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Determination of the alkaloids in *Evodiae fructus* by capillary electrophoresis

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Abstract

A total of ten *Evodia* alkaloids was separated successfully by micellar electrokinetic capillary chromatography (MECC) and capillary zone electrophoresis (CZE). The MECC method based on SDS was applied to analyze three indolequinazoline alkaloids (evodiamine, rutaecarpine and carboxyevodiamine) and six quinolone alkaloids (1-methyl-2-nonyl-4(1*H*)-quinolone, 1-methyl-2-[(*Z*)-6- undecenyl] -4(1*H*)-quinolone, 1-methyl-2-undecyl-4(1*H*)-quinolone, evocarpine, 1- methyl-2-[(6Z,9Z)-6,9-pentadecadienyl]-4(1*H*)-quinolone and dihydroevodiamine) in 15 min and CZE technique was used to determine the dehydroevodiamine in 5 min. Linearity over one order of magnitude of concentration was generally obtained and limits of detection for the alkaloids were in the range of 35–47 μg/ml. The relative standard deviations were less than 4% ($n=6$). Contents of *Evodiae* alkaloids in a methanol–water extract of *Evodia fructus* sample could easily be determined by this method. The effects of pH, surfactant concentration and organic modifier concentration of the carrier on the migration behaviour of the solutes are discussed.

Keywords: *Evodiae fructus*; Alkaloids; Indolequinazolines; Quinolones

1. Introduction

Evodiae fructus, the unripe fruit of *Evodia rutaecarpa* (Juss.) Benth or *E. rutaecarpa* (Juss.) Benth. var. *officinalis* (Dode) Huang, is a Chinese herbal drug which contains alkaloids, essential oils, carboxylic acids and limonoids as its main components. According to pharmacological tests, the alkaloids were found to have anti-fungal, analgesia, cardiotonic and body-temperature maintaining effects [1]. This study considers dehydroevodiamine (1), evodiamine (2), rutaecarpine (3), carboxyevodiamine (4), 1-methyl-2-nonyl-quinolone (5), 1-methyl-2-[(Z)-6-undecenyl)-4(1*H*)-quinolone (6), 1-methyl-2-undecyl-4(1*H*)-quinolone (7), evocarpine (8), 1-methyl - 2 - [(6Z,9Z) - 6,9 - pentadecadienyl) - 4(1*H*)-quinolone (9) and dihydroevocarpine (10), as shown in Fig. 1. Several methods have been established to determine some of the alkaloids contained in the crude drug such as thin layer chromatography (TLC) [2] and high-performance liquid chromatography (HPLC) [3,4]. Capillary electrophoresis is a widely applied technique in separation science on account of its high efficiency, rapid rate of separation and small sample requirement and has offered satisfactory results in the analysis of some Chinese herbs [5–8]. We used MECC to determine the content of three indolequinazoline alkaloids (2, 3 and 4) and six quinolone alkaloids (5, 6, 7, 8, 9 and 10) and used CZE to measure the amount of one indolequinazoline

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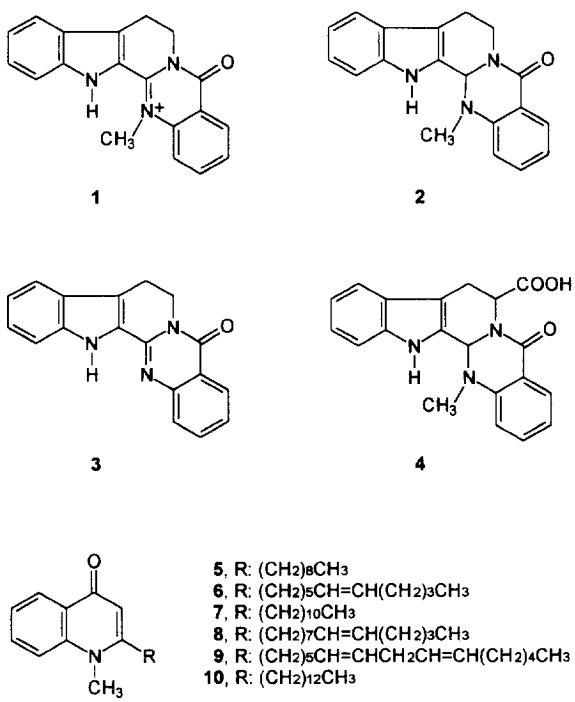

