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Analysis of Gamma-Hydroxybutyric Acid (GHB) in Spiked Water and Beverage Samples Using Solid Phase Microextraction (SPME) on Fiber Derivatization/Gas Chromatography-Mass Spectrometry (GC/MS)*

ABSTRACT: Gamma-Hydroxybutyric acid (GHB) is a CNS depressant that has been abused recreationally for its purported euphoric and relaxation effects and for the purposes of drug facilitated sexual assault due to its sedative and amnesic effects at higher doses. The dramatic increase in the abuse of GHB and association in criminal investigations over the past decade has created the need for forensic laboratories to develop analytical methods to detect GHB in a variety of matrices. The method developed in this work used solid-phase microextraction (SPME) to extract GHB from aqueous samples followed by on-fiber derivatization and analysis by gas chromatography/mass spectrometry (GC/MS). This method detected GHB in aqueous matrices with good sensitivity, high precision, excellent linearity from 0.01 mg/mL to 0.25 mg/mL, and without the need for sample manipulation that could cause interconversion between GHB and its lactone, GBL. The method was successfully applied for detection of GHB in spiked water and beverage samples.

KEYWORDS: forensic science, gamma-hydroxybutyric acid, gamma-butyrolactone, solid phase microextraction, on-fiber derivatization, gas chromatography/mass spectrometry, drug facilitated sexual assault, interconversion

Gamma-Hydroxybutyric Acid (GHB) is an endogenous compound found in the central nervous system (CNS) and peripheral tissues (1). GHB, a CNS depressant, has been abused recreationally for its purported euphoric and relaxation effects and for the purposes of drug facilitated sexual assault due to its sedative and amnesic effects at higher doses.

Throughout the past decade, there has been a dramatic increase in the abuse of GHB and related substances (2). GHB has a very sharp dose response curve (1). Users are often unaware of the exact amount of GHB they are consuming due to differences in manufacturing and hence purity and also because GHB is often diluted to varying degrees with water. The combined effects of a sharp dose response curve and the fact that users are often unaware of the amount they are consuming has led to an abundance of emergency room cases related to use of this drug. GHB has been associated with severe side effects including nausea, vomiting, disorientation, seizures, coma and even death.

GHB has become a drug of choice for rapists; it is odorless, colorless and easily dissolved into an alcoholic beverage. The ease with which GHB salt can be obtained has decreased due to its legal status. However, aqueous GHB produced from its corresponding lactone, GBL (an industrial solvent) and GHB substitute drugs are still relatively easy to purchase. The onset of the effects of GHB occurs within 10–15 min and the effects last for 3–4 h (3).

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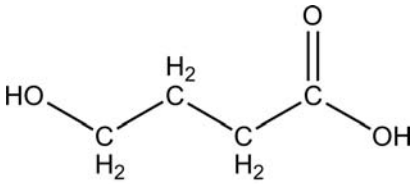
Gamma-Butyrolactone (GBL) is the corresponding lactone of GHB and is rapidly hydrolyzed to GHB in vivo with a half-life of less than 1 min (1). GBL is used extensively in chemical manufacturing and is therefore much harder to control than GHB, which is not used in industry. GHB was added to the list of DEA schedule I controlled substances in February 2000, GBL was designated as a list I chemical. While GBL was not explicitly scheduled under federal law, if it is intended for human consumption and meets the criteria of a Controlled Substance Analog (21 USC 802(32)), it can and has been treated as a schedule I controlled substance. At the state level scheduling status varies. Some states schedule both GHB and GBL while others carry legal distinctions.

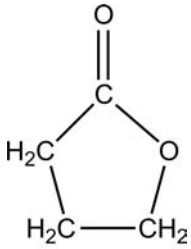
The dramatic increase in the abuse of GHB over the past decade has created the need for analytical methods to detect GHB in a variety of matrices. Specifically, the growing use of GHB for the purposes of drug facilitated sexual assault calls for the development of a method to determine whether GHB is present in a drink that is suspected of having been spiked. Current methods of analysis utilize a variety of extraction and sample preparation steps followed by GC/MS analysis (4–8), HPLC/UV analysis (9) or CE analysis (10).

