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Determination of quinolizidine alkaloids in traditional Chinese herbal drugs by nonaqueous capillary electrophoresis

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Abstract

A rapid method for the determination of quinolizidine alkaloids by nonaqueous capillary electrophoresis was developed. A total of 10 alkaloids (matrine, sophocarpine, oxymatrine, oxysophocarpine, sophoridine, cytisine, sophoramine, aloperine, lehmannine and dauricine) could be easily separated within 18 min. A running buffer composed of 50 m*M* ammonium acetate, 10% tetrahydrofuran and 0.5% acetic acid in methanol was found to be the most suitable for this separation. Five of these alkaloids were selected for further studies. The linear calibration ranges were 2.51–50.1 μg/ml for sophoridine and sophocarpine, 2.71–54.2 μg/ml for matrine, 3.30–65.9 μg/ml for oxymatrine, and 3.10–62.0 μg/ml for oxysophocarpine. The recovery of the five alkaloids was 98.0–101.3% with relative standard deviations from 1.03 to 2.68% ($n=5$). The limits of detection for all 10 alkaloids were over the range 0.93–2.31 μg/ml. The method was successfully applied to the phytochemical analysis of alkaloid extracts from three commonly used traditional Chinese herbal drugs: *Sophora flavescens* Ait. (Kushen), *S. alopecuroides* L. (Kudouzi or Kugancao) and *S. tonkinensis* Gapnep (Shandougen).

Keywords: *Sophora* spp.; Nonaqueous capillary electrophoresis; Pharmaceutical analysis; Alkaloids; Quinolizidines

1. Introduction

Quinolizidine alkaloids are the largest single group of legume alkaloids which appear to be restricted in distribution to species in the more primitive tribes of the Papilionoideae. These compounds are important due to their toxicity in humans and livestock as constituents of poisonous plants, and ironically some of them exhibit potentially useful pharmacological activities [1]. Therefore, both sensitive and specific detection methods for quinolizidine alkaloids are of considerable interest.

The roots of *Sophora flavescens*, *S. alopecuroides* and *S. tonkinensis* are frequently used traditional Chinese herbal drugs. These herbs are known to contain quinolizidine alkaloids as their bioactive constituents [2–5]. Two types of quinolizidine alkaloids, matrine-type and pyridone quinolizidine bases, are found in *Sophora* species and can be used for the identification of *Sophora* species in commercial preparations.

Several methods such as high-performance liquid chromatography (HPLC) and thin-layer chromatog-

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raphy (TLC) have been used to analyze these three crude drugs for the presence of quinolizidine alkaloids [6–9]. However, TLC lacks quantitative precision, while HPLC has lower efficiency and takes longer analyzing time. Therefore, it is necessary to establish a rapid and effective method for the quantitative analysis of these alkaloids.

In the field of natural product analysis, capillary electrophoresis (CE) has gained acceptance as an analytical technique, especially for complex mixtures of secondary constituents. It is particularly useful for solving separation problems in cases that are difficult or too time-consuming to be solved by HPLC [10]. CE is applied to nearly all groups of plant natural products including the analysis of alkaloids from poppy and opium preparation, isoquinoline alkaloids of different plant sources, and a few protoalkaloids from ephedra [11–16]. However, there is no report on the application of CE method for the analysis of quinolizidine alkaloids.

The present paper describes a newly developed nonaqueous CE method which makes a simultaneous separation of a total of 10 alkaloids (nine quinolizidine alkaloids and one bis-benzyl isoquinoline alkaloid) possible. In addition, the contents of the main quinolizidine alkaloids in the three herbs were also investigated using this method.

2. Experimental

2.1. Chemicals

Nine quinolizidine alkaloids namely matrine (MT), sophocarpine (SC), sophoridine (SRI), sophoramine (SA), aloperine (ALP), lehmannine (LEM), oxymatrine (OMT), oxysophocarpine (OSC) and cytisine (CYT), and one bis-benzyl isoquinoline alkaloid dauricine (DUR), together with scopolamine hydrochloride as an internal standard (I.S.), were supplied by the National Institute for the Control of Pharmaceutical and Biological Products of China (NICPBP, Beijing, China). Ammonium acetate was analytical-reagent grade and purchased from BDH (Poole, UK). Glacial acetic acid (AG, 99.8%) was purchased from Riedel-de Haën. Tetrahydrofuran (THF, HPLC grade) was purchased from E. Merck (Darmstadt, Germany) and methanol (HPLC grade) from Labscan (Dublin, Ireland). The Chinese herbs, *S. flavescens* (Kushen), *S. alopecuroides* (Kugancao) and *S. tonkinensis* (Shandougen) were purchased from the Hebei and Zhejiang province of China. The reference herbal drug of *S. flavescens* was provided by the NICPBP.

