ANALYSIS OF -HYDROXYBUTYRATE (GHB) AND -BUTYROLACTONE (GBL) IN LIQUIDS PERFORMED AT NATIONAL LABORATORY OF FORENSIC SCIENCE (SKL), SWEDEN

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ABSTRACT: GHB is classified as a narcotic drug in Sweden but GBL is not. GHB is totally and fast transformed to GBL at high temperatures and at a low pH. The transformation proceeds more gradually at a moderate acidic pH. Several methods is used at the forensic laboratory in Sweden (SKL) for the estimation of GHB and GBL in different seizures. Analytical methods used for the determination of GHB and GBL is both screening and verification analysis with GC-FID, GC-MS (with or without derivatisation with silylation (TMS) reagents), FT-IR or HPLC techniques.

KEY WORDS: GHB; GBL; 1,4-butanediol.

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INTRODUCTION

GHB is an endogenous substance found in most mammalian tissues including the brain. The substance has been used clinically since 1960 as an anesthetic and hypnotic agent. The drug has a legal use in some European countries for treatment of insomnia, narcolepsy, and in the treatment of alcohol withdrawal syndrome. Despite the fact that GHB is an endogenous substance it is far from harmless. The substance has both exciting and depressing effects. Its central nervous system depressant effects appear to be potentiated by other psychoactive drugs, particularly alcohol.

The great danger with GHB is that the difference between the desired dose and an overdose is so small.

At least five deaths have been reported in Sweden after GHB abuse, and many young people have been rescued in hospitals after respiratory depression. No clinically effective antidote is so far available.

Unfortunately GHB is easy to manufacture. It is illicitly manufactured by adding sodium hydroxide (NaOH) to -butyrolactone, an organic solvent that is easy to obtain.

ABUSE

GHB is used as an alternative to ecstasy and speed at techno parties and it is also used as an alternative to steroids by bodybuilders due to the induction of growth hormone release. However, the drug is also used by young people for the euphoric effect and by amphetamine abusers to "turn off" and get a relaxing sleep.

GHB is often abused by ingestion of a "capful" of liquid (5–10 ml corresponding to 1–6 g of the salt) and the desired effects appear within 15–60 min.

In low doses, effects of GHB can be euphoric, higher doses may lead to sleepiness and high doses can result in dangerous conditions as unconsciousness and cessation of breathing.

METABOLISM

The function of GHB in peripheral tissue is unknown but in the brain and neuronal tissue it is believed to be a neuromodulator. It temporarily inhibits the release of dopamin.

GHB stimulates both growth hormone and prolactin release. It is both a metabolite and precursor to the neurotransmitter GABA (Figure 1).

PRECURSORS, RELATED SUBSTANCES, PRODRUGS

Both -butyrolactone (GBL) and 1,4-butandiol are products used in big quantities by the chemical industry as solvents and/or diluters in various products and processes.

Enzymes in the blood and liver in humans rapidly convert GBL to GHB. GBL has a half-live of less than 1 minute in this conversion. It is less polar then GHB and therefore better absorbed after oral administration than GHB. In fact, GBL is converted to GHB so rapidly after absorption that the bioavailability of GHB is actually greater after administration of GBL than after administration of an equivalent dose of GHB.

As for GBL, there is a rapid conversion of 1,4-butandiol to GHB in humans. After intravenous administration of 1,4-butandiol, GHB levels peaked and began to decay within 2 min.

SEIZURES AND STATISTICS

The seizures of GHB in Sweden have increased every year since 1995 (Figure 2) and a rising tendency is seen even in the year of 2000 despite the

