Extraction of *gamma*-Hydroxybutyric Acid (GHB) from Urine Using ISOLUTE® SLE+ Prior to GC/MS Analysis

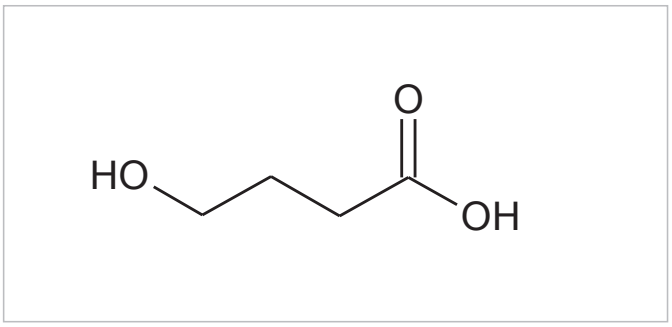

Figure 1. Structure of *gamma*-Hydroxybutyric acid (GHB)

Introduction

This application note describes the extraction of GHB from urine using supported liquid extraction and subsequent analysis by GC/MS.

ISOLUTE® SLE+ Supported Liquid Extraction plates and columns offer an efficient alternative to traditional liquid-liquid extraction (LLE) for bioanalytical sample preparation, providing high analyte recoveries, no emulsion formation, and significantly reduced sample preparation.

Analytes

Gamma-Hydroxybutyric acid (GHB) & GHB-D6

Sample Preparation Procedure

Sample Pre-treatment:	Dilute pre-treated urine (0.2 mL) with 0.2% formic acid (aq) (0.2 mL). Spike GHB-D6 internal standard and vortex mix thoroughly.
Format:	**ISOLUTE® SLE+ 200 µL Fixed Well Plate, part number 820-0200-P01**
Sample Loading:	Load the pre-treated urine (200 µL total volume) onto each well and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.
Analyte Extraction:	Apply ethyl acetate (1 mL) and allow to flow under gravity for 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent (5–10 seconds).
Format:	**ISOLUTE® SLE+ 400 µL Sample Capacity columns, part number 820-0055-B**
Sample Loading:	Load the pre-treated urine (200 µL total volume*) onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes. ***Note:** Column is underloaded
Analyte Extraction:	Apply ethyl acetate (1 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of ethyl acetate (1 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure to pull through any remaining extraction solvent (5–10 seconds).
Post Elution, Reconstitution and Derivatisation:	Dry the extract in a stream of air or nitrogen at ambient temperature using a SPE Dry (20 to 40 L/min) or TurboVap (1.0 bar) for 30 mins. Upon dryness, reconstitute with ethyl acetate (50 µL) and BSTFA/1% TMCS (50 µL) and vortex for 20 seconds. Transfer to a high recovery glass vial.

Biotage®

GC Conditions

Instrument:	Agilent 7890A with QuickSwap
Column:	Phenomenex Zebron ZB-35, 30 m x 0.25 mm ID x 0.25 µm
Carrier	Helium 1.2 mL/min (constant flow)
Inlet:	250 °C, Split (ratio 10:1), 12 mL/min, septum purge flow: 3 mL/min
Injection:	1 µL
Wash Solvent:	Ethyl acetate
Oven:	Initial temperature 70 °C
	Ramp 10 °C/min to 140 °C
	Ramp 120 °C/min to 350 °C
Post Run:	Back-flush for 1.6 minutes (2 void volumes)
Transfer Line:	280 °C

MS Conditions

Instrument:	Agilent 5975C
Source:	230 °C
Quadrupole:	150 °C
MSD mode:	SIM

SIM Parameters

Table 1. Ions acquired in the selected Ion Monitoring (SIM) mode

SIM Group	Analyte	Quantifier Ion	Quantifier Ion
1	GHB-D6	239	240
1	GHB	233	117

Results

The optimized protocols for ISOLUTE SLE+ plates and columns demonstrated reproducible recoveries between three unique donors, as shown in Figure 2. Percentage recovery was 52–69%. RSDs for GHB were below 9% on the 200 µL capacity plate format and below 4% on 400 µL capacity column format. **Figure 3**. shows linearity data for plate and column formats (r^2>0.999 for both formats). This application is unusual because of the relatively high concentration cut-off requirement (10 µg/mL) for illicit GHB. The low matrix volume used and relatively low recoveries do not adversely affect the required assay performance and sufficient sensitivity was achieved.

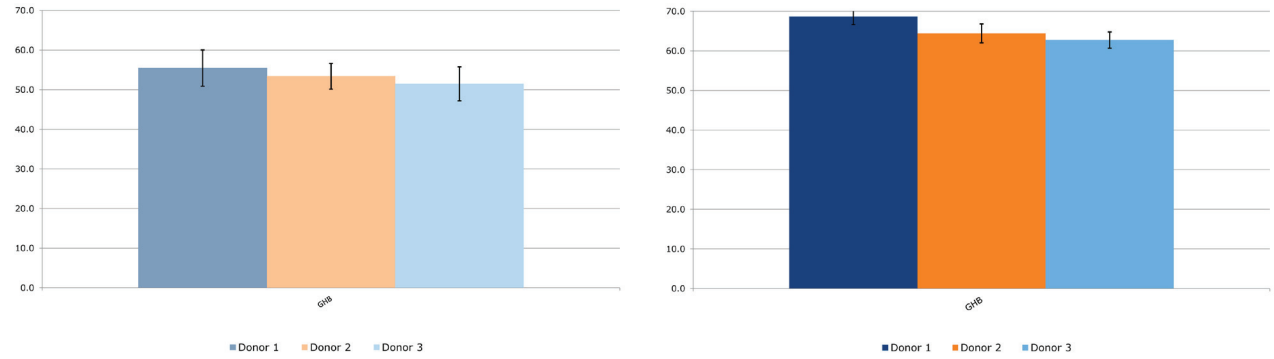

Figure 2. Typical analyte % extraction recoveries (n=7) using the ISOLUTE® SLE+ protocol using 200 µL capacity plate format (left) and 400 µL capacity column format (right)