2.2. Instrumentation

All CE separations were conducted on a Beckman P/ACE system 2200 HPCE instrument (Beckman, Palo Alto, CA, USA) coupled to a AST Bravo LC 4/66d PC with system control and data captured by System Gold software (Beckman Instruments). The electrophoretic separation was performed on a fused-silica capillary of 47.6 cm (40 cm effective length)× 50 μm I.D. (Beckman). All buffer solutions were filtered through a 0.25-μm filter. Sample injection was performed hydrodynamically at 0.5 p.s.i. for 5 s from the positive side of the capillary (corresponding to a sample volume of ca. 7 nl) (1 p.s.i.=6894.76 Pa). For CE analysis at the optimum conditions, the applied voltage was 30 kV and the average current was about 35 μA. The capillary temperature was controlled at 25°C and the detection wavelength was set at 200 nm. The running buffer was composed of 50 m*M* ammonium acetate, 10% THF and 0.5% acetic acid in methanol.

The capillary was conditioned with 1 *M* NaOH for 20 min followed by 0.1 *M* NaOH for 5 min and then by water for 5 min at 25°C, prior to use. After each run, the capillary was washed with 0.1 *M* NaOH and then with water for 4 min, respectively, then rinsed with running buffer for 4 min before the next run. The electroosmotic flow (EOF) was tested with acetone.

2.3. Preparation of reference solutions

Reference stock solutions of the 10 alkaloids and the internal standard (I.S.) solution were prepared by dissolving about 10 mg of the alkaloids in 10-ml volumetric flasks with methanol. All solutions were found to be stable when stored at 4°C for one month.

2.4. Preparation of herbal drug extracts

A 50-mg amount of pulverized *S. flavescens*, *S.*

alopecuroides or *S. tonkinensis* was weighed accurately in a 10-ml volumetric flask, about 8 ml of methanol and 0.2 ml of I.S. solution was added. After treatment with ultrasonication at room temperature (about 20°C, the temperature of the solution would be increased to 45°C after sonication) for 35 min, the solution was cooled down to ambient temperature. Then made up to the volume with methanol and mixed up. This extract was passed through a 0.25-μm filter for assay.

3. Results and discussion

3.1. Analytical conditions

The structures of these alkaloids are very similar, with two diastereomers (1 and 2) and two structural isomers (3 and 4, Fig. 1). Isomers possess the same static charge-to-mass ratio, which means that in a free solution the isomers should possess the same mobility. However, the effective charge-to-mass ratio can be different, which is caused by different spatial arrangements of the atoms (different densities and intramolecular interactions). Moreover, the mobilities of the isomers may face different resistance to the flow due to these different shapes. These small differences combined with a high efficiency of separation in CE may be sufficient to obtain the baseline separation.

An aqueous electrolyte solution was initially used to separate the 10 alkaloids mentioned above. The next step of this project was to develop a suitable method for further CE–MS–MS analysis. So involatile buffer salts were not used. The first electrolyte tested was 10 m*M* ammonium acetate (aqueous), where poor resolution and poor reproducibility of migration time were observed. When acetic acid was added to adjust the pH values from 6.0 to 4.0 or 2.0, slightly better separation efficiencies were observed. However, it limited the separation to just four peaks. Efforts to improve their separation by adding different organic solvents such as methanol and acetonitrile were found to be invalid. Therefore nonaqueous buffers based on some organic solvents including methanol, ethanol, isopropanol, acetonitrile, THF, *N*,*N*-dimethylformamide (DMF) and formamide were tested. When ethanol, isopropanol or THF was used, poor separation and long analysis time were observed due to the longer EOF attributed to their higher viscosities and lower permitivities of these solvents. When acetonitrile was used, the EOF and migration time of solutes were shortened and the separation was not satisfactory. DMF and formamide were found to be unsuitable due to their higher UV background at the wavelength of 200 nm. Separation efficiency was found to be greatly improved in methanol-based buffer compared to other buffer systems. However, in this condition baseline separations for two peak pairs of MT–SC and OMT–OSC which possess very similar chemical structures were not achieved simultaneously even by changing the content of ammonium acetate or of acetic acid. As the volume fraction of acetic acid was increased over 1%, OMT was separated from OSC; however, other peaks such as MT–SC and CYT–SRI were not separated well. No improvement was made by adding organic solvents such as ethanol, propanol and acetonitrile as modifiers. When THF was added to the buffer, different migration behavior between OMT and OSC was obtained. Although the changes in the viscosity, dielectric constant of the running buffer and the properties of the inner surface of capillary were factors to influence the separation, the different interaction between solvent and solutes probably plays a more important role [17,18]. THF, a commonly used dipole interaction solvent [19], may have dipole–dipole interactions with the N-oxide of OMT and OSC in the CE separation. In this study, when the content of THF varied from 0% to 20%, the mobilities of all tested alkaloids were decreased. However, the variations of mobilities of OMT and OSC were smaller than those of other components. Baseline separation of OMT and OSC was achieved when THF was 10%. As the concentration of THF was increased above 15%, peak SC and peak OMT became fused into one (Fig. 2A).

