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Application Note

Simutaneous Analysis Of GHB And Its Precursors In Urine Using LC-MS/MS

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Abstract

The method presented here is the first demonstration of the use of LC-MS/MS for the simultaneous analysis of GHB and its precursors in urine samples. The method is simple and rapid (total analysis time of <12 min). It offers sufficient sensitivity to enable the measurement of endogenous levels of GHB and to identify exogenous ingestion.

Benefits

- It enables the simultaneous quantification of the GHB and the precursors in a single analysis
- It involves fewer manipulations and is less time-consuming

Introduction

Gamma-hydroxybutyrate (GHB) is a metabolite of gamma-aminobutyric acid (GABA) and plays the role of a central neurotransmitter and neuromodulator. Since GHB is a normal component of mammalian metabolism, it is present in all tissues of the body. Typical urinary GHB concentrations are < 10 mg/L[1,2]. In some countries GHB is used clinically as an intravenous anaesthetic and as a treatment for narcolepsy, alcoholism and opiate withdrawal. Over the last few years, GHB has been gaining popularity amongst club-goers as the recreational drug (Figure 1) where it is taken for its ability to produce feelings of euphoria and to enhance sexuality[3-5]. As a result of its potent prosexual effects, GHB has also been increasingly implicated in drug-facilitated sexual assault [6,7]. Ingestion of the chemical precursors of GHB i.e. gamma-butyrolactone (GBL) and 1,4-butanediol (1,4-BD) also results in similar physiological effects since they are rapidly converted to GHB in the body[8]. Raised awareness of the effects of these drugs and their potential for misuse, in addition to their ease of availability, has resulted in a dramatic increase in the demand for their analytical determination in both biological specimens and putative drug preparations. The purpose of this study was to develop and validate a sensitive LC-MS/MS procedure that would enable the simultaneous quantification of GHB, GBL and 1,4-BD in urine.

Figure 1. GHB.

Experimental

Samples

Calibrators and quality control (QC) samples.

Control urine was spiked with GHB, GBL and 1,4-BD to yield a series of calibrators at the following concentrations; 0, 1, 2, 5, 10, 20, 50 and 80 mg/L. Low and high QC samples were prepared by spiking control urine with the drugs to yield concentrations of 4 and 40 mg/L, respectively.

Authentic Samples

One hundred and eighty two authentic human urine samples were collected from club-goers attending a post dance-club 'chill-out' venue and were the result of 2 separate raids by the Belgian Police Department. The samples were analysed for GHB and the precursors using the newly-developed LC-MS/MS procedure. For comparative purposes, the samples were also analysed for GHB using a routinely used GC-MS procedure.

Sample Preparation

Urine samples were diluted (1:20) with an internal standard solution (GHB-d6 and GBL-d6, at a

concentration of 2 mg/L in deionised water).

LC Conditions

LC system:	Alliance 2795
Column:	Atlantis dC_{18} column (3 x 100 mm, 5 µm) at 35 ˚C
Mobile phases:	(A): 0.1% aqueous formic acid (B): methanol
Isocratic elution:	90:10 (A:B)
Flow rate:	200 µL/min
Inj. Volume:	20 µL

MS Conditions

Mass spectrometer:	Quattro micro mass spectrometer
Ionisation mode:	ES+
Capillary voltage:	3.5 kV
Source Temperature:	120 ˚C
Desolvation gas:	Nitrogen at 700 L/Hr, 350 ˚C
MS/MS:	Collision gas (argon) at 5 x 10^{-3} mbar

Results and Discussion

Multiple reaction monitoring (MRM) transitions were determined for GHB, the precursors and the internal standards i.e. GHB-d6 and GBLd6 (Table 1). Figure 2 shows some examples of product ion spectra.

Compound	Precursor ion (m/z)	Product ion (m/z)	Cone Voltage (V)	Collision energy (eV)
GHB	105	87	10	7
GHB-d6	111	93	10	7
GBL	87	45	25	15
GBL-d6	93	49	25	15
1,4-BD	91	73	12	5

Table 1. MRM transitions and conditions for the measurement of G HB, GBL, 1,4-BD and their deuterated internal standards.