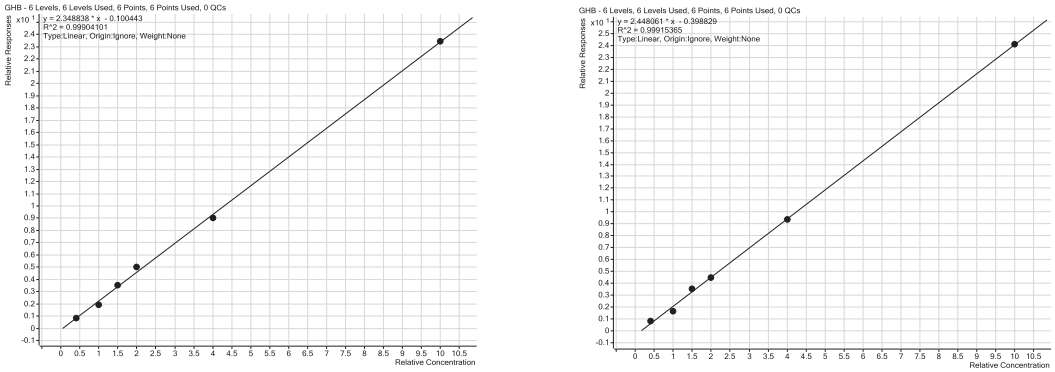

Figure 3. Calibration curves for extracted levels of spiked urine, using ISOLUTE® SLE+ protocol on 200 µL capacity plate format (left) and 400 µL capacity column format (right). Concentrations are 2.5, 5, 7.5, 10, 20 and 50 µg/mL. r^2 values are greater than 0.999.

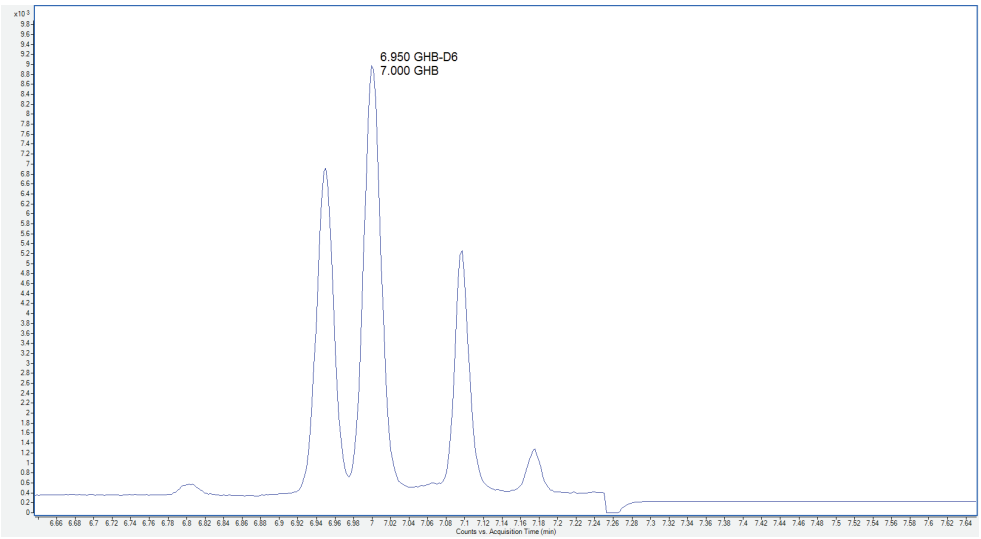

Figure 4. GC/MS chromatography for urine (Cal 1) spiked at 2.5 ng/mL. Compared to other more traditional solid phase extraction procedures, this ISOLUTE® SLE+ method results in a baseline with reduced noise and thus offers greater confidence when qualifying GHB.

Additional Information

0.2% formic acid (aq) is prepared by adding 100 µL concentrated formic acid (commercially available 98%) to 49.9 mL HPLC grade water

Ordering Information

Part Number	Description	Quantity
820-0200-P01	ISOLUTE® SLE+ 200 µL Supported Liquid Extraction Plate	1
820-0055-B	ISOLUTE® SLE+ 400 µL Sample Volume Columns	50
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold	1
SD-9600-DHS-EU	Biotage® SPE Dry Sample Concentrator System 220/240 V	1
SD-9600-DHS-NA	Biotage® SPE Dry Sample Concentrator System 100/120 V	1
C103198	TurboVap® LV, 100/120V	1
C103199	TurboVap® LV, 220/240V	1

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EUROPE
Main Office: +46 18 565900
Toll Free: +800 18 565710
Fax: +46 18 591922
Order Tel: +46 18 565710
Order Fax: +46 18 565705
order@biotage.com
Support Tel: +46 18 56 59 11
Support Fax: + 46 18 56 57 11
eu-1-pointsupport@biotage.com

NORTH & LATIN AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
Order Fax: +1 434 296 8217
ordermailbox@biotage.com
Support Tel: +1 800 446 4752
Outside US: +1 704 654 4900
us-1-pointsupport@biotage.com

JAPAN
Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com
jp-1-pointsupport@biotage.com

CHINA
Tel: +86 21 2898 6655
Fax: +86 21 2898 6153
cn_order@biotage.com
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