Service of Forensic Chemistry and Toxicology

QUANTIFICATION OF GHB (γ-HYDROXYBUTYRATE) IN WHOLE BLOOD BY SPE AND GC-MS-MS FOR FORENSIC PURPOSES

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INTRODUCTION AND AIMS

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 (hipocampus, 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*), for forensic purposes.

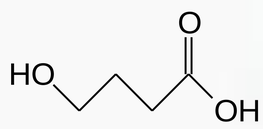

Figure 1 – GHB chemical structure

Figure 2 – GHB consumption

MATERIAL AND METHODS

The applied methodology included:

✓ a solid phase extraction (SPE) procedure with Oasis MCX® 3cc columns from WATERS (Figure 3).

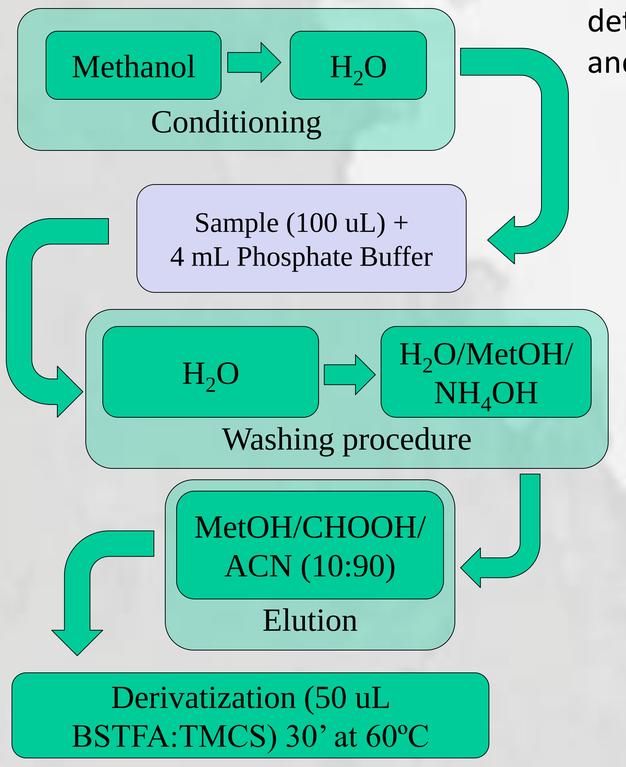

Figure 3. Sample preparation and extraction scheme.

✓ an analytical technique using a gas chromatograph GC-450, coupled to a mass spectrometer MS-300 with a triple quadrupole detector from BRUKER (Figure 4 and Tables 1 and 2).

Figure 4. GC-MS-MS triple quadrupole

✓ Analytical Standards were GHB 1 mg/mL (LIPOMED) e 6-hidroxicaproic Acid (HCA) 10 g (SIGMA) as Internal Standard (Figure 5)

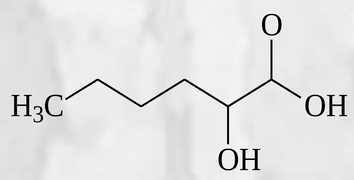

Figure 5 –HCA Chemical Structure.

TABLE 1. Analytical parameters of GC-MS-MS system

Injector	Temperature	250ºC
	Sample Volume	2.0 µL
	Injection Mode	Splitless
Column	Agilent J & W HP-5MS	0.25 mm / 30 m / 0.25 µm
	Constant Flow	1.3 mL/min
Column Oven	Initial Temperature	60ºC (2')
	Temperature "Ramp" 1	10ºC/min (8')
	Final Temperature	120ºC
	Temperature "Ramp" 2	30ºC/min (2')
	Final Temperature	300ºC
	Total Run Time	16,0 min
Mass Spectrometer	Transfer Line	280ºC
	Quadrupole Temperature	40ºC
	Ionization Source Temperature	260ºC
	Ionization	EI (*Electron Impact*)
	Solvent Delay	9 min
	Acquisition Parameters	Quad1: SIM Mode (*Selected Ion Monitoring*) Quad3: SIM Mode (*Selected Ion Monitoring*)

TABLE 2. Mass Spectrometer Acquisition parameters. The quantification ion is underlined.

