LABORATORY OF FORENSIC CHEMISTRY AND TOXICOLOGY

Endogenous GHB concentrations in whole blood postmortem samples as a biomarker for postmortem interval estimation – A set of real cases analysis

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AIMS & INTRODUCTION

Gamma-hydroxybutyric acid (GHB) is an endogenous compound which has a story of clinical use and illicit abuse since the 1960's. Its postmortem behaviour, namely regarding degradation and metabolism, has been increasingly studied to be used as a putative biomarker for postmortem interval (PMI) estimation. Previous studies have suggested a correlation between GHB concentrations in whole blood and the corresponding PMI. However, most of these studies were developed in *in vitro* conditions.

To evaluate this possible usefulness, the postmortem concentration of GHB of 32 GHB unsuspicious cases was evaluated in whole blood samples for PMI interval estimation purposes.

MATERIAL & METHODS

Whole blood postmortem GHB levels were obtained from thirty two real cases with previous information on death and autopsy data. The samples were treated through sample methanolic precipitation followed by GC-MS/MS analysis (Table 1 and Figure 1), as previously described [1].

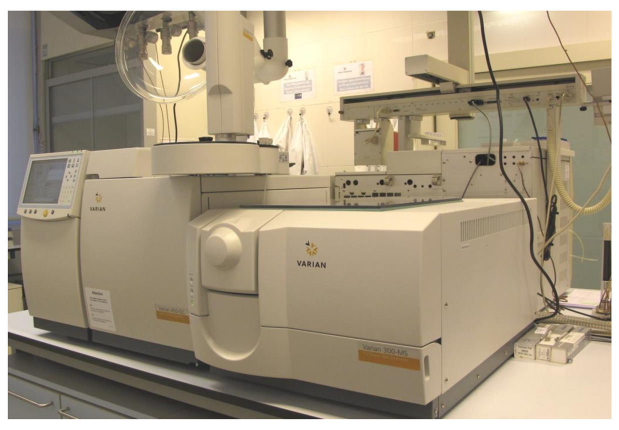

Table 1 – MS/MS parameters for GHB and GHB-D_6 (Internal Standard)

GHB	**Precursor ion**	**233**
	Product ion	**143; 131**
GHB-D_6	**Precursor ion**	**239**
	Product ion	**149**

Figure 1 - GC-MS/MS instrument: Bruker GC-450 gas chromatograph coupled to a 300-MS triple quadrupole detector.

RESULTS

All the analyzed samples were positive for GHB. Figure 2 shows a typical chromatogram positive for GHB at a concentration of 0,1 mg/L (LLOQ). GHB-D_6 was added at a concentration of 1 mg/L.