Fig. 1. Structures of the ten *Evodia* alkaloids. 1: dehydroevodiamine; 2: evodiamine; 3: rutaecarpine; 4: carboxyevodiamine; 5: 1-methyl-2-nonyl-quinolone; 6: 1-methyl-2-[(*Z*)-6-undecenyl]-4(1*H*)-quinolone; 7: 1-methyl-2-undecyl-4(1*H*)quinolone; 8: evocarpine (1-methyl-2-[(*Z*)-8-tridecenyl]-4(1*H*)-quinolone); 9: 1-methyl-2-[(6*Z*,9*Z*)-6,9-pentadecadienyl]-4(1*H*)-quinolone; 10: dihydroevocarpine.

alkaloid (1) in *Evodiae fructus* sample. The effects of pH, surfactant and acetonitrile concentrations of the carrier on the migration behaviour of the solutes in MECC and the effect of phosphate and organic modifier concentrations of the electrolyte on the peak-shape improvement of dehydroevodiamine in CZE were studied.

2. Experimental

2.1. Reagents and materials

The alkaloids (1–10) were isolated from *Evodiae fructus* [9–13] and their structures were elucidated on the basis of spectral data, such as infrared (IR), ^{1}H NMR (PMR), ^{13}C NMR (CMR) and mass spectrometry (MS). The purity of these compounds was checked by HPLC. Sodium dodecyl sulphate (SDS) was purchased from Sigma (St. Louis, MO, USA), sodium dihydrogenphosphate from Yoneyama (Osaka, Japan) and sodium borate from Kanto (Kyoto, Japan). Acetonitrile and methanol were of LC grade (Mallinckrodt, Paris, KY, USA). Deionized water from a Milli-Q system (Millipore, Bedford, MA, USA) was used to prepare all buffers and sample solutions. *Evodiae fructus* was purchased from the Chinese herbal market in Taipei (Taiwan).

2.2. Preparation of Evodiae fructus extracts

A 0.5-g sample of pulverized *Evodiae fructus* was extracted with 70% methanol (7 ml) by stirring at room temperature for 30 min, then centrifuged at 1500 *g* (Universal, Hettich Zentrifugen) for 5 min. Extraction was repeated three times. The extracts were combined and filtered through a No.1 filter-paper. After adding a 2-ml aliquot of internal standard solution (2 mg of 18-β-glycyrrhetinic acid in 1 ml of 70% methanol for MECC, 20 mg of benzyltriethylammonium chloride in 1 ml of 70% methanol for CZE), the extract was diluted to 25 ml with 70% methanol. This solution was passed through a 0.45-μm filter and the filtrate was then injected into the capillary electrophoresis system.

2.3. Apparatus and conditions

The analysis was carried out on a Waters Quanta 4000 capillary electrophoresis system equipped with a UV detector set at 254 nm and a 70 cm×75 μm I.D. fused-silica capillary tube (Polymicro, Phoenix, AZ, USA) with the detection window placed 62.5 cm from the injection. The conditions were as follows: injection time, 2 s, hydrostatic (injection volume, 3.4 nl); applied voltage, 25 kV (constant voltage, positive to negative polarity); and temperature, 24.5–25.0°C. In MECC, the electrolyte was a pH 7.31 buffer solution of 40 m*M* SDS, 20 m*M* NaH_2PO_4 and 9 m*M* $Na_2B_4O_7$–acetonitrile (3:2); run time, 15 min. In CZE, the electrolyte was a buffer solution of 40 m*M* NaH_2PO_4–acetonitrile (9:1); run time, 5 min. Before each run, the capillary was washed with 0.1 *M* NaOH for 2 min, with water for 2 min and then with buffer for 2 min.

