A rapid method to measure exogenous gamma-hydroxybutric acid in beer for drug facilitated sexual assault investigation using hydrophilic interaction liquid chromatography electrospray ionization tandem mass spectrometry

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Abstract

Gamma-hydroxybutyric acid (GHB) is a commonly used drug of abuse often associated with drug facilitated sexual assault (DFSA). In this study, a hydrophilic interaction liquid chromatography electrospray ionization tandem mass spectrometry (HILIC-ESI/MS/MS) method has been validated for the quantification of GHB in beer, a commonly used alcoholic drink. The method was validated using the ISO 17025 parameters which were determined to be 1 to 50 µg/mL for dynamic range, 8.6 % relative standard deviation (RSD) or less for precision, 91-108 % for accuracy, 0.015 µg/mL for limit of detection (LOD), and 0.049 µg/mL for limit of quantification (LOQ). In addition, a conducted interference study affirmed that related structural isomers of GHB, including alpha-hydroxybutyric acid (AHB) and beta-hydroxybutyric acid (BHB), did not interfere with the quantification of GHB. Furthermore, conducted matrix effect and recovery studies yielded an average of 82.1% and RSD% of 0.9% for matrix effect and an average of 16.1% and RSD% of 11.8% for recovery. The low recovery by liquid-liquid extraction (LLE) is probably due to the high polarity of the GHB molecule.

Introduction

First introduced medically in 1960 as an anesthetic, GHB served as a powerful depressant of the central nervous system (CNS) through its ability to induce deep sleep and temporary coma. In the mid-1990s, GHB's popularity as an illicit party drug has already been ascertained primarily because of its notoriety as a date rape drug that can immobilize victims if administered at high doses thereby leading to high profile DFSA incidents.

In DSFA investigations, the most frequently analyzed specimen is urine [1-6]. However, traces of GHB become undetectable in urine after 12 hours of ingestion. Drinks can be useful specimens due to an unlimited detection window, but they have been infrequently analyzed so far.

GHB quantification is complicated due to the endogenous presence of BHB and AHB, so chromatographic baseline separation of GHB from AHB and BHB is required. Currently, most studies used GC-MS methods to quantify GHB in urine [2-6]. While GC-MS methods were able to achieve chromatographic baseline separation of GHB from BHB and AHB, a derivatization step is required to convert the GHB molecule from polar and nonvolatile to nonpolar and volatile.

Reverse phase liquid chromatography (RPLC) allowed omission of the derivatization step required by GC methods [1]. However, with RPLC an acidified mobile phase is required to improve the retention of GHB because RPLC uses a nonpolar stationary phase and an aqueous organic mobile phase. Thus, ESI must be performed in the positive-ion mode, although GHB prefers negative-ion ESI due to a carboxylic group in its structure. HILIC is a type of normal phase liquid chromatography (NPLC) that uses a polar stationary phase and an aqueous organic mobile phase, while NPLC generally uses organic solvents only. Chromatographic separation of small polar molecules can be achieved using HILIC with a neutral mobile phase when GHB is negatively charged, allowing negative-ion ESI to be performed so that analytical sensitivity can be improved.

Experimental

Calibration samples: Six calibration samples with 1, 2, 5, 10, 25, and 50 µg/mL of GHB and 5 µg/mL GHB-d_6 were prepared in beer.

Quality control (QC) samples: Three QC samples were prepared in beer with a concentration of 1, 5, or 25 µg/mL of GHB and 5 µg/mL GHB-d_6.

Liquid-liquid extraction: First, 400 µL of 0.1 M HCl was added to a 2.0 mL centrifuge tube containing 200 µL of either a calibration or a QC sample. After vortex mixing for 30 seconds, 1 mL of ethyl acetate was added into the centrifuge tube. After vortex mixing for 30 seconds again, it was centrifuged at 12,000 rpm for 5 minutes. Then, 750 µL of the upper layer was transferred to another 2.0 mL centrifuge tube. After evaporated to dryness under gentle nitrogen, 150 µL of water was added into the 2.0 mL centrifuge tube.

