Idaho State Police
Forensic Services
Toxicology Section

Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for GC/MSD Confirmation

2.3.9 Qualitative Confirmation of Gamma Hydroxybutyrate in Urine Samples and GHB Containing Products

2.3.9.1 BACKGROUND

This method provides a solid phase extraction option for the extraction of Gamma-hydroxybutyrate/Gamma-Hydroxybutyric Acid (GHB). GHB occurs naturally in minute quantities as a result of the metabolism of the inhibitory neurotransmitter, GABA.[6,8] The drug GHB is a potent tranquilizer that was previously used as an anesthetic and as a treatment for major depressive illnesses, alcohol withdrawal, and narcolepsy.[1-8] Legitimate use is limited due the side effects associated with the drug. GHB can produce visual disturbances, nausea, vomiting, drowsiness, dizziness, severe respiratory depression, unconsciousness and involuntary muscle spasms.[1,5,6] Overdoses can require emergency medical treatment including intensive care due to the respiratory depression, bradycardia and coma.[5,6,8]

The use of GHB has been increasing since the 1980s and currently is popular among body builders, teenagers and on the club/dance scene. Body builders use the drug due its alleged role as a growth hormone releasing agent to enhance muscle growth.[5,6,8] GHB has not been proven to possess any anabolic effects.[6] GHB has gained notoriety as a date rape drug due to its ability to produce short-term amnesia and decreased inhibitions.[1-8] The FDA banned the use of GHB in 1990 except for FDA approved physical supervised protocols.[6,8]

A dose of GHB consists of a capful that is usually approximately one teaspoon. This results in a dose anywhere from 2.5 to 4.0 grams of GHB. The taste of GHB has been described as salty or soapy, the odor is said to be mothball-like.[5,6] Due to the short half-life of GHB (0.3 to 1.0 hours[5], 27 ± 5 minutes[7]) the person will re-administer every 45 minutes to 1 hour. The onset of effects is 15 to 60 minutes. The effects of the drug will be detectable during a DRE exam for 4 to 6 hours. GHB is classified as a central nervous system depressant. The observed effects include horizontal and vertical nystagmus, lack of convergence, body tremors, and slowed breathing. The person will also exhibit a lowered pulse, blood pressure, and body temperature. In addition, the muscle tone will exhibit flaccidity and the person may be in a trance-like state, the pupils will exhibit a lack of reaction to light.[5] Lower doses will promote an agitated, combative state however their pulse and other vitals will be depressed.[5,6]

Combining GHB with alcohol plus a stimulant or marihuana allows the user to remain conscious during use. This allows them to experience the euphoric "buzz" that is the desired effect of its abuse. The desired effect is a state of relaxation and tranquility, a pleasant drowsiness, mild euphoria, hallucinations and a release of inhibitions. Combining GHB with alcohol or other central nervous system depressant will provide an additive depressant effect.[5]

GHB is detectable in blood for up to eight hours and in urine for up to 12 hours [5,6,7]. Peak plasma levels are obtained in 20 to 45 minutes.[7] Peak urine GHB concentrations on the order of 1100 µg/mL are observed within the first four hours after a 100 mg/kg oral dose.[3,7]

GHB is manufactured by reacting butyrolactone with sodium hydroxide in an aqueous solution in the presence of a low molecular weight alcohol (methanol, ethanol).[5,6] The average yield of GHB is 70%. If the yield of the manufacturing process is low, there will be significant amounts of the lactone present in the product. Abuse of this compound will continue due to its relatively simple synthesis and the availability and low cost of starting materials.[5] Users can ingest Gamma butyral lactone (GBL), a degreaser and floor stripper, and it is converted in vivo into GHB. GBL is therefore diverted from legitimate sources to either be taken directly or converted to GHB.

This compound has been referred to by a variety of names as listed in the table below.

Street Names	Marketing Names
"G"	Revitalize
"G" caps	Rejuvenate
Liquid X	Renewtrient
Soap	Revivarant
Easy Lay	Blue Nitro
	Thunder Nectar
	Rest-Eze
	Energy Drink

There are thousands of documented GHB overdoses with numerous deaths. The danger in GHB use stems from its steep dose-response curve. A small increase in dose can create a dramatic difference in adverse effects. This makes the potential of overdosing with GHB very high. This is compounded by the fact that GHB effects users so differently. A dose that one individual uses could adversely effect another, thus word of mouth is a poor determiner of how much of the substance to use.

