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Short communication

Determination of matrine and oxymatrine in *Sophora subprostata* by CE

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Abstract

Shan-dou-gen is the dried roots of *Sophora subprostata* (Leguminosae) and a commonly used Chinese herbal drug in Taiwan. It possesses antipyretic, anti-inflammatory, analgesic effects and is used to treat sore throat and acute pharyngolaryngeal infections. To evaluate the quality of *S. subprostata*, a simple, rapid and accurate high-performance capillary electrophoresis (HPCE) method was developed for the assay of two alkaloids: matrine and oxymatrine. The electrolyte was a buffer solution containing 75% 130 mM phosphate buffer (NaH_2PO_4/H_3PO_4, pH 3.5) and 25% acetonitrile. Applied voltage was 10 kV and temperature was 30 °C. 2-(4-Hydroxyphenyl)ethylammonium chloride was used as an internal standard and detector set at 200 nm. The contents of matrine and oxymatrine of *S. subprostata* in several different samples of crude drugs and commercial concentrated preparation have also been determined.

Keywords: Matrine; Oxymatrine; High-performance capillary electrophoresis (HPCE); Pharmaceutical analysis

1. Introduction

Shan-dou-gen is the dried roots of *Sophora subprostata* (Leguminosae) and a commonly used Chinese herbal drug in Taiwan. It possesses antipyretic, anti-inflammatory, analgesic effects and is used to treat sore throat and acute pharyngolaryngeal infections [1]. Matrine and oxymatrine are the major quinolizidine alkaloids in the roots of *S. subprostata*. The chemical structures of two constituents are shown in Fig. 1. Quinolizidine alkaloids are important due to their toxicity in humans and livestock as constituents of poisonous plants and ironically some of them exhibit potentially useful pharmacological activity [2]. There are several herbal drugs been used as substituted for *S. subprostata*. Kan-chih-ma (*Helicters angustifolia*, Sterculiaceae) usually substituted or adulterated of Shan-dou-gen on Taiwan market. Analysis of marker constituents in *H. angustifolia* by high-performance liquid chromatography (HPLC) was developed [3]. It is shown that these two herbal drugs were different.

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There are several methods such as HPLC and high-performance capillary electrophoresis (HPCE) have been established for analysis of matrine and oxymatrine in the same genus, *S. flavescens*, *S. alopecuroides* and *S. tonkinensis* [4–7]. HPLC methods [4,5] have lower efficiency and take longer analysis time. HPCE requires a short analysis time, a small amount of sample, and the capillary column can be thoroughly cleaned with ease. After several trials, the conditions of the related known HPCE methods [6,7] gave bad resolution, oxymatrine especially, for *S. subprostata*. In our laboratory, we have already developed several HPLC [3,8] and HPCE [9,10] methods for the determination of marker constituents in herbal drugs.

In this study, we developed an HPCE method for the determination of these two alkaloids of *S. subprostata*. The contents of these two constituents in five commercial concentrated products from Taiwan markets have also been determined.

2. Experimental

2.1. Reagents and materials

Matrine and oxymatrine were isolated from the roots of *S. subprostata* and identified spectroscopically [11]. 2-(4-Hydroxyphenyl)ethylammonium chloride was purchased from E. Merck (Darmstadt, Germany). Acetonitrile (HPLC grade) was purchased from Labscan (Dublin, Ireland). Sodium dihydrogenphosphate was purchased from Nakalai (Kyoto, Japan). *ortho*-Phosphoric acid was analytical reagent grade. Ultra-pure distilled water with a resistance greater than 18 MΩ was used. Two samples of *S. subprostata* and five commercial concentrated products were obtained from markets in Taipei, Taiwan. Crude plant samples were identified by comparative microscopy studies.

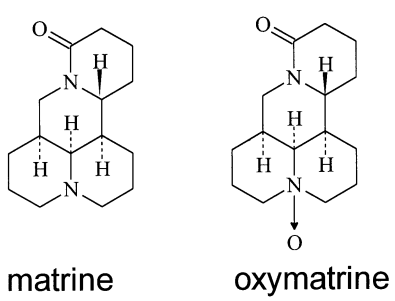

Fig. 1. The chemical structures of matrine and oxymatrine.

