TECHNICAL NOTE

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Detection of Gamma-Butyrolactone (GBL) as a Natural Component in Wine

REFERENCE: Vose J, Tighe T, Schwartz M, Buel E. Detection of gamma-butyrolactone (GBL) as a natural component in wine. J Forensic Sci 2001;46(5):1164–1167.

ABSTRACT: The compound gamma-butyrolactone (GBL) was found in extracts from samples of unadulterated wines. This finding indicates that GBL is a naturally occurring component in some wines and may be present in similar products. The concentration detected was approximately 5 μg/mL and was easily observed using a simple extraction technique followed by GC/MS analysis. These results illustrate the need to carefully examine an allegedly adulterated sample's matrix before determining a sample was laced with GBL.

KEYWORDS: forensic science, gamma-butyrolactone, gamma-hydroxybutyric acid, wine

Gamma-butyrolactone (GBL) is an industrial solvent and a List 1 chemical under federal regulation that may be used to illegally produce gamma-hydroxybutyric acid (GHB), which is federally controlled as a Schedule I controlled substance. Originally developed as a surgical anesthetic, the use of GHB was discontinued in the United State due to its many side effects although it is still used in Europe as an adjuvant to anesthesia. The U.S. Food and Drug Administration banned over the counter sales of GHB in 1990, but it continues to be illicitly used as a purported releaser of human growth hormones, as a sleep aid and for weight control. Recently, GHB has become popular for abuse and much media attention has been given to its use as a "date rape" drug. In this use, GHB may be added to a drink and when consumed, render the victim helpless and open for attack. It is the submission of this mixture of drug and drink to the forensic laboratory that may pose analytical problems. The analysis of such possible mixtures must be undertaken with care and with an understanding of the matrix to which GBL or GHB was added.

GHB is a small four-carbon molecule with both an acid and alcohol functional group. The molecule resembles the neurotransmitter gamma-aminobutyric acid (GABA) where the alcohol group in GHB is substituted for an amino group, Fig. 1. During normal brain metabolism, GABA is converted to GHB (1) where it may have some physiological function (2). GHB is also formed through the conversion of ingested GBL (2). In addition to brain tissue, GHB, has also been found in other body tissues including kidney, heart, and skeletal muscle (3).

A variety of techniques have been detailed for the analysis of GHB and GBL (4–8). The inter-conversion of the compounds during analysis has also been raised as a concern (9). This inter-conversion in aqueous solutions is pH dependent with solutions at seven and above favoring the formation of GHB from GBL, with a true equilibrium established at pH 2 (10). The conversion of GBL to GHB under pH conditions between two and seven was studied but no equilibrium conditions were observed under the time frame examined (10). The analysis of GHB (or the salt form) or GBL may be performed using infrared spectroscopy, or mass spectrometry when GHB is derivatized. Some analytical techniques convert any GHB present in a sample to GBL for analysis by GC/MS. When conducting these analyses, one must be aware of the matrix in which the material is present. If the matrix is a complex mixture of natural occurring components, one must assess whether the drug detected has been added or is in fact naturally occurring. We report the analysis of a variety of wines in which GBL was detected as a naturally occurring constituent.

Materials and Methods

Wines were obtained from laboratory staff or purchased. Gamma-butyrolactone was purchased from Sigma, St. Louis, MO. Approximately 6 mL of wine were extracted with 0.2 mL of chloroform containing 50 μg/mL octadecane, which served as a gas chromatography retention time reference. The chloroform extract was then analyzed by gas chromatography-mass spectrometry without further preparation.

GC/MS Analysis

Samples were analyzed using a Hewlett Packard 5890 Gas Chromatograph equipped with a Model 5970 Mass Selective Detector, (Hewlett Packard, Palo Alto, California). Hewlett Packard Chemstation Software, version A.03.00, was used to collect and analyze the data. The instrumental conditions were as follows:

Column: 20 m by 0.18 mm, DB-5 (J and W Scientific, Folsom, CA)
Injector temperature: 250°C
Injector volume: 1 μL
Transfer line temperature: 280°C

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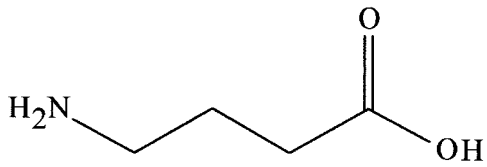
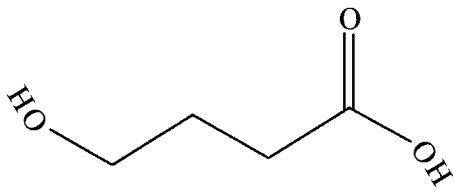
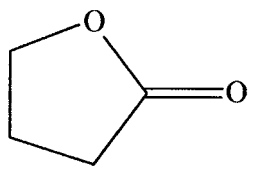
Received 16 June 2000; and in revised form 29 Aug. 2000; accepted 3 Jan. 2001.