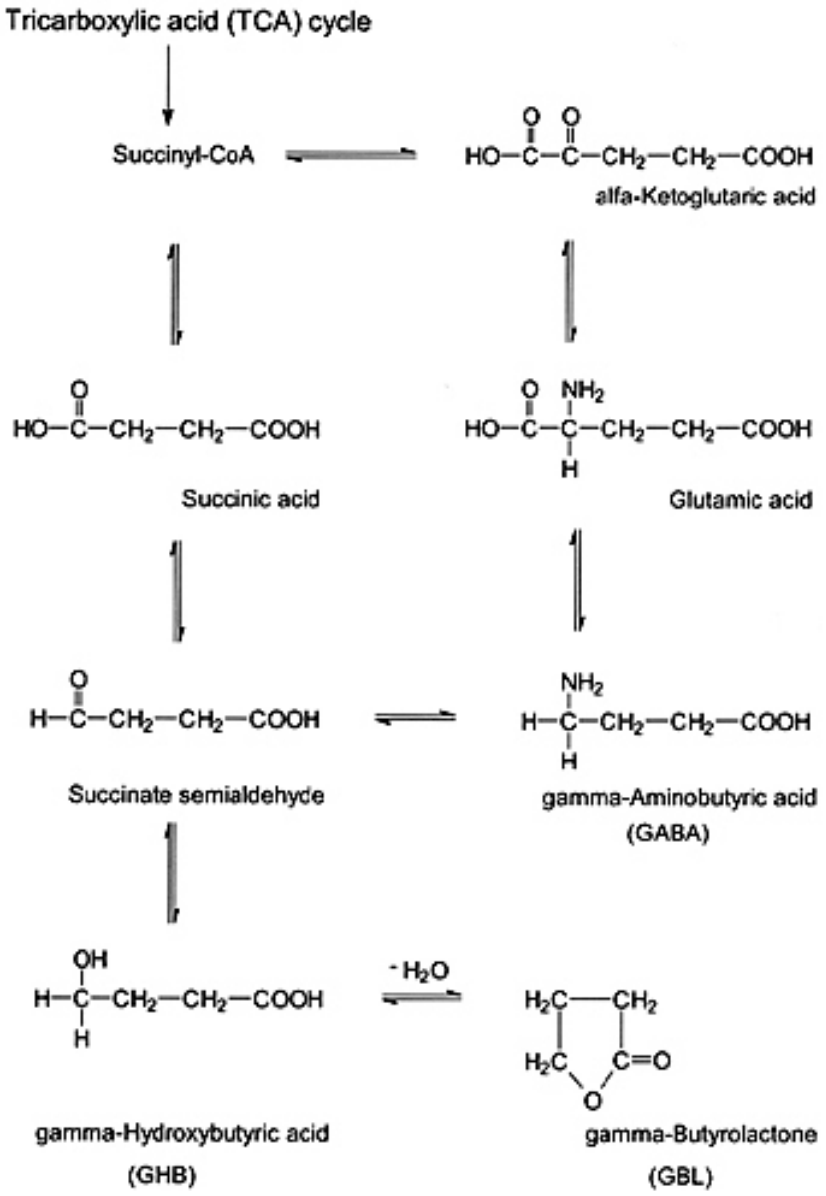


Fig. 1. Metabolic pathway for GHB.

fact that (or because?) GHB was classified as a narcotic drug in the 1st of February 2000.

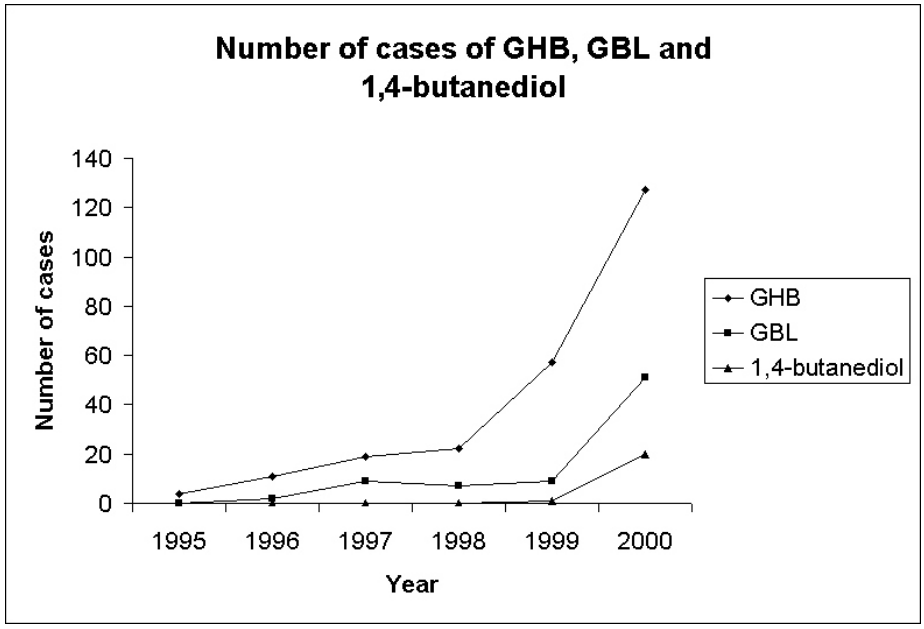


Fig. 2. The seizures of GHB, GBL and 1,4-butanediol in Sweden since 1995.

