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Determination of Gamma-Hydroxybutyrate (GHB) and Gamma-Butyrolactone (GBL) by HPLC/UV-VIS Spectrophotometry and HPLC/Thermospray Mass Spectrometry

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ABSTRACT: This laboratory frequently receives samples that are suspected of being gamma-hydroxybutyrate (GHB), its lactone, gamma-butyrolactone (GBL), or a mixture of the two. We have developed an HPLC/UV-VIS spectrophotometric method for the separation and quantitation of GHB and GBL in illegal preparations that are available on the black market. The estimated detection limit is 50 ng injected onto the column. We are also reporting a simple and fast HPLC/thermospray mass spectrometric method for the confirmation of these compounds in illegal preparations. The characteristic mass spectrum for each compound could be obtained from as low as a 5 μg injection.

KEYWORDS: forensic science, gamma-hydroxybutyrate, high-performance liquid chromotography, thermospray liquid chromotography/mass spectrometry, substance abuse detection

γ-hydroxybutyrate (GHB) is an endogenous constituent of the mammalian brain where it is believed to play a role in neurotransmission (1). It is a suppressant of the central nervous system used in some European countries as an anesthetic adjunct and has also found some use in treating sleep disorders and alcoholism (2–5). Until the early 1990s, GHB was classified as an ''unapproved food additive'' by the FDA and was sold in health food stores as a food supplement under the brand names GHB, Gamma-OH, 4-Hydroxybutyrate, Gamma Hydrate, Somatomax and Sodium Oxybate, with reported effects of increasing growth hormone. GHB became popular among athletes as a ''steroid alternative'' because of its perceived anabolic benefits (6). In the late 1980s, it became apparent that GHB was associated with severe side effects such as extreme seizures, nausea, vomiting, dizziness, and disorientation. There were poisoning reports in several states (6–8). As a result, the U.S. Food and Drug Administration (FDA) designated GHB as an ''investigational new drug'' and banned its sale as a food supplement in 1990, following a rush of reports of adverse health effects. The FDA also called for its voluntary recall. Since its ban, GHB has been marketed illicitly to bodybuilders and athletes and to a lesser extent for weight control and as a sleep aid (9).

Recent reports indicate that GHB use is on the rise and it has been implicated in an increasing number of sexual assault cases and at least one death (10). Our laboratory has been involved in three GHB-related death cases. Reportedly, nearly 10% of all driving-under-the-influence (DUI) cases in a Florida community are the result of GHB ingestion. GHB is readily available because it is made from readily available components by following a simple recipe. This laboratory frequently receives samples that are suspected of being gamma-hydroxybutyrate, GHB, its lactone, gamma-butyrolactone (GBL), or a mixture of the two. These samples are typically white powders or clear liquids or both. The analysis of GHB and GBL (Fig. 1) is complicated by the small, polar nature of the molecules which results in short retention times on reversed phase columns. The absence of a strong chromophoric group makes detection by UV-VIS difficult at best. We have developed a high-performance liquid chromatography/ultraviolet-visible spectrophotometric (HPLC/UV-VIS) method for the separation and quantitation of GHB and GBL in illegal preparations that are available on the black market. Current mass spectrometric (MS) methods for these compounds involve derivatization followed by gas chromatography (GC/MS) (11,12), but these methods are not capable of distinguishing between GHB and GBL. We are also reporting a simple and fast HPLC/thermospray mass spectrometric method for the confirmation of these compounds in illegal preparations that are available on the black market.

Experimental

Reagents—HPLC-grade methanol and ammonium acetate were obtained from Fisher Scientific, Pittsburgh, PA. HPLC-grade water was prepared using a Milli-Q water purification system, from Millipore Corporation, Bedford, MA, and a Culligan Aqua-Cleer Reverse Osmosis system from Culligan Corporation, Northbrook, IL. GHB sodium salt (99.9% pure), γ-hydroxybutyric acid lactone (98.9% pure) and triethylamine were obtained from Sigma Chemical Co. St. Louis, MO. Twenty-five mM triethylammonium acetate was prepared by mixing equimolar amounts of triethylamine and acetic acid in water.

