

## Case Reports

## GAMMA-HYDROXYBUTYRIC ACID (GHB) MEASUREMENT BY GC-MS IN BLOOD, URINE AND GASTRIC CONTENTS, FOLLOWING AN ACUTE INTOXICATION IN BELGIUM

Q. Bodson, R. Denooz, P. Serpe, C. Charlier

**Key words :** GHB, gamma-hydroxybutyrate, intoxication, GC-MS, gas chromatography-mass, spectrometry

### ABSTRACT

Gamma-hydroxybutyrate (GHB, sodium oxybate) is a compound related to neuromodulator gamma-aminobutyric acid (GABA), emerging as a recreational drug of abuse and as a rape drug. GHB-related emergencies have dramatically increased in the 1990s, but a decrease is observed since 2000. We describe the case of an acute GHB intoxication in a 28-year-old male who fell unconscious after ingestion of a mouthful of an unknown beverage, and required medical support for 2 days. A cocaine abuse was also detected by preliminary toxicological screening, but the clinical presentation was not typical of cocaine intoxication.

A simple liquid-liquid extraction was used for quantitation of GHB, followed by disilyl-derivatiza-

tion and analysis in selective ion monitoring (SIM) mode by gas chromatography-mass spectrometry (GC-MS), using GHB-d6 as internal standard.

High concentrations of GHB were detected in urine (3020 mg/L) and gastric contents (71487 mg/L) at admission. After a 6-hours delay, GHB was still present in urine at 2324 mg/L and in blood at 43 mg/L.

The clinical symptoms of cocaine intoxication were diminished by GHB consumption, and the cerebral scan was modified. Attention must thus be paid to acute intoxications with surprising clinical symptoms, and GHB has probably to be added to the preliminary toxicological screening.

Data available regarding GHB are briefly reviewed, and our results are compared with previously published reports of non-fatal GHB intoxication.

Laboratoire de Toxicologie,  
Université de Liège,  
CHU Sart-Tilman, Belgium

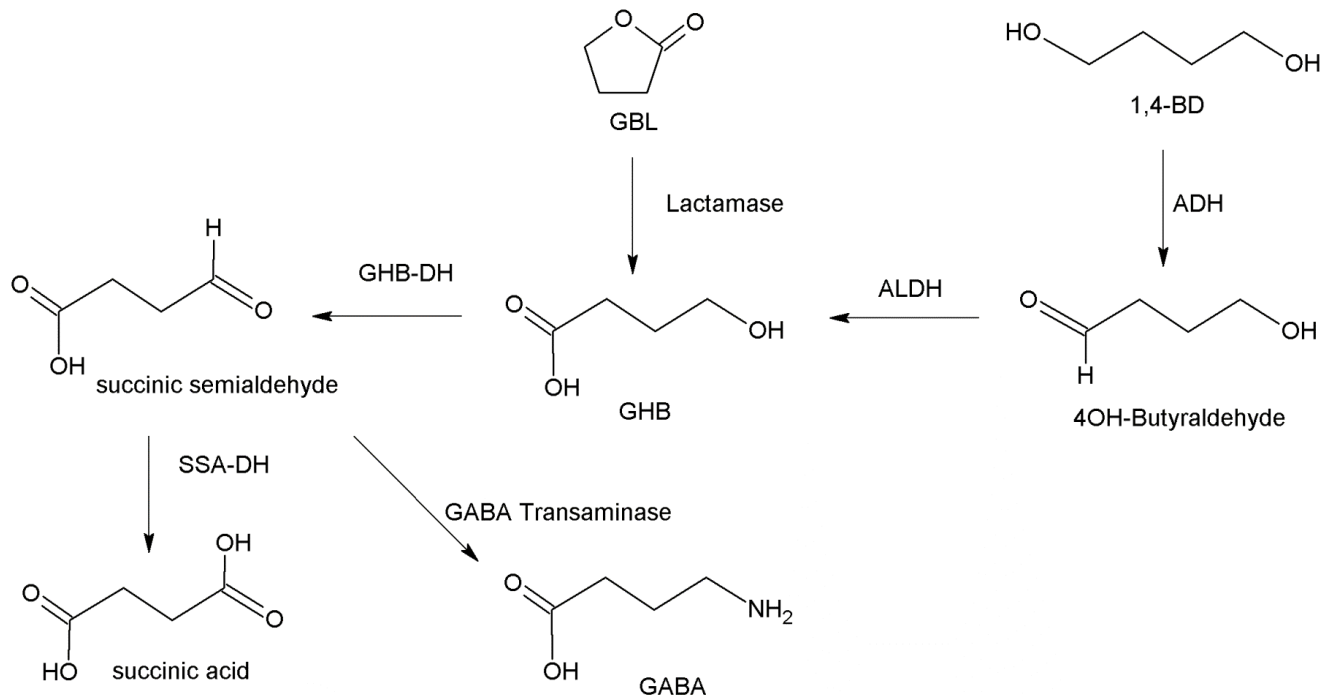
#### Address for Correspondence:

Phn. Quentin Bodson,  
Laboratoire de Toxicologie Clinique  
Tour II, +5  
CHU Sart-Tilman  
4000 Liège  
Belgium.  
Tel: +32 (0)4 366 76 79  
Fax: +32 (0)4 366 88 89  
E-mail: Q.Bodson@ulg.ac.be

### INTRODUCTION

Gamma-hydroxybutyrate (GHB, sodium oxybate), a compound structurally related to the human depressant neurotransmitter gamma-aminobutyric acid (GABA) (Figure 1), is naturally present at nanomolar concentrations in the mammalian brain (1). It has been shown to act on specific receptors, called GHB receptors (2), and also on GABA-B receptors (3;4).

