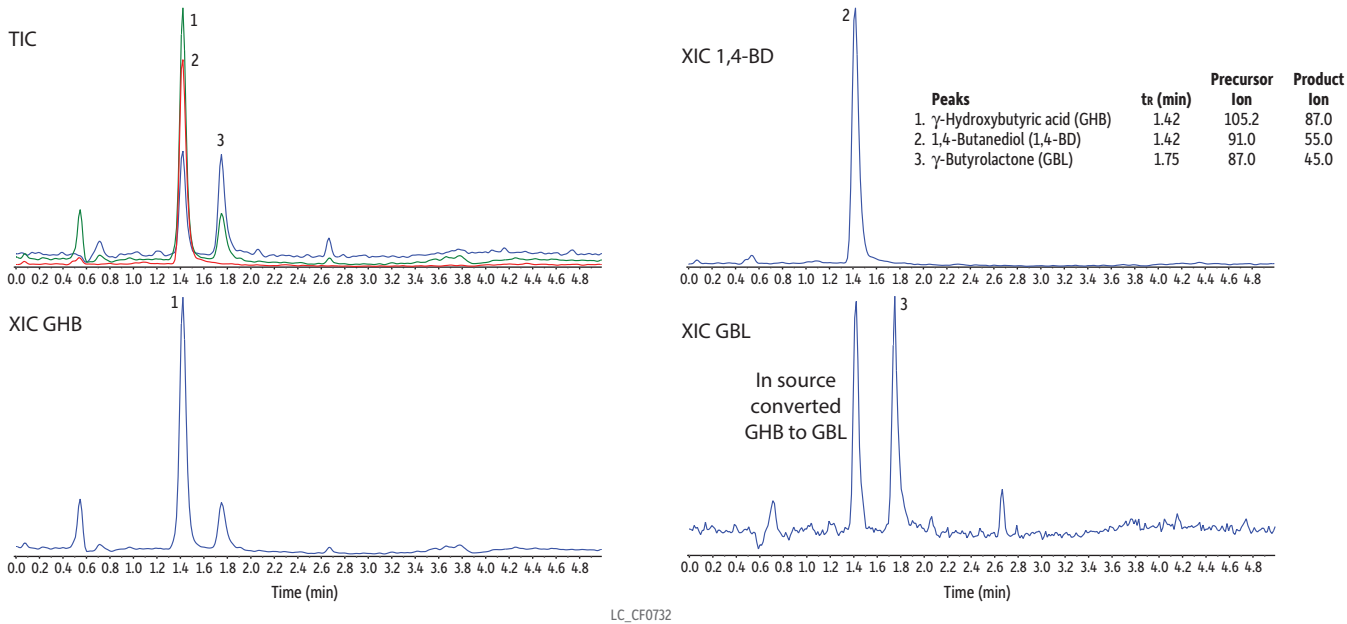
Featured Application: *Gamma-Hydroxybutyrate (GHB) in Whole Blood on Force C18*

Fast, Sensitive LC-MS/MS Analysis of GHB and Related Compounds in Human Blood for Forensic Testing

- Simultaneously analyze GHB, GBL, and 1,4-BD in whole blood samples in a fast, 5-minute cycle time.
- Chromatographic separation of actual GBL and GBL produced from GHB in-source conversion.
- Sufficient sensitivity to measure endogenous levels of GHB and to identify exogenous drug ingestion.

Recently, gamma-hydroxybutyrate (GHB) and its precursors gamma-butyrolactone (GBL) and 1,4-butanediol (1,4-BD) have been gaining popularity as recreational drugs because of their euphoric properties. In addition, due to its potent pro-sexual effects, GHB is increasingly implicated in drug-facilitated sexual assault. Because of their easy availability and potential for misuse, demand is growing for analytical determination of these drugs in both urine and blood samples. Analysis can be challenging because these compounds are small molecules that are difficult to retain, so many methods employ time-consuming derivatization and require long analysis times. Measurement is further complicated by both endogenous levels of GHB, which is present in all tissues as a naturally occurring neurotransmitter, and by the very rapid metabolism and elimination of ingested GHB.

