



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

By Amanda Rigdon and Kristi Sellers

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 [1]. This procedure reduces sample preparation time and minimizes both column contamination from derivatization reagents and contamination from sample matrix caused by liquid injections.

Related Products

- *Rtx-BAC1 GC Capillary Column*
- *Rtx-5MS GC Capillary Column*

Eliminate Derivatization and Reduce System Contamination

Samples were spiked in urine and extracted according to the procedure detailed 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 1 mL of sample (urine) to each tube.
3. Add 50 μ L of AMGB (internal standard) to each tube.
4. Add 150 μ L 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 5 mL methylene chloride to each tube. Shake 10 minutes to extract.
7. Centrifuge samples at 3000 rpm for 5 minutes.
8. Transfer bottom (methylene chloride) layer to a clean test tube for drying.
9. Concentrate samples to \sim 100 μ L at 30 $^{\circ}$ C under nitrogen.
10. For headspace analysis, inject 15 μ L 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 ($r^2 = 0.9992$, 10-100 $\mu\text{g/mL}$ 4-point curve) and converted GHB in matrix ($r^2 = 0.9910$, 20-200 $\mu\text{g/mL}$ 4-point curve) with AMBG at 50 $\mu\text{g/mL}$

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.

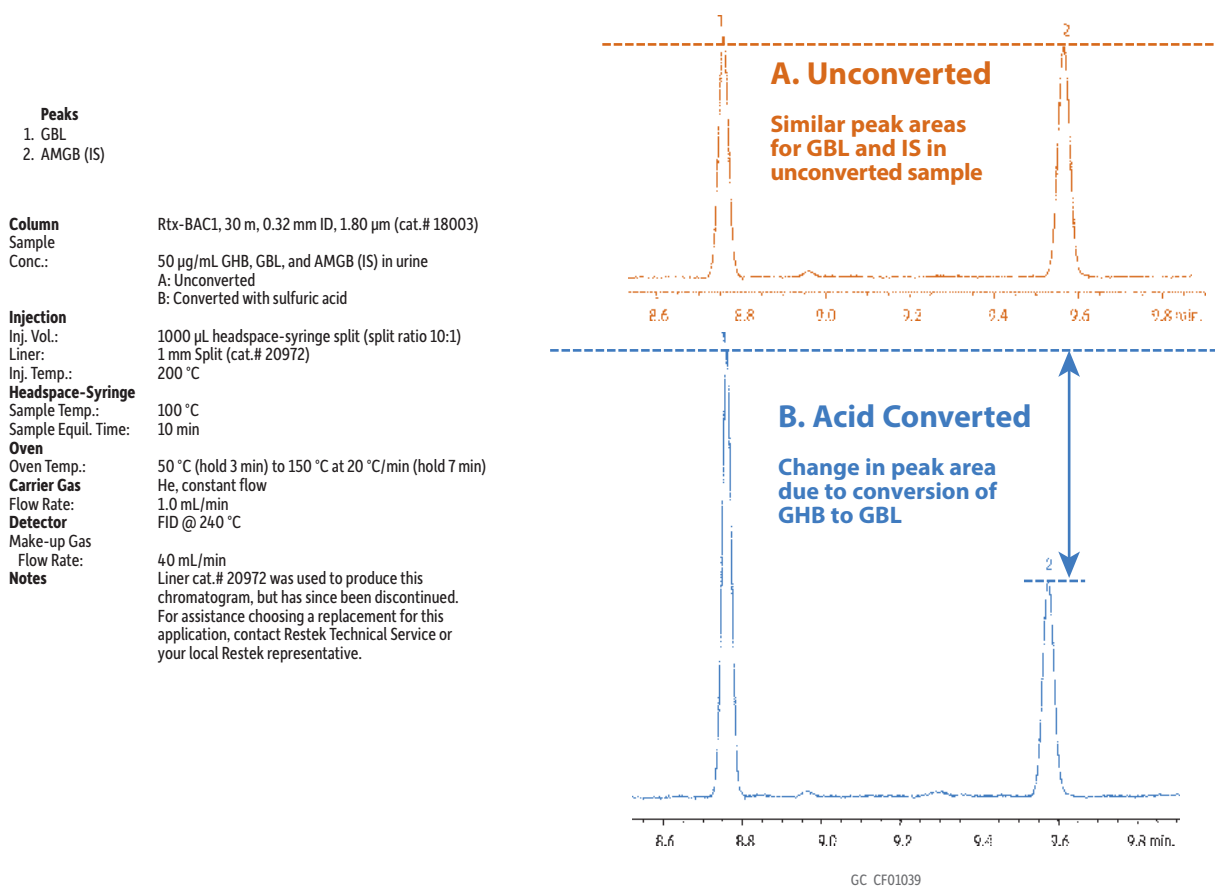


Figure 2: GHB (Analyzed as GBL) Confirmation Method Calibration Curve for Headspace GC-MS Analysis (10-300 µg/mL in Urine)

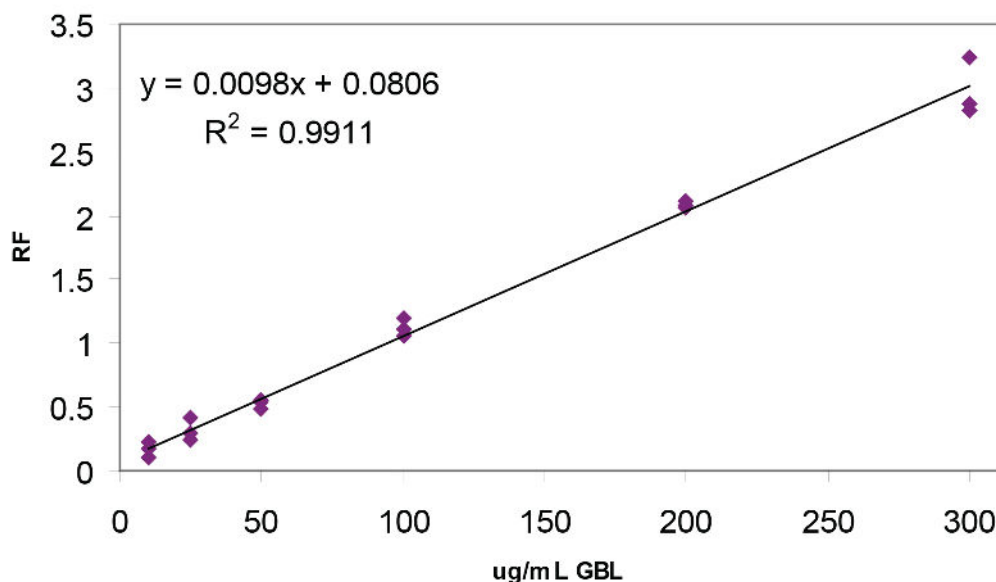
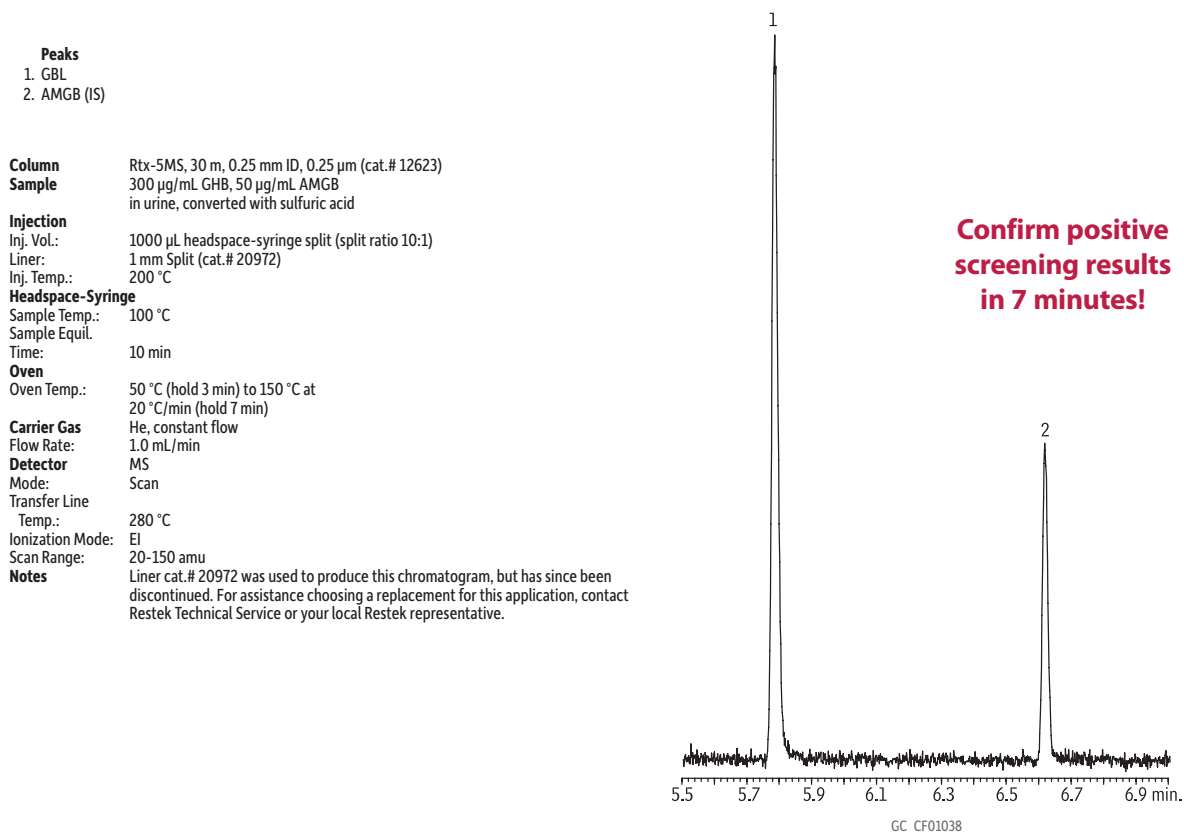


