Determination of Camphor and Borneol in *Flos Chrysanthemi Indici* by UAE and GC–FID

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Abstract

In the work, ultrasonic-assisted extraction (UAE) followed by gas chromatography with flame ionization detector (GC–FID) is developed for the quantitative analysis of the bioactive components of camphor and borneol in a traditional Chinese medicine (TCM) of *Flos Chrysanthemi Indici*. The extraction parameters are investigated. The optimum extraction conditions found are: solvent, methanol; solvent to sample ratio, 12:1 (v/w); extraction time, 15 min. Camphor and borneol are determined using this extraction method in *Flos Chrysanthemi Indici* samples from 5 different growing areas. The relative standard deviation values for camphor and borneol are 8.4% and 5.6%, respectively. The recoveries for camphor and borneol are 89% and 95%, respectively, and the method detection limits are lower than 0.23 µg/mL. To demonstrate the method feasibility, steam distillation is also used to analyze camphor and borneol in *Flos Chrysanthemi Indici* samples from these different growing areas. The statistical comparison by t-test (95% confidence level) showed no significant difference between these results. It has been shown that the proposed UAE–GC–FID is a simple, rapid, and reliable method for quantitative analysis of camphor and borneol in TCM, and a potential tool for quality assessment of *Flos Chrysanthemi Indici*.

Introduction

Traditional Chinese medicines (TCMs) are invaluable drug resources. Due to their high pharmacological activity, low toxicity, and rare complications, they have been used in the clinical therapy of many diseases for several thousand years (1). In recent years, more and more interest has been placed on this field. *Flos Chrysanthemi Indici*, anthotaxy of *Chrysanthemum indicum* L. (Asteraceae) is used as a heat-clearing and detoxification herb. It can also inhibit the agglutination of blood platelet and promote the myocardial blood circulation and white cell phagocytosis; thus, it has been used to treat many diseases, such as furuncle and skin nodules (2,3). Lately, it has been found to show inhibitory activity against nitric oxide (NO) production in lipopolysaccharide-activated macrophages as well (4). Camphor and borneol are the main active components present in the essential oil of *Flos Chrysanthemi Indici*, which play an important role in the drug effect of the TCM (5–13). Recently, Zhu et al. demonstrated that the two compounds have significant antimicrobial activity (14).

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Before the volatile fractions in TCM can be analyzed, they have to be extracted from the matrix. Various extraction techniques can be used [e.g., hydro-distillation (steam distillation), Soxhlet extraction, and solvent extraction (15–20)]. However, these conventional techniques require a long time and large amount of organic solvents for the extraction of the TCM essential oils. Moreover, losses of some volatile compounds, low extraction efficiency, and toxic solvent residues in the extract may be encountered using these extraction methods. These shortcomings have led to the consideration of the new techniques for volatile oil extraction, which typically use less solvent, time, and energy, such as supercritical fluids, ultrasound, and microwave extraction. There has recently been widespread interest in the application.

Modern techniques, such as ultrasonically assisted extraction (UAE), can be used to get bioactive components from plants (21,22). Ultrasounds produce cell disruption, particle size reduction, and ultrasonic jet towards a solid's surface leading to a greater contact area between solid and liquid phase, and better access of solvent to valuable components, compared with traditional methods (23). This type of extraction has been applied to biological matrices such as plant materials (24,25) and even animal tissues (26). The application of supersonic technique to plants has produced satisfactory results (27).

In this work, UAE followed by gas chromatography with flame ionization detector (GC–FID) was developed for quantitative analysis of camphor and borneol in the TCM of *Flos Chrysanthemi Indici*. The experimental parameters were studied, and the method precision, recovery, detection limit, and linearity were investigated. The method was tested by the application to the determination of camphor and borneol in *Flos Chrysanthemi Indici* samples.

Experimental

Reagent and samples

Analytical grade acetone, methanol, ethyl acetate, methylene chloride, camphor, and borneol were obtained from the National Institute for the Control of Pharmaceuticals and Biological Products (Beijing, China). Stock solutions (5.0 mg/mL) of the two standards were prepared by dissolving camphor and borneol into ethyl acetate. Decane, obtained from Supelco (Bellefonte, PA), was used as the internal standard (IS) in the study, for it was not detected in the samples of *Flos Chrysanthemi Indici*. The IS solution with a concentration of 10 mg/mL was made in ethyl

acetate. Calibration solutions ranged from 0.1 to 2000 µg/mL and were prepared by the dilution of the stock solution with ethyl acetate, and 100 µg of IS was added to each solution. The dry *Flos Chrysanthemi Indici* samples from 5 growing areas (Hubei, Fujian, Anhui, Henan, Jiangxi) in China were purchased from Lengyingshang (Shanghai, China). After being ground to fine powder with particle size of 120 mesh, the TCM samples were used in the study.

