Quantification of the Date Rape Drug Gamma-Hydroxybutyric Acid in Drinking Water by Hydrophilic Interaction Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry

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Abstract

Gamma-hydroxybutyric acid (GHB) is a Schedule I controlled substance that is frequently used by predators for the purpose of drug facilitated sexual assault (DFSA), and it is commonly found in drinks served at social gatherings. In this study, a hydrophilic interaction liquid chromatography electrospray ionization tandem mass spectrometry (HILIC-ESI/MS/MS) method for the analysis of GHB in drinking water was developed. The method eliminated the derivation step which is required by traditional gas chromatography mass spectrometry (GC-MS) methods. It also achieved baseline resolution of GHB with its structural isomers, i.e. alpha-hydroxybutyric acid (AHB) and beta-hydroxybutyric acid (BHB), and allowed the use a neutral mobile phase, resulting in the use of negative-ion ESI to achieve a lower LOD and LOQ, 0.0198 and 0.0660 µg/mL, respectively. In comparison, it is unusual for negative ESI to be used by reverse phase liquid chromatography (RPLC). Furthermore, the method used isocratic elution with 84% acetonitrile in the mobile phase, resulting in a rapid analysis within 2.5 minutes.

Introduction

In the 1960s, GHB was used as an anesthetic in surgery due to its strong effects on the central nervous system. Later, it was used as a treatment for depression and sleep disorders, and a way to improve athletic performance. In the 1990s, GHB was found to have an increasing presence in overdose, driving under the influence, and DFSA cases.

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.

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

Sample preparations

1. **Calibration samples:** A total of six calibration samples with 0.2, 0.4, 1, 2, 5, and 10 µg/mL of GHB and 1 µg/mL GHB-d6 (internal standard) were prepared in drinking water.
2. **Quality control (QC) samples**: A total of three QC samples was prepared in drinking water with a concentration of 0.2, 1, or 10 µg/mL of GHB and 1 µg/mL GHB-d6.

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

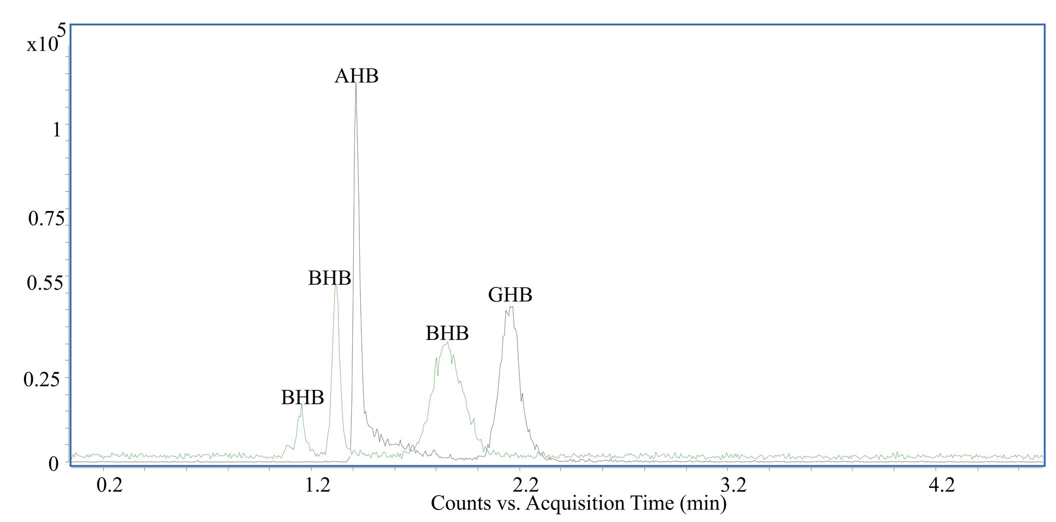

Figure 1. MS/MS EIC chromatogram of a mixture of AHB, BHB, and GHB at 1 µg/mL individual concentration in water. Black trace: m/z 103.040 → 57.035; green trace: m/z 103.040 → 59.014.

