

# Fast Screening and Confirmation of Gamma-Hydroxybutyrate (GHB) in Urine

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*Maximize your analytical options with this versatile GHB extraction method. No derivatization means faster sample preparation. Extracts are amenable to both liquid injection GC/FID and headspace GC/MS methods.*

Gamma-hydroxybutyrate (GHB) and its precursor, gamma-butyrolactone (GBL), are controlled substances associated with drug-facilitated sexual assault. Criminal cases often hinge on lab results, which can include screening urine samples and then quantifying GHB using GC/MS. In its native state, GHB is extremely difficult to chromatograph and must be analyzed as a trimethylsilyl derivative or converted to GBL. The headspace (HS) procedure described here (adapted from an FBI Chemistry Unit method) eliminates time-consuming derivatization.<sup>1</sup> This procedure reduces sample preparation time and minimizes both column contamination from derivatization reagents and contamination from sample matrix caused by liquid injections.

**Improve lab efficiency and reduce contamination and matrix effects by eliminating derivatization and moving to a headspace technique.**

## Eliminate Derivatization and Reduce System Contamination

Samples were spiked in urine and extracted according the procedure in Table I, using alpha-methylene-gamma-butyrolactone (AMGB) as an internal standard. GHB is converted to GBL with sulfuric acid, eliminating the need for derivatization (Figure 1). Note the unconverted sample shows comparable levels of GBL and AMGB, whereas GBL levels in the converted sample are significantly higher, due to the conversion of GHB to GBL.

**Table I** Extraction procedure for GHB and GBL.

1. Label two screw top test tubes per specimen. One for total GHB, the other for GBL only.
2. Add 1mL of sample (urine) to each tube.
3. Add 50µL of AMGB (internal standard) to each tube.
4. Add 150µL concentrated sulfuric acid only to tubes used for analysis of total GHB.
5. Vortex all tubes and allow them to sit 5 minutes.
6. Add 5mL methylene chloride to each tube. Shake 10 minutes to extract.
7. Centrifuge samples at 3,000 rpm for 5 minutes.
8. Transfer bottom (methylene chloride) layer to a clean test tube for drying.
9. Concentrate samples to ~100µL at 30°C under nitrogen.
10. For headspace analysis, inject 15µL of sample into a capped headspace vial. Or, for liquid injection, transfer extract to a limited volume insert.

## Reliably Screen Samples Using Existing Blood Alcohol Testing Set-Up

Headspace injections (using the total vaporization technique) of the final urine extracts were screened by GC/FID using an Rtx®-BAC1 column in a blood alcohol headspace GC system. This system is commonly used in clinical/forensic labs, eliminating the need for additional equipment. Excellent linear response was obtained from both unconverted ( $r^2 = 0.9992$ , 10-100µg/mL 4-point curve) and converted GHB in matrix ( $r^2 = 0.9910$ , 20-200µg/mL 4-point curve) with AMBG at 50µg/mL.

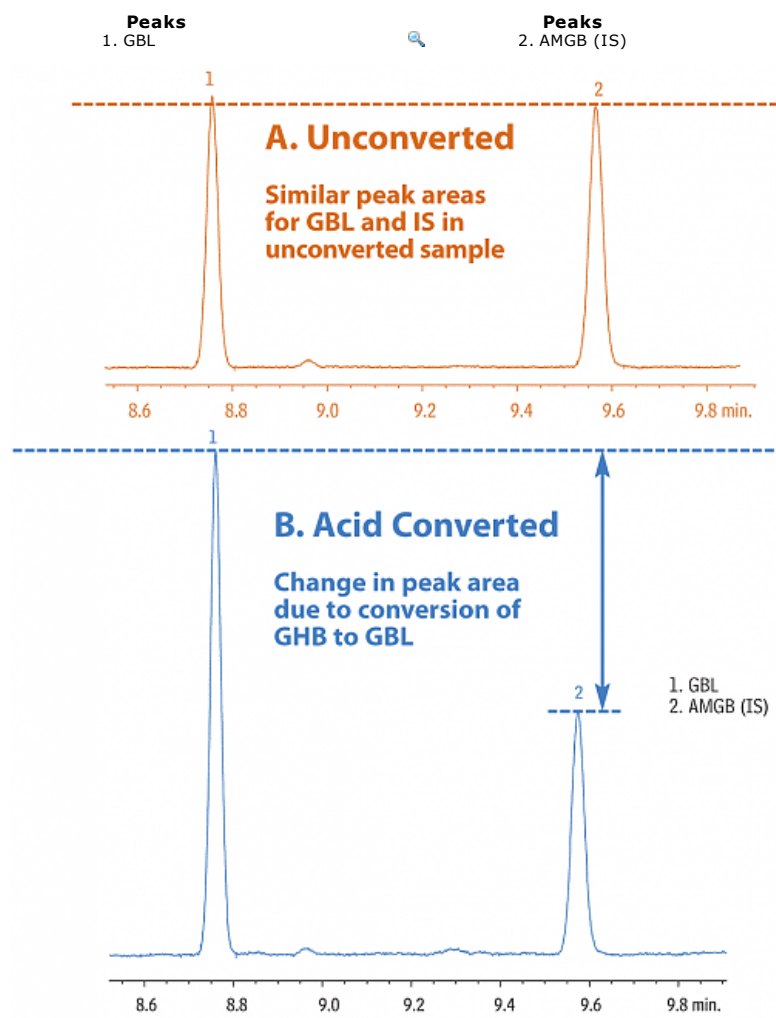
## Fast, Definitive Confirmation Analysis by Headspace GC/MS

