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Case Report

Quantification of GHB and GHB-GLUC in an 1,4-Butanediol Intoxication: A case report

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Manuscript's Highlights

- Description of a case report concerning an intoxication by 1,4-BD.
- Description and interpretation of the obtained results.
- Discussion about GHB-GLUC metabolite, found in the sample.

Abstract

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 drug-facilitated crimes, specifically in sexual assaults. Besides the misuse of the main compound, there are precursors like Gammabutyrolactone (GBL) and 1,4-butanediol (1,4-BD), usually non controlled substances, becoming a much easier way to obtain the target-compound.

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

A suspicious liquid and a serum sample were sent by an hospital ER and analysed by GC-MS-single quadrupole and GC-MS/MS TQD, respectively. A methodology including protein precipitation and GC-MS/MS TQD analysis was used to detect and quantify GHB and GHB-GLUC in serum.

Toxicological analysis revealed the presence of 1,4-Butanediol in the liquid and GHB [171 mg/L] and GHB-GLUC [13,7 mg/L] in serum. The victim reverted the coma with no neurological *sequelae*.

This was the first detected case, in Portugal, with 1,4-Butanediol, suggesting that it is important to be aware that consumers have different options to obtain illicit compounds, such as GHB. On the other hand, GHB-GLUC was identified and quantified for the first time in a real case, due to intoxication. This case highlights the importance of analysing all samples for active compounds, precursors and metabolites that can lead to the main intoxication origin.

*Key-words***:** 1,4-Butanediol, GHB, GHB-GLUC, GC-MS/MS, forensic toxicology

1. Introduction

Gamma-hydroxybutyric acid (GHB) is an endogenous compound, with known action at the neural level [1,2]. Its psychoactive effects led to an illicit use context, including recreational purposes, muscle building effects in bodybuilders and drug-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 get the target-compound. Both precursors are rapidly and easily metabolized in GHB, leading to all the desired (and undesired) psychoactive effects (Figure 1) [1,3]. Illicit users of GHB, GBL and/or 1,4-BD take these drugs in order to achieve euphoria, recreation/relaxation, increased sociability, and increased sexual arousal [4].

Considering that GBL and 1,4-BD are metabolised endogenously to GHB, all their main effects result from this conversion [4].

1,4-BD was firstly discovered in 1890 and, since then, has been legally used as an industrial solvent [4]. Its fast absorption, peak plasma rate and extensive (practically complete) conversion to GHB poses a challenge in terms of analytical detection [2,4]. Thus, detection and quantification of GHB remains almost the solely way to clearly identify an intoxication context. However, 1,4-BD is eliminated at a slower rate than GHB, suggesting that its effects last longer than those of GHB [5]. It is also known that 1,4-BD undergoes two-stage conversion in the liver *in vivo* via enzymatic biotransformation; firstly, to gamma-hydroxy butyraldehyde by alcohol dehydrogenase (ADH), and then to GHB through aldehyde dehydrogenase [5]. Also, both the half-life elimination of 1,4-BD and time to maximal GHB concentration are similar, suggesting that the psychoactive effects of 1,4-BD are associated to its conversion *in vivo* into GHB. Finally, the pharmacokinetics of 1,4-BD, including

distribution and rate of conversion to GHB, may influence its desired and undesired pharmacological actions [4,5].

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.

2. Case Report

A 25 years old male entered at the hospital emergency room (ER) in a coma (Glasgow Coma State = 7), with the suspicion of intoxication due to an unknown liquid ingestion. The forensic toxicology laboratory was asked to collaborate, in order to identify the possible substance(s) involved. Both 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.