The effects of the contents of ammonium acetate and acetic acid, as well as applied voltage and capillary temperature on the migration behavior, were also examined to obtain an optimum separation. As the concentration of ammonium acetate increased from 20 to 100 m*M*, the mobilities of all compounds were decreased and some peaks broadened at high concentrations of ammonium acetate. Within the

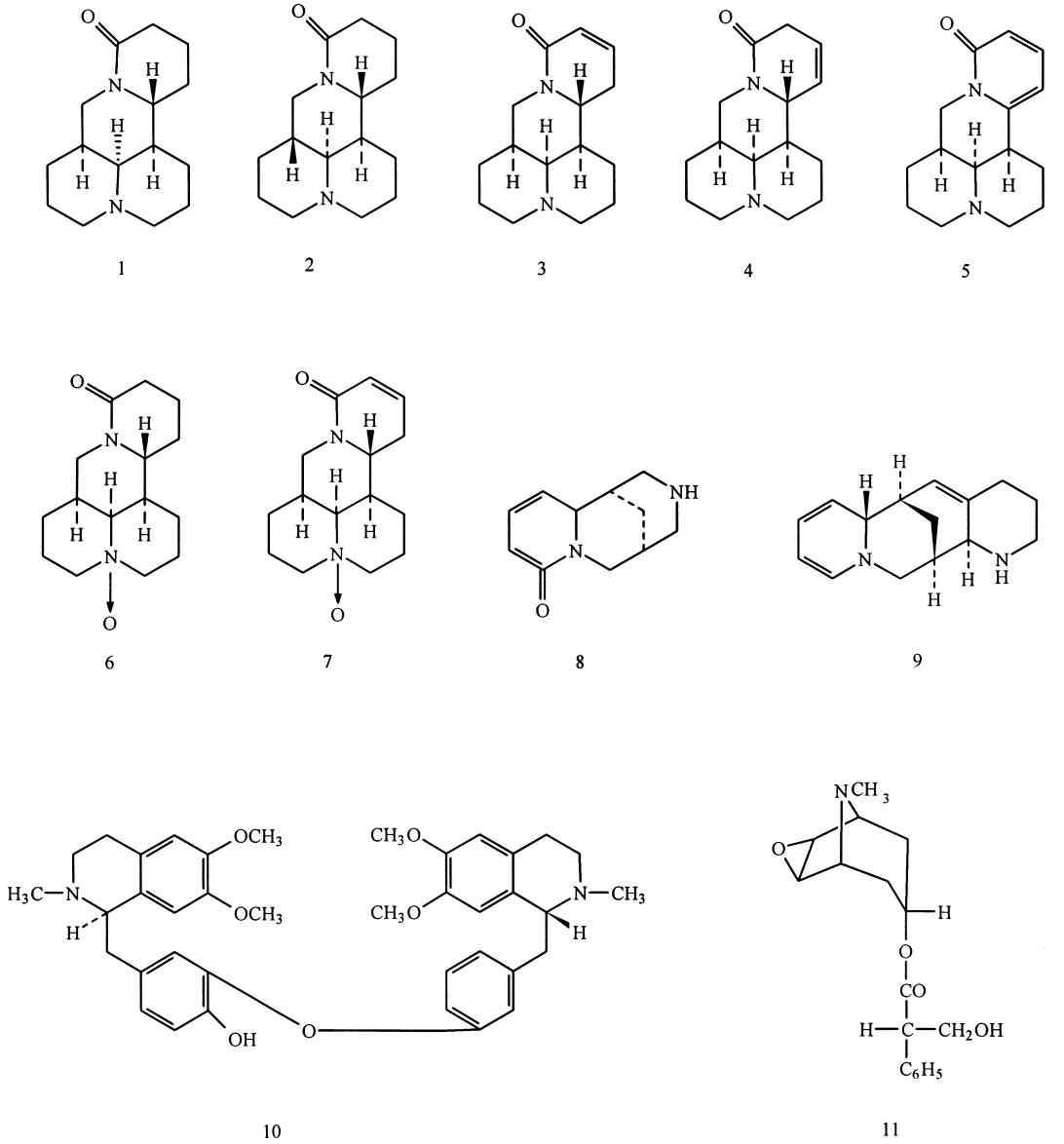

Fig. 1. Structural formulae of nine quinolizidine alkaloids (1–9), a bis-benzyl isoquinoliue alkaloid (10) and the internal standard (11). 1=Matrine (MT), 2=sophoridine (SRI), 3=sophocarpine (SC), 4=lehmannine (LEM), 5=sophoramine (SA), 6=oxymatrine (OMT), 7=oxysophocarpine (OSC), 8=cytisine (CYT), 9=aloperine (ALP), 10=dauricine and 11=scopolamine (internal standard).

concentration range of 40 to 60 m*M*, both the separation and the current were acceptable (Fig. 2B).

The concentration of acetic acid was found to significantly affect the separation. When the concentrations increased from 0 to 0.5%, all peaks migrated faster except ALE. When the content of acetic acid was at 0.5%, all peaks were separated completely. However, the separation worsened when acetic acid content was increased to over 1.0% (Fig. 2C).

The separation of OMT and OSC was also found to be affected slightly by the applied voltage and the