UAE procedure

An ultrasonator model SB3200 (Shanghai Branson Ultrasound Co. Ltd., China) was used in ultrasonic extraction procedure. To begin with, 0.5 g *Flos Chrysanthemi Indici* sample and 6-mL methanol were put into a 10-mL glass vial, then the vial was sealed with an aluminum cap with PTFE-silicon septa. The vial with sample was sonicated with continuous power for 15 min in a water bath in the sonicator, whose depth of immersion is 5 cm. The solution of extracting liquid was filtered, and the filtrate was collected for GC–FID analysis.

Steam distillation

Fifty grams of *Flos Chrysanthemi Indici* was ground to fine powder, and then put into a 1000-mL distillation flask. Five hundred mL of distilled water and 10-mL ethyl acetate was added, and a volatile oil distillation apparatus was set according to the Chinese pharmacopoeia (Chinese Pharmacopoeia Committee Publishing House of People's Health, 2000, Appendix 64). The mixture was distilled for 6 h. Oil was collected from the condenser dried over anhydrous sodium sulfate.

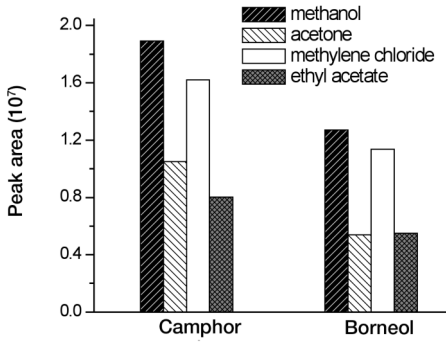

Figure 1. Effect of extraction solvent on the peak areas of the two active compounds (camphor and borneol) in *Flos Chrysanthemi Indici*.

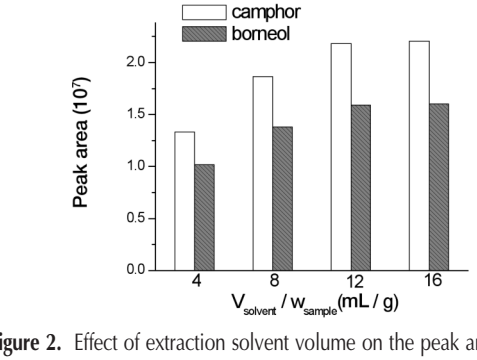

Figure 2. Effect of extraction solvent volume on the peak areas of the two active compounds (camphor and borneol) in *Flos Chrysanthemi Indici*.

Instruments

Analyses of camphor and borneol in the extracts were performed using a GC system CP-3800 (Varian, Palo Alto, CA) with FID. The GC–FID system was equipped with a CP-8510 capillary column (15 m × 0.25 mm i.d., 0.25 µm film thickness), and nitrogen (99.999%, purity) was used as the carrier gas at 1 mL/min flow rate. The injection port temperature was set at 250°C, and the detector temperature was 300°C. The injector was operated in splitless mode and 1 µL of concentrated extract was injected. The oven program was as follows: initial temperature, 60°C (hold for 1 min), ramp at 12°C/min up to 160°C; then ramp up at 30°C/min to 300°C.

Linearity, precision, recovery, and detection limit

To obtain the method linearity, replicate analyses of calibration solutions (from 0.1 to 2000 µg/mL) spiked with 20 µg/mL decane (IS) were directly analyzed with the GC–FID system. The method precision was studied by five replicate analyses of camphor and borneol in the *Flos Chrysanthemi Indici* sample from Hubei by UAE. The relative standard deviation (RSD %) was calculated on the basis of the peak areas. Recovery was also investigated by adding 20 µL of standard stock solution (5.0 mg/mL) and 10 µL IS solution (10 mg/mL) to a *Flos Chrysanthemi Indici* sample (500 mg) containing known amounts of camphor and borneol. The peak height of each compound of the lowest concentration was used to calculate the detection limit (S/N = 3) of this work. Triplicate measurements were performed by UAE–GC–FID.

