

*Prikaz slučaja /
Case report*

CONFIRMATION OF GAMMA-HYDROXY-
BUTYRATE POISONING – *Case report*

POTVRDA TROVANJA GAMA-HIDROKSI-
BUTIRATOM – *Prikaz slučaja*

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Ključne reči

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Abstract

As an endogenous substance, gamma-hydroxybutyrate (GHB) is naturally present in the central nervous system (CNS) of mammals as well as in peripheral tissues. It also acts as a minor metabolite and precursor of GABA, a major inhibitory neurotransmitter in the CNS. Interest in GHB began in the 1960s when it was initially used in anesthesiology. However, due to negative patient reactions, this use was quickly discontinued. In subsequent years, GHB saw renewed interest, particularly in the fields of nutrition and bodybuilding. By the end of the 20th century, GHB's popularity surged as a „club” drug. It can appear as a white powder or a colorless liquid, with the latter often being dyed to conceal its presence and make it easier to add to drinks. The effects of GHB start 10-20 minutes after consumption and can last up to 11 hours. Chromatography coupled with tandem mass spectrometry (LC-MS/MS) has proven to be an effective method for detecting GHB in biological samples such as urine and blood.

This article presents case of young man after acute ingestion of GHB, who was unconsciousness at admission with data about recreational abuse of GHB. He had hypooxygenation and hypotension. Urine test for standard psychoactive substances was negative. The liquid chromatography coupled with tandem mass spectrometry was used for detection of GHB in blood and urine samples.

GHB poisonings are not common, but they can have severe clinical picture. In most cases, diagnosis is made on the basis of the clinical picture, but analytical confirmation can facilitate it.

INTRODUCTION

Gamma-hydroxybutyric acid is a short-chain adipose acid, which acts as a precursor and a metabolite of the inhibitory neurotransmitter gamma-aminobutyric acid, GABA. It is approved and registered in some countries as a remedial agent (Xyrem®) for the treatment of cataplexy associated with narcolepsy, while in the other countries it's an adjunct in the treatment of drunkenness [1]. In recent years, GHB has made its way onto the market illegally as a recreational „club drug” because of its ability to induce feelings of euphoria or enhance libido [2]. Consumption of its analogue gamma-butyrolactone (GBL), also leads to similar

physiological effects, as it is rapidly converted in the body to GHB [3]. The endogenous nature of GHB can occasionally beget difficulty in taking analysis of natural samples. An arrestment of 10 mg/L is recommended for demarcation between endogenously formed and exogenously introduced GHB in antemortem urine samples[4]. For the determination of GHB in biological samples, it is most often used a reliable and rapid method of liquid chromatography with tandem mass spectrometry. During the analysis itself, preparation for different biological matrices is very fast. Also, analysis takes only a few minutes, and requires a small amount of the examined sample and can be applied in various clinical and forensic cases.

CASE REPORT

A 34-year-old man was brought to the emergency room at the National Control Center after recreational GHB abuse. Shortly after using GHB became aggressive. He ran out of the club and was found unconscious on the street. At the reception in the NPCC, he had hypotension (90/60) and pulse 40 beats per minute, respiratory rate 8/min. and saturation of 55% of oxygen. EKG showed sinus rhythm with a pulse of 110/min without another change. After intubation, mechanical ventilation was used for 19 h. Standard urine test for the presence of psychoactive substances (opioids, cocaine, amphetamines, MDMA, cannabinoids) was negative. Screening for medical drugs was negative and 0.28 % ethanol was detected in blood. Liquid chromatography with mass spectrometry was used for the identification of GHB in biological samples. GHB was detected in blood and urine. Two days later, after a complete recovery, the patient was discharged from the clinic. The patient had two episodes of GHB poisoning within a month.

MATERIAL AND METHODS:

Biological samples were prepared by solid-phase extraction on Waters MCX cartridges.

HPLC-MS/MS analysis was performed using UPLC Waters Acquity chromatography coupled to a quadrupole tandem mass spectrometer WatersXevo TQ on Acquity UPLC HSS reverse phase column C18 (1.8 μ m, 2.1 \times 150 mm). During the analysis, the column temperature was maintained at 30°C. Isocratic elution was performed at a flow rate of 0.3 mL/min. with a mixture of 5 mM ammonium acetate-acetonitrile (20:80) as a mobile phase. Total run time was 5 min. Ionization is achieved using electrospray in positive mode (ES+). GHB was detected according to ions: 103, 85 and 57 m/z.

The obtained result, MRM chromatograms of urine, are shown in Figure 1.

DISCUSSION

Gamma-Hydroxybutyrate (GHB) is a central nervous system depressant that is both naturally occurring and synthetically produced. It is abused due to its euphoric and sedative properties, but also as a steroid alternative in athletes in gym. Due to its ability to induce unconsciousness and impair memory it is known as a rape drug.

