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Short communication

Determination of honokiol and magnolol in Cortex Magnoliae Officinalis by capillary electrophoresis with electrochemical detection

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Abstract

Capillary electrophoresis with electrochemical detection has been employed for the determination of honokiol and magnolol in Cortex Magnoliae Officinalis (i.e. Magnolia Bark) for the first time. Effects of several important factors such as the concentration and the acidity of the running buffer, separation voltage, injection time, and detection potential were investigated to acquire the optimum conditions. The detection electrode was a 300 μm diameter carbon disc electrode at a working potential of +0.90 V (versus saturated calomel electrode (SCE)). The two analytes can be well separated within 6 min in a 40 cm length fused silica capillary at a separation voltage of 18 kV in a 50 mM borate buffer (pH 9.2). The relation between peak current and analyte concentration was linear over about three orders of magnitude with the detection limits (S/N = 3) of 0.38 and 0.51 μM for honokiol and magnolol, respectively. The proposed method has been successfully applied to monitor the two bioactive constituents in the real plant samples with satisfactory assay results.

Keywords: Cortex Magnoliae Officinalis; Honokiol; Magnolol; Capillary electrophoresis; Electrochemical detection

1. Introduction

Traditional Chinese medicine (TCM), Cortex Magnoliae Officinalis (i.e. Magnolia Bark) is the dried bark of the trunk or root of *Magnolia officinalis* Rehd. et Wils. or *Magnolia officinalis* Rehd. et Wils. var. biloba Rehd. et Wils., which belongs to the Magnoliaceae family. It can alleviate gastric and abdominal distension and other digestive disturbances, reduce the symptom of cough and asthma due to the accumulation of phlegm in the lung, and treat syndromes caused by emotional distress, digestive disturbance, and emotional turmoil [1]. Its antibacterial properties have been recognized and may be responsible for its ability to alleviate digestive discomforts due to some intestinal bacterial infections [2]. Cortex Magnoliae Officinalis has been frequently used as an important ingredient in many traditional prescriptions [1,2]. As two important phenolic lignans, honokiol and magnolol are the two major bioactive constituents in Cortex Magnoliae Officinalis. Recent pharmacological investigations show that honokiol and magnolol have a broad range of physiological activities such as anti-inflammatory [3], antioxidant [4], anti-tumor [5], anti-bacteria [6], anti-arrhythmia [7], anti-platelet [8], etc. The total content of honokiol and magnolol is an important parameter for evaluating the quality of Cortex Magnoliae Officinalis. The Pharmacopoeia of China requires the total content of honokiol and magnolol in Cortex Magnoliae Officinalis to be no less than 2.0% [1]. Moreover, some concentrated composite herbal preparations that contain Cortex Magnoliae Officinalis in their prescriptions are widely used in oriental countries for their convenient use. Hence, it is interesting to establish some rapid, simple, and accurate approaches for the determination of honokiol and magnolol in Cortex Magnoliae Officinalis.

Liquid chromatography (LC) was the most commonly used method for the determination of honokiol, magnolol, and some coexistent constituents in Cortex Magnoliae Officinalis [9,10], composite herbal preparations with Cortex Magnoliae Officinalis as their ingredient [11], and biological samples such as rat plasma [12,13]. Separation and determination of various constituents in plant drugs is always a complicated and challenging task. Nowadays, the application of capillary electrophore-

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sis (CE) for the separation of various bioactive constituents in medicinal plants has become increasingly widespread because of its minimal sample volume requirement, short analysis time, and high separation efficiency [14,15]. Electrochemical detection (ECD) typically operated in the amperometric mode can be coupled with CE to provide high sensitivity and selectivity for the determination of electroactive substances in the herbal drugs [16,17]. Nowadays, it is of high importance to control the quality of herbal drugs based on their active constituents and some co-existent substances. In 2000, the U.S. Food and Drug Administration (FDA) published a draft of Guidance for Industry Botanical Drug Products. Before a plant drug can become legally marketed, its spectroscopic or chromatographic fingerprints and chemical assay of the characteristic markers are required. CE-ECD should find more applications in this area. CE [18–20] has been employed for the quantitative determination of honokiol and magnolol in Cortex Magnoliae Officinalis coupled with ultraviolet (UV) detectors. Because the absorbance path length of the capillary (the typical inner diameter, 25–100 μm) is very short, the low sensitivity of the UV detector used results in poor detection limit. Usually, the content of the constituents in the TCMs is very low (typically 0.01–10 mg/g). High sensitive detection methods are highly demanded.