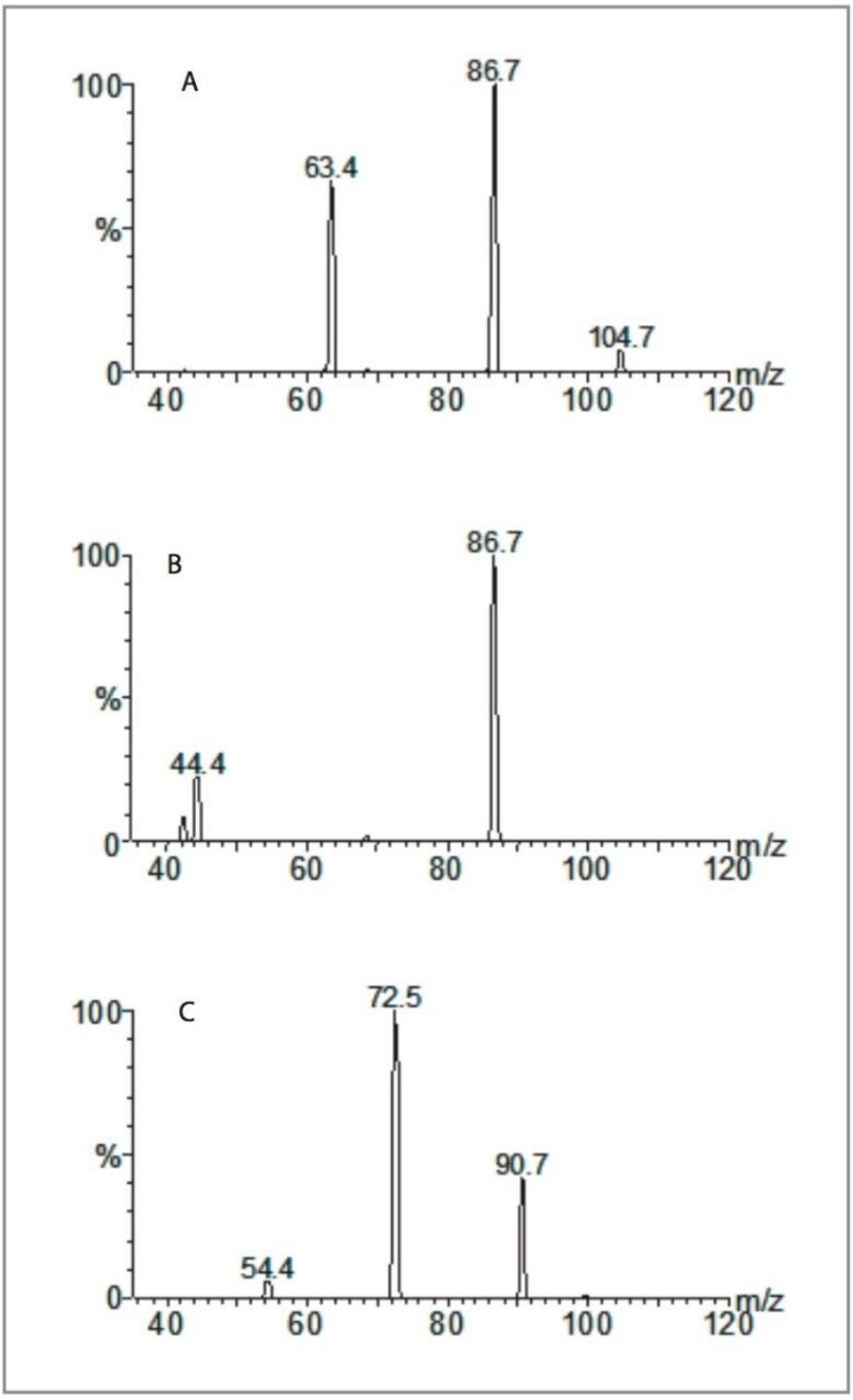

Figure 2. Product ion spectra for GHB (A), GBL (B) and 1,4-BD (C). Pure standards (5 mg/L) were infused into the mass spectrometer and the cone voltage (CV) optimised for the precursor ion. CID was then performed and product ion spectra acquired under optimum conditions for the most abundant product ion.*

A series of urine calibrators was prepared. Following preparation i.e. simple dilution, the samples were analysed using LC-MS/MS. Figure 3 shows the MRM chromatograms obtained following the analysis of a

control urine sample and the same sample enriched with GHB, GBL and 1,4-BD.

