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Original communication

Quantitative analysis of the endogenous GHB level in the hair of the Chinese population using GC/MS/MS

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ABSTRACT

Endogenous production complicates interpretation when gamma-hydroxybutyrate (GHB) is measured in hair for forensic purposes. A method capable of quantifying the endogenous concentration of GHB in human head hair was developed and validated using GC/MS/MS. Hair was digested under alkaline conditions (1 mol/L NaOH, 90 °C 10 min), and GHB-d6 was used as an internal standard. Before derivatization with BSTFA and ethyl acetate, a liquid–liquid extraction with ethyl acetate under acidic conditions was performed. GHB-TMS derivatives were detected using GC/MS/MS in the multiple-reaction monitoring mode. This method exhibited good linearity ($y = 0.018x + 0.038$, $R^2 = 0.9998$), and the limit of detection was 0.02 ng/mg. The extraction recoveries were more than 60%, and the inter-day and intra-day relative standard deviations (RSD) were less than 15%. This method has been applied for the analysis of the endogenous GHB in hair samples from 66 drug-free Chinese donors. The mean measured concentration for 0–3 cm hair was 1.93 ± 1.40 ng/mg (n = 66), and extreme values were in the range of 0.28 –4.91 ng/mg. The mean male endogenous GHB level was 2.95 ng/mg (0.92–4.91 ng/mg, n = 35), while the mean female level was 0.77 ng/mg (0.28–1.95 ng/mg, n = 31). This method was applied to a forensic case for the determination of GHB in hair samples but it is hard to make a reasonable "cut off" in hair. The solution is to use each subject as his own control.

1. Introduction

Gamma-hydroxybutyrate (GHB) is a powerful central nervous system depressant and was used in legal medicine as an anaesthetic agent prior to it being phased out because of its very steep dose–response curve and various side effects.[1,2] Since 2000, GHB has received more attention as an abused drug and a "club drug", including its use as a "date rape" drug in drug-facilitated sexual assaults (DFSA).[3–6] However, the endogenous presence of GHB, the single dose, its short half-life makes it extremely difficult to document these drugging from an analytical point of view. The victims' blood and urine samples are usually unable to provide decisive evidence of drug intake because the time between sampling and incident is too long. In this situation, hair was suggested as a valuable specimen because of its long detection window, which plays an important role in drug-assisted criminal cases, and sometimes, it even becomes the only means to provide evidence.[7–9]

Due to the presence of endogenous GHB and the fact that the endogenous and exogenous GHB levels are often of the same order of magnitude, differentiation between endogenous and exogenous GHB in hair is of great importance in forensic cases. Thus, the accumulation of a basic endogenous level of GHB in hair and the establishment of a reporting "cut-off" for endogenous GHB was suggested. Goullé et al. have reported that the average concentration of endogenous GHB in the Caucasian population is 0.90 ng/mg (n = 19, SD = 0.37 ng/mg),[10] while a study by Shen et al. showed a concentration of 1.02 ± 0.27 ng/mg (n = 20)in the Chinese population.[11] However, these data were still quite limited because of individual differences and some other factors, and therefore it is necessary to accumulate more sample data to allow the analysis of the GHB in hair to be applied in practice.

When the analysis of the GHB in hair is necessary as part of the investigation of a drug-facilitated assault, the challenge is even greater because the detection of single dose of GHB is needed. Due

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to its high polarity and low concentration in hair, GHB analysis requires analytical methods capable of the highest sensitivity. Analytical methods based on GC-MS[10,12–14] and LC-MS-MS[15–18] were developed, and the limits of detection (LOD) and quantification (LOQ) have been improving over time. Our team had already developed a GC-MS method to detect the endogenous GHB in hair, and the LOD is 0.5 ng/mg.[11] Gas chromatography-tandem mass spectrometry (GC-MS/MS) is uniquely able to eliminate background interference and perform ion-selective analysis. Therefore, it is particularly suitable when the sample has a low target concentration and high background from the matrix.

Thus, the purpose of this study was the development and validation of qualitative and quantitative procedures to detect the GHB in hair through the use of gas chromatography-tandem mass spectrometry (GC/MS/MS) and the quantitative analysis of the endogenous gamma-hydroxybutyrate (GHB) level in the hair of the Chinese population.

2. Materials and methods

2.1. Chemicals and reagents

GHB and deuterated GHB (GHB-d6) standards as well as N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS), which was used for the derivatization, were obtained from Cerilliant (Texas, USA). HPLC grade methanol was purchased from Sigma–Aldrich (Missouri, USA). Ethyl acetate, acetic acid and other chemicals were of analytical grade (China). Deionized water was purified using a Milli-Q system (Millipore, Massachusetts, USA).

