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Journal of Chromatography A, 802 (1998) 225–231

JOURNAL OF CHROMATOGRAPHY A

Analysis of *Puerariae radix* and its medicinal preparations by capillary electrophoresis

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Abstract

This study presents a high-performance capillary electrophoresis (CE) method to analyze five constituents of *Puerariae radix*, an important crude herb used in Chinese medicine. Puerarin, daidzin, daidzein, genistein and biochanin A are the bioactive constituents of *Puerariae radix*. Herein, those analytes were successfully separated within 6 min using a pH 10.1 borax–NaOH buffer. The effects of pH value and concentration of the running buffer on the separation of the five analytes were also examined. The relative standard deviations of the analytes' migration times were less than 0.38% under the optimized separation conditions. Notably, the correlation coefficients of the analytes' linear calibration graphs exceeded 0.998. Moreover, the amounts of the five constituents in three different *Puerariae radix* samples were determined by the CE method with a relatively simple extraction procedure. © 1998 Elsevier Science B.V.

Keywords: *Puerariae radix*; Pharmaceutical analysis; Puerarin; Daidzin; Daidzein; Genistein; Biochanin A

1. Introduction

Traditional Chinese medicine has been extensively used to prevent and cure many diseases that have inflicted humans for over a millennium. In particular, the merits of low toxicity and rare complications have subsequently led to considerable attention in various fields. Among pertinent investigations include the analysis of active ingredients and/or major components of the medicine, treatment of diseases and the search for alternative drugs.

Puerariae radix, referred to as ''Ge-gen'' in Chinese, is an important crude herb used in Chinese medicine [1–5]. This herb has been used primarily to treat the common cold, influenza and wrist or shoulder stiffness. The fact that *Puerariae radix* comprises puerarin, daidzin, daidzein, genistein, and biochanin A as the major constituents accounts for why this investigation selects the five compounds for analyzing *Puerariae radix*.

Thin-layer chromatography (TLC) is the conventional means of analyzing traditional Chinese medicinal preparations. However, this technique can only analyze one or two components in a crude herb or in a concentrated preparation [3]. Several studies have demonstrated that high-performance liquid chromatography (HPLC) can individually or simultaneously determine puerarin, daidzin and daidzein in *Puerariae radix* or in traditional Chinese medicinal preparations [4–8]. However, the HPLC method takes more than 40 min if puerarin, daidzin and daidzein are simultaneously determined in the traditional preparations [6,7]. Therefore, this study focuses primarily on establishing a rapid and efficient analytical method to analyze the constituents of *Puerariae radix* and its medicinal preparations.

Capillary electrophoresis (CE) is highly effective in analyzing many kinds of compounds in various

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0021-9673/98/$19.00
PII S0021-9673(97)01070-4

fields [9–14]. CE offers the advantages of excellent separation efficiency, high resolution and rapid analysis. In addition, the amount of the sample and solvent usage of CE and the hazardous waste solution produced by CE is minimum, thereby offering advantages over the HPLC method. Consequently, CE has been employed to analyze Chinese crude herbs and Chinese medicinal preparations [15–18].

In the present study, CE was employed to separate and determine puerarin, daidzin, daidzein, genistein and biochanin A in *Puerariae radix*. The influences of pH value and concentration of buffer solution on the separation of the analytes were also investigated. The reproducibility of the CE method was also examined. Moreover, the five analytes in *Puerariae radix* and its medicinal preparations were determined by the optimized CE method.

2. Experimental

2.1. Apparatus

The experiments were performed on Beckman P/ACE 2000 and P/ACE 5500 CE systems (Beckman Instruments, Fullerton, CA, USA). A personal computer controlled the P/ACE instrument. Data analyses were performed on System Gold software. A 47 cm (40 cm to the detector)×50 μm I.D. fused-silica capillary tube (Polymicro Technologies, Phoenix, AZ, USA) was used. The capillary column was assembled in a cartridge format. Temperature of the capillary tube during electrophoresis was maintained at 25°C by the P/ACE thermostatting system. The applied voltage of the electrophoresis separation was set at 21 kV. Samples were pressure injected at 0.034 bar (0.5 p.s.i.; 1 p.s.i.=6894.76 Pa) for 2 s.