GHB is well absorbed after oral ingestion, and effects occur after 15 min. Maximal conc. in blood is achieved after 20-40 min. Half-life in plasma is 1-2 hours. It is metabolized in liver through succinate and Krebs cycle and eliminate as carbon dioxide and water. Less than 5% is eliminated via kidney unchanged. It has short time of detection. Only 3-10 hours. [1] [5] [6]

GHB is an extremely dangerous drug as the dose range between safe and toxic is very narrow. At low doses, GHB produces effects such as relaxation, euphoria, increased sociability, and mild sedation. At higher doses, it can cause deep sedation, confusion, memory impairment, and disorientation, which can persist for hours. Overdose can cause deep sedation and loss of consciousness, sometimes with amnesia of the event [7].

In users that coingested alcohol with GHB, risk of adverse effects and hospital treatment is increased. Both

alcohol and GHB depress the central nervous system, and their combined use can lead to severe respiratory depression, loss of consciousness and coma [8].

We registered 34-year-old man, who was admitted in Emergency room in National Poisoning Control Centre due to data about recreational abuse of drugs. He was aggressive in club. After some time, he lost consciousness, and transported to the hospital. At the reception he had hypotension (90/60 mmHg), bradycardia (40 bpm), low respiratory rate (8 respiration per minute) and oxygen saturation (50%). He was intubated and nonspecific detoxication therapy was applied. Routine toxicological analysis showed presence only ethanol in blood in concentration of 0.28 mg/mL. Urine was negative on standard psychoactive substances. Because clinical picture indicated poisoning, we performed analysis on GHB.

In the case presented by YS Sia and YT Wong [9], a 39-year-old man who had taken GHB with alcohol displayed symptoms of fatigue and convulsions during an exercise session. He had isolated limb jerks and had a Glasgow Coma Score of 12/15. His vital signs stayed stable, and his CT scan came back normal. After taking diazepam for two hours, he improved even though he was still twitching somewhat, and he was released against medical advice. Coma and respiratory depression are among the most severe effects that are produced by higher dosages of GHB, however they usually disappear after seven hours.

GHB poisoning is present in both situations, despite differences in its intensity and presentation. The 34-year-old man required 19 hours of mechanical breathing after experiencing severe respiratory depression, hypotension, and bradycardia following recreational GHB use. Two days after he fully recovered, he was released. The first patient experienced a more severe episode that necessitated continuous breathing support, while the second patient had a milder, self-limiting episode. The 39-year-old man, however, only needed a short exposure to GHB, as indicated by his fatigue, limb twitches, and significant respiratory issues after consuming GHB with alcohol. These two cases highlight the unpredictable nature of GHB poisoning, as symptoms can vary from mild to severe, underscoring the importance of seeking medical help promptly.

Diagnosing of GHB poisoning is very important especially when there are not any data about toxic agent. It not just clinical, but also analytical challenge due to its short half-life and rapid metabolism. Additionally, GHB is not routinely included in standard toxicology screens, which makes it harder to detect without specific tests. However, a combination of clinical evaluation, symptom recognition, and laboratory testing can help healthcare. Because of its rapid metabolism and clearance, blood and urine samples should be collected as soon as possible after suspected ingestion.

Different method is used for detection of GHB in biological samples. There are test strips for detection of GHB, but they are not reliable [10].

Different authors suggest liquid chromatography with mass spectrometry for determination of GHB in biological samples [11] [12] [13].

