

1,4-BUTANEDIOL INTOXICATION: A CASE REPORT

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AIMS & INTRODUCTION

Gamma-hydroxybutyric acid (GHB) is an endogenous compound, with known action at the neural level. Its psychoactive effects led to an illicit use context, including recreational purposes, muscle building effects in bodybuilders and drugs-facilitated crimes, specifically, in sexual assaults. Besides the use of the main compound, there are precursors, like Gamma-butyrolactone (GBL) and 1,4-butanediol (1,4-BD), usually non controlled substances, which can be obtained, allowing a much easier way to obtain the target-compound. Both precursors are rapidly and easily metabolized in GHB, leading to all the desired (and undesired) psychoactive effects (Figure 1).

The authors present the first reported intoxication case in Portugal with 1,4-Butanediol, including quantification of GHB and GHB-GLUC in serum, by GC-MS/MS-TQD.

A 25 years old male entered in coma state (GCS = 7) at the hospital emergency room, with the suspicion of an intoxication due to an unknown liquid ingestion. The forensic toxicology lab was asked to collaborate, in order to identify the possible substance(s) and to allow a diagnostic for the patient treatment. The suspected liquid and a serum sample were sent by the hospital, and were analysed by GC-MS-single quadrupole and GC-MS/MS TQD, respectively. A methodology by protein precipitation and GC-MS/MS-TQD was performed, to detect and quantify GHB and GHB-GLUC in serum.