GHB, a small polar molecule (Fig. 1), has presented some analytical difficulties in forensic laboratories. This compound undergoes thermal conversion to GBL (Fig. 2) in the heated injection port of a gas chromatograph. In order to avoid this conversion, GHB has often been derivatized before analysis by GC/MS (4–6). Prior to derivatization it is necessary to extract GHB from the aqueous environment in which it is dissolved. pH has been shown to have a significant effect on the rate and extent of interconversion between GHB and GBL in solution as published in the Journal of Forensic Sciences (11) as well as in the NMR interconversion work conducted by the authors of this study (Almirall, Meyers). Therefore,



MW=104

FIG. 1—*Structure of Gamma-Hydroxybutyric Acid (GHB).*



MW=86

FIG. 2—*Structure of Gamma-Butyrolactone (GBL).*

adjustment of pH during the extraction process may cause inadvertent conversion between GHB and GBL and should be avoided, if possible. Techniques that do not allow differentiation between GHB and GBL may be problematic in the legal arena, particularly at the state level, due to differences in scheduling between the two substances.

Solid Phase Microextraction (SPME) has been applied to the analysis of a variety of forensic samples (12–15) and the method presented, SPME-on-fiber derivatization/GC-MS was developed with the above-mentioned analytical difficulties in mind. The method was applied to the analysis of GHB in water as well as to several spiked beverages.

Experimental

Materials

Gamma-hydroxybutryric acid sodium salt and gamma-butyrolactone (≥99%) were purchased from Sigma chemical (St. Louis, MO). High purity deionized water (>18 $M\Omega cm^{-1}$) was obtained with a Nanopure Infinity purification system (Barnstead, Dubuque, IA). Deuterated GHB standard in methanol (1 mg/mL) was purchased from Cerilliant (Round Rock, Tx.). N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was purchased from Supelco (St. Louis, MO). Alcoholic and non-alcoholic beverages were purchased from a local Publix grocery store.

All SPME fibers and the SPME sampling apparatus were purchased from Supelco (St.Louis, MO). The fibers used for analysis were: 100 μm Polydimethylsiloxane (PDMS), 70 μm stableflex Carbowax/Divinylbenzene (CW/DVB), 65 μm stableflex Polydimethylsiloxane/Divinylbenzene (PDMS/DVB), 85 μm Polyacrylate (PA), 50/30 μm stableflex Carboxen/Divinylbenzene/Polydimethylsiloxane (Car/DVB/PDMS), and 85 μm stableflex Carboxen/Polydimethylsiloxane (Car/PDMS).

Instrumentation

Data were collected using an Agilent gas chromatograph 6890 series interfaced with an Agilent 5973 mass selective detector. A 30-m HP5-MS column with a 0.25 μm film thickness and 0.25 mm internal diameter was used for separation of components. The gas chromatograph was operated in splitless mode and equipped with a narrow SPME insert (0.75 mm). The injection port temperature was set to 220°C for the desorption of the analytes from the CW/DVB fibers and to 250°C for all other fibers. The carrier gas (helium) flow rate was set at 1.0 mL/min. A solvent delay of 7 min was employed due to the presence of derivatizing agent, which elutes in high abundance throughout the first 7 min.

An initial oven temperature of 50°C was held for 2 min. The temperature was then ramped at a rate of 10°C/min to 150°C, held for 4 min, followed by a temperature ramp of 35°C/min to a final temperature of 265°C where it was held for 2 min. The total run time was 21.29 min. The MS quadrupole temperature was 150°C and the MS source temperature was 230°C. The method of ionization was electron impact (E.I.) and the mass range analyzed was from 42 to 255 m/z.

Sample Preparation

Solid samples of GHB were weighed into 4 mL glass vials fitted with double sided teflon reinforced septa. The samples were diluted with deionized water to a total volume of 2.5 mL. A small teflon stir bar (8 mm × 1.5mm) was added to each vial.

SPME on-Fiber Derivatization Method

Solid phase microextraction (SPME), a fast, simple, and solvent free method for the extraction of drugs directly from aqueous samples was used for the extraction and pre-concentration of GHB. The sorbent phase of a SPME fiber was immersed in a solution of aqueous GHB for 15 min with moderate stirring. Fifteen minutes was determined to be long enough to reach equilibrium between the amount of analyte in the sample, the amount of analyte in the headspace above the sample, and the amount of analyte in the SPME fiber coating. Following the 15-min extraction, the fiber was exposed to the atmosphere for 1 min before being retracted (in order to dry the fiber coating).