Because both honokiol and magnolol contain phenolic hydroxyl groups that are electroactive at modest oxidation potential on carbon electrode, CE-ECD should be an assistant, alternative, and complement technique for the constituent investigation of Cortex Magnoliae Officinalis. Moreover, ECD can provide higher selectivity as only electroactive substances can be detected so that the electropherograms are greatly simplified, which is important for the analysis of medicinal plants because their constituents are usually complex. In this study, CE-ECD was employed for the determination of honokiol and magnolol (their molecular structures are shown in Fig. 1) in Cortex Magnoliae Officinalis without derivatization. This method is simple, sensitive, selective and efficient, providing not only a way for evaluating the quality of Cortex Magnoliae Officinalis and plant medicines made from Cortex Magnoliae Officinalis in marketplaces, but also an alternative approach for quality control in medicinal factories and constituent investigations of other related plants. To our best knowledge, there are no earlier reports published on the determination of active constituents in Cortex Magnoliae Officinalis by CE-ECD. The optimization, detailed characterization, and advantages of the CE-ECD approach are reported in the following sections in connection to the measurement of honokiol and magnolol in the crude drugs.

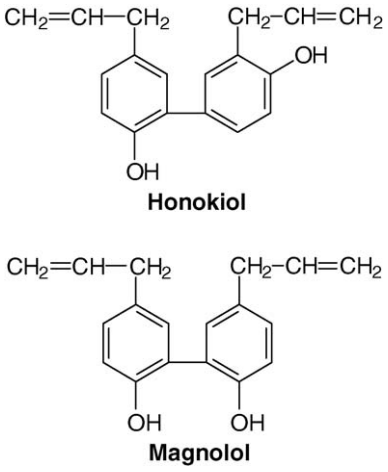

Fig. 1. Molecular structure of honokiol and magnolol.

2. Experimental

2.1. Reagent and solutions

Honokiol and magnolol were both supplied by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All aqueous solutions were made up in doubly distilled water. Other chemicals were of analytical grade.

Stock solutions of honokiol and magnolol (10 mM) were prepared in ethanol and were kept in a 4 °C refrigerator. They were stable for at least 1 month. The running buffer was 50 mM borate buffer (pH 9.2) unless mentioned otherwise. The stock solutions were diluted to the desired concentration with the running buffer just prior to use.

2.2. Apparatus

The CE-ECD system used has been described previously [21–23]. A ±30 kV high-voltage dc power supply (Shanghai Institute of Nuclear Research, China) provided a separation voltage between the two ends of the capillary. The inlet of the capillary was held at a positive potential and the outlet of capillary in the detection cell was maintained at ground. The separations were carried out in a 40 cm length of 25 μm i.d. and 360 μm o.d. fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA).

A three-electrode electrochemical cell consisting of a laboratory-made 300 μm diameter carbon disc working electrode, a platinum auxiliary electrode and a saturated calomel electrode (SCE) as the reference electrode, was used in combination with a BAS LC-4C amperometric detector (Bioanalytical Systems Inc., West Lafayette, IN, USA). The filter of the detector was set at 0.1 Hz. The working electrode was positioned carefully opposite the outlet of the capillary with the aid of a micromanipulator (CORRECT, Tokyo, Japan) and arranged in a wall-jet configuration. The distance between the tip of the working electrode and the capillary outlet was adjusted to ∼50 μm by comparison with the bore (25 μm) in the capillary while being viewed under a microscope. The electropherograms were recorded using a LKBREC 1 chart record (Pharmacia, Sweden). A YS 38-1000 220 V alternate constant-voltage power supply (Shanghai Instrumental Transformer Factory, Shanghai, China) was employed to suppress the voltage fluctuation of the power line. The whole system was assembled in a laboratory that was air-conditioned at 25 °C to minimize the temperature fluctuation.

2.3. Sample preparation

Three samples of Cortex Magnoliae Officinalis were obtained from Sun-Tian-Tang Traditional Chinese Medicine Store

(Shanghai, China). They were all dried at 60 °C for 2 h and then were pulverized. About 2.0 g of the powder was weighed accurately and dispersed in 100 ml of methanol. The mixture was kept in a 60 °C water bath for 3 h. After cooling, it was sonicated for 30 min and filtered through a filter paper. The extract was diluted using 50 mM borate buffer (pH 9.2) at a ratio of 10 (1–10) just prior to CE analysis. Because electrokinetic injection was employed in this work, the stock solution and the extract of herbal drug were both diluted in running buffer to minimize the difference between the running buffer and the sample solution.