Positive screening results were quickly confirmed on an Rtx®-5MS column by headspace GC/MS; several quantification and qualifier ions were identified for each compound (GBL: 42, 56, 86; AMBG: 40, 68, 98). Again, excellent linearity was achieved (Figure 2) and analysis time was less than 7 minutes (Figure 3).

In summary, the versatile extraction and headspace method shown here saves lab time and minimizes contamination by eliminating the need for derivatization and by reducing matrix effects. Rapid screening is accomplished on commonly used blood alcohol GC columns, allowing labs to reduce costs by using existing equipment. Confirmation testing using the Rtx®-5MS column, provides the definitive results needed in court with a fast analysis time of less than 7 minutes.

**Figure 1** GHB can be converted to GBL for analysis, saving time by eliminating

derivatization.



GC\_CF01039

**Column** Rtx®-BAC1, 30 m, 0.32 mm ID, 1.80 µm (cat.# 18003)  
**Sample**  
Conc.: 50 µg/mL GHB, GBL, and AMGB (IS) in urine  
A: unconverted  
B: converted with sulfuric acid

**Injection**  
Inj. Vol.: 1000 µL headspace-syringe split (split ratio 10:1)  
Liner: 1mm Split (cat.# 20972)  
Inj. Temp.: 200 °C

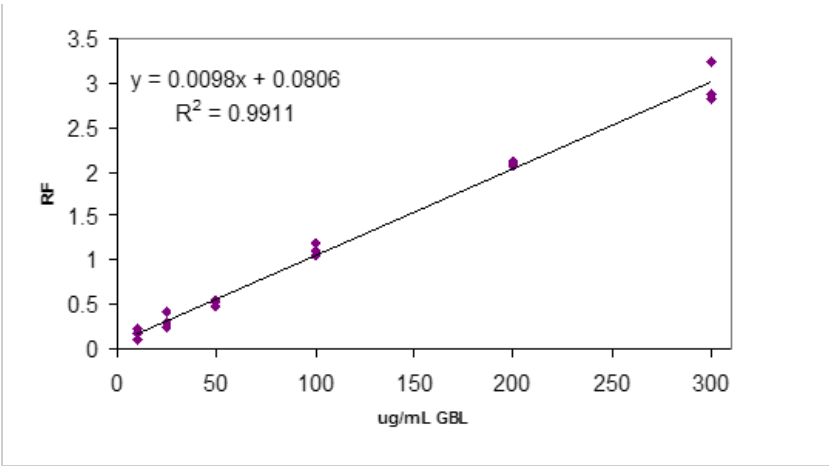
**Headspace-Syringe**  
Sample Temp.: 100 °C  
Sample Equil. Time: 10 min

**Oven**  
Oven Temp.: 50 °C (hold 3 min) to 150 °C at 20 °C/min (hold 7 min)

**Carrier Gas**  
Carrier Gas: He, constant flow  
Flow Rate: 1.0 mL/min

**Detector**  
Detector: FID @ 240 °C  
Make-up Gas Flow Rate: 40 mL/min

**Figure 2** GHB (analyzed as GBL) confirmation method calibration curve for headspace GC/MS analysis (10-300µg/mL in urine).



