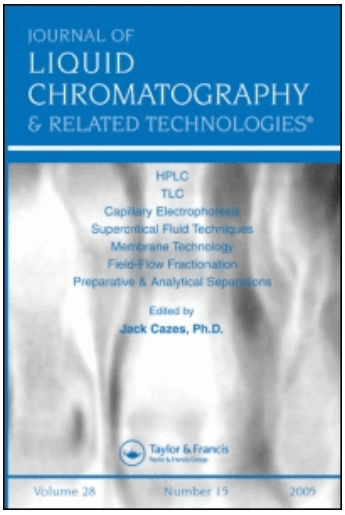

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Optimized Separation of Isoquinoline Alkaloids in *Thalictrum* Herbal Medicine by Microemulsion Electrokinetic Chromatography

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ABSTRACT

Microemulsion electrokinetic chromatography (MEEKC), as a promising separation technique providing good selectivity and high efficiency, was used for the separation of seven isoquinoline alkaloids in *Thalictrum* plant. The optimized separation was studied with regard to pH, the compositions of a microemulsion system, the additions of organic solvents and sodium cholate (SC), applied voltage, and column temperature. The baseline separation was successfully achieved using the microemusion system at pH 8.0, consisting of 10 mM phosphate,

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140 mM sodium dodecyl sulfate (SDS), 100 mM *n*-heptane, 12% (v/v) *n*-butanol, 1% (v/v) methanol, and 5 mM SC under 25 kV applied voltage and 25°C column temperature within 20 min. Meanwhile, the effects of variables on separation selectivity were discussed.

Key Words: Microemulsion electrokinetic chromatography; Isoquinoline alkaloid; *Thalictrum*.

INTRODUCTION

Plants of the genus *Thalictrum* L., with more than 200 species worldwide, are mainly distributed in Asia, Europe, Africa, South and North America, and totally 67 kinds of this herb have been found in China, mostly in the southwestern region. The isoquinoline-type alkaloids are mainly pharmacologically active constituents that contribute to their antitumor activity of these plants.[1] Among all *Thalictrum* species, *T. AFG* is one of the most important drugs because of its high alkaloid contents, which makes it an official representative of *Thalictrums*. The roots of the plant called "Ma-Wei-Lian" or "Shui-Huang-Lian" are used for the treatment of infectious hepatitis, carbuncles, dysenteric, and certain gastro enteric disorders.[1]

More than 20 isoquinoline alkaloids have been isolated from aerial part (stems and leaves) of *Thalictrum* plant. Up to now, thin-layer chromatography (TLC) scanning and high performance liquid chromatography (HPLC) have been involved in the separation and determination of isoquinoline alkaloids in *Thalictrum* plant.[2–6] However, TLC lacks quantitative precision and HPLC has lower separation efficiency, higher cost, and tends to be suffered from column contamination.

Capillary electrophoresis (CE) for the analysis of active compounds in Chinese traditional medicine is a very active field[7–12] due to its high separation efficiency, high-speed analysis, and easy column regeneration. Capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), and nonaqueous capillary electrophoresis methods have been established for the determination of isoquinoline alkaloids in *Thalictrum* plant.[13–15]

As a variant of MEKC, microemulsion electrokinetic chromatography (MEEKC) has attracted more and more chromatographers because of its high separation efficiency, capacity, and selectivity.[16–22] Up to date, MEEKC has been used for the separation of many different analytes, such as water soluble and insoluble vitamins, analgesics, and steroids.[16] However, the number of literatures on MEEKC is still limited, and there is no publication about the application of MEEKC in the separation of alkaloids.