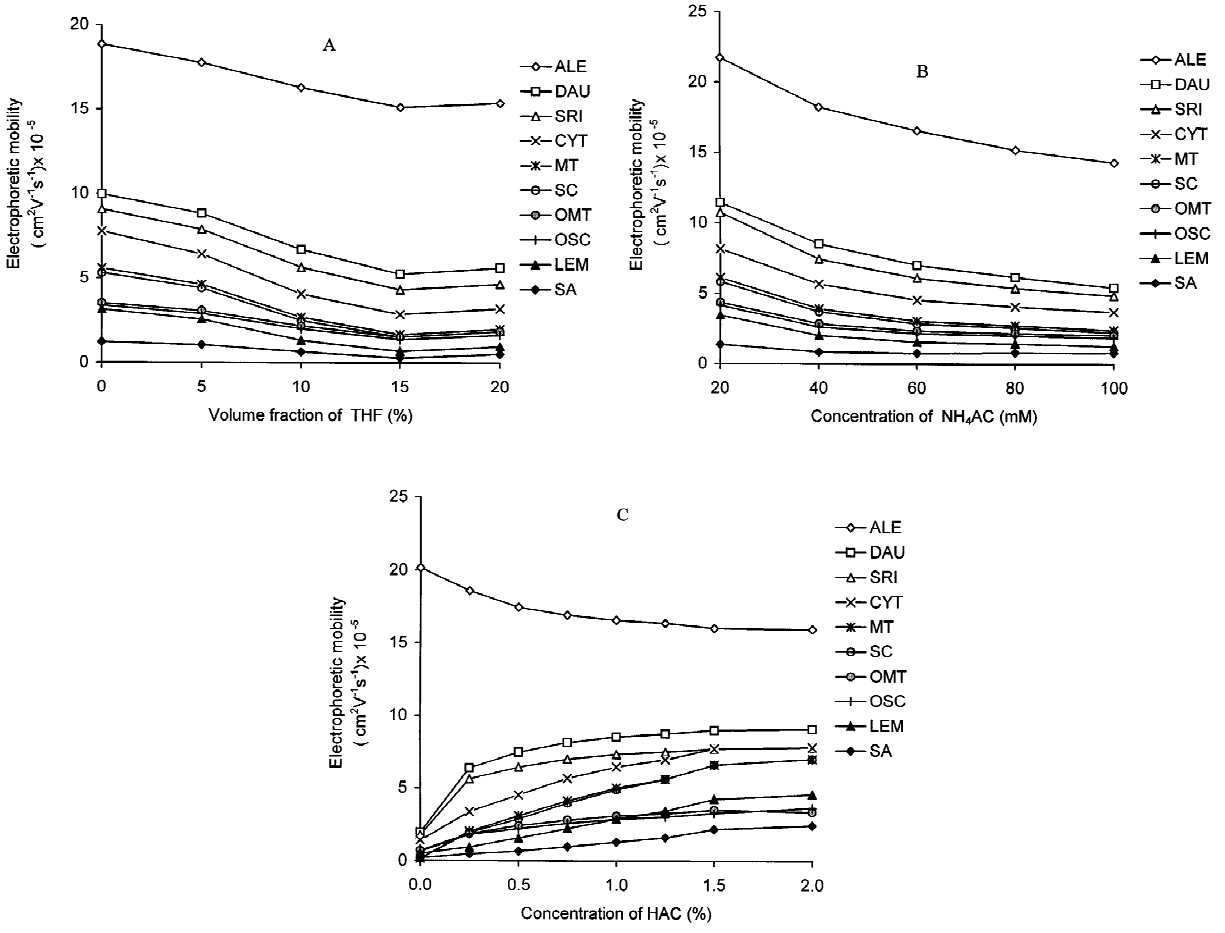

Fig. 2. Effect of varying the concentration of THF (A), ammonium acetate (B) and acetic acid (C) on mobilities of alkaloids. Column: 47.5 cm (40 cm effective length)×50 μm of uncoated fused-silica capillary. Voltage: 20 kV. Temperature: 25°C. Buffer: (A) 50 m*M* ammonium acetate, 0.5% acetic acid and varying amount of THF in methanol; (B) 0.5% acetic acid, 10% THF and varying amount of ammonium acetic acid in methanol, and (C) 50 m*M* ammonium acetate, 10% THF and varying amount of acetic acid. The electrophoretic mobility was calculated as

$$\mu_e = \frac{lL}{V}\left(\frac{1}{t_m} - \frac{1}{t_o}\right)$$

where l is the effective capillary length, t_m is the migration time of solute, t_o is the EOF, V is the applied voltage and L is the total length of capillary.

capillary temperature. As the voltage increased from 20 kV to 30 kV, the migration times of all alkaloids were shortened and the resolution of peak OMT and peak OSC was better at 30 kV. When the capillary temperature varied from 20 to 30°C, all peaks migrated faster. At 30°C, peak OMT and peak OSC were partly fused; at 20°C, peak OMT and peak OSC were separated, but peak LEM was closer to peak OSC. The optimum separation was found to be at 25°C.

3.2. Linearity

Linearities for five selected alkaloids which are commonly used as target compounds to qualitate and quantitate the three mentioned herbs [7–9] were