During the year of 2000, a screening method was used in the qualitative analysis of the samples suspected to be GHB/GBL (Figures 3–5). Approximately 30% were tentatively identified as GHB/GBL. In the following verification analysis about 90% of these samples were identified as GHB/GBL.

QUALITATIVE ANALYSIS

Because of the conversion of GHB to GBL in the presence of heat, a direct analysis using gas chromatography (GC) is not possible. The problem can be circumvented by an extraction that separates the compounds prior to the analysis or by a derivatisation with a suitable reagent that prevents the conversion.

In the lack of hyphenated techniques like LC-MS and CE-MS, we have developed several methods for the determination of GHB and GBL (Figure 6). A simple GC-FID method, that demands no cleaning up procedure, is used for qualitative analysis. The method is the same that currently is used in the analysis of illicitly manufactured alcohol.

Fig. 3. A kit for "measurement of pH" included 3 bottles with 360 ml of GBL, 3 bottles with 180 g sodium hydroxide and pH-sticks. The package was sent from Canada and it was confiscated by the Swedish customs.

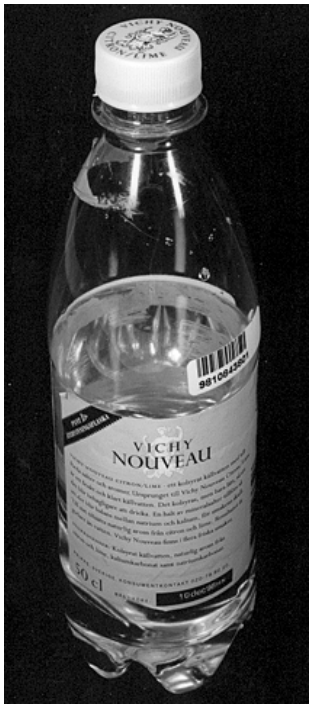


Fig. 4. A "normal" seizure of liquid GHB.

Fig. 5. A bottle with very alkaline GHB solution (pH 12).