3. Material and Methods

3.1. Materials, standards and chemicals

GHB-β-O-glucuronide was synthesized by Petersen *et al.* [6] and provided by the Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen. Pure GHB and deuterated internal standard (GHB-D_6) were purchased from Cerilliant Corporation (Round Rock, TC, USA). Stock standard solutions and working solutions at concentrations of 1 mg/L and 10 mg/L were prepared in methanol and properly stored at -20ºC. Methanol (gradient grade) was purchased from E. Merck (Darmstadt, Germany). The derivatization reagent [BSTFA (N,O-bis(trimethylsilyl)-trifluoroacetamide) + 1% TMCS (trimethylchlorosilane] was purchased from Sigma Aldrich (St Louis, MO).

2.2. Samples preparation and analysis

Both the suspicious liquid and the serum sample were received from the ER Service of the requesting Hospital. An aliquot of the suspicious liquid was diluted in methanol in a crimp cap vial. After drying and derivatization with 60µL of BSTFA:TMCS (99:1) – 30 minutes – it was directly analysed using a GC-MSD Agilent 6890/5973N using SCAN Mode (Table 1). Serum samples were analysed according to Castro *et al.* [7] and Dias *et al.* [8], for GHB and GHB-GLUC, respectively. Briefly, a 100 uL serum aliquot was subjected to a protein precipitation extraction procedure, after Internal Standard addition, namely 5 µL of GHB-D6 (1 mg/L), using 300 µL of methanol in an eppendorf tube. After derivatization with 60 µL of BSTFA+TMCS (99:1), the sample was analysed in a BRUKER 450-GC/300-MS GC-MS/MS-TQD apparatus in MS/MS mode (Table 1), using transitions 233>143 m/z and 233>131 m/z for GHB, 305>143 m/z and 305>149 m/z for GHB-GLUC and 239>149 m/z for GHB-D6. Quantitation ions for both compounds are marked in bold.

4. Results and discussion

The direct analysis of the suspicious liquid allowed a positive identification: 1,4-butanediol (1,4-BD), based on the full scan MS spectrum, compared to Wiley® MS Spectrum library [Figure 2 a) and b)].

As mentioned above, 1,4-BD is known for being a GHB precursor. However, and due to the fast conversion to GHB, the presence of 1,4-BD in the sample was not expected. Thus, the obtained serum sample was analysed for GHB and GHB-GLUC (Figure 3 and 4).

The sample was positive for both compounds. The obtained quantification values were 171 mg/L for GHB and 13.7 mg/L for GHB-GLUC.

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. In fact, only a few cases have been found in the literature, involving outcomes from full recovery to death [9,10]. Throughout these cases, whenever there was a full recovery after ER intervention, 1,4-BD was absent in all the blood samples. On the other hand, when death was the final outcome, 1,4-BD was found in two of the four post-mortem cases [9,10]. Concerning to GHB concentration values, it ranged from 317 mg/L to 415 mg/L in in vivo samples, and from 432 mg/L to 837 mg/L in post-mortem cases. In the present case, the obtained value for GHB (171 mg/L) 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) [1].

On the other hand, and for the first time, GHB-GLUC was also detected and measured (13.7 mg/L). The significance of this so-called metabolite of GHB is still under discussion, considering that several studies suggest its absence in GHB endogenous context in different samples [8,11,12,13,14]. The same studies suggest that this glucuronidated metabolite does not provide any diagnostic information regarding to GHB exposure, namely in the detection window increase [11,12,13,14]. Interestingly, Busardó et al. describes and evaluates the results obtained for a group of clinical cases, based on narcoleptic patients under sodium oxybate treatment. It is noticeable that GHB-GLUC values were always between LOD (0.2 mg/L) and LOQ (0.5 mg/L), comparable with endogenous values [13]. However, the obtained GHB values were also lower [8.12 – 32.35 mg/L], due to the controlled administration context, with therapeutic aims, than the obtained in the present case [171 mg/]. As so, the presence and detection of GHB-GLUC may be GHB-dose dependent and, in this specific

case, also dependent on 1,4-BD metabolic pathway. Moreover, its presence may suggest that this metabolite may be a possible biomarker in terms of exogenous intoxication context, as to GHB precursors is concerned.