Once the analyte was adsorbed onto the SPME fiber, the fiber was exposed to the headspace of 50 μL of BSTFA/TMCS (99:1) at 60°C for 40 min. The amount of derivatizing agent employed, temperature of the reaction, and time in the headspace were optimized to achieve the highest yield of product with a low background. The cap was then removed and the fiber was exposed to the atmosphere for 1 min. The fiber was then placed in the GC injection port to desorb for 12 min. This was determined to be a long enough desorption time to eliminate nearly all carryover. A blank fiber analysis was always conducted between samples to further ensure no carryover on the fiber before the next analysis. The GC/MS conditions described above were used for the separation and detection of derivatized GHB.

In order to determine which sorbent phase would be most effective for the extraction of GHB from an aqueous environment, each fiber was immersed in a 1000 ppm (1mg/mL) solution of GHB for 15 min with moderate stirring. The derivatization step was skipped and the fibers were analyzed using the SPME on-fiber derivatization method detailed previously.

A linear calibration curve was obtained by extracting GHB standards from spiked water samples with concentrations ranging from 10 ppm (0.01 mg/mL) to 250 ppm (0.25 mg/mL). To each solution,

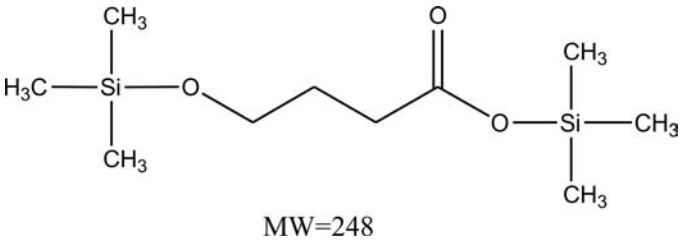


FIG. 3—*Structure of derivatized GHB (GHB-diTMS).*

125 μL of deuterated GHB (GHB-d_6) was added as the internal standard. The samples were prepared and analyzed in triplicate. All samples were analyzed in full scan mode, but ion 233 was extracted from the total ion chromatogram for quantification in order to minimize background area.

Results and Discussion

GHB was derivatized by silylation using BSTFA/TMCS (99:1). In the silylation process an alkylsilyl group, trimethylsilyl (TMS), replaces each active hydrogen of GHB. The structure of derivatized GHB is shown in Fig. 3. The silyl derivative is more volatile, less polar, and more thermally stable than underivatized GHB (sodium salt). The mass spectrum obtained from a 1 μL liquid injection of a neat derivatized GHB solution is shown in Fig. 4 with some important fragments labeled.

Molecular ions of TMS ethers are often weak or not detected at all, with the fragment corresponding to the molecular ion less a methyl group ($[M-15]^+$), which results from the cleavage of a methyl to silicone bond, being more prominent (16). Under the conditions used for analysis, an ionization voltage of 70 eV, no molecular ion was detected. Therefore, the peak at 233 m/z ($[M-15]^+$ peak) was used to indicate the presence of derivatized GHB. Another ion indicative of derivatized GHB is 159 m/z. The base peak, ion 147 m/z, is commonly found in polyhydroxy TMS compounds containing two or more TMS groups either on adjacent carbons or brought near to each other through expulsion of the central part of the molecule (16). Another prominent peak, ion 73, results from the TMS fragment itself. Ions 147 m/z and 73 m/z are consistent with the mass spectrum of the TMS derivative of GHB, but would be present in most TMS compounds and therefore cannot be used alone for the identification of derivatized GHB.

By incorporating SPME into the sample preparation process, the use of expensive and potentially hazardous solvents was minimized. The use of SPME allows for the extraction and pre-concentration in a single step, thus eliminating the need for complicated apparatus or multiple step liquid extractions. In addition, this method of extraction does not require sample manipulation such as pH adjustments that could cause conversion between GHB and GBL. Derivatization of GHB offers several advantages. It imparts thermal stability so that conversion of GHB to GBL in the heated injection port of the GC will not occur. Also, by derivatizing GHB, a less polar and more volatile compound with better chromatographic properties was analyzed.

The CW/DVB fiber was found to be, by far, the most effective fiber for extraction of GHB from an aqueous environment followed by the Car/PDMS and 100 μm PDMS fibers. In general, the results were as expected since polar fibers like CW/DVB tend to work best for the extraction of polar compounds such as GHB. Also, Car/PDMS tends to be very effective for the extraction of low molecular weight compounds like GHB (MW = 104) (17). The CW/DVB fiber was chosen for analysis due to its superior extraction capabilities and hence higher yield of final product and also due to the absence of co-eluting peaks resulting from fiber bleed and/or peaks resulting from the interaction between the derivatizing agent and the fiber.