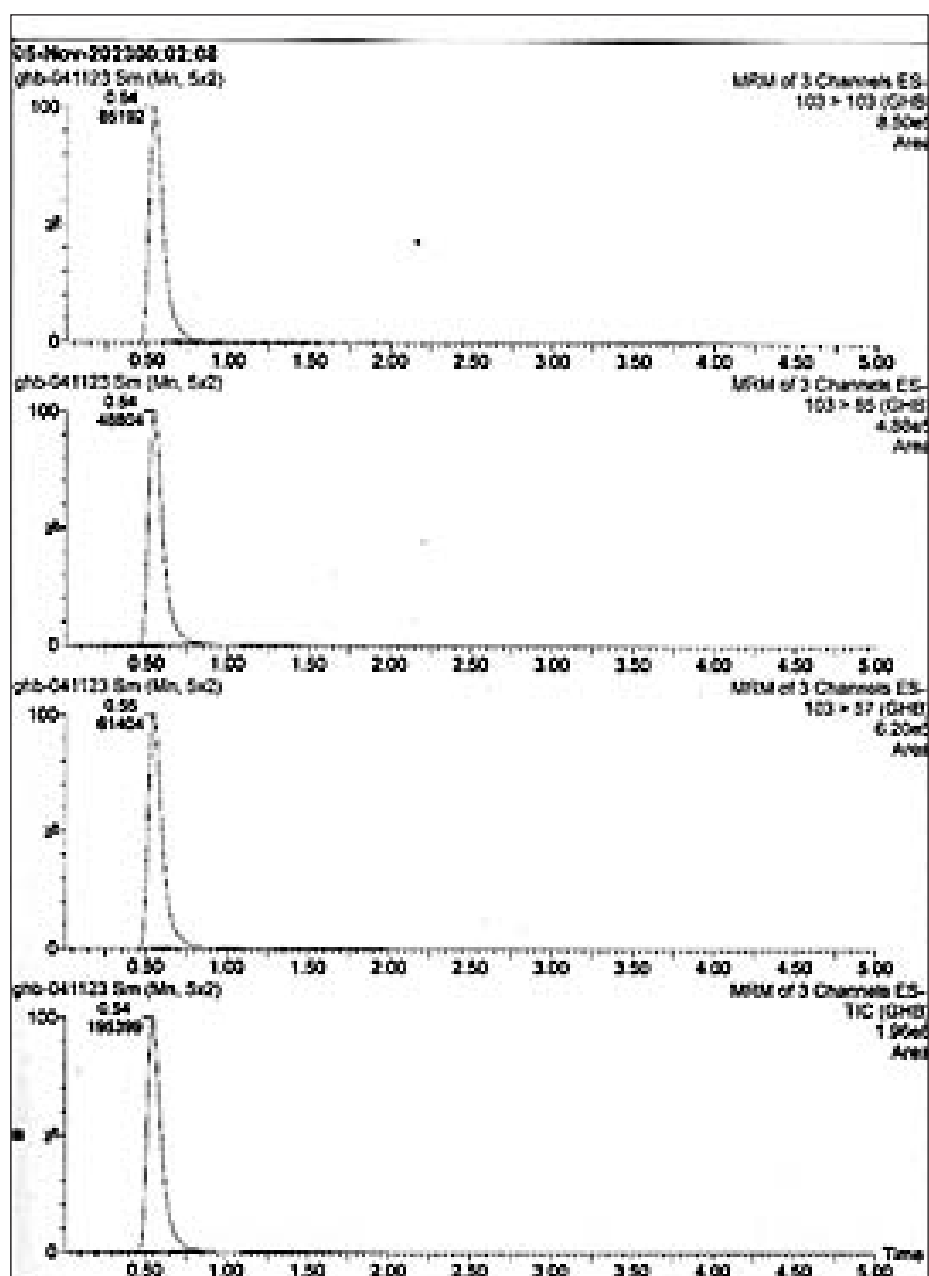


Figure 1. Chromatograms obtained after the UPLC-MS/MS analysis of the patient's urine

In our laboratory we developed method of liquid chromatography with tandem mass spectrometry for reliable determination of GHB in biological samples. This method confirmed presence of GHB in patients' blood and urine samples. He was on mechanical respiration for 19 h and after two days he was discharged fully recovered.

Compare to other method [13], our method is more target-specific and directed. It makes use of solid-phase extraction to prepare the samples, which works best for the sensitivity and specificity for GHB in urine and blood. The strategy also makes use of HPLC-MS/MS with an accurate setup, of which the lowest limit of quantification is (1 mg/kg), which works very well on the sensitivity as well as on accuracy for detecting GHB. Compared to it, the initial method [13], even though effective for some drugs, is less optimized for the analysis of GHB and hence our method being the best choice for accurate and efficient GHB analysis in forensic examinations.

CONCLUSION

It pinpoints the severity of GHB intoxication alongside the needed rapid intervention. Although HPLC-MS/MS is perfectly accurate, it takes a processing time of up to 5 minutes and, because of that, lacks improvements for quicker outcomes. Oftentimes, improved extraction and automated systems can help speed up results. GHB overdoses are sometimes severe and can be fatal, especially when combined with CNS depressants. That's why diagnosis is important, because treatment can be directed adequately. In our case, tandem mass spectrometry unequivocally confirmed the ingestion of GHB. Improved diagnosis and treatment to minimize complications become crucial as a result of this case.

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Sažetak

Kao endogena supstanca, gama-hidroksibutirat (GHB) je prirodno jedinjenje prisutno u centralnom nervnom sistemu (CNS) sisara, kao i u perifernim tkivima. Deluje i kao minorni metabolit i prekursor GABA, glavnog inhibitorynog neurotransmitera u CNS-u. Interesovanje za GHB počelo je 1960-ih kada je prvobitno korišćen u anesteziologiji. Međutim, zbog negativnih reakcija pacijenata, ova upotreba je brzo prekinuta. U narednim godinama, interesovanje za GHB se ponovo javilo, posebno u oblasti ishrane i bodibildinga. Do kraja 20. veka, popularnost GHB-a je porasla kao „klubske“ droge. Može se pojaviti kao beli prah ili bezbojna tečnost, pri čemu je ova druga često obojena kako bi se prikrilo njegovo prisustvo i olakšalo dodavanje u piće. Efekti GHB počinju 10-20 minuta nakon konzumiranja i mogu trajati do 11 sati. Hromatografija u kombinaciji sa tandem masenom spektrometrijom (LC-MS/MS) se pokazala kao efikasna metoda za detekciju GHB u biološkim uzorcima kao što su urin i krv.