3. Results and discussion

3.1. Analytical conditions

3.1.1. MECC

Following the method for analyzing the flavonoids in *Scutellariae Radix* [14], a buffer solution of 20 m*M* SDS,10 m*M* NaH_2PO_4 and 12.5 m*M* $Na_2B_4O_7$ was applied to separate the *Evodiae fructus* extract. However, only carboxyevodiamine gave good resolution under these conditions. Using higher SDS concentration and adding acetonitrile as organic modifier, the ten *Evodia* alkaloids could be successfully separated in a single run. The separation was achieved by optimizing the pH of the buffer, the SDS concentration and acetonitrile concentration.

Effect of pH value

Electrolyte systems containing 40 m*M* SDS and 40% acetonitrile at six different pH values ranging from 6.61–7.52 (prepared by mixing 20 m*M* NaH_2PO_4 with 5, 6, 7, 8, 9 and 10 m*M* of $Na_2B_4O_7$, respectively) were used in order to study the effect of pH on the resolution. In Fig. 2, the migration times for the ten alkaloids obtained at different pH values are shown. The migration times of all compounds varied as the pH of the buffer changed and almost paralleled that of the EOF except carboxyevodiamine (4) which lengthened remarkedly even if there was a small increase in pH. From the results, a buffer solution of pH 7.31 (20 m*M* NaH_2P0_4 and 9 m*M* $Na_2B_4O_7$) or higher was found to produce a good separation, with the shortest run time at pH 7.31. At pH values lower than 7.31, however, compound 4 overlapped with the components 5–10 completely or partially, with one of them each time.

Effect of SDS concentration

Electrolyte systems containing 40% acetonitrile at six different SDS concentrations ranging from 0–50 m*M* at pH 7.31 were used to study the effect of SDS concentration on the resolution. The results obtained are shown in Fig. 3, where the migration times are plotted against SDS concentrations. The migration times of the neutral compounds (2, 3 and 5–10) were increased with the increasing SDS concentration. The migration order of these compounds could mostly be explained in terms of degree of hydrophobicity and agreed with the results obtained by reversed-phase HPLC [4]. Usually, the migration times of quinolone alkaloids (5–10) were more influenced by SDS concentration than that of indolequinazoline alkaloids (2 and 3). When SDS was absent or at a concentration lower than 30 m*M*, the six quinolone alkaloids overlapped with each other. At a concentration of 40 m*M*, all compounds to be examined were completely resolved.

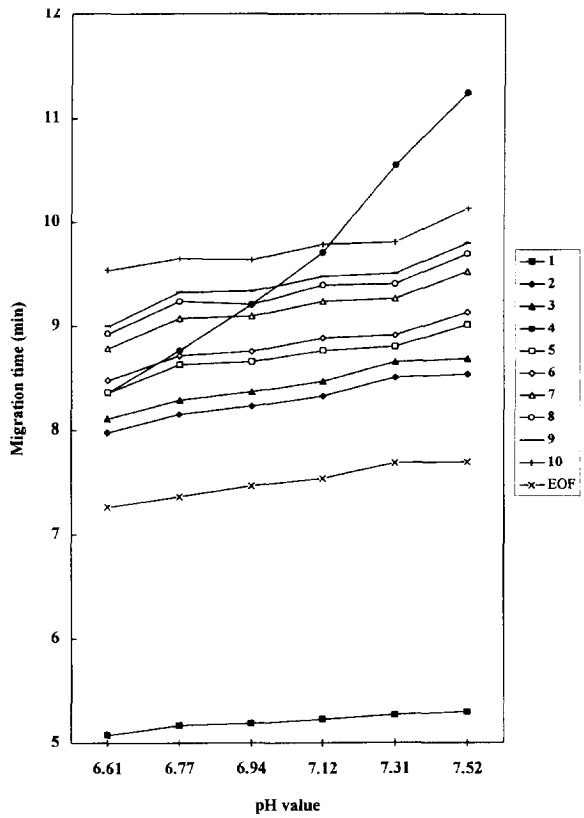

Fig. 2. Effect of pH on migration time. All these experiments were conducted at a voltage of 25 kV across the 70 cm×75 μm I.D. separating tube filled with 60% of phosphate–borate buffers of different pH values containing 40 m*M* SDS and 40% of acetonitrile; temperature 24.5–25.0°C; detection wavelength, 254 nm. Symbols are the same as those in Fig. 1.

