

# APPLICATIONS

## Quantitative Analysis of Gamma Hydroxybutyrate (GHB) in Whole Blood Using Fast SPE and LC-MS/MS

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### Introduction

As a drug of abuse, Gamma-hydroxybutyric Acid (GHB) is often overlooked in forensic toxicological analysis unless it is specifically requested in the screen. Testing for GHB can be difficult due to inaccurate/unreliable response in immunoassays or chromatographic screens and due to the stability of the compound. The time sensitive nature of this assay demands for an analytical procedure that is quick, efficient, and one that preserves the authenticity of the biological specimen. The goal of this application is to present a comprehensive method for the quantitation of GHB in human blood using solid phase extraction (SPE) in conjunction with LC-MS/MS analysis. A quick, two step sample preparation protocol combining protein precipitation (PP) with SPE was employed. The SPE method did not require any conditioning or equilibration of the SPE cartridge, thus allowing for significant time savings. A Luna<sup>®</sup> 3  $\mu$ m 150 x 2.0 mm HILIC LC column was utilized for chromatographic analysis, enabling direct injection of the extracted sample in a MS friendly 75% organic. The prescribed protocol circumvents the need for a dry down or reconstitution step and makes it quick and easy to implement in a laboratory workflow, all while providing accurate results.

### Materials and Methods

#### Reagents and Chemicals

Analytical reference standards and internal standards were purchased from Cerilliant Corporation (Round Rock, TX, USA). Pooled human whole blood with disodium EDTA was purchased from Bioreclamation/IVT (Westbury, NY). All other reagents and chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Ultrapure D.I water was obtained from Sartorius arium comfort II, courtesy of Sartorius Corporation (Bohemia, NY).

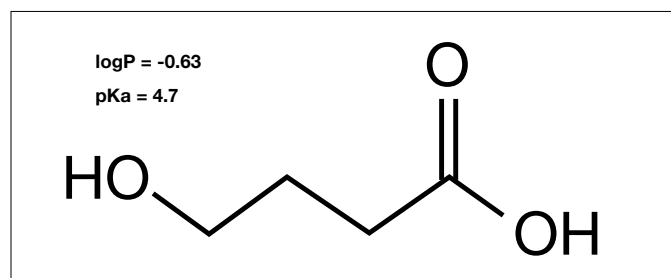
### SPE Protocol

#### Sample Pre-treatment

To 500  $\mu$ L whole blood add 100  $\mu$ L 5 % (w/v) ZnSO<sub>4</sub> and, vortex 3-5 seconds. Add 1.5 mL of chilled (~0 °C) 90:10 Acetonitrile/Methanol while vortexing. Centrifuge samples at 6000 rpm for 10 minutes and then collect supernatant.

### SPE Conditions

- 96-Well Plate:** Strata-X PRO, 30mg/well  
**Part No.:** 8E-S536-TGA  
**Load:** Pass the supernatant collected from pre-treatment, apply vacuum and collect extract.  
**Direct Injection:** 5  $\mu$ L of the above sample was injected directly on LC-MS/MS (bypass dry-down and reconstitution).



### LC Conditions

#### Quantitative Analysis for GHB

<b>Column:</b>	Luna <sup>®</sup> 3 $\mu$ m HILIC	
<b>Dimensions:</b>	150 x 2.0 mm	
<b>Part No.:</b>	<a href="#">00F-4449-B0</a>	
<b>Mobile Phase:</b>	A: Acetonitrile B: 100 mM Ammonium Formate	
<b>Gradient:</b>	<b>Time (min)</b>	<b>% B</b>
	0	20
	1	20
	1.5	50
	2	50
	2.01	20
	7	20
<b>Flow Rate</b>	0.4 mL/min	
<b>Injection Volume:</b>	5 $\mu$ L	
<b>UHPLC Instrument:</b>	Agilent 1260	
<b>MS/MS Instrument:</b>	SCIEX <sup>®</sup> API Triple Quad 4500 <sup>™</sup> , ESI Source (+)	

