionized water was obtained from a Purite RO200-Stillplus HP System (Purite Oxon). Separate solutions of 12 M NaOH and 0.5 M hydroxylamine HCl were prepared by dissolving the appropriate masses in deionized water and were found to be stable for up to 2 weeks at room temperature. Primary GBL stock solutions (Sigma-Aldrich), of 10 mg/ml were prepared by dissolving the required mass in deionized water. These were then diluted with sufficient deionized water to give working standards solutions over the concentration range 0.1–1.2 mg/ml. Beverage samples were obtained from local commercial outlets.

2.2 | Gamma-hydroxybutyrate and gamma-butyrolactone hydroxamate derivatization procedure

Scheme II shows an overview of the reaction mechanism to give the purple-colored complex. A 0.3 ml aliquot of sample was taken and added to a 30ml Universal glass vial with a 24mm polypropylene screw cap. The sample was then adjusted to pH 1 by the addition of H_2SO_4 , to convert the GHB (I) present to the lactone, GBL (II) via intramolecular esterification. A 1.0 ml aliquot of a 1.0 M hydroxylamine

HCl solution and 0.2 ml of 12 M sodium hydroxide solution was then added. The resulting mixture was then mixed gently by hand to form the hydroxamate derivative (III). Upon the addition of 0.2 ml of ferric chloride, a purple-colored complex (IV) is then formed instantly.

2.3 | UV/Vis spectrographic investigations of the ferric-hydroxamate derivative

Spectrophotometric investigations were undertaken using an Unicam UV 500 spectrophotometer (Thermo Electron Corporation) using Vision 32 software for data acquisition and processing. Behavior of the GBL derivative was studied over the wavelength range 200–700nm with a bandwidth of 2 nm and a path length of 1 cm. Single wavelength analysis was undertaken at 499 nm.

2.4 | Smartphone RGB sampling of colorimetric GBL standards and samples

The image of the samples and standard solutions were obtained using an iPhone SE 2020 running iOS 14.8.1, with an Apple A13 Bionic Processor. The smartphone used the standard fitted 12 MP (rear-facing) camera with a six-element lens and a sapphire crystal lens cover, with a f/1.8 aperture. The glass vials containing the samples and those containing the standard solutions under investigation were placed on an A4 sheet of white paper. Individual smartphone images of the standard and sample solutions were taken after 2 min at a distance of 10 cm under ambient lighting conditions. The images were then processed using Color picker and helper, version 1.1.6 software obtained from the Apple app store (<https://apps.apple.com/gb/app/color-picker-and-helper/id1555136705>). The effect of distance of the smartphone camera to the subject was investigated using a modification of the calibration method described by Merli et al. [37]. A rectangle was drawn in Microsoft Word® and filled with color (R 181; G 83; B 111) using the "other filling colors" option. This was then displayed on the screen of the computer and using the smartphone camera, photos of this were taken at varying distances between 2 and 120 cm in triplicate. The RGB values of the resulting images were then recorded using the Color picker and helper, version 1.1.6 software.

3 | RESULTS AND DISCUSSION

3.1 | Visible spectroscopy investigation of the ferric-hydroxamate iron complex of GBL

Initial studies were undertaken to investigate the possibility of quantitatively determining GBL and GHB as their purple-colored ferric-hydroxamate iron complexes. Previous studies had shown it possible to qualitatively show the presence of GBL and GHB using a similar approach [26]. We initially studied a 0.5 mg/ml solution of GBL and

were able to form a purple-colored solution, in agreement with previous studies, which gave an absorption maximum of 499 nm. The color was produced instantly following the addition of ferric chloride. The resulting color was found to give a linear response with its absorption at 499 nm over the range 0.1 to 1.12 mg/ml GBL using visible spectroscopy. A slope of 0.246 absorbance units per mg/ml (R^2 value of 0.9994) was recorded using a 1 cm path length. Figure 1 shows the resulting solutions obtained in (a) in the presence of 1.12 mg/ml of GBL and (b) in the absence of GBL. The developed color was found to be stable for over 2 weeks for a 1.0 mg/ml GBL solution. However, previous studies have shown that the color developed immediately [41] remained unchanged for at least 5 min, and decreased to 76% of its original value at 25 min [42]. Figure 1C shows the image obtained via the color picker and helper. The crosshairs show the area of the image sampled to obtain the RGB data.