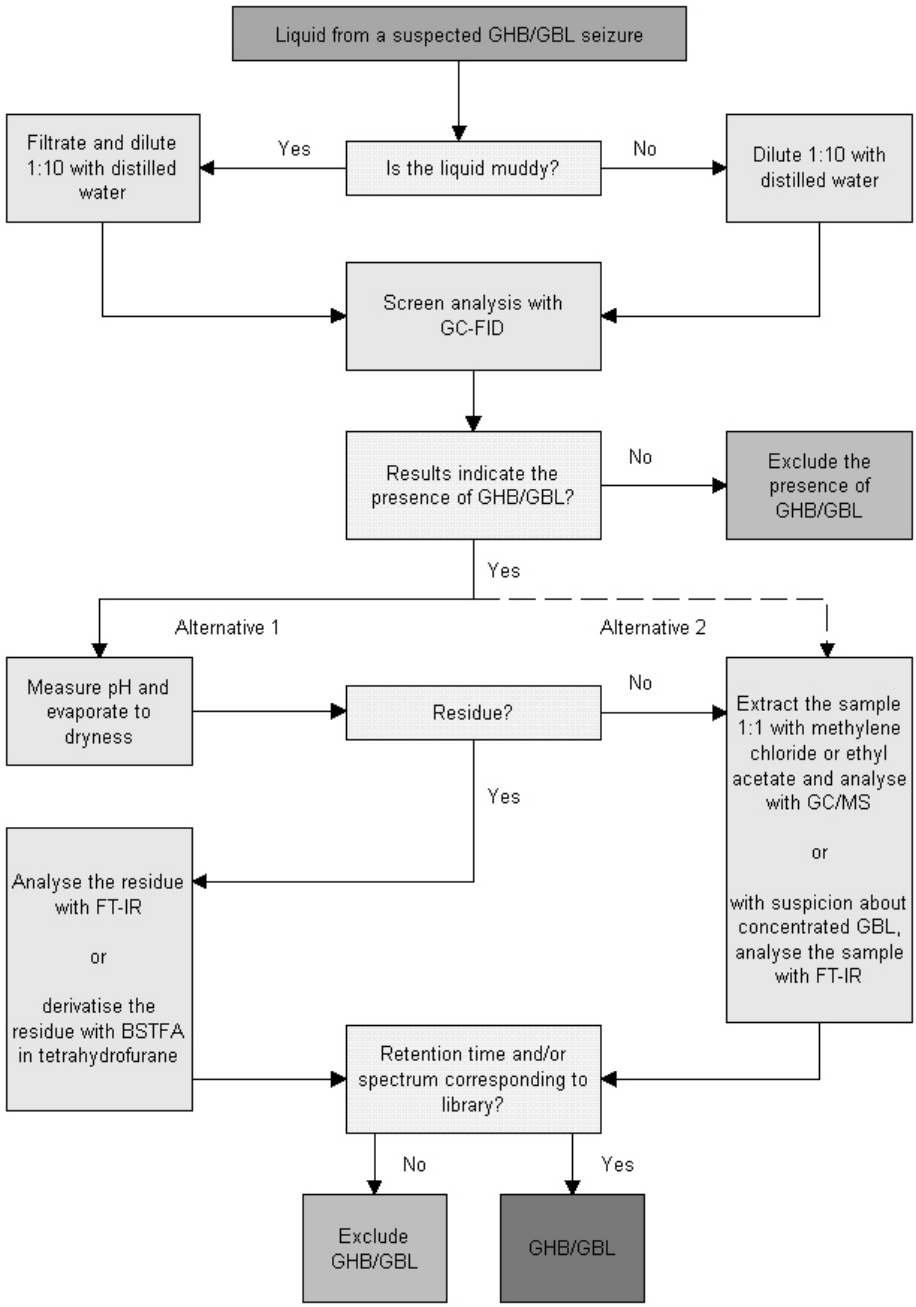


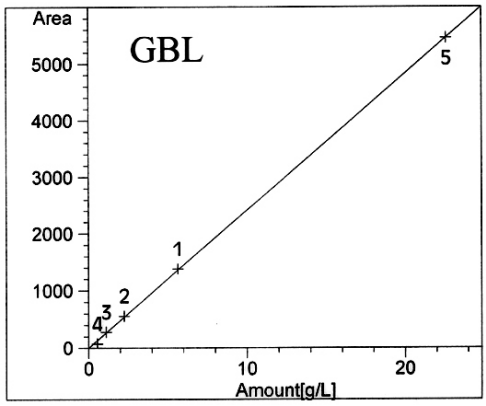
Fig. 6. Flow chart for analysis of "normal" GHB/GBL seizures.

When the screening analysis gives a positive result, pH is measured to estimate whether GHB or GBL is the dominating substance in the sample. Extraction combined with evaporation to dryness is performed and the identification is made by analysis with FT-IR or GC-MS.

Samples with a limit amount of material is derivatised with BSTFA –*N, O*-bis(trimethylsilyl)trifluoroacetamide – before analysis with GC-MS.

QUANTITATIVE ANALYSIS

A quantitative method for determination of GHB/GBL has been developed. The analysis of the samples is performed on a Hewlett Packard 1100 HPLC instrument. Standardcurves for GHB and GBL is linear (Figures 7, 8). No cleaning up procedure is needed. The retention of GHB and GBL is rather poor, and the method needs improvement of the chromatographic conditions.

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GBL at exp. RT: 0.696
DAD1 B, Sig=215,4 Ref=350,50
Correlation:              0.99993
Residual Std. Dev.:      33.33278
Formula: y = mx
      m:      241.53943
      x: Amount[g/L]
      y: Area
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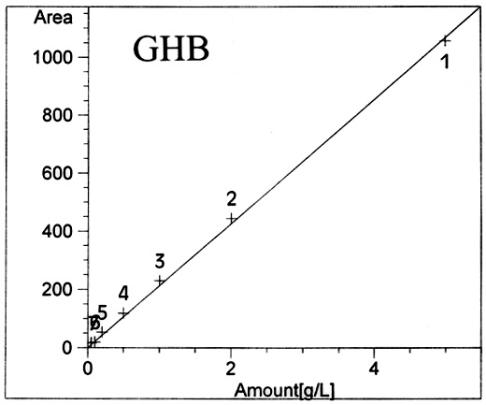
Fig. 7. Standardcurve for GHB.

