

TECHNICAL NOTE

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Comparative Analysis of Gamma-Hydroxybutyrate and Gamma-Hydroxyvalerate Using GC/MS and HPLC*

ABSTRACT: This paper describes two analytical techniques used to separate and quantify gamma-hydroxybutyrate (GHB) and gamma-hydroxyvalerate (GHV). The first technique was a *N,O*-bis(trimethylsilyl)trifluoroacetamide-trimethylchlorosilane derivatization, followed by gas chromatography/mass spectrometry analysis using an HP-5 capillary column at a rate of 1.0 mL/min with a run time of 9.25 min. This technique was found to be sensitive (LOD 1 pg on column) and gave a low average error (5%) in a beverage study. When supplemented by a surrogate spike, the method yielded 97% analyte recovery from beverages. The second technique was high-performance liquid chromatography/UV (HPLC/UV) using a C-18 column with a (20:80% v/v) methanol:dibasic phosphoric buffer (10 mM, pH 3) at a rate of 1.00 mL/min with a run time of 7.5 min. UV detection occurred at 254 nm. This method was found to be less sensitive (LOD 0.05 µg on column) for direct analysis of aqueous samples. To remove interferences seen in the beverage study, a liquid-liquid extraction before HPLC analysis was tested. However, a decreased sensitivity (LOD 100 µg on column) and irreproducible peak profiles resulted.

KEYWORDS: forensic science, drug analysis, gamma-hydroxyvalerate, 4-methyl gamma-hydroxybutyrate, gamma-hydroxybutyrate, beverage, drug-facilitated sexual assault, gas chromatography, surrogate spike, high-performance liquid chromatography

Gamma-hydroxybutyrate (GHB) has become well known for its illicit uses, including its use as a date rape drug. Gamma-hydroxyvalerate (GHV) is the 5-carbon analog of GHB. GHV has been shown to have effects similar to GHB (namely sedation, catalepsy, and ataxia), but requires a higher dosage, potentially increasing its toxic and/or lethal effects (1). GHV is listed by the Food and Drug Administration as generally regarded as safe (GRAS) and may be found as an additive in commercial products or available as a dietary supplement. Given the increased dosage level, the inherent toxicity of GHV is of significant concern, and its detection and characterization will become an important issue in forensic toxicology and solid dose analysis. Like GHB, GHV may be abused in recreational settings by mixing with water or alcoholic beverages or may be used in drug facilitated sexual assault (DFSA) (2). An average dose or spike is anecdotally reported to be between 3 and 8 g in a 12 oz (355 mL) serving, correlating to between 0.8% and 2.3% w/v (8400–22,500 p.p.m.) (3).

The similarities between GHB and GHV extend to synthesis from their analogs. When reacted with sodium hydroxide, gamma-valerolactone (GVL), and gamma-butyrolactone (GBL) undergo a

saponification conversion to the sodium salts (NaGHB and NaGHV, respectively) (4). These chemical similarities should be exploited to formulate accurate and sensitive confirmatory tests for simultaneous identification and quantification of GHB and GHV. Several analytical methods have been reported for the confirmation of GHB. In this study, a gas chromatography/mass spectrometry (GC/MS) method and a high-performance liquid chromatography/UV (HPLC/UV) method were evaluated to detect GHV, discriminate GHV from GHB, and quantitate both drugs and their lactones in beverages.

GC/MS is readily available to most forensic laboratories and is an effective method for the detection of GHB and GBL (5–9). Sample preparation for GC analysis is generally more time consuming than LC because GHB should be derivatized to counter the thermal degradation of the small molecule. In addition, derivatization followed by GC/MS is not sufficient for simultaneous detection of GHB and GBL because derivatization induces ring opening of the lactone. Derivatized products of GHB and GBL would be identical (10–12). This study also found that using a surrogate spike improved the GC/MS method by providing a means of tracking analyte recovery.

Surrogate spikes are used in environmental analytical methods to measure analyte recovery for individual samples. A surrogate compound is one that is chemically similar to the analyte and is thus expected to respond to analytical conditions similar to the analytes. Surrogates must be compounds that are not expected to be found in samples themselves. These criteria are the same as those used to select an internal standard; the difference is in how the two compound types (surrogates and internal standards) are

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integrated into the analysis and how the resulting data are interpreted. An internal standard is used as the basis of a ratio of concentrations, and responses generate a calibration curve. The surrogate is treated as an analyte and the percent recovery of the surrogate, determined using an internal standard calibration method, is used to gauge the recovery for a particular individual sample. A surrogate spike provides an additional level of quality assurance and can identify intrinsically difficult matrices within a sample batch. This capability is valuable in the context of GHB and related analyses, given the known difficulties associated with these samples and matrices (6,13,14).

