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原　　報

SIMPLE EXTRACTION OF GAMMA-HYDROXYBUTYRATE IN HUMAN WHOLE BLOOD BY HEADSPACE SOLID-PHASE MICROEXTRACTION

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ヘッドスペース固相マイクロ抽出を用いた全血中ガンマヒドロキシ酪酸（GHB）の簡便抽出法

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Summary

We have developed a simple method for the extraction of gamma-hydroxybutyrate (GHB) in human whole blood using headspace solid-phase microextraction (SPME). The procedure involves the conversion of GHB to gamma-butyrolactone (GBL) with acid catalysis; gamma-valerolactone (GVL) was used as internal standard (IS). After heating a vial containing a whole blood sample with GHB and IS at 80℃ for 5 min in the presence of H_3PO_4 solution, a Carboxen/polydimethylsiloxane -coated fiber was exposed to the headspace to allow adsorption of GBL and IS. The fiber needle was then injected into a capillary gas chromatography port. Although the extraction efficiency of GHB was no more than 0.68%, the calibration curve showed good linearity in the range of 10−200 μg/ml, and intra-day and inter-day assay coefficients of variation were 3.29 and 4.14%, respectively. The detection limit was about 2 μg/ml. The present SPME method for GHB is sensitive enough to be adopted in forensic toxicology and clinical pharmacology.

Key words: Solid-phase microextraction; Headspace method; Gas chromatography; Gamma-hydroxybutyrate; Gamma-butyrolactone; Gamma-valerolactone

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Introduction

Gamma-hydroxybutyrate (GHB) is an endogenous substance which is thought to be a neurotransmitter [1]. When administered as a drug, it produces diverse pharmacological effects, such as sleep induction [2], increased brain dopamine [3] and glucose levels [4], euphoric reactions [5] and stimulating the release of growth hormone and prolactin [6].

Due to these effects, GHB has been used for various purposes. In Europe it was once used as an anaesthetic, but now it is used for treatments of narcolepsy [7, 8], alcoholism [9, 10] and opiate withdrawal syndrome [11]. In the United States, it had been available as an over-the-counter medicine for inducing sleep and sold in health food stores as an aid for arriving at euphoric state, muscular development, weight loss and anti-aging, before Food and Drug Administration (FDA) of the United States banned its marketing in 1990 [12]. In spite of its serious side effects, such as dizziness, vomiting, disorientation, seizures, hypotension, bradycardia, coma and respiratory depression, GHB abuse is on the rise and is associated with driving under influence cases [13, 14] and sexual assaults [15]. Deaths caused by GHB intoxication were also reported [16–18]. Thus, simple and accurate methods to analyze GHB are needed.

Solid-phase microextraction (SPME), a new and simple extraction method, was first introduced by Arthur and Pawliszyn in 1990 [19]. Our group has applied the method to analyses of many drugs and poisons [20–24]. In this report, we present a simple method for extraction of GHB from whole blood by SPME prior to analysis by gas chromatography (GC).

Experimental

Materials

GHB sodium salt was purchased from Sigma (St. Louis, MO, USA); gamma-butyrolactone (GBL) and gamma-valerolactone (GVL) from Aldrich (Milwaukee, WI, USA). SPME devices and their five types of fiber assemblies, *i.e.*, 65 μm Carboxen/polydimethylsiloxane (CAR/PDMS), 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 85 μm polyacrylate and 65 μm Carbowax/divinylbenzene (CW/DVB) coated fibers, were purchased from Supelco Inc. (Bellefonte, PA, USA), and an Rtx-Volatiles fused silica capillary column (30 m×0.32 mm ID, film thickness 1.5 μm) from Restek (Bellefonte, PA, USA). Other common chemicals used were of the analytical grade. Whole blood was obtained from healthy volunteers.

