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Chromatography Products

Clinical, Forensic, & Toxicology Applications

Versatile GHB Method

For Headspace or Liquid Injection

System contamination is a significant issue for labs analyzing gamma-hydroxybutyrate (GHB) as typical methods include derivatization and liquid injection. Here, an acid conversion solvent extraction method is evaluated for compatibility with both headspace and liquid injection techniques. This extraction method can improve lab efficiency and reduce contamination by eliminating the use of derivatization reagents. Contamination can be further reduced by using a headspace technique instead of liquid injection, thus minimizing the introduction of matrix into the analytical system.

Introduction

For many years, gamma-hydroxybutyrate (GHB) and its related products (1,4-butanediol and gamma-butyrolactone [GBL]) have been identified as abused substances in cases of driving under the influence and drug-facilitated sexual assault. Currently, GHB is regulated as a federally controlled Schedule I drug and is analyzed by clinical and forensic labs. In its native state, GHB is extremely difficult to chromatograph. Therefore, it often is analyzed as a trimethylsilyl (TMS) derivative (Figure 1).[1] Typical gas chromatography/mass spectrometry (GC/MS) analysis methods for derivatized GHB require liquid injections, which can quickly contaminate the injector and column with sample matrix and excess derivatization reagent. This buildup of contaminants results in added maintenance and downtime.

As an alternative to derivatization, GHB can be easily converted to GBL, which is much easier to chromatograph than GHB (Figure 2). The FBI Chemistry Unit's solvent-based extraction procedure incorporates acid conversion of GHB to GBL, eliminating the system contamination introduced by derivatization reagents.[2] This acid conversion solvent extraction procedure further reduces system contamination by allowing headspace injection to be used instead of liquid injection, which reduces contamination from matrix components.

Here we demonstrate the compatibility of this extraction technique with both liquid injection and headspace injection. The liquid injection procedure uses a common blood alcohol testing set-up, allowing samples to be quickly screened on existing equipment. Confirmation testing is performed on samples with positive screening results using total vaporization technique (TVT) headspace injection. TVT headspace analysis (a modification to the FBI method) reduces run-to-run variation caused by pressure from vaporized extraction solvent in the headspace vials.

Procedure

Urine samples were spiked with known concentrations of GHB, GBL, and the internal standard AMGBL. Samples were extracted according to the FBI Chemistry Unit's solvent extraction procedure (Table I). Note that GBL can be lost during

Figure 1 Trimethylsilyl (TMS) derivatization of GHB.

Figure 2 Sulfuric acid conversion of GHB to GBL.

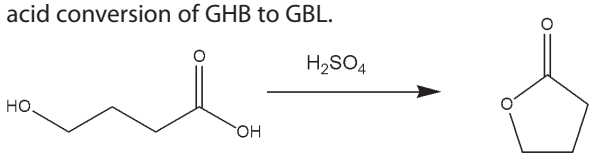
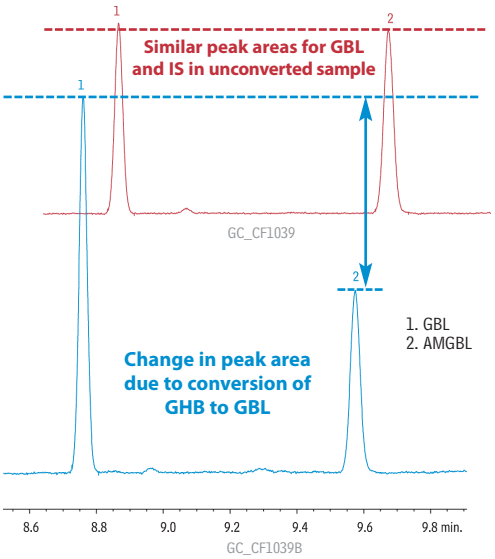


Table I Acid conversion solvent extraction procedure from FBI method for GHB analysis.

