

	TX-PM 3.4 Preliminary Screening of Gamma-hydroxybutyrate (GHB) in Urine by QTRAP 4500	
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	Document Manager: Nicholas Fillinger	Approved By: Jeffrey Nye

3.4 Preliminary Screening of Gamma-hydroxybutyrate (GHB) in Urine by QTRAP 4500

3.4.1 Analyte

Gamma-hydroxybutyrate

3.4.2 Specimen Requirements

100 µL of urine

3.4.3 General Description of Method

A preliminary analysis of gamma-hydroxybutyrate in urine using liquid chromatography/tandem mass spectrometry.

3.4.4 Equipment and Reagents

- QTRAP 4500 equipped with validated column
- 0.1% Formic Acid in Water
- 0.1% Formic Acid in Methanol
- 1.7 mL micro-centrifuge tube
- 12x75 mm culture tube
- 11 mm autosampler vial with conical insert and appropriate caps
- The usual assortment of laboratory glassware, pipettes, vortexers, centrifuges and turbovaps
- HPLC grade, or higher, acetonitrile
- High purity formic acid
- LCMS grade water
- Certified reference materials

3.4.5 Sample Preparation

3.4.5.1 Internal Standard

- Pipette 10 µL of internal standard into each centrifuge tube

3.4.5.2 Standards and Controls

- Pipette 10 µL of the GHB standard, 5 µg/mL control and 10 µg/mL control to appropriately labeled centrifuge tubes

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- Pipette 100 µL of blank urine into a centrifuge tube to be used as a negative control
- Pipette 100 µL of water into each centrifuge tube
- Pipette 100 µL blank urine into each centrifuge tube

3.4.5.3 Unknowns

- Pipette 100 µL of water into each centrifuge tube
- Pipette 100 µL of sample urine into appropriately labeled centrifuge tube

3.4.5.4 Additional Sample Preparation

- Close centrifuge tube and vortex
- Centrifuge for 10 minutes at 13000 RPM
- Transfer 150 µL of supernatant into autosampler vials described in 3.4.4 and crimp caps
- Tap vials or vortex to remove any existing air bubbles at bottom of vial

3.4.6 Instrument Preparation

3.4.6.1 Maintenance

- Check to ensure that the following maintenance has been completed
 - Curtain plate has been cleaned within the last 7 days
 - Guard column has been changed within the last 2 months
 - Mobile phases have been prepared within the last 30 days and are of sufficient volume to complete the analytical run
- HPLC is connected to MS
- Correct mobile phases are being used
- Waste container is not full
- Pump valves are closed

3.4.6.2 Equilibrate

- Equilibrate until operating temperature (40°C) is obtained
- Ensure that operating pressures are stable and record in log book
- Run at least two blanks to warm up the system

3.4.6.3 Retention Time Update

- Run appropriate standard and update retention times in acquisition and quantitation methods

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3.4.7 Run Samples

- Subsequent to all steps in 3.3.6, samples may be analyzed on the QTRAP 4500

3.4.7.1 Controls

- In addition to casework samples, each analytical batch shall contain a standard, control, cutoff control A, cutoff control B and negative control.
- Control values \pm 30% of the target value to be considered acceptable.
- If one control is out of range, no action is necessary.
- If two controls are out of range, the batch shall be repeated.

Analyte	Standard $\mu\text{g/mL}$	Control $\mu\text{g/mL}$	Cutoff Control $\mu\text{g/mL}$
GHB	10	10	5

3.4.8 Data Analysis

3.4.8.1 Cutoff levels

Analyte	Cutoff + $\mu\text{g/mL}$	Cutoff ++ $\mu\text{g/mL}$
GHB	5-24.9	≥ 25

3.4.8.2 Unknowns

- Precursor and product ions have been validated and shall not be changed.
- Retention times listed are intended to aid the analyst in data interpretation, however may need to be adjusted from batch to batch.
- Each standard, control and unknown shall be monitored for the following analyte product ions:

Analyte	Precursor Ion	Product Ion 1	Product Ion 2	Retention Time
GHB	103	57.1	85	1.78
D6-GHB	109	90	61.1	1.76

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3.4.8.3 Multiquant

- All data analysis shall occur in the MultiQuant software

3.4.9 Bibliography

Alan D. Brailsford, David A. Cowan and Andrew T. Kieman; Urinary γ -hydroxybutyrate Concentrations in 1126 Female Subjects: Journal of Analytical Toxicology, Vol. 32, November/December 2010

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