Results

Optimization of ultrasonic extraction

The effects of organic extraction solvent, solvent volume, and extraction time of UAE were investigated in the study. *Flos Chrysanthemi Indici* samples (0.5 g) from Hubei were treated through the UAE method described earlier. The extraction efficiency of camphor and borneol in the sample were investigated, and the analytical results obtained by GC–FID measurement under different conditions were compared to obtain the optimum extraction conditions.

Selection of extraction solvent

As solvents, acetone, methanol, ethyl acetate, and methylene chloride were tested to extract camphor and borneol from *Flos Chrysanthemi Indici* under sonication [extraction time: 30 min; solvent/sample ratio: 8/1 (v/w)]. The results are shown in Figure 1. As seen from Figure 1, we can find that methanol has the best extraction efficiency of camphor and borneol. Therefore, methanol was chosen as the best solvent in the following extraction experiments.

Selection of extraction solvent volume

In general, a larger solvent volume can dissolve constituents more effectively, leading to an enhancement of the extraction yield. The influence of solvent volume on the extraction efficiency of camphor and borneol from *Flos Chrysanthemi Indici* was evaluated (Figure 2). The results indicated that an

increase of extraction efficiency of camphor and borneol could be observed with the increase of the ratio of solvent volume/sample mass. Hence, the extraction efficiency did not increase when the ratio of the solvent volume/sample weight increased from 12 to 16. So, the solvent volume/sample weight ratio of 12 was selected for the following work.

Selection of extraction time

The influence of extraction time on the extraction efficiency of camphor and borneol from *Flos Chrysanthemi Indici* is shown in Figure 3. The results indicate that the extraction amount of camphor and borneol increases proportionally to the extraction time for the first 15 min; the extraction efficiency did not increase when extraction time increased from 15 min to 60 min. So an extraction time of 15 min was selected for the following work. Considering these described results, the following experimental conditions were selected as optimum: methanol with a solvent/sample ratio 12/1 (v/w); extraction time, 15 min.

Method validation

Several levels of standard solutions (from 0.1 to 2000 µg/mL) spiked with 20.0 µg/mL decane (IS) were directly analyzed with the GC–FID system. The calibration curves and correlation coefficients for camphor and borneol in TCMs were obtained by the analytical results of GC–FID measurement. The linear range was 0.05–10 µg/mL for both borneol and camphor. The calibration curve for quantifying camphor was: $Y = 24.40X - 0.40$, with a correlation coefficient of 0.9994 (Y = Peak area ratio of camphor to IS; X = camphor concentration, mg/mL), and for borneol was: $Y = 25.01X - 0.20$, with a correlation coefficient of 0.9990 (Y = Peak area ratio of borneol to IS; X = borneol concentration in TCMs, mg/mL). The method precision was expressed by the RSD value. Five replicate analyses of *Flos Chrysanthemi Indici* from Hubei by UAE were performed. By using their peak areas, the calculated RSD values for camphor and borneol were 8.4% and 5.6%, respectively. Recoveries were obtained by comparing the real values of the added camphor and borneol amounts with those by calculation; the recoveries for camphor and borneol were achieved. As seen from Table I, the recoveries for camphor and borneol were 89% and 95%, respectively.

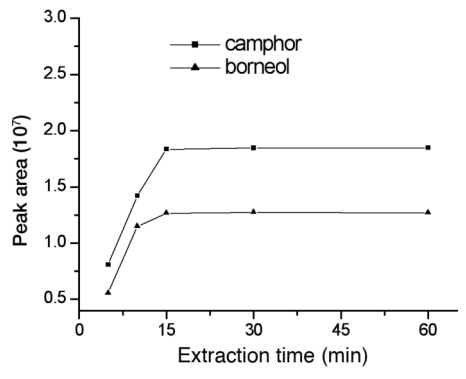

Figure 3. Effect of extraction time on the peak areas of the two active compounds of camphor and borneol in *Flos Chrysanthemi Indici*.