At low doses (about 10 mg/kg), it induces anxiolysis, somnolence, amnesia, hypotonia; whereas it is hypnotic with powerful sedative properties at higher doses (30 to 60 mg/kg) (5;6). GHB might lead to euphoria, dependence, and shows an unfortunate effectiveness



**Figure 1.** Metabolic pathway of GHB and related substances.

GHB is almost completely metabolized by GHB dehydrogenase (GHB-DH) into succinic semialdehyde, which is then converted by succinic semialdehyde dehydrogenase (SSA-DH) into succinic acid that enters Krebs cycle and leads to the production of CO<sub>2</sub> + H<sub>2</sub>O. GHB has thus no specifically identifiable oxidation products.

Conversion of GHB into neurotransmitter gamma-aminobutyric acid (GABA) is ensured by GHB-DH and GABA transaminase.

Gamma-butyrolactone (GBL) is converted in vivo into GHB by lactamase, which simply breaks open the lactonic ring. 1,4-butanediol (1,4-BD) is oxidized in 4-OH-butaldehyde by ADH (alcohol dehydrogenase), and then converted into GHB by aldehyde dehydrogenase (ALDH).

as a "drug-facilitated sexual assault" agent ("rape drug") (7;8).

GHB, isolated in 1874 (9), was first synthesized by Laborit in the early 1960s (10). GHB was then used for years in Europe and the United States as an anaesthetic agent, as an hypnotic (11) and in the treatment of alcohol withdrawal (12). It was originally considered as a food supplement, sold in health food stores as a sleep aid, as a weight loss drug and as muscle enhancer. GHB was alleged to increase production of growth hormone (13;14), which lead to its abuse by bodybuilders as a steroid alternative when steroid use became illegal in the late 1980s (15).

In the 1990s, GHB spread from the sports world over the club world (16;17). Due to its stimulant, anxiolytic and euphoric effects at low doses, GHB became a popular drug of abuse in clubs, where it is often called "liquid ecstasy", "liquid X", "G", "Grievous Bodily Harm", "liquid E", "Georgia Home Boy", "gamma-oh", "easy lay", etc. Consequently, GHB-related emergencies in the US increased dramatically from 56 cases in 1994 to about

5000 in 2000 (18), but a decrease was observed when GHB was scheduled in 2000 (19-21).

Since the United Nation's decision to put GHB under control in March 2001, GHB has been a controlled substance in most European Union member states. The European Medicines Agency (EMA) has designated it as an "Orphan Medicinal Product" in February 2003, and delivered a marketing authorisation, valid throughout the EU, in October 2005 (22), for the treatment of cataplexy in adult patients with narcolepsy (23;24). GHB is now legally available for this indication, under controlled conditions, in most of the EU member states as in the US (Xyrem®) (22). There are important differences in GHB seizures in Europe, but the global trend seems to be stabilization or decrease in GHB abuse since 2002-2003 (25-27). In Belgium, the trend in GHB use in regard of total drug abuse in recreational settings varied between 1% to 2% from 2002 to 2005 (28).

Gamma-butyrolactone (GBL) and 1,4-butanediol (1,4-BD), converted in vivo to GHB (Figure 1), are not controlled substances (e.g. industrial solvents) (29).

Easily available on the Internet, they are often used for GHB preparation or as GHB substitute (30;31), with an increasing risk of adverse effects due to increased bio-availability (32-34).

Difficulties to find one's personal dose range (an extra 10% can lead from euphoria to vomiting) (35), concentrations variability of available powders and solutions, confusion between GHB, 1,4-BD and GBL, and frequent dilution with alcohol are associated with an elevated occurrence of adverse effects, although it has been shown that there are minimal pharmacokinetic interactions between GHB and ethanol (36).

The half-life of GHB is approximately 30 min and GHB can be totally excreted from the body within 10-12 h. GHB is often undetectable in plasma after 6-8 h, in living patients (37;38).

Moreover, GHB is an endogenous compound and post-mortem production might be significant and become a severe artefact, particularly in blood (39-41). Urine, easily available and less susceptible to post-mortem modification, is the recommended sample (41;42).

In forensic cases (i.e. overdose, death, driving under the influence (DUI), sexual assault), case history, delay of analysis, post-mortem interval (39;43), storage (42;44), clinical presentation (levels of sedation or coma, agitation, combativeness, self-injurious behaviours, etc.) have to be considered for an accurate interpretation of the toxicological findings (45).

## CASE HISTORY

A 28-year-old white male was found in comatose on the public way, in the lateral recovery position, on a Saturday night. He was transported to an emergency department. Friends reported that he had vigilance disorders 10 minutes after ingestion of a mouthful of an unknown beverage. Clinical presentation did not agree with morphinic or cocaine intoxication. The patient was in an ethylic state. The subject's eyes were bloodshot, with a strong bilateral miosis and fixed pupils. He was haemodynamically stable with a pulse of 66 bpm and a blood pressure of 120/80 mm Hg. His respiratory rate was of 19 breaths per minute.

Propofol and remifentanyl were administered upon arrival at the emergency department to allow patient's intubation, and to calm him down. Urine, blood and gastric aspiration were collected for toxicology. Gastric

lavage and activated charcoal administration were performed. A cerebral CT scan showed a cerebral oedema and a suspicion of meningeal haemorrhage. The patient did not show any trace of cutaneous or intravenous injection. Electrocardiogram and cardio-pulmonary examination were normal. Blood tests showed a slight elevation of white blood cell count (15440/mm<sup>3</sup>) with normal leukocyte differential. Electrolyte profile was normal. A mild respiratory acidosis (pH 7.29) was present, and lactic acid was elevated (26.4 mg/dL). Renal, liver and pancreatic function tests were normal.

Toxicology screening on Abbott AxSYM® and Roche Cobas Integra 400®, based on "Fluorescence Polarization Immunoassay" (FPIA), were performed on urine, blood and gastric aspiration.