2.4. Procedures

Before use, the carbon disc electrode was successively polished with emery paper and alumina powder, and sonicated in doubly distilled water. The capillary used for the separation were treated before use by flushing with 0.1 M NaOH and doubly distilled water for 10 min each. Subsequently, the capillary was filled with the running buffer and was conditioned with the running buffer for at least 10 min at the voltage of 18 kV between the two ends of the capillary. CE was performed at a separation voltage of 18 kV, unless otherwise indicated. The potential applied to the working electrode was +0.90 V (versus SCE). Before injection, both the anode end of the capillary and the platinum-wire anode were moved from the anode solution to the sample solution. After an injection voltage of 18 kV was applied between the two ends of the capillary for 6 s, the sample solution could be introduced into the capillary. The anode end of the capillary together with the anode was then quickly returned to the anode solution. A voltage of 18 kV was subsequently applied in the constant-voltage mode for CE separation. The amperometric detector was on during the injection procedures. Note that the cathode solution in the electrochemical detection cell, the anode solution, and the sample solution were all at the same level. Moreover, sample solutions, standard solutions, and running buffer were all filtered through a polypropylene filter (0.22 μm, Shanghai Bandao Industry Co. Ltd., Shanghai, China) prior to their use. Peak identification was performed by the standard-addition method.

3. Results and discussion

3.1. Hydrodynamic voltammograms (HDVs)

Honokiol and magnolol both contain phenolic hydroxyl groups, which are electroactive on carbon electrode. In this work, a carbon disc electrode was used for their detection. The potential applied to the working electrode directly affects the sensitivity and the detection limits of this method, and it is necessary to determine the hydrodynamic voltammograms (HDVs) for the analytes to obtain the optimum potential. Fig. 2 depicts the HDVs for the oxidation of honokiol and magnolol using the carbon disc-electrode detector. The curves were recorded pointwise from +0.1 to +1.1 V (versus SCE) in steps of 0.1 V using a separation voltage of 18 kV. Both analytes displayed similar profiles, with an increase of the response starting at +0.5 V (versus SCE). The current rose rapidly upon raising the potential above +0.6 V (versus SCE). Although an applied potential greater than +0.90 V (versus SCE) resulted in higher peak currents, both the baseline noise and the background current increase substantially. The high background current led to an unstable baseline, which is a disadvantage for the sensitive and stable detection. Considering the sensitivity and background current, subsequent amperometric detection work employed a detection potential of +0.90 V (versus SCE), which offered the most favorable signal-to-noise characteristics. The stability of the working electrode was good and the reproducibility was high at the optimum potential.

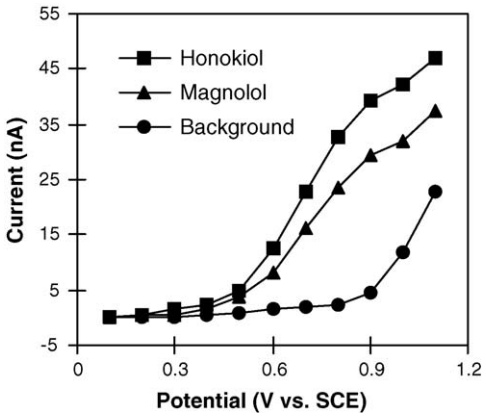

Fig. 2. Hydrodynamic voltammograms (HDVs) for 0.5 mM of honokiol and magnolol in CE. Fused-silica capillary, 25 μm i.d. × 40 cm length; working electrode, 300 μm diameter carbon disc electrode; running buffer, 50 mM borate buffer (pH 9.2); separation and injection voltage, 18 kV; injection time, 6 s.

3.2. Effects of the concentration and the acidity of the running buffer

In order to enhance the resolution and solubility of analytes, alkaline borate buffer was employed in this study. Although the two phenolic lignans are position isomers (as shown in Fig. 1), their pK_a values are quite different. The pK_{a1} and pK_{a2} values are 9.92 and 10.71 for honokiol and 7.01 and 10.64 for magnolol, respectively [24]. Honokiol and magnolol are partially negative charged in alkaline borate buffer because their phenolic hydroxy groups can dissociate to form anions. In this work, capillary zone electrophoresis (CZE) was employed for the separation of the two analytes based on their difference in pK_{a1} value.