Apparatus—The liquid chromatography system consisted of a Model 1090 liquid chromatograph, equipped with an autosampler and a Rheodyne injector and photodiode array detector, all from Hewlett Packard, Palo Alto, CA. The mass spectrometer is a Model

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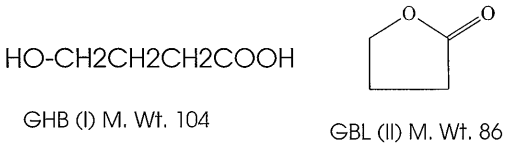

FIG. 1—*Structures of gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL).*

5989A equipped with a thermospray probe, from Hewlett Packard, Palo Alto, CA.

Sample preparation—Solid samples are prepared by dissolving about 20 mg of sample in 4 mL of 50% methanol in water, while liquid samples are typically prepared by diluting them 50-fold with 50% methanol in water. All samples were mixed 1 to 1 with the appropriate mobile phase before injection.

Preparation of standard—A 4000 μg mL^{-1} stock standard in 50% methanol in water was prepared. Working standards were prepared from the stock standards by further dilutions in the mobile phase.

Chromatographic conditions—The column was a 3.9 mm × 30 cm, 10 μm particle size, C-18 μBondapak from Waters Chromatography Division, Millipore Corporation, Bedford, MA. The binary mobile phase consisted of 70% buffer, 30% methanol at a flow rate of 1.0 mL min. The buffer is 10 mM KH_2PO_4 its ph adjusted to 3 with H_3PO_4. Duplicate injections of 1 or 5 μL each were made. The absorbance of GHB and its lactone were measured at 215 nm. For the mass spectrometric experiments the mobile phase consisted of 85% acetate buffer, 15% methanol at a flow rate of 0.75 mL/min. The acetate buffer is 25 mM ammonium acetate or triethylammonium acetate with the pH adjusted to 4 with acetic acid. We had to use different chromatographic conditions for the LC/UV-VIS and LC/MS experiments because of the incompatibility of the phosphate buffer with the thermospray LC/MS interface and the high UV cutoff of the acetate buffer.

Mass spectrometric conditions—Injection volume: 10 μL or as shown on the chromatogram; ion source temperature: 250°C; quadrupole temperature: 100°C; ionization mode: discharge on; thermospray probe stem temperature 115°C; multiplier voltage: 1632V, scan threshold: 30; mass range scanned: 80 to 220 amμ.

Results and Discussion

Figure 2*a* shows the chromatogram of a standard mixture that contains both GHB (2 μg) and GBL (1.5 μg). The two compounds are well resolved. The chromatogram for a sample that contains a mixture of the two compounds is shown in Fig. 2*b*. The relatively clean nature of these samples provides a simple chromatogram despite the minimal sample preparation. The calibration curve is linear from 500 ng to 1500 μg on-column for GBL, with a correlation coefficient of 0.99999 for a five-point calibration. For GHB the linear range is much narrower, 500 ng to 30 μg, with a correlation coefficient of 0.99999 for a three-point calibration. Injection of amounts in excess of 30 μg of GHB resulted in severe peak splitting. The percent relative standard deviation of five determinations at 16 μg of GHB and 35 μg of GBL injected was less than 2% in both cases. The determination limit, defined as the amount of analyte that will produce a signal higher than ten times the standard deviation of the signal for the blank, is 150 ng for GHB and 110 ng for GBL.

Figure 3 shows the total ion chromatogram for a standard that contains 14 μg of GHB and 20 μg of GBL. The total ion chromatogram for a sample that contains a mixture of the two compounds