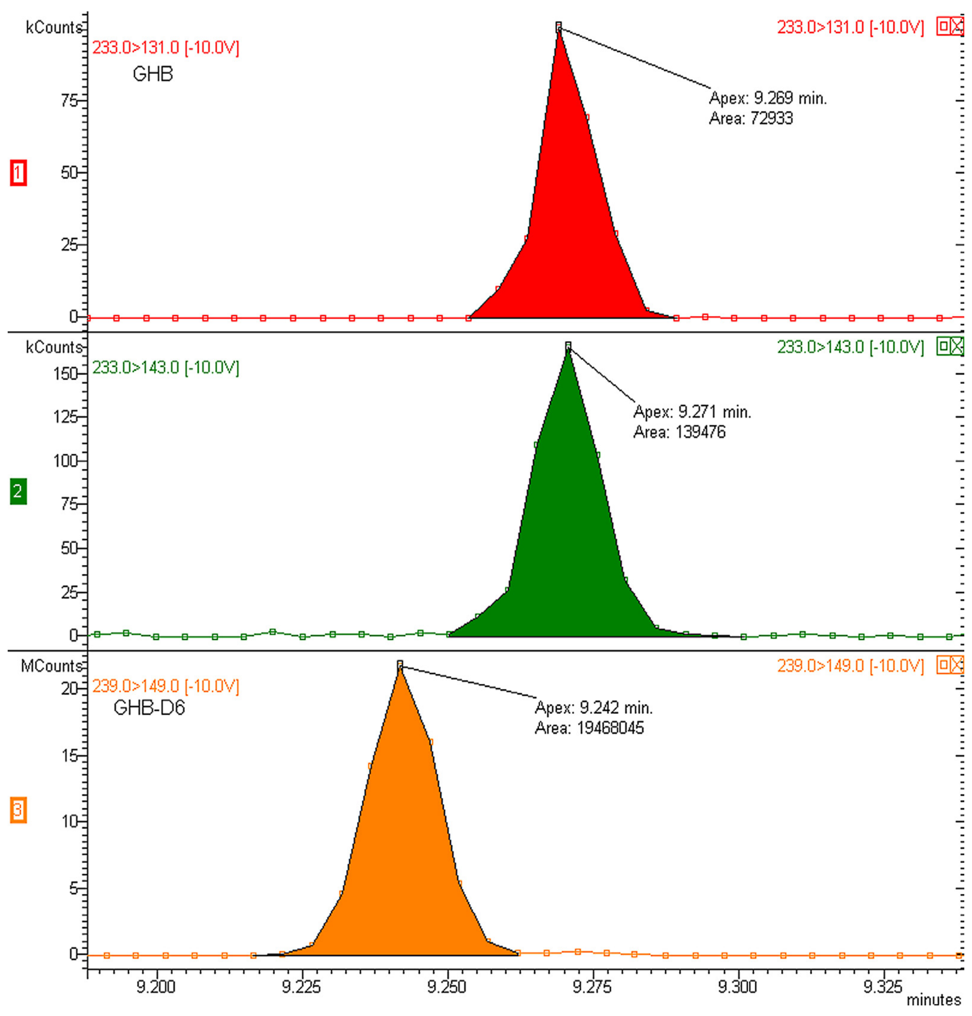

Figure 2 - GC-MS/MS chromatogram for a positive sample.

Statistical analysis combined a Shapiro–Wilk test to confirm the normal distribution of the samples' values, the Student's t test or ANOVA to compare more than two different data groups, as well as the Tukey test for mean values evaluation for the different sub-groups.

Whole blood GHB concentrations obtained for all the cases analyzed were lower than 30 mg GHB/L (1.82 to 15.80 mg GHB/L) and may be considered as endogenous values. A normal distribution was found, with a mean value of 8.43 mg GHB/L and a median of 7.06 mg GHB/L.

The results were grouped and statistically analysed according to gender (Table 2), age group (Table 3), medico-legal etiology (Table 4), presence or absence of other substances (Table 5) and postmortem interval (Table 6 and Figure 3). Data are presented as mean ± standard deviation.

Table 2 – GHB levels according to gender

Gender	n (%)	[GHB] (mg/L)
Fem	8 (24%)	6.716 ± 2.603
Masc	26 (76%)	7.794 ± 5.039

Table 3 – GHB levels according to age groups

Age group	n	[GHB] (mg/L)
1 - 44 years	5	7.872 ± 2.064
45 - 60 years	14	6.799 ± 3.666
> 65 years	13	5.721 ± 2.391

Table 4 – GHB levels according to the medico-legal etiology

Medico-Legal etiology	n	[GHB] (mg/L)
Accident	8	7.956 ± 2.255
Natural / unknown	19	6.745 ± 3.215
Suicide	7	5.140 ± 2.958

Table 5 – GHB levels according to the presence or absence of other substances

Other substances	n	[GHB] (mg/L)
Absence	15	6.37 ± 2.607
Presence	19	6.96 ± 3.381

Table 6 – GHB levels according to the postmortem interval (PMI)