1. Add 1mL of sample (urine) to each tube.
2. Add 50µL of internal standard (50µg/mL AMGBL in methanol).
3. Add 150µL concentrated sulfuric acid.
4. Vortex and allow tubes to sit 5 minutes. Add 5mL methylene chloride to each tube. Shake by hand or on a mechanical shaker for 10 minutes to extract.
5. Centrifuge samples at 3,000 rpm for 5 minutes.
6. Transfer bottom (methylene chloride) layer to a clean test tube for drying.
7. Concentrate samples to ~100µL at 30°C under nitrogen. Use a gentle gas flow to prevent the loss of GBL.
8. For headspace analysis, inject 15µL of sample into a capped headspace vial.
9. For liquid injection, transfer extract to a limited volume insert.

Note: To demonstrate the effectiveness of the acid conversion of GHB to GBL, a second set of tubes was prepared according to Table I, except step 3 (acid conversion) was omitted.

Figure 3 GBL peak area increases in acid treated samples due to conversion of GHB to GBL.



Column:	Rtx®-BAC1, 30m, 0.32mm ID, 1.80µm (cat.# 18003)
Sample:	urine spiked with 50µg/mL each GHB, GBL, and AMGBL (IS), extracted according to procedure in Table I, and analyzed using headspace (total vaporization technique) A: unconverted B: converted with sulfuric acid
Inj.:	1mL 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
Oven temp.:	50°C (hold 3 min.) to 150°C @ 20°C/min. (hold 7 min.)
Det.:	FID @ 240°C
Hydrogen:	40mL/min.
Air:	400mL/min.
Makeup:	40mL/min.

sample evaporation, so care must be taken when concentrating samples. Extracts were analyzed first by liquid injection/FID as a high-throughput screening method. Then, samples with positive screening results were analyzed by TVT headspace/MS for confirmation.

Equipment used for method evaluation included Shimadzu and Agilent GCs, an Overbrook syringe headspace autosampler, and a Tekmar HT3 headspace loop/trap autosampler. An Rtx®-BAC1 column (30m, 0.32mm, 1.8µm) and an Rtx®-5MS column (30m, 0.25mm, 0.25µm) were used for the GC/FID screening analysis and the GC/MS confirmation analysis, respectively.

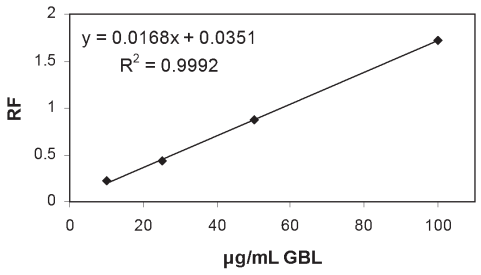
Results and Discussion

First, conversion of GHB to GBL was verified by comparison of peak heights from converted and unconverted samples (Figure 3). Note, the unconverted sample shows similar levels of GBL and internal standard; whereas, in the converted sample, GBL levels are significantly higher than AMGBL levels. This difference in peak height represents the conversion of GHB to GBL in the acid conversion solvent extraction procedure. Conversion efficiency is maintained across the concentration range shown in Figure 4. Linearity was established for both unconverted (GBL only) and acid converted (GHB + GBL) samples analyzed by headspace/FID. This acid conversion solvent extraction procedure offers an effective alternative for labs currently using a derivatization procedure who wish to reduce system contamination and related maintenance resulting from the use of derivatization reagents.

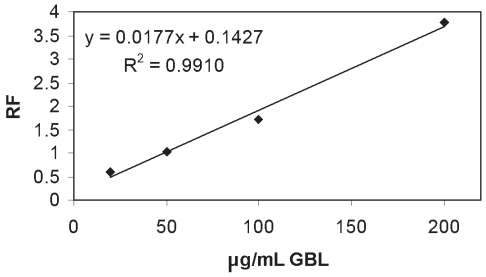
Next, recognizing that many labs currently use liquid injection systems, but are interested in headspace analysis as another means to reduce contamination, we evaluated the compatibility of the acid conversion solvent extraction samples with both liquid and headspace injection techniques. Linearity was established with both injection techniques using FID detection (Figure 5). Chromatographic evaluations were then done using both FID and MS detection to demonstrate versatility. Two system configurations where chosen to illustrate the range of analytical options that are compatible with the FBI acid conversion solvent extraction method: liquid injection/FID and headspace/MS. These analytical options can be used together as an FID screening and MS confirmation procedure, or they may be used independently, based on lab resources and requirements.