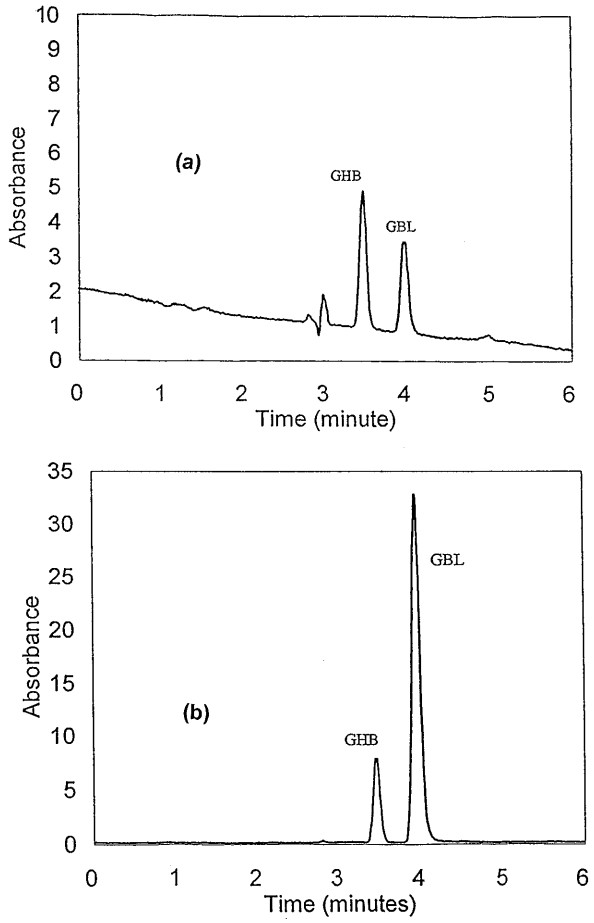

FIG. 2—*(a) Chromatogram of a UV-VIS trace of 5 μL injection containing 0.4 mg mL^{-1} of GHB and 0.3 mg mL^{-1} of GBL. (b) Chromatogram of a UV-VIS trace of 2 μL injection of a sample containing both GHB and GBL.*