Table 1
Linearity studies of five selected alkaloids

Alkaloids	Concentration range (μg/ml)	Slope	Intercept	R^2
SRI[a]	2.51–50.1	0.048	−0.014	0.9996
MT	2.71–54.2	0.056	−0.012	0.9998
SC	2.52–50.4	0.068	−0.015	0.9998
OMT	3.30–65.9	0.044	−0.060	0.9996
OSC	3.10–62.0	0.041	−0.068	0.9996

[a] For abbreviation of alkaloids, please see text.

assessed over a concentration range of about 20-fold magnitude (Table 1). Varying volumes of standard solutions were spiked into the 10-ml volumetric flasks to give concentrations of 2.51–50.1 μg/ml of SRI and SC, 2.71–54.2 μg/ml of MT, 3.30–65.9 μg/ml of OMT and 3.10–62.0 μg/ml of OSC. Triplicate injections were made at each concentration. The linearity of each standard curve was confirmed by plotting the peak area ratio of the alkaloid and I.S. peak areas versus the concentration of the alkaloid (in μg/ml). Results obtained from the experiments were shown in Table 1.

3.3. Limits of detection (LODs) and quantitation (LOQs)

The LODs for all 10 alkaloids, on the basis of a signal-to-noise ratio of 3 ($S/N=3$) were determined from a range of 0.93 to 2.31 μg/ml. The LOQs based on a signal-to-noise ratio of 10 for five alkaloids mentioned above were found to be in the range 0.97–7.49 μg/ml. Results corresponding to six detected alkaloids are shown in Table 4 and the others are listed in Section 3.5.2.

