Characterization of Flavonoids in Extracts from Four Species of *Epimedium* by Micellar Electrokinetic Capillary Chromatography with Diode-Array Detection

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Abstract

A micellar electrokinetic capillary chromatographic (MEKC) method with diode-array detection is developed for the characterization of pharmacologically active flavonoids in extracts prepared from *Epimedium brevicornum, E. hunanense, E. coactum,* and *E. truncatum.* The pK_a values of icariin, epimedin B, and epimedin C are determined by spectrophotometry. Optimal separation of icariin, epimedin B and C, and eight other compounds is achieved by determining pK_a values and by systematically optimizing electrolytic and instrumental parameters. The repeatability of analyses and the reliability of identifications are evaluated by the marker technique. Calculated for relative migration times of flavonoids in the extracts, the repeatability of the analyses varies from 0.7 to 6.4% (nine replicates). For migration indices calculated with two markers, however, the repeatability almost falls below 0.5%. The distribution of the flavonoids is found to differ both qualitatively and quantitatively among the four species. The MEKC technique appears to provide a powerful tool for the identification and quality control of plant drugs and for phytotaxonomic investigations.

Introduction

Herba Epimedii from several species of *Epimedium* (of the family *Berberidaceae*) is a popular Chinese medicine used against chronic and cardiovascular diseases. Flavonoids are active compounds and the major constituents of the genus. The flavonoid extracts from *Epimedium* are pharmacologically effective on dilation of the coronary artery, inhibition of platelet aggregation, and delay of thrombus formation. They can also improve humoral and cellular immunity, increase synthesis of DNA, and delay aging (1). It would be a great benefit, therefore, to be able to separate and identify the individual flavonoids in the extracts for the purpose of further pharmaceutical and pharmacological studies.

Such methods as pulse polarography, coulometric titration, fluorometry, and liquid chromatography (2,3) have been reported for the determination of total flavonoids and of icariin. The latter is thought to be the major active compound of *Epimedium.* However, none of these methods is entirely adequate because of the long time required for analysis, the limited resolution, and the low sensitivity. As a modern separation method, capillary electrophoresis (CE) has won increasing acclaim for its extremely high efficiency, small sample volume, high speed, and good resolution (4). CE has been applied to the separation of flavonoids since 1991 (5–15). Nevertheless, most reports have focused on the potential of the technique for standard solution separations and have paid little attention to the potential for the systematic validation and characterization of pharmaceuticals in real plant samples.

In our later study, 17 flavonoids and one phenylethanoid glucoside from *Epimedium* were separated by CE. Fifteen of them were completely resolved by micellar electrokinetic capillary chromatography (MEKC), but icariin and epimedin B and C partially co-eluted (16). Selecting the optimum pH for the separation of these three compounds requires determination of their pK_a values, but these values have not yet been reported.

Icariin and epimedin B and C have been isolated by preparatory chromatography as major constituents of *E. hunanense* Handz-Mazz. *Epimedium coactum* H.R. Liang and *E. truncatum* H.R. Liang were recently discovered as new species and are believed to have better medical efficacy than other known species (17). However, their chemical constituents have not yet been elucidated. *E. brevicornum* was the first *Epimedium* species to be used and the first of five species of the genus to be accepted into the Chinese Pharmacopoeia. Only icariin was isolated from *E. brevicornum.* Moreover, there have been no reports so far on the determination of flavonoids other than total flavonoids or icariin. Because icariin is a secondary glycoside of epimedin B and C, the simultaneous determination of these three compounds, along with other flavonoids, is essential to the reliable quality control and phytotaxonomy of the different species of *Epimedium.*

The aims of this study were (*a*) to determine the pK_a values

of icariin and epimedin B and C; (*b*) to optimize the separation of icariin, epimedin B and C, and eight other components from *Epimedium* by MEKC; (*c*) to validate the new method with the aid of the two-marker technique; and (*d*) to apply the method to the qualitative and quantitative analysis of flavonoids in extracts of *E. brevicornum, E. hunanense, E. coactum*, and *E. truncatum*.

Experimental

Chemicals

All chemicals were of analytical-reagent grade; they were sodium dodecyl sulfate (SDS) (Sigma, St. Louis, MO), disodium tetraborate decahydrate (borate) and *o*-phthalic acid (FA) (E. Merck, Darmstadt, Germany), xanthene-9-carboxylic acid (XA) (EGA-Chemie, Steinheim, Germany), meso-2,3-diphenyl succinic acid (MA) (TCI, Tokyo, Japan), triphenylacetic acid (TFA) (Aldrich, Steinheim, Germany) KCl (2M), NaOH (0.1M) and HCl (0.1M). Water used for dilution and buffer solutions was distilled, deionized, and filtered through 0.45-µm membranes.

The flavonoids and the phenylethanoid glucoside (Figure 1) were isolated from the aerial parts of *Epimedium* species by preparatory chromatography. Their structures have been identified by spectroscopic methods (UV, IR, FABMS, MS–MS, ^{1}H- and ^{13}C-NMR, ^{1}H-^{1}H COSY, DEPT, HMQC, HMBC, and TOCSY) (17).

Plant materials

E. brevicornum Maxim., *E. coactum* H.R. Liang, *E. hunanense* Handz-Mazz., and *E. truncatum* H.R. Liang were collected in China (1994). The materials were identified, and their voucher specimens were deposited in the herbarium of the Beijing University of Traditional Chinese Medicine.