2.3.9.2 PRINCIPLE

GHB is extracted first into acetone, which is dried and reconstituted with phosphate buffer (pH 6.0). This sample extract is applied to a pretreated/conditioned SPE column. This method requires no heating to create the GHB-TMS derivative. The SPE elutant is evaporated and a di-

TMS derivative of GHB is prepared. The resulting derivative can be analyzed by either full scan or SIM GC/MSD in EI mode.

2.3.9.3 EQUIPMENT

2.3.9.3.1 Evaporative Concentrator (Zymark Turbo-Vap or equivalent).

2.3.9.3.2 Laboratory Centrifuge

2.3.9.3.3 Gas chromatograph equipped with a mass selective detector (HP 6890/5973 or equivalent) and a nonpolar capillary column with a phase composition capable of efficiently separating GHB and its analogs in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5%diphenyl)

2.3.9.4 SUPPLIES

2.3.9.4.1 Glassware

Tapered tip 16X144 centrifuge tubes (Fisher catalog 05-538-41C or equivalent)
Snap caps (Fisher 05-538-41N or equivalent)
GC/MS vials (HP 5182-0865 or equivalent)
GC/MS vial microinserts (HP 5183-2088 or equivalent)
2.3.1.11.3.2 1.5mL snap cap centrifuge tubes (Fisher # or equivalent).

2.3.9.4.2 CLEAN SCREEN® GHB SPE columns (United Chemical Technologies, Inc., #ZSGHB020 or equivalent)

2.3.9.5 REAGENTS

2.3.9.5.1 Hexane (Fisher #H303 or equivalent)
2.3.9.5.2 Sodium phosphate monobasic (Fisher #S369 or equivalent)
2.3.9.5.3 Sodium phosphate dibasic (Fisher #S374 or equivalent)
2.3.9.5.4 Dimethylformamide (Fisher #D119 or equivalent)
2.3.9.5.5 Ammonium hydroxide (Fisher #A669 or equivalent)
2.3.9.5.6 Methanol (Fisher #A454 or equivalent)
2.3.9.5.7 Ethyl Acetate (Ansys #203 or Fisher #E145-1 or equivalent)
2.3.9.5.8 Silylating Agent (select from)
BSTFA/1% TMCS (Pierce #38842ZZ or equivalent)
MSFTA (Pierce #48910 or equivalent)
BSTFA with 1%TMCS (Pierce #38831 or equivalent)

2.3.9.6 STANDARDS AND SOLUTIONS

Refer to section 2.6 for buffer solution preparation.

2.3.9.6.1 GHB Stock Solution
1.0mg/mL (Radian International #G-001 or equivalent)

2.3.9.6.2 0.1M Phosphate Buffer

2.3.9.6.3 99:1 CH_3OH:NH_4OH
Place ~80mL of methanol in a 100mL volumetric flask. Add 1mL of ammonium hydroxide, QS to 100mL.
Prepare fresh daily.

2.3.9.7 PROCEDURE

2.3.9.7.1 Initial set-up

2.3.9.7.1.1 Label GHB SPE extraction columns as follows:

- *GHB-NC* (Negative Control)
- *GHB-PC* (Positive Control)
- Laboratory numbers of samples without prefix.

2.3.9.7.1.2 Label Tapered-end centrifuge tubes and GC/MS vials as follows:

- *GHB-NC* (Negative Control)
- *GHB-PC* (Positive Control)
- Laboratory numbers of samples without prefix
- *GHB-NES* (Non-extracted GHB standard)