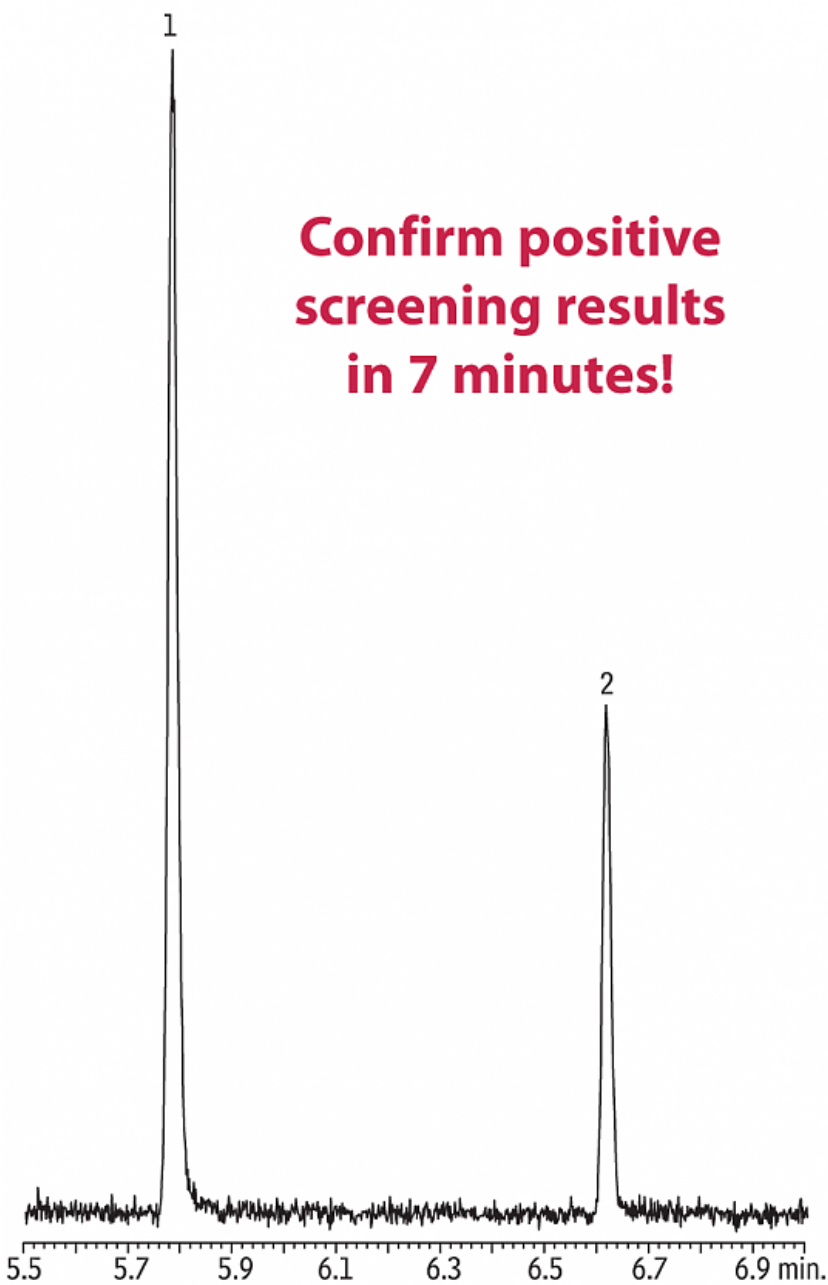
**Figure 3** Confirmation headspace GC/MS analysis of 300µg/mL converted GHB (analyzed as GBL) standard in urine.

**Peaks**  
1. GBL



**Peaks**  
2. AMGB (IS)





**Confirm positive  
screening results  
in 7 minutes!**

GC\_CF01038

**Column** Rtx®-5MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 12623)  
**Sample** 300 µg/mL GHB, 50 µg/mL AMGB in urine, converted with sulfuric acid  
**Injection**  
Inj. Vol.: 1000 µL headspace-syringe split (split ratio 10:1)  
Liner: 1mm Split (cat.# 20972)  
Inj. Temp.: 200 °C  
**Headspace-Syringe**  
Sample Temp.: 100 °C  
Sample Equil. Time: 10 min  
**Oven**  
Oven Temp.: 50 °C (hold 3 min) to 150 °C at 20 °C/min (hold 7 min)  
**Carrier Gas** He, constant flow  
Flow Rate: 1.0 mL/min  
**Detector** MS  
Mode: Scan  
Transfer Line Temp.: 280 °C  
Ionization Mode: EI  
Scan Range: 20-150 amu

#### REFERENCES

1. M.A. LeBeau, M.A. Montgomery, M.L. Miller, S. G. Bumeister, J. Anal. Toxicol. 24 (2000) 421.

#### RELATED SEARCHES

[GHB](#), [Gamma-Hydroxybutyrate](#), [gamma-butyrolactone](#), [gbl](#), [date rape](#), [date rape drug](#), [AMGB](#), [sexual assault](#), [drug-facilitated](#), [urine](#), [headspace](#)