Effect of acetonitrile concentration

Electrolyte systems containing 40 m*M* SDS, 20 m*M* NaH_2PO_4 and 9 m*M* $Na_2B_4O_7$ (pH 7.31) were used to study the effect of six acetonitrile concentrations on the resolution. The results obtained

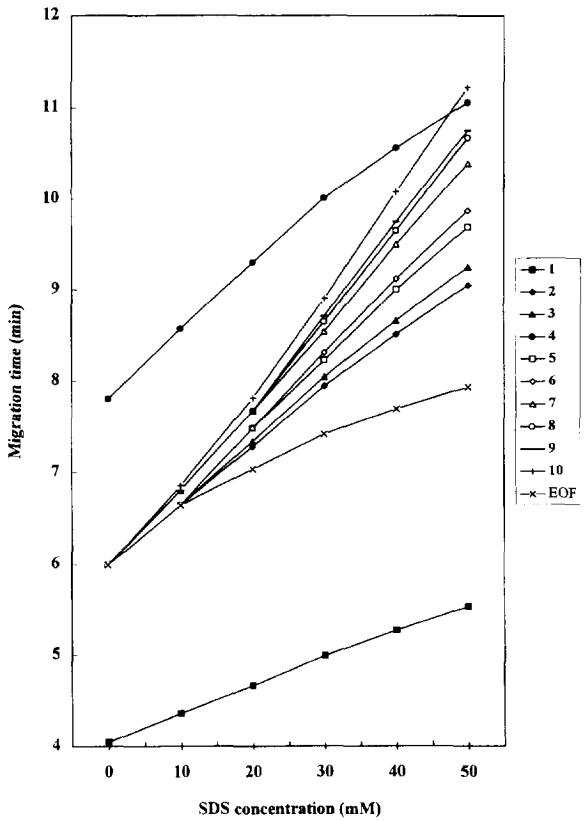

Fig. 3. Effect of SDS concentration on migration time. The carriers were 60% of phosphate–borate buffer (20 m*M* NaH_2PO_4 and 9 m*M* $Na_2B_4O_7$, pH 7.31) containing 0–40 m*M* SDS and 40% of acetonitrile. Other conditions were the same as those in Fig. 2.

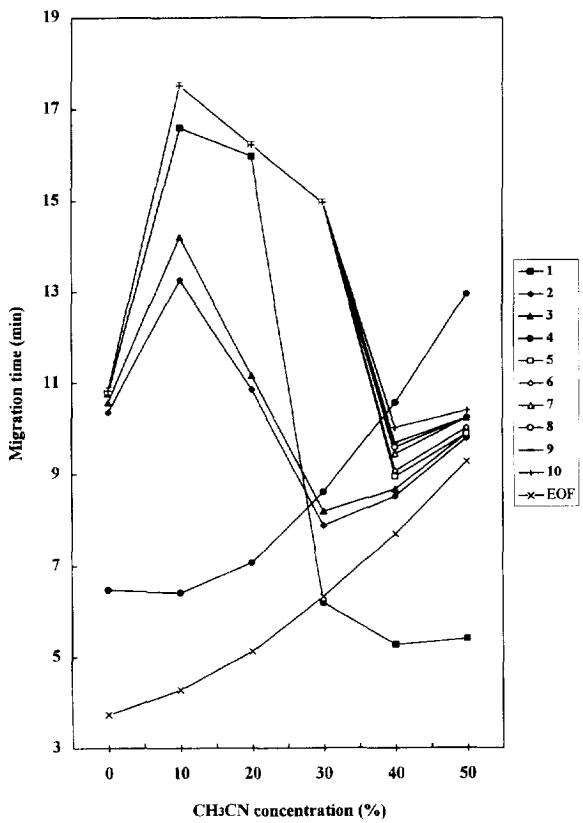

Fig. 4. Effect of acetonitrile concentration on migration time. The carriers were buffer solutions (40 m*M* SDS, 20 m*M* NaH_2PO_4 and 9 m*M* $Na_2B_4O_7$) mixed with different amounts of acetonitrile. Other conditions were the same as those in Fig. 2.

