Pharmacokinetic Study of Borneol and Menthol in Rats after Oral Administration of Qingyan Drop Pills

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Key words
- borneol
- menthol
- simultaneous determination
- pharmacokinetics
- gas chromatography

Abstract

Both borneol and menthol are bioactive substances derived from Chinese herbal medicines. In order to understand the pharmacokinetics of borneol and menthol in Qingyan drop pills, a rapid, sensitive, and simple gas chromatographic (GC) method with flame ionization detection (FID) was developed for the simultaneous determination of borneol and menthol in rat plasma. Sample preparations were carried out by liquid-liquid extraction (LLE) with an internal standard solution of naphthalene. The analytes and internal standard (IS, naphthalene) were separated well on an HP-1 capillary column. The pharmacokinetic parameters were estimated by a compartmental method using the Phoenix WinNonlin software program (Version 6.0). The standard curves were linear over a wide concentration range of 2.5–50.0 ng/μL ($r = 0.9963$), 8.7–62.2 ng/μL ($r = 0.9994$) for both borneol and menthol in plasma, respectively. The limits of quantification (LOQ) of borneol and menthol in plasma were 2.4 ng/μL and 5.0 ng/μL, respectively. The intra-day precisions for borneol and menthol were < or = 10.0% R.S.D. at the LOQ and < or = 6.0% at higher concentrations. The average value of C_{max} was 18.97 ± 2.71 ng/μL with a T_{max} at 20.00 ± 0.00 min for borneol after oral administration of the drop pills; for menthol, the average value of C_{max} was 79.02 ± 11.40 ng/μL with a T_{max} at 25.00 ± 4.40 min. This validated assay method was successfully applied to a pharmacokinetic study of borneol and menthol after oral administration of Qingyan drop pills in rat. The results showed that the kinetics of borneol and menthol can be described by an open one-compartment model. The pharmacokinetic parameters provide some information for clinical administration of Qingyan drop pills.

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Bibliography
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Introduction

Medication can exert its therapeutic effect on the living body through different dosage forms. Proper dosage forms should be selected on the basis of medicamentous properties and goals of treatment. Drop pills of Chinese herbal medicines, developed in recent years, are a novel dosage form which results from the application of contemporary preparation technology; its high oral absorption rate and bioavailability can quickly alleviate the symptoms of the patients.

For example, Qingyan drop pills are Chinese formulated products in a novel form of medication, which is composed of several Chinese medicinal herbs, including myrobalan, bezoarbovis, indigo naturalis, and licorice, and two other volatile chemical components, borneol and menthol. It was found that Qingyan drop pills exhibit therapeutic activities, including detumescence, alleviating pain, desintoxication, relieving sore throat, and expelling wind, which are widely used in treating acute and chronic inflammation in clinical practice. It has also been reported that the pills can exert anti-inflammation and moderate antiblastic effects on *Staphylococcus aureus, Candida albicans, Escherichia coli,* and *Klebsiella pneumoniae* [1,2].

Borneol occurs naturally in monoterpene alcohols. Some studies showed that borneol could improve some drugs oral bioavailability, accelerate the passage of the blood–brain barriers (BBB), and enhance the distribution of drugs in brain tissue [3,4]. Studies also indicated that borneol has some antibacterial activity, anti-inflammatory action, and analgesic effect, among others [5]. Menthol is a monocyclic terpene alcohol that occurs naturally in more than 100 essential oils, including spearmint and peppermint. Several studies demonstrated that menthol has anti-inflamma-

tory, analgesic, and cooling and relieving itching effects, as well as other properties such as relieving cough [6, 7]. The characteristic minty aroma and cooling qualities of synthetic *d,l*-menthol and the natural *l*-menthol isomer have resulted in their use in a variety of commercial products such as toothpaste, foods, and oral pharmaceutical preparations. Drugs that contain menthol are widely used for the treatment of symptoms caused by the common cold.

Borneol and menthol are aroma ingredients which are commonly employed in Chinese *Fufang*. *Fufang* in Chinese herbal medicine means a formula comprising multiple herbs. In a Qingyan drop pill, the content of borneol and menthol were 3.04 mg and 0.43 mg, respectively. Although several analytical methods [8–11] were used to measure the concentration of borneol in the plasma such as, GC-MS^n, GC-MSD, GC-FID, to date, there are few reports on the simultaneous determination and pharmacokinetics of borneol and menthol in Chinese *Fufang*. From this point of view, the aim of the present study is to establish an analytical method to simultaneously determine borneol and menthol in rat plasma after oral administration of Qingyan drop pills and to apply the method to a preliminary pharmacokinetic study. The pharmacokinetic parameters can provide some information for the clinical administration of Qingyan drop pills.