Cocaine metabolite was found highly positive in urine, above the limit of linearity (LOL: 5.0 mg/L). Presence of cocaine in urine (benzoylecgonine >1.2 mg/L) and blood (benzoylecgonine: 142 µg/L) was confirmed by GC-MS. Benzoylecgonine was detected positive in gastric aspiration by HPLC-DAD, but not quantified. Ethanol was found positive at 1.60 g/L on Abbott AxSYM®, based on "Radiative Energy Attenuation" (REA) technology.

Urine<sup>1</sup> and serum<sup>2</sup> "FPIA"-screening were negative for salicylates, acetaminophen, barbiturates, benzodiazepines, tricyclic antidepressants, cannabinoids, opiates and amphetamines. GC-MS and HPLC-DAD techniques confirmed these findings. Gastric aspiration screening was also negative, after treatment with Subtilisin A (Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

The patient was transferred to the Intensive Care Unit, where he was extubated rapidly. At day +1, the patient was still complaining about persistence of moderate pulsatile holocranial cephalgias. He also reported a total amnesia of the past events. Electroencephalogram was normal. The patient was discharged 2 days after arrival.

<sup>1</sup> Limits Of Quantitation (LOQ) for urine (in our lab).  
Cocaine metabolites : 0.10 mg/L; barbiturates : 0.12 mg/L;  
benzodiazepines : 0.20 mg/L; tricyclic antidepressants : 40.0 µg/L; salicylates : 5.0 mg/L; cannabinoids : 13.0 µg/L; opiates : 0.06 mg/L; amphetamines : 0.50 mg/L

<sup>2</sup> Limits Of Quantitation (LOQ) for serum (in our lab).  
Tricyclic antidepressants : 75.0 µg/L; salicylates : 5.0 mg/L;  
acetaminophen : 1.0 mg/L; barbiturates : 0.03 mg/L; benzodiazepines : 0.003 mg/L.

Due to intoxication history and clinical evolution (46;47), a specific determination of GHB was performed afterwards on the available samples, i.e. urine obtained at admission and 6 hours after admission, serum obtained 6 hours after admission (blood sample at admission was insufficient), and gastric contents obtained at admission.

To measure GHB in our patient's samples, a conventional technique was used: liquid-liquid extraction followed by gas chromatography coupled with mass spectrometry (GC-MS), with direct derivatization of GHB (48-50).

## MATERIAL (APPARATUS)

### Chemical and reagents

GHB sodium and GHB-d6 sodium were purchased from Cerilliant (Texas, USA) as 1.0 mg/ml solutions in methanol (as salt). Methanol and acetonitrile were analytical High Performance Liquid Chromatography (HPLC) grade (both purchased from Lab-Scan Analytical Sciences, Dublin, Ireland). Ethyl acetate was Normapur analytical reagents from BDH-ProLabo-VWR. The derivatization reagent was a BSTFA/TMCS mix (99:1, V/V). BSTFA was N,O-bis(trimethylsilyl)-trifluoroacetamide for gas chromatography from Merck KGaA (Darmstadt, Germany). TMCS was trimethylchlorosilane for synthesis from Merck Schuchardt OHG (Hohenbrunn, Germany). 0.1N sulphuric acid solution used for extraction was prepared by dilution of sulphuric acid min 95%, purchased from VEL (Leuven, Belgium).

### Instrumentation

The automated injector was a Combi/Liquid PAL System Autosampler from CTC Analytics – Interscience. The GC model was an Interscience Thermo Electron Corporation Trace GC Ultra Gas Chromatograph with a split/splitless injector. The carrier gas was He (purity grade N60, 99.9999%) and the column used was a VF-5 MS capillary column ((5%-Phenyl)-dimethylpolysiloxane, 30m x 0.25 mm i.d., 0.25 µm film thickness) (Factor Four Capillary Column from Varian, The Netherlands). A Thermo Electron Corporation DSQ Mass Selective Detector was coupled to the GC for quantitative analysis.

## Methods

### Extraction

The extraction procedure was the same for urine, serum and gastric aspiration. Gastric contents were centrifuged. All samples were stored at -18°C (44). Each assay included a blank and an in-house quality control of 30 mg/L. To be in the linearity range of the GC, the patient's samples were appropriately diluted with bi-distilled water.

A 6-points calibration curve was built for urine, serum and gastric liquid, respectively. Twenty µl of blank matrix (fresh urine, fresh serum or fresh gastric liquid) were spiked with appropriate volume of GHB working solutions in methanol to obtain concentrations of 2.5, 5.0, 10.0, 20.0, 40.0 and 80.0 mg/L. In order to take into consideration the "matrix effect" in the extraction procedure, pure methanol was added to the mix to systematically reach the volume of 40 µl of methanol in every sample.

Similarly, 20 µl of the samples were vortex mixed with 40 µl of methanol. 10 µl of GHB-d6 methanolic solution (40 mg/L) were added as internal standard, followed by 200 µl of H<sub>2</sub>SO<sub>4</sub> 0.1N and vortex mixed. Ethyl acetate (4 ml) was added, and the compounds were extracted for 10 minutes on a rotating mixer, followed by a centrifugation at 2500 rpm for 5 minutes. The supernatant organic layer was transferred to a clean extraction tube and evaporated to dryness under N<sub>2</sub> gentle flow at 30 °C. The extracts were derivatized with 30 µl of a BSTFA/TMCS mix (99:1, V/V) for 30 min at 70°C. After cooling, the samples were transferred in GC vials.