Figure 3: Confirmation Headspace GC-MS Analysis of 300 µg/mL Converted GHB (Analyzed as GBL) Standard in Urine



References

M.A. LeBeau, M.A. Montgomery, M.L. Miller, S. G. Burmeister, Analysis of biofluids for gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL) by headspace GC-FID and GC-MS, J. Anal. Toxicol. 24 (6) (2000) 421-428. <https://doi.org/10.1093/jat/24.6.421>



Rtx-BAC1 GC Capillary Column

- Application-specific columns for blood alcohol analysis—achieve baseline resolution in less than 3 minutes. Also excellent for abused inhalant anesthetics, γ -hydroxybutyrate (GHB)/ γ -butyrolactone (GBL), glycols, and common industrial solvents.
- Rtx-BAC2 confirmation column provides four elution order changes under the same conditions.
- Stable to 260 °C.

| Catalog No. | df (Film Thickness) | Internal Diameter (ID) | Length | Units |
|-------------|---------------------|------------------------|--------|-------|
| 18003 | 1.80 μ m | 0.32 mm | 30 m | ea. |

Rtx-5MS GC Capillary Column

- General purpose columns for drugs, solvent impurities, pesticides, hydrocarbons, PCB congeners (e.g., Aroclor mixes), essential oils, semivolatiles.
- Column specifically tested for low-bleed performance.
- Temperature range: -60 °C to 350 °C.
- Equivalent to USP G27 and G36 phases.

| Catalog No. | df (Film Thickness) | Internal Diameter (ID) | Length | Units |
|-------------|---------------------|------------------------|--------|-------|
| 12623 | 0.25 μ m | 0.25 mm | 30 m | ea. |