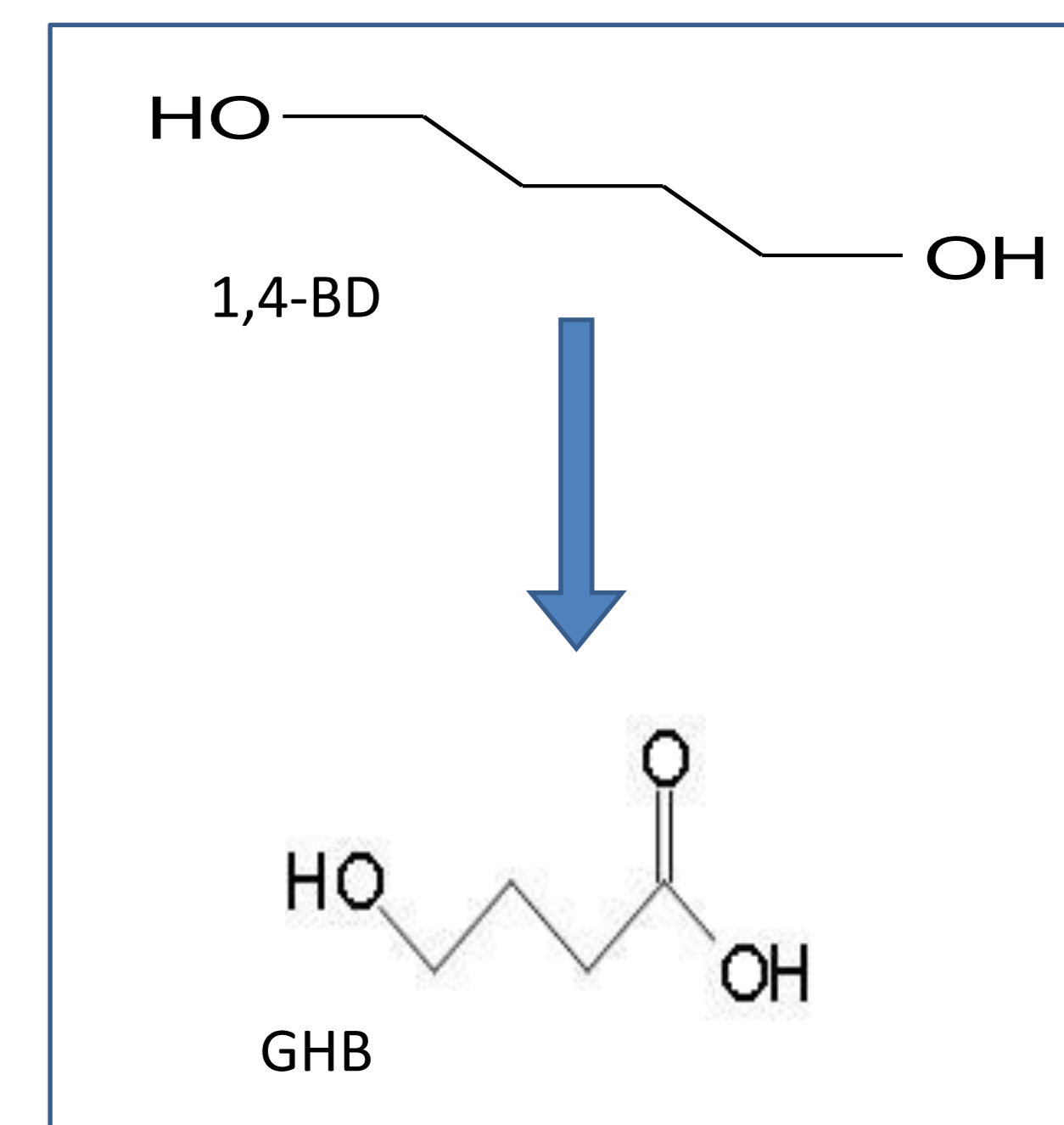


Figure 1 – Biotransformation of 1,4-BD into GHB.

MATERIAL & METHODS

After the samples reception, **the suspected liquid** was analysed directly through GC-MS single quad, after derivatization with BSTFA:TMCS (99:1) (Figure 2).



Figure 2 – GC-MS single quad.

Instrumental conditions are described in Table 1.

Table 1 – GC-MS Instrument conditions.

GC	AGILENT 6890N
MS	AGILENT 5973N
Autosampler	AGILENT 7683
Chromatographic column	J&W Scientific 5-ms, 30 m x 0,25 mm x 0,25 µm 100°C for 5 min, to 290°C at 5°C/min, plateau for 8 min
Detector	Direct interface; Internal Ionization by IE ; T _{transferline} : 280°C T _{quadrupole} : 150°C; SCAN mode, dwell time 1 sec/scan; T _{ionization} : 230°C.

Serum samples were subjected to a protein precipitation extraction procedure, using Methanol in an eppendorf tube (Table 2).

Table 2 – Serum Sample Preparation.

Sample volume	100 µL of Serum
Methanol	200 µL
Vortex	15 seconds
Centrifugation	10' 4000 RPM

After derivatization with BSTFA+TMCS (99:1), the sample was processed in a GC-MS/MS TQD (Figure 3), using the conditions described in Table 3 and 4 [1,2].

Table 3 – GC-MS/MS Instrument conditions.

GC	BRUKER 450-GC
MS	BRUKER 300-MS
Autosampler	VARIAN CP-8400
Chromatographic column	J&W Scientific 5-ms, 30 m x 0,25 mm x 0,25 µm 60°C for 2 min, to 120°C at 10°C/min, plateau 8 min, to 300°C at 30 °C/min, plateau 6 min
Detector	Direct interface; Internal Ionization by IE ; T _{transferline} : 280°C; T _{ionization} : 260°C; MRM mode.



Figure 3 – GC-MS/MS: Bruker GC-450 GC coupled to a 300-MS TQD.

Table 4 – MRM conditions.

Compound	Transitions
GHB	233 > 143 233 > 131
GHB-D6	239 > 149
GHB-GLUC	305 > 143 305 > 149

RESULTS

The direct analysis of the suspicious liquid allowed a positive identification: 1,4-butanediol (1,4-BD), based on the full scan MS spectrum (Figure 4).