are given in Fig. 4. As the acetonitrile concentration increased, the migration times of all compounds became longer at first and then became shorter. This behaviour can be explained by the decrease of electroosmotic flow (EOF) and tendency to form micelles as the acetonitrile concentration increased. When acetonitrile was not added or was present at a low concentration, all compounds were not separated except the anionic carboxyevodiamine. At 30% acetonitrile, the peaks for indolequinazoline alkaloids (2, 3 and 4) were well separated, but compound 1 eluted with EOF and quinolone alkaloids (5–10) were found to overlap together. Analysis at 40% acetonitrile could offer good separation with peak 1 coming before the EOF. When 50% acetonitrile was used, all neutral compounds were migrating with the EOF as the micelles formation was prevented by the organic modifier.

From the above results, the best resolution was obtained with an electrolyte containing 60% of buffer solution (40 m*M* SDS, 20 m*M* NaH_2PO_4 and 9 m*M* $Na_2B_4O_7$) and 40% of acetonitrile. Fig. 5B is an electropherogram showing the separation of the ten *Evodia* alkaloids with the following migration times: 1, 5.24 min; 2, 8.37 min; 3, 8.52 min; 4, 10.34 min; 5, 8.81 min; 6, 8.92 min; 7, 9.27 min.; 8, 9.41 min; 9, 9.95 min; 10, 9.81 min (plate numbers for 2–10: 169 170–224 052; 1: 82 456).

3.1.2. CZE

Although the MECC conditions listed above could offer good resolution for compounds 2–10, it is not

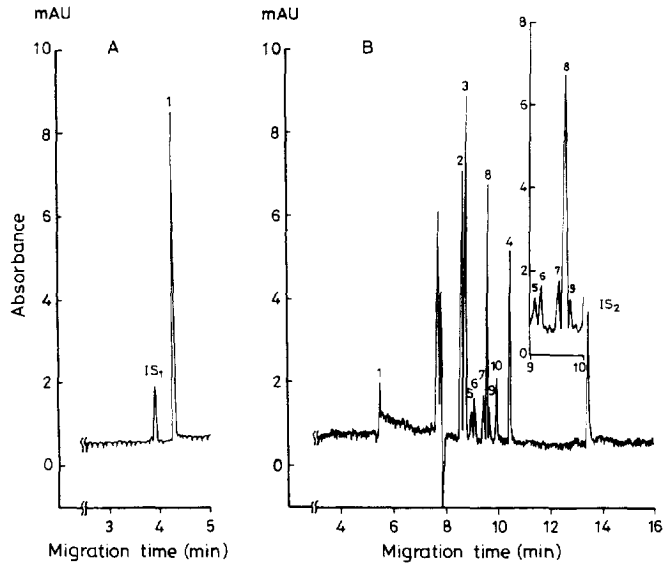

Fig. 5. Capillary electropherograms of a mixture of the ten *Evodia* alkaloids in (A) CZE and (B) MECC. IS_1 = benzyltriethylammonium chloride; IS_2 = 18-β-glycyrrhetinic acid; other symbols are the same as those in Fig. 1.

suitable for 1 owing to a serious tailing phenomenon. The tailing phenomenon would be worse if higher pH values were used, as shown in Fig. 6. It is therefore necessary to develop some other methods for the determination of this compound. The quaternary alkaloids in *Coptidis rhizoma* have been well separated by a simple CZE technique with the use of acetate as counter ion [6]. However, such separation technique was not suitable for the analysis of dehydroevodiamine. Using dihydrogenphosphate to substitute acetate and adding some acetonitrile to the carrier, a symmetrical peak for compound 1 could finally be achieved.

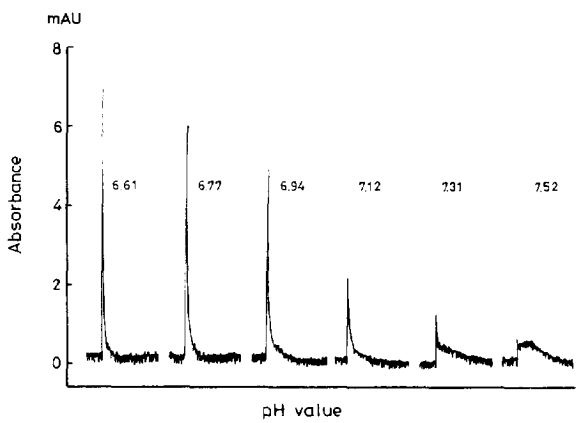

Fig. 6. The tailing phenomenon for dehydroevodiamine (1) at different pH values.

