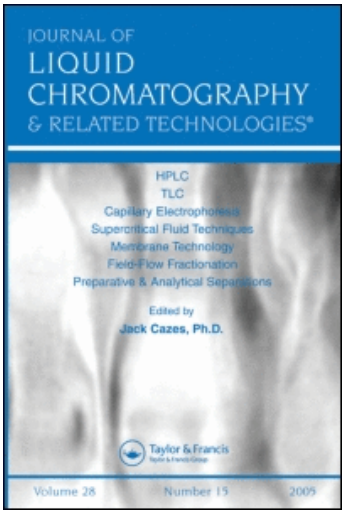

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SIMULTANEOUS DETERMINATION OF HONOKIOL AND MAGNOLOL IN MAGNOLIA OFFICINALIS BY CAPILLARY ZONE ELECTROPHORESIS

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ABSTRACT

A simple and rapid capillary electrophoretic method was developed for the simultaneous determination of honokiol and magnolol in *Magnolia officinalis* extracts and dextrorphan was used as the internal standard. The running buffer was composed of 22.5 mM Na_2HPO_4 and 10 mM $Na_2B_4O_7$ (pH 9.1-9.2). The linear calibration range was 2-20 μg/mL for honokiol and 5-50 μg/mL for magnolol. It was found that 0.95 ± 0.02 mg of honokiol and 4.37 ± 0.08 mg of magnolol were contained in the ethanol (50%) extracts of 1 g of *Magnolia officinalis*. The contents of these two active principles in *Magnolia officinalis* was successfully determined within 12 min.

INTRODUCTION

The stem bark of *Magnolia officinalis* (Hou-Po) has been used as a folk medicine in China for the treatment of thrombotic stroke, typhoid fever and headache.[1] It has been reported that Hou-Po possesses antimicrobial activities[2] and central depressant effects.[3] Honokiol and magnolol (Fig. 1), isomers of neolignans, have been isolated from the bark of this plant and other Magnoliaceae.[4] These compounds inhibit intracellular calcium mobilization in platelets,[5] relax vascular smooth muscle,[6] inhibit collagen-induced platelet serotonin release[7,8] and have antihemostatic and antithrombotic effects.[9]

Recent studies indicated that magnolol has antiinflammatory and analgesic effects,[10] and modulates central serotonergic activities.[11] These two compounds are also effective in inhibition of 11 beta hydroxysteroid dehydrogenase,[12] acetyltransferase[13] and hydroxyl radicals,[14,15,16] and have antiemetic activities.[17] Several methods for the determination of honokiol and magnolol have been described, including ion pair HPLC,[18] HPLC photodiode array detection,[19] and liquid chromatography-mass spectrometry.[20,21] However, none of the methods has been described to the determination of honokiol and magnolol by capillary electrophoresis.

In this work, we developed a simple and rapid capillary zone electrophoretic method, using dextrorphan as the internal standard, for the simultaneous determination of these compounds in *Magnolia officinalis*. The proposed technique is a viable alternative to HPLC and should be useful for the quality control of *Magnolia officinalis*.

MATERIALS AND METHODS

Materials and reagents

Magnolia officinalis was purchased from a traditional Chinese herbal drug store in Taipei. Authentic honokiol and magnolol were obtained from Nacalai Tesque (Kyoto, Japan), disodium hydrogen phosphate, sodium tetraborate, ethanol (99.5%) and NaOH from E. Merck (Darmstadt, Germany). Triple deionized water (Millipore Corporation, Bedford, MA, U.S.A.) was used for all preparations.

Extraction

A 5 g powder of *Magnolia officinalis* was boiled with 50 mL of extraction solvents [water, ethanol (50%), 0.1 M NaOH] for 5 min. Extraction was repeated twice. The two extracts were combined and diluted to 100 mL in a

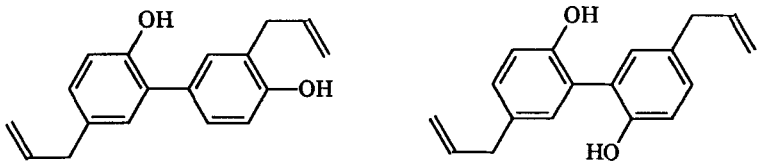

Figure 1. Chemical structures of honokiol (left) and magnolol (right).

volumetric flask. A 1 mL aliquot of this solution was filtrated using 0.2 μm disposable syringe filters, followed by adding a known concentration of dextrorphan (20 μg/mL) as internal standard. A 30 nL (5 sec pressurized injection) of this reconstituted sample was injected into the capillary electrophoresis system directly.

Apparatus andCondition

All measurements were performed on a Beckman P/ACE 2200 (Fullerton, CA, U.S.A.) capillary electrophoresis system, equipped with a UV detector set at 214 nm. A 75 μm ID uncoated fused-silica capillary of 57 cm total length (Polymicro Technologies, Phoenix, AZ, U.S.A.), was employed. Sample injection was done by introducing a pressure of 0.5 Psi for 5 sec to the sample. The applied voltage was a constant 15 kV (positive to negative polarity), the temperature was set at 25 °C and the running time was 12 min. The electrophoresis buffer was 22.5 mM disodium hydrogen phosphate and 10 mM sodium tetraborate buffer (pH 9.1-9.2). Prior to each run, the capillary was rinsed for 2 min with running buffer. After each run, the capillary was washed for 3 min with 0.1 M NaOH followed by deionized water.