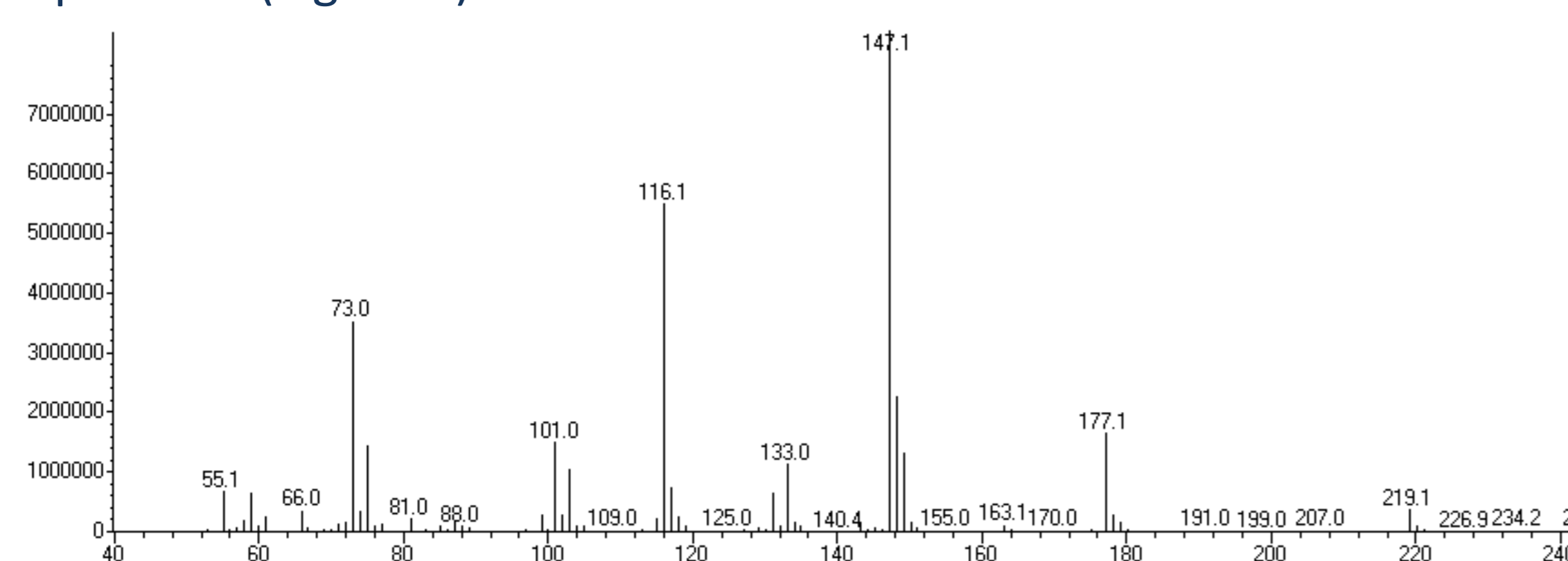


Figure 4 – GC-MS full scan spectrum of the suspicious liquid.

As referred above, 1,4-BD is known for being a GHB precursor. However, and due to the fast conversion to GHB, serum sample did not show the presence of 1,4-BD.

As so, the obtained serum sample was analysed for GHB and GHB-GLUC (Figure 5 and 6).