Assessment of recovery and matrix effect: The control is water with drug spiked in it, extended matrix is blank matrix extract with drug spiked before extraction, and extended extract is blank matrix extract with drug spiked after extraction.

$$Recovery\% = \frac{A_{extended\ matrix} - A_{blank\ matrix\ extract}}{A_{extended\ extract} - A_{blank\ matrix\ extract}} \times 100$$

$$Matrix\ effect\ \% = \frac{A_{extended\ extract} - A_{blank\ matrix\ extract}}{A_{control}} \times 100$$

Table 1. Agilent 1260 Infinity II LC conditions

Parameter	Value
Column	SeQuant® ZIC®-HILIC 150 mm × 2.1 mm, 3.5 µm
Column temperature	30 °C
Injection volume	2 µL
Mobile phase	A: Water/ACN 80/20 + 1 mM ammonium formate B: ACN
Flow rate	0.300 mL/min
Gradient program	
0.0 minute	80% B
3.0 minute	80% B
Stop time	3.0 minute
Post time	Off

Table 2. Agilent 6545 Q-TOF MS and MS/MS parameters

Parameter	Value
System tune	Standard 3200 m/z; 2 GHz Extended dynamic range; high resolution slicer mode
Transmission tune	50–1700 m/z; 2 GHz Extended dynamic range; high sensitivity slicer mode
Mass calibration	50 - 250 m/z; 2 GHz Extended dynamic range; high sensitivity slicer mode
Ion source	Dual AJS ESI
MS acquisition mass range	100–150 m/z
MS acquisition rate	5 spectra/s
MS/MS acquisition mass range	50–150 m/z
MS/MS acquisition rate	5 spectra/s
Drying gas temperature	325 °C
Drying gas flow	11 L/min
Nebulizer pressure	30 psi
Sheath gas temperature	350 °C
Sheath gas flow	12 L/min
Ionization mode	Negative
Capillary voltage	1500 V
Nozzle voltage	800 V
Fragmentor	100 V
Skimmer	45 V
Oct1 RF Vpp	750 V
MS reference mass ions	68.995758, 121.050873

Table 3. Agilent 6545 Q-TOF MS and MS/MS parameters

Parameter	Precursor (m/z)	RT (min)	Δ_{RT} (min)	Isolation width	CE	Quantifier ion	Quanlifier ion
GHB	103.0401	2.0	1	~ 1.3 m/z	10	57.0346	85.0289
GHB-d_6	109.0772	2.0	1	~ 1.3 m/z	10	61.0592	90.0630

Results

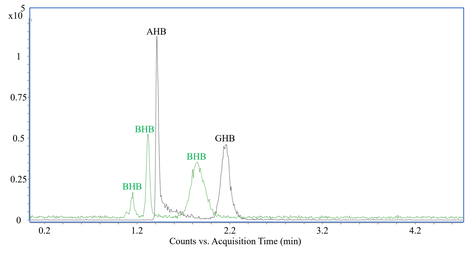


Figure 1. MS/MS EIC chromatogram of a mixture of AHB, BHB, and GHB at 1 µg/mL individual concentration in water. Black trace: m/z 103.040 → 57.035; green trace: m/z 103.040 → 59.014.