HPLC is an instrument common to toxicology laboratories but less common in other forensic settings. HPLC offers a simple and fast analytical method for the separation and confirmation of GHB and GBL (14–17). A key advantage of HPLC is the ability to analyze directly solutions of GHB and GBL while simultaneously detecting both the free acid and lactone species.

The goal of this study was to investigate accepted analyses for GHB and determine the method best suited for the simultaneous detection and quantitation of the sodium salts of GHB and GHV (NaGHB and NaGHV), as well as the lactones GBL and GVL, while still offering a valid means for analyzing suspect beverages.

Materials and Methods

Solutions of 99% GVL and GBL were obtained (Fluka, Ronkonkoma, NY) as was solid NaGHB (Sigma-Aldrich, St. Louis, MO). NaGHV was synthesized via the hydrolysis of GVL with NaOH. The GHV synthesis method was validated using ^1H nuclear magnetic resonance (NMR) to elucidate the structure of the reaction product and to assess its purity.

GC/MS

The internal standard was 1,5-pentanediol and the surrogate spike was 1,2-hexanediol; both were obtained from Sigma-Aldrich. The derivatizing agent was *N,O*-bis(trimethylsilyl) trifluoro-acetamide (BSTFA) with trimethylchlorosilane (TMCS; Supelco, Bellefonte, PA). The derivatization reaction was catalyzed by *N,N*-dimethylformamide (DMF) obtained from EMD (Gibbstown, NJ). The GC–MS used in this study was an Agilent gas chromatograph model 6890 (Agilent, Santa Clara, CA) with mass-selective detector model 5973.

The GC was operated in the splitless injection mode with an injection port temperature of 250°C. The carrier gas was helium and the flow rate was maintained at 1.0 mL/min. An initial oven temperature of 50°C was held for 5 min. The temperature was then ramped at a rate of 10°C/min to 100°C, held for 1 min, followed by a temperature ramp of 40°C/min to a final temperature of 230°C. The total analysis time was 9.25 min including a 6-min solvent delay. An HP-5 capillary column was used (30 m × 0.25 mm i.d. × 0.25 μm film). Quadrupole mass spectrometry was used for detection.

To create a calibration curve, standard solutions containing dissolved NaGHB and NaGHV were spiked with the internal standard 1,5-pentanediol (PD) and the surrogate 1,2-hexanediol (HD). Nine replicate calibration curves were analyzed and averaged. For the beverage study, a variety of beverages were used, including: water, Tropicana[®] cranberry juice cocktail with Barton[®] vodka (1.5 oz vodka and 10.5 oz cranberry juice), Coca Cola[®], Guinness Stout[®] beer, Coors Light[®] beer, and Willi Haag[®] Riesling (2003). All beverages were obtained from local stores. These beverages were spiked with NaGHB and NaGHV at

10,000 p.p.m. The spiked beverages were diluted 1:100 with the internal standard solution. All solutions, standards (for the calibration curve), and beverages (for the beverage study) were prepared for GC analysis by derivatization with BSTFA—TMCS; this reaction was catalyzed with DMF. Derivatized solutions were then capped, vortexed, and heated at 70°C for 15 min. The derivatized sample was directly injected into the GC/MS. Quantitation was based on an internal standard method; derivatized GHB was quantitated using the peak area of the ion at 233 *m/z* and derivatized GHV was quantitated using the peak area of the ion at 117 *m/z*. Beverage samples were analyzed in triplicate.

HPLC/UV

The binary mobile phase was made using methanol (HPLC grade, J. T. Baker, Phillipsburg, NJ) and 10 mM dibasic phosphoric buffer (KH₂PO₄; Sigma-Aldrich) adjusted to pH 3 using phosphoric acid (J. T. Baker) in a 20:80 methanol:buffer ratio. HPLC solutions were filtered with a Millipore[®] filtration system (Millipore, Billerica, MA). The HPLC–UV used in this study was a Perkin Elmer 200 series (Perkin Elmer, Wellesley, MA) with an autosampler, UV/VIS detector (set to 254 nm), a pump, and a data handling system. A laboratory shaker (model RKVSD) was obtained from Appropriate Technical Resources (Laurel, MD).