Extraction procedure

To 0.5 ml of whole blood containing 50 μg GHB and 12.5 μg GVL, which had been placed in

a 7-ml vial with a small Teflon-coated stirring bar, were added 1.1 ml distilled water, 1.5 g Na_2SO_4 and 0.4 ml of 50% H_3PO_4. The vial was sealed with a silicone septum cap and placed on an aluminum block heater set at 80℃. After 5 min, the needle of the SPME fiber holder was passed through the septum. Then the fiber in the needle was pushed out and exposed to the headspace of the vial at 80℃ for 30 min before the fiber was pulled back into the needle. The needle containing the fiber was then quickly pulled out of the septum, and inserted into the gas chromatograph port; the fiber was again pushed out. The needle with the exposed fiber was held there for 3 min before being pulled out of the chromatograph.

GC conditions

GC analysis was performed on a Shimadzu (Kyoto) GC-14B equipped with a flame ionization detector (FID). The column temperature was held at 100℃ for 1 min after injection and then programmed to 250℃ at 10℃/min. Injector and detector temperatures were 240 and 280℃, respectively, and the helium flow rate was about 2.0 ml/min. The injection port was set in the splitless mode, and the splitter was opened 1 min after the insertion of the SPME fiber.

Results

Figure 1 shows the typical gas chromatograms. The left panel shows the authentic GBL and IS dissolved in methanol (200 ng each on-column), and the right one shows the headspace SPME extract from human whole blood containing spiked GHB (100 μg/ml) and IS (25 μg/ml). The retention times were 8.7 min for GBL and 9.3 min for IS. No endogenous GHB was detected as shown in whole blood blank (middle panel).

The extraction efficiencies for GHB were 0.68% for 10 μg/ml and 0.60% for 100 μg/ml GHB; they were calculated by comparing the peak areas for the SPME extract with that for the authentic GBL dissolved in methanol.

We tested other types of SPME fibers, such as PDMS, PDMS/DVB, polyacrylate and CW/DVB; their extraction efficiencies were less than 10% as compared with that of the CAR/PDMS-coated fiber (Fig. 2). We also tried some salts to obtain the highest efficiency; Na_2SO_4 gave higher efficiency than $CaCl_2 \cdot 2H_2O$ or NaCl. The optimum amount of Na_2SO_4 was found to be 1.5−2.0g in a vial. We tested various temperatures, such as 60, 65, 70, 75, 80, 85, 90 and 95℃, and got the best GHB efficiency at 80℃ (data not shown).

The calibration curve was drawn by plotting six different points in the range of 10−200 μg/ml. It gave good linearity, and the equation was $y = -0.00102 + 0.00926\ x$ with the r value of 0.995. The detection limit of GHB was about 2 μg/ml.

The coefficients of variation (CVs) for intra-day measurements (n=4) were 2.67% (20 μg/ml

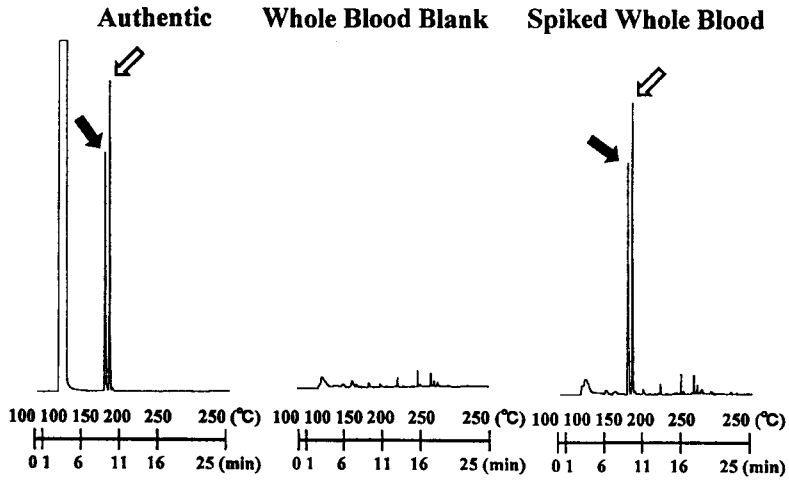

Fig. 1. Gas chromatograms for 200 ng of the non-extracted authentic GBL (filled arrow) and IS (open arrow) dissolved in methanol with direct injection (left panel), the headspace SPME extracts from human whole blood containing spiked 100 μg/ml of GHB (filled arrow) and 25 μg/ml of IS (open arrow) (right panel) and whole blood blank (middle panel).

