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CASE REPORT

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Drug-Facilitated Sexual Assault Involving Gamma-Hydroxybutyric Acid

ABSTRACT: The first case involving an alleged sexual assault linked to the use of gamma-hydroxybutyric acid (GHB) in Oklahoma is reported. A-48-year-old Caucasian woman taking amitriptyline was known to have voluntarily ingested a sports drink containing a relaxing health product. She purportedly experienced unconsciousness that persisted for approximately 4 h. The toxicological testing on urine identified GHB, amitriptyline, and nortriptyline using a capillary Hewlett-Packard 6890 gas chromatograph coupled to a Hewlett-Packard 5973 mass selective detector (MSD). The GHB concentration in urine was 26.9 μg/mL. Urine concentrations of amitriptyline and nortriptyline were not determined. The analytical method used for identifying and quantitating GHB can be applied to matters of forensic interests.

KEYWORDS: forensic science, abuse drugs, drugs, drug-facilitated, gamma-hydroxybutyric acid (GHB), toxicology

Gamma-hydroxybutyrate (4-hydroxybutyrate, GHB) is a naturally occurring metabolite for gamma-aminobutyric acid (GABA) found in the central nervous system and peripheral tissues at a concentration less than 1.0 μg/mL in living persons (15). GHB is metabolized by oxidative enzymes and has a short plasma half-life of 18 min to 1 h. Less than 5% of an oral dose of GHB is excreted unchanged in the urine, and GHB is not detectable in urine after 12 h (1–3). Its effects include euphoria, drowsiness, reduced inhibitions, dizziness, nausea and sedation (3,14). Unconsciousness associated with GHB is usually manifested approximately 15 min after oral administration and persists for about 3 h on average (4). The prodrug, gamma-butyrolactone (GBL), is also rapidly converted to GHB by enzymes in the blood and liver (2,3,5,6). GBL is converted to GHB on an equimolar basis and has a half-life of less than 1 min in the conversion. GBL is less polar, better absorbed, and more rapidly absorbed than GHB after an oral administration (6,8).

Recently, GHB has received media attention associated with its use to facilitate sexual assaults and has frequently been detected in victims of drug-facilitated sexual assault or date-rape (7,12,13). As a result, there has been an increase in toxicological requests by Oklahoma law enforcement agencies for GHB analysis. In Oklahoma, GHB is a Schedule I controlled dangerous substance (9) and GBL is a Schedule I controlled dangerous substance for human consumption (10). The legal distinction between GHB and GBL, with the potential for GBL to undergo conversion to GHB, has raised important issues within the criminal justice community (13). Analytical difficulties surrounding GHB include a short half-life, interpretation of results, and it requires a targeted analysis for detection (7).

This paper reports a case involving an alleged sexual assault linked to the use of gamma-hydroxybutyric acid and emphasizes the need for employing selected ion monitoring (SIM) mode gas chromatography-mass spectrometry (GC/MS) for the determination of GHB and its lactone form, gamma-butyrolactone (GBL), which may serve as a source of GHB (6).

Case Histories

A 48-year-old Caucasian woman, with a history of taking the tricyclic antidepressant amitriptyline, met with a 43-year-old man through the Internet and agreed to get together at her home with plans to go on a date. He presented a bottle that he claimed to contain a relaxing health product, which he mixed with a sports drink. Following absorption of the mixture the victim experienced unconsciousness, which persisted for about 4 h. During that time the victims physical perception was that she was subjected to nonconsensual sexual intercourse while she was unconscious due to the effects of the mixture she had consumed.

Urine samples, the specimen of choice for toxicological investigation of drug-facilitated sexual assault (7), were collected less than 12 h after the reported incident during the hospital rape examination and sent to the toxicological laboratory of the Oklahoma State Bureau of Investigation for analysis. Blood specimens were not available for toxicological analysis.

Material and Methods

The initial screening of the urine sample in this case was by a comprehensive procedure using a modified protocol from the previously published method (11). All confirmations were performed by GC/MS.

GHB and GBL were extracted according to the method of Lettieri and Fung (5), with minor modifications. Briefly, two 200 μL aliquots of the victim's urine were used for the identification and determination of GHB and GBL. The 200 μL aliquot of urine designated for the determination of GHB was spiked with 50 μL of a 10

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μg/mL solution of internal standard (d6-Gamma Hydroxybutyrate, Radian Corporation, Austin, TX). The mixture was treated with 10 drops of concentrated H_2SO_4 for the purpose of hydrolyzing GHB to GBL. The samples were then agitated with a vortex mixer for 10–15 s and allowed to cool to room temperature. Ten drops of 1.2N KOH were added to all the tubes and they were agitated with a vortex mixer for 10–15 s, followed by the addition of 1.0 mL of chloroform. The mixture was shaken vigorously and centrifuged at 3000 rpm for 15 min. The aqueous layer was removed and discarded from each mixture. The organic phase was evaporated to approximately 100 μL with a nitrogen stream and no heat. The sample was transferred to a glass sample vial, sealed with a snap cap and 1 μL aliquots were injected into the chromatograph. GC/MS analysis was performed in the selected ion-monitoring (SIM) mode.

