| REVIEWED | |
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| By Celena Shrum at 9:37 am, May 06, 2021 | J |

SC

5/6/2021

Worklist: 4953

| LAB CASE | <u>ITEM</u> | ITEM TYPE | DESCRIPTION |
|------------|-------------|-----------|----------------|
| M2021-1056 | 2 | UCK | AM 6 Urine GHB |



AM 6: Urine GHB Screening Extraction

Extraction Date: 5/5/21

Analyst: Sarah Collins

Mobile phase B: 0.1% Formic Acid in MeOH

Mobile phase A: 0.1% Formic Acid in Water **Column**: Phenomenex Phenyl Hexyl (4.6x50mm, 2.6um) Blank Urine Lot: POC031319 LCMS-QQQ ID: 069901 **GHB-D6**: FE07031801Cerilliant GHB: FE04111903 Cerilliant

Pre-Analytic:

☑ 1. Positive Control Working Solution Preparation Instructions:

- Working Solution: Preparation of 200,000 ng/mL Positive Control Working Solution: Add 200µL of GHB 1 mg/mL stock solution to 800µL negative urine.
- Preparation of 10,000 ng/mL Positive Control: Add 10µL of GHB 20,000 ng/mL working solution to 190 • μL negative urine.
- \boxtimes 2. Check levels of mobile phases and needle wash refill as needed. Ensure waste is not full.
- \boxtimes 3. Ensure correct column is installed and begin mobile phase flow allow to equilibrate ~ 30 minutes.

Analytic:

- ⊠ 1. Remove working solutions, controls, and samples from cold storage.
- \boxtimes 2. Label centrifuge tubes for positive control, negative control and case samples.
- Label ALS or LCMS vials for positive control, negative control, and case samples. Place insert in all vials. ⊠ 3.
- \boxtimes 4. Place on tube rocker at ambient temp for approx. 10 minutes.
- ⊠ 5. Pipette positive and negative controls (for negative control, 200 µL urine will be added to the appropriate tube). Add 200µL urine to each centrifuge tube for case samples.
- ⊠ 6. Add 100µL of the GHB-D6 Internal Standard Working Solution to each tube.
- ⊠ 7. Add 900µL of 0.1% formic acid in methanol to each tube. Vortex.
- ⊠ 8. Centrifuge at ~3400 rpm for 15 minutes.
- Add 100µL 0.1% formic acid in water to each vial insert. ⊠ 9.
- ⊠ 10. Transfer 10µL of sample from each centrifuge tube to the corresponding vial insert (avoid disturbing the pellet at the bottom). Vortex.

Post-Analytic

- ⊠ 1. Open quantitation software and create a new quantitation batch.
- ⊠ 2. Using the positive control, a 1-point calibration curve will be established. The curve will be set to linear, nonweighted and origin set to force.
- If a sample gives a response that is greater than 10,000 ng/mL, a statement on the report will be included ⊠ 3. saying that preliminary testing indicated a possible presence of an elevated level of GHB and that it is recommended that the sample be sent to a private lab for quantitation. If a sample gives a response between 7,000 and 10,000 ng/mL, an inconclusive statement can be added to the report.
- ⊠ 4. The S/N for samples and controls at and over 10,000 ng/mL must be 5 or greater
- Case samples and negative controls will generally be considered negative if the calculated concentration is \times 5 less than 7,000 ng/mL.
- ⊠ 6. Central File Packet to include: LIMS Worklist, Method Checklist, Working solution prep sheet(s), Calibration and Control Reports

COMMENTS: The working solutions for the GHB positive control and internal standard were made using the lots above on 5/4/21.

AM #6 GHB Screen Results



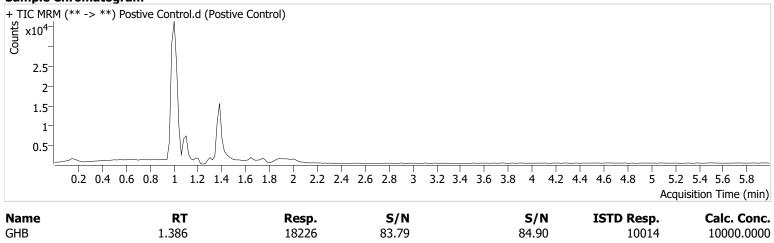
 Batch results
 D:\MassHunter\Data\2021\GHB\050521 GHB SC\QuantResults\GHB.batch.bin

 Calibration Last Update
 5/6/2021 9:00:56 AM

InstrumentFalcoTypeCalAcq. MethodGHB urine screen.mSample PositionP1-A2Injection Volume2.5Acq. Date-Time5/5/2021 2:34:31 PMSample Info.Falco

Data File Sample Operator Comment Postive Control.d Postive Control Sarah Collins

Sample Chromatogram



AM #6 GHB Screen Results



D:\MassHunter\Data\2021\GHB\050521 GHB SC\QuantResults\GHB.batch.bin **Batch results** Calibration Last Update 5/6/2021 9:00:56 AM

Falco Instrument Sample Туре Acq. Method GHB urine screen.m Sample Position P1-A1 **Injection Volume** 2.5 Acq. Date-Time 5/5/2021 2:27:56 PM Sample Info.

Data File Sample Operator Comment Negative Control.d **Negative Control** Sarah Collins

Sample Chromatogram