2.2. Chemicals

Sodium hydroxide was purchased from Fluka (Buchs, Switzerland). Borax, biochanin A and genistein were purchased from Sigma (St. Louis, MO, USA). Daidzin and daidzein were obtained from Extrasynthese (Genay, France). Puerarin was purchased from Yoneyama (Osaka, Japan). Methanol was obtained from Merck (Darmstadt, Germany). Chinese medicinal preparations of *Puerariae radix* and Ge-gen-tang samples manufactured by GMP medicinal companies and *Puerariae radix* root were purchased from local drug stores. The manufacturing procedure for the two commercial products includes boiling in water, drying and powdering. Water was purified with a Milli-Q water system (Millipore, Bedford, MA, USA) and filtered through a 0.22-μm filter.

2.3. Procedure

5 mg/ml standard solutions of five analytes were prepared in methanol. Sample solutions with various concentrations were prepared by diluting the standard solutions with methanol. Borax–NaOH buffer solutions were prepared by mixing appropriate amounts of 0.1 *M* borax with 0.1 *M* NaOH in deionized water.

3 g of *Puerariae radix* root and each concentrated commercial medicinal preparation were accurately weighed. The weighed sample was mixed and extracted with 20 ml methanol for 15 min in an ultrasonic bath. The extract was then filtered through a filter paper. The extraction procedure was repeated three times. Next, a total of 60 ml extracted solution was concentrated to dryness. Methanol was added to dissolve the residue to a final volume of 4.0 ml before analysis by CE.

3. Results and discussion

Fig. 1 illustrates the molecular structures of the five analytes. Those analytes have similar primary structures with different functional groups at their benzene rings. Among those analytes, the hydroxyl group is the common functional group for those analytes; two of the analytes have glucosyl moiety. The difference in the functional group among the analytes is attributed primarily to the number and position of the hydroxyl groups at the benzene ring. Based on UV absorbance spectra of those analytes, 200 nm was selected herein for detection.

3.1. Effects of buffer pH value and buffer concentration on the separation

Fig. 2 summarizes the effects of buffer pH value,

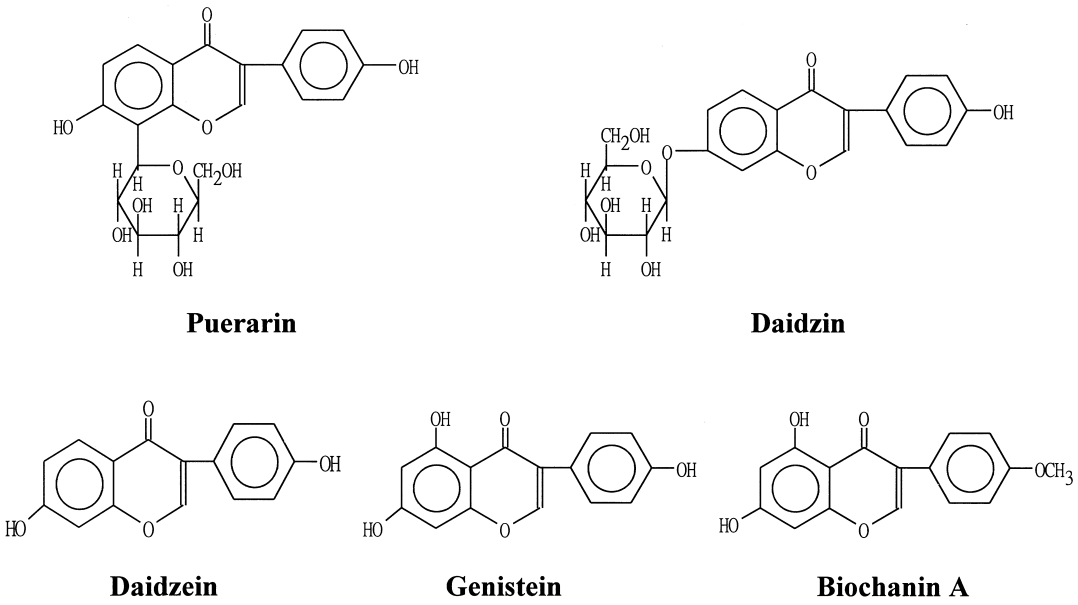

Fig. 1. Molecular structures of the five analytes.

