Idaho State Police
Forensic Services
Toxicology Section

Section Two
Urine Toxicology

2.4 Liquid-Liquid Extraction Methods for GC/MSD Confirmation

2.4.2.1 Qualitative Confirmation of Gamma-Hydroxybutyrate (GHB) in Urine Samples and GHB Containing Products

2.4.2.1.1 BACKGROUND

This method provides two qualitative analysis options for the liquid-liquid extraction of samples suspected of containing γ-Hydroxybutyrate/γ-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 physician 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.

2.4.2.1.2 PRINCIPLE - EXTRACTION OPTION ONE

GHB is isolated from an acidified solution into methylene chloride and heptane with zinc chloride to facilitate the extraction process. The extraction is achieved with an Ansys Toxi-B extraction tube. The

extraction is followed by the creation of a di-TMS derivative of GHB. The derivative is analyzed by full scan GC/MS in EI mode. This method may not provide adequate sensitivity for weaker concentrations of GHB.

2.4.2.1.3 EQUIPMENT EXTRACTION OPTION ONE

2.4.2.1.3.1 Tube Rocker (Fisher Scientific or equivalent)

2.4.2.1.3.2 Evaporative Concentrator (Zymark Turbo-Vap or equivalent)

2.4.2.1.3.3 Laboratory Centrifuge (Fisher Marathon or equivalent)

2.4.2.1.3.4 Glassware

2.4.2.1.3.4.1 Tapered tip 16X144 centrifuge tubes (Fisher catalog 05-538-41C or equivalent)

2.4.2.1.3.4.2 Snap caps (Fisher 05-538-41N or equivalent)

2.4.2.1.3.4.3 GC/MS vials (HP 5182-0865 or equivalent)

2.4.2.1.3.4.4 GC/MS vial microinserts (HP 5183-2088 or equivalent)

2.4.2.1.3.5 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)

2.4.2.1.4 REAGENTS EXTRACTION OPTION ONE

2.4.2.1.4.1 ANSYS TOXI-TUBES B (109B-100)

2.4.2.1.4.2 Silylating Agent (select from)

MSFTA (Pierce #48910 or equivalent)

BSTFA with 1%TMCS (Pierce #38831 or equivalent)

2.4.2.1.5 STANDARDS EXTRACTION OPTION ONE

2.4.2.1.5.1 Stock Standard Solution

1.0mg/mL (Radian International #G-001 or equivalent).

2.4.2.1.6 PROCEDURE EXTRACTION OPTION ONE

2.4.2.1.6.1 Initial set-up

2.4.2.1.6.1.1 Label TOXI-TUBES B as follows:

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

2.4.2.1.6.1.2 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)

2.4.2.1.6.2 Preparation of Controls

2.4.2.1.6.2.1 **Spiked Urine**

GHB Positive Control [200µg/mL]
Add 900uL of GHB 1mg/mL stock to 3600uL negative urine. Vortex.

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

2.4.2.1.6.3 Extraction procedure

2.4.2.1.6.3.1 Extract 4.5 mL of specimen, negative or spiked urine in TOXI-TUBE B (acidic extraction @pH=4.5).

2.4.2.1.6.3.2 Rock TOXI-TUBE for 15 minutes.

2.4.2.1.6.3.3 Centrifuge tube at 2500 rpm for 15 minutes.

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

2.4.2.1.6.3.5 Evaporate solvent to approximately 50µL with nitrogen at 40°C in TurboVap apparatus.

2.4.2.1.6.4 Derivatization Procedure

2.4.2.1.6.4.1 Add 40µL silylating agent to evaporated extracted samples, spiked standards and non-extracted standard. Cap tube with snap cap.

2.4.2.1.6.4.2 Vortex tube.

2.4.2.1.6.4.3 Place tube in 60°C sandbath for 15 minutes.

2.4.2.1.6.4.4 Remove tube from sandbath. Allow sample to cool. Transfer derivative to labeled GC/MS ALS vial for analysis.

2.4.2.1.6.4.5 Inject 1 µL into GC/MS.

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

2.4.2.1.6.5.1 Refer to following method for oven program, and injector and interface temperatures.

2.4.2.1.6.5.2 Sample should be analyzed in full scan acquisition. Refer to attached GC/MSD method printout for current parameters to be employed for analysis.

2.4.2.1.6.6 Detection and Identification Criteria

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

2.4.2.1.6.6.2 Acceptable retention time window is ± 2%.

2.4.2.1.7 PRINCIPLE - EXTRACTION OPTION TWO

GHB is isolated from an acidified solution into ethyl acetate. The extraction is followed by the derivatization of GHB with BSTFA/1% TMCS and 60µL acetonitrile. The derivative is analyzed by SIM and/or full scan GC/MS in EI mode.