U ovom članku prikazan je slučaj mladića nakon akutnog uzimanja GHB, koji je bio u nesvesti pri prijemu sa podacima o rekreativnoj zloupotrebi GHB. Imao je hipooksigenaciju i hipotenziju. Test urina na standardne psihoaktivne supstance bio je negativan. Za detekciju GHB u uzorcima krvi i urina korišćena je tečna hromatografija u kombinaciji sa tandem masenom spektrometrijom. Trovanja GHB nisu česta, ali mogu imati tešku kliničku sliku. U većini slučajeva dijagnoza se postavlja na osnovu kliničke slike, ali analitička potvrda to može olakšati.

REFERENCES

1. Busardò FP, Jones AW. GHB pharmacology and toxicology: acute intoxication, concentrations in blood and urine in forensic cases and treatment of the withdrawal syndrome. *Curr Neuroparmacol.* 2015;13(1):47-70. doi:10.2174/1570159X13666141210215423
2. Bellis MA, Hughes K, Bennett A, Thomson R. The role of an international nightlife resort in the proliferation of recreational drugs. *Addiction.* 2003;98(12):1713-1721. doi:10.1111/j.1360-0443.2003.00554.x
3. Palmer RB. Gamma-butyrolactone and 1,4-butanediol: abused analogues of gamma-hydroxybutyrate. *Toxicol Rev.* 2004;23(1):21-31. doi:10.2165/00139709-200423010-00003
4. Busardo FP, Kyriakou C. GHB in Biological Specimens: Which Cut-off Levels Should be Taken into Consideration in Forensic Toxicological Investigation?. *Recent Pat Biotechnol.* 2014;8(3):206-214. doi:10.2174/1872208309666150504143155
5. Felmler MA, Morse BL, Morris ME. γ -Hydroxybutyric Acid: Pharmacokinetics, Pharmacodynamics, and Toxicology. *AAPS J.* 2021;23(1):22. Published 2021 Jan 8. doi:10.1208/s12248-020-00543-z
6. White CM. Pharmacologic, Pharmacokinetic, and Clinical Assessment of Illicitly Used γ -Hydroxybutyrate. *J Clin Pharmacol.* 2017;57(1):33-39. doi:10.1002/jcph.767
7. Steele MT, Watson WA. Acute poisoning from gamma hydroxybutyrate (GHB). *Mo Med.* 1995;92(7):354-357.
8. Thai D, Dyer JE, Benowitz NL, Haller CA. Gamma-hydroxybutyrate and ethanol effects and interactions in humans. *J Clin Psychopharmacol.* 2006;26(5):524-529. doi:10.1097/01.jcp.0000237944.57893.28
9. Sia Y, Wong Y. Case Report: Gamma-Hydroxybutyrate Poisoning. *Hong Kong Journal of Emergency Medicine.* 2000;7(3):166-168. doi:10.1177/102490790000700308
10. Smits TA, Gresnigt FMJ, van Groen BD, Franssen EJF, Attema-de Jonge ME. Prospective Investigation of the Performance of 2 Gamma-Hydroxybutyric Acid Tests: DrugCheck GHB Single Test and Viva-E GHB Immunoassay. *Ther Drug Monit.* 2020;42(1):139-145. doi:10.1097/FTD.0000000000000677
11. Haller C, Thai D, Jacob P 3rd, Dyer JE. GHB urine concentrations after single-dose administration in humans. *J Anal Toxicol.* 2006;30(6):360-364. doi:10.1093/jat/30.6.360
12. Sørensen LK, Faldborg KB, Andersen CU, Hasselstrøm JB. Determination of endogenous GHB in ante-mortem whole blood, urine, and oral fluid by LC-MS/MS: The effect of different additives and storage conditions on the stability of GHB in blood. *Forensic Sci Int.* 2024;365:112286. doi:10.1016/j.forsciint.2024.112286
13. Johansen SS, Windberg CN. Simultaneous determination of γ -Hydroxybutyrate (GHB) and its analogues (GBL, 1,4-BD, GVL) in whole blood and urine by liquid chromatography coupled to tandem mass spectrometry. *J Anal Toxicol.* 2011;35(1):8-14. doi:10.1093/anatox/35.1.8

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