Each step in the method was optimized for the highest yield of derivatized product with the least amount of interfering background. The optimized conditions are detailed in the experimental section. In Fig. 5, the total ion chromatogram and extracted ion chromatogram are shown for the analysis of a 100 ppm GHB sample in deionized water spiked with 50 ppm of deuterated GHB

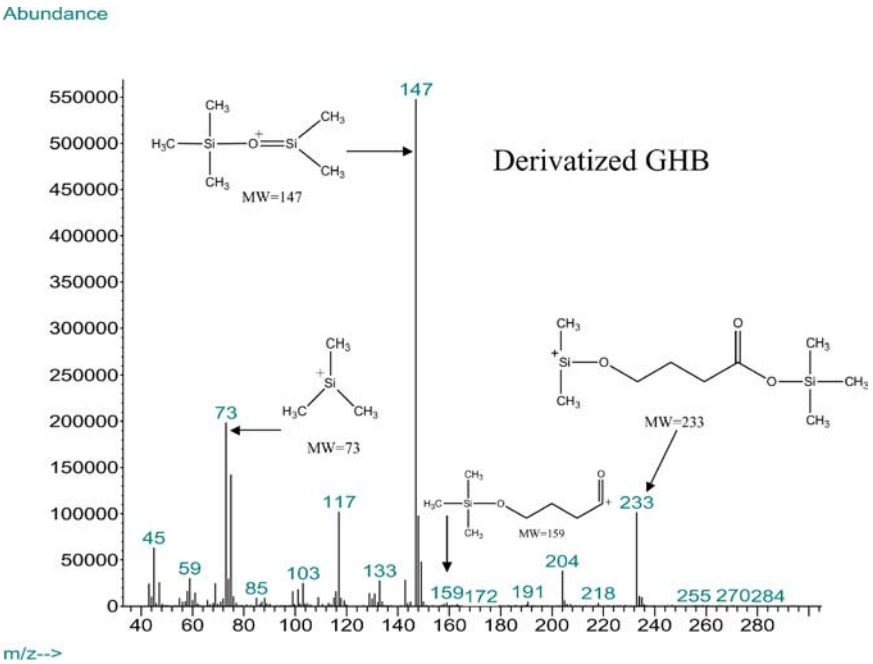


FIG. 4—*Mass spectrum of a 1 μL injection of neat, derivatized GHB.*

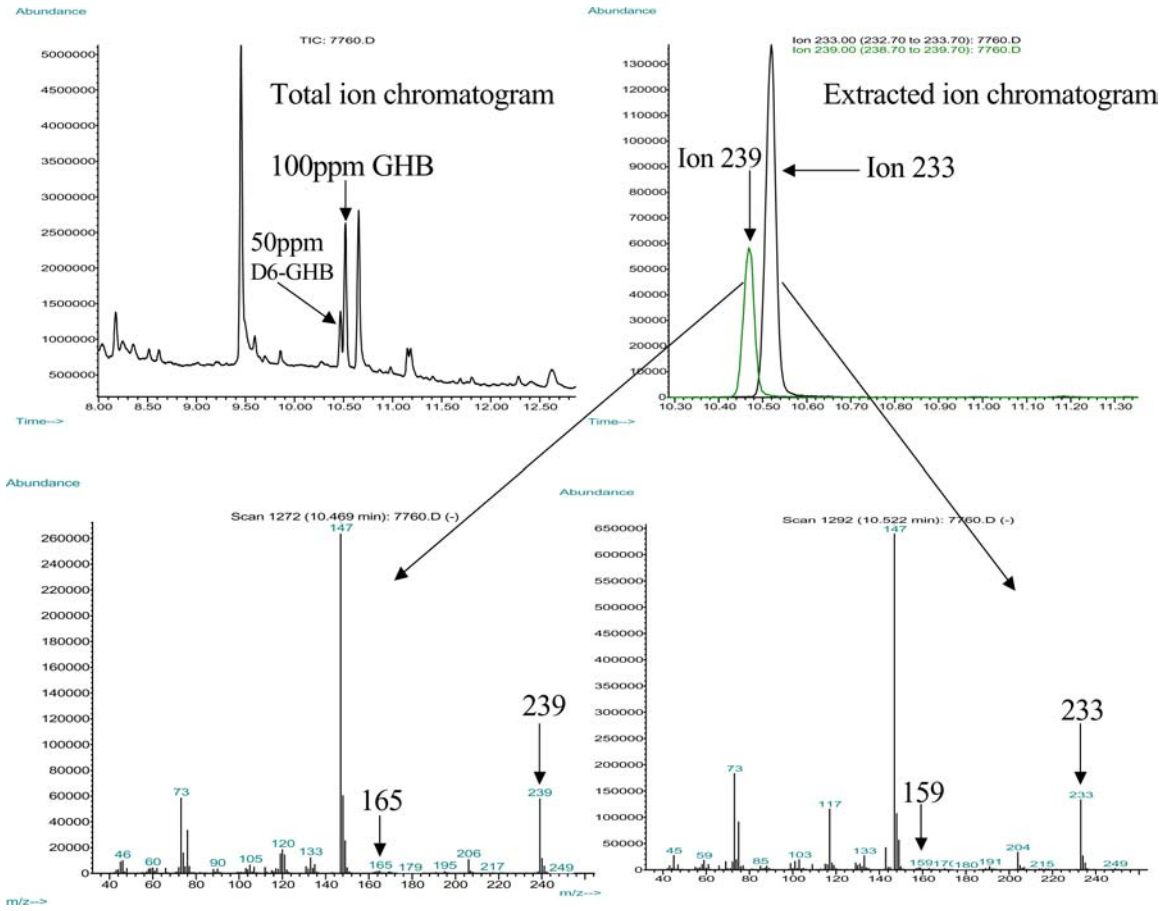


FIG. 5—*Ion chromatograms and mass spectra of a 100 ppm GHB standard in DI water spiked with 50 ppm GHB-d_6 (IS).*

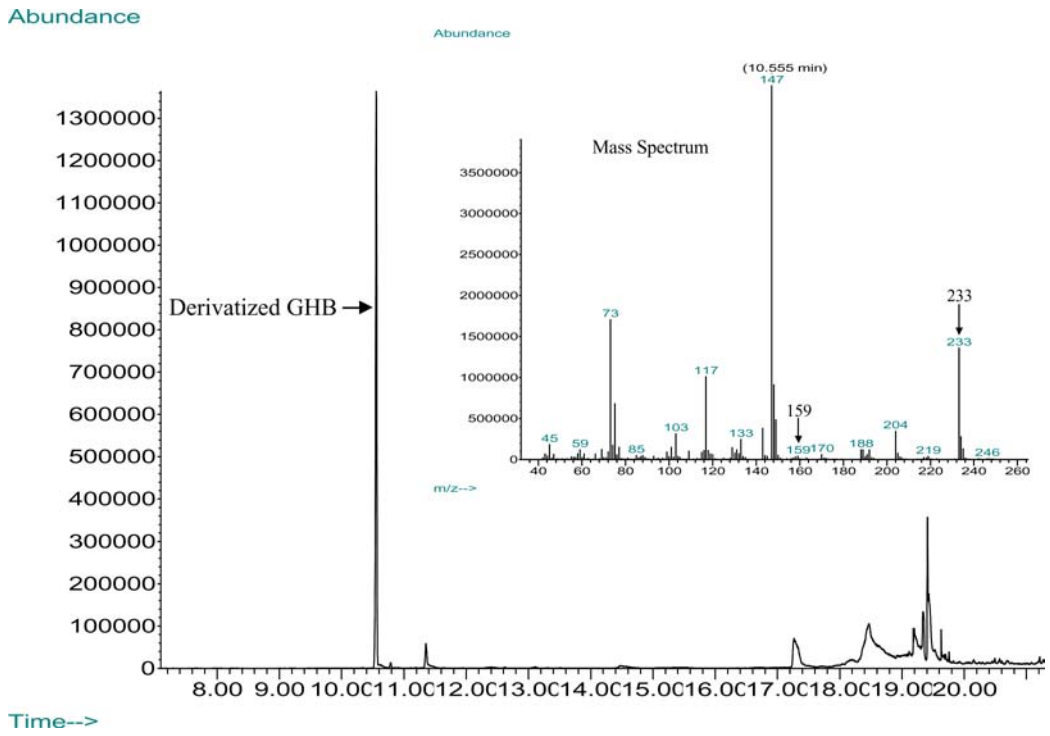


FIG. 6—*Extracted ion chromatogram and mass spectrum of GHB (di-TMS) extracted from Coke®.*