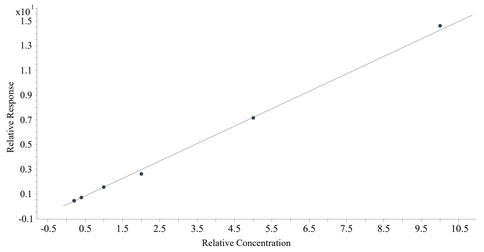


Figure 2. Representative internal standard calibration curve

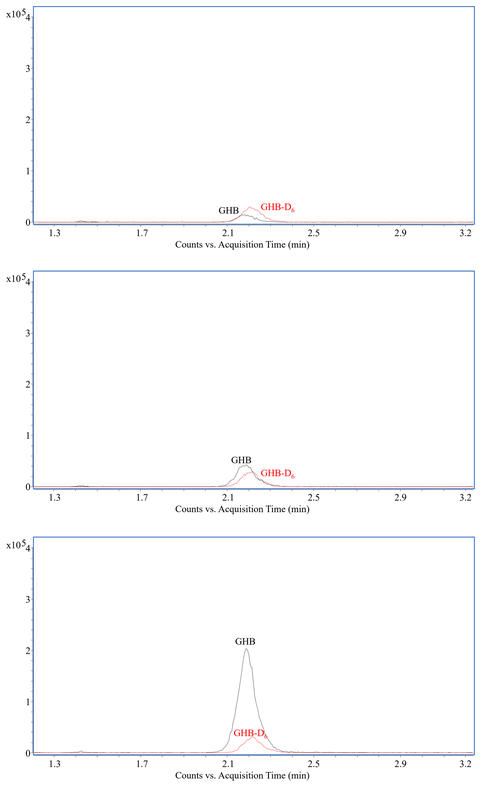
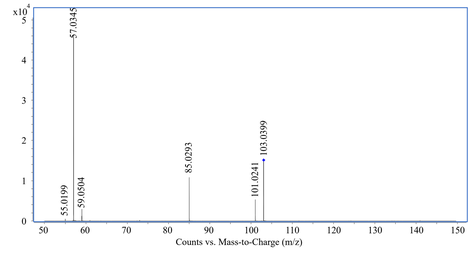


Figure 3. MS/MS EIC chromatogram of the QC samples with 1, 5, and 25 µg/mL GHB (black trace: m/z 103.040 → 57.035) and 5 µg/mL GHB-d_6 (red trace: m/z 103.040 → 59.014).



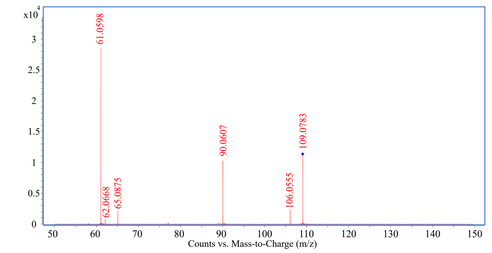


Figure 4. MS/MS mass spectra of GHB (black) and GHB-d_6 (red).

Table 4. The intraday and inter-day accuracy of the QC samples

QC Conc.	1 µg/mL	5 µg/mL	25 µg/mL
Intra-day 1	105	91	95
Intra-day 2	103	108	101
Intra-day 3	108	98	95
Inter-day	105	97	97

Table 5. The intraday and inter-day precision of the QC samples

QC Conc.	1 µg/mL	5 µg/mL	25 µg/mL
Intra-day 1	8.6	4.7	1.2
Intra-day 2	3.7	4.4	3.3
Intra-day 3	4.7	0.5	3.4
Inter-day	5.7	3.0	2.6

Table 6. Results of recovery and matrix effect at 5 µg/mL GHB level*

Conc.	Day 1	Day 2	Day 3	Average	%RSD
Recovery	14.0	16.7	17.7	16.1	11.8
Matrix effect	82.6	82.6	81.3	82.1	0.9

*The recovery was low by LLE, probably due to the high polarity of the GHB molecule. During sample analysis the method further used an isotopically labeled internal standard to normalize both the recovery and the matrix effect, which should ideally reach 100% with even better repeatability.

Conclusions

- A HILIC-ESI/MS/MS method to measure exogenous GHB for DFSA investigation has been validated for the analysis of GHB in beer, a matrix that have been infrequently studied so far.
- One advantage of the method is the elimination of a cumbersome derivatization step that are required by traditional GC-MS methods to make the polar and non-volatile GHB molecule nonpolar and volatile.
- Another advantage of the method is the use of a neutral mobile phase that are suitable for negative ESI, which cannot be usually used by RPLC, resulting in a lower LOQ.
- Furthermore, the method uses isocratic elution with a high content of organic mobile phase, i.e. 16% water and 84% acetonitrile, resulting in a rapid analysis of only 3 minutes.

References

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