

REVIEWED

By Anne Nord at 1:04 pm, Nov 17, 2020

11/12/2020 BW

Worklist: 4609

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2020-2120	2	UCK	AM 6 Urine GHB



AM 6: Urine GHB Screening Extraction

Extraction Date: 11/6/2020

Analyst: Britany Wylie

Mobile phase A: 0.1% Formic Acid in Water
0.1% formic acid in methanol

Mobile phase B: 0.1% Formic Acid in MeOH
0.1% formic acid in water

Blank Urine Lot: 10120

Column: Phenomenex Phenyl Hexyl (4.6x50mm, 2.6um)

LCMS-QQQ ID: 69679

GHB Control Lot: 11620

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.
- ☒ 2. Check levels of mobile phases and needle wash refill as needed. Ensure waste is not full.
- ☒ 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.
- ☒ 2. Label centrifuge tubes for positive control, negative control and case samples.
- ☒ 3. Label ALS or LCMS vials for positive control, negative control, and case samples. Place insert in all vials.
- ☒ 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.
- ☒ 9. Add 100µL 0.1% formic acid in water to each vial insert.
- ☒ 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, non-weighted and origin set to force.
- ☒ 3. If a sample gives a response that is greater than 10,000 ng/mL, a statement on the report will be included 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
- ☒ 5. Case samples and negative controls will generally be considered negative if the calculated concentration is 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:

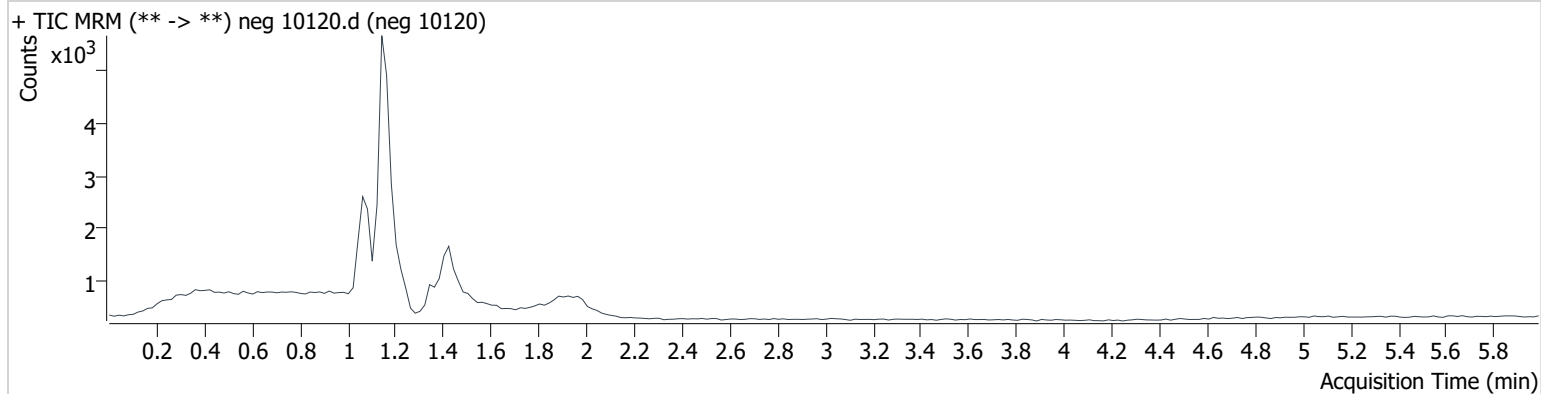
GHB Screen results

BW

Batch results D:\MassHunter\Data\2020 Data\ghb 11-6-20\QuantResults\GHB 11-17-20.batch.bin
Calibration Last Update 11/17/2020 10:44:31 AM

Instrument	69679	Data File	neg 10120.d
Type	Sample	Sample	neg 10120
Acq. Method	GHB urine screen.m	Operator	Britany Wylie
Sample Position	P5-B1	Comment	
Injection Volume	1		
Acq. Date-Time	11/6/2020 9:45:31 AM		
Sample Info.			