2.2. Apparatus and conditions

The analysis was carried out on a Beckman P/ACE 2200 CE system equipped with a UV detector. Detector was set at 200 nm and a 47 cm × 75 μm I.D. uncoated capillary (Beckman) with the detection window placed at 40 cm. The conditions were as follows: sampling time, 1.0 s, hydrostatic; run time, 15 min; applied voltage, 10.0 kV (constant voltage, positive to negative polarity); and temperature, 30 °C. The electrolyte buffer was a solution containing 75% 130 mM sodium phosphate buffer (NaH_2PO_4/H_3PO_4, pH 3.5) and 25% acetonitrile. The electrolyte was filtered through a 0.45 μm syringe filter (Gelman) before use. Between each sample throughout the experiment, the capillary was cleaned with 1.0% sodium hydroxide, 2 min and water, 2 min, successively. The capillary was rinsed with buffer for 2 min before each experiment. The Gold software (Beckman) for system control and data processing was used.

2.3. Preparation of standard solution

To prepare standard solutions (containing matrine and oxymatrine), an accurately weighed amount of matrine and oxymatrine standard which was dissolved in water for HPCE. Five concentrations were chosen, with the range 22.0–440.0 and 10.8–216.0 μg ml^{-1}, respectively. To each solution a suitable amount of internal standard was added to yield a final concentration of 25.0 μg ml^{-1} of 2-(4-hydroxyphenyl)ethylammonium chloride. Calibration graphs were plotted subsequently for linear regression analysis of the peak area ratios versus concentrations. All solutions were found to be stable when stored at 4 °C for one month.

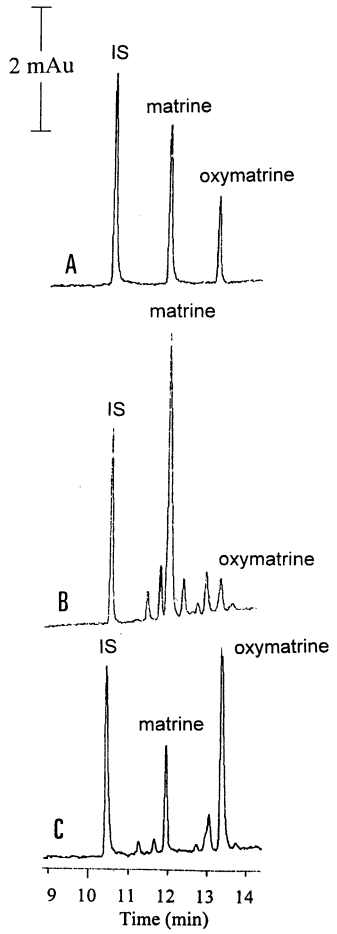

Fig. 2. Chromatograms of blank (A); water extract of *S. subprostata* (B); and commercial concentrated product (C). HPCE conditions, CE system: Beckman P/ACE 2200; wavelength: 230 nm; capillary: 47 cm × 75 μm I.D. uncoated capillary (Beckman), the detection window placed at 40 cm; applied voltage: 10.0 kV; temperature: 30 °C; buffer: 75% 130 mM sodium phosphate buffer (NaH_2PO_4/H_3PO_4, pH 3.5) and 25% acetonitrile. IS = 2-(4-hydroxyphenyl)ethylammonium chloride.

2.4. Preparation of sample solution

Twenty grams of the roots of *S. subprostata* (bark and wood) were cut to pieces and mixed well. Two grams of this sample was extracted two times (15 and 10 ml, successively) with water by ultrasonic at 40 °C, each half-hour. The extracts were combined and filtered while hot into a volumetric flask. Water was added to 25 ml and used as stock solution. The sample solution was prepared by 1.0 ml of above solution and 0.5 ml of 2-(4-hydroxyphenyl)ethylammonium chloride solution (250 μg ml^{-1}) into a 5 ml volumetric flask and then adjusting the volume to 5 ml with water. This solution was filtered through a 0.45 μm syringe filter (Gelman Sciences, Ann Arbor, MI, USA) before use.