3.4. Recovery assessment

The recovery was evaluated by comparing the peak area ratio of a solute and the I.S. of the test solution with that of the control solution. Suitable amounts of five alkaloids mentioned in Section 3.2 were added to an accurately weighed fine powder of *S. flavescens* or *S. alopecuroides*. Similarly, three alkaloids were added to a sample of *S. tonkinensis*. The mixture of the alkaloids was extracted and analyzed using the proposed method. The control solution was prepared by extracting the same amount of the three samples without adding the alkaloids. Results of recovery studies are presented in Table 2.

3.5. Determination of quinolizidine alkaloids in the crude drugs

3.5.1. Effect of the ultrasonicating time on the extracting efficiency

Commonly, alkaloids in crude drugs were extracted by chloroform after alkaline treatment with ammonium hydroxide solution [20]. Highly polar organic solvents such as methanol, ethanol or organic–aqueous mixtures were also used as extracting solvents [7,14,16,21]. This paper proposed a simple method in which only several mg of crude drugs and a small volume of methanol was needed. Extracting efficiency was evaluated by determining the amount of alkaloids dissolved in the herb sample at regular time intervals. It was shown that the concentration of

Table 2
Recovery studies of five selected alkaloids

Samples	Recovery (%, ±RSD, %; $n=5$)				
	SRI[b]	MT	SC	OMT	OSC
SF[a]	98.69±2.33	99.83±2.48	100.7±2.68	98.00±1.27	99.34±1.59
SL	99.14±1.97	101.3±1.82	98.31±2.15	99.37±1.11	101.1±1.03
ST	–	98.77±1.44	–	100.5±1.68	98.14±2.05

[a] SF=*S. flavescens*, SL=*S. alopecuroides* and ST=*S. tonkinensis*.
[b] For abbreviation of alkaloids, please see text.

Table 3
Effect of extraction methods

Methods	Concentration of alkaloids measured (mg/g, ±RSD, %; $n=3$)				
	SRI[b]	MT	SC	OMT	OSC
I[a]	0.74±2.02	0.48±1.98	0.35±2.04	4.12±1.23	4.96±1.49
II	0.72±3.85	0.48±4.17	0.32±3.88	3.99±2.31	5.01±2.05

[a] I=Method presented in Experimental, II=chloroform extraction after NH_4OH treatment.
[b] For abbreviation of alkaloids, please see text.

each dissolved alkaloid remained stable after 30 min sonication. Results obtained using the method presented in this report were compared with results obtained using chloroform after treatment with ammonium hydroxide [20]. No significant difference between the two methods was found, but the precision was higher in the method presented in this report (Table 3).

3.5.2. Results of determining the contents of alkaloids

When the test solutions of *S. flavescens*, *S. alopecuroides* and *S. tonkinensis* were analyzed by the proposed method, the electropherograms shown in Fig. 3 were obtained. Peaks 1–3, 6–8 were identified as matrine, sophoridine, sophocarpine, oxymatrine, oxysophocarpine and cytisine by com-