Because the buffer concentration influences the viscosity coefficient of the solution, the diffusion coefficient of analytes and the zeta-potential (ζ) of the inner surface of capillary tube as well, it affects not only the resolution and effective mobility of the two analytes, but also the peak current. Fig. 3A indicates that the effective mobility decreases with increasing buffer concentration (pH 9.2). Upon raising the concentration above 50 mM, the effective mobility decreases much slower while the peak current was low and the peak shape became poor because the electric current in the capillary also increased, resulting in Joule heating and peak broadening. In this experiment, a 50 mM borate buffer (pH 9.2) was chosen as the running buffer in consider-

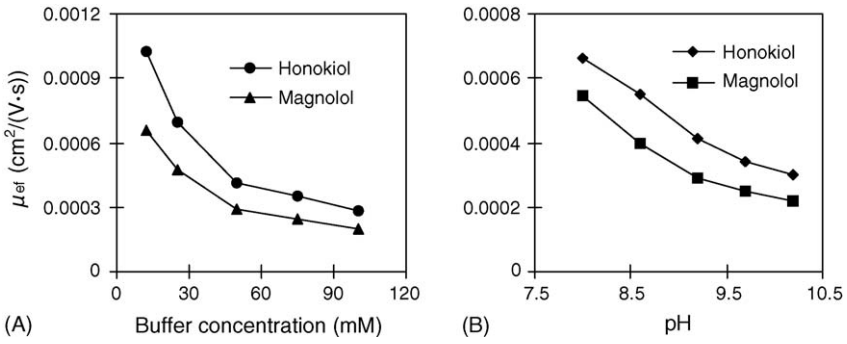

Fig. 3. Effect of (A) the concentration and (B) the acidity of the running buffer on the effective mobility (μ_{ef}) of honokiol and magnolol. Working potential, +0.90 V (vs. SCE); other conditions, as in Fig. 2.

ing the peak current, resolution, analytical time and the buffer capacity.

The effect of the running buffer pH on the effective mobility of the analytes is shown in Fig. 3B. The running buffers were 50 mM borate buffers at five different pH values (8.0, 8.6, 9.2, 9.7 and 10.2). As shown in Fig. 3B, the effective mobility decreases with increasing the pH value of the running buffer due to the dissociation of the hydroxyl groups for both analytes. At pH 9.2, honokiol and magnolol can be well separated within a relatively short time. Meanwhile, the peak current is low and the peak shape becomes poor at pH value above 9.7. In this experiment, 50 mM borate buffer at pH of 9.2 was chosen as the running buffer in considering the peak current, resolution, the analytical time, and the stability of the running buffer.

3.3. Effect of separation voltage and injection time

Increasing the voltage gives shorter migration time for both compounds, but also increases the baseline noise, resulting in poorer detection limits. It is found that higher separation voltages are not beneficial to the resolution of honokiol and magnolol and can result in higher Joule heating, which directly affects the separation efficiency of this method. Separation voltages, which are too low, will increase the analysis time considerably; this in turn causes peak broadening. On the basis of experiments, 18 kV was chosen as the optimum voltage to accomplish a good compromise.

In this study, samples were introduced into the capillary electrokinetically. The injection time directly affected the amount of sampling, which affected the peak height and the peak shape. The effect of the injection time on CE separation was investigated by changing the sampling time from 2 to 10 s in increments of 2 s at an injection voltage of 18 kV. It was found that both the peak current and the peak width increased with increasing the sampling time. When the injection time exceeded 6 s, the peak current leveled off and peak broadening became more severe. In this experiment, 6 s (at 18 kV) was selected as the optimum injection time, considering the separation and sensitivity.

Through the above-mentioned experiments, the optimum conditions for determining honokiol and magnolol were acquired. The typical electropherogram for a mixture containing 0.5 mM honokiol and magnolol is shown in Fig. 4A. Baseline separation for honokiol and magnolol could be achieved within 6 min.

3.4. Repeatability, linearity and detection limits

The intra-day precision of the peak current was examined from a series of seven repetitive injections of a sample mixture containing 0.5 mM honokiol and magnolol under the optimum conditions. Reproducible signals were obtained with R.S.D. of 3.1% (honokiol) and 2.4% (magnolol) for the peak currents. Nine determinations of a mixture containing 0.5 mM honokiol and magnolol over 3 days (three times a day) resulted in the inter-day precisions of 4.2% and 4.8%, respectively. Such good repeatability reflects the reduced surface fouling of the carbon electrode, and indicates that this approach is suitable for the analysis of real samples.