Preparation of plant samples

Dried and powdered aerial parts (200 mg) from *Epimedium brevicornum, E. coactum, E. hunanense*, and *E. truncatum* were refluxed with 95% ethanol for 3 h (three times). The concentrated extracts were suspended in water and partitioned with petroleum ether, chloroform, and *n*-butanol. The stock solutions of 1000-ppm *n*-butanol fractions in methanol were filtered and used as the plant samples for CE analyses.

Capillary electrophoresis

Apparatus

The separations were performed on a Waters Quanta 4000 CE system (Millipore Corporation, Waters Chromatography Division, Milford, MA) and a Hewlett-Packard Chemstation 3D CE equipped with an HP diode-array detector (DAD) (Hewlett-Packard, Avondale, PA) using a 0.050-mm-i.d. × 360-mm-o.d. fused-silica capillary (Composite Metal Services Ltd., Worcestershire, UK). Migration indices were computed with in-house programs developed for MATLAB (19). The data were analyzed with Microsoft Excel version 5.0.

The pH of the buffers was adjusted with a Jenway 3030 pH meter connected to a Jenway electrode (Jenway, Felsted, UK) containing 4M KCl in saturated AgCl. The electrode system was calibrated with potassium hydrogen phthalate (pH 4.0) and borate–sodium hydroxide solutions (pH 11.0). A water system from Gelman Sciences (Ann Arbor, MI) was used for ion-exchange of the distilled water.

Procedure

New capillary tubes were purged with 0.1M NaOH, water, and buffer before use. The capillaries were conditioned daily by washing with 0.1M NaOH, water, and buffer successively for 3–5 min. Between consecutive analyses, the capillary was purged for 1.5 min with NaOH, and then with water and the buffer solution for 2 min each.

Standards were dissolved in methanol (1000 ppm) and further diluted with 30% aqueous methanol to obtain reference solutions (20–200 µg/mL). Stock solutions of 1000 ppm of each marker (XA and MA) were prepared in methanol. All solutions were filtered through 0.45-µm pore size membranes (Gelman Sciences). Twenty microliters of XA and 150 µL of MA were added to tubes containing 400 µL of the sample solutions.

Samples were introduced into the capillary from its anodic end by hydrostatic mode for 8 s. The total length of the capillary was varied from 53 to 73 cm, the effective length was varied from 45 to 65 cm, and the running voltage was varied from 16 to 25 kV. Detection was performed at wavelengths of 200, 254, 280, 330, and 360 nm. All experiments were carried out at ambient temperature.

Spectrophotometric determination of pK_a values for icariin and epimedin B and C

Apparatus

The pK_a values were measured by spectrophotometric titration at 25°C with an ultraviolet–visible (UV–vis) spectrophotometer (Perkin-Elmer model 554 printer RPS-10) (Perkin-Elmer, Ueberlingen, Germany) with 1-cm path length cuvettes, and values of pH were measured with a potentiometer (Radiometer PHM 64) (Radiometer, Copenhagen, Denmark).

Procedures

The pH meter was calibrated with standard buffers of 0.05M potassium hydrogen phthalate (pH 4.008) and disodium tetra

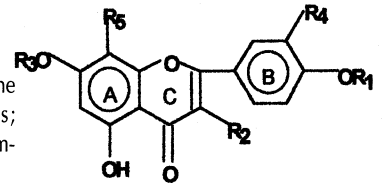


Figure 1. Structures of the investigated flavonoids; glu = glucose, rha = rhamnose, and xyl = xylose.

No.	Name	R_1	R_2	R_3	R_4	R_5
1	Thalictoside					
2	Luteolin-7-O-glucoside	H	H	glu	OH	H
3	Icariin I	CH_3	OH	glu	H	prenyl
4	Quercitrin	H	O-rha	H	OH	H
5	Quercetin	H	OH	H	OH	H
6	Tricin	H	H	H	OCH_3	H(5'-OCH_3)
7	Icariin II	CH_3	O-rha	H	H	prenyl
8	Luteolin	H	H	H	OH	H
9	Epimedin C	CH_3	O-rha-rha	glu	H	prenyl
10	Epimedin B	CH_3	O-rha-xyl	glu	H	prenyl
11	Icariin	CH_3	O-rha	glu	H	prenyl

borate decahydrate (pH 9.180) at 25°C.

Recrystallized materials (icariin [molecular weight, 676], epimedin C [molecular weight, 822], and epimedin B [molecular weight, 808]) were lyophilized overnight, weighed, and dissolved in distilled and deionized water to provide reference stock solutions. Three different sample solutions of each compound were prepared by mixing 20 mL of the stock solution with 10 mL of 0.1M NaOH and 15 mL of 2M KCl (A), or with 10 mL of 0.1M HCl and 15 mL of 2M KCl (B), or with 15 mL of 2M KCl only (C). Solutions A, B, and C were then each diluted to 100 mL (0.07379mM icariin, 0.074mM epimedin C, and 0.05596mM epimedin B).

Results and Discussion

Optimization of flavonoid separation

Optimized conditions for extracts

The primary purpose of the method development was to separate as many flavonoids of *Epimedium* as possible. After studies (17) on the effects of borate, SDS, organic modifier, voltage, and effective capillary length on the migration of 17 standard compounds from *Epimedium,* 15 of the analytes could be completely separated within 20 min with 20mM borate and 48mM SDS containing 1mM 1,3-diamino propane at pH 8.5. Icariin and epimedin B and C partially overlapped, however (17). Flavonoid extracts prepared from the four species of the present study were analyzed under the optimized conditions. The results showed that the contents (percentages of peak areas) of the three flavonoids in the extracts of *Epimedium* were high, so complete separation of the three analytes is of significance.

