

DETERMINATION OF GAMMA-HYDROXYBUTYRATE (GHB) BY HEADSPACE-GC/MS (FID) IN FORENSIC SAMPLES

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ABSTRACT: A fast, simple, and selective method for the determination of gamma-hydroxybutyrate (GHB) in whole blood and urine is developed. GHB is converted to its lactonic form gamma-butyrolactone (GBL) under acidic conditions and high temperatures. The analysis is done by headspace GC with a flame ionisation detector or coupled to a mass spectrometer. The detection limit of GHB is low ppm. The method is linear using FID or MS over a range of several factors over 3 orders of magnitude in whole blood and urine. The reproducibility is below 10%.

KEY WORDS: GHB; GC/MS; GC.

*Problems of Forensic Sciences, vol. XLIII, 2000, 126–130
Received 9 September 1999; accepted 16 May 2000*

INTRODUCTION

GHB is of interest for forensic chemists due to the compounds illegal use, where several deaths have been observed. GHB is used by bodybuilders/athletes, as new hot club drug (“fantasy”) and implicated in sexual assault cases (“easy lay”) [3, 4].

Most published methods involve high amounts of organic solvent and use of derivatization [2, 4, 5]. Here is a simple, fast, and selective method be presented.

MATERIALS AND METHODS

Sample preparation

0.5 g NaHSO₄ was added to 1 ml blood or urine in a headspace vial sealed with PTFE-coated silicone rubber septa.

Chromatographic conditions

Method development was done using PE Autosystem GC equipped with a HS 40, a CPSil 19 CB column (50 m, 0.32 mm, 1.2 μm) and a flame ionisation detector. The HS heating conditions was: thermostating time 20 min at 125°C, pressurizing time 4 min.,

injection time 0.5 min, withdrawal time 0.2 min, jet needle at 135°C, transfer line at 140°C. A PE cryofocusing accessory was used with pre-cryotime of 2 min. The GC settings were: injector temp. 140°C, detector temp. 220°C, oven temp. 50°C in 5 min then 15°/min to 200°C held for 5 min, carrier gas (nitrogen) 1 ml/min. Turbochrom Navigator was used for data handling.

Verification was done using PE Autosystem XL GC equipped with QMASS 910 mass spectrometer. The MS is operated in full scan mode (TIC). GBL m/z 86, 56 and 42.

RESULTS AND DISCUSSION

GHB is converted to its lactonic form gammabutyrolactone (GBL) under acidic conditions and high temperatures (Figure 1). This reaction takes place in a headspace

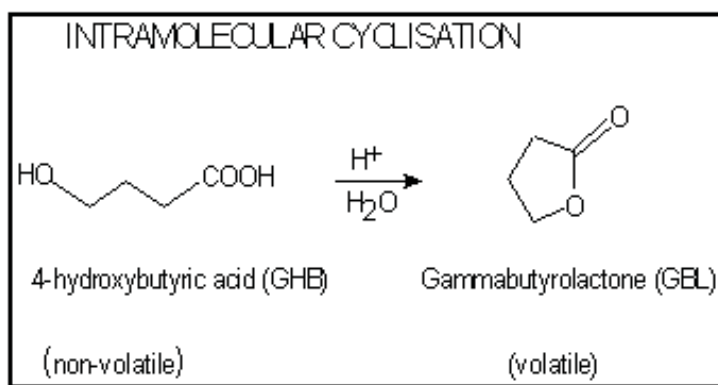


Fig. 1. Reaction of GHB under acidic conditions at 125°C.

vial during the thermostating of the sample in the autosampler HS storing the semi-volatile product GBL.

Sampling conditions for GHB/GBL

A sample temperature of 125°C was chosen due to problems with the signal/FID: At higher temperatures (> 130°C) the flame vent out (Figure 2).

At this sample temperature no significant difference in equilibrium time between 15 and 60 min was observed and therefore 20 min was used. Furthermore cryofocusing resulted in a sensitivity increase by a factor of 3.

Conversion of GBH to GBL

The highest response was achieved under saturated conditions with NaHSO₄ due to the acidic pH and a "salting out" effect (Table I).

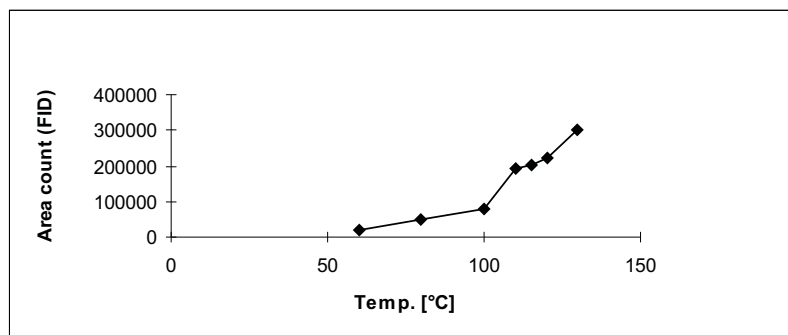


Fig. 2. The effect of sample temperature on detection of GBL.