Gamma-aminobutyric acid



Gamma-hydroxybutyric acid



Gamma-butyrolactone

FIG. 1—*Structures for GABA, GHB, and GBL.*

TABLE 1—*Summary of examined wines.*

Red Pinoir Noir	Red Cabernet Savignon
Red Merlot	Blended Red Wine
White Chablis	Dry Sherry
Zinfindel	Burgundy
Chardonnay	White Blend
Italian White	

Temperature program: 60°C for the first minute, then 15°C/min to 300°C, MS solvent delay 3 min, total run time 23 min.
Carrier gas: Helium at 10.2 psi
Ion range: 15 to 550 aμ

Retention time consistency was established by including octadecane in the extraction solvent and converting all retention times into relative retention times by dividing the retention time of the compound of interest by the octadecane retention time.

Results and Discussion

All but one of the wines tested contained a small amount of GBL. No detectable GBL was observed in the Italian white wine. The wine extracts yielding a positive GBL result contained a peak that had both the relative retention index and mass spectrum matching the standard GBL. Table 1 details the wines examined. No attempt was made to quantitate possible differences in GBL concentrations between wine samples. The observation that these wines contained GBL was demonstrative of the need to run proper controls in the analysis of GBL. A quantitative estimate revealed that the Red Merlot contained approximately 5 μg/mL of GBL. Based on simple