In this paper, a MEEKC method to simultaneously separate seven isoquinoline alkaloids (see Fig. 1 for their structures) in *Thalictrum* plant

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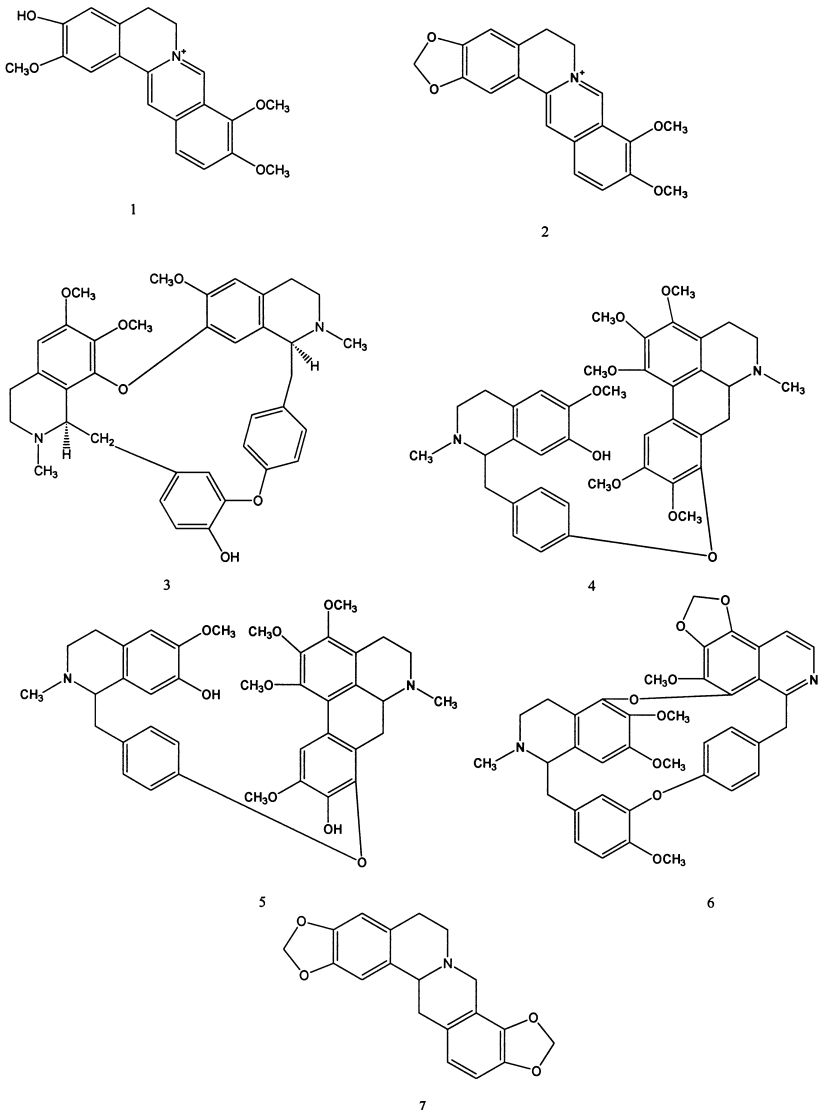

Figure 1. Chemical structures of the seven isoquinoline alkaloids. 1 = Jatrorrhizine, 2 = Berberine, 3 = Berbamine, 4 = Thalifaricine, 5 = Thalifaretine, 6 = Northalfine, 7 = Tetrahydrocoptisine.

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was developed. The baseline separation of seven analytes was successfully achieved under the optimal conditions within 20 min.

EXPERIMENTAL

Materials and Reagents

Standards of berberine, jatrorrhizine, berbamine, thalifaricine, thalifaretine, northalfine, tetrahydrocoptisine, along with four unknown compounds extracted from *T. AFG*, were kindly donated by the Institute of Medical Plant Development, Peking Union Medical College and Chinese Academy of Medical Sciences (Beijing, P.R. China). The unknowns were speculated to be analogues of these alkaloids based on their mass spectra and NMR results, although their structures have not been elucidated at present due to the extremely low amount of these unknown compound available.

Sodium dodecyl sulfate (SDS) was purchased from Fluka (Buchs, Switzerland). Sulfated-β-CD was kindly donated by BioAnalytical Systems (West Lafayette, IN), and other CDs were provided by Advanced Separation Technologies Inc. (Whippany, NY). All other chemicals were analytical-reagent grade and purchased from Beijing Chemical Factory (Beijing, China). Redistilled water was used to prepare all buffers and other solutions.

