Determination of γ-hydroxybutyrate in urine by an HILIC-MS/MS method generating multiple transition products

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Introduction

The drug γ-hydroxybutyric acid (GHB) (Fig. 1) is a powerful central nervous system depressant that is abused recreationally and found in body fluids and hair from suspected victims of drug-facilitated sexual assaults. Testing for GHB in biological matrices is typically performed by gas chromatography after derivatisation because the polar properties of GHB make direct reverse-phase liquid chromatography (LC) less advantageous. In this study, a reliable hydrophilic interaction liquid chromatography (HILIC) tandem mass spectrometric (MS/MS) method was developed for the determination of un-derivatised GHB in urine.

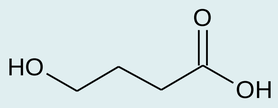

Fig. 1
Molecular structure of GHB.

Experimental

Reagents

Standard solutions: GHB in MeOH; 10, 100, 200, 300, and 400 mg/L. Internal standard solution (IS): GHB-D6 in methanol (MeOH), 100 mg/L. Phosphate buffer: di-potassium hydrogenphosphate (K_2HPO_4), 10 mM. Ammonium acetate (NH_4Ac), 1 M. Mobile phases A /B: 1 mM NH_4Ac/acetonitrile (MeCN).

Equipment

Mass Spectrometer: Xevo TQ MS triple-quadrupole instrument with an ESI ion source (Waters). LC system: Acquity UPLC system (Waters). Solid phase extraction (SPE) cartridges: Strong anion exchange (SAX) sorbent, 100 mg IST Isolute (Biotage, Uppsala, Sweden). HPLC column: SeQuant ZIC HILIC (5 µm, 200 Å, 2.1 mm I.D. × 100 mm) column (Merck SeQuant, Umeå, Sweden).

Clean-up

A 100 µL volume of urine was transferred to a disposable 2 mL centrifuge tube. Then, 200 µL phosphate buffer, 150 µL of MeOH and 50 µL of the IS were added, and the tube contents were mixed and centrifuged at 10,000 g for 5 min. A 100 µL volume of the supernatant was applied to the bottom of a SAX SPE cartridge. The cartridge had previously been conditioned with 1 mL of MeOH, followed by 1 mL of 1 M NH_4Ac and 1 mL of water. The cartridge was washed with 1 mL of 80% MeCN and then eluted with 2 mL of 0.1% formic acid in MeCN. All drainage through the cartridge was driven by gravity. The eluate was evaporated to bare dryness at 30°C under a steam of nitrogen. The residue was redissolved in 250 µL 90% MeCN.

Calibration

Calibrants were based on a mixture of blank urine samples from at least 5 persons. The samples were treated using the same procedure with the exception that 50 µL of MeOH was replaced with 50 µL of the standard solutions of GHB. Sample concentrations were obtained at 5, 50, 100, 150 and 200 µg/L of GHB.

Chromatography and mass spectrometry

A volume of 10 µL extract was injected onto the column, which was maintained at 30°C, and elution was performed with a gradient (Table 1). The GHB concentration was measured in both the negative and positive ESI ion modes (Table 2).

Table 1
Chromatographic conditions.

Time (min)	Mobile phase A (%)	Flow (µL/min)	MS bypass (min)
0 - 5	5 > 42 linear	200	0 - 3
5 - 5.2	42 > 95 linear	200	
5.2 - 6	95	200	
6 - 6.5	95 > 5 linear	200	6 - 11
6.5 - 11	5	200	

Table 2
Mass spectrometric conditions. The source and desolvation temps. were 150 and 600°C respectively. The bold Q3 ions were used for quantification. The underlined ions were used as qualifiers.

ESI mode	Q1 *m/z*	Q3 *m/z*	Relative abundance	Capillary voltage (kV)	Cone voltage (V)	Collision energy (eV)
-	103	**85**/57/101	100/93/32	2.5	20	8/12/12
+	105	**87**/69	100/2	3.0	12	7/8

Results and discussion

Diluted urine can be analysed directly by LC-MS/MS; however, the numerous polar components in urine and the great variation in its composition make it difficult to obtain reproducible low limits of detection (LODs) across samples. The use of clean-up by SAX produced a more robust analysis method that generated reproducible results, although some ion suppression was still observed. GHB was separated from α- and β-hydroxybutyrate by chromatography (Fig. 2), and four abundant transition products were obtained under optimal HILIC conditions.

Because the endogenous concentration of GHB in urine may be up to at least 4 mg/L, cut-off levels of 5-10 mg/L have been proposed for the discrimination between endogenous and exogenous GHB [1]. The LODs obtained by the developed method were at least ten times less (Table 3).

The relative intra-laboratory reproducibility standard deviations ($RSD_{R,intra\text{-}lab}$) were approximately 15% at a concentration of 2 mg/L and 8% at concentrations of 20-150 mg/L (Table 4). These values are acceptable according to the Horwitz equation [2]. The relative repeatability standard deviations (RSD_r) were approximately a factor two less. The mean true recovery and the trueness were close to 100% (Table 5).