Materials and Methods

Chemical and reagents

Qingyan drop pills, borneol (99.9% purity), and menthol (99.9% purity) were kindly donated by Tianjin Zhongxin Pharmaceuticals; naphthalene (internal standard, IS; 99.9% purity) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The other reagents and solvents were of analytical grade and obtained from Tianjin Chemical Company.

Animals

Male rats weighting 230–250 g were obtained from the Chinese Academy of Medical Sciences, Institute of Radiation Medicine. All animals were clinically healthy and biochemically normal throughout the experimental period. Food, but not water, was withheld for 10 h before drug administration. All animal experiments were based on the guiding principles in the care and use of animals and approved by the ethical committee of the Tianjin Traditional Chinese Medicine University.

Instruments and chromatographic conditions

The GC-FID system consisted of an Agilent 6890N gas chromatographic (GC) system, a flame ionization detector (FID), and Agilent Chemstation software for data analysis. Separation of borneol, menthol, and naphthalene was performed on an HP-1 capillary column (30 m × 0.25 mm × 0.25 µm). Nitrogen, at a flow rate of 30 mL/min, was used as the carrier gas. The oven temperature was kept at 110 °C for 13 min and then increased to 200 °C by post run for 6 min. Injection port temperature was 250 °C; detector temperature was maintained at 250 °C. One µL of samples were injected automatically.

Preparation of stock solution and calibration standard solution

The reference standards (borneol 6.3 mg, menthol 12.5 mg) were accurately weighed into a 25-mL volumetric flask and dissolved in a mixed solution with *n*-hexane : dichloromethane (9 : 1 v/v) to make a stock solution. A series of standard solutions were obtained by further dilution of the stock solution with *n*-hexane : dichloromethane (9 : 1 v/v). The IS solution was prepared in *n*-hexane : dichloromethane (9 : 1 v/v) at a concentration of 95.0 ng/µL. 28 µL of the standard solution and 20 µL of sodium chloride solution were transferred into 150 µL of blank rat plasma to obtain two calibration standards in concentrations of 2.5, 8.8, 12.5, 17.5, 22.5, 37.5, 50.0 ng/µL borneol and 8.7, 12.4, 14.9, 17.4, 22.4, 37.3, 62.2 ng/µL menthol.

Preparation of samples

150 µL of each plasma sample were transferred to a 1.5-mL polyethylene centrifuge tube. 20 µL of the sodium chloride solution and 28 µL of the IS solution were added together to each plasma sample and vortex mixed for approximately 0.5 min. 550 µL mixed solution of *n*-hexane : dichloromethane (9 : 1 v/v) were added to each plasma sample and vortex mixed for 5 min. The denatured protein precipitate was separated by centrifugation at 12 000 rpm for 10 min. After centrifugation, the upper organic phases were collected and evaporated to dryness under a stream of nitrogen flow at 30 °C. The dried residue obtained was dissolved in 100 µL mixed solution of *n*-hexane : dichloromethane (9 : 1 v/v), vortex mixed for 1 min, centrifuged at 12 000 rpm for 5 min, and 1 µL of the supernatant was directly injected into the GC-FID apparatus for analysis.

Validation of assay method

The specificity of the method was investigated by comparing the FID chromatograms of blank plasma; plasma spiked with IS (17.7 ng/µL), borneol (12.5 ng/µL), and menthol (37.3 ng/µL); and a plasma sample 10 min after oral administration of the Qingyan drop pills (see **Fig. 1**).

The linearity of each calibration curve was determined by plotting the peak-area ratio (*Y*) of the analyte to IS versus the nominal concentration (*X*) of either borneol or menthol. The intraday accuracy and precision of borneol and menthol determinations were analyzed by performing the determination three times in the same day at concentrations of 3.5, 15.5, and 50.0 ng/µL for borneol and 8.7, 17.4, and 62.6 ng/µL for menthol. Assay precision was expressed as the relative standard deviation (RSD) (coefficient of variation). Accuracy (expressed as percent of nominal values) was determined by comparing the concentration calculated from the calibration curve with the known concentration (see **Table 1**).

The recoveries of borneol and menthol

The extraction recovery was determined by comparing the peak areas obtained from quality control (QC) samples subtracting blank plasma with the standard working solutions at the same concentrations (see **Table 2**).

