Section Two
Urine Toxicology

2.4 Liquid-Liquid Extraction Methods for Qualitative GC/MSD Confirmation

2.4.2 Screening or Confirmation for Gamma-Hydroxybutyrate (GHB) in Urine Utilizing TOXI-B or DE-TOX Extraction Tubes

2.4.2.1 BACKGROUND

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 to 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 to 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 is anywhere from 2.5 to 4.0 grams in approximately 1 teaspoon ("capful") of liquid. 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
Georgia Home Boy	Thunder Nectar
G-riffic	Rest-Eze
Grievous Bodily Harm	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 affects users so differently. A dose that one individual uses could adversely affect another, thus word of mouth is a poor determiner of how much of the substance to use.

2.4.2.2 SCOPE

This method provides an efficient qualitative analysis option for the liquid-liquid extraction of urine samples suspected of containing γ-Hydroxybutyrate/γ-Hydroxybutyric Acid (GHB). 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 a De-Tox 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. This method should only be used for driving under the influence of drugs (DUID) situations where GHB is suspected or for screening purposes for DFSA. Presently DFSA case urine that indicates a positive result by this method must be outsourced for quantitative confirmation.

2.4.2.3 EQUIPMENT

2.4.2.3.1 Tube Rocker (Fisher Scientific or equivalent)
2.4.2.3.2 Evaporative Concentrator equipped with a nitrogen tank
2.4.2.3.3 Laboratory Centrifuge
2.4.2.3.4 Drybath
2.4.2.3.5 Fixed and adjustable volume single channel air displacement pipetters, and appropriate tips, capable of accurate and precise dispensing of volumes indicated.
2.4.2.3.6 16X100mm centrifuge tubes
2.4.2.3.7 {Optional} 16X144mm tapered-end centrifuge tubes
2.4.2.3.8 Caps for 16mm O.D. Tubes
2.4.2.3.9 Automated Liquid Sampler (ALS) vials
2.4.2.3.10 ALS vial microinserts
2.4.2.3.11 Gas Chromatograph equipped with a mass selective detector 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)

2.4.2.4 REAGENTS

2.4.2.4.1 De-Tox B Tubes (or equivalent Toxi-B tube)
2.4.2.4.2 MSFTA or BSTFA with 1% TMCS

2.4.2.5 REFERENCE MATERIAL

2.4.2.5.1 Stock Solution
1mg/mL (1000ng/µL) GHB

2.4.2.5.2 GHB Spiked Urine Positive Controls
Use the same lot of negative urine to prepare both the negative and spiked positive controls.

2.4.6.5.2.1 **100µg/mL**
Add 450µL of GHB 1mg/mL stock to 4050µL negative urine. Vortex.

2.4.6.5.2.2 **200µg/mL**
Add 900µL of GHB 1mg/mL stock to 3600µL negative urine. Vortex.

2.4.2.5.3 Non-Extracted GHB Reference Material [10µg]
Place 10µL of GHB stock into tube.

2.4.2.5.4 Negative Control
Negative Urine can be commercially obtained or in-house urine verified to be negative for drugs of interest.

2.4.2.6 PROCEDURE

2.4.2.6.1 Initial set-up

2.4.2.6.1.1 Label De-Tox B Tubes for positive controls, negative control and case samples.

2.4.2.6.1.2 Label tubes and ALS vials for positive controls, negative control, case samples and non-extracted reference material.

2.4.2.6.2 Extraction procedure

2.4.2.6.2.1 Extract 4.5 mL of specimen, negative and spiked urine in De-Tox B Tube (acidic extraction @pH=4.5). Less than 4.5mL may be used if sample is limited.

2.4.2.6.2.2 Rock De-Tox tube for ≅10 minutes.

2.4.2.6.2.3 Centrifuge TOXI-TUBE at ≅2500-3000 rpm for ≅10 minutes.

2.4.2.6.2.4 Transfer solvent from De-Tox Tube into tapered-end centrifuge tube.

2.4.2.6.2.5 Evaporate solvent to approximately 50µL with nitrogen at ≤40°C. Non-extracted reference material must be evaporated to dryness.

2.4.2.6.3 Derivatization Procedure

2.4.2.6.3.1 Add 40µL MSFTA or BSTFA with 1%TMCS to evaporated extracted samples, spiked control(s) and non-extracted reference material. Cap tube with snap cap. Vortex.

2.4.2.6.3.2 Place tube in 60°C drybath for 15 minutes.

2.4.2.6.3.3 Remove tube from drybath. Allow sample to cool.

2.4.2.6.3.4 Transfer derivative to labeled GC/MS ALS vial for analysis.

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

2.4.2.6.4.1 Key parameters are specified below. Parameters not specified are at the discretion of the analyst and should be optimized for the both gas chromatographic and mass spectral characteristics of an instrument. Refer to GC/MS METHOD for current parameters for analysis. Each laboratory shall maintain a centrally

stored current METHOD printout or electronic copy.

2.4.2.6.4.2 **ALS Parameters**
Injection Volume: 1µL

2.4.2.6.4.3 **Acquisition Mode**
Sample must be analyzed full scan acquisition mode.

2.4.2.6.5 Detection and Identification Criteria

2.4.2.6.5.1 **Chromatographic Criteria**
The retention time of the analyte should fall within ±2% of the retention time exhibited by GHB reference material and control(s).

2.4.2.6.5.2 **Mass Spectral Criteria**
Full scan mass spectral data should be compared against within run GHB reference material and control(s). No significant differences should be apparent.

2.4.2.7 QUALITY ASSURANCE

2.4.2.7.1 General

2.4.2.7.1.1 Refer to toxicology analytical methods 5.8 and 5.10 for additional quality assurance and reference material authentication requirements.

2.4.2.7.2 Per Analysis Run Control and Reference Material Requirement
Each run should include, at a minimum, a 100µg/mL or 200µg/mL GHB control, a negative control and a non-extracted GHB reference material.

2.4.2.8 ANALYSIS DOCUMENTATION

2.4.2.8.1 Original data for controls will be compiled for each analysis run and stored centrally in the laboratory where the analysis was performed until archiving.

2.4.2.8.2 A copy of controls need not be included in individual case files. When necessary, a copy of control printouts can be prepared from the centrally stored document.

2.4.2.9 REFERENCES

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

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

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Revision History

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2.4.2 Qualitative Confirmation of Gamma-Hydroxybutyrate (GHB) in Urine Utilizing TOXI-B Extraction Tubes

Revision #	Issue Date	Revision
1	02/05/02	Original Issue in SOP Format
2	10/18/02	Refinements
3	05/20/04	Refinements Renamed from 2.4.2.1 Extraction option two moved to 2.4.6 GHB products moved to SOP 6.1 and 6.2 No extraction method modifications therefore no revalidation pursued.
4	05/07/2007	Updated QA measures and reformatting.
5	07-28-2008	Clarified that negative urine used to prepare positive control is the same lot as used for negative control.
6	12-16-2011	Adjusted volume of non-extracted ref material used to prevent overloading instrument, removed qc requirements that are covered in other methods. Clarified that samples must be run in full acquisition mode. Removed injection parameters that are not needed. Reduced centrifuge time from 15 minutes to 10 minutes.
7	09-06-2013	Replaced Toxi B tubes with De-Tox B tubes and allowed for either tube to be used. Reduced tube rocking time to 10 minutes from 15 and expanded centrifuge speed from 2500 rpm to 2500-3000 rpm.