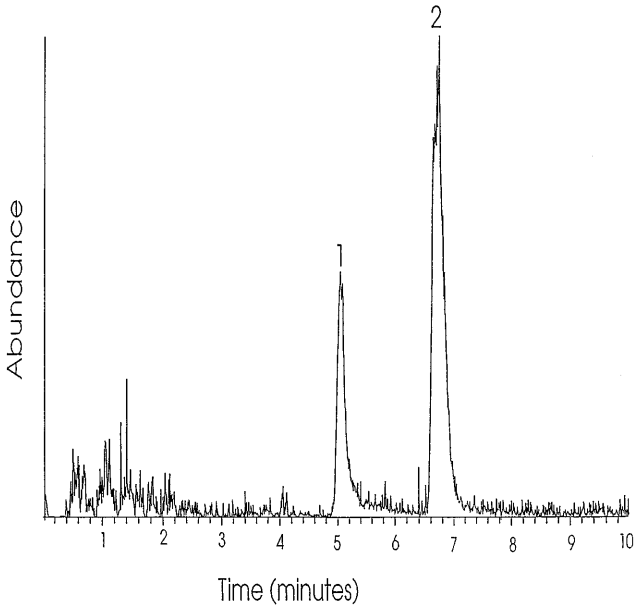

FIG. 3—*Total ion chromatogram of a standard containing GHB and GBL.*

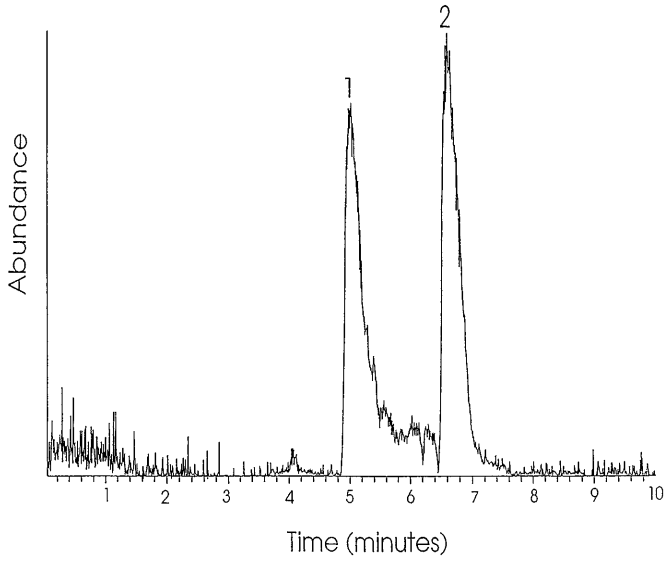

FIG. 4—*Total ion chromatogram of a sample containing GHB (1) and GBL (2).*

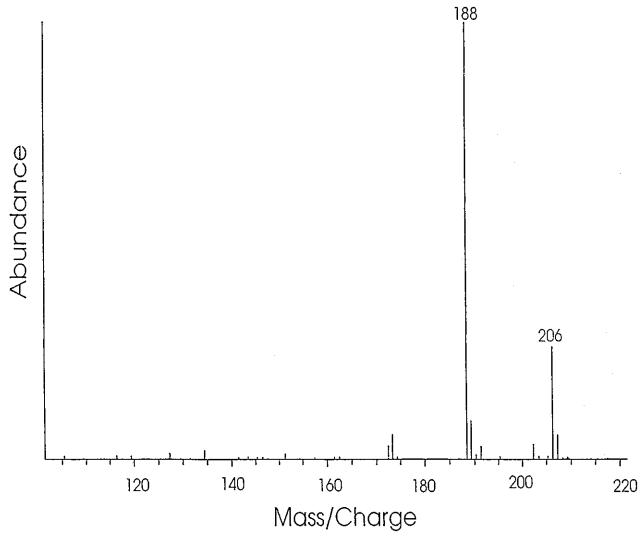

FIG. 6—*Mass spectrum corresponding to GHB obtained with triethylammonium acetate buffer.*

is shown in Fig. 4. Figure 5*a,b* shows the mass spectra for the two peaks, corresponding to GHB and GBL in the sample. The ion at mass 104 in the GHB spectrum corresponds to the $[M + NH_4]^+$ ion for the lactone form and the ion at 122 corresponds to the $[M + NH_4]^+$ ion for the GHB. The ion at 105 probably corresponds to $[M + H]^+$ for gamma-hydroxybutyric acid. The presence of the ion that corresponds to the lactone in the GHB peak is a result of the conversion of GHB to its lactone in the ion source. This is evident from Fig. 6 where changing the adduct ion from ammonium to triethylammonium gives ions at 188 and 206 for GHB standard, corresponding to the $[M + \{NH(C_2H_5)_3\}]^+$ ions for the lactone and GHB, respectively. The characteristic mass spectrum for each compound could be obtained from as low as 5 μg of analyte. In the Forensic Chemistry Center, additional spectral information is obtained by GC-MS or Fourier Transform Infrared Spectroscopy (FTIR) if needed, though neither method is capable of differentiating between GHB and GBL.

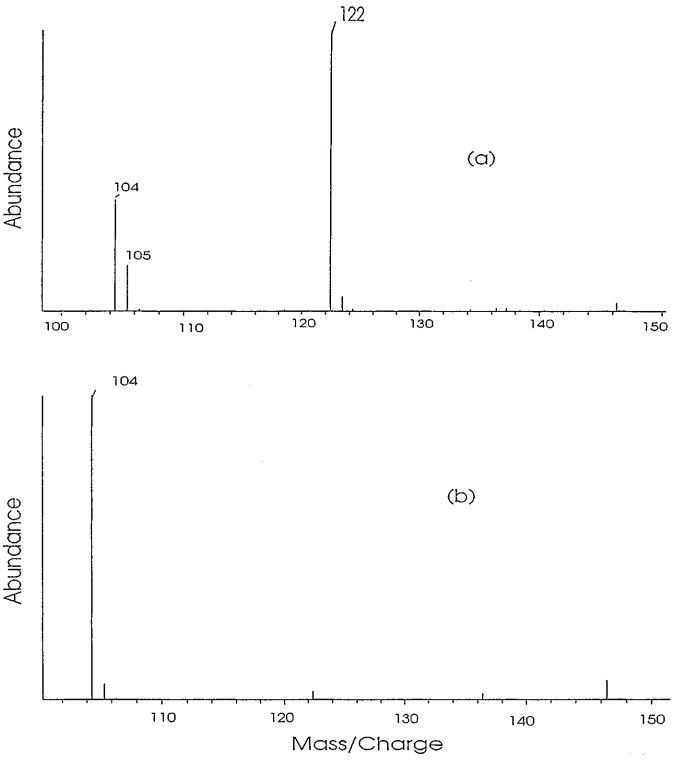

FIG. 5—*Thermospray mass spectra corresponding to GHB (a) and GBL (b) in a sample.*

Conclusions

This method provides a simple, fast, and sensitive procedure for the determination of GHB and its lactone in illegal preparations that are available on the black market. It provides also a simple and sensitive procedure for the confirmation of GHB and its lactone in black market preparations. The method is capable of differentiating between GHB and its lactone, GBL. The apparent lack of sensitivity is not a problem as the levels of GHB and its lactone in these preparations are in the percent range.

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