Pharmacokinetic study of borneol and menthol in rat

Twenty male rats were fasted for 10 h with free access to water. 30 of the Qingyan drop pills were dissolved in 10 mL distilled water and then orally administered to the rats (drug equivalent to borneol 173 mg/kg bw and menthol 693 mg/kg bw). After oral administration, rats were killed after blood collection at time in-

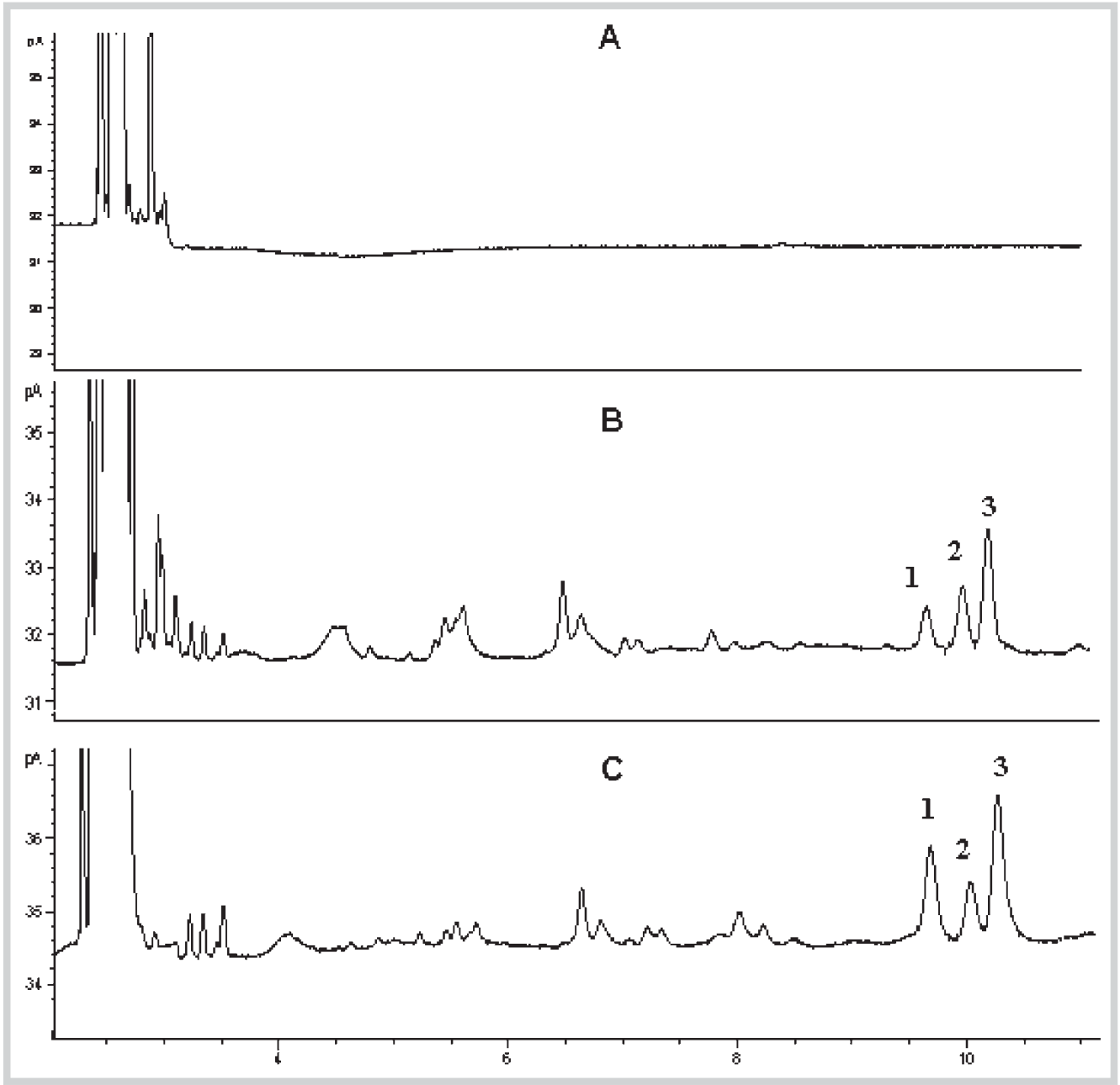

Fig. 1 Typical gas chromatograms of blank rat plasma (**A**), plasma spiked with IS (17.7 ng/μL), borneol (12.5 ng/μL), and menthol (37.3 ng/μL) (**B**), and a plasma sample 10 min after oral administration of the Qingyan drop pills (**C**) (1. IS; 2. borneol; 3. menthol).