2.3.9.7.2 Preparation of GHB Controls and Standards

2.3.9.7.2.1 Spiked Urine - GHB Positive Control [200µg/mL]
Add of GHB 1mg/mL stock to negative urine. Vortex.

2.3.9.7.2.2 Non-Extracted Standard [200µg]
Place 200uL of GHB stock into taped-end centrifuge tube.

2.3.9.7.3 Extraction procedure

2.3.9.7.3.1 To 200uL of specimen, calibrators, negative and positive controls.

2.3.9.7.3.2 Add 25uL GHB-D6 internal standard.

2.3.9.7.3.3 Add 1mL of acetone, vortex for 15 seconds.

2.3.9.7.3.4 Centrifuge tube at ≈3300rpm for 10 minutes.

2.3.9.7.3.5 Transfer solvent from tube into tapered-end centrifuge tube.

2.3.9.7.3.6 Evaporate solvent with nitrogen at 80°C in TurboVap apparatus.

2.3.9.7.3.7 Reconstitute the evaporated extracts with 200uL of 0.1 M phosphate buffer (pH 6.0). Vortex 15 seconds.

2.3.9.7.4 Column Conditioning

Prepare CLEAN SCREEN® GHB SPE column as follows:

2.3.9.7.4.1 Apply 3mL of MeOH; aspirate at ≤ 3 inches of Hg.

2.3.9.7.4.2 Apply 3mL of DI H2O; aspirate ≤ 3 inches of Hg.

2.3.9.7.4.3 Apply 3 mL of 0.1M Phosphate Buffer (pH 6.0), aspirate ≤ 3 inches of Hg.

2.3.9.7.5 Sample Application

Add sample to prepared column with air displacement pipet (MLA, Eppendorf). Aspirate at 1 inch Hg.

2.3.9.7.6 Collection of Extract

Place tapered bottom centrifuge tube into collection rack. Add 1mL of MeOH/NH_4OH (99:1) to original sample centrifuge tube (from step 6.3.5), vortex.
Decant onto column and collect extract.

2.3.9.7.7 Concentration of Extract

Place tubes from vacuum manifold into TurboVap apparatus. Evaporate solvent with nitrogen at 70°C.

2.3.9.7.8 Derivatization Procedure

2.3.9.7.8.1 Add 100µL of Ethyl acetate and 100µL of BSTFA with 1% TCMS to evaporated extracted samples, spiked standards and non-extracted standard.

2.3.9.7.8.2 Transfer derivative to GC/MS ALS vial for analysis.

2.3.9.7.8.3 Inject 1 µL into GC/MS.

2.3.9.7.9 Gas Chromatography/Mass Spectrometry (GC/MS) Parameters

2.3.9.7.9.1 Oven program, Injector and Interface Temperatures:
Refer to Method print-out that follows SOP.

2.3.9.7.9.2 Sample should be analyzed in full scan acquisition. Refer to attached GC/MSD method print-out for current parameters.

2.3.9.7.10 Detection and Identification Criteria
The qualitative presence of GHB can be established if there are no significant differences in the retention time and mass spectra for the sample versus standards.

2.3.9.8 REFERENCES

12.3.9.8.1 Frommhold, S. *Gamma-Hydroxybutyrate (GHB): What's "the Scoop"? in:* Toxi-News 16(1), 1997; pp. 3-8.

12.3.9.8.2 Ferrare, S.D., Tedeschi, L. Frison, G., et. al, *Therapeutic gamma-hydroxybutyric acid monitoring in plasma and urine by gas chromatography-mass spectrometry.* J Pharm. Biomed Anal, 1993, 11(6):483-487.

12.3.9.8.3 Stephens, B. and Baselt, R.C. *Driving Under the Influence of GHB?* J Anal Tox, 1994, 18:357-358.

12.3.9.8.4 ElSohly, M.A. and Salamone, S.J. *Prevalence of Drugs used in Cases of Alleged sexual Assault* J Anal Tox, 1999, 23:141-146.

12.3.9.8.5 Chase, D.A., *Gamma Hydroxy Butyrate,* "*GHB*", Presentation at IACP DRE Conference, Minnesota, 1999.

12.3.9.8.6 Good, P.J., *Selected Abuse Substances,* Presentation at IACP DRE Conference, Portland, Oregon, 1998.

12.3.9.8.7 Determination of Gamma-Hydroxybutyric Acid by GC/MS, Dade County Medical Examiner's Toxicology Lab SOP.

12.3.9.8.8 Microgram, Volume XXXI, No. 3, March 1998.