Finally, a pitfall that must be mentioned is that the analyzed metabolite was the one glucuronidated at the hydroxyl moiety of GHB and not the one glucuronidated at the carboxyl moiety, due to the unavailability of the standard compound [6,8].

In terms of metabolism, GHB is mainly metabolized to water and carbon dioxide in the citric acid cycle, via the conversion to succinic semialdehyde and succinic acid. Another pathway includes the metabolization to GABA by GABA transaminase [4,5]. Also, it is suggested that β-oxidation is also a possible elimination route [5]. Considering that ethyl glucuronide is a common marker of alcohol intake [15], we must be aware that UDP-Glucuronosiltransferase enzyme converts both ethanol in ethyl glucuronide and GHB in GHB-GLUC. This enzymatic path could then explain the presence of the metabolite. Obviously, this suggestion needs further studies for proper confirmation or rebuttal.

5. Conclusion

In Portugal, this is the first time that an intoxication by 1,4-BD was detected and reported in in vivo samples. The misuse of its precursors (1,4-BD, GBL) seems to be a new trend on GHB consumption, namely in "Chemsex" misuse, as both are easier to obtain, due to their non-controlled substances context [16]. Due to the specificity of the compounds, the presence of the suspected liquid and a fast collection of biological samples were pivotal to the conclusion of the process. As to the victim clinical state, the coma was reverted entirely, with no neurological *sequelae* or whatsoever. Toxicological analysis was crucial for the compound identification, and the presence in the serum sample, not only of GHB, but also of GHB-

GLUC may deserve further discussion on this metabolite role in GHB and precursors metabolic pathway.

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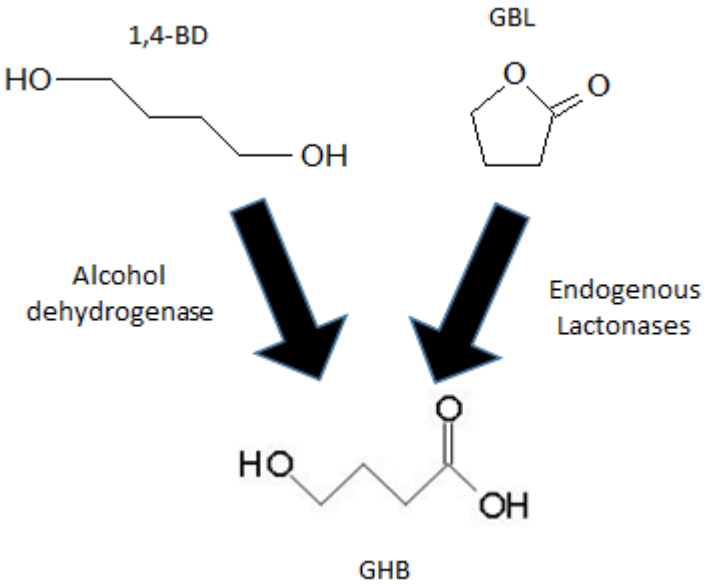