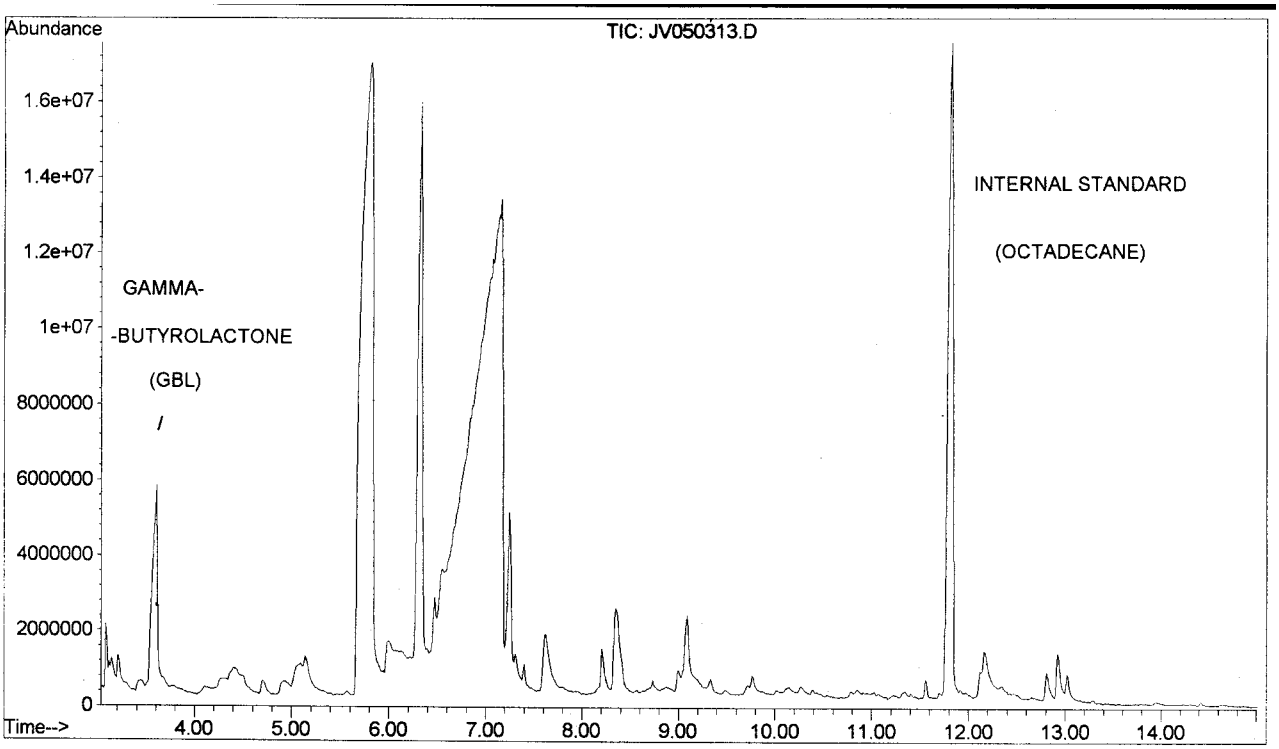


FIG. 2—*Total ion chromatogram for typical wine extract.*

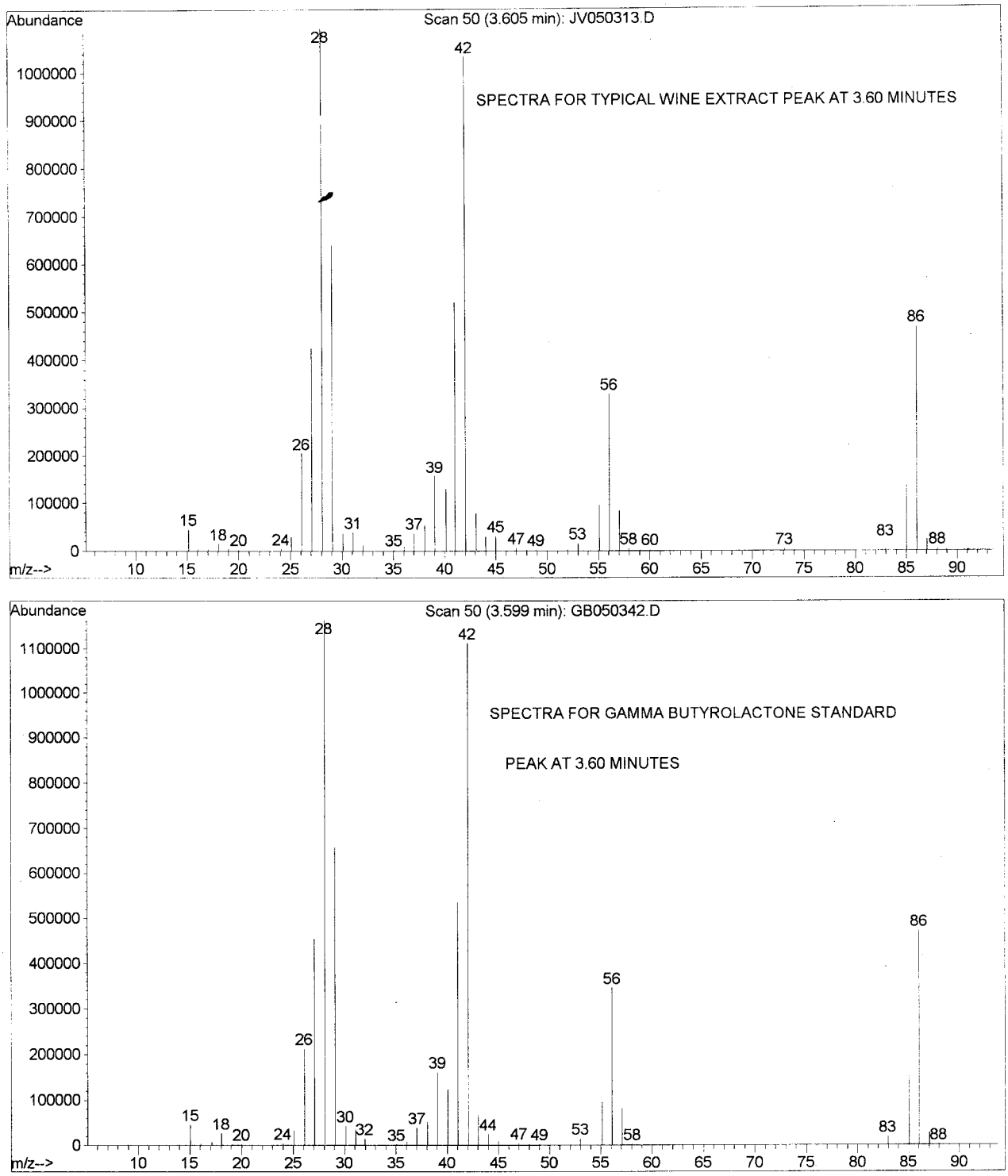


FIG. 3—*Mass spectrum for peak in wine extract at a relative retention index of 0.30 compared to the mass spectrum for gamma-butyrolactone standard.*

comparisons of peak height, the other wines contained similar concentrations of GBL. None of the wines tested contained significantly higher levels of GBL. Figure 2 shows the total ion chromatogram obtained after GC/MS analysis of an extract. Many components are present in the extract, with GBL eluting at 3.60 min under the described conditions. The relative retention index to octadecane was 0.30. The mass spectrum for the component eluting at 3.60 min is compared to the GBL standard in Fig. 3.

The approximate pH of the wines examined was between three and four. One would expect that under these conditions measurable amounts of GHB would be present due to the equilibrium that exists between these two compounds in an aqueous solution. Addition of GHB to the wines would result in the formation of GBL and vice versa. We made no attempt to ascertain if GHB was present in these wines. However, finding GHB would not be unexpected. The simple chloroform extraction procedure used here is not suitable

for the extraction of the salt forms of GHB, which are insoluble in chloroform. Alternative extraction procedures or the direct examination of the solution by high pressure liquid chromatography (HPLC) could be attempted to identify the relative concentrations of both GHB and GBL (10).

The initial wine sample that was analyzed by this laboratory was obtained from a case in which it was suspected that an adulterant had been added to the wine. Through routine drug analysis extraction procedures, no drugs were found in the sample except for the presence of a small amount of GBL. An extraction of the same vintage of wine from an unopened bottle revealed a similar amount of GBL. Through further study it was concluded that a variety of wines contain GBL, which may interfere with the analysis of wines, adulterated with this drug. When considering a suspected adulterated wine, it may be possible to conduct comparative quantitative analyses to assess whether the amount of GBL present is at a natural occurring or elevated level.

Further preliminary work has shown that grape juice does not contain GBL, indicating GBL may be produced during the fermentation process. Both GBL and GHB are relatively small molecules where GHB is a fatty acid alcohol, and GBL its condensation product. Both compounds are naturally occurring and should be considered during the analysis of allegedly adulterated samples before rendering a decision that the material has been tainted.

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