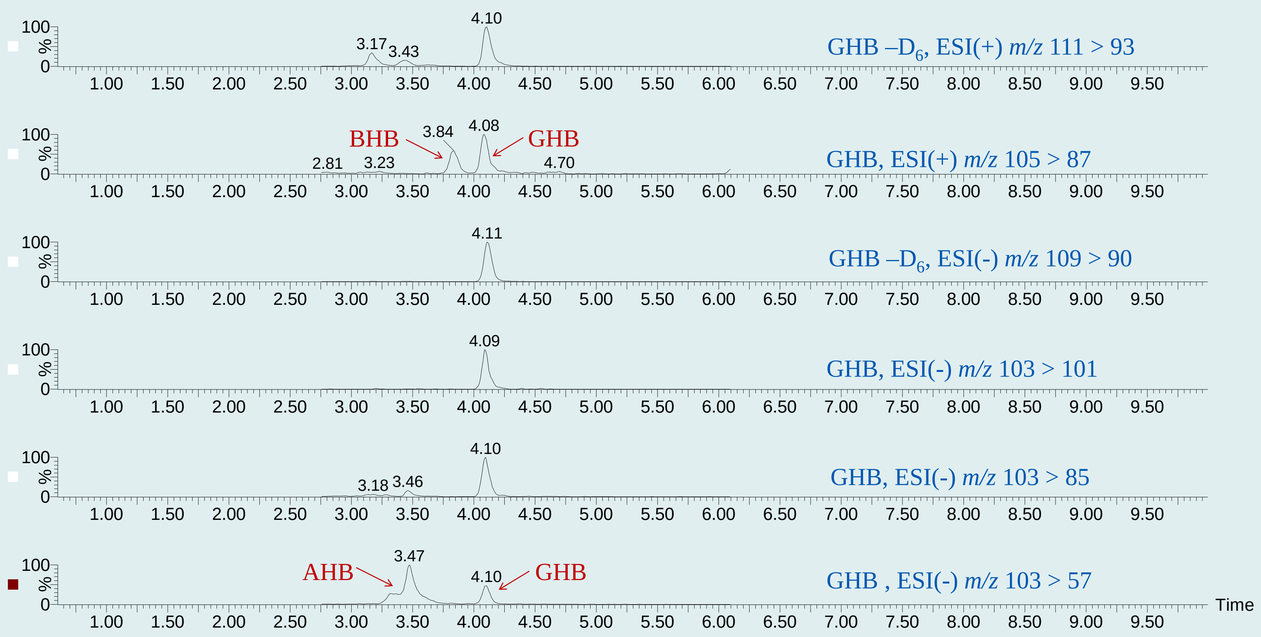

Fig. 2
Chromatograms of urine spiked with 4 mg/L of GHB. The retention times of α-hydroxybutyrate (AHB) and β-hydroxybutyrate (BHB) are shown on the chromatograms.

Table 3
Limits of detection determined using a random selection of 20 different urine samples.

ESI mode	Transition	Spiked conc. (mg/L)	Analysed conc. ‡ mean (± SD), (mg/L)	LOD (mg/L)
ESI (-)	*m/z* 103 > 85	0.2	0.20 (± 0.07)	0.2
	m/z 103 > 57	0.2	0.26 (± 0.11)	0.3
	m/z 103 > 101	0.2	0.31 (± 0.13)	0.4
ESI(+)	*m/z* 105 > 87	0.2	0.22 (± 0.13)	0.4

‡: The endogenous content of GHB (mean 0.5, range <0.2-1.8 mg/L) was subtracted before calculation.

Table 4
Precision values obtained using two different urine samples each spiked to three concentration levels. The samples were analysed by duplicate analyses in eight independent series.

Transition	Conc. level (mg/L)	RSD_r (%)	$RSD_{R,\ intra\text{-}lab}$ (%)
ESI (-) *m/z* 103 > 85	2/20/150	7/3/6	15/9/7
ESI(+) *m/z* 105 > 87	2/20/150	9/5/5	14/13/8

Table 5
True recovery and trueness determined using 20 different urine samples spiked with 20 mg/L GHB.

	Mean result (%)	SD (%)
True recovery ‡	102	11
Trueness	102	8

‡: Determined from samples spiked before and after extraction. Calculations were performed without IS.

Conclusion

A robust and selective HILIC-ESI-MS/MS method was developed for the determination of GHB concentrations in urine samples. By monitoring several ion transition products a high degree of confidence was obtained in the interpretation of the results.

References

[1] H. Andresen, N. Sprys, A. Schmoldt, A. Mueller, S. Iwersen-Bergmann, Forensic Sci. Int. 200 (2010) 93-99.
[2] W. Horwitz, Anal. Chem. 54 (1982) 67A-76A.