APPLICATIONS

Product Manager
Sample Preparation
Matt Brusius is an avid ice hockey player. He likes skating backwards and taking slapshots from the point.

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 over looked 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® 3 µm 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 BioreclamationIVT® (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 Sartorious Corporation (Bohemia, NY).

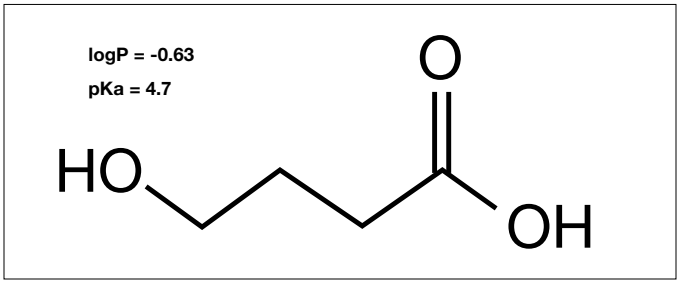
SPE Protocol

Sample Pre-treatment

To 500 µL whole blood add 100 µL 5 % (w/v) $ZnSO_4$ 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 µL of the above sample was injected directly on LC-MS/MS (bypass dry-down and reconstitution).



LC Conditions

Quantitative Analysis for GHB

Column:	Luna 3 µm HILIC	
Dimensions:	150 x 2.0 mm	
Part No.:	00F-4449-B0	
Mobile Phase:	**A:** Acetonitrile **B:** 100 mM Ammonium Formate	
Gradient:	**Time (min)**	**% B**
	0	20
	1	20
	1.5	50
	2	50
	2.01	20
	7	20
Flow Rate	0.4 mL/min	
Injection Volume:	5 µL	
IHPLC Instrument:	Agilent® 1260	
MS/MS Instrument:	SCIEX® API Triple Quad 4500™, ESI Source (+)	

Qualitative Analysis for Phospholipids

Column:	Kinetex® 2.6 µm C18	
Dimensions:	50 x 2.1 mm	
Part No.:	00B-4462-AN	
Mobile Phase:	**A:** 0.1 % Formic acid in Water **B:** 0.1 % Formic acid in Methanol	
Gradient:	**Time (min)**	**% B**
	0	40
	0.5	95
	11.5	95
	11.51	40
	13.5	40
Flow Rate	0.4 mL/min	
Injection Volume:	1 µL	
HPLC Instrument:	Agilent 1260	
MS/MS Instrument:	SCIEX API Triple Quad 4500, ESI Source (+)	

Revision: 0
PHEN-RUO-00093

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®-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.

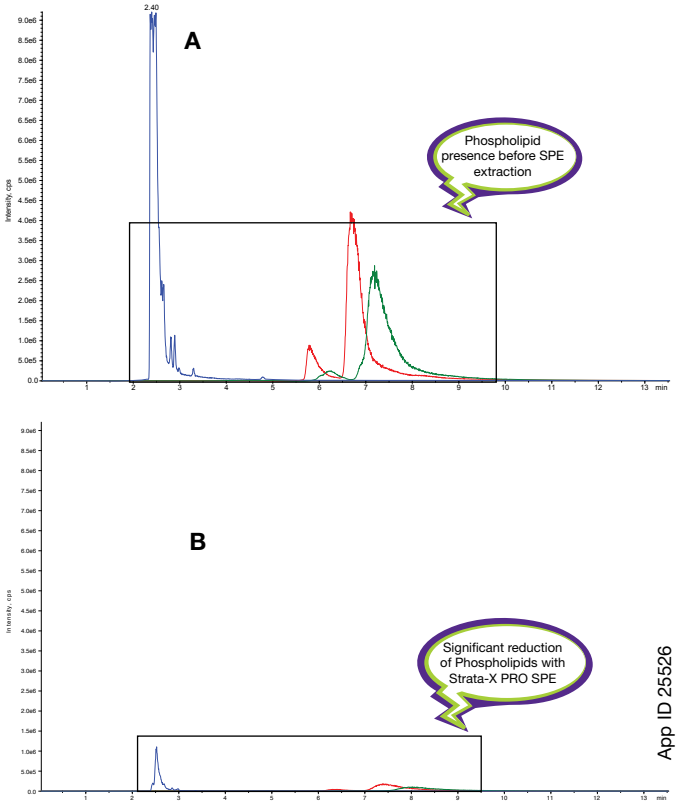


Figure 1.
Representative Chromatogram of GHB Extracted Whole Blood Analyzed by a Luna® 3 µm 150 x 2.0 mm HILIC column