Determination of Honokiol and Magnolol

Calibration graphs for 4 concentrations of honokiol (2, 5, 10, 20 μg/mL) and 4 concentrations of magnolol (5, 10, 20, 50 μg/mL) were analysed by capillary electrophoresis. The contents of honokiol and magnolol in the extract of *Magnolia officinalis* were determined from the peak area ratio by using the equation for linear regression from the calibration curve.

RESULTS AND DISCUSSION

The structures of honokiol and magnolol suggested that could be analysed as anions. We found that a buffer solution containing 22.5 mM Na_2HPO_4 and

Table 1

Intra- and Inter-Assay Precision and Accuracy in Honokiol and Magnolol Determination (n=5)

	Nominal Concentration (μg/mL)					
	Honokiol			Magnolol		
	2	10	20	5	20	50
Intra-Assay						
Mean	2.15	9.86	20.07	4.92	20.23	49.92
S. D.	0.12	0.14	0.06	0.25	0.47	0.15
% C. V.	5.7	1.4	0.3	5.0	2.3	0.3
Accuracy (%)	7.6	-1.3	0.3	-1.6	1.1	-0.2
Inter-Assay						
Mean	1.76	10.11	19.20	5.505	19.92	50.01
S. D.	0.19	0.24	0.12	0.31	0.55	0.15
% C. V.	10.7	2.4	01.6	6.2	2.8	0.3
Accuracy (%)	-11.8	1.1	-0.5	1.0	-0.3	0.0

Precision (% C. V.) = [standard deviation (S. D.) / mean concentration] x 100.
Accuracy (%) = (mean conc. - actual conc.) / actual conc.] x 100.

10 mM $Na_2B_4O_7$ (pH 9.1-9.2) could separate these two compounds without interference with other peaks. Fig. 2 (A) shows typical electropherogram of the standard mixtures. Fig. 2 (B) shows the ethanol (50%) extracts of *Magnolia officinalis*. The migration times of internal standard (dextrorphan), honokiol and magnolol were found to be 4.8, 6.4, 8.5 min, respectively. All measurements were completed within 12 min.

The equations of the calibration curve for honokiol and magnolol were $y = 5.5929x - 0.0411$ ($r^2 = 0.999$) and $y = 6.6869x + 0.2178$ ($r^2 = 0.999$), respectively. Where x is the response in peak area ratio and y is the amount of compound analyzed. The linearity ranges were 2-20 μg/mL for honokiol and 5-50 μg/mL for magnolol. The lower detection limits for honokiol and magnolol, at a signal-to-noise ratio of 3, were 0.2 and 0.5 ng, respectively.

The reproducibility of the method can be defined by examining both intra-assay and inter-assay variabilities. Table 1 shows the intra- and inter-assay precision and accuracy in honokiol and magnolol determination (n=5). Table 2

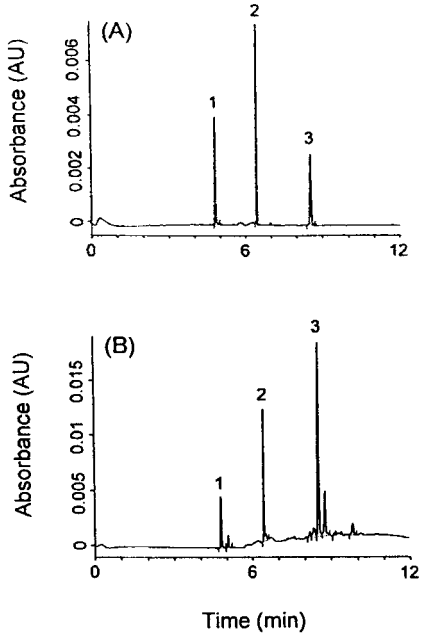

Figure 2. (A) Capillary electropherogram of a standard mixture. (B) Capillary electropherogram of the ethanol (50%) extracts of *Magnolia officinalis*. 1: dextorphan (internal standard); 2: honkiol; 3: magnolol.

Table 2

Contents of Honokiol and Magnolol in Different Extracts of 1 g of Magnolia Officinalis (mg/g)

Extraction Solvent	Honokiol	Magnolol
Water	0.25 ± 0.01	0.98 ± 0.02
Ethanol (50%)	0.95 ± 0.02	4.37 ± 0.08
0.1M NaOH	3.68 ± 0.09	27.49 ± 0.43

Results are mean ± S. D. (n=6)

gives the contents of honokiol and magnolol in extracts of *Magnolia officinalis* obtained with different solvents. It appears that 0.1 M NaOH is the best solvent for the extraction of honokiol and magnolol. In conclusion, the proposed

technique is suitable for the simultaneous determination of honokiol and magnolol by capillary zone electrophoresis, and should be useful for the quality control of *Magnolia officinalis*. The short analysis time, the small amount of samples and easily cleaned column, make this method a potential alternative to HPLC.

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