



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



*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.*

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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.

## Eliminate Derivatization and Reduce System Contamination

Samples were spiked in urine and extracted according to the procedure in Table I, using alpha-methyl-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.

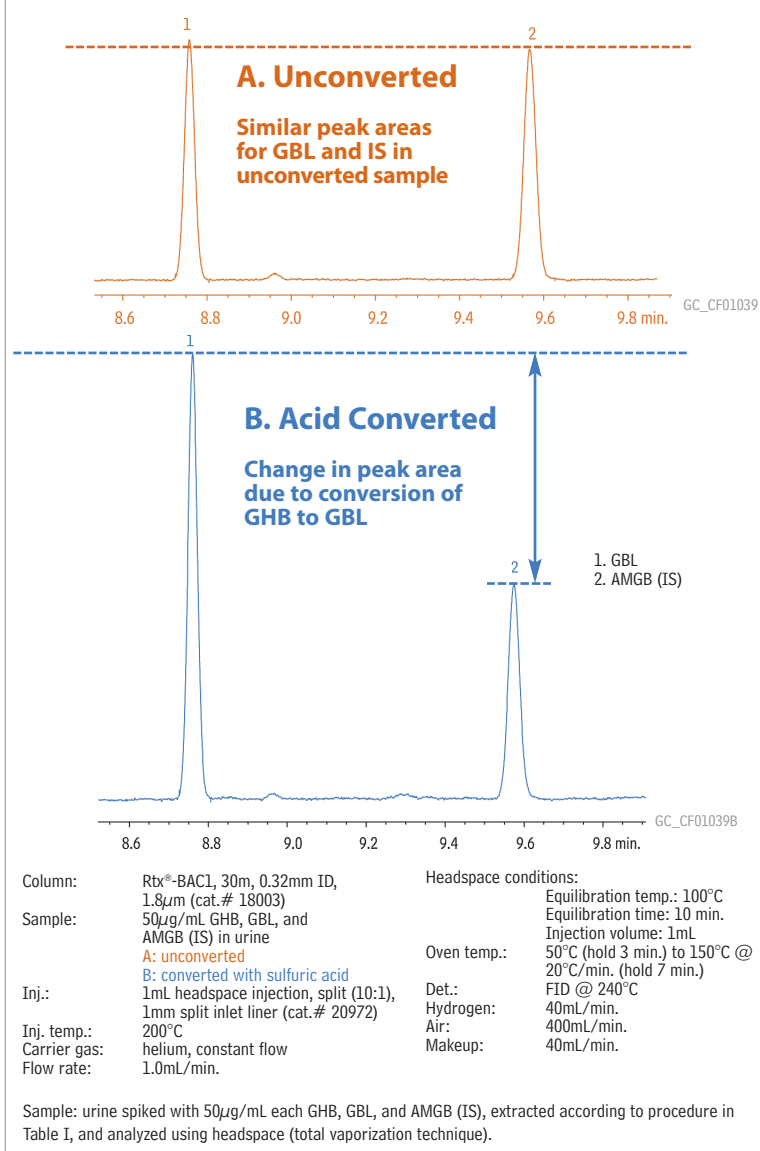
## Reliably Screen Samples Using Existing Blood Alcohol Testing Set-Up

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 com-

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

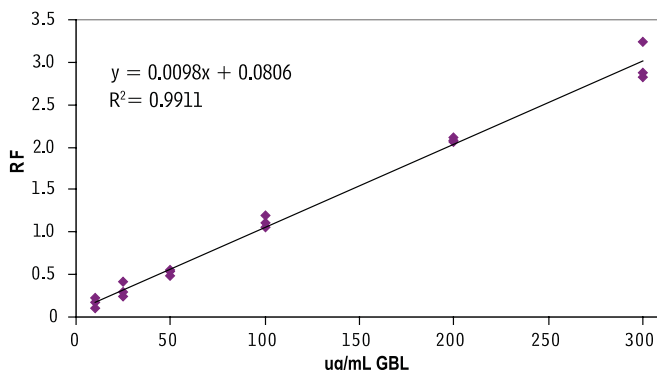
- Label two screw top test tubes per specimen. One for total GHB, the other for GBL only.
- Add 1mL of sample (urine) to each tube.
- Add 50µL of AMGB to each tube.
- Add 150µL concentrated sulfuric acid only to tubes used for analysis of total GHB.
- Vortex all tubes and allow them to sit 5 minutes.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