Figure 4 Linear results were obtained for both unconverted and acid converted solvent extracted urine samples (headspace analysis/FID).

A. Unconverted (represents GBL content only)



B. Converted sample (represents GBL and GHB as GBL)



The liquid injection/FID analysis was done on a commonly used blood alcohol testing system using an Rtx®-BAC1 column (Figure 6). This configuration allows labs to use an existing set-up to quickly screen samples. Confirmation analysis can then be performed on positive samples using a headspace/MS system with an Rtx®-5MS column, as shown in Figure 7. The headspace injection was optimized using total vaporization technique (TVT), which eliminates matrix effects by completely vaporizing the sample. TVT headspace is especially useful for the analysis of volatile or difficult matrices, but the sample amount must be kept small (10-15µL) since the entire sample is in the gaseous phase in the headspace vial and higher pressure in the vial from larger sample volumes can cause irreproducible results. Both the liquid injection/FID and TVT headspace/MS systems provided excellent chromatographic results with short analysis times.

Conclusions

Results demonstrate that the FBI acid conversion solvent extraction method is compatible with both headspace and liquid injection gas chromatographic systems, including existing blood alcohol testing systems. This method is advantageous compared to derivatization procedures, as it eliminates the use of derivatization reagents, which are a common system contaminant for GHB analysis. Contamination can be further reduced by using a TVT headspace procedure for analysis. The acid conversion solvent extraction procedure is a highly versatile method that should be considered by labs interested in reducing contamination resulting from either derivatization or liquid injection.

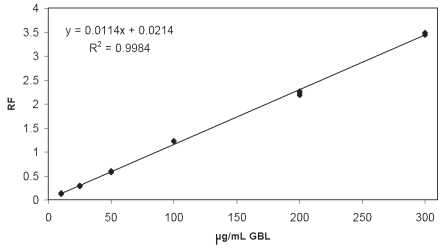
References

[1] A.A. Elian, Forensic Science International. 109 (2000) 183.

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

Figure 5 Linearity was established for both liquid injection and TVT headspace techniques (n=3, FID detection).

A. Liquid injection



B. TVT headspace injection

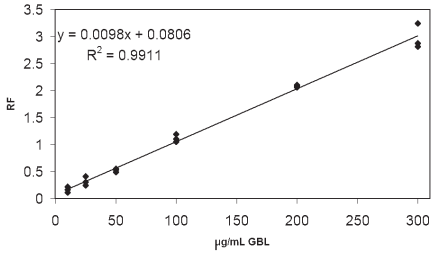
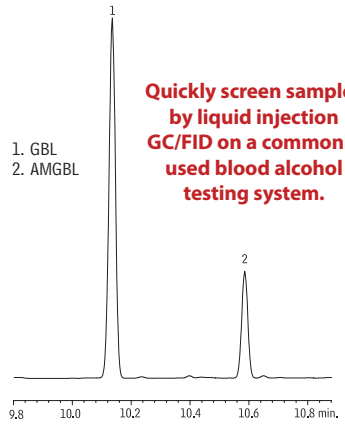


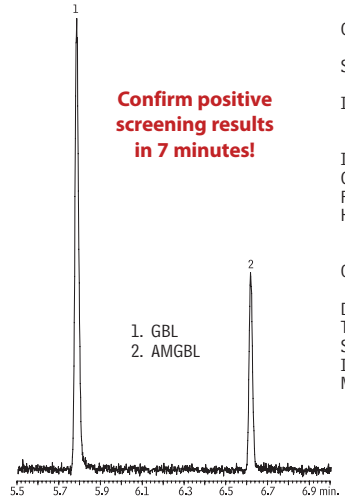
Figure 6 Liquid injection/FID screening assay of 300µg/mL GHB in urine (acid conversion solvent extraction procedure, analyzed as GBL).