2.5. Commercial concentrated sample determination

0.5 grams of the *S. subprostata* commercial concentrated sample was extracted two times (15 and 10 ml, successively) with water by ultrasonic bath at 40 °C and then processed as above.

2.6. Precision

The intra- and interdays variabilities at three typical assay concentrations were evaluated for five replicates within one day and over 5 successive days.

2.7. Preparation of recovery studies

Three different concentrations of markers: 20.6, 41.2 and 82.4 μg ml^{-1} for matrine and 10.5, 21.0 and 42.0 μg ml^{-1} for oxymatrine were added to the plant material samples, respectively. Sample was extracted with water by ultrasonic bath at 40 °C and then processed as above. All samples were filtered through a 0.45 μm syringe filter (Gelman) and injected for HPCE analysis to calculate the concentration of matrine and oxymatrine from their calibration graphs.

3. Results and discussion

The detection wavelength of 200 nm was chosen because these two constituents have reasonable absorption only at this wavelength. In this low wavelength, phosphate salt was chosen for the buffer solution due to its lower absorbance. 2-(4-Hydroxyphenyl)ethylammonium chloride was used as an internal standard which migrated out before matrine and oxymatrine.

Table 1
Intra- and interdays assay variations of two marker constituents

Constituent	Concentration (μg ml^{-1})	Intraday R.S.D. (%)[a]	Interday R.S.D. (%)[a]
Matrine	22.0	2.99	1.68
	110.0	1.75	1.72
	440.0	3.98	2.95
Oxymatrine	10.8	1.27	2.82
	43.2	3.23	3.30
	216.0	3.63	2.63

[a] $n = 5$.

Table 2
Recoveries of two marker constituents in *H. angustifolia*

Constituent	Amount added (μg ml^{-1})	Recovery (%)	Mean ± S.D. (%)	R.S.D. (%)[a]
Matrine	20.6	90.4	90.7 ± 1.2	1.3
	41.2	89.7		
	82.4	92.5		
Oxymatrine	10.5	87.6	88.3 ± 2.2	2.5
	21.0	91.3		
	42.0	86.1		

[a] $n = 3$.

In order to study the influence among pH, acetonitrile concentration and voltage, serial experiments were carried out. Preliminary experiments were first conducted at pH 3.5 of 80, 100, 120 and 130 mM NaH_2PO_4 without acetonitrile in the electrophoretic medium. In all instances, matrine and oxymatrine standard were successfully separated, but these two constituents overlapped with interferences in sample. The 130 mM NaH_2PO_4 buffer with more than 20% acetonitrile gave the best resolution in sample analysis. The 130 mM NaH_2PO_4 buffer was used with different acetonitrile concentrations (20, 25 and 30%) to study the effect of organic solvent on the separation. The 25% acetonitrile solution gave the best resolution. Different voltages (8, 10, 12, 15 and 18 kV) were also used to study the effect of supplied voltage on the selectively of the separation. The result indicated that matrine and oxymatrine were best resolved with no co-eluting constituents at 10 kV. Finally, the separation was optimized only pH 3.5, 25% of acetonitrile and 10 kV for best resolution and none of interferences in sample analyses.

Fig. 2A and B present chromatograms showing the separation of the constituents with the migration times of 10.4 min for internal standard; 11.9 min for matrine and 13.3 min for oxymatrine. When the sample solution was injected directly and analyzed, the whole analysis was finished within 14 min.

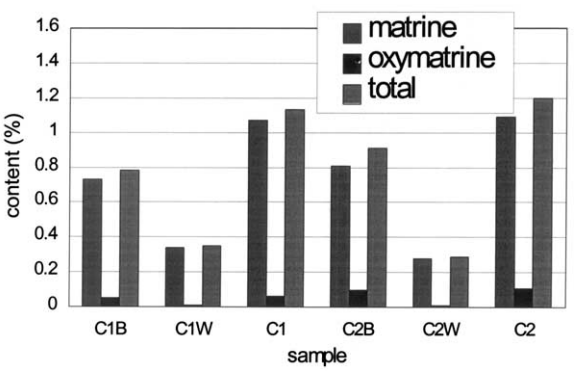

Fig. 3. The contents of two constituents in two *S. subprostata*. B: bark, W: wood.

