16 GAMMA-HYDROXYBUTYRATE QUANTITATION AND CONFIRMATION BY GCMS

16.1 Summary

16.1.1 GHB is extracted from biological samples by acidifying samples with sulfuric acid followed by extracting twice with ethyl acetate. The extract are derivatized with BSTFA and analyzed by selective ion monitoring on a gas chromatograph with a mass selective detector.

16.2 Specimen Requirements

16.2.1 Approximately 200 µL of blood, biological fluids or tissue homogenates.

16.3 Reagents and Standards

16.3.1 Gamma hydroxybutyrate (GHB), 1 mg/mL

16.3.2 GHB-d_6, 1 mg/mL

16.3.3 Ethyl acetate

16.3.4 Acetonitrile

16.3.5 BSTFA

16.3.6 Sulfuric acid

16.3.7 Methanol

16.4 Solutions, Internal Standard, Calibrators and Controls

16.4.1 GHB working solution (200 µg/mL): Pipet 1 mL GHB standard (1 mg/mL) into a 5 mL volumetric flask and QS to volume with methanol.

16.4.2 GHB-d_6 internal standard working solution (200 µg/mL): Pipet 1 mL GHB-d_6 internal standard (1 mg/mL) into a 5 mL volumetric flask and QS to volume with methanol.

16.4.3 0.1 N sulfuric acid: Pipet 300 µL concentrated sulfuric acid into a 100 mL volumetric flask and QS to volume with dH_2O.

16.4.4 Blood calibrator and control preparation:

16.4.4.1 Add the following volumes of GHB working solution to appropriately labeled 16 x 125mm screw cap tubes for the following final concentrations of GHB:

16.4.4.1.1 400 mg/L GHB: 400 µL GHB Working Solution
16.4.4.1.2 200 mg/L GHB: 200 µL GHB Working Solution
16.4.4.1.3 100 mg/L GHB: 100 µL GHB Working Solution
16.4.4.1.4 40 mg/L GHB: 40 µL GHB Working Solution
16.4.4.1.5 20 mg/L GHB: 20 µL GHB Working Solution
16.4.4.1.6 10 mg/L GHB: 10 µL GHB Working Solution

16.4.4.2 Evaporate standards to dryness under nitrogen. Add 200 µL blank blood to each standard.

16.4.4.2.1 Negative control. Blood bank blood (or comparable) determined not to contain GHB.

16.4.4.2.2 Positive control. Control may be from an external source or prepared in house using drugs from different manufacturers, lot numbers or prepared by a chemist different than the individual performing the extraction.

16.5 Apparatus

16.5.1 Agilent GC/MSD, Chemstation software, compatible computer and printer

16.5.2 Test tubes, 16 x 125 mm round bottom, screw cap tubes, borosilicate glass with Teflon caps

16.5.3 Test tubes, 16 x 114 mm, glass centrifuge, conical bottom

16.5.4 Centrifuge capable of 2,000 – 3,000 rpm

16.5.5 Vortex mixer

16.5.6 Heating block

16.5.7 Evaporator/concentrator

16.5.8 GC autosampler vials and inserts

16.5.9 GC/MSD Parameters. Instrument conditions may be changed to permit improved performance.

16.5.9.1 Acquisition Mode: SIM (quantitation ions)

GHB: 233, 117, 204
GHB d_6: 239

16.5.9.2 Column: HP5 MS 25 m x 0.25 mm x 0.25 µm

16.5.9.3 Detector Temperature: 280° C

16.5.9.4 Oven Program

- Equilibration time: 0.50 minutes
- Initial temp: 80° C
- Initial time: 2 minutes
- Ramp: 17° C/min
- Final Temp: 280° C
- Final Time: 5 minutes
- Run Time 15 minutes

16.5.9.4.1 Inlet

- Mode: Split, ratio 10:1
- Temperature: 250° C
- Injection volume: 1.0 µL
- Purge Time: ON at 2.0 minute

16.6 Procedure

16.6.1 Label clean 16 x 125 mm screw cap tubes accordingly, negative, calibrators, controls and case sample IDs.

16.6.2 Prepare calibrators and controls.

16.6.3 Pipet 200 µL of each case sample into appropriately labeled tubes.

16.6.4 Add 200 µL GHB-d_6 internal standard working solution (200µg/mL) to each tube.

16.6.5 Add 250 µL cold 0.1 N sulfuric acid to each tube.

16.6.6 Add 6 mL ethyl acetate to each tube.

16.6.7 Vortex each sample for 30 seconds.

16.6.8 Centrifuge at approximately 2500 rpm for 10 minutes.

16.6.9 Transfer upper organic layer (ethyl acetate) to labeled conical bottom tubes. Save bottom layer.

16.6.10 Add 6 mL ethyl acetate to each sample again.

16.6.11 Vortex each sample for 30 seconds.

16.6.12 Centrifuge at approximately 2500 rpm for 10 minutes.

16.6.13 Transfer upper organic layer (ethyl acetate) to conical bottom tubes containing the first extract.

16.6.14 Evaporate samples under nitrogen at 55° C

16.6.15 Add 60 µL acetonitrile to each sample

16.6.16 Add 30 µL BSTFA to each sample. Mix briefly.

16.6.17 Heat samples at 70° C for 15 minutes.

16.6.18 Transfer samples to GC autosampler vials.

16.6.19 Inject samples on GCMS

16.7 Calculation

16.7.1 Calculate the concentrations by interpolation of a linear plot of the response curve based on ratios (using target ions listed under GCMS conditions) versus calibrator concentration.

16.8 Quality Control and reporting

16.8.1 If positive, the analyte must be confirmed in a second extract.

16.8.2 See Toxicology Quality Guidelines

16.9 References

16.9.1 A solid phase method for GHB in urine without conversion to GBL, developed by United Chemical Technologies.

16.9.2 F. Couper and B. Logan. Determination of GHB in biological specimens by GCMS. J Anal Tox 24:1-7, 2000.