### Analytical conditions

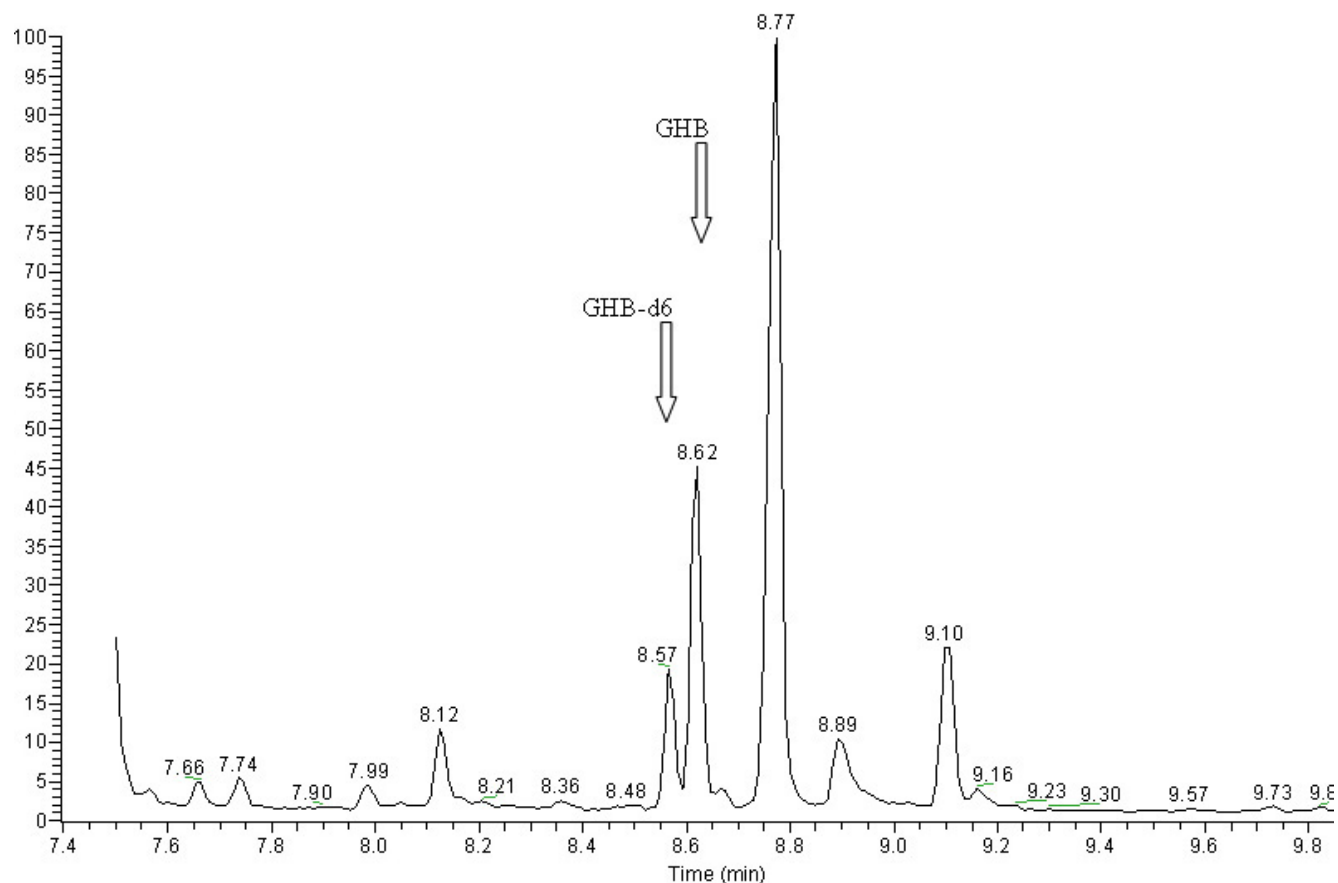
A 1 µl aliquot of the sample was automatically injected into the column, through which was applied a constant flow of 1.8 mL/min of carrier gas. Splitless mode was used for injection (Splitless time 1 min), and split vent remained opened all analysis long. The injector temperature was 230°C, and the oven temperature was initially set at 60°C, hold for 2 min, increase to 150°C at 10°C/min, then increase to 300°C at 30°C/min, and hold at 300°C for 5 min. The GC-MS transfer line temperature was set at 300 °C and the ion source at 250 °C. The classical electron impact mode at 70 eV was used for the ionization of the compounds. The electron multiplier was operated at 1370V and the

detector gain set at  $1.10^5$ . Dwell times were set at 50 ms for each ion and the total scan time was of 0.27 sec. Prior to all analyses the MS was tuned with the automated DSQ Tune programme.

Pure derivatized GHB and GHB-d6 were injected to define analytical conditions. Data were recorded in full scan mode, for the identification of GHB and GHB-d6 specific ions and to identify appropriate retention time (Figure 2). Retention times were established at 8.57 min for derivatized GHB-d6 and 8.62 for derivatized GHB. Ions selected for Single Ion Monitoring (SIM) of GHB were  $m/z$  233, 204 and 117, and GHB-d6 specific ion was  $m/z$  239 (Figure 3), as previously described (48;51) (underlined ions were used for quantitation).