Optimization of electrolyte solutions

In a study on the effect of SDS concentration from 0 to 54mM, we found the best concentration for the separation of icariin and epimedin B and C to be 12mM at pH 8.5 (17). This was probably because the difference in hydrophobic property of the analytes was greatest at that concentration; because they differed only in terminal sugar moieties, they showed little difference at higher SDS concentrations. To further optimize the separation at 12mM SDS, seven buffer solutions of borate ranging from 10 to 36mM were used. Of these, 30mM borate proved to be the best, but the resolution was still not good enough to separate the extracts. Accordingly, we next studied the effect of adding 2-propanol to the electrolyte solution in concentrations ranging from 1 to 15% (v/v). When 15% 2-propanol was added to a buffer of 12mM SDS and 30mM borate, icariin and epimedin C were completely resolved, but their migration times were increased to over 40 min. This time is too long for routine analysis, and 2-propanol was thus dropped from the further studies.

Table I. Determination of pK_a Values for Icariin and Epimedin B and C (25°C)

	pH	A	A_I-A	$A-A_M$	$\log\frac{A_I-A}{A-A_M}$	pK_a	I	pf_{H^+}	pK_a
Analyte: icariin, λ = 280 nm, A_I = 0.384 (pH 11.9), A_M = 0.214 (pH 3.56)									
1	10.18	0.235	0.149	0.021	0.85	10.83	0.300	0.129	10.70
2	10.54	0.268	0.116	0.054	0.33	10.87	0.300	0.129	10.74
3	10.69	0.287	0.097	0.073	0.12	10.81	0.300	0.129	10.68
4	11.02	0.315	0.069	0.101	–0.17	10.85	0.300	0.129	10.72
5	11.10	0.326	0.058	0.112	–0.29	10.82	0.300	0.129	10.69
6	11.20	0.336	0.048	0.122	–0.50	10.80	0.300	0.129	10.67
7	11.31	0.348	0.036	0.134	–0.57	10.79	0.300	0.129	10.66
8	11.51	0.362	0.022	0.148	–0.63	10.87	0.300	0.129	10.74
					pK_a = 10.70 ± 0.03				
Analyte: epimedin C, λ = 280 nm, A_I = 0.386 (pH 11.5), A_M = 0.240 (pH 3.5)									
1	10.10	0.262	0.124	0.022	0.75	10.85	0.300	0.129	10.72
2	10.54	0.291	0.095	0.051	0.27	10.81	0.300	0.129	10.68
3	10.79	0.307	0.079	0.067	0.07	10.86	0.300	0.129	10.73
4	11.02	0.330	0.056	0.090	–0.21	10.81	0.300	0.129	10.68
5	11.20	0.340	0.046	0.100	–0.34	10.87	0.300	0.129	10.74
6	11.30	0.348	0.038	0.108	–0.45	10.85	0.300	0.129	10.72
7	11.40	0.359	0.027	0.119	–0.60	10.80	0.300	0.129	10.67
8	11.51	0.371	0.015	0.131	–0.74	10.77	0.300	0.129	10.64
					pK_a = 10.70 ± 0.03				
Analyte: epimedin B, λ = 280 nm, A_I = 0.245 (pH 11.8), A_M = 0.100 (pH 3.51)									
1	10.23	0.129	0.116	0.029	0.602	10.83	0.300	0.129	10.70
2	10.47	0.144	0.101	0.044	0.361	10.83	0.300	0.129	10.70
3	10.56	0.154	0.091	0.054	0.227	10.79	0.300	0.129	10.66
4	10.67	0.164	0.081	0.064	0.102	10.77	0.300	0.129	10.64
5	10.88	0.181	0.064	0.081	–0.102	10.77	0.300	0.129	10.64
6	10.81	0.172	0.073	0.072	0.006	10.82	0.300	0.129	10.69
7	11.20	0.200	0.045	0.100	–0.347	10.85	0.300	0.129	10.72
8	11.20	0.207	0.038	0.107	–0.450	10.75	0.300	0.129	10.62
					pK_a = 10.67 ± 0.04				

Determination of pK_a values for icariin and epimedin B and C

To find the optimum pH for effective separation of icariin and epimedin B and C, we determined their ionization constants. Flavonoid compounds are weak acids, and their apparent charge depends on their pK_a values. Their pK_a values are thought to vary between 7.3 and 12.5 because of the presence of phenyl hydroxyl groups (20). Icariin and epimedin B and C have only one phenyl hydroxyl group at C-5, and they do not dissolve well in water; 5-hydroxyl flavone dissociates only at high pH values because of its strong intramolecular hydrogen bond. Spectrophotometry is thus preferred to potentiometry for the determination of pK_a values.

The maximum absorbance of ionized species was found at 277 nm for icariin and

epimedin C and at 278 nm for epimedin B (at both pH 11.9 and pH 13.0), whereas the maximum absorbance of neutral species was found at 268 nm for all these compounds (at both pH 3.56 and pH 1.0). A wavelength of 280 nm was accordingly selected for the analytical wavelengths of these compounds. The analytical wavelengths and maximum wavelengths (λ_{max}) of neutral molecules of icariin and epimedin B and C were the same.