2.4.2.1.8 EQUIPMENT EXTRACTION OPTION TWO

2.4.2.1.8.1 Tube Rocker (Fisher Scientific or equivalent)

2.4.2.1.8.2 Evaporative Concentrator (Zymark Turbo-Vap or equivalent)

2.4.2.1.8.3 Laboratory Centrifuge (Fisher Marathon or equivalent)

2.4.2.1.8.4 Glassware

2.4.2.1.8.4.1 Screw-top 16x100mm centrifuge tubes (Fisher #14-959 or equivalent)

2.4.2.1.8.4.2 Screw caps (Fisher 14-930-15E or equivalent)

2.4.2.1.8.4.3 Tapered tip 16X144 centrifuge tubes (Fisher #05-538-41C or equivalent)

2.4.2.1.8.4.4 Snap caps (Fisher #05-538-41N or equivalent)

2.4.2.1.8.4.5 GC/MS vials (HP 5182-0865 or equivalent)

2.4.2.1.8.4.6 GC/MS vial microinserts (HP 5183-2088 or equivalent)

2.4.2.1.8.5 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)

2.4.2.1.9 REAGENTS EXTRACTION OPTION TWO

2.4.2.1.9.1 Concentrated Sulfuric Acid (Fisher #A300S-500 or equivalent) diluted to 0.1N.

2.4.2.1.9.2 Ethyl Acetate (Ansys #203 or Fisher #E145-1 or equivalent)

2.4.2.1.9.3 Acetonitrile (Fisher #A996-1 or equivalent)

2.4.2.1.9.4 BSTFA with 1%TMCS (Pierce #38831 or equivalent)

2.4.2.1.10 STANDARDS EXTRACTION OPTION TWO

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

2.4.2.1.11 PROCEDURE EXTRACTION OPTION TWO

2.4.2.1.11.1 Preparation of Controls

2.4.2.1.11.1.1 100µg/mL and 200µg/mL Spiked Urine Positive Controls

100µg/mL: Add 100uL of GHB 1mg/mL stock to 950uL negative urine. Vortex.

200µg/mL : Add 200uL of GHB 1mg/mL stock to 800uL negative urine. Vortex.

2.4.2.1.11.1.2 Non-Extracted Standard [100µg]
Place 100uL of GHB stock into taped-end centrifuge tube.

2.4.2.1.11.2 Extraction procedure

2.4.2.1.11.2.1 Place 1.0mL of specimen, negative or spiked urine in round bottom centrifuge tube.

2.4.2.1.11.2.2 Add 250uL of cold 0.1N H_2SO_4 and vortex.

2.4.2.1.11.2.3 Add 6mL ethyl acetate. Cap.

2.4.2.1.11.2.4 Rock tube for 15 minutes.

2.4.2.1.11.2.5 Centrifuge tube at 2500 rpm for 10 minutes.

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

2.4.2.1.11.2.7 Re-extract urine with an additional 6mL ethyl acetate. Cap.

2.4.2.1.11.2.8 Rock tube for 15 minutes.

2.4.2.1.11.2.9 Centrifuge tube at 2500 rpm for 15 minutes.

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

2.4.2.1.11.2.11 Evaporate the combined solvent with nitrogen at ≤40°C in TurboVap apparatus.

2.4.2.1.11.3 Derivatization Procedure

2.4.2.1.11.3.1 Add 30µL BSTFA/1 % TMCS and 60µL acetonitrile to evaporated samples, spiked standards and non-extracted standard. Cap tube with snap cap.

2.4.2.1.11.3.2 Vortex tube.

2.4.2.1.11.3.3 Place tube in 70°C sandbath for 15 minutes.

2.4.2.1.11.3.4 Remove tube from sandbath and allow to cool. Transfer derivative to GC/MS ALS vial for analysis.

2.4.2.1.11.3.5 Inject 2 µL into GC/MS.

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

2.4.2.1.11.4.1 Refer to attached GC/MSD method printout for current parameters for analysis and quantitation.

2.4.2.1.11.4.2 Sample should be analyzed full scan acquisition mode.

2.4.2.1.11.5 Detection and Identification Criteria

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

2.4.2.1.11.5.1 Chromatographic Criteria

The retention time of the analyte should fall within ±2% of the retention time exhibited by GHB standards.

2.4.2.1.11.5.2 Full Scan Acquisition

Full scan data should be compared against within run GHB standards.

2.4.2.1.12 REFERENCES

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