## RESULTS

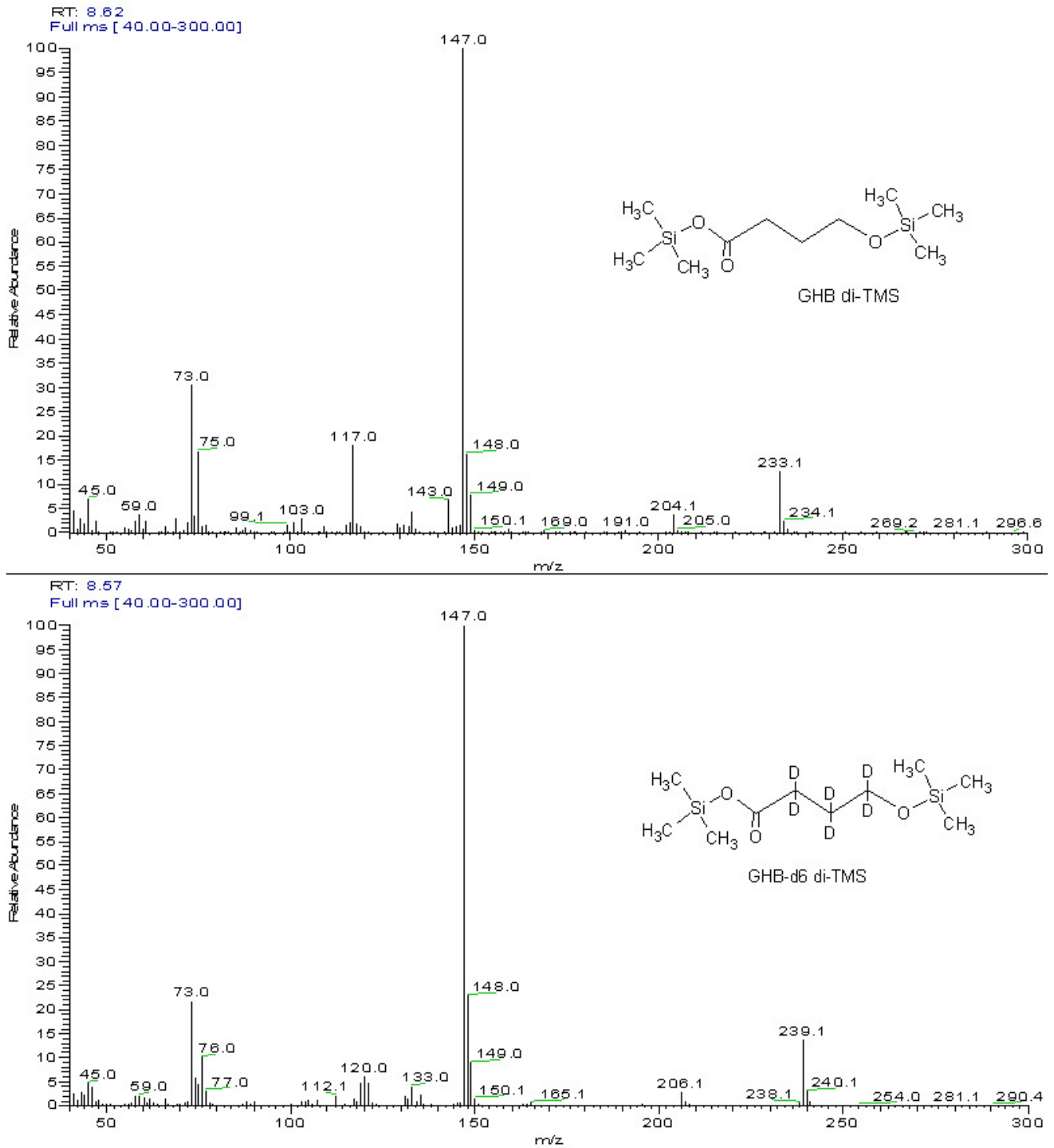
For all samples, a weighted ( $1/x^2$ ) linear regression analysis of the ratio "GHB peak area to internal standard (GHB-d6) peak area" was performed. The 6-points calibration curves were linear over the concentration range assayed (2.5 to 80 mg/L), and regression fits ( $R^2$ ) were excellent ( $R^2=0.9975$  for urine,  $R^2=0.9971$  for serum and  $R^2=0.9982$  for gastric contents). Independently of the matrix, coefficients of variation were of less than 9% and accuracy ranged between 90.1% and 104.2%. Limit of detection (LOD) was determined as 1 mg/L, and limit of quantitation (LOQ) was defined as the lowest calibrator point, i.e. 2.5 mg/L.



**Figure 2:** GC-MS profile of a derivatized GHB-spiked urine.

Retention times were established at 8.57 min for derivatized GHB-d6 (internal standard) and 8.62 for derivatized GHB. (RT 8.77 = urea; RT 9.10 = phosphate).





**Figure 3:** Mass spectra of the trimethylsilyl derivative of GHB (GHB-di-TMS) and deuterated-GHB (GHB-d6-di-TMS as internal standard). Ions selected for Single Ion Monitoring (SIM) of GHB di-TMS are m/z 233, 204 and 117, and GHB-d6-di-TMS specific ion is m/z 239.

Three-hydroxybutyrate (BHB), an endogenous beta-isomer of GHB known to have a very similar ion fragmentation pattern to GHB (52), had a retention time more than 1 min shorter than GHB, and did not interfere. Moreover, ion ratios were different between GHB and BHB.

Urine collected at admission was positive for GHB at the concentration of 3020 mg/L, a high concentration not frequently reported in the literature (Figure 4). Urine collected 6 hours after admission was still positive, at the concentration of 2324 mg/L.

In spite of the short half-life of GHB in humans (37;53), serum obtained 6 hours after admission was still positive for GHB, at the concentration of 43 mg/L.

Gastric contents collected at admission were positive for GHB at concentration of 71487 mg/L.