PMI (h)	n	[GHB] (mg/L)
0-24	3	6.359 ± 2.420
24-48	15	6.592 ± 2.723
48-72	8	8.288 ± 4.184
72-96	5	6.357 ± 2.121
96-120	2	4.572 ± 0.812

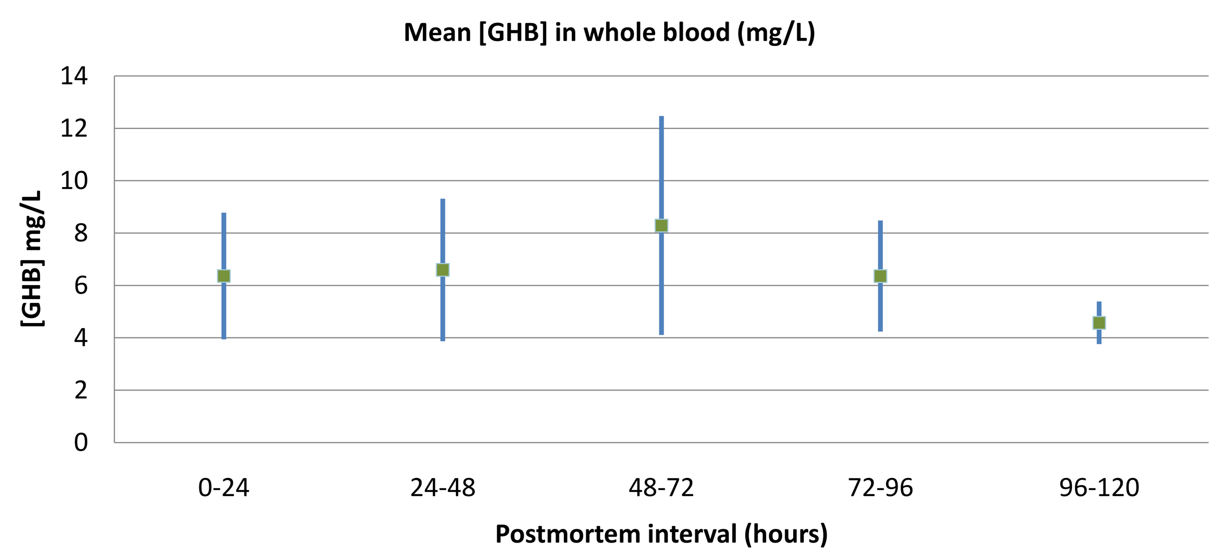

Figure 3 – GHB levels according to the postmortem interval (PMI).

DISCUSSION & CONCLUSIONS

No differences were found regarding gender (p=0.273, Student's t test followed by post-hoc Tukey test), cause of death (p≥0.05, ANOVA followed by post-hoc Tukey test) and presence or absence of substances (p=0.405, Student's t test followed by post-hoc Tukey test). As to age is concerned, a trend to reduced GHB levels accompanying the increment of age was found; however, the differences did not achieve statistical significance (p≥0.05, ANOVA followed by post-hoc Tukey test).

On the other hand, the results obtained suggest that the PMI (until 5 days between death and sampling) may influence GHB whole blood concentration, namely between 48 and 72 hours [24 - 48 hours (p=0.893), 48 - 72 hours (p<0.05); 72 - 96 hours (p=0.123)]. It should be noticed that the small number (n= 5) of cases included in the subgroup of PMI with 72–96 hours may have nullified a possible distinction.

This study brings additional data regarding the usefulness of GHB levels in forensic toxicology, which might be further strengthened with larger, but comparable, studies from other laboratories and institutions in the forensic toxicology context.

REFERENCES

[1] Castro A, Tarelho S, Dias M, Reis F, Teixeira HM. A fast and reliable method for GHB quantitation in whole blood by GC-MS/MS (TQD) for forensic purposes. *J Pharm Biomed Anal* 2016; 119: 139–144.