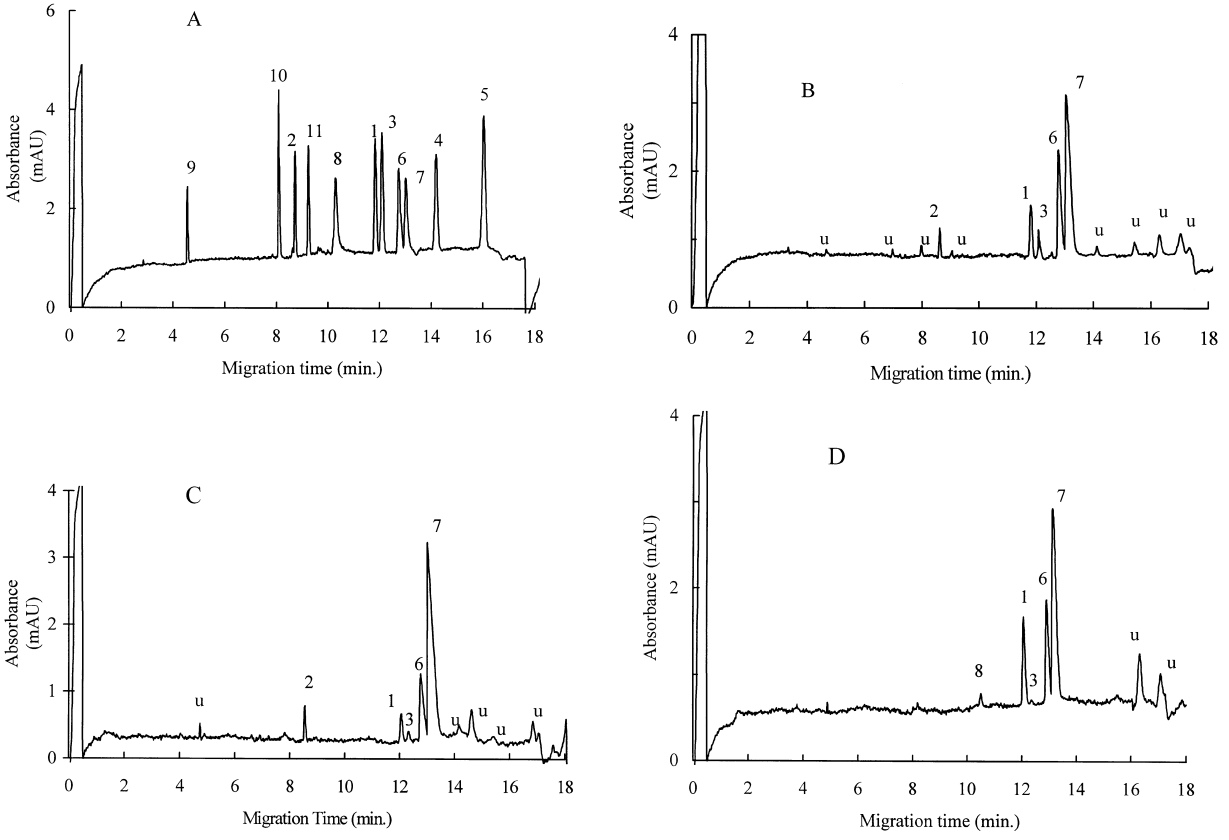

Fig. 3. Electropherograms of (A) 10 alkaloids and the I.S. mixture, (B) *S. flavescens*, (C) *S. alopecuroides* and (D) *S. tonkinensis*. u=Unknown. Applied voltage: 30 kV, temperature: 25°C. Other conditions as in Fig. 2.

Table 4
Contents (mg/g) of alkaloids found in eight samples of herbal drugs

Sample	Content of alkaloids in herbal drugs (mg/g, ±RSD, %; $n=3$)					
	SRI	CYT	MT	SC	OMT	OSC
SF No.1	0.75±2.01	ND	0.47±1.59	0.34±2.92	4.09±0.98	4.98±1.86
SF No.2	2.75±1.63	ND	0.54±2.78	0.41±2.02	2.76±1.54	2.75±1.09
SF No.3	0.36±2.11	ND	0.54±1.94	0.32±2.34	4.56±1.98	13.1±1.23
SF No.4	0.74±1.93	ND	0.46±2.72	0.31±2.42	4.71±1.80	14.4±0.86
SF Ref.	0.69±2.53	ND	1.20±1.25	0.37±2.01	5.96±1.13	16.4±1.02
SL No.1	0.61±1.86	ND	1.68±1.04	0.59±1.81	4.45±1.07	15.2±1.06
SL No.2	0.60±2.22	ND	1.49±1.51	2.58±1.06	5.93±0.97	1.93±1.94
ST No.1	ND	Trace	3.83±1.07	Trace	2.85±1.32	0.92±2.20
ST No.2	ND	Trace	3.15±1.11	Trace	2.31±1.95	0.75±2.45
LOD (μg/ml)	1.18	2.31	1.27	1.05	2.24	2.11
≈ppm[a]	23.8	46.6	25.6	21.2	45.2	42.5
LOQ (μg/ml)	3.91	7.65	4.24	3.50	7.49	7.04
≈ppm[a]	78.8	154	85.5	70.6	151	142

ND = Not detected.
[a] Equivalent to ppm contained in the herbal drugs.

parison with the standard alkaloids. So far, various alkaloids have been isolated from *S. flavescens* (MT, SC, OMT, OSC, SRI and SA) [23], *S. alopecuroides* (MT, SC, OMT, OSC, SRI, ALP, LEM and CYT) [2], and *S. tonkinensis* (MT, OMT, SC, CYT and DUR) [24]. However, in this study, only some of these alkaloids previously reported in each of these herbs were detected. For example, LEM and ALP were not detected in *S. alopecuroides,* and DUR was not detected in *S. tonkinensis* (LODs for these alkaloids were 0.29–1.55 μg/ml). The results suggested that the contents of these alkaloids may be beyond the detection limit of our method.