### DISCUSSION

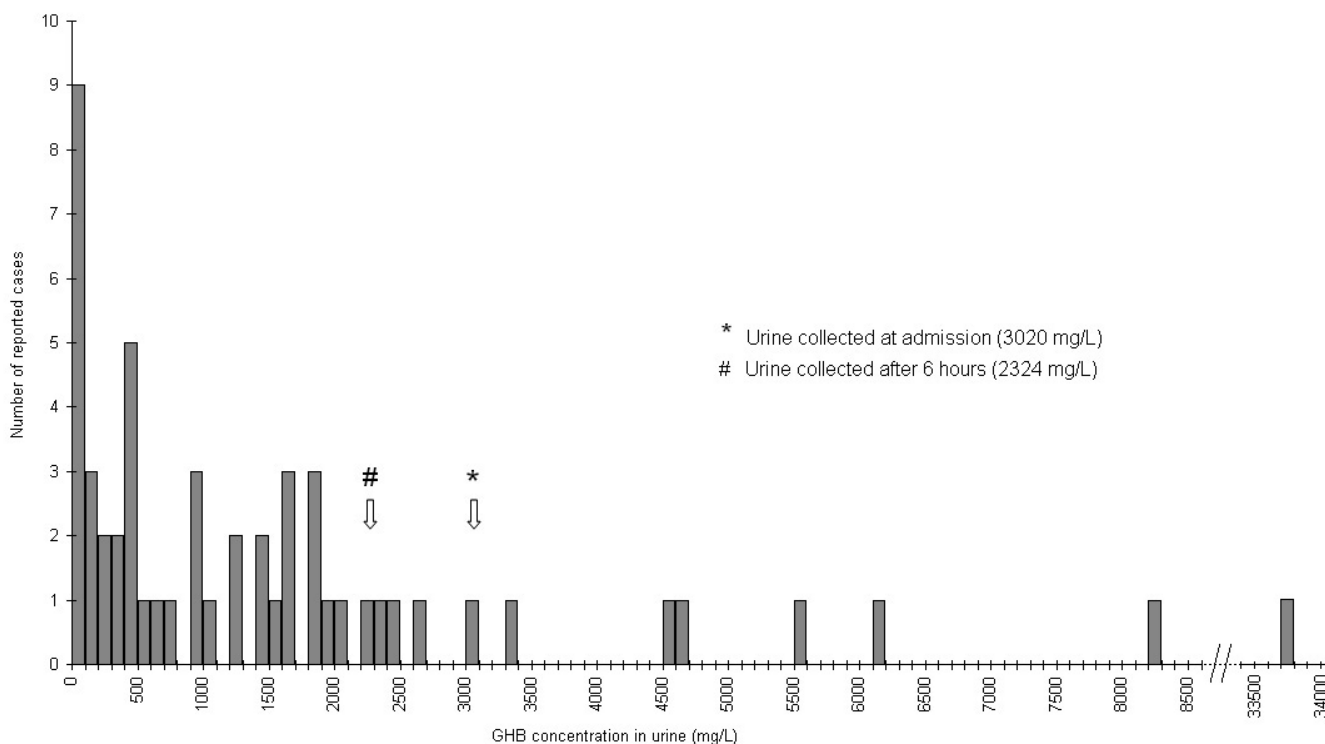
The combination of a stimulant drug (cocaine) and a depressant drug (GHB) leads to a clinical feature quite

difficult to manage for emergency physicians. The antagonism of effects between the 2 drugs, moreover associated with an alcoholic state, can explain this situation.

We found a high concentration of GHB in urine at admission (3020 mg/L), and the urine concentration was still elevated after 6 hours, at the concentration of 2324 mg/L. Despite the short half-life of GHB in body, serum was still positive after 6 hours, at the concentration of 43 mg/L. The concentration measured in gastric contents (71487 mg/L) is comparable to the value reported by Kintz et al (70800 mg/L) (54).

### CONCLUSION

This paper reports on a case of an acute intoxication with GHB, together with cocaine. This is, to our knowledge, one of the most serious GHB overdose reported in Belgium, after Louagie et al in 1997 (55) and Segers et al in 2002 (56). Moreover, GHB concentration in urine is a high concentration not frequently reported in the medical literature (Figure 4).



**Figure 4:** Patient's GHB concentration in urine, in comparison with data reported in the literature, i.e. 53 cases > 10 mg/L (48;54;56-67). Fifty-one per cent of reported cases are below 1000 mg/L (27 cases on 53), 75% of reported cases are below 2000 mg/L (40 cases on 53), and 85% of reported cases are below 3000 mg/L (45 cases on 53). One extremely high concentration of 33727 mg/L has been reported by Kintz et al (54).

What is quite interesting is that the clinical presentation was not typical of cocaine intoxication, even if cocaine and benzoylecgonine have been ingested.

The clinical symptoms of cocaine intoxication were diminished by GHB consumption, and the cerebral scan was modified. Attention must thus be paid to acute intoxications with surprising clinical symptoms, and GHB has probably to be added to the preliminary toxicological screening.