Apparatus and Conditions

All separations were performed on a HP 3D CE system with air cooling and a diode-array detector (Agilent Technologies, Palo Alto, CA). An uncoated fused-silica capillary, 50 μm I. D. × 375 μm O. D. (Reifeng Co., Hebei, P.R. China) was utilized with total length of 48.5 cm and 40 cm to the detector. The other conditions are as follows: capillary temperature 25°C, applied voltage 25 kV, UV detection at 230 nm. Sample solution was injected by applying a pressure of 50 mbar for 5 s.

Before use, new capillaries were flushed with 0.5 M sodium hydroxide (NaOH) for 30 min and then flushed with redistilled water and background electrolyte, each for 10 min. Between consecutive runs, the capillary was conditioned by washing with 0.5 M NaOH solution for 1 min, then with water for 2 min, and background electrolyte for 3 min.

Preparation of Microemulsions and Samples

Three buffer systems, citrate, phosphate, and borate, each at 10 mM were adjusted to the desired pH by 1 M NaOH solution. Microemulsions were

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prepared by mixing SDS, *n*-butanol, *n*-heptane, and additives with buffer solutions, and then the mixtures were exposed to ultrasound in an ultrasonic bath with the power of 60 W for 30 min, to obtain clear and highly stable solutions. A mixed solution of alkaloid standards was prepared by dissolving the alkaloids in methanol or the optimal microemulsion system, and then by ultrasonication for 30 min. Before use, all the solutions were filtered through a 0.45 μm membrane filter.

RESULTS AND DISCUSSION

Optimization of Microemulsion Forming Solutes

The pH of the microemulsion system has a pronounced impact on MEEKC separation as it affects both solute ionization and the magnitude of EOF generated. The effect of pH on separation was studied in the range of 6.0–10.0 using 10 mM citrate buffer (for pH 4.0–5.5), phosphate buffer (for pH 6.0–8.0) and borate buffer (for pH 8.5–10.0), each containing 120 mM SDS, 10% (v/v) *n*-butanol, 100 mM *n*-heptane under 20 kV applied voltage, and 35°C column temperature. The experiments showed that the migration times of analytes decreased with the increase of pH from 6.0 to 9.0 (see Fig. 2) since the enhancement of EOF was dominant. As peak shape, overall resolution, and analysis time were concerned, pH 8.0 was selected to perform further optimization. Under the optimal pH value, the isoquinoline alkaloids have a low extent of ionization according to the dissociation constants of four typical alkaloids (see Table 1).[23]

The variation of surfactant concentration will alter the separation selectivity of the analytes in MEEKC as it can influence the negative charge density on the surface of oil droplets. A series of SDS concentrations from 60 mM to 160 mM were employed to investigate the effect of SDS by using the microemulsion system at pH 8.0, consisting of 10 mM phosphate, 10% (v/v) *n*-butanol, and 100 mM *n*-heptane under 20 kV applied voltage, and 35°C column temperature. The results indicated that the migration times of seven solutes decreased with the increase of SDS concentration due to the reduced EOF. The study also demonstrated that higher SDS concentration contributed to better resolution. When SDS concentration decreased below 100 mM, the separation deteriorated and all alkaloids co-eluted, while the analytical time was too long at higher than 150 mM SDS concentration. As a result, 140 mM SDS was selected as the optimal concentration, under which a best separation trend was obtained (see Fig. 3).