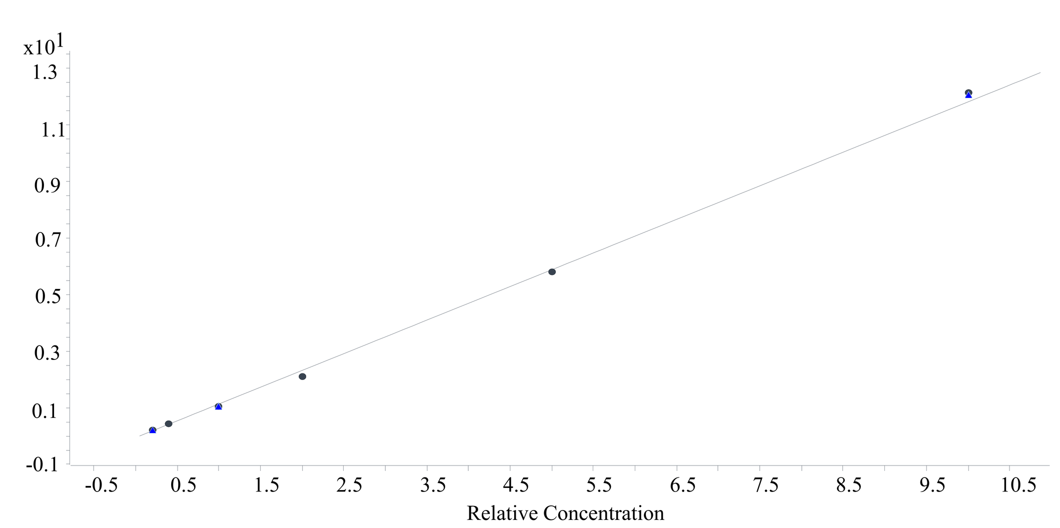

Figure 2. Representative internal standard calibration curve

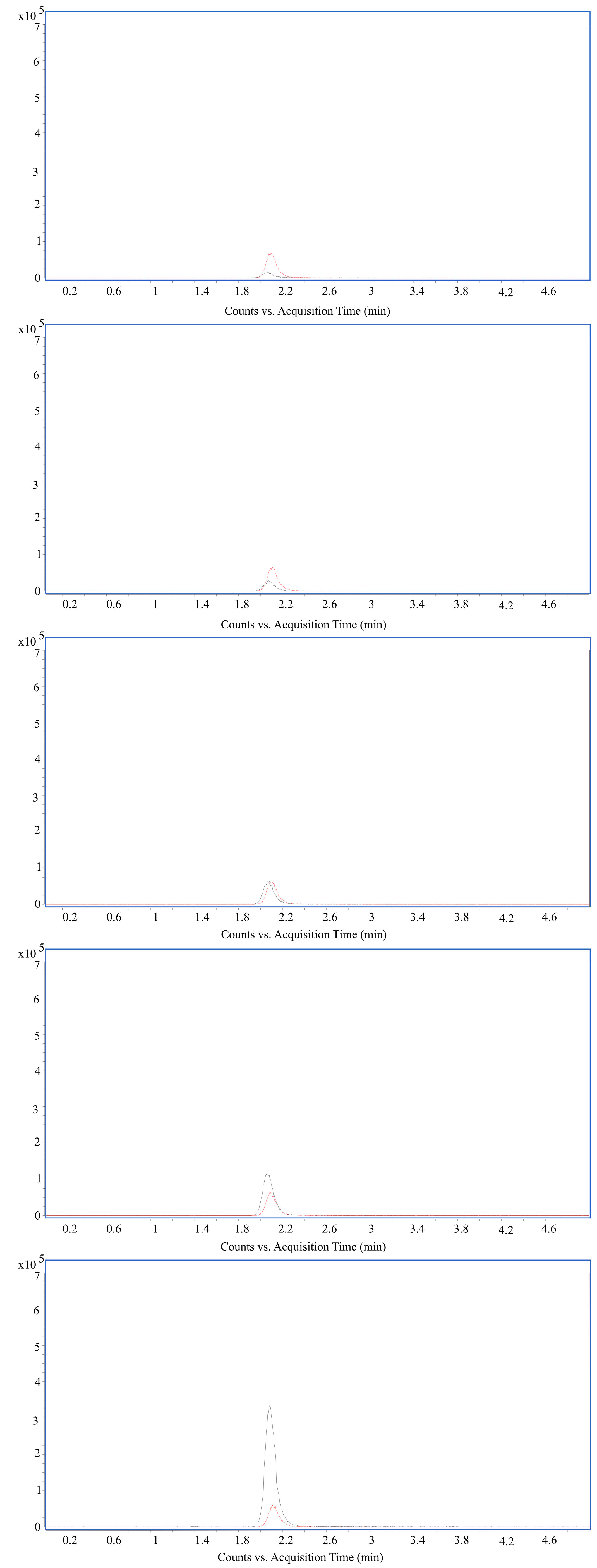

Figure 3. MS/MS EIC chromatogram of the calibration samples with 0.2, 0.4, 1, 2, and 5 µg/mL GHB (black trace: m/z 103.040 → 57.035) and 1 µg/mL GHB-d6 (red trace: m/z 103.040 → 59.014).