A series of the standard mixture solutions of honokiol and magnolol with concentrations ranging from 1 μM to 1 mM were tested to determine the linearity at the carbon disc electrode in this method. The carbon electrode detector offers a well-defined concentration dependence. The results of a regression analysis

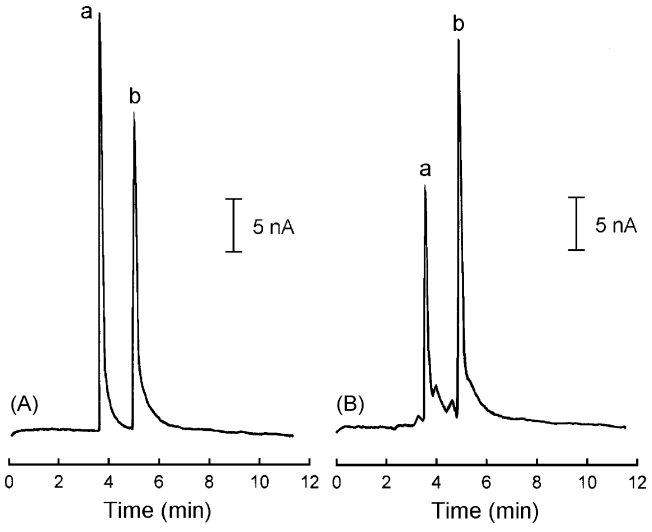

Fig. 4. (A) Electropherogram for a mixture containing 0.5 mM of honokiol (a) and magnolol (b) and (B) typical electropherogram for a diluted extract from a sample of Cortex Magnoliae Officinalis. Working potential, +0.90 V (vs. SCE); other conditions, as in Fig. 2.

Table 1
The results of regression analysis on calibration curves and the detection limits[a]

Compound	Regression equation, $Y = a + bX$[b]	Correlation coefficient	Linear range (mM)	Detection limit[c] (μM)
Honokiol	$Y = 0.1211 + 78.283X$	0.9995	0.001–1.0	0.38
Magnolol	$Y = 0.1631 + 58.889X$	0.9993	0.001–1.0	0.51

[a] Working potential is +0.90 V (vs. SCE). Other conditions are the same as in Fig. 2.
[b] Where the Y and X are the peak current (nA) and concentration of the analytes (mM), respectively.
[c] The detection limits correspond to concentrations giving a signal-to-noise ratio of 3.

Table 2
Results from assay of honokiol and magnolol in Cortex Magnoliae Officinalis[a] ($n = 3$, mg/g)

Sample	Honokiol	Magnolol
1	37.33 (3.2)[b]	60.49 (2.2)
2	28.74 (2.9)	45.05 (2.7)
3	27.03 (3.6)	33.04 (4.1)

[a] Working potential is +0.90 V (vs. SCE). Other conditions are the same as in Fig. 2.
[b] The data in the parentheses are the R.S.D.s (%).

on the calibration curves and the detection limits are presented in Table 1. The detection limits were evaluated based on a signal-to-noise ratio of 3. The calibration curves exhibit a satisfactory linear behavior over the concentration range of three orders of magnitude with the detection limits of 0.38 and 0.51 μM for honokiol and magnolol, respectively.

3.5. Sample analysis and recovery

Under the optimum conditions, CE-ECD was applied for the determination of honokiol and magnolol in traditional Chinese medicine, Cortex Magnoliae Officinalis. The typical electropherogram for the diluted extract from a plant sample are shown in Fig. 4B. Peak identification was performed by the standard addition method. The assay results are summarized in Table 2. The determined contents of honokiol and magnolol in Cortex Magnoliae Officinalis are well in agreement with previous reports (8.0–68.7 mg/g for honokiol and 12.2–96.8 mg/g for magnolol, respectively) [10,25].

Recovery experiments were performed by adding accurate amounts of honokiol and magnolol to the diluted extract of Cortex Magnoliae Officinalis in the running buffer. Subsequently, the standard-spiked sample solution was analyzed under the optimum conditions. The average recoveries and the corresponding R.S.D. were 97.1% and 3.7% for honokiol and 98.5% and 2.4% for magnolol, respectively ($n = 3$). The results demonstrated that this method had both high recovery and good precision for honokiol and magnolol.

4. Conclusions

For the first time, CE-ECD was employed for the determination of honokiol and magnolol in Cortex Magnoliae Officinalis. It is characterized by its higher resolution and sensitivity, lower expense of operation, and less amount of sample. Because of the short absorbance path length of the capillary, the sensitivity of the UV detector coupled with CE is low with the typical detection limit of 10 μM. CE-ECD is an efficient approach for the determination of electroactive constituents in plant drugs due to its higher sensitivity and selectivity in comparison with CE-UV because only electroactive substances can be detected. Another advantage of CE as an analytical technique for the analysis of plant samples is that capillary is much easier to clean. Because honokiol and magnolol are directly detected on carbon electrode, samples do not need derivatization before determination. It is concluded that CE-ECD is an efficient approach for the constituent and fingerprint study of plant drugs due to its special attributes.

Acknowledgments

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