Effect of phosphate concentration

Electrolyte systems of ten different phosphate concentrations (10–100 m*M* sodium dihydrogenphosphate, pH 4.71–4.28) were used to study the effect of phosphate concentration on the zone spreading. The number of theoretical plates obtained in the different phosphate concentrations were $5.79 \cdot 10^4$ in 10 m*M*, $1.18 \cdot 10^5$ in 20 m*M*, $1.47 \cdot 10^5$ in 30 m*M*, $1.75 \cdot 10^5$ in 40 m*M*, $1.68 \cdot 10^5$ in 50 m*M*, $1.64 \cdot 10^5$ in 60 m*M*, $1.52 \cdot 10^5$ in 70 m*M*, $1.47 \cdot 10^5$ in 80 m*M*, $1.43 \cdot 10^5$ in 90 m*M* and $1.33 \cdot 10^5$ in 100 m*M*, respectively. These data indicated that the plate number was highest at 40 m*M*, increasing with the increasing of phosphate concentration from 10–40 m*M* and decreasing with the increasing of phosphate concentration from 40–100 m*M*. Therefore, concentration of 40 m*M* NaH_2PO_4 was found to be the best for dehydroevodiamine not only in getting the highest resolution but also in eliminating the serious peak-tailing (tailing factor=2.02).

Effect of acetonitrile concentration

Electrolyte systems containing 40 m*M* NaH_2PO_4 at four different acetonitrile concentrations were used to study the effect of the acetonitrile concentration on the zone spreading. The addition of acetonitrile to the phosphate buffer provided not only a better peak-symmetry for dehydroevodiamine but also a lower background noise. After a series of experiments, a solution containing 10% acetonitrile gave the best symmetrical peak (tailing factor 1.19). At 5% acetonitrile, 1 overlapped partially with an unidentified component of *Evodiae fructus*. At 20% or higher, the tailing phenomenon for compound 1 became evident.

From the above results, a buffer solution consisting of 40 m*M* NaH_2PO_4–acetonitrile (9:1) was chosen. Under these conditions, the migration time for 1 was 4.27 min and the plate number was $1.89 \cdot 10^5$ (Fig. 5A)

3.2. Calibration graphs for Evodia alkaloids

Calibration graphs (peak-area ratio, y, vs. concentration, x, mg/ml) were constructed in the ranges: 0.206–2.060 mg/ml for 1, 0.089–0.890 mg/ml for 2, 0.085–0.850 mg/ml for 3, 0.041–0.410 mg/ml for 4, 0.013–0.130 mg/ml for 5, 0.011–0.110 mg/ml for 6, 0.017–0.170 mg/ml for 7, 0.071–0.710 mg/ml for 8, 0.016–0.160 mg/ml for 9 and 0.021–0.210 mg/ml for 10. The regression equations of these curves and their correlation coefficients were calculated as follows: 1, $y=6.897x-0.391$ ($r=0.9995$); 2, $y=2.611x+0.034$ ($r=0.9999$); 3, $y=2.180x-0.102$ ($r=0.9999$); 4, $y=3.567x-0.064$ ($r=0.9997$); 5, $y=3.803x+0.006$ ($r=0.9990$); 6, $y=3.485x+0.005$ ($r=0.9985$); 7, $y=3.463x+0.005$ ($r=0.9982$); 8, $y=3.197x+0.005$ ($r=0.9987$); 9, $y=2.969x+0.004$ ($r=0.9991$); 10, $y=3.178x+0.005$ ($r=0.9989$).