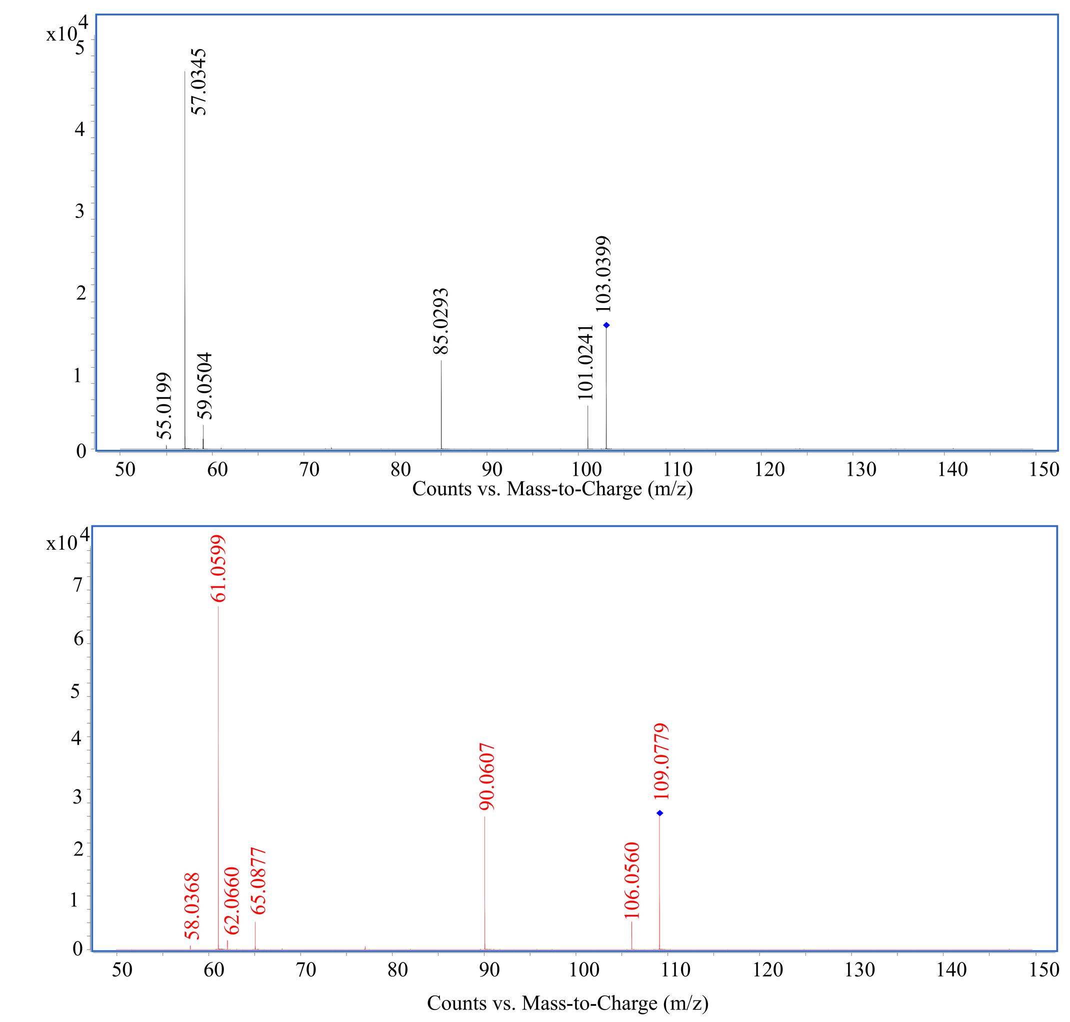

Figure 4. MS/MS mass spectra of GHB (black) and GHB-d6 (red).

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

Conc.	0.2 µg/mL	1 µg/mL	10.0 µg/mL
Intraday 1	110.9	94.4	103.8
Intraday 2	110.9	94.4	103.8
Intraday 3	107.3	93.1	103.8
Inter-day	109.7	94.0	103.8

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

Conc.	0.2 µg/mL	1 µg/mL	10.0 µg/mL
Intraday 1	3.244	1.366	1.513
Intraday 2	3.074	1.890	1.404
Intraday 3	3.377	2.731	1.322
Inter-day	4.555	1.912	1.273

Conclusions

- A HILIC-ESI/MS/MS method was developed for the analysis of drinking water to measure GHB during DFSA investigations.
- It eliminates the derivatization step seen in traditional GC-MS methods to make the GHB molecule nonpolar and volatile.
- It uses a neutral mobile phase that is acceptable for negative ESI, resulting in a lower LOD and LOQ, 0.0198 and 0.0660 µg/mL, respectively. In comparison, it is unusual for negative ESI to be used by RPLC.
- It uses isocratic elution with 84% acetonitrile in the mobile phase, which results in a rapid analysis that occurs within 2.5 minutes.

References

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