#### Qualitative Analysis for Phospholipids

<b>Column:</b>	Kinetex <sup>®</sup> 2.6 $\mu$ m C18	
<b>Dimensions:</b>	50 x 2.1 mm	
<b>Part No.:</b>	<a href="#">00B-4462-AN</a>	
<b>Mobile Phase:</b>	A: 0.1 % Formic acid in Water B: 0.1 % Formic acid in Methanol	
<b>Gradient:</b>	<b>Time (min)</b>	<b>% B</b>
	0	40
	0.5	95
	11.5	95
	11.51	40
	13.5	40
<b>Flow Rate</b>	0.4 mL/min	
<b>Injection Volume:</b>	1 $\mu$ L	
<b>HPLC Instrument:</b>	Agilent 1260	
<b>MS/MS Instrument:</b>	SCIEX <sup>®</sup> API Triple Quad 4500, ESI Source (+)	

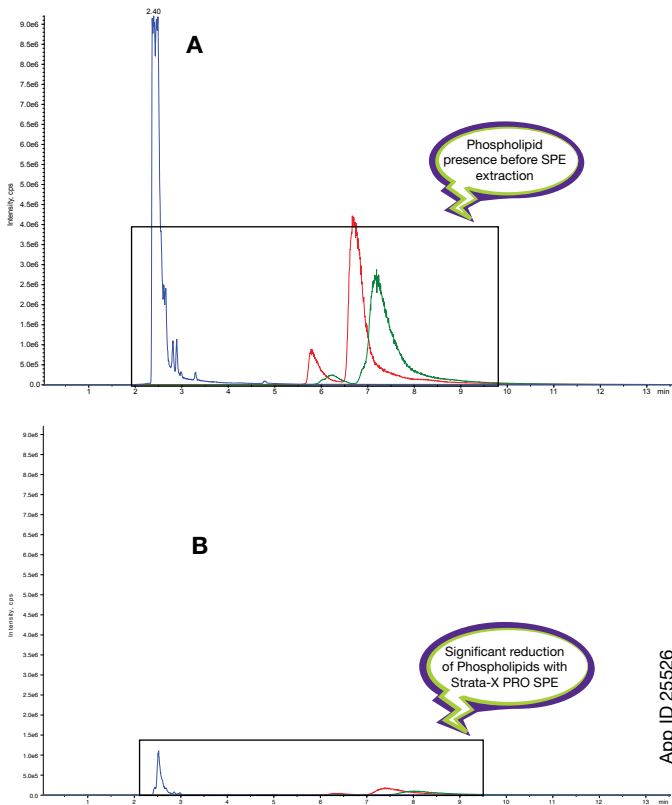
**Table 1.**  
Retention time (RT) and MRM Transition for Analytes

Analyte	RT (min)	Q1	Q3
GHB	1.7	104.9	86.9 68.9
GHB-d6	1.7	111	93
Lyso PC	2.4	496.4	184.2
PC-1	6.7	760.7	184.2
PC-2	7.2	786.8	184.2

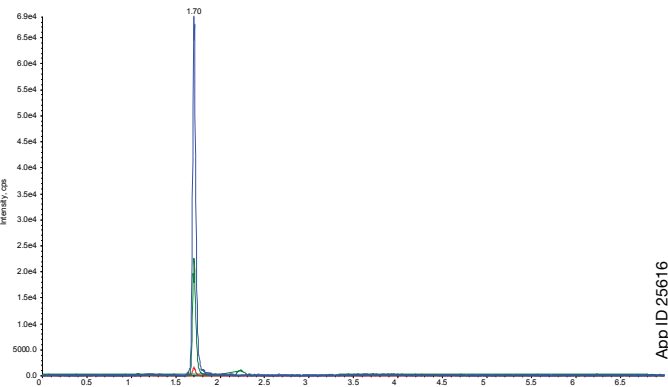
**Table 2.**  
% Absolute Recovery of GHB from Extracted  
Whole Blood Sample (N=4) Using Strata<sup>®</sup>-X PRO SPE

Spiked Conc.	% Recovery	% CV
10.0 µg/mL	98 %	1.7 %

**Figure 2.**  
Representative Chromatogram Showing Phospholipid Trace of Blood  
Matrix in pre (A) and post (B) SPE (Strata-X PRO) Extracted Sample.



