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SHORT COMMUNICATION

Simultaneous determination of borneol and its metabolite in rat plasma by GC–MS and its application to pharmacokinetic study

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KEYWORDS

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Abstract A gas chromatography mass spectrometry (GC–MS) method has been developed and fully validated for the simultaneous determination of natural borneol (NB) and its metabolite, camphor, in rat plasma. Following a single liquid–liquid extraction, the analytes were separated using an HP-5MS capillary column (0.25 mm × 30 m × 0.25 μm) and analyzed by MS in the selected ion monitoring mode. Selected ion monitor (*m/z*) of borneol, camphor and internal standard was 95, 95 and 128, respectively. Linearity, accuracy, precision and extraction recovery of the analytes were all satisfactory. The method was successfully applied to pharmacokinetic studies of NB after oral administration to Wistar rats.

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1. Introduction

Borneol (Fig. 1A), usually used as an important adjuvant in "Su-Xiao-Jiu-Xin-Wan", "Su-He-Xiang-Wan", and "Liu-Shen-Wan" for preventing and curing cardiovascular and cerebrovascular diseases [1,2]. It is classified into natural borneol (NB) and synthetical borneol (SB). Because SB is much easier to get and cheaper than NB, it has been widely used in Chinese formulas. But due to its unstable quality in storage, it can be transformed into camphor and result in higher reproductive toxicity [3–5]. Camphor is reported to cause seizures, respiratory failure, myocarditis and hepatotoxicity; fetuses and infants are specially susceptible to camphor [6–8]. Therefore, NB is becoming more and more widely used. However, there are few reports about its in vivo study, and let alone its metabolite.

Several papers about the pharmacokinetic study of NB have been reported. These results indicate that camphor is the main metabolite of NB, too [9–13]. Therefore, it is necessary to study the pharmacokinetics of camphor after oral administration of NB and to discuss the conversion ration from NB to camphor in vivo for the purpose of safety. In this paper, we hereby developed a systematic gas chromatography mass spectrometry (GC–MS) method for the simultaneous determination of borneol and camphor in rat plasma. It was successfully applied to the pharmacokinetic study of NB in Wistar rats.

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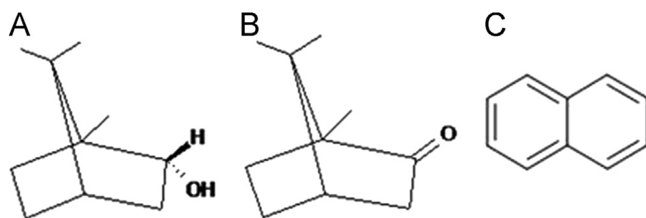

Fig. 1 Structures of borneol (A), camphor (B), and naphthalene (C).

2. Experimental

2.1. Chemicals and reagents

NB reference standard (purity >99.9%) and camphor (purity >99.3%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Naphthalene (purity ≥98%, internal standard, IS, Fig. 1C) was purchased from Damao Chemical Reagent Factory (Tianjin, China). NB (99.4% purity, batch no. 20060800) was purchased from Linke NB Factory (Jiangxi, China) for animal experiments.

2.2. Apparatus and operating conditions

A Thermo DSQ IITrace GC–MS system (Thermo Scientific, USA) with Xcalibular station was used in the study. GC separation was accomplished on HP-5MS column (30 m × 0.25 mm i.d., 0.25 μm particle size). The inlet temperature was set at 210 °C. The oven temperature program was started at an initial temperature of 80 °C, lasting for 1.0 min, then increased to 145 °C at 30 °C/min and retained for 3.0 min. Samples were injected in the split mode (1/10), using helium as carrier gas (99.9%, 1 mL/min). In the MS system, both the ion source temperature and inlet temperature were 280 °C. Electron impact ionization (EI) mode was used with nominal electron energy (70 eV). Selected ion monitor (SIM, *m/z*) of NB, camphor and IS was 95, 95 and 128, respectively. Processing data were acquired from 3.00 to 6.18 min.

