



**A Solid Phase Extraction Method for The Determination
of GAMMA-HYDROXYBUTYRATE (GHB)
in Urine Without Conversion to
GAMMA-BUTYROLACTONE (GBL)**

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INTRODUCTION

Gamma-hydroxybutyric acid has become widely known as the “Sex drug for the 1990’s.” Upon ingestion, this drug reduces inhibitions and reportedly increases libido. Other popular street names include “Scoop” and “Liquid Ecstasy.” This drug has been classified as a date rape drug along with lorazepam, ketamine and flunitrazepam.

GHB is an endogenous metabolite found in human metabolism structurally similar to the neurotransmitter gamma-aminobutyric acid (GABA) (1,2). It was first synthesized and used in Europe as an anesthetic, but was later discontinued because it often caused seizures. This drug was widely sold in health food stores as a weight control drug (functionally similar to L-tryptophan) and to induce the secretion of growth hormone for body building. In 1990 the sale and distribution of GHB was federally banned due to its potentially harmful effects (3).

GHB is easily synthesized from gamma-butyrolactone, a compound that is found in some commercial products. The internet has popularized this drug by giving recipes on how to manufacture it. Clandestine preparations vary significantly in their concentrations leading to problems in overdosage. There may also be harmful contaminants from potentially low quality ingredients. More than 20 deaths in the United States in the 1990’s were attributed to the use of GHB and are generally in conjunction with alcohol ingestion (4).

GHB is a small polar molecule that is very difficult to separate for a qualitative determination. Many methods extract it following conversion to butyrolactone and chemically derivatize by silylation (5,6,7). Our method allows for the chemical derivatization of the parent compound without the formation of the lactone. Instrumental analysis is performed by GC/MS.

MATERIALS AND METHODS

Reagents

Certified ACS grade hexane, sodium phosphate monobasic, sodium phosphate dibasic, dimethylformamide, ammonium hydroxide and HPLC grade methanol and ethyl acetate were all purchased from Mallinckrodt (Phillipsburg, PA). Distilled water was prepared using a Millipore purification system.

BSTFA (Bis(trimethylsilyl)trifluoroacetamide) with 1% TMCS (Trimethylchlorosilane) were from United Chemical Technologies (Bristol, PA).

Standards and Solutions

4-Hydroxybutyric acid, sodium salt (GHB) was purchased from Aldrich (Milwaukee, WI).

GHB-D6 was purchased from Radian International (Austin, TX).

The GHB and GHB-D6 were prepared to 0.1 mg/mL in methanol.

The 0.1 M phosphate buffer (pH6.0) was prepared by dissolving 1.70 g Na₂HPO₄ and 12.14 g NaH₂PO₄·H₂O in 800 mL DI H₂O. Dilute to 1000 mL using DI H₂O. Mix. The solution is stable for at least 1 month.

Adjust pH to 6.0 ± 0.1 with 0.1 M monobasic or dibasic sodium phosphate.

The CH₃OH/NH₄OH (99/1) is prepared fresh daily.

Sorbent

The extraction columns were CLEAN SCREEN[®] ZSGHB020 containing 200 mg of sorbent in a 10 mL column and were made by United Chemical Technologies, Inc. (Bristol, PA).

Instrumentation

A Hewlett Packard 5971A Mass Selective Detector, 7673 Autosampler and a 5890 Gas Chromatograph fitted with a 30 m, 0.25 mm i.d., 0.25 µm film thickness Rtx-5 (comparable to a DB-5 or HP-5) from Restek (Bellefonte, PA).

GC Conditions

Column Head Pressure	8 psi
Injection Port Temp.	250° C
Transfer Line Temp.	280° C
Split Vent Flow	50 mL/minute
Septum Purge Flow	2.6 mL/minute
Equilibration Time	0.5 minutes
Purge On Time	0.1 minutes
Injection Volume	1 µL, Splitless injection

Oven Temperature Program

Initial Temperature 70° C, hold 1.00 minutes.
Ramp to 100° C @15° C/minute.
Ramp to 175° C @25° C/minute.
Ramp to 280° C @35° C/minute.

Total Run Time= 9.00 minutes

Run time is extended past the elution of the GHB to eliminate any residual BSTFA or urine by-products.