The LC-MS/MS analysis of GHB and its precursors shown here uses a quick and simple protein crash procedure for whole blood samples. Excellent retention and chromatographic separations are reliably obtained in a fast, 5-minute analysis without the need for derivatization or extensive sample preparation. All compounds were chromatographically separated on a Force C18 column, which is critical for this analysis because GHB can convert to GBL in the instrument's ion source. In addition, method sensitivity is sufficient to measure low endogenous levels of GHB and to identify exogenous ingestion of these drugs. This LC-MS/MS analysis of GHB and its precursors is a faster alternative to typical approaches found in the literature and provides an effective means of testing and reporting levels in biological specimens.



Peaks	t_R (min)	Precursor Ion	Product Ion
1. γ-Hydroxybutyric acid (GHB)	1.42	105.2	87.0
2. 1,4-Butanediol (1,4-BD)	1.42	91.0	55.0
3. γ-Butyrolactone (GBL)	1.75	87.0	45.0

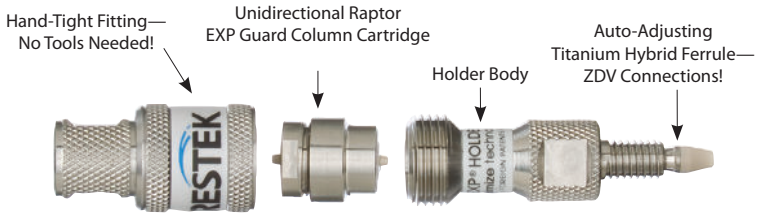
LC_CF0732

Column: Force C18 (cat.# 963431E); Dimensions: 100 mm x 3.0 mm ID; Particle Size: 3 µm; Pore Size: 100 Å; Guard Column: Force C18 EXP guard column cartridge 5 mm, 3.0 mm ID, 3 µm (cat.# 963450253); Temp.: 30 °C; **Sample:** Diluent: Water; Conc.: 500 ng/mL; Inj. Vol.: 10 µL; **Mobile Phase:** A: 0.5% Formic acid in water; B: 0.5% Formic acid in methanol; **Gradient (%B):** 0.00 min (5%), 0.50 min (5%), 3.00 min (50%), 3.01 min (5%), 5.00 min (5%), **Flow:** 0.7 mL/min; **Detector**: MS/MS; Ion Mode: ESI+; Mode: MRM; **Instrument:** HPLC; **Notes:** 100 µL of whole human blood was fortified at 50 µg/mL with GHB, GBL, 1,4-BD, and GHB-D6 (IS) using 5 µL of 1 mg/mL solutions. The blood was precipitated with 380 µL methanol. The sample was then vortexed at 1000 rpm for 10 seconds and centrifuged at 3000 rpm for 10 minutes at 10 °C. 50 µL of the supernatant was removed and diluted to 1 mL with water. The sample was then vortexed and subjected to LC-MS/MS analysis. (Internal standard not shown on chromatogram.)

Force C18 LC Columns (USP L1)

Length	2.1 mm cat.#	3.0 mm cat.#	4.6 mm cat.#
1.8 µm Columns			
30 mm	9634232		
50 mm	9634252	963425E	
100 mm	9634212	963421E	
3 µm Columns			
30 mm	9634332		
50 mm	9634352	963435E	
100 mm	9634312	963431E	9634315
150 mm	9634362	963436E	9634365
5 µm Columns			
50 mm	9634552	963455E	
100 mm	9634512	963451E	9634515
150 mm	9634562	963456E	9634565
250 mm			9634575

EXP Guard Cartridges



Protect your investment and extend the life of our already-rugged LC columns and change guard column cartridges by hand without breaking fluid connections—no tools needed!

EXP Direct Connect Holder

Description	qty.	cat.#
EXP Direct Connect Holder for EXP Guard Cartridges (includes hex-head fitting & 2 ferrules)	ea.	25808

Maximum holder pressure: 20,000 psi (1,400 bar)

Force EXP Guard Column Cartridges

Description	qty.	5 x 2.1 mm cat.#	5 x 3.0 mm cat.#	5 x 4.6 mm cat.#
Force C18 EXP Guard Column Cartridge	3-pk.	963450252	963450253	963450250

Maximum cartridge pressure: 600 bar/8,700 psi.

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