3.3. Determination of the alkaloids in Evodiae fructus sample

When the test solution was analysed by CE under the selected conditions, the electropherograms shown in Fig. 7A and B was obtained. The peaks were identified by comparison of the migration times and by spiking the mixture with a single alkaloid in a subsequent run. By substituting the peak-area ratios of the individual peaks for y in the above equations, the contents of the individual alkaloids in the *Evodiae fructus* were obtained: 1, 40.13±0.02; 2, 12.34±0.05; 3, 18.49±0.09; 4, 5.43±0.05; 5, 1.29±0.13; 6, 1.24±0.05; 7, 1.84±0.07; 8,

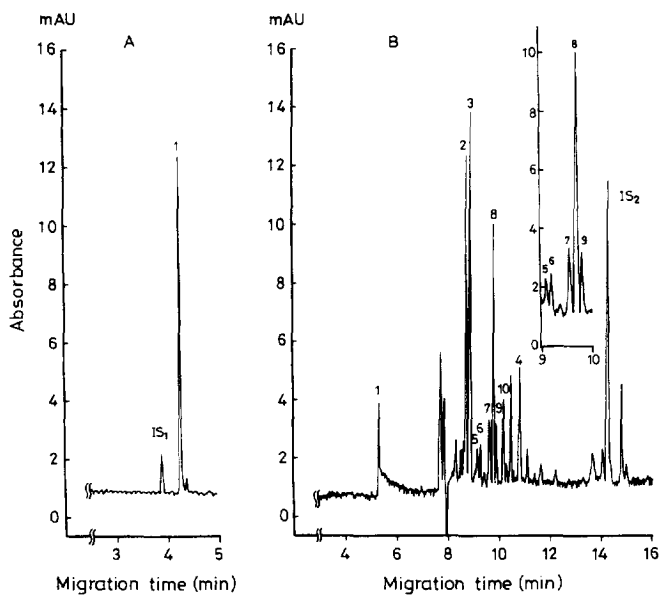

Fig. 7. Capillary electropherograms of the extract of an *Evodiae fructus* sample in (A) CZE and (B) MECC. Peaks as in Fig. 5.

7.95±0.14; 9, 1.85±0.09; 10, 2.39±0.08 mg/g (mean±S.D.; $n=3$).

Suitable amounts (0.25–4.50 mg) of the ten alkaloids were added to a sample of *Evodiae fructus* of known alkaloid content and the mixture was extracted and analysed using the proposed procedure. The recoveries of the alkaloids were 96.57–104.55% in MECC and 95.62% in CZE. The reproducibility (relative standard deviation) of each compound for six replicated injections was 0.67–3.43% in MECC and 0.68–2.31% in CZE. R.S.D. of the migration time of each compound was below 1.9% ($n=6$). The detection limits ($S/N=3$) for these alkaloids were 16.50–47.33 μg/ml (column I.D.=75 μm), as shown in Table 1.

This work has successfully demonstrated that by optimizing parameters such as pH, surfactant and organic modifier concentration in MECC and phosphate and acetonitrile concentration in CZE, high resolution separations of a complicated mixture can easily be achieved. Meanwhile, the results obtained indicate that the proposed CE methods are suitable for the determination of alkaloids in the crude extracts of *Evodiae fructus* sample.

Table 1
Detection limits and reproducibility of migration time (T_m) and peak area (A_p) ($n=6$) of the alkaloids[a]

Compound	Detection limit ng (μg/ml)	Intra-day RSD (%)		Inter-day RSD (%)	
		T_m	A_p	T_m	A_p
1	0.13 (38.63)	0.91	0.68	1.83	2.31
2	0.13 (39.50)	0.69	0.99	1.52	2.64
3	0.16 (47.33)	0.69	1.41	1.53	3.43
4	0.06 (16.50)	0.66	1.03	1.36	2.87
5	0.12 (35.85)	0.75	0.82	1.51	2.85
6	0.12 (35.88)	0.79	1.06	1.51	2.49
7	0.12 (35.65)	0.83	1.38	1.46	2.95
8	0.12 (35.25)	0.89	0.72	1.46	2.28
9	0.12 (35.91)	0.91	1.39	1.47	2.76
10	0.12 (35.85)	0.92	0.67	1.41	2.39

[a] Compound 1 was examined by CZE and 2–10 were examined by MECC.

Acknowledgments

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