The column was a 3.9 mm × 300 mm 10 μm particle size C-18 μ Bondapak from Waters Chromatography Division, Millipore Corporation (Bedford, MA). The binary mobile phase consisted of 80% buffer and 20% methanol at a flow rate of 1.00 mL/min. The total analysis time was 7.5 min.

Standard solutions contained all four analytes of interest (GHB, GHV, GBL, and GVL). A calibration curve generated from area under the peak versus concentration. The standards used to make the calibration curve were analyzed nine times and averaged to create the curve. The following beverages were spiked with all analytes at a concentration of 10,000 p.p.m.: deionized water, Tropicana[®] cranberry juice cocktail with Barton[®] vodka (1.5 oz vodka and 10.5 oz cranberry juice), Coca Cola[®], Guinness Stout[®] beer, Coors Light[®] beer, and Willi Haag[®] Riesling (2003). Beverage samples were analyzed in triplicate by direct injection. The following extraction scheme was implemented to reduce interferences. One milliliter of solution was extracted with 5 mL of chloroform and shaken for 5 min at 40 r.p.m. One milliliter of chloroform was removed, evaporated to dryness, and reconstituted in 100 μL of mobile phase to remove lactones for separate identification and quantitation. From the chloroform extraction tube, 0.5 mL of the

TABLE 1—Results of GC/MS and HPLC/UV calibration.

	Analyte	GC/MS	HPLC/UV
LOD	GHB	0.001 p.p.m.	5 p.p.m.
	GHV	0.001 p.p.m.	5 p.p.m.
	GBL		5 p.p.m.
	GVL		5 p.p.m.
Range	GHB	0.01–10 p.p.m.	10–750 p.p.m.
	GHV	0.01–10 p.p.m.	10–750 p.p.m.
	GBL		10–750 p.p.m.
	GVL		10–750 p.p.m.
<i>R</i> ²	GHB	0.999	0.998
	GHV	1.000	0.9987
	GBL		0.9991
	GVL		0.9982

GC/MS, gas chromatography/mass spectrometry; GHB, gamma-hydroxybutyrate; GBL, gamma-butyrolactone; GHV, gamma-hydroxyvalerate; GVL, gamma-valerolactone; HPLC/UV, high-performance liquid chromatography/UV.

aqueous phase was removed; the aqueous phase is believed to still contain the free acid species. To the aqueous phase, 0.5 g NaCl and 5 mL ethyl acetate were added and the sample was shaken at 40 r.p.m. for 5 min. One milliliter of ethyl acetate was removed, evaporated, and reconstituted in 100 μ L of mobile phase. This extraction was modified from Elliot and Burgess(5). Extracted samples were analyzed in triplicate on two separate occasions.

Results

GC/MS

The results of the GC analysis of standard solutions are shown in Table 1. The derivatization efficiencies of GHB and GHV range from 88.5% to 100% and 92.9% to 100%, respectively, when the GC/MS analytical procedure is used on beverages. Efficiencies were

calculated by dividing the resulting ion peak areas for derivatized analytes to the ion peak areas obtained during calibration. The average error of the technique was found to be 5%. Figure 1 shows a typical total ion chromatogram (1) for derivatized GHB, derivatized GHV, and internal standards in water compared with the TIC for the analytes in Coors Light[®]. Table 2 shows the results of the beverage analysis. The surrogate was added at a known concentration and has a molecular structure similar to the analytes of interest; thus it is useful for forensic beverage analysis to monitor analyte recovery from inconsistent and complicated matrices.

HPLC/UV

Figure 2 shows a typical chromatogram of all four analytes in an aqueous solution. HPLC analysis of a solution containing