Table 1 Intra-day precision of borneol and menthol in rat plasma (n = 3).

	Concentrations (ng/μL)	$\bar{X}$ ($A_{borneol}/A_{naphthalene}$)	RSD (%)
Borneol	3.5	0.22	5.12
	15.5	0.48	4.13
	50.5	1.37	6.93
Menthol	8.7	0.17	5.24
	17.4	0.41	4.26
	62.2	1.23	3.43

tervals of 5, 10, 20, 30, 35, 50, 60, 90, and 120 min, respectively. Plasma samples were collected after centrifugation at 3000 rpm for 10 min and immediately prepared as described above.

Peak concentration (C_{max}) and peak times (T_{max}) of borneol and menthol were derived directly from the experimental points. The other pharmacokinetic parameters were estimated by a compartmental method using the Phoenix WinNonlin software program (Version 6.0; Pharsight Corp.).

Results

Under the chromatographic conditions described above, optimized separation and detection conditions were achieved in plasma. The chromatograms (**Fig. 1**) were free of interference from other compounds and showed retention times of 10.0 min and 10.8 min for borneol and menthol, respectively. In conclu-

Table 2 Recoveries of borneol and menthol in rat plasma (n = 3).

	Concentrations (ng/μL)	Recovery (%)	Mean ± SD
Borneol		78.7	
	3.5	78.0	77.42 ± 1.60
		75.7	
		81.4	
	15.5	82.4	82.11 ± 0.71
		82.6	
		86.2	
	50.0	86.6	86.83 ± 0.62
		87.4	
Menthol		72.0	
	8.7	72.4	72.14 ± 0.32
		71.9	
		83.1	
	17.4	82.7	83.12 ± 0.41
		83.5	
		87.9	
	62.6	88.4	88.54 ± 0.70
		89.3	

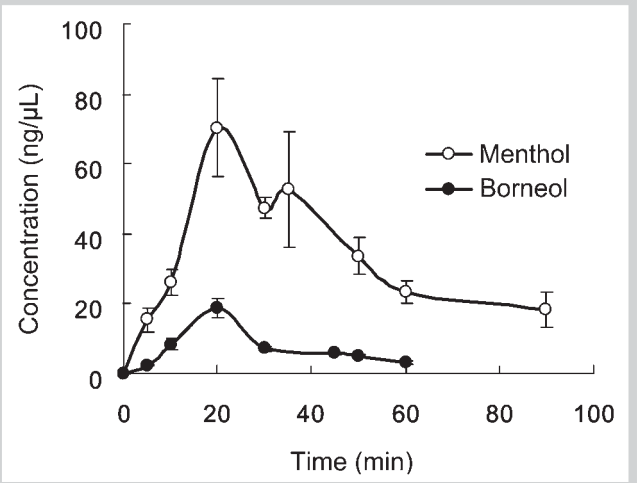

Fig. 2 Mean concentration-time profiles of borneol and menthol in rat plasma after oral administration of Qingyan drop pills equivalent to 173 mg/kg bw borneol and 638 mg/kg bw menthol. Each point represents the mean ± SEM of five experiments.

sion, these observations indicated that the specificity of the assay was adequate.

Under the experimental conditions used, the lower limit of detection (LOD) of borneol in plasma was 0.9 ng/μL at a signal-to-noise ratio of 2.8, and for menthol 1.4 ng/μL at a signal-to-noise ratio of 3.2. The limit of quantification (LOQ) of borneol was found to be 2.4 ng/μL, and for menthol, 5.0 ng/μL.

The standard curves for both borneol and menthol in the plasma were linear in the range of 2.5–50.0 ng/μL and 8.7–62.2 ng/μL, respectively. The calibration line of borneol was $Y = 24.69\ X_1 + 0.1554$ with $r = 0.9963$. For menthol, the calibration line was $Y = 20.34\ X_2 + 0.0078$ with $r = 0.9994$.

The summary of intraday precision at low, medium, and high concentrations of borneol and menthol is shown in **Table 1**. These data indicate that RSDs are less than 10%.

The extraction recoveries of both borneol and menthol in plasma were determined by comparing peak areas from plasma samples with those from standard solutions at the same levels. The summary of the mean recoveries is presented in **Table 2**. The results show that the extraction recoveries of borneol ranged from 77.4% to 86.7% and from 72.1% to 88.6% for menthol.

The plasma profile of borneol and menthol after oral administration to rats is shown in **Fig. 2**. It is demonstrated that the absorption of both borneol and menthol is rapid, with an absorption rate constant (k_a) of 0.09 ± 0.01 L/min and 0.08 ± 0.01 L/min for borneol and menthol, respectively.