Compound Name	Precursor-ion (m/z)	Product-ion (m/z)	Collision Energy	Retention time (min)
GHB	233	143 , 131	15 V	9,30
HCA	159	103	16 V	9,63

RESULTS

This analytical methodology validation was accomplished with the assessment of some parameters. 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 Figures 6, 7 and 8 and described, in brief, in table 3.

TABLE 3. Analytical validation parameters results.

Compound Name	LOD	LOQ	Work range	Intra-run precision (n=5)	Inter-run precision (n=2)
GHB	200 ng/mL	200 ng/mL	200 – 5000 ng/mL	12,7 % at 200 ng/mL	9,80 % at 200 ng/mL

As to carryover is concerned, it has not been noticed, even with samples positive over 5000 ng/mL. Intra-run precision was studied at 200 ng/mL, contemporarily with the LOQ testing. Inter-run precision was studied also at 200 ng/mL. CV was less than 20% for both parameters.

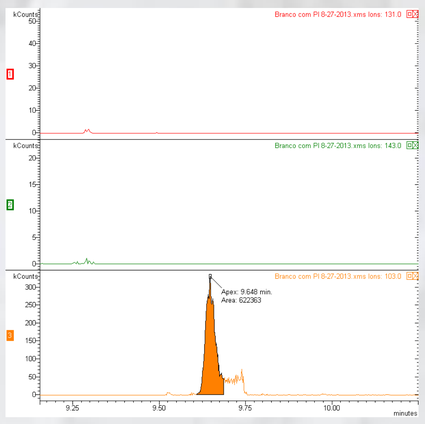

Figure 6. Blank sample

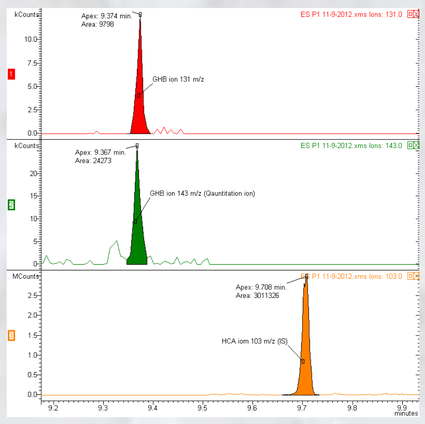

Figure 7. Sample with GHB at 500 ng/mL

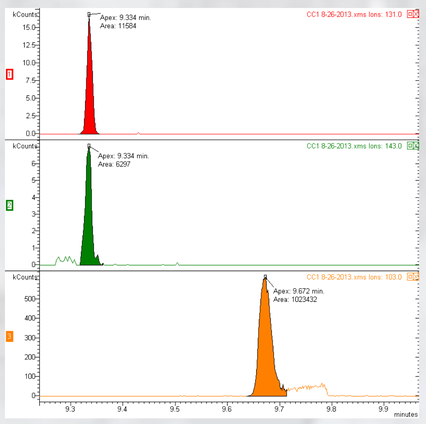

Figure 8. Sample with GHB at 200 ng/mL (LDQ)

CONCLUSIONS

The described method shows good fitness for purpose for whole blood samples. The obtained LOD and LOQ were 200 ng/mL, for 100 uL of sample. This increase in sensitivity was obtained due to an optimized SPE procedure and an instrumental technique state-of-the-art. The work range started at 200 ng/mL, far below the suggested cut-off for whole blood samples (5-10 mg/L). These results will allow the possibility to distinguish *post-mortem* production, endogenous values and external consumption, whenever this diagnosis is needed and requested, being applicable to forensic purposes.