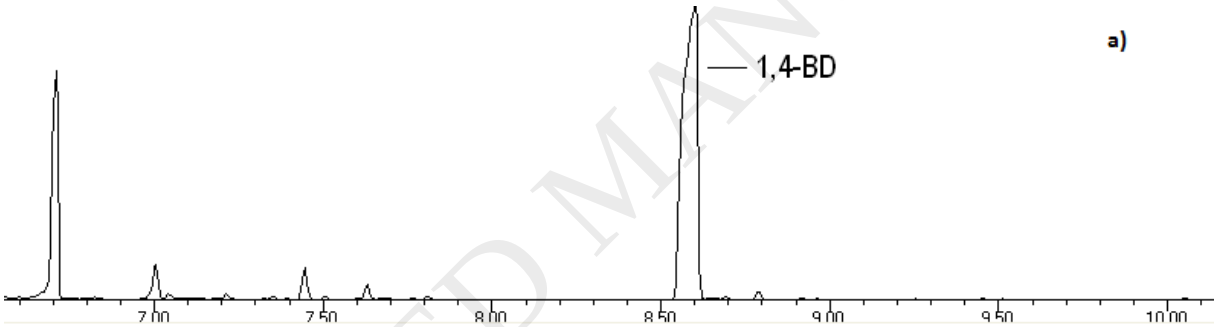

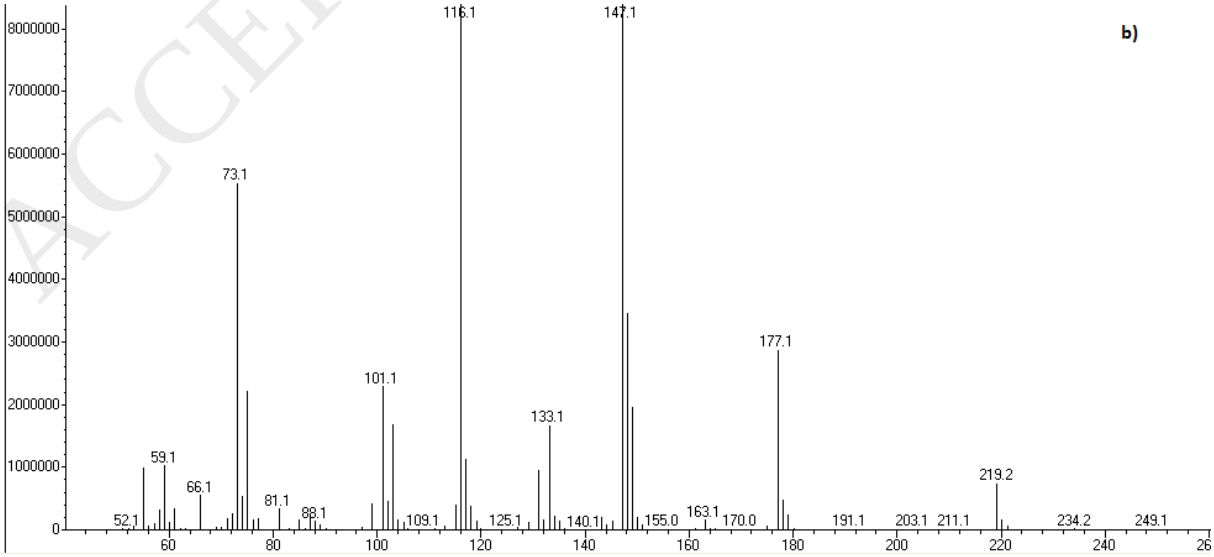

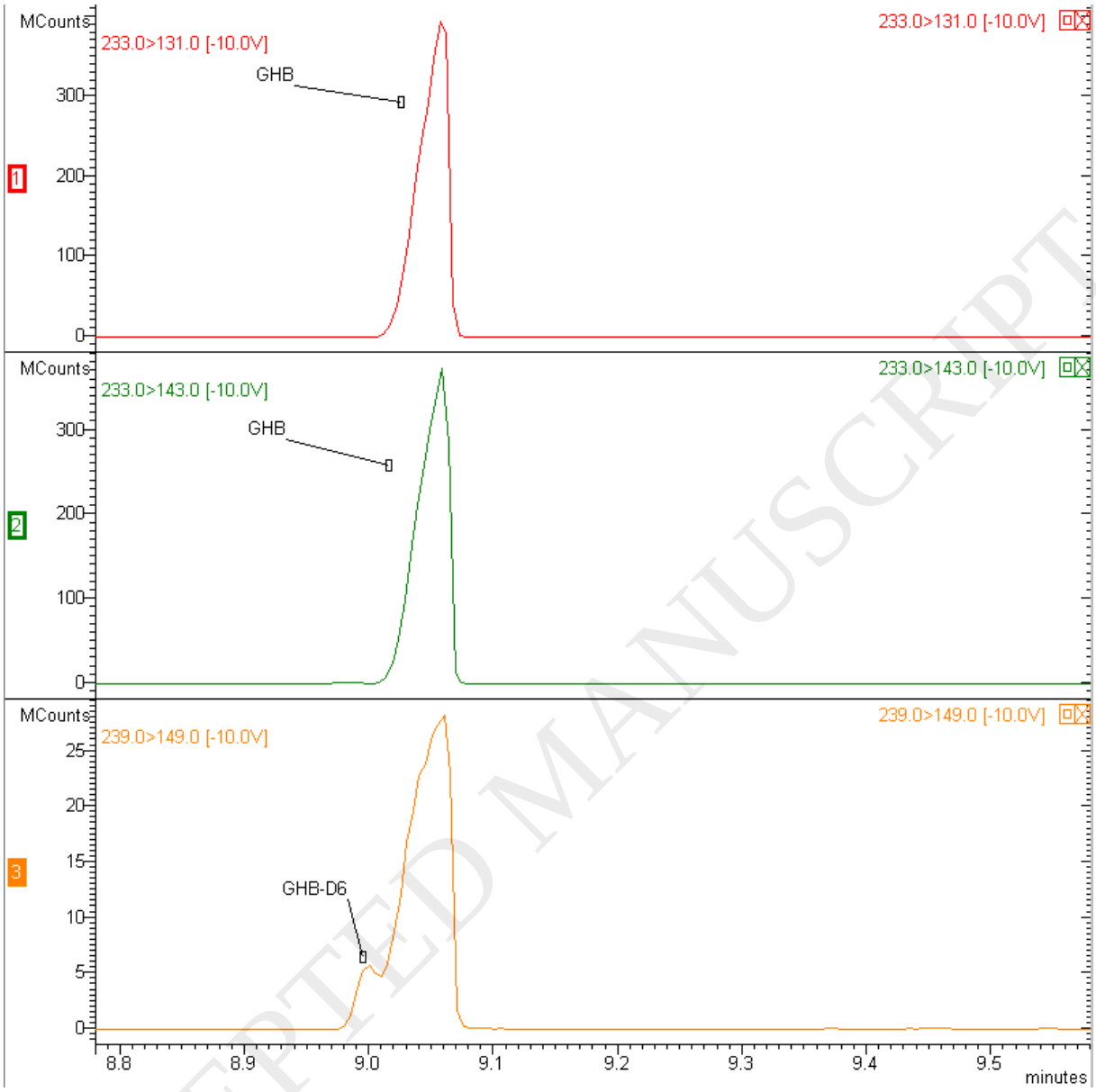

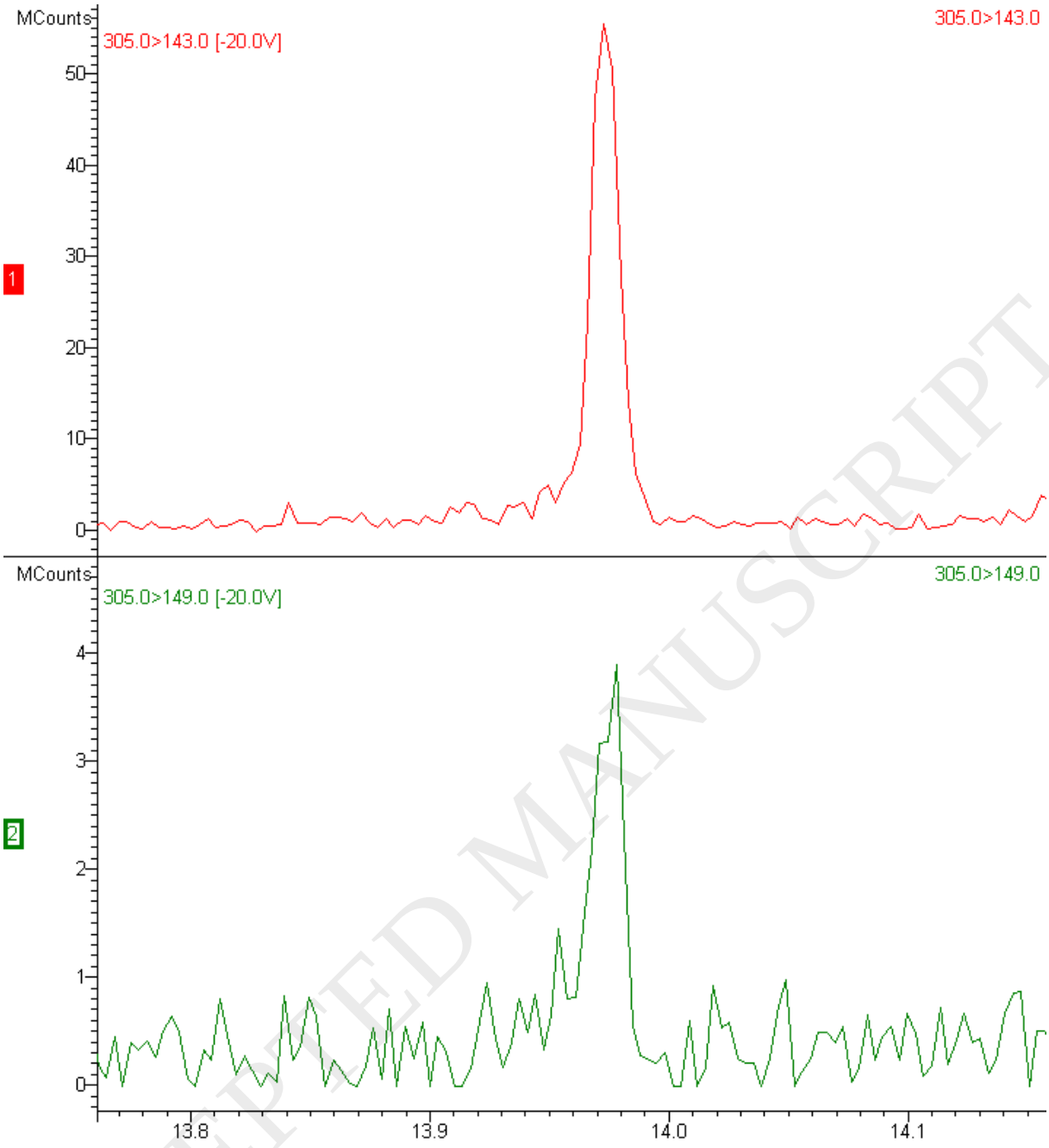

Table 1 - Instrumental conditions.

	GC-MSD Single Quad	GC-MS/MS TQD
GC	AGILENT 6890N	BRUKER 450-GC
MS	AGILENT 5973N	BRUKER 300-MS
Chromatographic column	J&W Scientific 5-ms, 30 m x 0,25 mm x 0,25 µm 100ºC for 5 min, to 290ºC at 5ºC/min, plateau for 8 min	J&W Scientific 5-ms, 30 m x 0,25 mm x 0,25 µm 60ºC for 2 min, to 120ºC at 10ºC/min, plateau 8 min, to 300ºC at 30 ºC/min, plateau 6 min
Detector	Direct interface; Internal Ionization by EI; $T_{transferline}$: 280ºC $T_{quadrupole}$: 150ºC; SCAN mode, dwell time 1 sec/scan; $T_{ionization}$: 230ºC.	Direct interface; Internal Ionization by EI ; $T_{transferline}$: 280ºC; $T_{ionization}$: 260ºC; MRM mode.