The pK_a values of icariin and epimedin B and C were calculated as follows:

$$pK_a = pH + \log\frac{(A_I - A)}{(A - A_M)} - pf_{H+} = pK_{a'} - pf_{H+}$$

$$pf_{H+} = \frac{0.509 \times I^{1/2}}{(1 + 2.201^{1/2} \times I)} - 0.129 \times I$$

(at 25°C)

$$I = 1/2\ \Sigma\, Ci \times (Zi)^2$$

where A_I is the absorbance of the pure ionized species, A_M is the absorbance of the pure molecular species, A is the observed absorbance at the analytical wavelength, I is the ionic strength, f is the activity coefficient, Ci is the concentration of all ionized species, and Zi is the chemical valence of ionized species.

The pK_a values (Table I) of these compounds were almost the same because they differed only in their terminal sugar moieties. In addition, the magnitudes of their pK_a values depended on the phenolic hydroxyl group at C-5, which does not dissociate as easily as the hydroxyl group at the other position because of its strong intramolecular hydrogen bridge.

Optimum pH for separation of icariin and epimedin B and C

The electrophoretic mobility of icariin and epimedin B and C depended on the ionization. For two compounds that differ only slightly in pK_a values, their best separation occured at a pH close to the mean pK_a of the two compounds (21). Terabe et al. have derived a valid equation for the determination of optimum pH for the separation of analytes whose K_{a1} and K_{a2} values are close to each other:

$$\frac{\Delta\alpha}{\alpha^{1/2}} = \frac{(r-1)[H^+](K_a)^{1/2}}{([H^+] + K_a)^{3/2}}$$

where $\alpha = K_a / ([H^+] + K_a)$ and $r = K_{a1}/K_{a2}$ by differentiating the equation. The optimum pH yielding the maximum value of $\Delta\alpha/(\alpha)^{0.5}$ was found as pH (optimum) = pK_a – log 2, so the calculated optimum pH for icariin and epimedin C was 10.40.

The effect of pH values 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, and 10.5 on the separation was investigated experimentally, and the value 10.5 was found to be the best. The results at pH 10.40, 10.45, 10.50, and 10.55 were then carefully compared, and pH 10.45 proved to be optimum. The calculated value and the experimental value obtained by optimization were thus almost the same. When pH values of the electrolyte solutions were near the pK_a values of analytes, minor differences in the pH values of electrolytic solutions resulted in changes in the separability of the analytes.