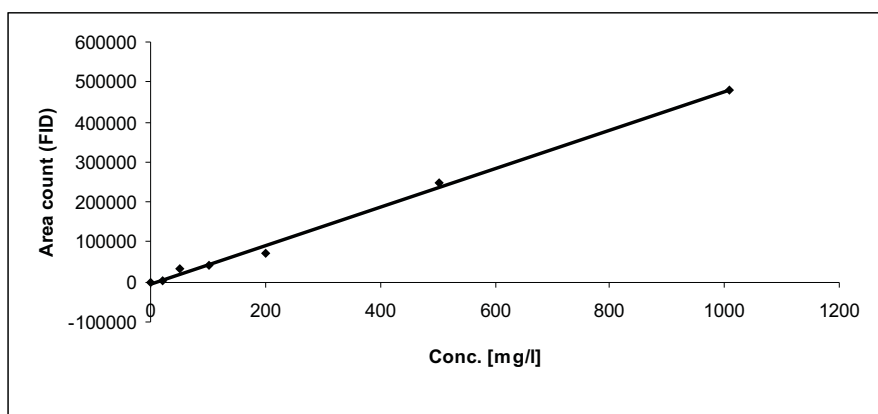


Fig. 3. A calibration curve for GHB in blood obtained by headspace-GC/FID.

TABLE I. THE EFFICIENCY OF DIFFERENT SAMPLE PREPARATION METHODS

Sample preparation	Slope of calibration curve (20–500 mg/l); corr. coef. > 0.999
GHB	–
GBL	58
GHB + 10 μ l 49% H ₂ SO ₄	62
GHB + 0.5g NaH SO ₄ (saturated)	220 => 80% conversion yield
GLB + 0.5g NaH SO ₄ (saturated)	280

Evaluation

A linear correlation was achieved from 10–1000 mg/l GHB (corr. coef. > 0.99) in blood (Figure 3). The reproducibility was 7.0% at 25 mg/l and 8.4% at 200 mg/l (n = 6).

A more specific and sensitive method was desired and therefore headspace-GC/MS was studied. However, due to break down of the equipment and PC, it was not possible

to produce a calibration curve in time, but a preliminary GC/MS study of a positive case is shown in Figure 4.

Future

Determine the limit of quantification of headspace-GC/MS by scan and selected ion mode, and the range of linearity.

Headspace-SPME (solid-phase microextraction) of GHB has been studied, but the sensitivity was not adequate. Recently Blair et al. [1] published a SPME-GC/MS

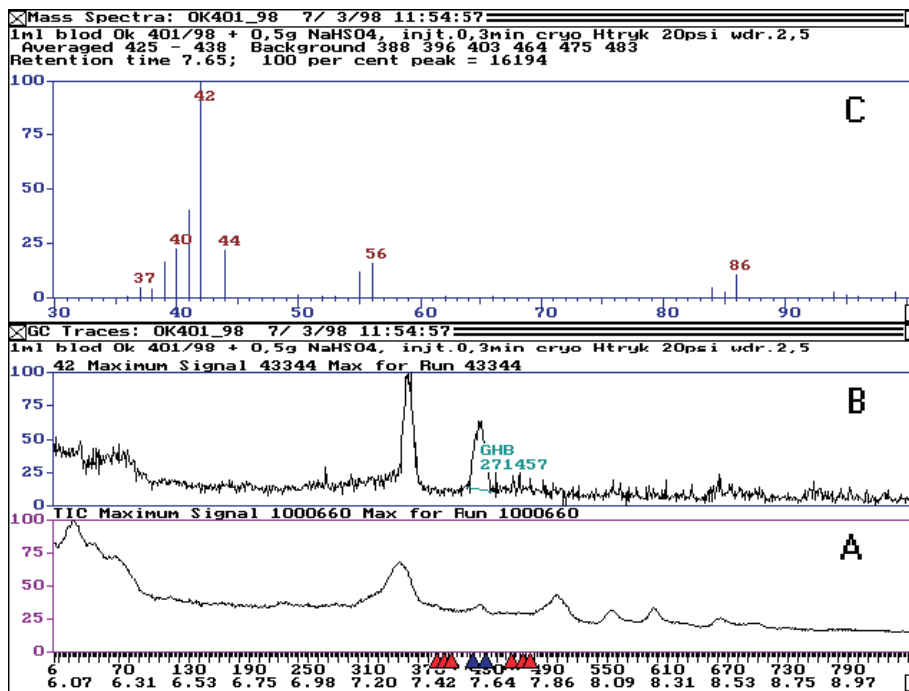


Fig. 4. A positive GHB case (~0.01 mg/kg) verified by headspace-GC/MS: a) TIC with GHB at 7.65 min; b) base peak m/z 42; c) the mass spectra.

method where GHB was derivatized before SPME. They achieved a LOQ of 0.2 mg/l urine. This might be a possibility although direct SPME from blood can be a problem.

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