3.2 | Smartphone RGB investigations

Little correlation with the concentration of the GBL color product was observable for the raw RGB data collected (Figure 2A). However, there would appear to be some pattern, which like the red and green diverge from each other, after 0.2 mg/ml GBL. The blue values also run parallel to red. Nevertheless, simple normalization of the RGB data was found to overcome this problem. A number of different methods have been reported to achieve this [43]. The simplest, and most readily achievable approach, which we have employed here, is to normalize the individual R, G, and B values as a percentage of the total RGB (Equations 1, 2, and 3). Normalizing the RGB data in such a way corrects for variables such as local illumination, viewing angle,

and distance. Once corrected, as a percentage, plots of %R, %G, and %B all showed good linear relationships (R^2 of 0.996 for the %R) with the concentration of GBL over the entire range investigated (Figure 2B).

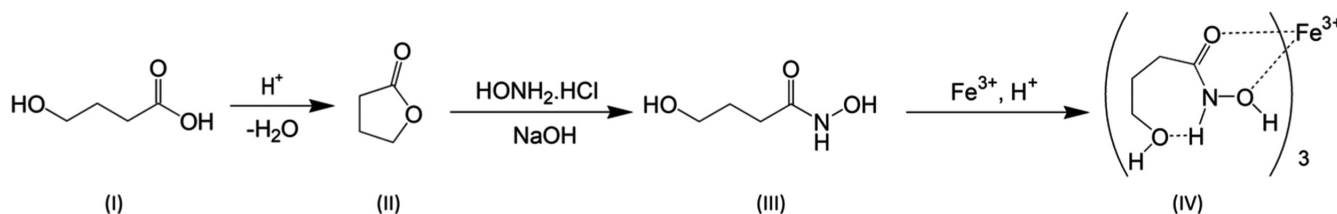
$$\%R = \left(\frac{R}{R + G + B} \right) \times 100 \quad (1)$$

$$\%G = \left(\frac{G}{R + G + B} \right) \times 100 \quad (2)$$

$$\%B = \left(\frac{B}{R + G + B} \right) \times 100 \quad (3)$$

In further studies of this approach, we investigated the effect of distance that the smartphone camera was from the color source had on the resulting percentage normalized RGB values (Figure 3). Using a color rectangle generated in Microsoft Word® [37], we acquired a series of images at increasing distances from the source. Once corrected using the equations described in Equations 1–3 we were able to show that the relative percentages of red (%R) did not change for up to at least 40 cm from the color source (%CV between 0.30% and 1.43%). Both the percentages of green (%G) and blue (%B) remained relatively constant for up to over one meter (%CV between 0.12% and 2.94%).

The color of the beer sample in the absence of GBL was found to be yellow, similar to that generally reported in the literature for this colorimetric test in the absence of the target lactone [26]. The %RGB values obtained from this solution were then subtracted from the sample values to blank correct the results, in a similar manner to zeroing with a blank solution for the spectrophotometer approach.



SCHEME II Reaction mechanism for GHB and GBL to form the purple-colored hydroxamate Fe(III) complex