Sample Chromatogram

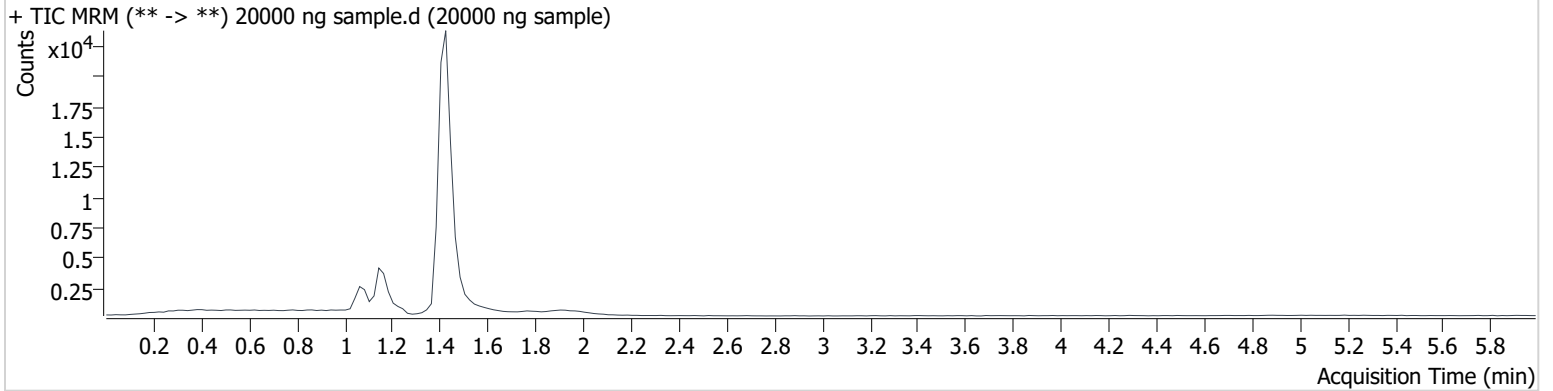


GHB Screen results

Batch results D:\MassHunter\Data\2020 Data\ghb 11-6-20\QuantResults\GHB 11-17-20.batch.bin
Calibration Last Update 11/17/2020 10:44:31 AM

Instrument	69679	Data File	20000 ng sample.d
Type	Cal	Sample	20000 ng sample
Acq. Method	GHB urine screen.m	Operator	Britany Wylie
Sample Position	P5-A1	Comment	
Injection Volume	1		
Acq. Date-Time	11/6/2020 9:38:56 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	S/N	ISTD Resp.	Calc. Conc.
GHB	1.426	60720	187.5	167.9	4252	200000.000

GHB controls

200000 ng/ml working solution 200 ul 1 mg/ml GHB into 800 ul neg urine (10120)

ppd 11/6/20 Exp 5/6/20 lot 11620 by baw

Drug	lot	expiration
GHB	FE04111903	5/1/2024

20000 ng/nl working internal standard solution 1ml 100ul/ml GHB D6 stock in 4000 ul methanol

Ppd 4/15/20 exp 4/15/2021 lot GHB-D6 41520 by amn

Drug	lot	expiration
GHB-D6	FE07031801	9/1/2023

Request for Departure from an Analytical Method or Quality Standard

Date of Request

11/13/20

Requestor

Britany Wylie

Analytical Method/Quality Standard

Toxicology Analytical Method 6

3.3.2.1 Preparation of 200,000 ng/mL Positive Control Working Solution

3.3.2.1.1 Add 200 μ L of GHB 1 mg/mL stock solution to 800 μ L negative urine. Vortex. This solution will be stored in the freezer and is good for approximately six months.

3.3.2.2 Preparation of 10,000 ng/mL Positive Control

3.3.2.2.1 Add 10 μ L of GHB 20,000 ng/mL working solution to 190 μ L negative urine. Vortex. This control should be made fresh with each run.

Request

On 11/6/20 I made a new positive control working solution following the method. The working control was used for the extraction and not diluted as defined in 3.3.2.2.1. The internal standard response for the one case sample run during this extraction had a significant internal standard response and no response for GHB, it is requested the sample be evaluated as negative. The positive control concentration is higher than required in the method, however does not affect the results of the sample. It will be noted in the control prep the additional step of dilution for future runs. A checklist was also not available for the method, however one was created after the extraction was completed.

Review

Departure approved

Comments:

Departure Not Approved

Comments:



Celena Shrum
Title: Toxicology Discipline Lead

Date: 11/13/20