GHB Detection in Biological Samples by GC-MS-MS with Forensic Purposes

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INTRODUCTION

Gamma-Hydroxybutyrate (sodium hydroxybutyrate; sodium oxybutyrate; GHB) is known to be an endogenous, naturally occurring, short-chained fatty acid found in mammalian tissues, with wide distribution and action in several brain areas (hipothalamus, basal ganglia).(Figure 1). Although it was first synthesised in 1960, it soon was noticed that it is no more than an endogenous compound. With more than 30 years of clinical use, both in Europe and the U.S.A, its illicit use includes recreational use, muscle building effects in bodybuilders and drug-facilitated sexual abuse. Used as a club drug, alone or mixed with other substances, it´s symptoms mimetize MDMA, ketamine and ehtanol (Figure 2). On the ohter hand, it is also used for drug-facilitated sexual abuse (DFSA) purposes. In this work, the authors aim to develop and validate an analytical procedure for GHB detection in whole blood (in vivo and *post-mortem*) and hair samples, for forensic purposes.

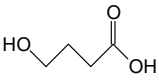


Figure 1 – GHB chemical structure

Figure 2 – GHB consumption

MATERIALS & METHODS

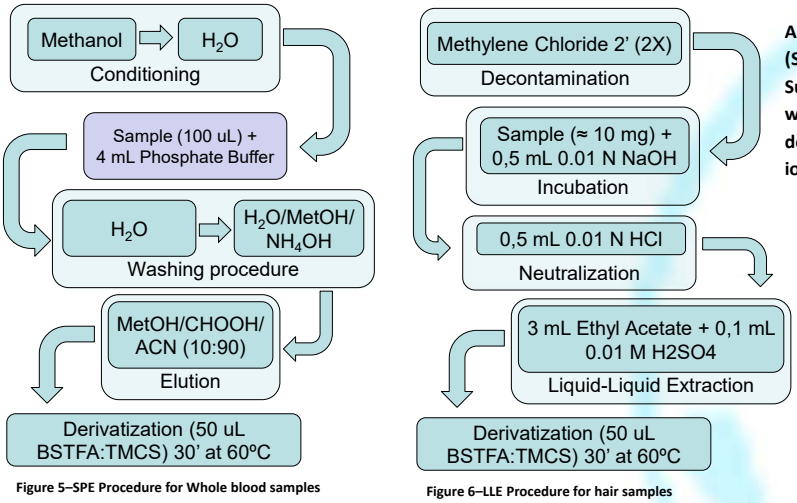


Figure 5–SPE Procedure for Whole blood samples

Figure 6–LLE Procedure for hair samples

Analytical Standards were GHB 1 mg/mL (LIPOMED) e 6-hidroxicaproic Acid (HCA) 10 g (SIGMA) as Internal Standard (Figure 3). Derivatizing agent was BSTFA:TMCS (99:1) from Supelco. SPE was done using OASIS MCX® (WATERS™) cartridges. The analytical apparatus was a GC-450 coupled to a MS-300 (BRUKER – Figure 4). SPE procedure for whole blood is described in figure 5. LLE procedure for hair samples is described in figure 6 and diagnostic ions are described in table 1.

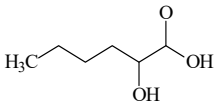


Figure 3 –HCA Chemical Structure.

Table 1 – Diagnostic ions (quantitation ion underlined).

GHB	Precursor-ion	233
	Product-ions	142 ; 143 ; 131
HCA	159 Product-ion	103

Figure 4– GC-MS-MS.

RESULTS

For whole blood samples, specificity and selectivity were studied. This parameter was firstly evaluated by testing Phosphate Saline Buffer and water with GHB (200 ng/mL) and HCA as Internal Standard (1500 ng/mL). This approach was intended to avoid "False Positives" linked to GHB endogenous status.

Nevertheless, it was also tested a blood sample, in order to evaluate the possible selectivity of the method to GHB.

The results are shown in table 2.

Table 2 – Validation results for whole blood samples

Parameter	Positive Samples	Negative Samples
Specificity / Selectivity	Fortified buffer samples Fortified whole blood samples [GHB]=200 ng/mL	Buffer Samples Whole Blood Samples [HCA]=1500 ng/mL
Linearity	200 ng/mL<= [GHB] <= 10000 ng/mL	
Limit of Detection	[GHB] = 100 ng/mL	
Limit of Quantitation	[GHB] = 200 ng/mL	
Carryover	6 samples ([GHB] = 200 ng/mL) 6 samples ([GHB] = 2000 ng/mL)	

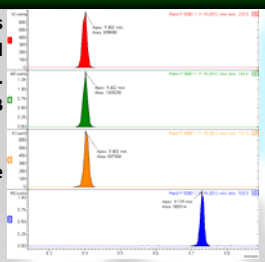


Figure 7 – Chromatogram of Positive samples (Whole Blood above – hair down).

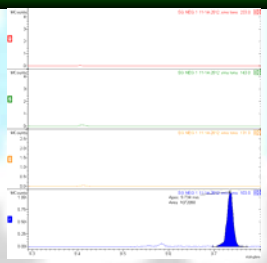
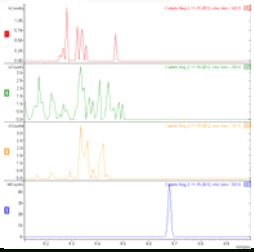
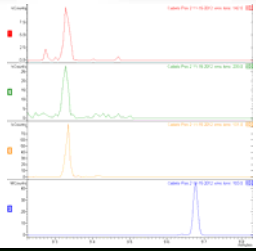


Figure 8 – Chromatogram of a Negative sample (Whole Blood above – hair down).





Spiked samples were prepared adding GHB [5 ng/mL], to 10 mg of blank hair sample, obtained from a volunteer (female, 38 years old) with no history of GHB consumption.

In hair samples, the extraction procedures tested have shown, by now, good results in terms of specificity and selectivity, with 0% of False Positives and 0% of False Negatives.

Once again, blank samples, only with methylene chloride, were also studied, in order to evidence the absence of interferences from the solvents and reagents used in the ectraction procedure.

Figures 7 and 8 show chromatograms obtained for both samples, either positive and negative.

DISCUSSION

GHB analytical detection in biological samples for forensic purposes became a part of routine analysis in many toxicological labs. Nevertheless, GHB levels obtained in casework, both in in vivo as in post-mortem samples require a careful interpretation, not only due to its endogenous condition, as to post-mortem production, linked to autolysis and microbial action phenomena.

Thus, the use of alternative biological samples, such as hair, is mandatory for such an objective medico-legal interpretation, allowing also an increase in the detection window, not only for regular consumers as for single consumption, linked, for example, to a DFSA case.

The described method shows good fitness for purpose for whole blood samples, and promisses good results also for hair samples, although the Limit of Detection is still being studied. It will allow the possibility to distinguish post-mortem production, endogenous values and external consumption.

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