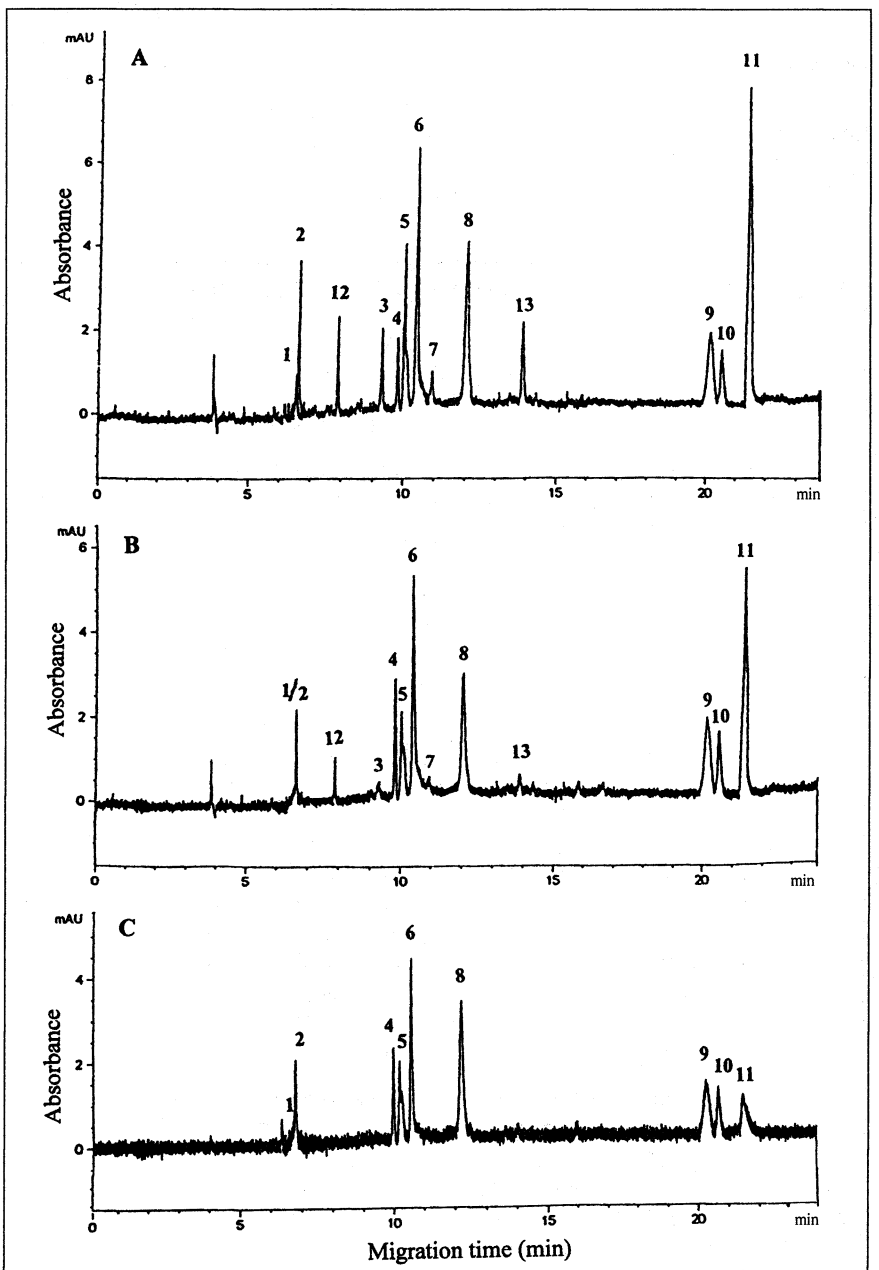
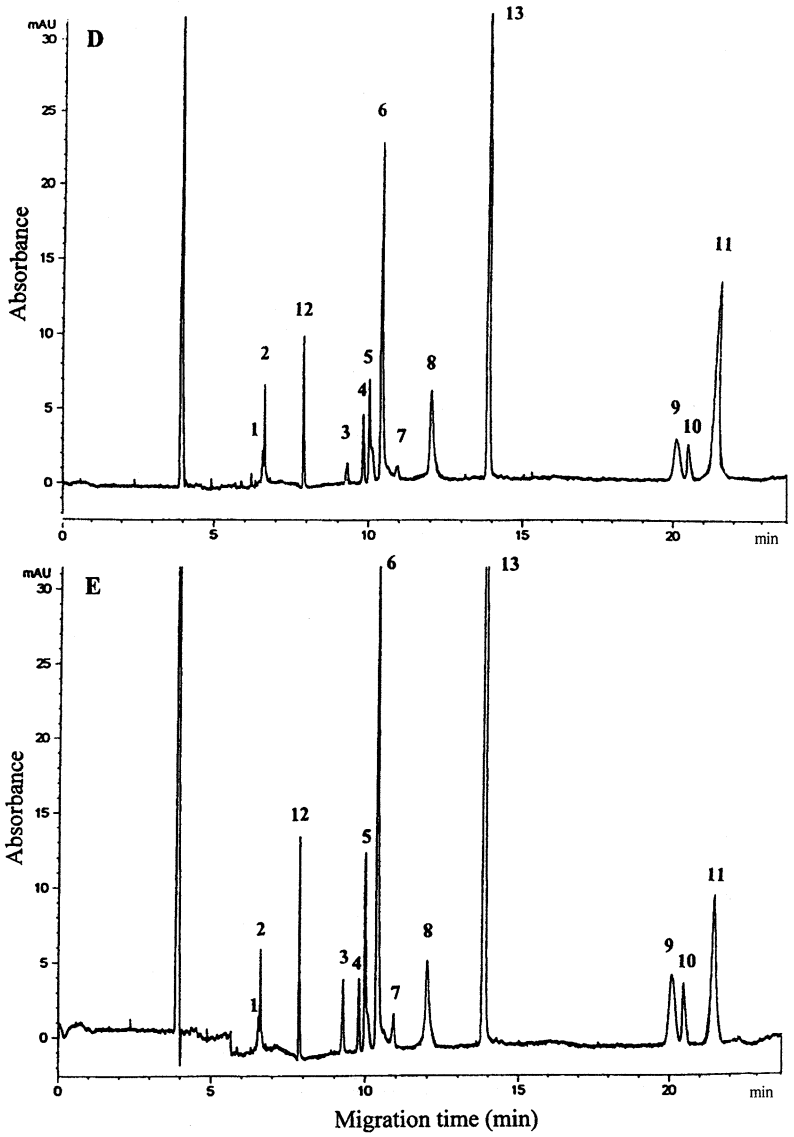


Figure 2. Electropherograms of the analytes at different detection wavelengths with the HP Chemstation 3D CE system. Conditions: 30 mM borate, 12 mM SDS, pH 10.45, and 22 kV with an effective capillary length of 50 cm. For explanation of numbers, see Figure 1. Peak 12, XA; peak 13, MA. A, 254 nm; B, 280 nm; C, 360 nm; D, 200 nm; and E, 210 nm (continued on next page).

Selection of instrumental parameters

A series of experiments with different capillary lengths and applied voltages showed an effective capillary length of 50 cm and an applied voltage of 22 kV to be best for the optimized separation. Detection was performed at wavelengths of 254, 280, 360, 200, and 214 nm (Figure 2). The detector was most sensitive for the analytes at 200 and 210 nm, and then most sensitive at 254 nm. Because of the strong absorption of solvent and electrolyte solutions and the strong interference from nonflavonoids in extracts at 200 and 214 nm, a wavelength of 254 nm was selected for the detection.

At this point, the optimum separation conditions for the analytes were 30mM borate and 12mM SDS at pH 10.45, a capillary with an effective length of 50 cm, and an applied voltage of 22 kV (Figures 2 and 3).



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Method validation

Absolute migration times in CE tend to be poorly repeatable, mainly due to the changes in electroosmotic flow velocity (v_{eo}) and effective electric field strength (E_{eff}) (19). In recent studies (16,22,23), we confirmed that the repeatability obtained with absolute migration times could be improved by introducing marker compounds and a migration index system. In the present study, we compared the repeatabilities achieved with relative migration times with those achieved with migration indices. The two-marker (XA/MA) technique (22,23) and the computer program based on MATLAB were applied to reveal the repeatability of analyses and the reliability of identifications between two successively migrating compounds (Figure 2A).

Table II shows that the migration indices provided better repeatability than the relative migration times in nine replicates. Whereas the repeatability of relative migration times of the analytes varied from 0.22 to 1.03% (nine replicates), the repeatability of migration indices calculated with the two-marker technique varied from 0.01 to 0.27%. Table III shows that the introduction of migration indices resulted in reliable identification of all analyte pairs.