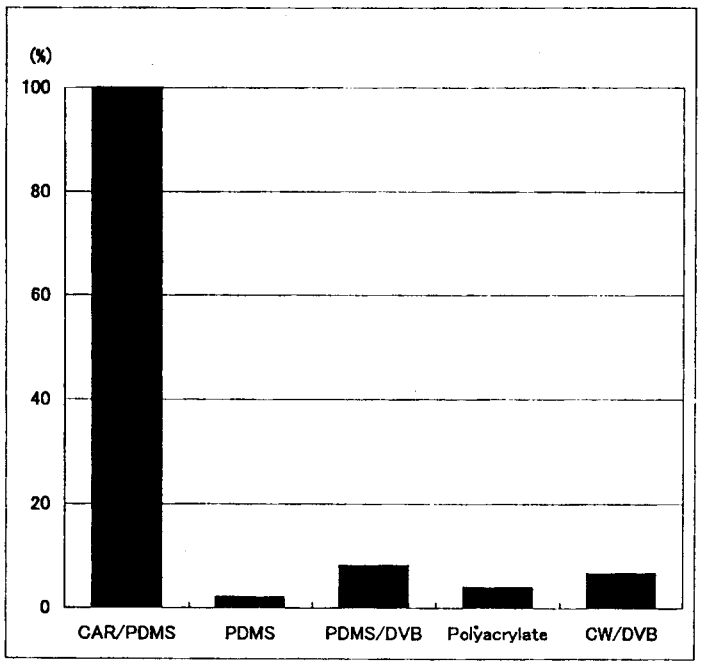

Fig. 2. Comparison of five SPME fiber coatings, *i.e.*, Carboxen/polydimethylsiloxane (CAR/PDMS), polydimethylsiloxane (PDMS), polydimethylsiloxane/divinylbenzene (PDMS/DVB), polyacrylate and Carbowax/divinylbenzene (CW/DVB) for extraction of GHB from whole blood (100 μg/ml). Each SPME fiber was exposed to the headspace of the sample solution for 30 min at 80℃. The amount of GHB extracted by the CAR/PDMS fiber was set at 100%.

GHB) and 3.29% (100 μg/ml GHB), and inter-day CVs (n=4) were 4.14% (20 μg/ml GHB) and 3.95% (100 μg/ml GHB).

Discussion

In this report, we have presented a simple method for extraction of GHB in human whole blood using SPME. Owing to the high polarity of GHB, it was converted to GBL by acid catalysis before extraction in many methods [25−30]. In some reports, the extraction of GHB is followed by derivatization for GC analysis to detect GHB without such conversion [14, 31−35].

It was reported that there were no significant levels of endogenous GBL in blood [28]; there was no enzymatic or chemical formation of GBL formation from GHB [28, 29]. Hence we have adopted the conversion technique by acid catalysis to avoid complicated preparations.

Very recently, Aspelund *et al.* [36] have demonstrated a method of GHB analysis using SPME with a Carbowax/templated resin-coated fiber and capillary gas chromatography with FID. The detection limit of our method is almost comparable to that of theirs.

It was reported that 33 and 34 μg/ml blood concentrations of GHB were associated with driving under influence (DUI), 130−220 μg/ml associated with toxic effects [14] and 300 μg/ml associated with lethal effects [18]. Thus the present method is sufficiently applicable to the measurements of the drug at therapeutic or toxic levels.

In conclusion, our method has proved to be recommendable for actual clinical and toxic cases, because of its simplicity, accuracy and sufficient sensitivity.

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