The second 200 μL aliquot of urine designated for the determination of GBL was spiked with 50 μL of a 10 μg/mL solution of internal standard (d6-Gamma Butyrolactone, Radian Corporation, Austin, TX) followed by the addition of 1.0 mL of chloroform. The mixture was shaken vigorously and centrifuge at 3000 rpm for 15 min. The aqueous layer was removed and discarded from the mixture. The organic phase was evaporated to approximately 100 μL with a nitrogen stream and no heat. The sample was transferred to a glass sample vial, sealed with a snap cap, and 1 μL aliquots were injected into the chromatograph. GC/MS analysis was performed as above in the selected ion-monitoring (SIM) mode. Ions monitored were: m/z 86, 56, 42, and 28 (GBL); and m/z 92, 60, 48, and 32 (GBL-D_6). Ions at m/z 86 and 92 were used for quantitation.

The GC/MS analysis was performed with a Hewlett-Packard 6890 gas chromatograph coupled to a Hewlett-Packard 5973 mass selective detector (MSD). Separation was carried out on a Hewlett Packard Ultra 1 cross-linked fused-silica capillary column (12 m × 0.2 mm, 0.33 μm film thickness) connected to the MSD through a direct capillary interface. The injector port was a capillary splitless injector with a splitless silanized glass insert. Carrier gas (He) flow was 1 mL/min. Injector and interface temperatures were 270°C and 280°C, respectively. The oven was programmed 30°C at 10°C/min to 80°C and increased at 30°C/min to 245°C. The MSD was used in the electron impact (70 eV) SIM mode and the electron multiplier was set 200 above the autotune voltage.

Results and Discussion

GHB can be detected effectively by the described GC/MS (SIM) method in biological fluids and tissues after administration of GHB, GBL, or precursors of the former (3). The described analytical method proved to be simple, specific, and accurate.

The urine level of GHB was 26.9 μg/mL, reported as gamma-butyrolactone. The urine samples for GBL determination gave negative results. The toxicological screening for basic drugs in the urine was found to contain amitriptyline and nortriptyline. GHB concentrations of 1.0 μg/mL or less are consistent with endogenous GHB of living persons (15). The GHB concentrations observed from the urine samples collected from the victim in this case are consistent with GHB being exogenously administered (15,16).

The symptoms manifested by the victim in this case report are consistent with the known pharmacological effects of GHB. GHB has been shown to act synergistically with other central nervous system depressants such as ethanol (2). There are no published reports of interactions of GHB and amitriptyline. The author is therefore unable to comment on the possible contribution of amitriptyline and nortriptyline to the victim's symptoms.

References

1. Baselt, RC, Cravey RH. Disposition of toxic drugs and chemicals in man. 4th ed. Foster City: Chemical Toxicology Institute 1995;348–9.
2. Baselt, RC, Stephens GB. Driving under the influence of GHB? J Anal Tox 1994;18:357–8.
3. Ferrara SD, Zotti S, Tedeschi L, Frison G, Castagna F, Gallimberti L, et al. Pharmacokinetics of gamma hydroxybutyric acid in alcohol dependent patients after single and repeated oral doses. BR. J Clin Pharmac 1992;34:231–5.
4. Ferrara SD, Tedeschi L, Frison G, Castagna F, Gallimberti L, Giorgetti R, et al. Therapeutic gamma-hydroxybutyric acid monitoring in plasma and urine by gas chromatography-mass spectrometry. J Pharmac and Biomedical Analysis 1993;11:6, 283–7.
5. Lettieri, TJ, Fung HL. Evaluation and development of gas chromatographic procedures for the determination of gamma-hydroxybutyric acid and gamma-butyrolactone in plasma. Biochemical Medicine 1978;20:70–80.
6. Irwin RD. NTP summary report on the metabolism, disposition and toxicity of 1,4-butanediol, U.S. Depart. Of Health and Human Services, Public Health Service, NIH Publication No. 96-3932, May 1996.
7. LeBeau M, Andollo W, Hearn WL, Baselt R, Cone E, Finkle B, et al. Recommendations for toxicological investigations of drug-facilitated sexual assaults. J Forensic Sci 1999;44(1):227–30.
8. Kohrs PF, Gamma-hydroxybutyrate intoxication and overdose. Ann Emerg Med 1999;33:475.
9. Uniform Controlled Dangerous Substance Act. 46th Legislature, 2nd Sess. (1998).
10. Uniform Controlled Dangerous Substance Act. 47th Legislature, 2nd Sess. (2000).
11. Foerester EH, Hatchett D, Garriott JC. A rapid, comprehensive screening procedure for basic drugs in blood or tissues by gas chromatography. J Anal Toxicol 1978;50–5.
12. Elsohly MA, Salamone SJ. Prevalence of drugs used in cases of alleged sexual assault. J Anal Toxicol 1999;23(3):141–6.
13. Ciolino LA, Mesmer MZ, Satzger RD, Machal AC, McCauley HA, Mohrhaus AS. The chemical interconversion of GHB and GBL: forensic issues and implications. J Forensic Sci 2001;46(6):1315–26.
14. Couper FJ, Logan BK. GHB and driving impairment. J Forensic Sci 2001;46(4):919–23.
15. Fieler E, Coleman D, Baselt R. Gamma-hydroxybutyrate concentration in pre- and postmortem blood and urine (comment). Clin Chem 1998;44:692.
16. McCusker RR, Paget-Wilkes H, Chronister CW, Goldberger BA, Elsohly MA. Analysis of gamma-hydroxybutyrate (GHB) in urine by gas chromatography-mass spectrometry. J Anal Toxicol 1999;23(3):141–6.

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