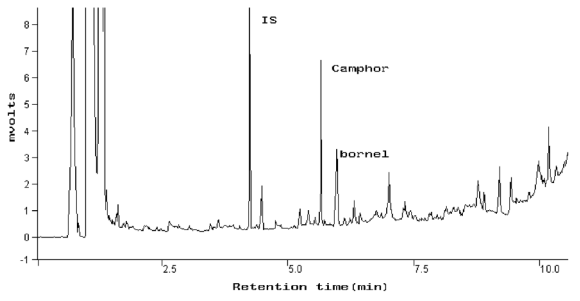

Figure 4. The GC–FID chromatogram of *Flos Chrysanthemi Indici* sample with UAE–GC–FID.

Determination of camphor and borneol in *Flos Chrysanthemi Indici* samples by UAE–GC–FID

Flos Chrysanthemi Indici samples from five different growing areas were extracted by UAE technique at the optimal conditions. The filtrate was analyzed by GC–FID. Figure 4 is the GC–FID chromatogram of *Flos Chrysanthemi Indici* sample with UAE–GC–FID. The retention times of IS, camphor, and borneol are 4.2, 5.6, and 5.9 min, respectively. According to the calibration curves, the concentrations of camphor and borneol in the *Flos Chrysanthemi Indici* from different areas were calculated, and the analytical results are listed in Table II.

Comparison of UAE–GC–FID and SD for analysis of camphor and borneol in *Flos Chrysanthemi Indici* samples

The steam distillation (SD) method was also used to quantitatively analyze camphor and borneol in *Flos Chrysanthemi Indici* from the same five growing areas in China. The concentrations of camphor and borneol in *Flos Chrysanthemi Indici* from the five growing areas were measured by the SD method, according to Huang's method (28). The analytical results are shown in Table II.

Discussion

As seen from Table I, the correlation coefficient value of more than 0.999 shows that the method has very good linearity. The precision ranged from 6–8%, and the recovery value of more than 89% shows that the method has an acceptable precision and high recovery. The results show that the proposed method for the analysis of camphor and borneol in *Flos Chrysanthemi Indici* is feasible.

The data in Table II show that the concentrations of camphor and borneol in TCMs from different growing areas were very different. It has been demonstrated in our previous studies (29,30) that quality assessment can be performed according to the concentrations of active components in TCMs. Camphor and

Table I. Precision, Detection Limit, and Recovery

Analytes	R^2	Precision (%)	Detection limit (µg/mL)	Recovery (%)
Camphor	0.9994	8.4	0.23	89
Berneol	0.9984	5.6	0.10	95

Table II. The Concentrations and Standard Deviations for Camphor and Borneol from Different Growing Areas by UAE–GC–FID and SD (n = 5)

Growing area	Camphor (mg/g)		Borneol (mg/g)	
	UAE–GC–FID	SD	UAE–GC–FID	SD
Hubei	0.77 ± 0.06	0.73 ± 0.04	0.65 ± 0.05	0.65 ± 0.04
Fujian	0.34 ± 0.02	0.35 ± 0.02	0.21 ± 0.01	0.22 ± 0.01
Anhui	0.49 ± 0.03	0.49 ± 0.04	0.34 ± 0.02	0.35 ± 0.02
Henan	0.38 ± 0.01	0.38 ± 0.02	0.20 ± 0.01	0.21 ± 0.01
Jiangxi	0.39 ± 0.02	0.39 ± 0.02	0.19 ± 0.01	0.20 ± 0.01

borneol have been shown to be the main active components in *Flos Chrysanthemi Indici*. In the study, the concentration of camphor and borneol was used to evaluate the quality of TCMs. Therefore, the proposed method has the potential for quality monitoring of *Flos Chrysanthemi Indici*.

Table II shows that very close results were obtained by SD and UAE–GC–FID. The paired t-test was applied to the data of Tables II and showed that the results of both methods are not significantly different at the 95% confidence level. This suggests that UAE-GC–FID is a reliable method for quantitative analysis of camphor and borneol in *Flos Chrysanthemi Indici*, and a potential tool for TCM quality assessment.

Conclusions

A UAE–GC–FID method for quantitative analysis of camphor and borneol in *Flos Chrysanthemi Indici* from different growing areas was proposed. Compared with SD, the proposed method needed very little sample amount (only 500 mg), little sample preparation time (less than 30 min), and it has easy operation. Therefore, UAE–GC–FID is a simple, rapid approach to quantitative analysis of camphor and borneol in TCMs. The experimental results demonstrate that UAE–GC–FID is an alternative tool for TCM quality assessment.

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