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## REFERENCES

- Roth RH, Giarmann NJ. Natural occurrence of gamma-hydroxybutyrate in mammalian brain. *Biochem Pharmacol* 1970; 19: 1087-93.
- Snead OC. Evidence for a G protein-coupled gamma-hydroxybutyric acid receptor. *J Neurochem* 2000; 75(5): 1986-96.
- Lingenhoehl K, Brom R, Heid J, et al. Gamma-hydroxybutyrate is a weak agonist at recombinant GABA(B) receptors. *Neuropharmacology* 1999; 38(11): 1667-73.
- Carai MAM, Colombo G, Brunetti G, et al. Role of GABA(B) receptors in the sedative/hypnotic effect of gamma-hydroxybutyric acid. *Eur J Pharmacol* 2001; 428(3): 315-21.
- Hornfeldt CS, Lothridge K, Upshaw Downs JC. Forensic science update: gamma-hydroxybutyrate (GHB). *Forensic Sci Comm* 2002; 4(1): 1-10.
- Drasbek KR, Christensen J, Jensen K. Gamma-hydroxybutyrate - a drug of abuse. *Acta Neurol Scand* 2006; 114(3): 145-56.
- Schwartz RH, Milteer R, LeBeau MA. Drug-facilitated sexual assault ("date rape"). *South Med J* 2000; 93(6): 558-61.
- Elian AA. A novel method for GHB detection in urine and its application in drug-facilitated sexual assaults. *Forensic Sci Int* 2000; 109(3): 183-7.
- Saytzeff A. Naturally occurring metabolite of GABA thought to function as a neurotransmitter or neuromodulator. The highest conc in humans is found in fetal cerebellum and adult hypothalamus. *Ann* 1874; 171: 258.
- Laborit H, Jouany JM, Gerard J, Fabiani F. Summary of an experimental and clinical study on a metabolic substrate with inhibitory central action: sodium 4-hydroxybutyrate. *Presse Med* 1960; 68: 1867-9.
- Hoes MJ, Vree TB, Guelen PJ. Gamma-hydroxybutyric acid as hypnotic. Clinical and pharmacokinetic evaluation of gamma-hydroxybutyric acid as hypnotic in man. *Encephale* 1980; 6(1): 93-9.
- Addolorato G, Caputo F, Capristo E, Stefanini GF, Gasbarrini G. Gamma-hydroxybutyric acid - Efficacy, potential abuse, and dependence in the treatment of alcohol addiction. *Alcohol* 2000; 20(3): 217-22.
- Takahara J, Yunoki S, Yakushiji W, Yamauchi J, Yamane Y. Stimulatory effects of gamma-hydroxybutyric acid on growth hormone and prolactin release in humans. *J Clin Endocrinol. Metab* 1977; 44(5): 1014-7.
- Rigamonti AE, Muller EE. Gamma-hydroxybutyric acid and growth hormone secretion studies in rats and dogs. *Alcohol* 2000; 20(3): 293-304.
- Krawczeniuk A. The occurrence of gamma-hydroxybutyric acid (GHB) in a steroid seizure. *Microgram* 1993; 26(7): 160-6.
- Rodgers J, Ashton CH, Gilvarry E, Young AH. Liquid ecstasy: a new kid on the dance floor. *Br J Psychiatry* 2004; 184: 104-6.
- Van Sassenbroeck DK, Calle PA, Rousseau FM, et al. Medical problems related to recreational drug use at nocturnal dance parties. *Eur J Emerg Med* 2003; 10(4): 302-8.
- Drug Abuse Warning Network. Trends in Drug-Related Emergency Department Visits, 1994-2002 At a Glance. <https://dawninfo.samhsa.gov>, 2003. Last accessed 10-3-2007.
- Anderson IB, Kim SY, Dyer JE, et al. Trends in gamma-hydroxybutyrate (GHB) and related drug intoxication: 1999 to 2003. *Ann Emerg Med* 2006; 47(2): 177-83.
- Drug Abuse Warning Network. Drug Abuse Warning Network, 2004: National Estimates of Drug-Related Emergency Department Visits. <https://dawninfo.samhsa.gov>, 2004. Last accessed 10-3-2007.
- Food and Drug Administration. FDA warns about products containing gamma-butyrolactone or GBL and asks companies to issue a recall. FDA Talk Paper. <http://www.fda.gov/bbs/topics/answers/ans00937.html>, 1999. Last accessed 15-2-2007.
- European Medicines Agency (EMA). Xyrem European Public Assessment Report. <http://www.emea.europa.eu/humandocs/Humans/EPAR/xyrem/xyrem.htm>, 2007. Last accessed 8-6-2007.
- Mamelak M, Scharf MB, Woods M. Treatment of narcolepsy with gamma-hydroxybutyrate. A review of clinical and sleep laboratory findings. *Sleep* 1986; 9: 285-9.
- Scharf MB, Lai AA, Branigan B, Stover R, Berkowitz DB. Pharmacokinetics of gamma-hydroxybutyrate (GHB) in narcoleptic patients. *Sleep* 1998; 21(5): 507-14.
- European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). **Annual report 2004: the state of the drugs problem in Europe**. Luxembourg: Office for Official Publications of the European Communities. 2004: 1-113.
- European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). **Annual report 2005: the state of the drugs problem in Europe**. Luxembourg: Office for Official Publications of the European Communities. 2005: 1-87.
- European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). **Annual report 2006: the state of the drugs problem in Europe**. Luxembourg: Office for Official Publications of the European Communities. 2006: 1-96.
- Epidemiology Unit - Scientific Institute of Public Health. Belgian National Report on Drugs 2006. Sleiman S, Roelands M, (eds). Brussels: Scientific Institute of Public Health. 2006: 1-151.
- Andersen MB, Netterstrom B. Unconsciousness after ingestion of nail varnish. *Ugeskr Laeg* 1992; 154(44): 3064.
- Dyer JE. Gamma-Hydroxybutyrate (GHB). In: Olson KR, ed. *Poisoning & Drug Overdose*. New-York, NY: McGraw-Hill/Apleton & Lange. 2004: 210-3.



