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Separation and determination of phenylpropanoid glycosides from *Pedicularis* species by capillary electrophoresis

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Abstract

Capillary zone electrophoresis, using 30 m*M* borate buffer (pH 9.00) with 10% (v/v) methanol, was established for the identification and determination of four phenylpropanoid glycosides (PPGs)—echinocoside (ECH), verbascoside (VER), pedicularioside *M* (PED-M) and pedicularioside A (PED-A)—in extracts of *Pedicularis longiflora* var tubiformis, *Pedicularis longiflora* and *Pedicularis Kansuensis*. Regression equations revealed linear relationships (correlation coefficients: 0.9993–0.9999) between the peak area of each compound (ECH, VER, PED-M and PED-A) and its concentration. The relative standard deviations of the migration times and peak areas were <1.93 and 4.54%, respectively. The recoveries of four PPGs ranged between 95.6 and 108.4%. The effects of several CE parameters on the resolutions were studied systematically.

Keywords: *Pedicularis*, spp.; Pharmaceutical analysis; Phenylpropanoid glycosides; Echinocoside; Verbascoside; Pedicularisides

1. Introduction

As a folk medicinal herb, the *Pedicularis* species, called 'native-ginseng' by local inhabitants living in northwestern China, has many therapeutic effects, such as in cardiac-tonic of collapse, in exhaustion, spontaneous sweating, seminal emission and senility, invigoration of blood circulation, aiding digestion, increasing vitality, and relieving uneasiness of body and mind [1]. Echinocoside (ECH), verbascoside (VER), pedicularioside M (PED-M) and pedicularioside A (PED-A), as phenylpropanoid glycosides (PPGs), have been isolated from the *Pedicularis* species. The four PPGs abound in plants of *Pedicularis* species, in which VER was found to be the best antioxidant—a scavenging reactive oxygen species which repairs DNA based oxidative damage [2,3]. PPGs extracted from *Pedicularis* species have been reported to have antiviral [4], antiplatelet activities [5], to inhibit leukotriene B_4 formation [6], to inhibit the growth of tumor cells [7,8] and to repair DNA damage [9]. Therefore, it is necessary to develop a simple and quality control method for identifying and determining PPGs in *Pedicularis* species.

Analytical methods described in the literature are

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mainly based on gas chromatography (GC) [10,11], thin-layer chromatography (TLC) [12] and liquid chromatography (LC) [13–15]. Recently, owing to its high resolving power, low solvent consumption and simple pretreatment, capillary electrophoresis (CE) has been used as an attractive method for separating and monitoring Chinese traditional medicines [16–21]. To the best of our knowledge, there are no reports on the separation and determination of the four PPGs (ECH, VER, PED-M and PED-A) in *P. longiflora* var tubiformis, *P. longiflora* and *P. Kansuensis*. by CE. This paper first developed a CE method for the identification and determination of ECH, VER, PED-M and PED-A in the three Chinese traditional medicines mentioned above.

2. Experimental

2.1. Instruments

All the experiments were carried out on a Bio-Focuis 3000 capillary electrophoresis system (Bio-Rad, USA). The applied voltage was held constant at 15 kV. The separation capillary was an untreated fused-silica capillary with a total length of 35 cm (effective length 30 cm)×50 μm I.D.×365 μm O.D. (Yongnian, Hebei Province, China). The UV detector was operated at 250 nm. The temperature of the capillary cartridge was maintained at 25 °C. Before use, the capillary was rinsed with 0.5 *M* NaOH for 10 min, then with deionized water for 4 min; it was then conditioned with running electrolyte for 4 min. Between runs the capillary was rinsed with electrolyte only for 2 min. Samples were introduced under pressurized injection at 8 p.s.i. s (1 p.s.i.= 6894.76 Pa).

2.2. Materials and reagents

The samples of *P. longiflora* var tubiformis, *P. longiflora* and *P. Kansuensis* collected in the Qinghai provinces (marked as samples 1, 2 and 3, respectively) were identified by Ji Ma of Cold and Arid Regions Environmental and Engineer Research Institute, Chinese Academy of Sciences, Lanzhou, China. ECH, VER, PED-M and PED-A (structures shown in Fig. 1) were the kind gifts from Zhong-Jian Jia of National Laboratory of Applied Organic Chemistry, Department of Chemistry of Lanzhou University, China. All chemicals were analytical grade and purchased from Beijing Chemical Reagents Plant. Deionized water was used throughout. All solutions and samples were filtered through a 0.45-μm syringe filter.

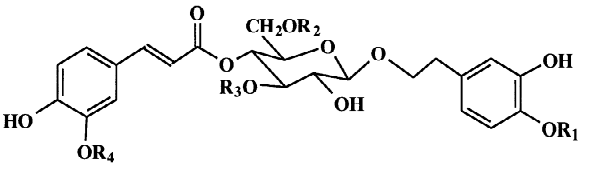

Fig. 1. Structures of phenylpropanoid glycosides. Echinocoside (ECH): $R_1=R_4=H$, $R_2=$glucosyl, $R_3=$rhamnosyl. Verbascoside (VER): $R_1=R_2=R_4=H$, $R_3=$rhamnosyl; pedicularioside M (PED-M): $R_1=H$, $R_2=$apiosyl, $R_3=$rhamnosyl, $R_4=CH_3$; pedicularioside A (PED-A): $R_1=R_4=H$, $R_2=$apiosyl, $R_3=$ rhamnosyl.