Column:	Rtx®-BAC1, 30m, 0.32mm ID, 1.80µm (cat.# 18003)
Sample:	300µg/mL GHB and 50µg/mL AMGBL in urine converted with sulfuric acid
Inj.:	1µL liquid injection, split (10:1), 1mm split inlet liner (cat.# 20972)
Inj. temp.:	200°C
Carrier gas:	helium, constant flow
Flow rate:	1.0mL/min.
Oven temp.:	50°C (hold 3 min.) to 150°C @ 20°C/min. (hold 7 min.)
Det.:	FID @ 240°C
Hydrogen:	40mL/min.
Air:	400mL/min.
Makeup:	40mL/min.

GC_CF01040

Figure 7 TVT headspace/MS confirmation analysis of 300µg/mL GHB in urine (acid conversion solvent extraction procedure, analyzed as GBL).



Column:	Rtx®-5MS, 30m, 0.25mm ID, 0.25µm (cat.# 12623)
Sample:	300µg/mL GHB, 50µg/mL AMGBL 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
Oven temp.:	50°C (hold 3 min.) to 150°C @ 20°C/min. (hold 7 min.)
Det.:	MS
Transfer line temp.:	280°C
Scan range:	20-150m/z
Ionization:	EI
Mode:	scan

GC_CF01038

Rtx®-BAC1 (proprietary Crossbond® phase)

- 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.
- Stable to 260°C.

ID	df (µm)	temp. limits	30-Meter
0.32mm	1.80	-20 to 240/260°C	18003
0.53mm	3.00	-20 to 240/260°C	18001

similar **phases**

DB-ALC1, DB-ALC2

Rtx®-5 MS (low-polarity phase; Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

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

The 5% diphenyl/95% dimethyl polysiloxane stationary phase is the most popular GC stationary phase and is used in a wide variety of applications. All residual catalysts and low molecular weight fragments are removed from the Rtx®-5 polymer, providing a tight monomodal distribution and extremely low bleed.

ID	df (µm)	temp. limits	15-Meter	30-Meter	60-Meter
0.25mm	0.10	-60 to 330/350°C	12605	12608	12611
	0.25	-60 to 330/350°C	12620	12623	12626
	0.50	-60 to 330/350°C	12635	12638	12641
	1.00	-60 to 325/350°C	12650	12653	
0.32mm	0.10	-60 to 330/350°C	12606	12609	12612
	0.25	-60 to 330/350°C	12621	12624	12627
	0.50	-60 to 330/350°C	12636	12639	12642
	1.00	-60 to 325/350°C	12651	12654	

similar **phases**

DB-5, HP-5, HP-5MS, Ultra-2, SPB-5, Equity-5, MDN-5, CP-Sil 8 CB

Note: The DB-5MS is a silarylene based polymer equivalent to the Rxi-5Sil MS.

Exempted Drug of Abuse Reference Materials

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

Compound	Solvent CAS#	Code	Conc.	cat.# (ea.)
γ-butyrolactone (GBL)	96-48-0	ACN	1,000	34077
α-methylene-γ-butyrolactone (AMGBL)	547-65-9	ACN	1,000	34079

ACN = acetonitrile

Silylation Derivatization Reagents

Compound	CAS#	cat.#
BSTFA w/1% TMCS (N,O-bis[trimethylsilyltrifluoroacetamide] w/1% trimethylchlorosilane)		
10-pk. (10x1g)	25561-30-2	35606
25g vial	25561-30-2	35607

1mm Split Liners for Agilent GCs

	ID* x OD & Length	qty.	cat.#
1mm Split	1.0mm x 6.3mm x 78.5mm	ea.	20972
Siltek 1mm Split	1.0mm x 6.3mm x 78.5mm	ea.	20972-214.1

*Nominal ID at syringe needle expulsion point.

Lit. Cat.# CFAN1107

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