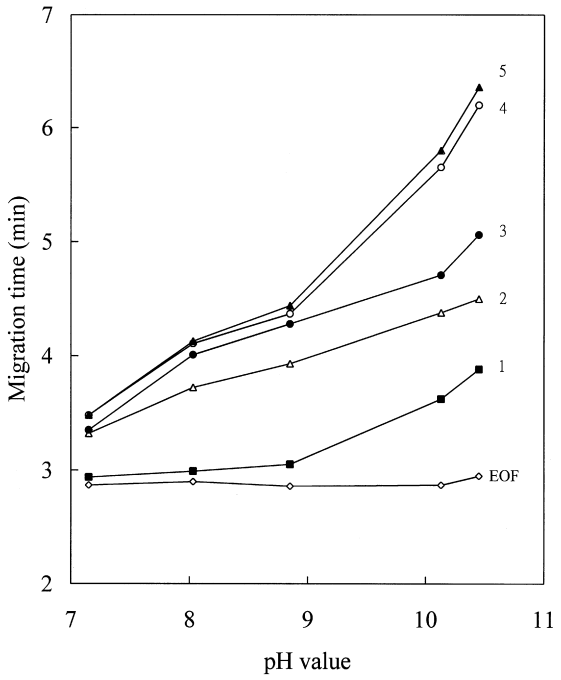

Fig. 2. Effect of buffer pH value on the analyte migration times. 1=Daidzin; 2=biochanin A; 3=puerarin; 4=daidzein; 5=genistein. Conditions: capillary, 47 cm (40 cm to detector)×50 μm I.D.; applied voltage, 21 kV; detection wavelength, 200 nm; pressure injection, 2 s; column temperature, 25°C.

ranging from 7.2 to 10.4, on the analyte migration behavior. Both phosphate and borate buffer systems were used as the running buffers. The migration times of analytes increased with an increasing pH value of the running buffer. This phenomenon can be attributed to that the hydroxyl group at the benzene rings of the five analytes could be dissociated and the degree of the dissociation of the hydroxyl group increased with a rising pH value. However, the migration sequence of the five analytes remained the same within the pH range.

As Fig. 2 indicates, the migration velocity of genistein was the lowest among those analytes. Genistein, having three hydroxyl groups at the benzene ring, could carry more negative charges than other analytes. In contrast, daidzin has the largest molecular mass with only one hydroxyl group, thus leading to the shortest migration time.

Experimental results indicate that the buffer pH value heavily influenced migration velocities of the five analytes. Also, the differences between the analyte migration velocities were altered with an increasing pH value. Daidzin and electroosmotic flow migrated at a similar velocity at pH 7.2. Biochanin A and puerarin similarly migrated with each other, as did as daidzein and genistein. Thus,

poor separation of the five analytes was observed at pH 7.2. The analyte resolutions enhanced with an increasing pH value. This enhancement is likely ascribed to the increasing difference in the hydroxyl group dissociation. The five analytes were adequately separated at pH 10.1 and 10.4 buffers. Moreover, the separation time at pH 10.4 surpassing that at pH 10.1 implied that the pH 10.1 borax–NaOH was the preferred choice to separate the analytes.

The buffer concentration plays a prominent role in electrophoretic separation. Consequently, more closely examining the effects of buffer concentration on the separation of the five analytes is highly desired. A borate buffer of pH 10.1 was employed with the concentration ranging from 5 m*M* to 25 m*M*. Fig. 3 summarizes the effects of buffer concentration on the analyte migration behavior. According to this Figure, the migration times of the five analytes increased with an increasing buffer concentration. Although the separation time could be reduced with a decreasing buffer concentration, the resolutions between the analytes deteriorated. Note that daidzein and genistein could not be separated when the buffer concentration was lower than 10 m*M*. While considering both resolution and separation time, the optimized condition of the pH 10.1 borax–NaOH buffer for separating the five analytes was at 20 m*M*.