Standard stock solutions of four PPGs at concentration of 5000 μg/ml were prepared in methanol, and various concentration of the sample solutions were prepared by appropriate dilution from the stock solution when needed. The pH of borate buffer solutions were adjusted by mixing 0.1 *M* HCl or 0.1 *M* NaOH solution with sodium tetraborate solution.

2.3. Sample preparation

A 1.0 g amount of powder of samples 1, 2 and 3 was extracted with 50 ml of methanol in Soxhlet extractor for 1.5 h. The residue was extracted three times by the same procedure, and the methanol extracts were combined and filtered. The methanol solution was dried by evaporation and the residue was dissolved in 20 ml of methanol for sample 1, but 10 ml for samples 2 and 3.

3. Results and discussion

The CE chromatogram of a mixture containing four PPGs found in *Pedicularis* is shown in Fig. 2 (see Fig. 1 for their structures). In order to optimize the separation conditions, the influences of several CE parameters on the migration times or resolution

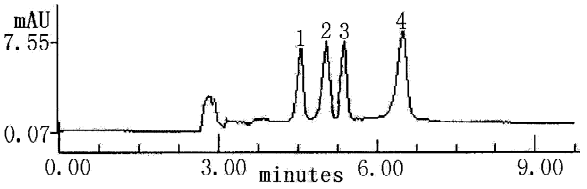

Fig. 2. Chromatogram of a phenylpropanoid glycosides standard mixture. 1=ECH; 2=VER; 3=PED-M; 4=PED-A. Analytical conditions: pH 9.00, borate 30 m*M*, methanol 10% and voltage 15 kV.

(R_s) were studied carefully. For figures in the paper, all of the data points are the results of average of three measurements, as are the results in the table.

3.1. Effect of buffer pH

To verify the effect of buffer pH on migration behavior, experiments were performed with 30 m*M* borate and 10% (v/v) methanol in electrophoretic medium, the effect of buffer pH on the migration times of the analytes are shown in Fig. 3. It can be observed that the co-migration of ECH and VER occurred at pH 7.00. With the increase in pH of the electrolyte, the separation of ECH, VER, PED-M and PED-A was improved. However, at pH 9.50, the migration times of four PPGs increased rapidly and the resolution decreased. Therefore, pH 9.00 was selected.

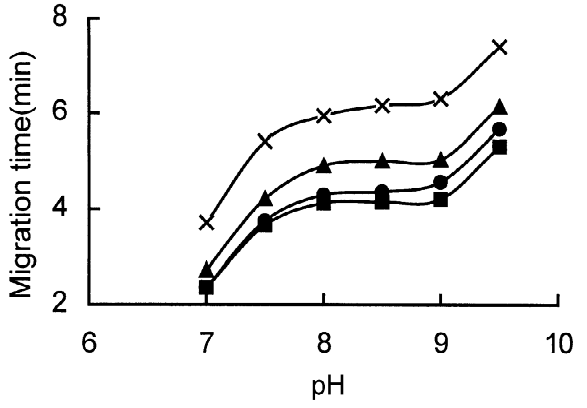

Fig. 3. Effect of pH on the migration time. ■, ECH; ●, VER; ▲, PED-M; ×, PED-A; other conditions as in Fig. 2.

3.2. Effect of organic modifiers

Methanol, isopropanol and acetone were used to improve the resolution, but a better result was obtained by methanol only. Fig. 4 shows the effect of methanol concentration on the separation of the four adjacent peaks. It can be seen that the organic modifier played an important role in a successful separation. With increasing concentration of methanol, the resolution of ECH–VER and PED-M–PED-A increased, but VER–PED-M decreased. The baseline separation was obtained when the concentration of methanol was 10% (v/v).

3.3. Effect of buffer concentration and applied voltage

The influence of the concentration of borate buffer in the range of 10–30 m*M* on the separation was examined with 10% (v/v) methanol at pH 9.00. When the borate buffer concentration increased, the resolution was improved, showing there should be a strong interaction between borate and the hydroxyl group of the four PPGs [22]. Therefore, 30 m*M* borate was chosen for the further experiments. The high voltage was necessary for rapid CE analysis. It was found that with the applied voltage ranging from 8 to 15 kV, the resolutions of four PPGs were not improved. But, when there was a lower voltage, the migration time increased; 15 kV was used as the run voltage.