Identification of flavonoids in extracts of the four species

The flavonoids in extracts of *E. brevicornum, E. coactum, E. hunanense*, and *E. truncatum* were comparatively studied to assess the suitability of the MEKC procedure for phytochemical taxonomic investigations. The results showed that the profiles of the extracts from these species were more or less similar and that such characteristic profiles could be used to distinguish this genus from other genera and to identify adulterates. Even though the concentrations of sample solutions from different species were the same, the total numbers of peaks that were separated were different: 23, 15, 18, and 12 from *E. hunanense, E. coactum, E. truncatum*, and *E. brevicornum*, respectively (Figure 4). Furthermore, the peak areas of the same compounds from different species were clearly different. These two features could support the development of an MEKC-based fingerprinting technique for the identification of different species within a genus. The separated peaks were identified through comparison of the relative migration times, migration indices (Table IV), and UV absorption spectra as well as by spiking of individual standards. The repeatability of the analysis of flavonoids in the extracts is shown in Table IV. Relative standard deviations (RSDs) with relative migration times varied from 0.4 to 6.4% (nine replicates),

whereas almost all values calculated with migration indices were below 0.5%.

Quantitative comparison of major flavonoids in the extracts

Because the Waters Quanta 4000 CE apparatus did not include a temperature control unit, an internal standard was necessary for quantitative MEKC work in order to minimize differences in the physical properties of sample solutions containing the same analytes. XA, TFA, MA, and FA, commonly used marker compounds in our laboratory, were evaluated for use as internal standards. XA was finally chosen because it migrated in the middle of the electrophorogram and it could be completely resolved from adjacent peaks because there were no interference effects. It also resembles the flavonoids in structure.

Quantitative studies were performed by adding 50 µg/mL of XA as the internal standard to 1000 ppm of sample. Use of the standard made it possible to evaluate the exact relative contents among the species. The content of flavonoids in extracts of the species was calculated according to the following equation:

$$x = \frac{\text{peak area/migration time of a compound}}{\text{peak area/migration time of the internal standard}}$$

where x is the mean of nine replicates. As Table V shows, icariin and epimedin B and C were present in all four species, but their contents varied from species to species (epimedin B and C were also present in *E. truncatum*, but their peak areas were too small to be integrated using the same integration parameters). Icariin was present in larger amounts than epimedin B and C except in *E. brevicornum*. The content of icariin was highest in *E. coactum* and second highest in *E. hunanense*, and the content in *E. truncatum* was 78% of that in *E. brevicornum*. The contents of epimedin B and C both were higher in *E. brevicornum* than in other species. The total amounts of these three compounds were highest in *E. coactum*. Evidently, *E. coactum* may have potential as a new pharmaceutical resource, and close study of its chemical constituents and pharmacological effects is warranted.

Conclusion

These findings demonstrated the successful applicability of MEKC to the analysis of pharmaceutical plant samples. The

Table II. Repeatability of Analysis in Terms of Relative Migration Times and Migration Indices for the Analytes (Nine Replicates)

	Relative migration times			Migration indices		
Analytes	t_{rel} (avg)	SD	%RSD	Ind (avg)	SD	%RSD
1	1.56	0.02	0.37	685.7	0.07	0.01
2	1.66	0.01	0.39	777.8	1.67	0.21
3	1.72	0.01	0.22	829.0	0.64	0.08
4	2.30	0.01	0.29	1162.3	1.07	0.09
5	2.45	0.01	0.30	1220.3	1.29	0.11
6	2.52	0.01	0.40	1248.5	2.29	0.18
7	2.69	0.02	0.73	1304.3	3.00	0.23
8	3.03	0.01	0.49	1397.4	2.96	0.21
9	4.72	0.04	0.93	1661.1	2.34	0.14
10	4.87	0.05	0.98	1684.4	2.78	0.17
11	5.05	0.05	1.03	1700.8	2.74	0.16