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GHB at exp. RT: 0.401
DAD1 B, Sig=215,4 Ref=350,50
Correlation:              0.99966
Residual Std. Dev.:      12.46347
Formula: y = mx
      m:      213.38266
      x: Amount[g/L]
      y: Area
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Fig. 8. Standardcurve for GHB.

The content of GHB and GBL has been determined in 12 seizures (Figure 9) and the concentrations were found to be in a wide range (39–600 g/l).

This situation may partly explain the difficulty for the abusers to get the desirable dosages. Due to the higher bioavailability to GBL as compared to GHB, differences in proportions of GHB and GBL in the seizures are likely to be a problem for the abusers.

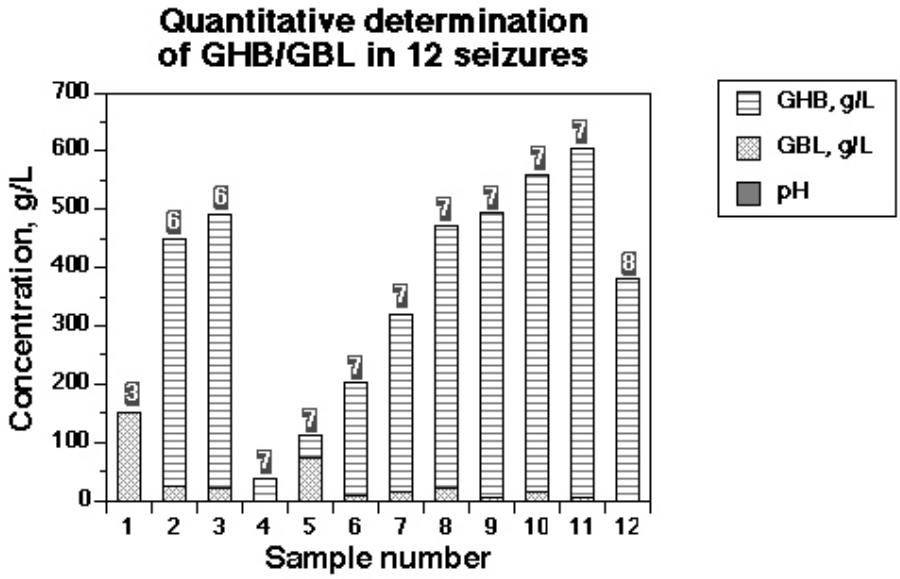


Fig. 9. The content of GHB and GBL determined in 12 seizures.

INSTRUMENTATION

GC-FID analysis was performed on a Hewlett Packard 6890 gas chromatograph (Figure 10).

Column:	Two columns in series
First:	Hewlett Packard-1 (cross-linked methylsiloxane), 30 m x 0,25 mm x 0,25μm
Second:	Hewlett Packard-INNOWAX (cross-linked polyethylene glycol), 30 m x 0,25 mm x 0,25 μm
Carrier gas:	Helium
Flow rate:	30 cm/s
Split:	15:1
Injection volume:	1 μl

The operating conditions for the analysis were:

Injection port:	25 C
Detector:	275 C
Initial oven temperature:	40 C, hold 5 min
Temperature increase:	Ramp 1: 8 C/min to 80 C Ramp 2: 30 C/min to 230 C, hold 2 min

Total time for analysis is 17 min.

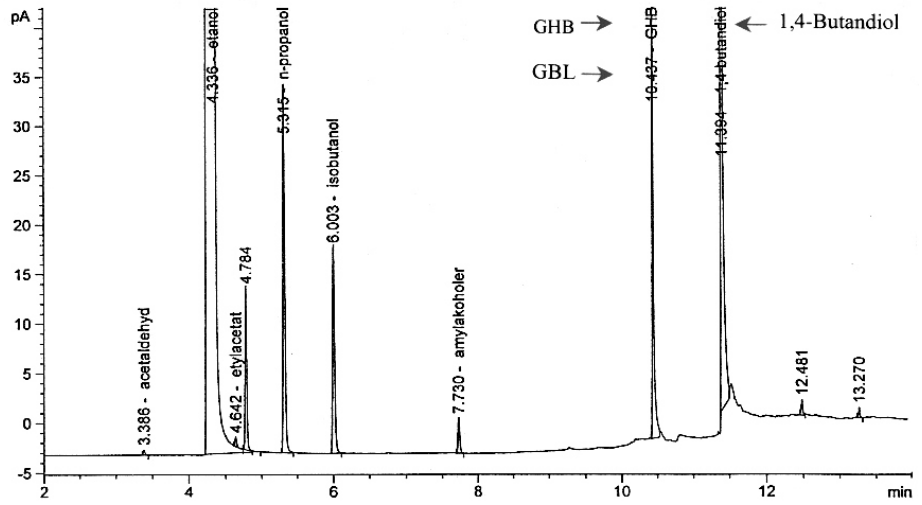


Fig. 10. Gas chromatogram of GHB, GBL and 1,4-butanediol.