The effect of *n*-butanol concentration was studied in the range of 8–14% (v/v) by using the optimal microemulsion system described above. It was

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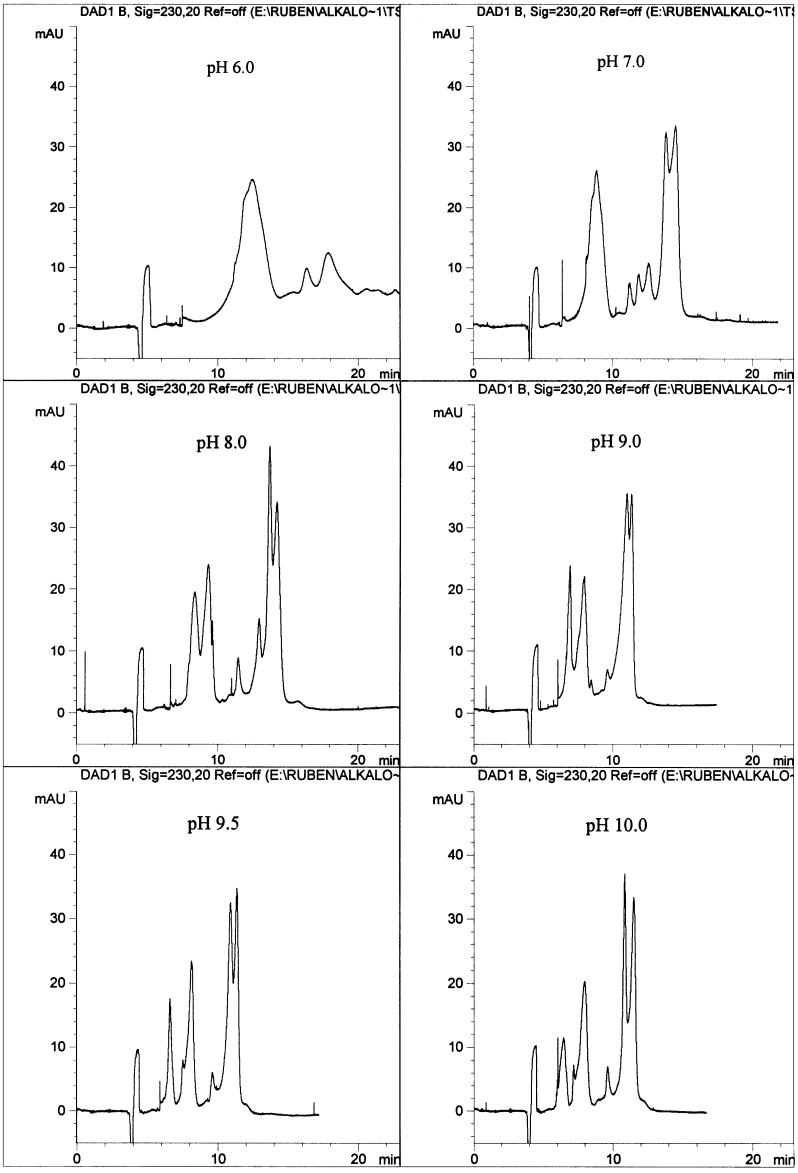

Figure 2. Influence of pH on MEEKC separation of the alkaloids. Conditions: 10 mM phosphate buffer (pH 6.0–8.0) or borate buffer (pH 8.5–10.0) at different pH in the range of 6.0–10.0, containing 120 mM sodium dodecyl sulfate, 10% (v/v) *n*-butanol, 100 mM *n*-heptane, under 20 kV, 35°C, 230 nm UV detection, and injection at 50 mbar for 5 s.

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Table 1. Dissociation constants of some alkaloids.[23]

Alkaloid	pK_a
Berbamine	7.45
Thalifaricine	6.32
Northalfine	6.67
Thalifaretine	6.58

observed that the separation could be improved with the increase of the *n*-butanol concentration in the range of 8–12% (v/v). However, further increasing of *n*-butanol concentration could enlarge the oil droplet scale, thus the negative charge density of the oil droplet would decrease, which resulted in the increase of the mobility of oil droplet. Hence, higher *n*-butanol concentration is not recommended. As an overall resolution was concerned, 12% (v/v) *n*-butanol concentration was the optimal condition.