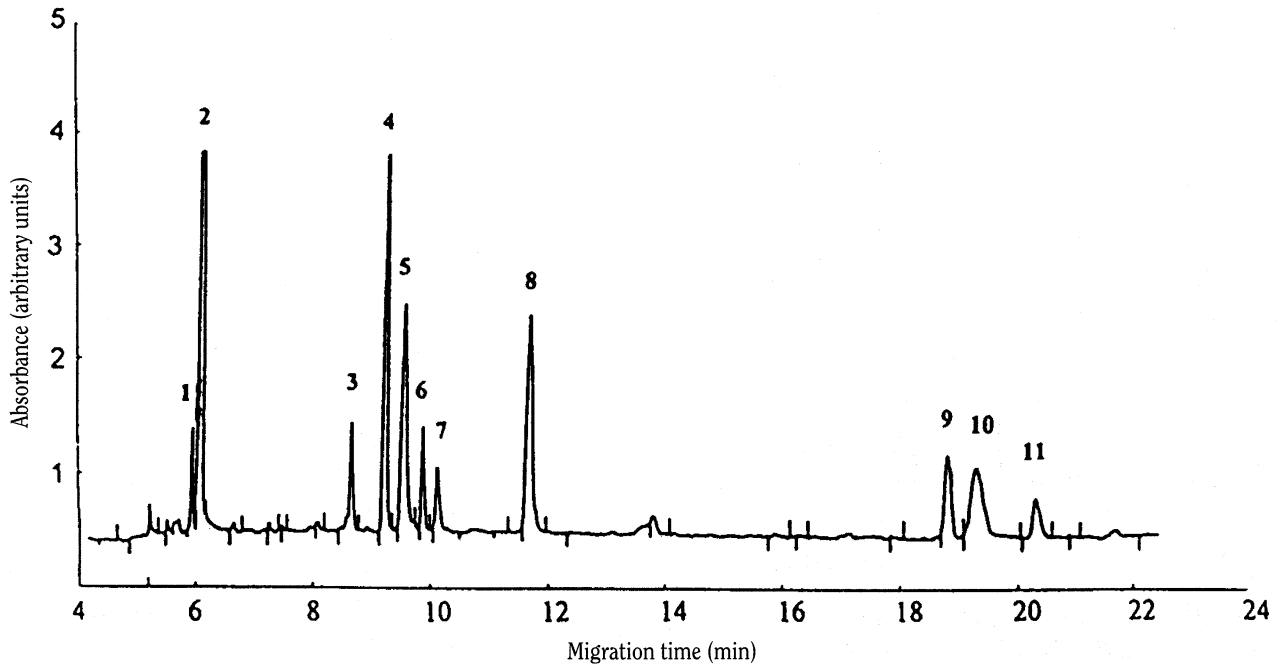

Figure 3. Electropherogram of the analytes recorded with a Waters Quanta 4000 CE system. Conditions and explanation of numbers are the same as in Figure 1.

Table III. Values of Coefficient for Identification (*Qid*) Obtained for Successive Peak Pairs

Analyte pair	Relative migration times *Qid* (t_{rel})	Migration indices *Qid* (ind)	Ratio *Qid* (ind)/*Qid* (t_{ab})
1/2	3.3	52.9	16
2/3	3.0	22.2	7
3/4	29.0	194.9	7
4/5	7.5	24.6	3
5/6	3.5	7.9	2
6/7	5.7	10.5	2
7/8	11.3	15.6	1
8/9	33.8	49.8	1
9/10	1.7	4.6	3
10/11	1.8	3.0	2

conditions for separating the flavonoids in various plant samples were easily and quickly changed, something that is necessary for the resolution of complicated mixtures of natural products. The MEKC method described allowed a simple, fast, effective, and simultaneous determination of the flavonoids in plant samples of *Epimedium*. Our work suggests that MEKC could be especially useful for the routine analysis of pharmaceutical plant products for quality control purposes and for phytochemical taxonomic studies.

Acknowledgments

This work was supported by a grant (to H.-R. Liang) from the Center for International Mobility in Finland. The authors are grateful to Dr. H.-W. Liu of Beijing University for his technical assistance.