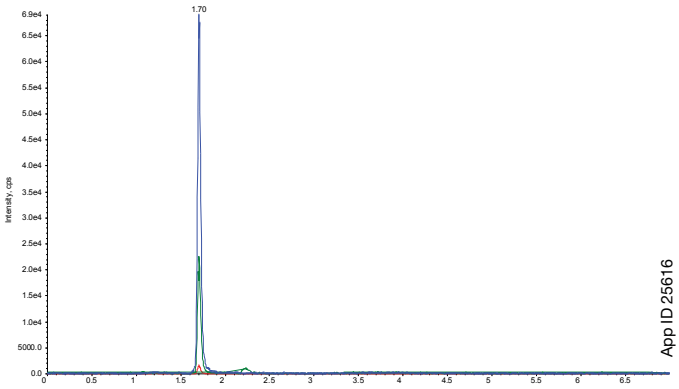
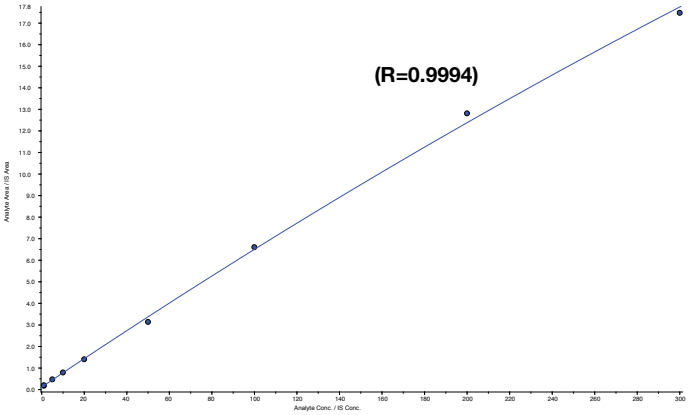


Table 3.
Precision and Accuracy Data for QC Samples

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® 3 µm 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 (< 5ug/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
Tube			
	10 mg	8B-S536-AAK	1 mL (100/box)
	30 mg	8B-S536-TAK	1 mL (100/box)
	30 mg	8B-S536-TBJ	3 mL (50/box)
	60 mg	8B-S536-UBJ	3 mL (50/box)
	200 mg	8B-S536-FBJ	3 mL (50/box)
	100 mg	8B-S536-ECH	6 mL (30/box)
	200 mg	8B-S536-FCH	6 mL (30/box)
	500 mg	8B-S536-HCH	6 mL (30/box)
96-Well Plate			
	10 mg/well	8E-S536-AGA	ea
	30 mg/well	8E-S536-TGA	ea
	60 mg/well	8E-S536-UGA	ea
96-Well Microelution Plate			
	2 mg/well	8M-S536-4GA	ea

Kinetex® Core-Shell LC Columns

2.6 µm 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	00A-4462-AN	00B-4462-AN	00C-4462-AN	00D-4462-AN	00F-4462-AN	AJ0-8782 for 2.1 mm ID

2.6 µm 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	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJ0-8775 for 3.0 mm ID

1.7 µm 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	00A-4475-AN	00B-4475-AN	00D-4475-AN	00F-4475-AN	AJ0-8782 for 2.1 mm ID

1.7 µm MidBore Columns (mm)			SecurityGuard ULTRA Cartridges‡
Phases	**50 x 3.0**	**100 x 3.0**	**3/pk**
C18	00B-4475-Y0	00D-4475-Y0	AJ0-8775 for 3.0 mm ID

‡SecurityGuard Ultra Cartridges require holder, Part No.: AJ0-9000

Presston™ 1000 Positive Pressure Manifold

Part No.	Description
AH1-7033	Presston 1000 Positive Pressure Manifold, 96-Well Plate

Phenomenex warrants the Presston 1000 will be free of defects in materials and workmanship under normal installation, use, and maintenance for a period of 12 months following delivery. **Please visit www.phenomenex.com/Presstonwarranty** for complete warranty information.

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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*
					/10pk
HILIC	00A-4449-B0	00B-4449-B0	00D-4449-B0	00F-4449-B0	AJ0-8328
					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*
					/10pk	/10pk
HILIC	00B-4449-Y0	00F-4449-Y0	00D-4449-E0	00F-4449-E0	AJ0-8328	AJ0-8329
					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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