The average value of C_{max} was 18.97 ± 2.71 ng/μL with a T_{max} at 20.00 ± 0.00 min for borneol after oral administration of drop pills; for menthol, the average value of C_{max} was 79.02 ± 11.40 ng/μL with a T_{max} at 25.00 ± 4.40 min. All the results are expressed as mean ± SEM (see **Table 3**).

Table 3 Pharmacokinetic parameters of borneol and menthol in rat plasma after oral administration of Qingyan drop pills (n = 5).

Parameters	Borneol	Menthol
	Estimate (mean ± SEM)	Estimate (mean ± SEM)
$t_{1/2\alpha}$ (min)	10.81 ± 1.30	23.10 ± 1.20
$AUC_{0-\infty}$ (mg/L · min)	494.32 ± 0.31	3235.23 ± 224.62
Vd/F (L)	1.05 ± 0.15	3.92 ± 0.31
CL/F (L/min)	0.07 ± 0.02	0.12 ± 0.03
C_{max} (ng/μL)	18.97 ± 2.71	79.02 ± 11.40
T_{max} (min)	20.00 ± 0.00	25.00 ± 4.40
k_e (1/min)	0.07 ± 0.02	0.03 ± 0.00
k_a (1/min)	0.09 ± 0.01	0.08 ± 0.01

Discussion

Qingyan drop pills are one of the widely used Chinese *Fufang* preparations for the treatment of pharyngitis and are composed of several Chinese medicinal herbs or their components, including bezoarbovis, borneol, menthol, myrobalan, licorice, and indigo naturalis. They have a rapid effect and a low dose of the medicaments is needed. Qingyan drop pills can produce effects which include relieving fever, analgesia, desintoxication, relieving sore throat, and expelling wind, and which are commonly needed for the treatment of acute and chronic inflammation in the clinical practice. Therefore, it is important to guide clinical medication by determining the disposition kinetics of the multiple active components of this Chinese *Fufang* preparation.

Kinetic changing rules of borneol and menthol (as volatile components) from Qingyan drop pills in the rat body were investigated in the present study. From the pharmacokinetic parameters, we found that the kinetics of an open one-compartment model was fitted to both borneol and menthol in the rat plasma concentration-time curve, which reached the maximum plasma concentration at 20.00 ± 0.00 and 25.00 ± 4.40 min, respectively. According to the drug plasma half-life ($t_{1/2\alpha}$), elimination rate constant (k_e, 1/min), and absorption rate constant (k_a, 1/min), it could be concluded that the absorption and elimination of borneol and menthol in rat body was rapid, which is consistent with the report by Kohlert et al. [12]. The plasma concentration of borneol and menthol could not be detected after 1 h and 1.5 h oral administration of Qingyan drop pills, respectively. Although the oral dose of menthol was only fourfold higher than that of borneol, the value of the area under the plasma concentration to

the time curve of menthol was fivefold higher than that of borneol. On one hand, the difference is probably related to the *in vivo* absorption characteristics of borneol and menthol. On the other hand, borneol presumably plays a certain role in promoting the absorption of menthol. Consequently, it was concluded that a drug interaction may have occurred during the absorption of the multiple components of the Chinese *Fufang* preparation.

Sample preparation played a key role for the determination of drugs in biological samples. At the beginning of the work, the organic solvent was evaporated after liquid-liquid extraction; however, the long drying process caused a significant loss of borneol and menthol due to the low volatility of the extraction liquid acetoacetate. Because of its high extraction efficiency and volatility, as well as the good recovery rate, we selected a mixed solvent of *n*-hexane and dichloromethane as solvent system, their best ratio (*n*-hexane : dichloromethane) being 9 : 1 (v/v). The amount of medicaments contained in the drop pills is small, so the plasma concentration of medicaments was low. In order to determine the plasma concentration, it was necessary to decrease the loss during the operation. Therefore, operation with controlled temperature was important for the sample preparation due to the low dose and volatility. An internal standard was necessary for the determination of analytes in biological samples and the measurement of GC. To enhance the reliability of the determination of medicaments in biological samples, we found that naphthalene was optimal for our work and selected it as the internal standard. A rapid, sensitive, and simple GC method using FID has been developed for the simultaneous analysis of borneol and menthol in rat plasma. The present study illustrated the pharmacokinetic process of borneol together with menthol in Qingyan drop pills in the rat body and provided important information for rational clinical administration of this medication.

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