Fig. 4 depicts the electropherogram of the five analytes under the optimized conditions. The five bioactive components were adequately separated within 6 min by the CE method. Table 1 lists the average migration times, the reproducibilities (R.S.D.s), slopes, intercepts, linearities of the calibration graphs and the detection limits of the five analytes. The R.S.D.s of the migration times were lower than 0.38%. Peak area of the electropherogram was employed for quantifying the analytes. The linearities of the calibration graphs for the analytes were at least two orders from 5 μg/ml to 500 μg/ml, in which correlation coefficients exceeded 0.998. The detection limits for those analytes ranged from 1.22 μg/ml to 1.77 μg/ml. Thus, the CE method has the advantages of high resolution, high efficiency and short separation time.

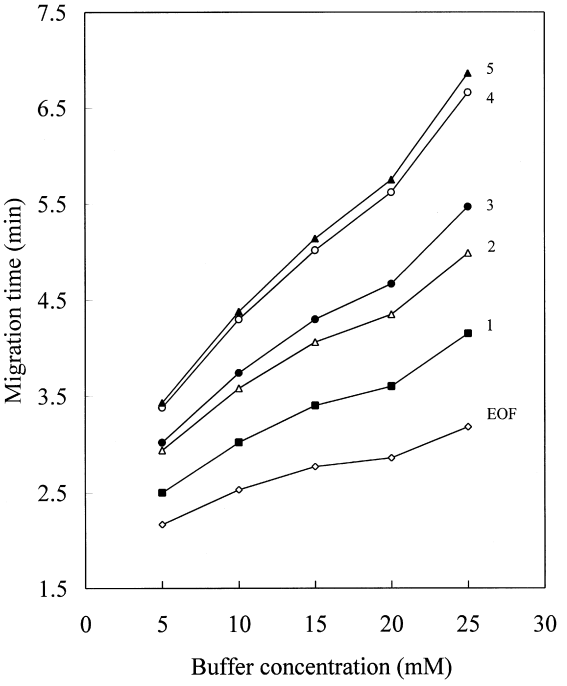

Fig. 3. Effect of buffer concentration on the analyte migration times. Conditions: separation solution, borax–NaOH buffer, pH 10.1. Other conditions as in Fig. 2.

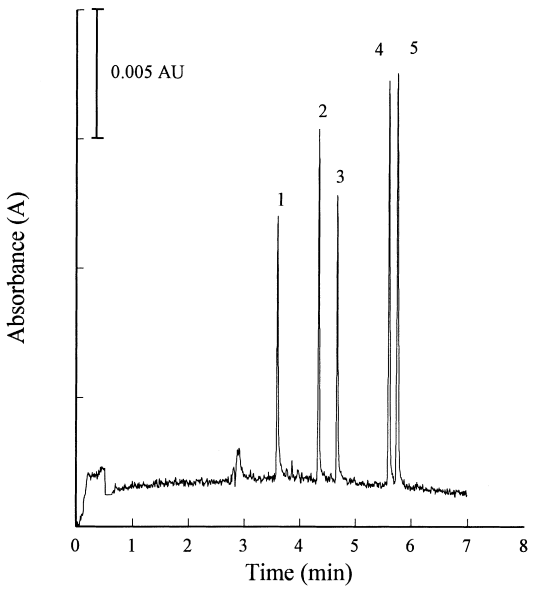

Fig. 4. Separation of the five analytes under the optimized conditions. Conditions: separation solution, 20 m*M* borax–NaOH buffer, pH 10.1; concentration of each analyte, 150 μg/ml. Other conditions as in Fig. 2.