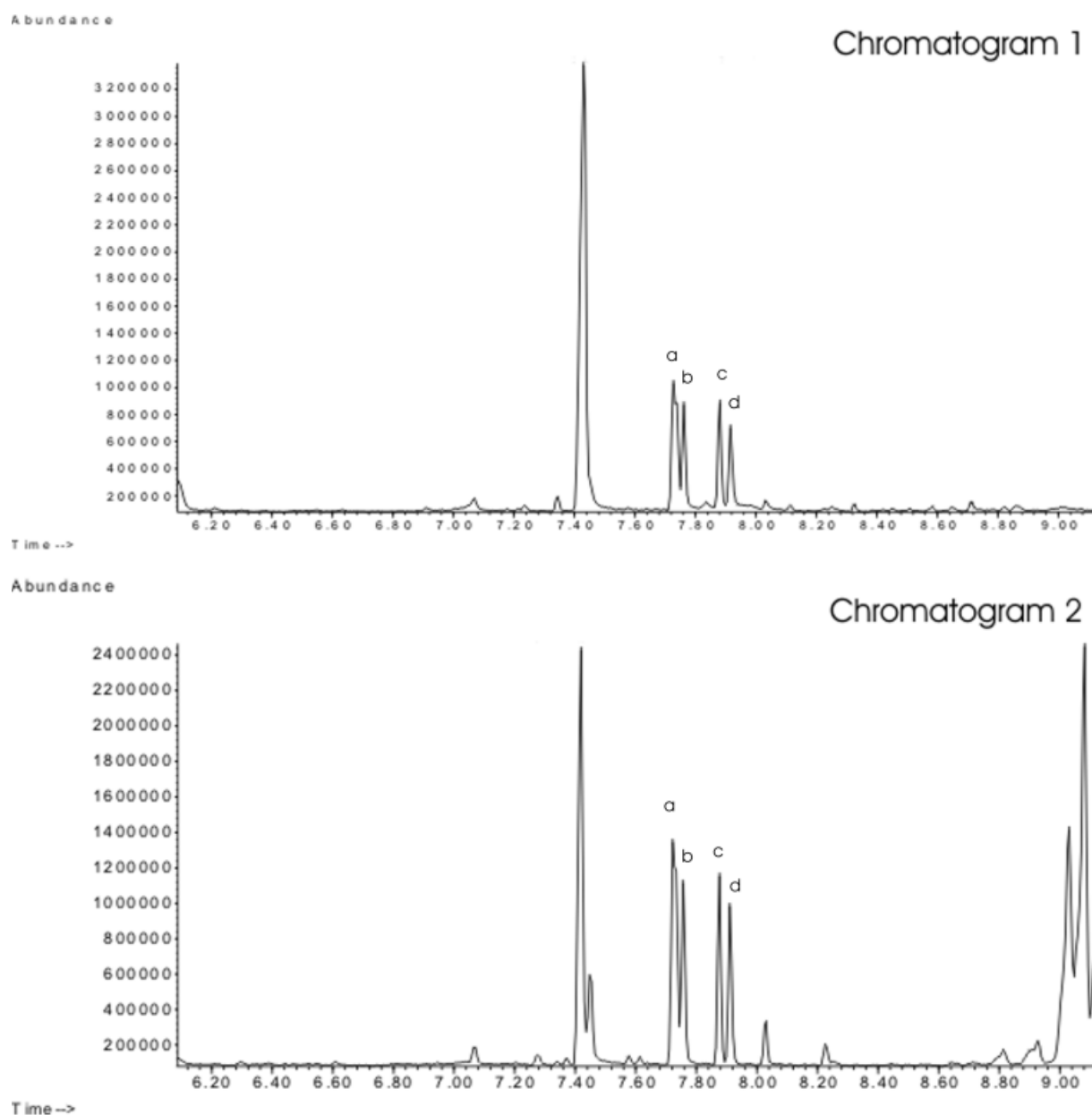


FIG. 1—Chromatogram 1 shows (a) 1,5-pentandiol, (b) gamma-hydroxybutyrate, (c) gamma-hydroxyvalerate, and (d) 1,2-hexanediol in deionized water; chromatogram 2 shows the same analytes in Coors Light[®]. No interfering peaks are seen. Peaks typical for the derivatizing agent are seen at earlier retention times than analytes, while peaks attributed to Coors Light[®] are seen at later retention times.

TABLE 2—Results of the beverage study by GC/MS.

Beverage	NaGHB		NaGHV		Surrogate Spike	
	Actual	Measured	Actual	Measured	Actual	Measured
Cran and Vodka	0.96	0.85 ± 0.04	1.29	1.23 ± 0.05	0.98	0.93 ± 0.01
Coors Light [®]	1.02	0.93 ± 0.11	1.10	1.10 ± 0.14	0.98	0.94 ± 0.02
Coca Cola [®]	1.06	0.96 ± 0.11	1.06	1.19 ± 0.07	0.98	0.99 ± 0.08
Guinness Stout [®]	1.01	0.92 ± 0.04	1.01	0.98 ± 0.05	0.98	0.94 ± 0.02
Riesling	0.96	0.97 ± 0.03	1.12	1.04 ± 0.04	0.98	0.94 ± 0.01
Water	0.98	1.00 ± 0.07	0.93	0.96 ± 0.04	0.98	0.98 ± 0.03

GC/MS, gas chromatography/mass spectrometry; GHB, gamma-hydroxybutyrate; GHV, gamma-hydroxyvalerate.
All units are p.p.m. ($\mu\text{g}/\text{mL}$).