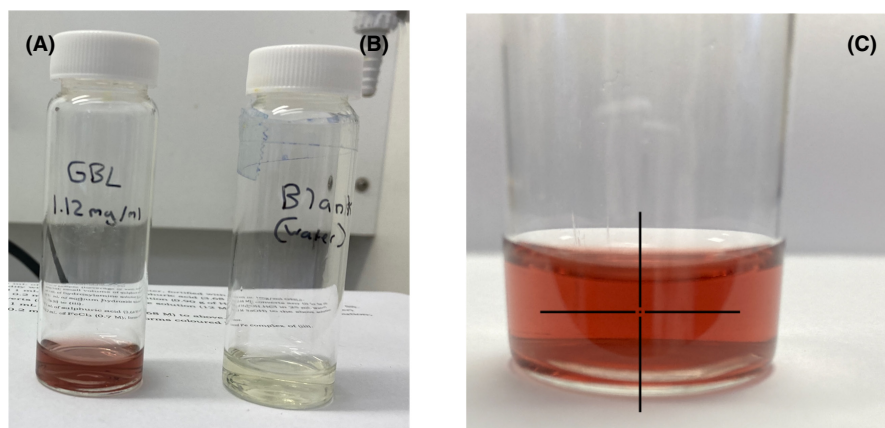


FIGURE 1 Color developed in the presence (A) and absence (B) of 1.12 mg/ml GBL, (C) 0.56 mg/ml GBL solution showing crosshairs of color picker and helper highlighting area where color was recorded. GBL, gamma-butyrolactone [Color figure can be viewed at wileyonlinelibrary.com]

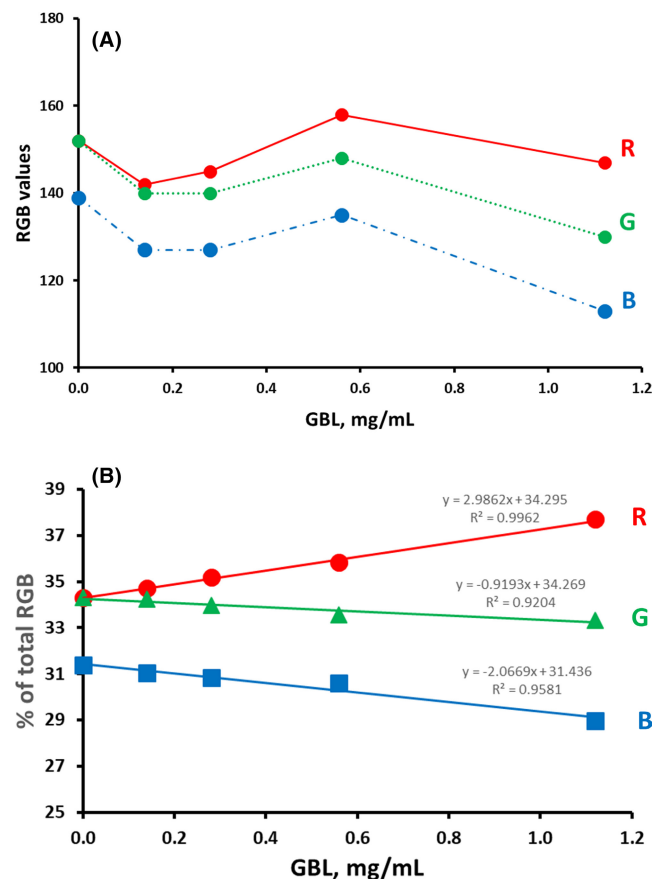


FIGURE 2 Plots of the relationship between the red (R), green (G) and blue (B) with GBL concentration as its hydroxylamine/ferric chloride derivative. Before normalization (A) and (B) after normalization. GBL, gamma-butyrolactone [Color figure can be viewed at wileyonlinelibrary.com]

In further investigations, we focused on the use of the R values for the determination of GBL as this was the most sensitive, giving the largest slope of the RGB values studied (Figure 2B). A limit of detection, based on 3σ , of 73.9 mg/L for GBL was found. Coefficient of variations of 1.90%, 1.10% and 2.28% for %R, %G and %B were obtained for a concentration of 0.56 mg/ml GBL. This is notably better than that reported by some other spectroscopy-based techniques applied to beverage samples [44,45]. Recent reports have also shown that similar results could be gained using cheaper camera devices [46].