Table 1
Average migration times, reproducibilities, slopes, intercepts, correlation coefficients of calibration graphs and the detection limits of the five analytes

Analyte	Migration time[a] (min)	R.S.D. (%)	Slope	Intercept	Correlation coefficient of calibration graph (r)	Detection limit (μg/ml)
Daidzin	3.57	0.21	0.0037	−0.0073	0.999	1.77
Biochanin A	4.34	0.38	0.0051	−0.0134	0.999	1.37
Puerarin	4.67	0.34	0.0032	0.0006	0.999	1.66
Daidzein	5.61	0.38	0.0060	−0.0731	0.998	1.22
Genistein	5.76	0.38	0.0027	−0.0098	0.999	1.33

[a] $n=10$.

3.2. Extraction and determination of the analytes in Puerariae radix and its medicinal preparations

The amounts of the five analytes were determined in three different samples, including *Puerariae radix*, medicinal preparation of *Puerariae radix*, and medicinal preparation of Ge-gen-tang. Methanol and methanol–water (7:3, v/v) were initially employed as the extraction solvents. According to experimental results, the five analytes solubility in methanol–water solution was inadequate. Also, the extract by methanol–water solution contained some interferences which intensified the background noise. Based on above results, we can infer that methanol is a better solvent to extract the samples.

Figs. 5–7 display the electropherograms of the extracts of *Puerariae radix*, medicinal preparation of *Puerariae radix* and medicinal preparation of Ge-gen-tang, respectively. As those Figures reveal, more than ten peaks appeared in the electropherograms. However, daidzin, biochanin A, puerarin, daidzein and genistein were adequately resolved from other unknown compounds and could be clearly identified. The five components could be determined in *Puerariae radix* and medicinal preparation of *Puerariae radix*. With the extract of the medicinal

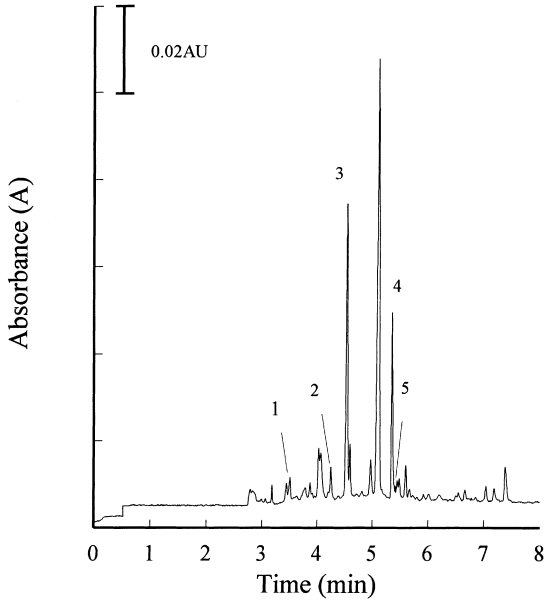

Fig. 5. Electropherogram of *Puerariae radix*. Conditions as in Fig. 4.

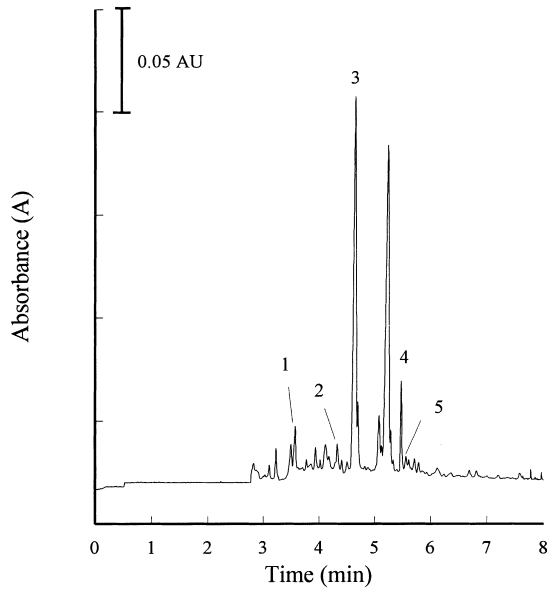

Fig. 6. Electropherogram of medicinal preparation of *Puerariae radix*. Conditions as in Fig. 4.