2.3. Preparation of standard and quality control (QC) samples

The stock solutions of NB (100 μg/mL), camphor (100 μg/mL) and IS (100 μg/mL) were prepared in hexane. A series of working solutions were obtained by diluting stock solutions with hexane. The IS working solution (100 ng/mL) was prepared by diluting stock solution with hexane. All solutions were stored at 4 °C and shielded from light.

Appropriate amounts of NB and camphor working solutions were added to 100 μL blank plasma to yield effective concentration in plasma. Effective concentrations in plasma samples were 15, 30, 150, 300, 1500, 3000 and 4000 ng/mL for NB, while 30, 60, 150, 300, 1500, 3000 and 4000 ng/mL for camphor. The QC samples used in the validation and during the pharmacokinetic study were prepared in the same way as the calibration standard. The three QC concentrations of NB (camphor) were 30 (60), 300 (300) and 3000 (3000) ng/mL.

2.4. Sample preparation

20 μL IS (100 ng/mL) working solution and 130 μL hexane were added to an aliquot of 100 μL rat plasma sample; the mixture was then vortex-mixed for 3 min. Then 100 μL supernatant was transferred to glass conical inserts after centrifugation for 10 min at 15,000 rpm (4 °C) and placed into 2 mL brown autosampler vials (22 °C) with screwtop caps and PTFE septa. One microliter of supernatant was injected into the GC–MS system.

2.5. Method validation

2.5.1. Selectivity

The selectivity of the method was investigated by comparing chromatograms of blank plasma, blank plasma sample spiked with standard at lower limit of quantification (LLOQ) and plasma sample at 0.25 h after oral administration of NB (90 mg/kg).

2.5.2. Linearity and LLOQ

The calibration curve consisted of six concentration levels. Each of these samples was prepared and assayed in triplicate on three separate days. The calibration curve was obtained by plotting the peak area ratio (Y) of the analyte to IS versus the nominal concentration (X) of either NB or camphor. These curves were described as $y=a+bx$ ($1/x^2$ weighted). LLOQ was defined as the lowest concentration whose precision (expressed as relative standard deviation, RSD) and accuracy (expressed as relative error, RE) were both ≤20%.

2.5.3. Accuracy and precision

The accuracy and precision of the method were assessed by analyzing three QC levels samples each in six replicates on three consecutive days. The inter- and intra-day precision is expressed as the relative standard deviation (RSD). Accuracy is defined as the relative error (RE) and is calculated using the formula RE (%)= [(measured value − theoretical value)]/theoretical value × 100%. Both accuracy (RE) and precision (RSD) were expected to be within ±15% as being acceptable.

2.5.4. Extraction recovery

Extraction recoveries of NB and camphor were assessed by comparing the peak responses from QC samples to those from post-extracted samples. QC samples were prepared according to previous descriptions; post-extracted samples were prepared as follows: blank plasma samples were extracted and then spiked with standard solutions to yield corresponding equivalent concentrations. Recoveries were tested at three QC levels each in five replicates and should be stable.

2.5.5. Stability

Samples at two concentrations levels (150 ng/mL and 3000 ng/mL for NB and camphor) were used in the stability test containing pre-treatment, post-treatment, three cycles of freeze–thaw, and long-term stabilities of the analytes. Pre-treatment stability was assessed by leaving samples at room temperature for 3 h before extraction. For post-treatment assessment, all processed samples were placed into autosampler (22 °C) for 10 h before injection. Freeze–thaw cycle plasma stability test was carried out via repeatedly freezing–thawing samples for three cycles before extraction. Long-term stability was evaluated by storing samples at −20 °C and −80 °C for 24 h before thawing and extraction.

2.5.6. Pharmacokinetic application of the GC–MS method

The study was approved by the Animal Ethics Committee of Guangdong Province. Six male Wistar rats weighing 200±20 g

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