Addition of Modifiers

For evaluating the effects of organic solvents, 5% (v/v) methanol, acetonitrile, isopropanol, and tetrahydrofurane were individually added to the microemulsion system at pH 8.0, consisting of 10 mM phosphate, 140 mM SDS, 10% (v/v) *n*-butanol, and 100 mM *n*-heptane under 20 kV applied voltage, and 35°C column temperature. It was observed that the addition of organic solvents obviously improved the separation. The migration times of analytes increased due to the reduction of the EOF, and the addition of acetonitrile led to the slowest mobility of alkaloids. Organic solvents could enhance the solubility of analytes, modify the physicochemical properties of the droplet including the surface charge density and scale, and accelerate the transportation of alkaloids from buffer to the droplet, thus greatly improving the separation. Especially, the improvement of overall resolution was more obvious for the analytes 3–7 which possess higher lipophilicity.

As a kind of surfactant with steroid structure, sodium cholate (SC) has better separation selectivity for solute with stronger lipophilicity. In our study, different concentrations of SC were added to the microemulsion system, showing that the obvious improvement of overall resolution of the analytes was achieved when 5 mM SC was added.

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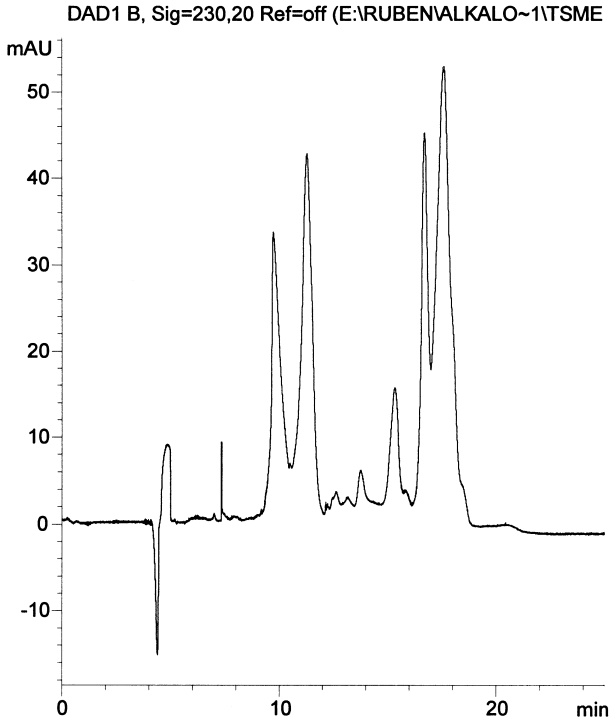

Figure 3. Typical chromatogram of the alkaloids by using the microemulsion system containing 140 mM SDS. Other conditions: 10 mM phosphate buffer at pH 8.0, containing 10% (v/v) *n*-butanol, 100 mM *n*-heptane, under 20 kV, 35°C, 230 nm UV detection, and injection at 50 mbar for 5 s.

Finally, the baseline separation of seven isoquinoline alkaloids was achieved by MEEKC, using the microemulsion system at pH 8.0, consisting of 10 mM phosphate, 100 mM *n*-heptane, 140 mM SDS, 12% (v/v) *n*-butanol, 1% (v/v) methanol, and 5 mM SC, under 25°C column temperature and 25 kV applied voltage [see Fig. 4(A)].

Effect of Applied Voltage and Column Temperature

The applied voltage ranging from 10 to 25 kV was tested, and the highest separation efficiency was obtained under 25 kV. The study on column temperature in the range of 25–45°C indicated that the migration times of