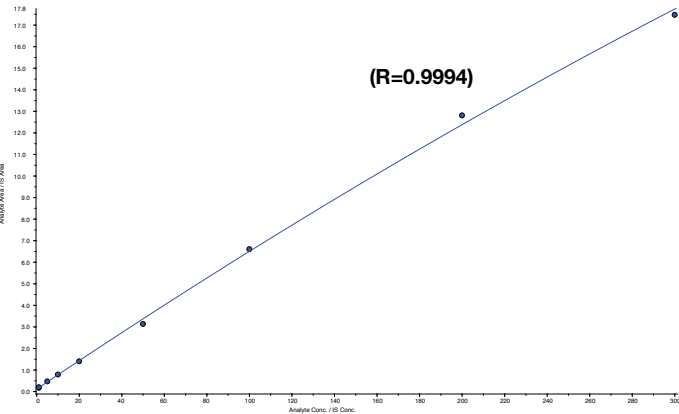
**Figure 1.**  
Representative Chromatogram of GHB Extracted Whole Blood  
Analyzed by a Luna<sup>®</sup> 3 µm 150 x 2.0 mm HILIC column



**Table 3.**  
Precision and Accuracy Data for QC Samples

Expected Conc. (µg/mL)	Sample	Replicates (N)	% CV	Accuracy
15	QC 1	4	11.4	104.2
75	QC 2	4	4.5	94.5

**Figure 3.**  
Linearity Curve for GHB Extracted Blood Matrix over 300-fold  
Dynamic Concentration Range



## Results and Discussion

The developed HILIC LC conditions utilizes a Luna® 3µm HILIC column (**Figure 1**) and allows for direct injection of extracted samples, bypassing the time consuming dry-down and reconstitution steps, before injection. The method utilizes a protein precipitation followed by a quick sample pass-through SPE method. The resulting extract yields cleaner background (**Figure 2B**) by selective removal of phospholipids. This is very important because phospholipids can be responsible for unpredictable, inaccurate results in an analytical run as well as causing increased MS instrument down time. The absolute recovery and % CV for extracted blood matrix reported were 98 % and 1.7 % respectively (**Table 2**). The linear regression value ( $R=0.9994$ ) of the extracted sample along with precision and accuracy data supports the sound extraction efficiency of the assay over 300-fold dynamic range (**Figure 3** and **Table 3**). The lowest point of linearity curve constructed was 1µg/mL, as the concentration range around that (< 5µg/mL) point generally is considered endogenous presence rather than GHB ingestion.

## Conclusion




The direct injection capability of the Strata®-X PRO extracted sample on the Luna HILIC LC column results in a simple, rapid identification and quantitation of GHB in whole blood. The prescribed method greatly benefits the time sensitive disposition of the assay and clean-up of whole blood matrix to provide an accurate analysis.

## References

1. Rachel R. McCusker. *Analysis of Gamma-Hydroxybutyrate (GHB) in urine by Gas Chromatography-Mass Spectrometry*. J. of Analytical Toxicology. Vol. 23, September 1999
2. Po-Chiao Liao a. *Clinical management of GHB withdrawal delirium*. J. of Formosan Medical Association 117, 1124-1127, 2018
3. Fiona J. Couper and Barry K. Logan. *Determination of g-Hydroxybutyrate (GHB) in Biological Specimens by Gas Chromatography-Mass Spectrometry*. J. of Analytical Toxicology, Vol. 24, January/ February 2000