10,000 p.p.m. GHB, GHV, GBL, and GVL in Coors Light[®] resulted in interfering peaks that prevented identification of the analytes (Fig. 3). To combat interferences, a modified extraction procedure was used in this method.

Early chloroform extractions of GHB and GBL in water yielded a predominant GBL peak with a small GHB peak, while the aqueous extraction yielded a GHB peak. However, when the extraction procedure was used on water samples containing all four analytes, insufficient peak areas were obtained and quantification was not viable. This was also the case for spiked beverages. The extractions also yielded results that were not reproducible. For example, a free acid peak was sometimes seen in the chloroform extract at the same intensity as the lactone peak. In the same manner, lactone peaks would sporadically appear in the aqueous extract. This could be due to an equilibrium shift between GHB and GBL occurring in the extraction solutions or due to partitioning of the analytes in the absence of interconversion. In addition, some beverages produced interfering peaks in the extracts. The liquid-liquid extraction, followed by HPLC analysis was irreproducible for all solutions containing 10,000 p.p.m. GHB, GHV, GBL, and GVL.

Discussion

GC/MS and HPLC/UV methods were optimized for the simultaneous detection and quantitation of GHB and GHV in aqueous solutions. Derivatization, followed by GCMS was found to be a suitable technique for differentiating and quantifying GHB and GHV in aqueous solutions and beverages.

Direct injection HPLC/UV was found to be suitable for the identification and quantitation of all analytes (GHB, GHV, GBL, and GVL) in aqueous solutions. However, the method was found to be difficult to adapt to beverage analysis due to matrix interference. An extraction procedure was developed before HPLC analysis, but was found to have a significantly higher LOD and led to peak profiles that could not be reproduced.

The derivatization of analytes, followed by the GC/MS analytical method presented here was found to be best suited for forensic analysis of seized beverages believed to contain GHB and GHV. The use of a surrogate, in addition to an internal standard, was found to be an ideal application for forensic analyses. A surrogate can ensure the effectiveness of forensic methods conducted on complex and inconsistent matrices.

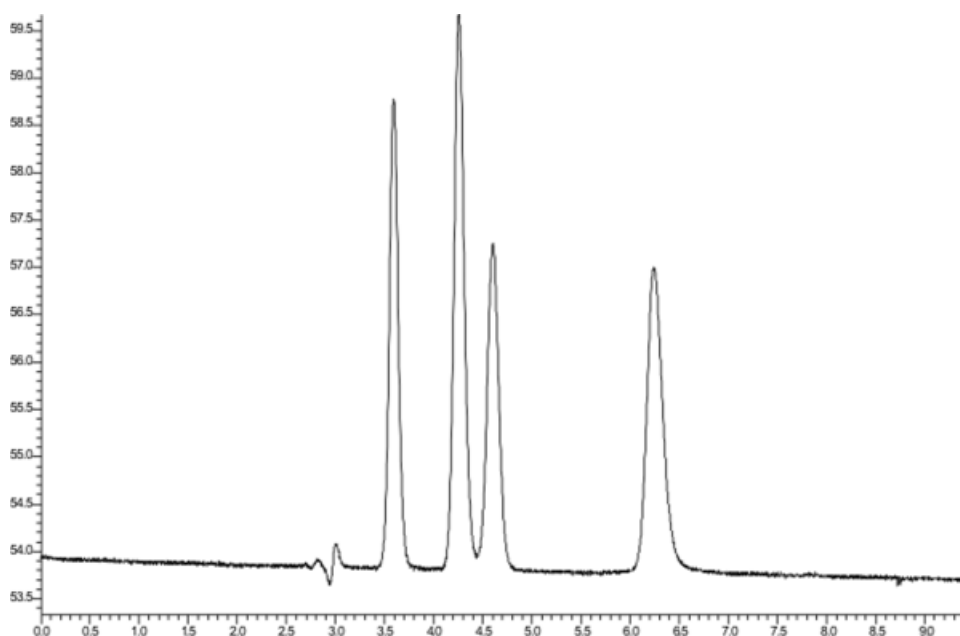


FIG. 2—An LC chromatogram of (a) gamma-hydroxybutyrate, (b) gamma-butyrolactone, (c) gamma-hydroxyvalerate, and (d) gamma-valerolactone in deionized water.