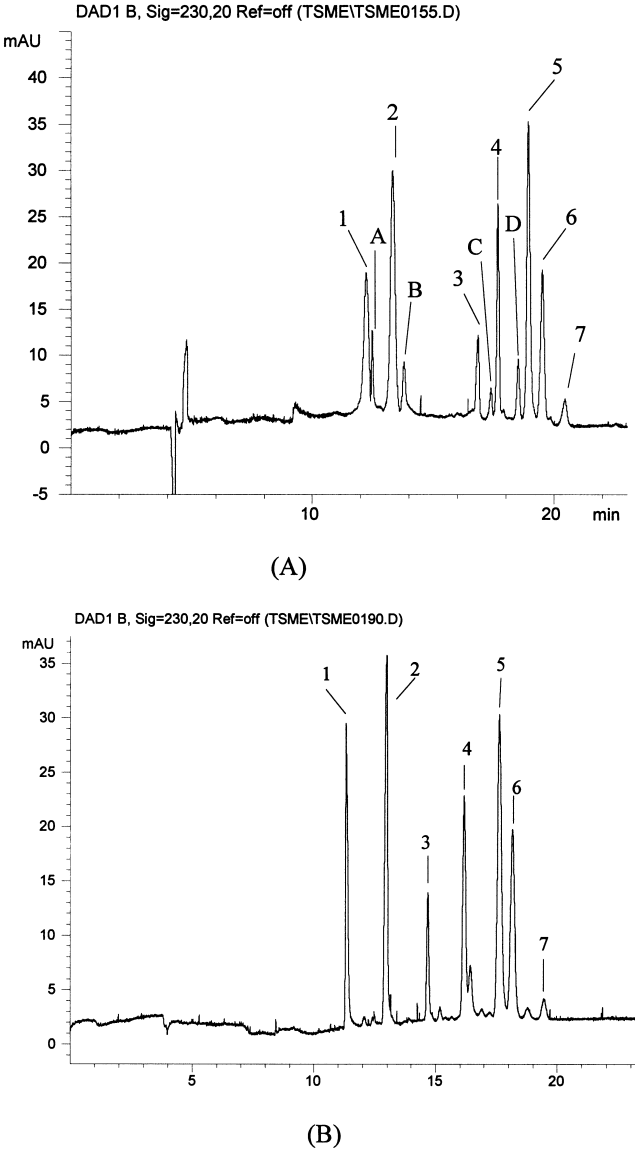

Figure 4. Typical chromatograms of seven alkaloids dissolved in (A) methanol and (B) microemulsion system under the optimal conditions. Other conditions: 10 mM phosphate buffer at pH 8.0, containing 140 mM SDS, 12% (v/v) *n*-butanol, 100 mM *n*-heptane, 5 mM sodium cholate, 1% (v/v) methanol, under 25 kV, 25°C, UV detection at 230 nm, and injection at 50 mbar for 5 s.

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Table 2. Theoretical plate number (n) with different solvents for sample dilution.

Alkaloids	Sample in microemulsion n (m^{-1})	Sample in methanol n (m^{-1})
Jatrorrhizine	183,625	40,315
Berberine	145,838	53,697
Berbamine	349,280	221,457
Thalifaricine	201,422	221,052
Thalifaretine	136,618	440,725
Northalfine	131,185	133,342
Tetrahydrocoptisine	140,215	308,087

analytes increased with the decrease of temperature due to the decrease of EOF, enhancing the separation selectivity. With regard to good separation selectivity and moderate analytical time, 25°C was selected as the optimal condition.

Sample Dilution

Our study proved that the dilution solvent for alkaloids also had essential impact on the separation efficiency. Instead of methanol, the optimal microemulsion system mentioned above was utilized to dissolve alkaloids, showing that the separation efficiency was greatly enhanced (see Table 2) and the peak shape of most analytes were also improved [see Fig. 4(B)].

CONCLUSIONS

As a variant of MEKC, MEEKC showed great separation potential for isoquinoline alkaloids. Based on the investigation with regard to pH, the composition of the microemulsion system, the addition of organic solvents and SC, applied voltage and column temperature, the baseline separation of seven isoquinoline alkaloids was successfully achieved within 20 min by employing the microemulsion system at pH 8.0, consisting of 10 mM phosphate, 140 mM SDS, 12% (v/v) n-butanol, 100 mM n-heptane, 5 mM SC, 1% (v/v) methanol, under 25 kV applied voltage, and 25°C capillary temperature, 230 nm UV detection, and the alkaloids were dissolved in optimal microemulsion system. This MEEKC method would be useful for the quality control of some herbal medicines containing these kinds of alkaloids.

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