

Determination of gamma-butyrolactone (GBL)

A tool for the detection of the administration of gamma-hydroxybutyric acid (GHB)

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Introduction

GHB is an endogenous compound. It is formed in the body as a metabolite of glutamic acid [1] and gamma-aminobutyric acid (GABA) [2]. GHB itself is converted into succinic acid [2]. GHB can also be produced synthetically. It is almost achromatic, odourless and tasteless. In detoxication of heroin or alcohol GHB is used to medicate withdrawal syndromes. Furthermore, it is used for the treatment of narcolepsy and as an anaesthetic in caesarean operation.

The effects of GHB strongly depend on the administrated amounts (table 1). At lower dosage sedative effects predominate. This clearly offers the potential for the abuse of GHB in sports. For instance, small doses of GHB can be used as a substitute for β -receptor blockers. Moreover, the sedative, as well as the anxiolytical and mood enhancing effects, will give rise to possible abuse in a large variety of disciplines. At higher dosage however, the effects of the drug increasingly become disadvantageous.

Table 1: Dose and related effects of GHB after oral application. Compiled from [3] and references therein.

Dose (g)	Effect
> 4	Coma, respiratory depression
↑	Unconsciousness
	Myoclonic jerks, obnubilation, vertigo
	Sickness and vomitus, brachicardia, hallucinations
0.7	Euphoria, relaxation, anxiolysis, sedation

GHB can be analysed by GC-MS following lactonisation to GBL at pH 1 and subsequent

liquid-liquid extraction. In the 1980s Donike and Kraft detected elevated concentrations of GBL in doping control samples taken after shooting events in modern pentathlon [4]. Because of its potential of abuse in certain sport disciplines, we developed a quick and simple screening method to detect GBL in urine. It is based on the above mentioned work of Donike and Kraft.

Materials and Methods

Sample preparation is described in table 2. The GC-MS parameters are described in table 3 and table 4.

Table 2: Sample preparation

Sample preparation
<ol style="list-style-type: none"> 1. 5 mL urine. 2. Add internal standard (D6-GHB, 10μg/mL). 3. Acidify to pH 1 (10 M HCl, 200 μL). 4. Lactonisation: 1 h, 60°C. 5. Adjust to pH 5.0 (KOH 5 M, 400 μL; sodium acetate buffer 4 M, 500 μL). 6. Add 0.2 mL of <i>tert.</i>-Butanol, vortex 30 sec. 7. Add 1 mL TBME, 5 g Na₂SO₄ 8. Shake for 20 min, centrifuge for 5 min at 1800 rpm. 9. Transfer organic layer into GC-vials.

Table 3: GC parameters

GC parameters
<ul style="list-style-type: none"> - Model: Hewlett-Packard 6890. - Column: Agilent HP-5MS (26 m \times 0.25 mm \times 0.25 μm). - Carrier gas: Helium, flow 1 mL/min. - Injector: 280°C, split 1:6, injection volume 5 μL. - Temperature: 70°C hold for 2 min and increased to 100°C at 15°C/min, then 35°C/min to 330°C, isothermal for 3 min.

Table 4: MS parameters

MS parameters
<ul style="list-style-type: none"> - Model: Hewlett-Packard 5973 - Signals: <ul style="list-style-type: none"> ⊗ Total-scan: m/z 40 to m/z 200. ⊗ SIM mode: m/z 42, 56, and 86 (GBL); m/z 48, 60, and 92 (D6-GBL).

Results and Discussion

Figure 1 shows GC-MS-chromatogram of a spiked urine sample (10 μ g/mL GHB and D6-GHB), and Figure 2 shows the corresponding mass spectrum. The recovery of GHB detected as GBL was estimated as ca. 60%.

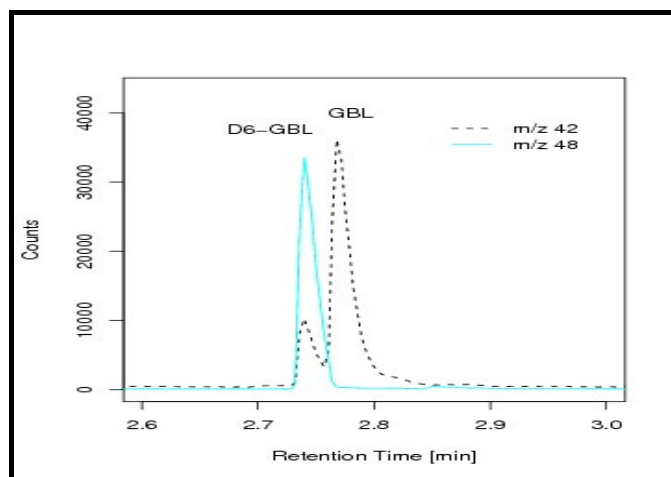


Figure 1: GC-MS-chromatogram of a urine sample spiked with GHB and D6-GHB (10 μ g/mL). SIM detection.

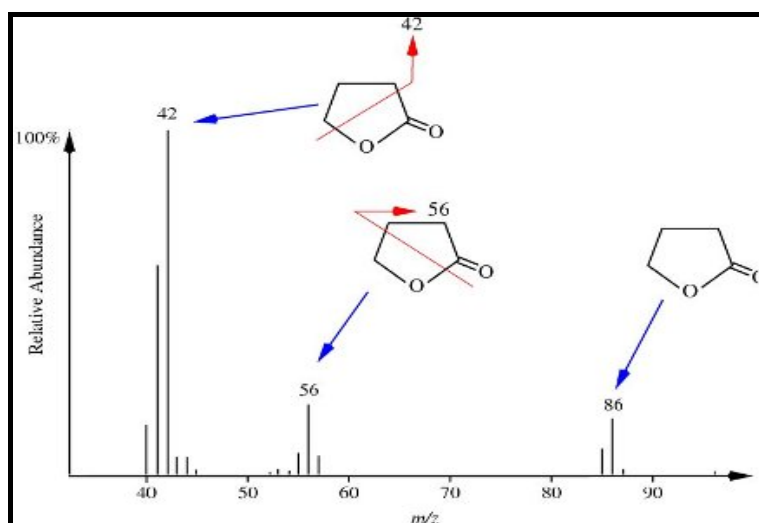


Figure 2: EI mass spectrum of GBL (molecular weight = 86).

Our work possibly bears relevance beyond the scope of doping control, because misuse also takes place in the party scene as a club drug or in the context of sexual assaults as a rape drug. Possibly, GBL is administered rather than GHB. GBL will be rapidly converted to GHB via serum lactonase [2]. Therefore the effects are mostly identical. In contrast to GHB, GBL is not legally prohibited. At the same time it is a widespread chemical in industry and household.

The advantages of GHB/GBL abuse compared to β -receptor blockers in sports are manifold. The decomposition of GHB in the body is faster. This yields shorter half-lives in urine and hence shorter duration of pharmacological effects compared to β -receptor blockers. This characteristic makes GHB/GBL especially interesting for abuse in modern pentathlon, where physically challenging disciplines follow the shooting event. The endogenous levels of GHB in urine generally seem not to exceed 10 $\mu\text{g/ml}$ [5]. However, the concentrations detected by Donike and Kraft [4] exceeded this concentration more than 10fold. A simple (semi-)quantitative method is the *conditio sine qua non* for any evaluation of reference samples. The establishment of threshold values represents a possible second step. Additionally to quantitative methods, analysis by IRMS in fact offers the potential for confirmative methodology [6].

References

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