31. Thai D, Dyer JE, Jacob P, Haller CA. Clinical pharmacology of 1,4-butanediol and gamma-hydroxybutyrate after oral 1,4-butanediol administration to healthy volunteers. *Clin Pharmacol Ther* 2007; 81(2): 178-84.
32. Lettieri J, Fung HL. Improved pharmacological activity via pro-drug modification: comparative pharmacokinetics of sodium gamma-hydroxybutyrate and gamma-butyrolactone. *Res Commun Chem Pathol Pharmacol* 1978; 22(1): 107-18.
33. Dupont P, Thornton J. Near-fatal gamma-butyrolactone intoxication - first report in the UK. *Hum Exp Toxicol* 2001; 20(1): 19-22.
34. Becker J. A case of gamma-butyrolactone overdose. *Clin Toxicol* 2000; 38(2): 227.
35. The Vaults of Erowid. GHB. <http://www.erowid.org/chemicals/ghb/>, 2007. Last accessed 15-3-2007.
36. Thai D, Dyer JE, Benowitz NL, Haller CA. Gamma-hydroxybutyrate and ethanol effects and interactions in humans. *J Clin Psychopharmacol* 2006; 26(5): 524-9.
37. Brenneisen R, Elsohly MA, Murphy TP, et al. Pharmacokinetics and excretion of gamma-hydroxybutyrate (GHB) in healthy subjects. *J Anal Toxicol* 2004; 28(8): 625-30.
38. Abanades S, Farre M, Segura M, et al. Gamma-hydroxybutyrate (GHB) in humans: pharmacodynamics and pharmacokinetics. *Ann NY Acad Sci* 2006; 1074: 559-76.
39. Moriya F, Hashimoto Y. Endogenous gamma-hydroxybutyric acid levels in postmortem blood. *Leg Med (Tokyo)* 2004; 6(1): 47-51.
40. Elliott SP, Lowe P, Symonds A. The possible influence of microorganisms and putrefaction in the production of GHB in post-mortem biological fluid. *Forensic Sci. Int* 2004; 139(2-3): 183-90.
41. Moriya F, Hashimoto Y. Site-dependent production of gamma-hydroxybutyric acid in the early postmortem period. *Forensic Sci Int* 2005; 148(2-3): 139-42.
42. Kerrigan S. In vitro production of gamma-hydroxybutyrate in antemortem urine samples. *J Anal Toxicol* 2002; 26(8): 571-4.
43. Elliott SP. Further evidence for the presence of GHB in postmortem biological fluid: implications for the interpretation of findings. *J Anal Toxicol* 2004; 28(1): 20-6.
44. LeBeau MA, Miller ML, Levine B. Effect of storage temperature on endogenous GHB levels in urine. *Forensic Sci Int* 2001; 119(2): 161-7.
45. Balikova M. Gamma-hydroxybutyrate (GHB) misuse or abuse and interpretation of toxicological findings. *Soud Lek* 2005; 50(1): 7-9.
46. Chin RL, Sporer KA, Cullison B, Dyer JE, Wu TD. Clinical course of gamma-hydroxybutyrate overdose. *Ann Emerg Med* 1998; 31(6): 716-22.
47. Dyer JE, Roth B, Hyma BA. Gamma-hydroxybutyrate withdrawal syndrome. *Ann Emerg Med* 2001; 37(2): 147-53.
48. Couper FJ, Logan BK. Determination of gamma-hydroxybutyrate (GHB) in biological specimens by gas chromatography-mass spectrometry. *J Anal Toxicol* 2000; 24(1): 1-7.
49. Elian AA. GC-MS determination of gamma-hydroxybutyric acid (GHB) in blood. *Forensic Sci. In.* 2001; 122(1): 43-7.
50. Villain M, Cirimele V, Ludes B, Kintz P. Ultra-rapid procedure to test for gamma-hydroxybutyric acid in blood and urine by gas chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2003; 792(1): 83-7.
51. Duer WC, Byers KL, Martin JV. Application of a convenient extraction procedure to analyze gamma-hydroxybutyric acid in fatalities involving gamma-hydroxybutyric acid, gamma-butyrolactone, and 1,4-butanediol. *J Anal Toxicol* 2001; 25(7): 576-82.
52. Shima N, Miki A, Kamata T, Katagi M, Tsuchihashi H. Urinary endogenous concentrations of GHB and its isomers in healthy humans and diabetics. *Forensic Sci Int* 2005; 149(2-3): 171-9.
53. Abanades S, Farre M, Segura M, et al. Gamma-hydroxybutyrate (GHB) in humans: pharmacodynamics and pharmacokinetics. *Ann NY Acad Sci* 2006; 1074: 559-76.
54. Kintz P, Villain M, Pelissier AL, Cirimele V, Leonetti G. Unusually high concentrations in a fatal GHB case. *J Anal Toxicol* 2005; 29(6): 582-5.
55. Louagie HK, Verstraete AG, De Soete CJ, Baetens DG, Calle PA. A sudden awakening from a near coma after combined intake of gamma-hydroxybutyric acid (GHB) and ethanol. *J Toxicol Clin Toxicol* 1997; 35(6): 591-4.
56. Segers S, Van Sassenbroeck DK, Verstraete AG, Martens P. Gamma-hydroxybuterzuur (GHB): van een anesthesieproduct naar illegale drug. *Tijdschr Geneeskd* 2002; 58(1): 49-54.
57. Mazarr-Proo S, Kerrigan S. Distribution of GHB in tissues and fluids following a fatal overdose. *J Anal Toxicol* 2005; 29(5): 398-400.
58. Stephens BG, Baselt RC. Driving under the influence of GHB? *J Anal Toxicol* 1994; 18(6): 357-8.
59. Caldicott DG, Chow FY, Burns BJ, Felgate PD, Byard RW. Fatalities associated with the use of gamma-hydroxybutyrate and its analogues in Australasia. *Med. J. Aust.* 2004; 181(6): 310-3.
60. Ferrara SD, Tedeschi L, Frison G, Rossi A. Fatality due to gamma-hydroxybutyric acid (GHB) and heroin intoxication. *J Forensic Sci* 1995; 40(3): 501-4.
61. Dyer JE, Isaacs SM, Keller KH. Gamma-hydroxybutyrate (GHB)-induced coma with serum and urine drugs levels. *Vet Hum Toxicol* 1994; 36: 339.
62. Sporer KA, Chin RL, Dyer JE, Lamb R. Gamma-hydroxybutyrate serum levels and clinical syndrome after severe overdose. *Ann Emerg Med* 2003; 42(1): 3-8.
63. Elliott SP. Nonfatal instances of intoxication with gamma-hydroxybutyrate in the United Kingdom. *Ther Drug Monit* 2004; 26(4): 432-40.
64. Couper FJ, Thatcher JE, Logan BK. Suspected GHB overdoses in the emergency department. *J Anal Toxicol* 2004; 28(6): 481-4.
65. Deveaux M, Renet S, Renet V, et al. Use of gamma-hydroxybutyric acid (GHB) at rave parties and in date rape in France: myth of reality? *Acta Clin. Belg Suppl* 2002; 1(1): 37-40.
66. WHO's Expert Committee on Drug Dependence (ECDD). Pre-review of gamma-hydroxybutyric acid (GHB). 2006; WHO ECDD 34th Meeting: 1-31.
67. McCusker RR, Paget-Wilkes H, Chronister CW, Goldberger BA. Analysis of gamma-hydroxybutyrate (GHB) in urine by gas chromatography-mass spectrometry. *J Anal Toxicol* 1999; 23(5): 301-5.