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

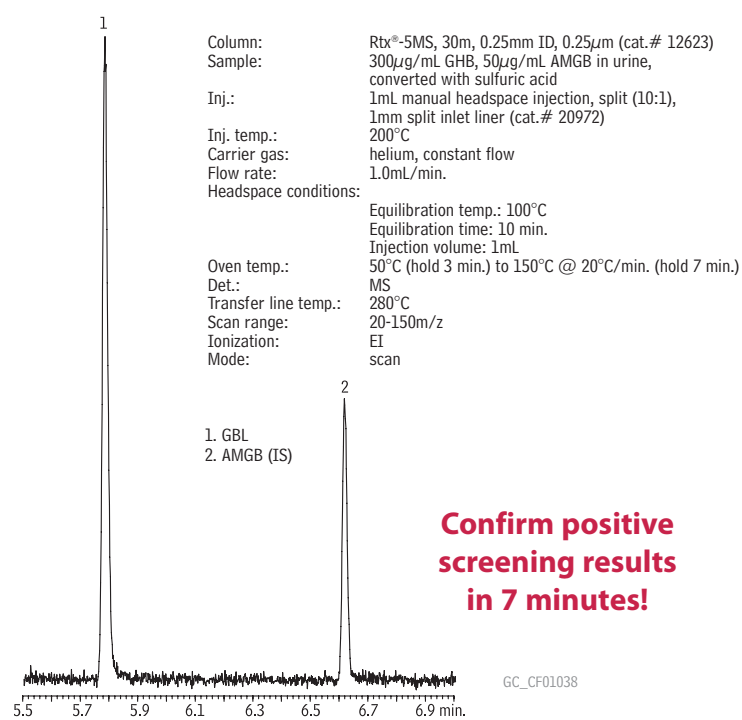


**This versatile extraction and headspace method improves lab efficiency and reduces both contamination and matrix effects by eliminating the need for derivatization and liquid injections.**

**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.



Column: Rtx®-5MS, 30m, 0.25mm ID, 0.25µm (cat.# 12623)  
 Sample: 300µg/mL GHB, 50µg/mL AMGB in urine, converted with sulfuric acid  
 Inj.: 1mL manual headspace injection, split (10:1), 1mm split inlet liner (cat.# 20972)  
 Inj. temp.: 200°C  
 Carrier gas: helium, constant flow  
 Flow rate: 1.0mL/min.  
 Headspace conditions: Equilibration temp.: 100°C  
 Equilibration time: 10 min.  
 Injection volume: 1mL  
 50°C (hold 3 min.) to 150°C @ 20°C/min. (hold 7 min.)  
 Oven temp.: MS  
 Det.: 280°C  
 Transfer line temp.: 20-150m/z  
 Scan range: EI  
 Ionization: scan  
 Mode:

monly 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.

#### References

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

## Product Listing

### Rtx®-BAC1 Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #	price
0.32mm	1.80	-20 to 240/260°C	30-Meter	18003	

### Rtx®-5MS—Low-bleed GC/MS Columns (fused silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	0.25	-60 to 330/350°C	30-Meter	12623	

### Exempted Drug of Abuse

#### Reference Materials

Concentration is µg/mL. Volume is 1mL/ampul.

Compound	CAS#	Solvent		cat.#	price
		Code	Conc.		
<b>GHB</b>					
γ-butyrolactone (GBL)	96-48-0	ACN	1,000	34077	
α-methylene-γ-butyrolactone (AMGBL)	547-65-9	ACN	1,000	34079	
ACN = acetonitrile					

### 1mm Split Liners for Agilent GCs

ID* x OD & Length	qty.	cat.#	price
1mm Split			
1.0mm x 6.3mm x 78.5mm	ea.	20972	

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