## Ordering Information

### Strata®-X PRO SPE

Format	Sorbent Mass	Part Number	Unit
<b>Tube</b>			
	10 mg	<a href="#">8B-S536-AAK</a>	1 mL (100/box)
	30 mg	<a href="#">8B-S536-TAK</a>	1 mL (100/box)
	30 mg	<a href="#">8B-S536-TBJ</a>	3 mL (50/box)
	60 mg	<a href="#">8B-S536-UBJ</a>	3 mL (50/box)
	200 mg	<a href="#">8B-S536-FBJ</a>	3 mL (50/box)
	100 mg	<a href="#">8B-S536-ECH</a>	6 mL (30/box)
	200 mg	<a href="#">8B-S536-FCH</a>	6 mL (30/box)
	500 mg	<a href="#">8B-S536-HCH</a>	6 mL (30/box)
<b>96-Well Plate</b>			
	10 mg/well	<a href="#">8E-S536-AGA</a>	ea
	30 mg/well	<a href="#">8E-S536-TGA</a>	ea
	60 mg/well	<a href="#">8E-S536-UGA</a>	ea
<b>96-Well Microelution Plate</b>			
	2 mg/well	<a href="#">8M-S536-4GA</a>	ea

## Kinetex® Core-Shell LC Columns

2.6µm Minibore Columns (mm)						SecurityGuard™ ULTRA Cartridges†
Phases	30 x 2.1	50 x 2.1	75 x 2.1	100 x 2.1	150 x 2.1	3/pk
C18	<a href="#">00A-4462-AN</a>	<a href="#">00B-4462-AN</a>	<a href="#">00C-4462-AN</a>	<a href="#">00D-4462-AN</a>	<a href="#">00F-4462-AN</a>	<a href="#">AJ0-8782</a>
						for 2.1 mm ID

2.6µm MidBore™ Columns (mm)						SecurityGuard ULTRA Cartridges†
Phases	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	3/pk
C18	<a href="#">00A-4462-Y0</a>	<a href="#">00B-4462-Y0</a>	<a href="#">00C-4462-Y0</a>	<a href="#">00D-4462-Y0</a>	<a href="#">00F-4462-Y0</a>	<a href="#">AJ0-8775</a>
						for 3.0 mm ID

1.7µm Minibore Columns (mm)					SecurityGuard ULTRA Cartridges†
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
C18	<a href="#">00A-4475-AN</a>	<a href="#">00B-4475-AN</a>	<a href="#">00D-4475-AN</a>	<a href="#">00F-4475-AN</a>	<a href="#">AJ0-8782</a>
					for 2.1 mm ID

1.7µm MidBore Columns (mm)			SecurityGuard ULTRA Cartridges†
Phases	50 x 3.0	100 x 3.0	3/pk
C18	<a href="#">00B-4475-Y0</a>	<a href="#">00D-4475-Y0</a>	<a href="#">AJ0-8775</a>
			for 3.0 mm ID

†SecurityGuard Ultra Cartridges require holder, Part No.: [AJ0-9000](#)

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## Luna® LC Columns

3 µm Minibore Columns (mm)					SecurityGuard™ Cartridges (mm)
Phases	30 x 2.0	50 x 2.0	100 x 2.0	150 x 2.0	4 x 2.0*
HILIC	<a href="#">00A-4449-B0</a>	<a href="#">00B-4449-B0</a>	<a href="#">00D-4449-B0</a>	<a href="#">00F-4449-B0</a>	<a href="#">AJ0-8328</a> /10pk

for ID: 2.0-3.0 mm

3 µm MidBore™ and Analytical Columns (mm)					SecurityGuard Cartridges (mm)	
Phases	50 x 3.0	150 x 3.0	100 x 4.6	150 x 4.6	4 x 2.0*	4 x 3.0*
HILIC	<a href="#">00B-4449-Y0</a>	<a href="#">00F-4449-Y0</a>	<a href="#">00D-4449-E0</a>	<a href="#">00F-4449-E0</a>	<a href="#">AJ0-8328</a> /10pk	<a href="#">AJ0-8329</a> /10pk

for ID: 2.0-3.0 mm      3.2-8.0 mm

\*SecurityGuard™ Analytical Cartridges require holder, Part No.: [KJ0-4282](#)

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