GC/MS ANALYSIS

The method uses Selected Ion Monitoring (SIM) for three ions for each analyte. The dwell times were set to 30 milliseconds per ion resulting in 3.62 cycles per second. The most prevalent ions for GHB-diTMS are 147, 233, 148, 149, 204, 143 and 234 m/z. The most prevalent ions for GHB-D6-diTMS are 147, 239, 148, 149, 206 and 240 m/z. Urea is also derivatized by BSTFA to form a diTMS derivative. It elutes near GHB and has many of the same ions including 147, 148 and 149 therefore some of the less abundant ions must be used for the SIM analysis. These include:

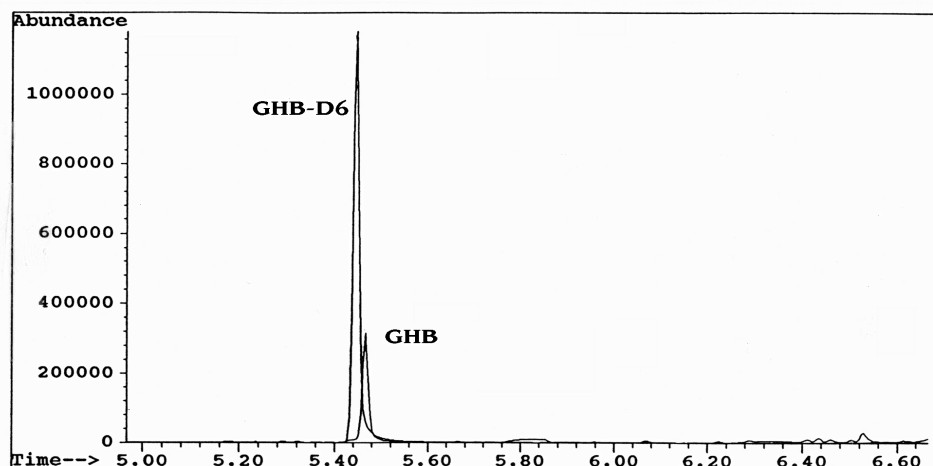
RT (Minutes)	Name	Target Ion (%of target ion)	2nd Ion (% of target ion)*	3rd Ion (% of target ion)*
5.40	GHB-D6-diTMS	239.2 (100)	240.2 (20)	241.1 (9)
5.43	GHB-diTMS	233.1 (100)	234.1 (19)	235.0 (7)

* Observed Values

RESULTS

Chromatography

Figure 1 Chromatogram of the selected target ions showing GHB and GHB-D6 from a 5 mg/L extracted urine standard.



Recovery

Five 50 mg/L standards were prepared by adding 10 µg of GHB to 200 µL of drug free urine. These standards were extracted using the previously described procedure. The internal standard (4 µg of GHB-D6) was added immediately prior to the evaporation of the DMF. Five 50 mg/L unextracted standards were prepared by adding 10 µg of GHB and 4 µg of GHB-D6 to a test tube which was dried at 50°C. All samples were derivatized using the procedure previously described. The recovery was calculated by comparing the area under the curve of the target ion for the extracted and the average of the unextracted standards. The average recovery was 67.2% (range=76-58%).

Linearity

The assay is linear from 1-100 mg/L with the upper range limited by the saturation of the detector. The method could be run with a split injection if higher concentrations need to be quantified.

Stability

The diTMS derivatives are stable for over 7 days at room temperature.

Sensitivity

The assay is sensitive to 1 mg/L.

Interferences

The only interference identified in this procedure was from urea. It is derivatized along with GHB to form urea-diTMS. Using the SIM ions listed above eliminates the interference.

DISCUSSION

UCT has developed a procedure that allows for the direct analysis of GHB and eliminates any possibility of forming or extracting gamma-butyrolactone (GBL). Conversion to GBL is problematic in forensic analysis where litigation is involved because GHB is a scheduled drug in many states and GBL is not. This method was originally designed to identify low levels of GHB in urine and requires only 200 μ L of sample. Expected concentrations in biological samples may be much higher and therefore a smaller sample size should be used (7). The method utilizes a novel copolymeric sorbent employing solid phase extraction (SPE) as a filter. A sample clean up step and silylation followed by GC/MS analysis is also incorporated into the method. The derivatization with BSTFA w/1% TMCS is accomplished without a lengthy heating process due to the high reactivity of the BSTFA to the GHB. The derivative is stable for more than a week while sitting on an autosampler tray. A procedure for blood and adulterated substances is currently in development.

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