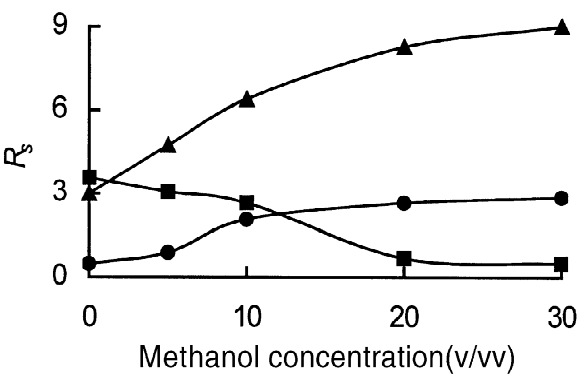

Fig. 4. Effect of methanol concentration on the resolution of the adjacent peaks. ●, ECH–VER; ■, VER–PED-M; ▲, PED-M–PED-A; other conditions as in Fig. 2.

3.4. Linear ranges of four PPGs

The linear relationships between the concentrations of four compounds and the peak areas were found in the concentration range of 20–2000 μg/ml for ECH and PED-A, 50–5000 μg/ml for VER and 10–1000 μg/ml for PED-M. The regression equations of these curves and their correlation coefficients (r) were calculated as follows: ECH, $y = 203.24x - 1159.55$ ($r = 0.9993$); VER, $y = 42.61x + 849.59$ ($r = 0.9999$); PED-M, $y = 442.13x + 1206.16$ ($r = 0.9997$); PED-A, $y = 397.91x + 5309.34$ ($r = 0.9999$); where y and x are the peak area and the concentration ($\mu g/ml^{-1}$) of the analytes, respectively.

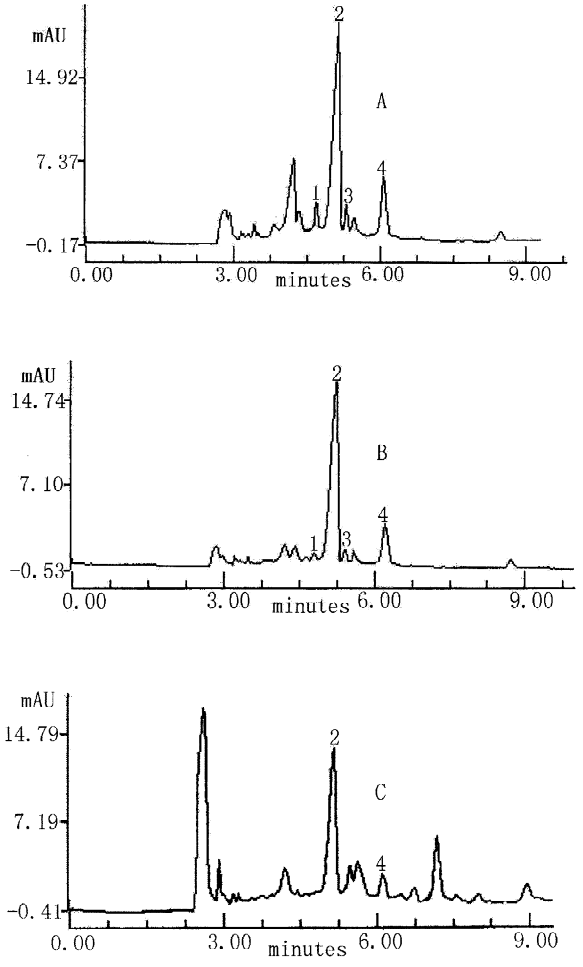

Fig. 5. Electropherograms of methanol extract of *P. longiflora* var tubiformis (A), *P. longiflora* (B) and *P. Kansuensis* (C). 1=ECH; 2=VER; 3=PED-M; 4=PED-A. Analytical conditions as in Fig. 2.

Table 1
Contents of the four phenylpropanoid glycosides in sample extracts

Samples	Components (%)			
	ECH	VER	PED-M	PED-A
1	0.16	3.06	0.13	0.61
2	0.069	1.37	0.028	0.23
3	Not detected	1.02	Not detected	0.074

3.5. System suitability and recovery of method

The method was validated for reproducibility of the migration time and the peak area of the analytes. The relative standard deviations of the migration time and the peak area of each of peak for six replicate injection were 1.03–1.93% and 1.74–4.54%, respectively. The accuracy and recovery of the method were determined with the standard addition method for ECH, VER, PED-M and PED-A. The results ranged from 95.6 to 106.7% for sample 1, 98.9 to 105.4% for sample 2 and 96.5 to 108.4% for sample 3.

3.6. Applications

Methanol solutions of extracts were injected directly and separated under the optimum condition described above. Typical electropherograms for samples 1, 2 and 3 are shown in Fig. 5 A, B and C, respectively. The PPGs and the other unknown compounds were well resolved within 7 min. Fig. 5 also shows that ECH, VER, PED-M and PED-A were all detected in samples 1 and 2, but only VER and PED-A were detected in sample 3. Peaks were identified by the addition of standard ECH, VER, PED-M and PED-A. The analytical results are summarized in Table 1.

4. Conclusion

The results demonstrate that CE is a useful, simple and rapid technique for the identification and de-

termination of ECH, VER, PED-M and PED-A in *Pedicularis* species. In addition, the proposed method also promises to be applicable to the quality control of traditional Chinese medicines.

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