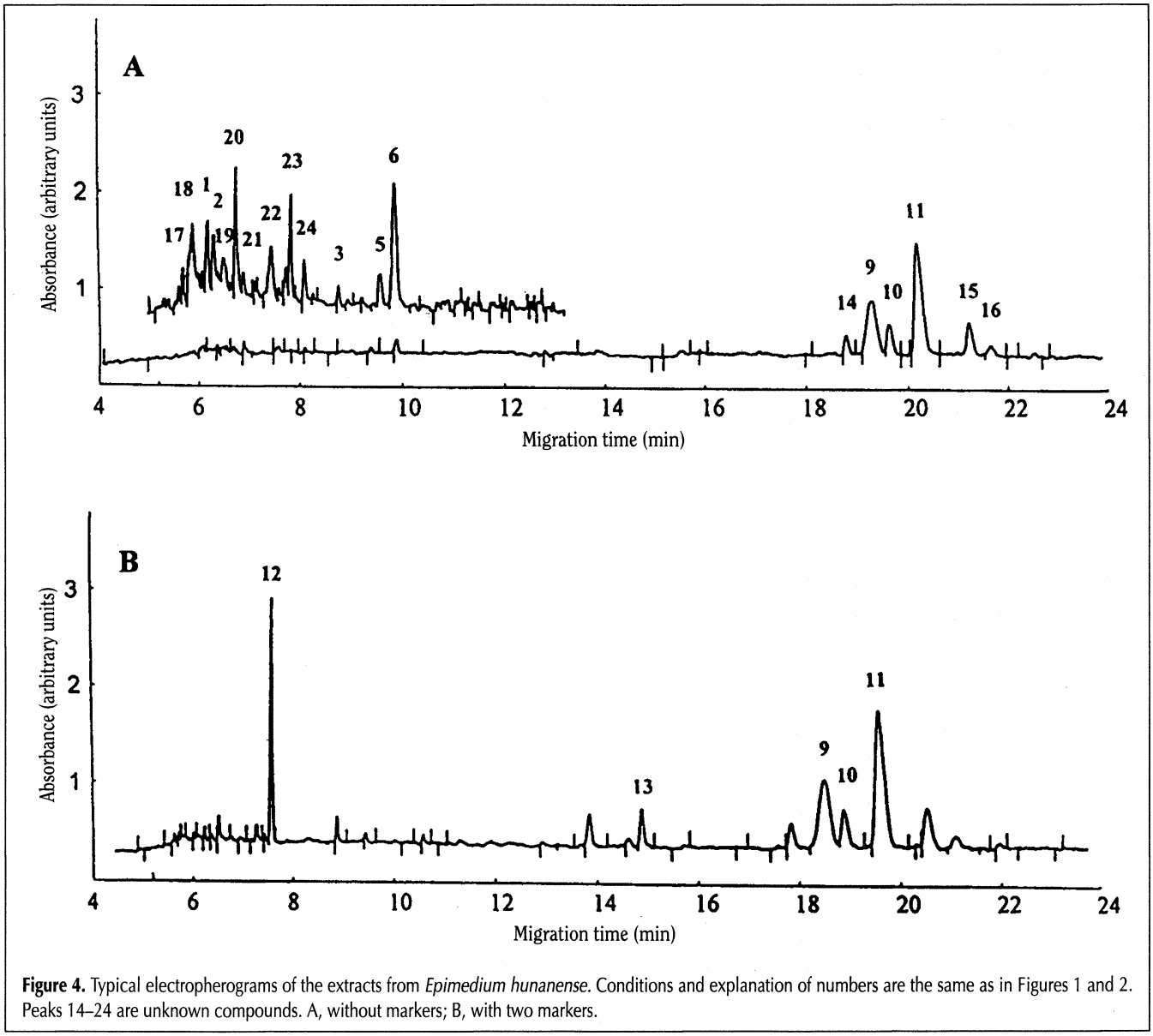

Figure 4. Typical electropherograms of the extracts from *Epimedium hunanense*. Conditions and explanation of numbers are the same as in Figures 1 and 2. Peaks 14–24 are unknown compounds. A, without markers; B, with two markers.

Table IV. Repeatability of Analysis in Terms of Relative Migration Times and Migration Indices for the Extracts (Nine Replicates)

	Relative migration times			Migration indices		
Flavonoid*	t_{rel} (avg)	SD	%RSD	Ind (avg)	SD	%RSD
E. brevicornum						
2	1.66	0.12	5.42	777.2	7.13	0.92
4	2.38	0.03	1.26	1147.8	3.77	0.33
6	2.59	0.02	0.77	1186.6	5.20	0.44
7	2.64	0.02	0.76	1244.8	4.11	0.33
	2.86	0.04	1.40	1285.9	1.30	0.10
	3.88	0.05	1.29	1496.4	3.91	0.26
9	4.81	0.02	0.42	1669.3	3.72	0.22
10	4.91	0.06	1.22	1687.8	6.00	0.36
11	5.09	0.08	1.57	1704.1	3.59	0.21
E. hunanense						
2	1.65	0.11	6.5	771.8	7.91	5.14
3	1.73	0.01	0.82	823.5	10.85	0.21
	1.88	0.02	0.9	838.5	1.52	0.18
7	2.69	0.03	1.03	1246.0	3.06	0.10
	2.88	0.05	1.74	1288.4	3.63	0.34
	4.59	0.06	1.35	1654.6	0.64	0.04
9	4.77	0.10	2.12	1668.4	1.50	0.09
10	4.88	0.10	2.06	1690.5	1.91	0.11
11	5.01	0.13	2.58	1702.1	4.37	0.26
	5.31	0.09	1.73	1730.0	3.24	0.19
E. coactum						
2	1.62	0.10	6.34	635.3	21.75	3.32
	1.70	0.02	1.35	683.7	6.15	0.90
3	1.73	0.03	1.49	692.1	6.02	0.87
	1.78	0.03	1.51	757.3	3.41	0.45
	1.85	0.01	0.66	836.2	7.02	0.84
	2.03	0.02	1.12	842.4	1.94	0.23
	2.20	0.06	2.69	948.0	7.49	0.79
4	2.38	0.09	3.69	1148.7	8.34	7.26
6	2.58	0.03	1.18	1185.6	9.84	0.83
7	2.66	0.02	0.8	1208.5	4.05	0.33
	4.61	0.09	1.93	1648.7	7.91	0.48
9	4.77	0.04	0.78	1669.8	10.85	0.65
10	4.87	0.06	1.21	1691.9	1.52	0.09
11	5.04	0.09	1.76	1702.5	3.06	0.18
	5.29	0.13	2.38	1728.0	3.63	0.21
E. truncatum						
3	1.70	0.05	2.9	683.3	13.71	2.10
	1.79	0.01	0.72	733.6	4.40	0.60
	1.95	0.02	0.85	832.6	3.76	0.45
	2.01	0.05	2.68	877.7	9.12	1.04
6	2.54	0.07	2.4	1197.2	1.68	0.14
	2.80	0.06	2.19	1212.4	1.33	0.11
	2.89	0.11	3.88	1245.1	2.74	0.22
8	3.03	0.02	0.53	1298.5	0.91	0.07
	4.59	0.12	2.68	1648.6	3.79	0.23
9	4.76	0.08	1.75	1660.7	3.49	0.21
10	4.88	0.14	2.86	1685.7	5.90	0.35
11	5.08	0.05	1.01	1697.5	3.40	0.20

* For explanation of numbers, see Figure 1.

Table V. Contents of Major Flavonoids in Extracts of *Epimedium*

Flavonoid extracts*	in *x*	SD	RSD	Relative contents	Total contents
E. brevicornum					
9	1.48	0.05	3.54	100	
10	0.54	0.03	5.78	100	
11	0.60	0.03	4.50	100	2.62
E. coactum					
Unknown 1	0.15	0.005	3.26		
9	0.46	0.022	4.81	31	
10	0.20	0.008	3.82	37	
11	2.22	0.082	3.70	370	3.03
E. hunanense					
Unknown 1	0.11	0.004	3.41		
9	0.66	0.016	2.45	45	
10	0.21	0.007	3.27	39	
11	0.98	0.025	2.60	163	
Unknown 2	0.22	0.010	4.56		2.18
E. truncatum					
9	0.47	0.016	3.34	78	0.47

* For explanation of numbers, see Figure 1.

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Manuscript accepted October 21, 1996.