3.3 | Analytical application

The possibility of determining the concentration of GBL in an alcoholic beverage was investigated. A 0.3 ml aliquot of the beer (Tyskie, a lager beer produced by Gronie) was taken and treated as described above using the optimized conditions. The concentration of GBL was determined by external calibration following blank correction using the normalized %R as part of the RGB color model. A mean recovery of 103% with an associated %CV of 0.70% intraday and

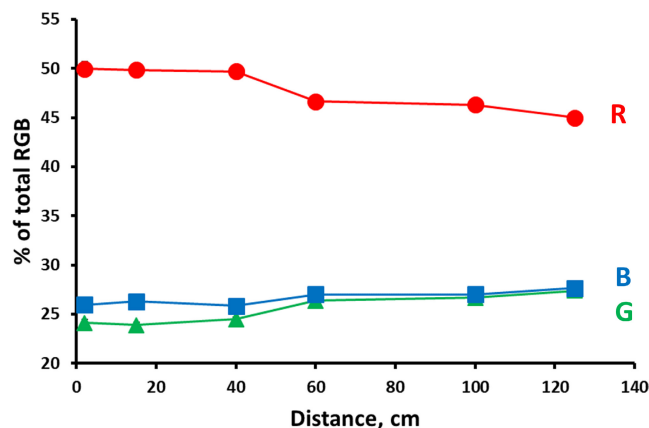


FIGURE 3 Plots of the relationship between the %red (%R), %green (%G) and %blue (%B) with distance of the smartphone camera from the color source. Each point is a mean of three of separate images [Color figure can be viewed at wileyonlinelibrary.com]

1.5% interday ($n = 5$) was obtained for the beer sample fortified at 0.56 mg/ml GBL. These data demonstrate that the proposed method has promise for the determination of GBL in such samples. It should be also mentioned that GHB can also be determined using the same approach. The analytical performance characteristics compared well with that obtained by a standard UV/Vis method utilizing a laboratory-based instrument. Using this approach, a mean percentage recovery of 82.4%, with an associated %CV of 12.7% ($n = 5$) was obtained for the same beer sample, fortified at 0.56 mg/ml GBL.

3.4 | Possible interferences

The method has been shown to be successful for the determination of GBL and GHB in a larger type beer. In future studies, we will explore the possibility of determining these and other drugs in a range of beverages. As with all colorimetric methods, the natural color of some beverages would prove to be a challenge. A number of other color models have been developed, such as the cyan, magenta, yellow and black (CMYK), and the hue, lightness, and saturation (HLS) models. However, these have generally not been applied analytically [47]. The possibility of determining more strongly colored beverages will be explored using technologies such as wavelength filters [48] and microfluidics [39], which could be used in combination with other detection systems such as electrochemical [49].

Beers, such as the lager we have studied here are reported [50] to contain a number of naturally occurring esters; principally ethyl acetate, at levels between 10 and 30 mg/L; with smaller levels of long chain and aromatic esters present. The levels of these are below the limit of detection of our developed method. Consequently, these levels will not interfere with our method when applied to such samples. The total ester content of distillates is reportedly notably higher and dependent on the spirit

in question [50]. Whiskies, depending on their origin, can have ester levels from 360mg/L up to 1010mg/L. Rums and brandies are also notably higher than beer, at 44 to 643mg/L and 300 to 6000mg/L, respectively. Bartos [51], has shown that the Fe-hydroxamate colored complexes of both ethyl acetate and GBL to have nearly equal molar extinction coefficients. As a result, our developed method will successfully work when applied to beer, but challenged by distillates such as whiskey and brandies.

4 | CONCLUSIONS

A simple and rapid method for the determination of GHB and GBL in a beverage sample using a colorimetric procedure and a smartphone has been successfully developed. Using the camera function of a smartphone and a free, readily available, downloadable App, forensically relevant concentrations could successfully be determined in beer. The method gives reliable results using an external calibration method with a mean percentage recovery of 103% (%CV = 0.70%, $n = 5$) obtained for a sample of beer fortified at a concentration of 0.56mg/ml. The determination of GBL levels required only a simple calculation of the percentage red component of the overall RGB determined. A theoretical detection limit of 73.9 mg/L was found and the developed method was shown to give similar performance to that gained by conventional, laboratory-based UV/Vis spectroscopy. To our knowledge, this is the first example of the application of this colorimetric procedure for the quantitative determination of GBL and the first employing smartphone technology. In future studies, we will investigate the possibility of using this technique to determine other drugs and compounds.

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