GC-MSD analysis was performed on a Hewlett Packard 5890 gas chromatograph (Figures 11–12).

Column:	Hewlett Packard 5 MS, 30 m x 0,25 mm x 0,25µm 5% phenyl methyl silicone cross-linked phase
Carrier gas:	Helium
Flow rate:	1 ml/min
Split:	Splitless
Injection volume:	1 µl

The operating conditions for the analysis were:

Injection port:	250 C
Detector:	280 C
Initial oven temperature:	50 C, hold 10 min
Temperature increase:	40 C/min to 280 C, hold 5 min

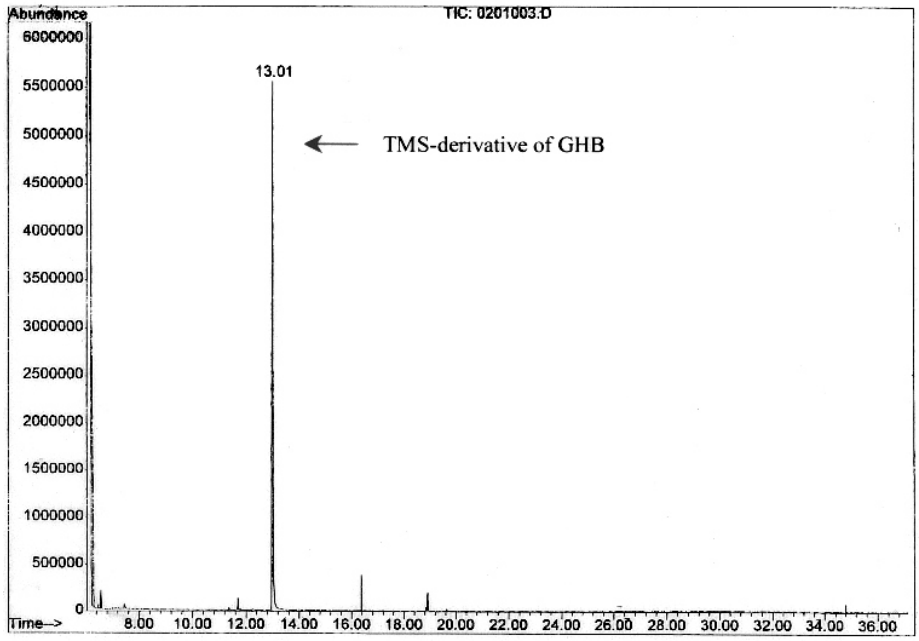


Fig. 11. Gas chromatogram for the TMS derivative of GHB.

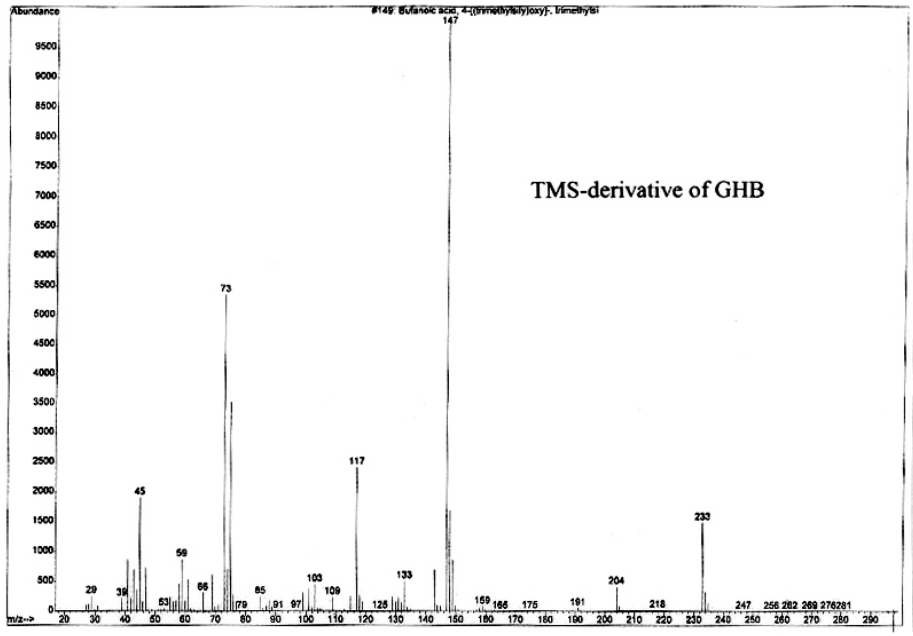


Fig. 12. GC-MS spectra for the TMS derivative of GHB.