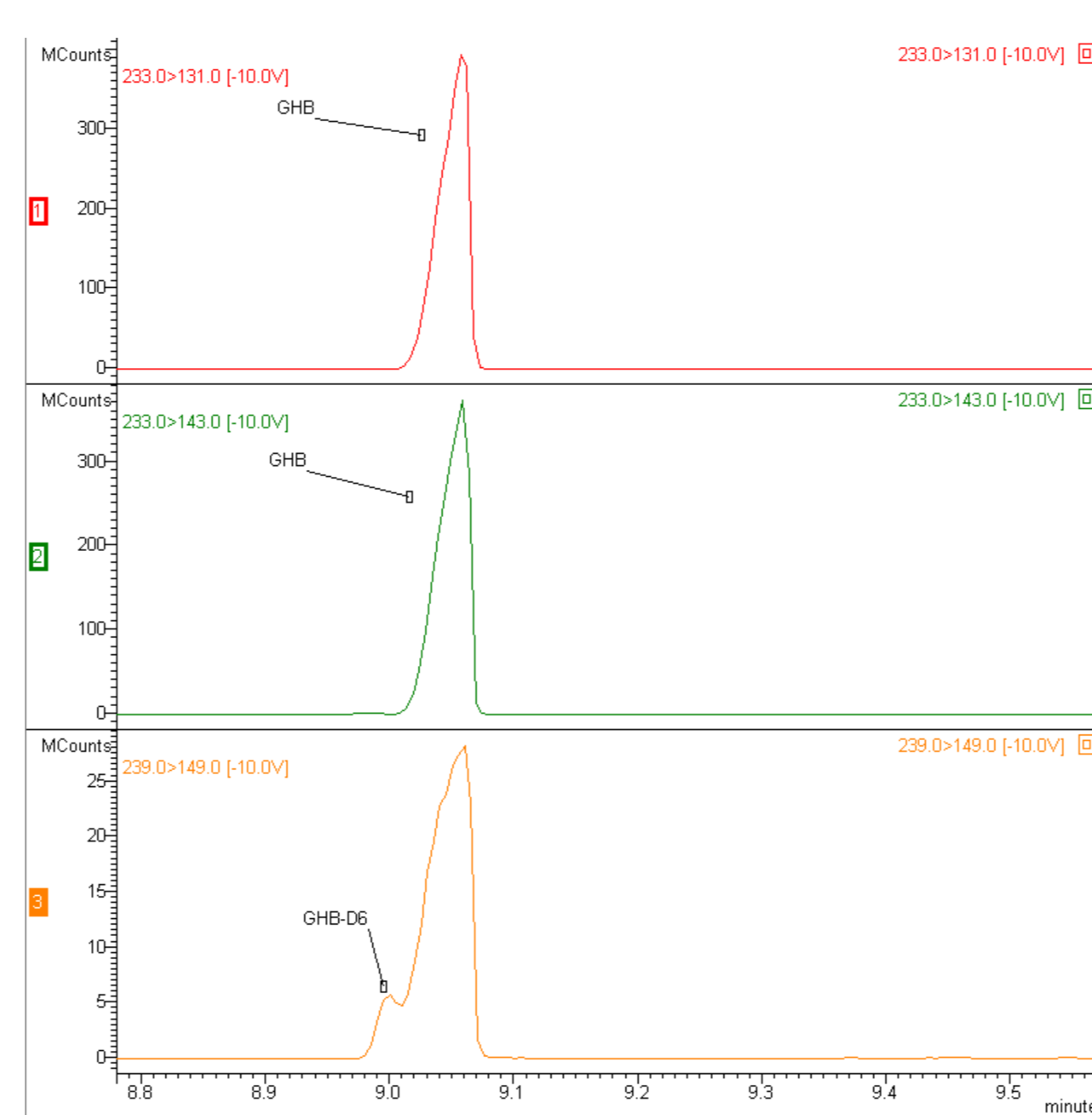


Figure 5 – Serum sample positive for GHB.

The sample was positive for both compounds. Quantitation values are described in table 5.

Table 5 – Quantitative values.