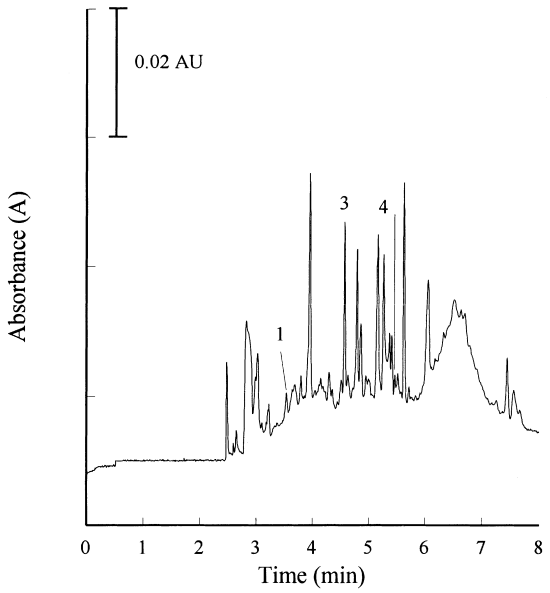

Fig. 7. Electropherogram of medicinal preparation of Ge-gen-tang. Conditions as in Fig. 4.

preparation of Ge-gen-tang sample, only daidzin, puerarin and daidzein were determined. Biochanin A and genistein could not be detected. Those analytes in actual samples were identified by comparing both the migration times and the UV spectra of standards with those in actual samples. Those analytes were further confirmed by spiking standards in actual samples. The specific constituents in actual samples could be adequately identified through those processes.

Table 2 lists the quantities of the five analytes in three actual samples and their relative standard deviations. The R.S.D.s were less than 5.83%. Notably, the amount of puerarin was the highest among those five analytes in the three samples. The amounts of the analytes in the medicinal preparation of *Puerariae radix* surpassed those in *Puerariae radix*. This finding is likely ascribed to that the medicinal preparations are concentrated and powdered from crude herbs. Since Ge-gen-tang includes other herbs, the amount of *Puerariae radix* in Ge-gen-tang is only 20% (w/w) [1]. Thus, the analyte contents in Ge-gen-tang were lowest among those samples. Also, the ingredients of the traditional Chinese medicinal preparation is quite complex and some components may alter during the manufacturing process. In addition, the relative ratios of the amounts for the five analytes varied among those actual samples, likely owing to different sources of crude herb or different manufacturing processes. Experimental results presented herein demonstrate that the CE method can rapidly and efficiently analyze constituents in crude herb and traditional Chinese medicinal preparation.

In summary, the five analytes were completely separated within 6 min using a 20 m*M* borax–NaOH (pH 10.1) buffer. The CE method successfully analyzed the five constituents in *Puerariae radix* and its traditional Chinese medicinal preparations. The extraction method for actual samples was relatively simple and efficient. Consequently, the CE method is a promising alternative to analyze other crude herbs and traditional Chinese medicinal preparations. Results presented herein can hopefully further advance current knowledge of Chinese traditional medicine.

Table 2
Content of the five analytes in actual samples

Analyte	*Puerariae radix*		Medicinal preparation of *Puerariae radix*		Medicinal preparation of Ge-gen-tang	
	Mean (μg/g)[a]	R.S.D. (%)[a]	Mean (μg/g)[a]	R.S.D. (%)[a]	Mean (μg/g)[a]	R.S.D. (%)[a]
Daidzin	63.0	1.37	292.1	0.32	52.1	4.96
Biochanin A	51.4	1.77	78.7	3.14	–[b]	
Puerarin	994.1	5.83	3238.4	0.91	298.5	0.82
Daidzein	54.7	1.29	207.9	5.12	45.7	1.58
Genistein	65.0	4.53	66.4	–[b]		

[a] $n=3$.
[b] Not detected.

Acknowledgements

This study was supported by Grant NSC 86-2113-M-009-016 from the National Science Council of Taiwan, R.O.C.

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