The contents of the main alkaloids detected in the three herbs are shown in Table 4. It was found that the alkaloid contents of herbal samples bearing the same name but collected from different locations were very different. The results suggested that the quality of these herbs can be more conveniently monitored and the identity of the herbs could be checked in case of herbal poisoning.

4. Conclusions

This is the first report to describe the determination of quinolizidine alkaloids by nonaqueous CE. All relevant operational parameters have been optimized for the nonaqueous CE experiments described in this work. A simple and precise method for the extraction was proposed and compared with the classical extraction method. This method may be used for the quantitative determination of quinolizidine alkaloids in some natural product samples and further applied in CE–MS–MS analysis.

Acknowledgements

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References

[1] A.D. Kinghorn, M.F. Balandrin, in: S.W. Pelletier (Ed.), Alkaloids: Chemical and Biological Perspectives, Vol. 2, Wiley, New York, 1984, p. 105.
[2] S.Y. Zhang, S.Y. Li, Chin. Pharm. J. 28 (1993) 328.
[3] A. Ueno, K. Morinaga, S. Fukushima, S. Okuda, Chem. Pharm. Bull. 26 (1978) 1832.
[4] B.G. Zhao, Acta Pharm. Sinica 15 (1980) 182.
[5] S. Shibata, Y. Nishikawa, Yakugaku Zasshi 81 (1961) 1635.

[6] K. Yamamoto, K. Arimoto, Y. Iwata, K. Ujita, K. Sakurai, Y. Shimaoka, Y. Shimada, A. Takagi, T. Taniyama, M. Nakahara, K. Nakajima, Y. Nunoura, M. Noguchi, Y. Hamashita, Y. Hisata, K. Hosoda, N. Mishima, Y. Yamamoto, M. Yoshizaki, Nat. Med. 48 (1994) 180.
[7] L.X. Jin, Y.Y. Cui, G.D. Zhang, Acta Pharm. Sinica 28 (1993) 136.
[8] J.F. Cui, G.D. Zhang, M.Z. Wang, Acta Pharm. Sinica 20 (1985) 59.
[9] C.S. Liu, W.Y. Liu, L.S. Sheng, Chin. Trad. Herbal Drugs 24 (1993) 405.
[10] F.A. Tomas-Barberan, Phytochem. Anal. 6 (1995) 177.
[11] I. Bjørnsdottir, S.H. Hansen, J. Pharm. Biomed. Anal. 13 (1995) 687.
[12] V.G. Trenerry, R.J. Well, J. Robertson, J. Chromatogr. A 718 (1995) 217.
[13] I. Bjørnsdottir, S.H. Hansen, J. Pharm. Biomed. Anal. 13 (1995) 1473.
[14] Y.M. Liu, S.J. Sheu, J. Chromatogr. 639 (1993) 323.
[15] P.G. Pietta, P.L. Mauri, C. Gardana, M.L. Colombo, F. Tomé, Phytochem. Anal. 6 (1995) 196.
[16] Y.M. Liu, S.J. Sheu, J. Chromatogr. 600 (1992) 370.
[17] M.T. Bowser, A.R. Kranack, D.D.Y. Chen, Trends Anal. Chem. 17 (1998) 424.
[18] S.H. Hansen, J. Tjørnelund, I. Bjørnsdottir, Trends Anal. Chem. 15 (1996) 175.
[19] R. Snyder, J. Chromatogr. Sci. 16 (1983) 233.
[20] Pharmacopoeia Commission of the Ministry of Public Health, in: 1995 edition, Pharmacopoeia of the Peoples Republic of China, Vol. I, Guangdong Sci. and. Tech. Press, 1995, p. 176.
[21] M. Unger, J. Stöckigt, J. Chromatogr. A 791 (1997) 323.
[22] F. Hirayama, C. Namiki, K. Sagara, Y.Y. Tong, L.Y. He, Y.H. Chen, Nat. Med. 49 (1995) 317.
[23] H.Z. Zheng, Z.H. Dong, J. She, in: Modern Study of Traditional Chinese Medicine, Vol. 3, Xue Yuan Press, Beijing, 1998, p. 2694.
[24] H.Z. Zheng, Z.H. Dong, J. She, in: Modern Study of Traditional Chinese Medicine, Vol. 1, Xue Yuan Press, Beijing, 1998, p. 547.