2.2. Hair samples

The hair of healthy Chinese volunteers (relatives and friends of laboratory workers) was cut at the posterior-vertex region as close to the scalp as possible and stored at room temperature in an envelope. Among the volunteers were 35 males and 31 females who have no history of GHB consumption. All written consent was given by the hair donors.

2.3. Sample preparation

0–3 cm hair samples were used. Hair samples were consecutively rinsed in deionized water and acetone. After air-drying, hair samples were cut into 1-mm lengths with clean scissors, wrapped with clean paper and preserved at room temperature.

Hair segments of 20 mg were precisely weighed and transferred into 10-mL tubes. A volume of 10 μL of GHB-d6 (2 μg/mL) and 0.3 mL of 1 mol/L NaOH were added to the tubes, and then, the samples were digested at 90 °C for 10 min. After cooling, the homogenate was neutralized with 0.3 mL of HCl (1 mol/L). Then, 3.0 mL of ethyl acetate and 50 μL of acetic acid were added. After agitation and centrifugation, the supernatant layer was transferred into a glass tube and evaporated to dryness under a N_2 flow at 60 °C. Following evaporation to dryness, 20 μL of ethyl acetate and 30 μL of BSTFA were added to the residue. The derivatization with BSTFA was performed at 80 °C for 20 min. After cooling the solution at room temperature for 10 min, 1 μL of the solution was injected into the GC/MS/MS.

2.4. Instrumental procedure

The GC/MS/MS instrument consisted in an Agilent 6890 gas chromatograph (California, USA) equipped with an Agilent 7683B series autosampler (California, USA) coupled to a Quatro Micro tandem mass spectrometer (Waters Inc., USA).

The analytical column was an Agilent HP1 capillary column (30 m × 250 μm × 0.1 μm). The carrier gas (He) flow was constant at 1 mL/min. The oven temperature program was as follows: initially held at 60 °C for 2 min, increased to 100 °C at a rate of 5 °C/min, increased to 280 °C at a rate of 30 °C/min and held for 1 min at 280 °C. The total run time was 17 min, and 1 μL of the derivatized extract was injected in the split mode (split ratio of 10:1).

The mass detector was operated at 70 eV in the electron impact (EI) ionization mode. The ion source and transfer line temperatures were 220 °C and 260 °C, respectively. The collision gas was argon, and the multiple reaction monitoring (MRM) mode was used. The ions of interest were 233 > 147, 233 > 149 for GHB-TMS and 239 > 147, 239 > 149 for GHB-d6-TMS. The ion pairs at m/z 233/147 and m/z 239/147 were used for quantification. The retention times were 11.30 min for GHB and 11.19 min for GHB-d6.

2.5. Method validation

The method was fully validated according to the recommendations of Peter.[19] A standard calibration curve was obtained by adding 1(0.05 ng/mg), 3 (0.15 ng/mg), 10 (0.5 ng/mg), 20 (1.0 ng/mg), 50(2.5 ng/mg), 100 (5 ng/mg), 200 (10 ng/mg)and 300 (15 ng/mg) ng of GHB to 20 mg of synthetic melanin. Melanin was analysed to check for the absence of the analyte ions. It is acknowledged that the use of melanin may not fully substitute for the use of real hair as a matrix. However, it was not possible to find hair that was free of endogenous GHB. For the determination of the analytical limits (limit of detection (LOD) and limit of quantification (LOQ)), blank melanin samples were spiked with six calibration standards near the expected LOD. The intra-day and inter-day precision were determined at four controlled concentrations by the analysis of six aliquots of the sample. A full set of calibration standards and a blank standard were run with each analysis.

3. Results

3.1. Optimization of the method

GHB can be released completely from hair samples under alkaline digestion.[10–12] For the determination of the stability of GHB, this study compares the digestion using NaOH solutions of different concentrations (1 mol/L and 0.1 mol/L) at 90 °C, and no significant differences were found between the two digestion conditions, except that the latter required more time.

GHB is a short-chain fatty acid with a low molecular weight and strong polarity, and it is necessary to improve its chromatographic behaviour by derivation. Based on the current literature, BSTFA and ethyl acetate were found to be frequently used as derivatization reagents.[11,12,20]

The optimum conditions for derivatization were investigated using a variety of solvent compositions, derivatization times and temperatures. As shown in Fig. 1a and b, when an acetate/BSTFA volume ratio of 2:3 at 80 °C for 20 min is selected, the highest GHB peak intensity can be achieved.

3.2. Validation results

There were no interfering peaks at the retention times characteristic of GHB and the IS in the melanin matrix (Fig. 2).

The calibration curve corresponds to linear regression analysis of the peak-area ratio of GHB to the IS and the final concentration of the drug in spiked melanin. The resulting calibration curve for GHB