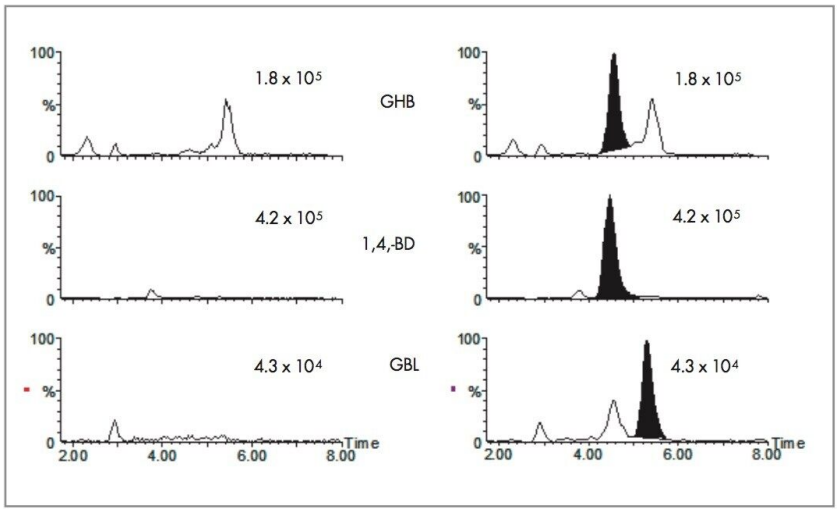

Figure 3. MRM chromatograms obtained with a single injection of a control urine sample (left-hand column) prepared by the dilution method and the same sample enriched with 10 mg/L of GHB, GBL and 1,4-BD (right-hand column). Peak intensity is shown in the top right-hand corner of each trace.

Quantification was achieved by integration of the area under the specific MRM chromatogram. For GHB and GBL, responses were calculated in reference to the integrated area of their respective deuterated internal standards. For 1,4-BD the response was calculated by reference to that of GHB-d6. Linear responses were obtained for GHB and 1,4-BD over the range investigated (1-80 mg/L). GBL produced a linear response over the range 1-50 mg/L.

Precision, intra-assay and interassay variation (as % CV) were all found to be highly satisfactory at < 7%. Analytical recoveries ranged from 90-107% (Table 2).

Compound	Low Control (4 mg/L)			High Control (40 mg/L)		
	Mean (mg/L)	Recovery (%)	%CV	Mean (mg/L)	Recovery (%)	%CV
Precision (n=5)						
GHB	4.0	100	3.0	41.0	103	0.5
GBL	3.8	95	4.2	40.2	101	3.9
1,4-BD	3.7	93	2.9	41.3	104	0.7
Intra-assay (n=5)						
GHB	3.9	98	3.2	42.7	107	3.5
GBL	3.7	93	3.2	36.1	90	2.9
1,4-BD	4.0	100	2.2	40.0	100	3.1
Interassay (n=5)						
GHB	4.1	103	5.3	40.0	100	3.4
GBL	4.0	100	6.6	39.8	100	6.3
1,4-BD	3.9	98	3.8	40.5	101	4.7

Table 2. Precision and analytical recovery data for GHB and its precursors in urine.

The limit of quantification was defined as the concentration of the lowest calibrator which was calculated to be with in ± 20% of the nominal value and with a % CV less than 20%. For all of the analytes of interest, this criteria was met by the 1 mg/L calibrator and was sufficient to determine endogenous levels of GHB in the urine.

To investigate any potential interference in GHB quantification by its naturally occuring isomers i.e. alpha and beta-hydroxybutyric acid, standards were analysed using the developed LC-MS/MS method. Both compounds were shown to be chromatographically resolved from GHB and thus would not interfere in the quantification of the latter (Figure 4).