HPLC analysis was performed on a Hewlett Packard 1100 with automatic injector Hewlett Packard 1050 (Figure 13).

Column:	Hypersil BDS C18 100 mm x 3 mm x 5µm
Column temp:	20 C
Mobile phase:	Phosphate buffer/EDTA pH 5.9 and acetonitrile
Detector:	Diode array detector

The operating conditions for the analysis were:

Gradient:	Isocratic, phosphatebuffer 99%, acetonitrile 1%
Flow rate:	1 ml/min
Injection volume:	20 µl
Wavelength:	215 nm

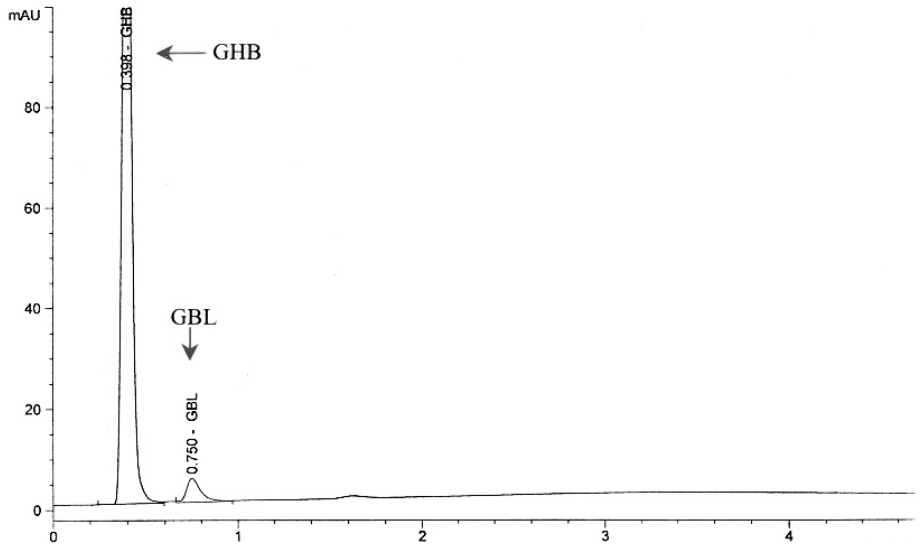


Fig. 13. HPLC chromatogram of GHB and GBL.

FT-IR analysis was performed on a Perkin-Elmer FT-IR spectrometer Spectrum 2000 using a Golden-Gate unit for sample application (Figures 14–15).

CE analysis was performed on a Hewlett Packard ^{3D}CE instrument equipped with a diode array detector (Figure 16).

