6/10/2024

REVIEWED
By Britany Wylie at 7:28 am, Jun 11, 2024

Worklist: 6840

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2024-0866	2	UCK	AM 6 Urine GHB

AM 6: Urine GHB Screening Extraction

Extraction Date: 6/10/24 Analyst: Anne Nord

Mobile phase A: 0.1% Formic Acid in Water **Mobile phase B:** 0.1% Formic Acid in MeOH
0.1% formic acid in methanol 0.1% formic acid in water
Blank Urine Lot: 6524 **Column**: Agilent poroshell 120 (4.6x50mm, 2.7um)
LCMS-QQQ ID: 69769 **GHB Control Lot**: 61024

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. Made fresh 100 ul 195725 formic acid fisher, 100 ml Honeywell lot ED456-US 01-4-24 AMN

☒ 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 controls

200000 ng/ml working solution 200 ul 1 mg/ml GHB into 800 ul neg urine (6524)

ppd 6/10/24 Exp 12/10/24 lot 61024 by AMN

Drug	lot	expiration
GHB	FE03012210	7/1/2027

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

Ppd 1/4/24 exp 1/4/25 lot GHB-D6 01424 by amn

Drug	lot
GHB-D6	FE07031801

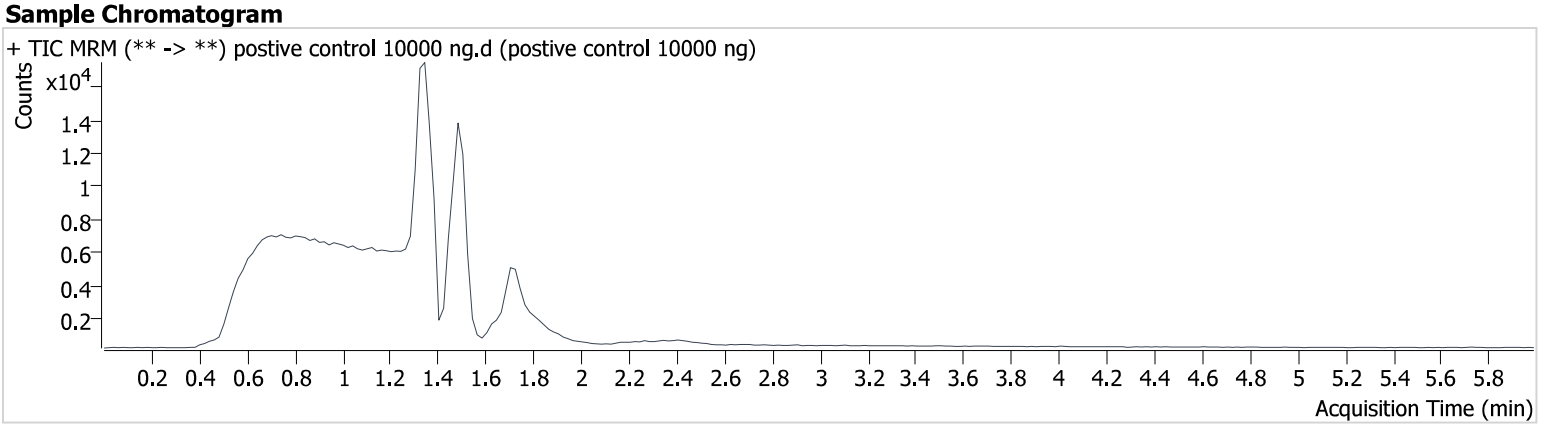
* AM 6 Control: add 10uL of working solution to 190uL negative urine and extract. Approx conc 10,000ng/mL

GHB Screen results

Batch results D:\MassHunter\Data\2024\ghb\061024\QuantResults\ghb.batch.bin
Calibration Last Update 6/10/2024 1:37:37 PM

Instrument	69679	**Data File**	postive control 10000 ng.d
Type	Cal	**Sample**	postive control 10000 ng
Acq. Method	GHB urine screen.m	**Operator**	Anne Nord
Sample Position	Vial 2	**Comment**	
Injection Volume	3		
Acq. Date-Time	6/10/2024 12:26:38 PM		
Sample Info.			

Sample Chromatogram



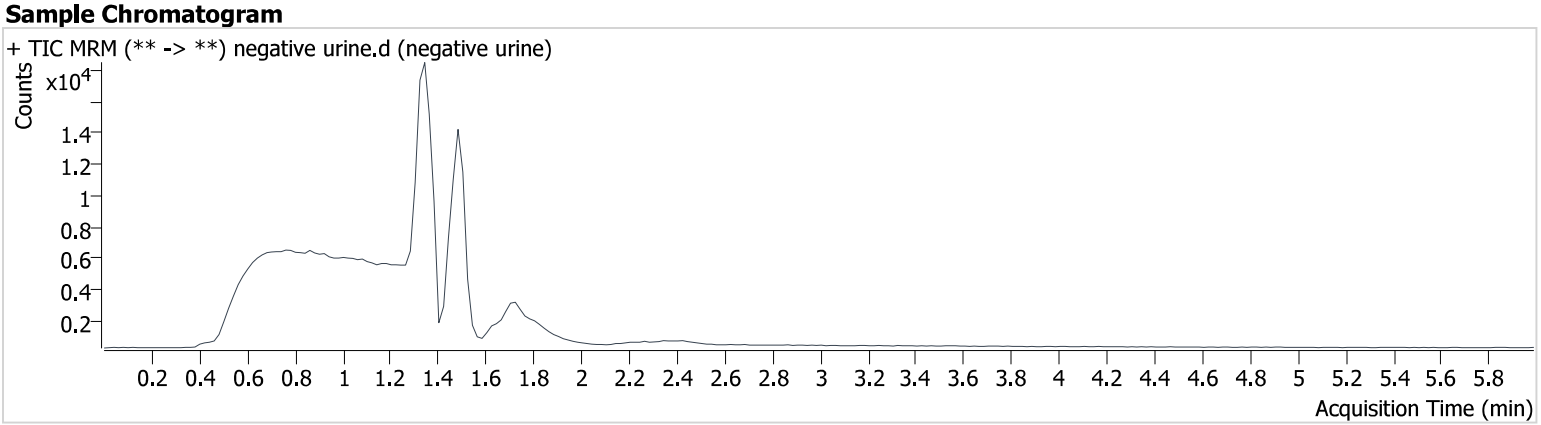
Name	RT	Resp.	S/N	S/N	ISTD Resp.	Calc. Conc.
GHB	1.727	8178	∞	∞	26513	10000.000

GHB Screen results

Batch results D:\MassHunter\Data\2024\ghb\061024\QuantResults\ghb.batch.bin
Calibration Last Update 6/10/2024 1:37:37 PM

Instrument	69679	**Data File**	negative urine.d
Type	Sample	**Sample**	negative urine
Acq. Method	GHB urine screen.m	**Operator**	Anne Nord
Sample Position	Vial 3	**Comment**	
Injection Volume	3		
Acq. Date-Time	6/10/2024 12:33:06 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	S/N	ISTD Resp.	Calc. Conc.	
GHB	1.807	2843	∞		23813	3871.177	<7000

Additionally peak shape is very poor, and qualifier is missing.