

*Approved by Director: Dr. Guy Vallaro*

## 1 PRINCIPLE/SCOPE

This procedure is used for detection of Gamma-hydroxybutyrate (GHB) in urine and liquid samples mainly within drug-facilitated crime (DFC) cases. A deuterated internal standard, Gamma-hydroxybutyrate-d<sub>6</sub> (GHB- d<sub>6</sub>) is added for quantitation purposes. Urine or liquid samples are diluted using water before instrumental analysis. After sample preparation, samples are analyzed by LC/MS/MS.

## 2 SPECIMENS

This procedure uses 20 µL of urine or liquid sample. No dilutions of samples shall be performed.

## 3 REAGENTS

3.1 If a part or item number listed is not available, an equivalent may be purchased.

NOTE: When possible, calibrators and controls are prepared with reference materials from different manufacturers. If different manufacturers are not available, a calibrator and control may be made using the same manufacturer provided a different analyst prepares each solution.

3.2 If the equivalent reference material purchased has a different concentration, an appropriate volume (µL) shall be used.

3.3 All reagents must be ACS grade or better.

3.3.1	Formic Acid	Fisher 27048
3.3.2	Water	Millipore, Deionized (DIW)
3.3.3	Methanol	Fisher A456
3.3.4	Drug-Free Human Urine	UTAK 88121-CDF(F)
3.3.5	GHB (1.0 mg/mL)	Cerilliant G-001-1ML
3.3.6	GHB (1.0 mg/mL)	Lipomed GHB-538-NA-1LM

3.3.7 GHB-d<sub>6</sub> (1.0 mg/mL)

Cerilliant G-006-1ML

**4 EQUIPMENT**

## 4.1 LC/MS/MS

4.1.1 Agilent 6475AA QQQ with a 1260 Infinity II LC stack or equivalent

## 4.2 LC Column

4.2.1 100 x 2.1 mm, 1.8 µm particle size, Zorbax Eclipse Plus C18, Agilent, or equivalent

## 4.3 Vortexer

## 4.4 Autosampler vials with inserts (1.8 mL or equivalent)

**5 SOLUTION PREPARATION**

Different volumes may be prepared if the concentration/ratio is consistent.

## 5.1 Mobile phase A (0.01% Formic Acid in water)

5.1.1 Combine 100 µL of formic acid to a final volume of 1000 mL water in a glass bottle.

5.1.1.1 Prepare fresh.

## 5.2 Mobile phase B (0.01% Formic Acid in Methanol)

5.2.1 Combine 100 µL of formic acid to a final volume of 1000 mL methanol in a glass bottle.

5.2.1.1 Prepare fresh.

## 5.3 Cutoff Solution (GHB 10,000 ng/mL)

5.3.1 Combine 10 µL of the 1.0 mg/mL Cerilliant GHB standard and 990 µL of drug-free urine; vortex.

5.3.2 Stable for 1 week when stored in the refrigerator.

## 5.4 Control Solution (GHB 30,000 ng/mL)

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5.4.1 Combine 30 µL of the 1.0 mg/mL Lipomed GHB standard and 970 µL of drug-free urine: vortex.

5.4.2 Stable for 1 week when stored in the refrigerator.

5.5 Internal Standard Solution (GHB 10,000 ng/mL)

5.5.1 Combine 50 µL of the 1.0 mg/mL Cerilliant GHB-d<sub>6</sub> standard and 4950 µL of methanol.

5.5.2 Stable for 6 months when stored in the freezer.

## 6 PROCEDURE

### 6.1 Sample Preparation

6.1.1 Label clean autosampler vials with inserts accordingly: negative control, cutoff, positive control, and case sample identifier.

6.1.2 Add 40 µL of Internal Standard Solution to all vials.

6.1.3 Add 180 µL of water to all vials.

6.1.4 Add 20 µL of sample(blank matrix (urine), cutoff solution (urine), control solution (urine) or case sample) to each vial.

For Liquid Cases: Additional water cutoff and control solutions will be run for matrix comparison. NOTE: The drug-free urine used for the negative control matrix must be the same lot as the cutoff and control solutions.

6.1.5 Cap the vials and mix.

6.1.6 Ensure the appropriate instrumental quality assurance/quality control (QA/QC) procedures were performed. The instrument must have passing QA/QC results prior to preparing and loading of samples.

6.1.7 Prepare the sequence and enter the samples in appropriate order. Negative controls will be analyzed prior to evidentiary samples. Blank samples (i.e., those containing just methanol) may be analyzed in between evidentiary samples to avoid carry-over.

6.1.8 Verify the sequence:

6.1.8.1 Print the sequence list.

6.1.8.2 Check that the physical placement of the autosampler vials and the vial positions within the instrument's sequence list match. Indicate completion of this check using sequence checked, sequence verified or similar on the sequence page along with analysts initials and date.

6.1.9 Print the instrument method and include both the method and sequence printouts with the batch documents.

## **7 INSTRUMENT PARAMETERS**

7.1 For complete method parameters see TX 45.1. Documentation of changes must be included with batch data so that any instrumental parameter change can be associated with data and casework until this procedure has been updated.

## **8 DATA PROCESSING**

8.1 Refer to TX 43 TOX Quality Control Manual for specific batch processing criteria.

8.2 GHB is endogenous and therefore may be present in negative control. If the concentration is greater than the cutoff, consult the FSE2 or above.

8.3 Ion ratios should compare favorably to ion ratios of an extracted calibrator or positive control at a comparable concentration. Generally, ion ratios are within the limits specific within the section procedure related to mass spectral comparisons.

## **9 STABILITY POST-EXTRACTION**

9.1 If a case sample was inadvertently not injected or needs to be re-injected, the cutoff and controls shall be re-injected along with the case sample.

NOTE: This would be considered a new analytical run and prepared separately from the original runs.

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- 10.1 Using the cutoff solution, a one-point calibration curve will be established. The curve will be set to linear, non-weighted and origin set to force.
- 10.2 GHB is endogenous and concentrations below the cutoff are considered normal.
- 10.3 Case samples equal to or above the cutoff solution are considered detected.
- 10.4 Gamma-butyrolactone (GBL) is observed in the GHB window, however, differs by retention time.

**11 SAFETY PRECAUTIONS**

Refer to the DSS GL 2 Safety Manual for precautions.

**12 REFERENCES**

S.S. Johansen and C.N. Windberg. Simultaneous Determination of  $\gamma$ -Hydroxybutyrate (GHB) and its Analogues (GBL, 1.4-BD, GVL) in Whole Blood and Urine by Liquid Chromatography Coupled to Tandem Mass Spectrometry. *Journal of Analytical Toxicology*, 35: 8-14 (2011).