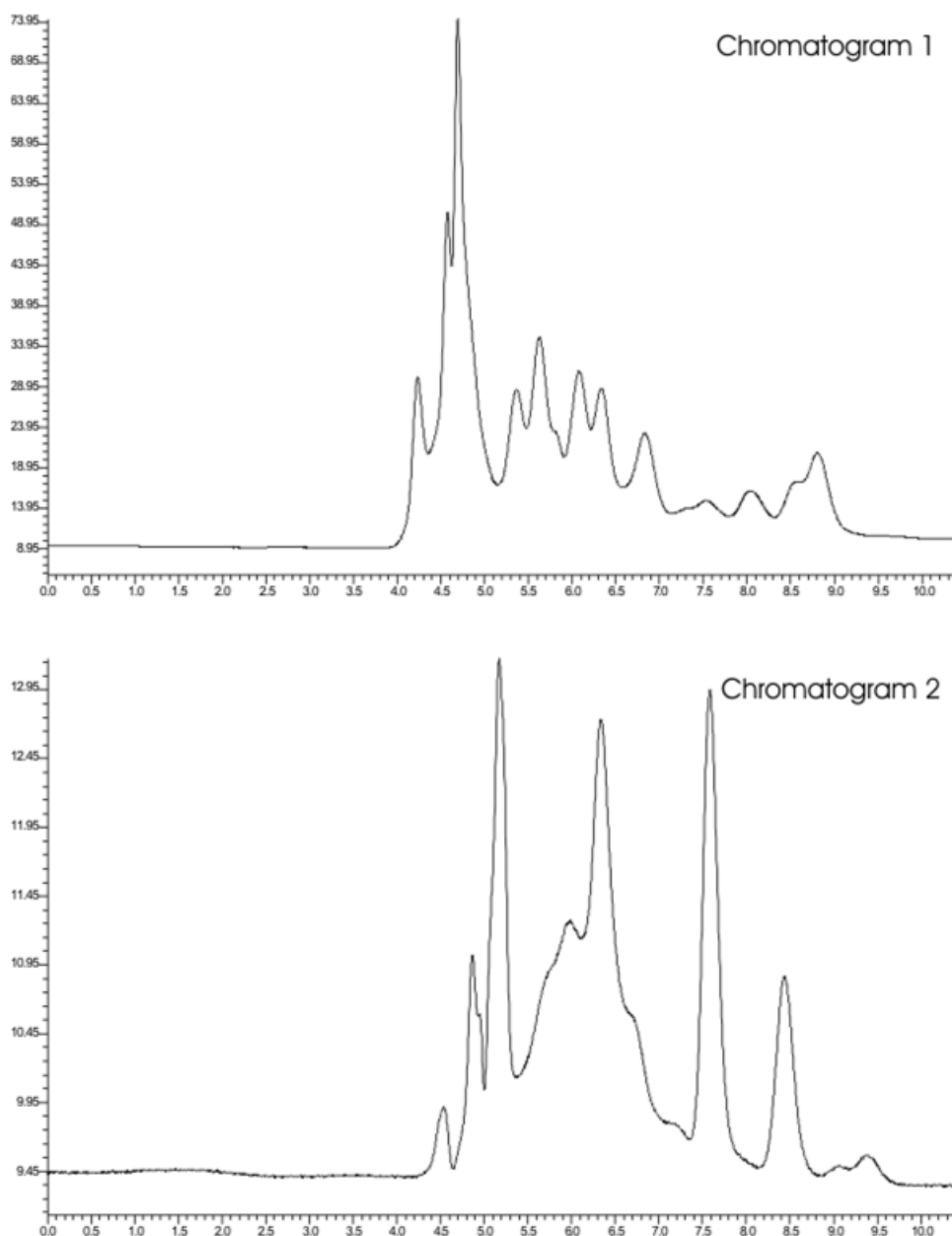


FIG. 3—Chromatogram 1 shows blank Coors Light[®], while chromatogram 2 shows Coors Light[®] containing 150 p.p.m. gamma-hydroxybutyrate, gamma-hydroxyvalerate, gamma-butyrolactone, and gamma-valerolactone.

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