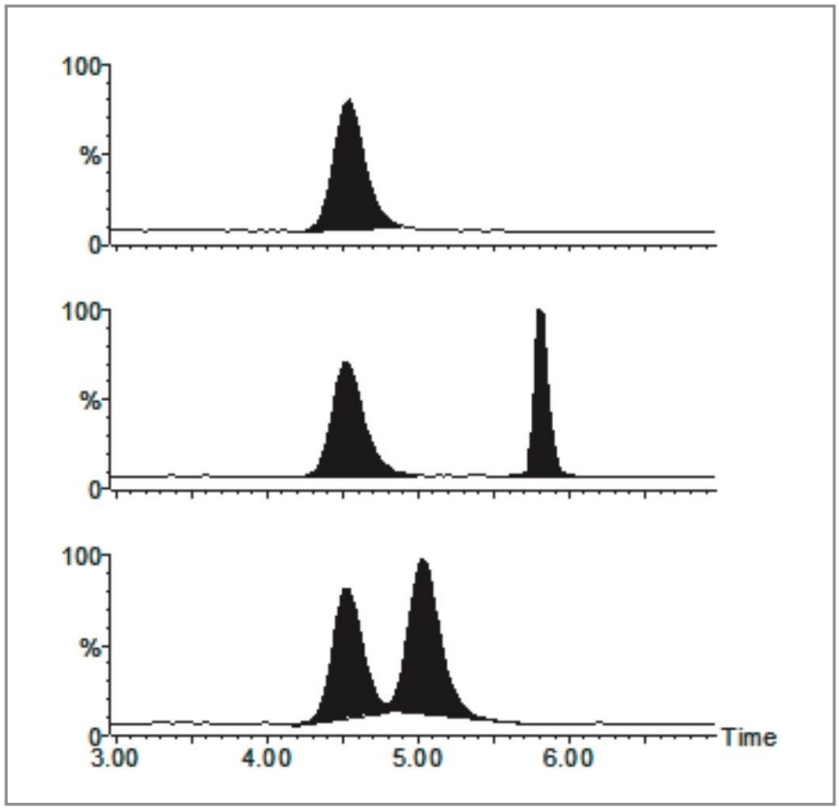

Figure 4. LC-MS analysis of the hydroxybutyric acid isomers. Ion chromatograms obtained following the analysis of gamma-hydroxybutyric acid (GHB) only (A) and GHB in the presence of alpha and beta-hydroxybutyric acid (traces B and C respectively). Peak intensity is shown in the top right-hand corner of each trace and is the sum of the responses obtained for both the protonated and the sodiated species species i.e. m/z 105 + 127.

The utility of the LC-MS/MS method was demonstrated by the analysis of one hundred and eighty-two authentic urine samples. Seven samples contained GHB at concentrations > 2 mg/L. The same seven samples were independently identified by the more time-consuming, labour-intensive GC-MS method.

Only two, of these seven samples, were above the recommended interpretive cut-off concentration of 10 mg/L and were 956 mg/L and 1411 mg/L, respectively. These two samples were also positive for GBL. None of the authentic urine samples contained 1,4-BD.

Conclusion

The method presented here is the first demonstration of the use of LC-MS/MS for the simultaneous analysis of GHB and its precursors in urine samples. The method is simple and rapid (total analysis time of <12 min). It offers sufficient sensitivity to enable the measurement of endogenous levels of GHB and to identify exogenous ingestion.

The LC-MS/MS results obtained following the analysis of authentic samples, correlated with the more labour-intensive, time-consuming (~1 hour) GC-MS method.

The procedure offers several advantages over other published techniques;

1. It enables the simultaneous quantification of the GHB and the precursors in a single analysis; this can facilitate the identification of the chemical basis of any seized putative drug preparations or if present in the biological specimen, can provide information of the chemical nature of the ingested drug.

2. It involves fewer manipulations and is less time-consuming.

Although the data presented here indicate that the actual prevalence of GHB-positives might be quite low, the hype and publicity surrounding these drugs has led to a dramatic increase in the number of requests for their analysis in biological samples (and particularly in urine). The simplicity and speed of the described LC-MS/MS technique, should prove a useful means to meet this current increased demand on laboratories.

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720001548, July 2007