

| LABORATORY SERVICES BUREAU | | |
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| Document: Toxicology Procedures | Policy Number: 1288 | Revision: 17 |
| Subject: TOX-SOP-22 Protocol for the Analysis of GHB in Blood and Urine | Approved: Gallegos, Amanda | |
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1. PROTOCOL FOR THE ANALYSIS OF GHB IN BLOOD AND URINE

PURPOSE

The following method describes the preliminary screening and qualitative confirmation of gamma-hydroxybutyric acid (GHB) in blood, serum, plasma and urine using a liquid/liquid extraction analyzed by GC/MS. Samples submitted for GHB analysis will follow the following protocol.

PLAN

A. Equipment:

- (1) GC/MS with a 5% diphenylpolysiloxane, 95% dimethylpolysiloxane, 15/30 meter, 0.25 micron film thickness column
- (2) Centrifuge
- (3) Heating block
- (4) Sample concentrator with UHP Nitrogen
- (5) Vortex mixer / Multi-tube vortex mixer

B. Reagents:

- (1) **Saturated ammonium chloride solution.** To 250ml of water add 50 grams of ammonium chloride. Stir until dissolved. Label reagent. Store at room temperature. Stable for two years.
- (2) **3N HCl.** To 250ml of water add 65ml of concentrated hydrochloric acid. Label reagent. Store at room temperature. Stable for two years.
- (3) **Ethyl acetate.** Prepare a 100ml transfer bottle of ACS/HPLC grade ethyl acetate. Label accordingly. Store in glass at room temperature. Stable until consumed.
- (4) **BSTFA with 1% TMCS.** Crimp cap and label appropriately if transferred. Store at room temperature. Stable until consumed.

C. Standards: (Store refrigerated)

- (1) **1 mg/ml GHB sodium salt standard** in methanol (Cerilliant G-001 or Cayman Item# 15661) Stable per manufacturer's recommendation.
- (2) **1 mg/ml D6-GHB sodium salt internal standard** in methanol (Cerilliant G-006) Stable per manufacturer's recommendation.

D. Calibrators and Internal Standard:

- (1) **100 µg/ml GHB calibrator stock solution.** In a 1.0 ml vial add 878 µl of methanol and 122 µl of 1 mg/ml GHB sodium salt standard. Store refrigerated. Stable for 2 years.
- (2) **100 µg/ml D6-GHB internal standard stock solution.** In a 10 ml vial add 7.26 ml of methanol and 1 ml of 1 mg/ml D6-GHB sodium salt internal standard. Store refrigerated. Stable for 2 years.

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- (3) **10 µg/ml GHB calibrator.** Prepare on day of use. Add 10 µl of 100 µg/ml GHB calibrator stock solution to 100 µl of negative urine or blood.
- (4) **25 µg/ml GHB calibrator.** Prepare on day of use. Add 25 µl of 100 µg/ml GHB calibrator stock solution to 100 µl of negative urine or blood.
- (5) **50 µg/ml GHB calibrator.** Prepare on day of use. Add 50 µl of 100 µg/ml GHB calibrator stock solution to 100 µl of negative urine or blood.
- (6) **100 µg/ml GHB calibrator.** Prepare on day of use. Add 100 µl of 100 µg/ml GHB calibrator stock solution to 100 µl of negative urine or blood.

E. Quality Controls. (Store Refrigerated)

- (1) **100 µg/ml GHB control stock solution.** In a 1.0 ml vial add 878 µl of methanol and 122 µl of 1 mg/ml GHB sodium salt standard (using a different source if possible than calibrator stock). Store refrigerated. Stable for 2 years.
- (2) **Positive Control(s) 15 µg/ml.** Add 15 µl of above 100 µg/ml GHB control stock solution to 100 µl of negative urine and/or blood (matrix matched controls).
- (3) **Negative Control(s).** Urine produced in house will be used as negative control if analyzing urine samples. Blank blood prepared in house consisting of 50% saline, 50% packed red blood cells, and 5 g sodium fluoride/1 g potassium oxalate (per 500 ml prepared blood) will be used as negative control if analyzing blood samples.

F. Extraction:

- (1) Prepare a set of calibrators, positive control(s) and pipette 100 µl of the negative control(s) as well as case samples into respectively labeled 16x100mm culture tubes. Add 50 µl of internal standard. (High GHB concentration samples from preliminary screen may be diluted, as an example x10 by adding 10 µl sample/ 90 µl deionized water.)

NOTE: Case Samples received in CITRATE BUFFERED TUBES will not be analyzed for GHB.

- (2) Add 500 µl of saturated ammonium chloride solution.
- (3) Add 20 µl of 3N HCl.
- (4) Add 2 ml of ethyl acetate.
- (5) Vortex until thoroughly mixed; centrifuge for five minutes at 3500 rpm. After tubes have been centrifuged, transfer top organic layer into appropriately labeled auto sampler vials.
- (6) Evaporate to near dryness (approximately 10µl). Reconstitute residue with 70 µl of ethyl acetate and vortex. Transfer to vial inserts if using screw cap autosampler vials.
- (7) To each of the vials or inserts add 30 µl of BSTFA (with 1% TMCS). Cap tightly and derivatize for at least 20 minutes at 70°C.

G. Data Acquisition and Analysis:

- (1) Make sure the Autotune was performed, rinse vials filled, etc.

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(2) Set up a sequence with the calibrators injected first in order to calibrate the instrument used. Subsequent injections to include positive and negative controls and solvent blanks (for preliminary screen at beginning and end of case samples; for confirmation between case samples). Load samples onto autosampler according to sequence and have it verified by another analyst before or after analysis but prior to unloading.

(3) The ion ratios and retention times should be set by a mid-level calibrator.

(4) Analyze using the appropriate method on GC/MS.

H. Results and Acceptability (Qualitative):

(1) Calibration $R^2 \geq 0.99$, lowest calibrator within 30% of set value and remaining calibrators within 20% of set value

(2) Positive control is positive ($\geq 10 \mu\text{g/ml}$)

(3) Negative control $< 5 \mu\text{g/ml}$

(4) Retention time within 2% as set or stored from calibrator

(5) Qualifier ion ratios within 20% as set or stored from calibrator

(6) Chromatographically acceptable; i.e. peak purity $\geq 90\%$ for target ion

(7) Positive results for GHB will be reported $\geq 10 \mu\text{g/ml}$