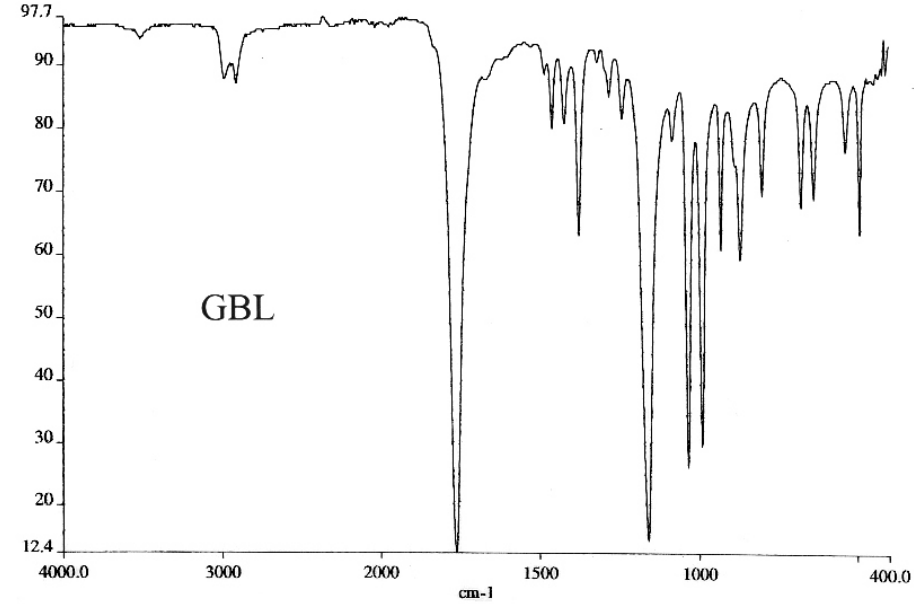


Fig. 14. FT-IR spectrum of GBL.

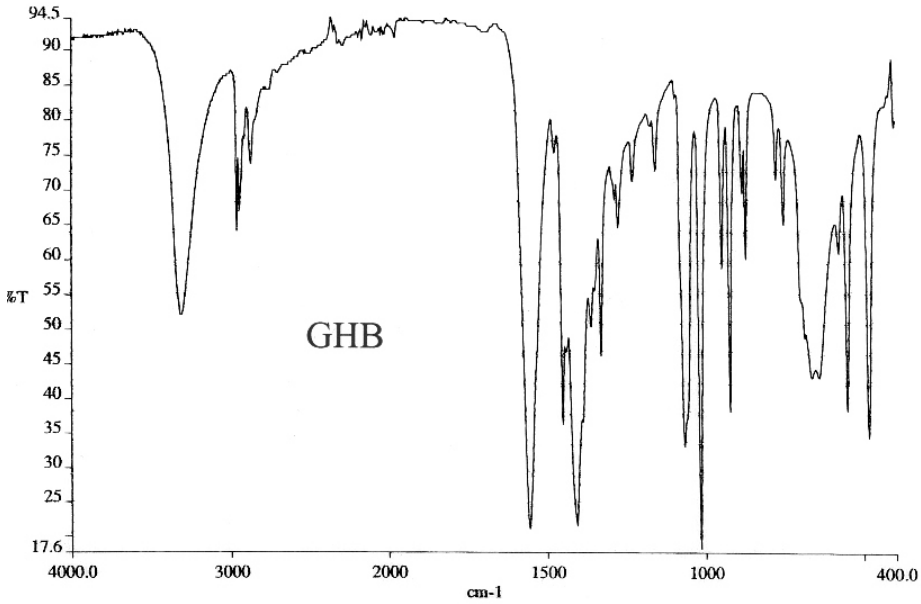
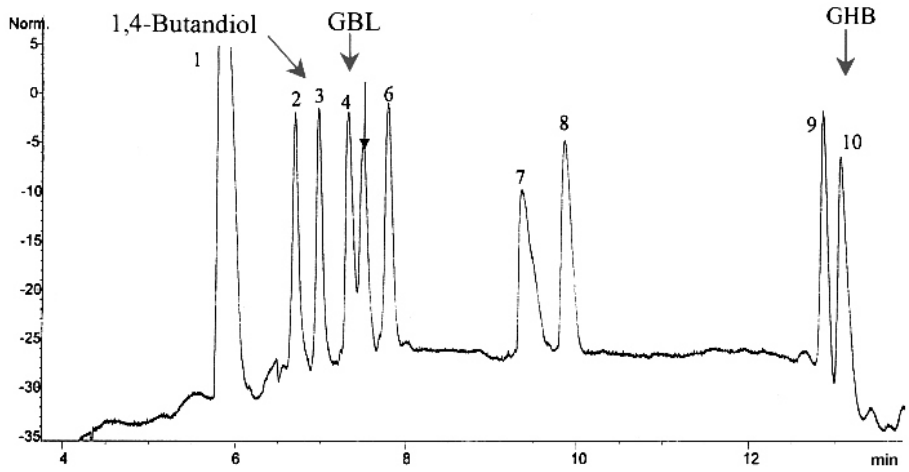


Fig. 15. FT-IR spectrum of GHB.



Peaks: (1) Water, (2) Methanol, (3) 1,4-butanediol, (4) GBL, (5) 2-propanol, (6) 1-propanol, (7) Ethyl acetate, (8) 1-butanol, (9) Valeric acid, (10) GHB

Fig. 16. CE chromatogram of GHB, GBL and 1,4-butanediol.