Compound [mg/L]	
GHB	171
GHB-GLUC	13.7

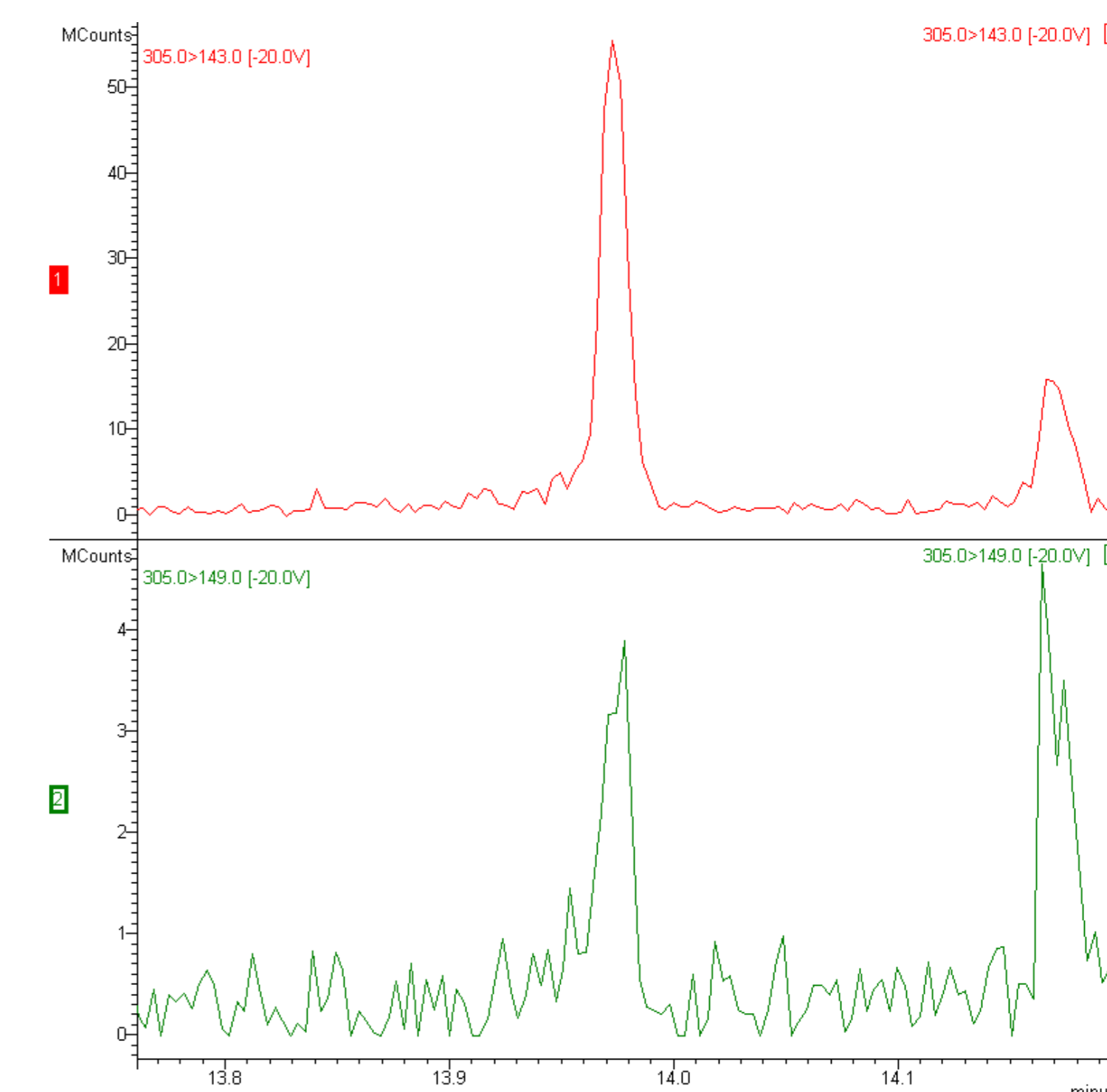


Figure 6 – Serum sample positive for GHB-GLUC.

DISCUSSION AND CONCLUSIONS

1,4-BD was firstly discovered in 1890 and, since then, has been legally used as an industrial solvent [3]. Its fast absorption and peak plasma rate (24' +/- 12') and extensive conversion to GHB poses a challenge in terms of analytical detection [3]. Thus, detection and quantitation of GHB remains almost the solely way to clearly identify an intoxication context. Nevertheless, the main issue remains: even though GHB is detected in biological samples, namely blood, the original ingested compound (GHB, GBL or 1,4-BD) may not be identified. As so, the suspected liquid analysis was crucial for a clear definition of the intoxicating compound. This may be an important reason for the lack of published references concerning intoxication by 1,4-BD. As a matter of a fact, only one case has been found in the literature, with a fatal conclusion [4]. In the present case, the obtained value for GHB is coherent with a reversible intoxication status, being the obtained GHB concentration well above the usually accepted cut-off value for endogenous origin of the compound (10 mg/L). On the other hand, and for the first time, GHB-GLUC was also detected and measured. The significance of this so-called metabolite of GHB is still under discussion, considering that several studies suggest its absence in GHB endogenous context [2].

However, its presence on this sample may suggest that this metabolite may be a possible biomarker in terms of intoxication context, even though some studies suggest that this glucuronated metabolite does not provide any diagnostic information regarding to GHB exposure.

In Portugal, this is the first time that an intoxication by 1,4-BD was detected and reported in *in vivo* samples. Due to the specificity of the compounds, the presence of the suspected liquid and a fast collection of biological samples was pivotal to the conclusion of the process. As to the victim clinical state, the coma was reverted entirely, with no neurological sequelae or whatsoever.

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