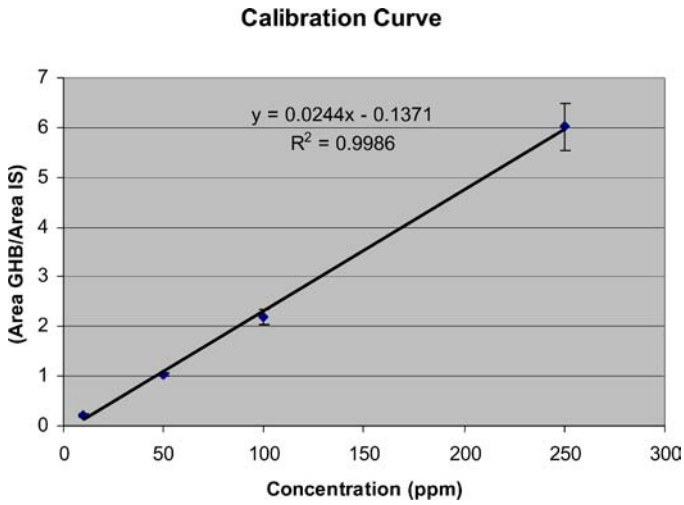


FIG. 7—*Four point calibration curve (unweighted) for the analysis of GHB standards in aqueous samples.*

(GHB-d_6). GHB-d_6 was spiked into samples at a concentration of 50 ppm for use as an internal standard when quantitative analysis was required.

The method developed was used to analyze several water blanks. There were no peaks detected (S/N ≥ 3) at the same retention time as derivatized GHB.

GBL is not derivatized by BSTFA/TMCS (99:1). Underivatized GBL eluted at approximately 5.9 min using the GC/MS conditions described above. Due to the long solvent delay required, GHB and GBL cannot be analyzed simultaneously using the method presented.

Spiked Beverage Analysis

GHB was spiked into several beverages (coke®, beer, lemonade) at a concentration of 100 ppm and the solutions were analyzed. A high yield of derivatized GHB was achieved for the extraction from all of the spiked beverages. The total ion chromatograms for the spiked beverage analyses were more complex than from pure water, however when ion 233 m/z was extracted, a very clean spectrum was obtained for all beverages. The extracted ion chromatogram and associated mass spectrum for analysis of GHB spiked into Coke are shown in Fig. 6. The blank beverage should always be analyzed since GBL has been detected in some unadulterated wines (18).

Quantification

The extraction method produced a linear calibration range from 10 ppm (0.01 mg/mL) to 250 ppm (0.25 mg/mL) with a correlation coefficient of 0.9986 for the 4-point calibration curve (Fig. 7). The average relative standard deviation for the samples prepared and analyzed in triplicate was 3.4%. The average signal to noise ratio at 10 ppm (0.01 mg/mL) was approximately 67. Based on the limit of quantification (LOQ) set at a signal to noise ratio of 10, the LOQ for the method developed was calculated to be approximately 1.5 ppm (1.5 mg/L). Sensitivity studies were not carried out experimentally since the concentrations expected in the analysis of spiked beverages would be well above the lowest concentrations tested, (0.01 mg/mL), and also because concentrations lower than 10 ppm (0.01 mg/mL) may be attributed to endogenous GHB when biological samples are analyzed (19,20).

Recommendations for Further Analysis

The major drawback of the method presented is the instability of the CW/DVB sorbent phase. The CW/DVB fiber was found to be less stable than other fibers, sometimes stripping away from the silica core when immersed in solution or heated in the injection port. There is a CW/Templated resin (TPR) fiber available which is reported to be more stable than the regular CW/DVB fiber because it contains no epoxy. The recommended use of this fiber is for HPLC, however a publication (21) indicates that it can be used for GC analysis once a spring is added to the fiber. Use of this more stable fiber could save time, money and increase the precision of the method presented.

The method thus far has only been applied to spiked water and beverage samples. Application of the method to biological samples could greatly increase the utility of the method. SPME has been applied successfully for the extraction of drugs from biological samples (22). The limit of detection and range of linearity for the method presented are similar to other methods that have been published for the analysis of GHB in biological samples (4–5,6,8,21,23).

Conclusion

In the method developed, SPME was used to extract GHB from aqueous samples followed by on-fiber derivatization and then analysis by GC/MS. The method detected GHB in aqueous matrices with good sensitivity, high precision, excellent linearity from 0.01 mg/mL to 0.25 mg/mL, and without the need for sample manipulation that could cause interconversion between GHB and its lactone, GBL. The method was successfully applied for detection of GHB in several alcoholic and non-alcoholic beverages.

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