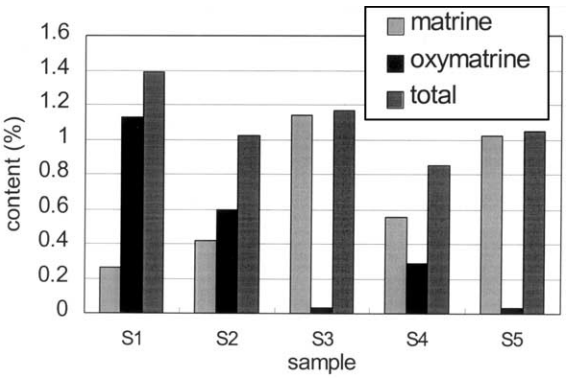

Fig. 4. The contents of two constituents in five *S. subprostata* of commercial concentrated products (S1–S5) obtained from different sources.

Calibration graphs were constructed in the range 22.0–440.0 μg ml^{-1} for matrine and 10.8–216.0 μg ml^{-1} for oxymatrine. The regression equations of these curves and their correlation coefficients (r), coefficients of determination (R^2) were calculated as follows: matrine, $y = 1.68E-2x-0.26$, 0.9984, 99.68% and oxymatrine, $y = 2.69E-2x-0.21$, 0.9975, 99.50%. It showed good linear relationships between the peak areas and the concentrations. Signals three and eight times higher than the peak noise height were regarded as the detection and quantification limit. The detection and quantification limits of these two constituents were 1.1 and 1.0 μg ml^{-1} for matrine and oxymatrine, 2.5 and 2.2 μg ml^{-1} for matrine and oxymatrine, respectively.

To assess the precision of these methods, we injected standard solutions of matrine and oxymatrine, respectively, five times on the same day and a 5-day period analysis. The coefficient variations of intra- and interdays studies were less than 4.0%, respectively. The precision as well as accuracy of this assay was satisfactory (Table 1). The results for the recoveries of matrine and oxymatrine ranged from 86.1 to 92.5% (Table 2). The R.S.D.s of recoveries of three constituents ranged between 1.3 and 2.5%. The stability of the analytes in solution was analyzed by the day-to-day experiment. The coefficient variations of matrine and oxymatrine for 5-days analysis were less than 3.0%.

When the sample solution was analyzed by HPCE, the peaks were identified by comparison of the migration time with those obtained from authentic samples of *S. subprostata*. Since after two times extraction, the two constituents yielded over 98%. Therefore, two times extraction was chosen. The heartwood and the bark of the roots were also separated for analysis. The extraction yields of two constituents in two samples (bark and wood) using different extraction methods were shown in Fig. 3. The yields of matrine and oxymatrine in bark were double than wood.

The comparison is depicted of the contents of five commercial concentrated products in Fig. 4. The contents of these two constituents ranged between 0.26–1.14 and 0.03–1.13% for matrine and oxymatrine, respectively. The total contents of matrine and oxymatrine ranged between 0.85 and 1.39%. It is found that variation of the content of oxymatrine was great. Since the total amounts of these two alkaloids were similar and oxymatrine can be obtained by air oxidation from matrine. Comparison of the ratio of freshly extracted samples and commercially available samples (Figs. 3 and 4) clearly showed that the quality of S3 and S5 should be better than S1 and S2.

In conclusion, we suggested that these two quinolizidine, matrine and oxymatrine is unique of Shan-dou-gen (*S. subprostata*) and can be used as markers to determine the quality of *S. subprostata* by HPCE. This technique offers high separation efficiencies, rapid analyses, lower running costs. Instead of organic solvent, aqueous solvent was adapted throughout the HPCE analysis. All of these are advantages over traditional chromatographic procedures.

References

[1] H.-M. Chang, P.P.-H. But, Pharmacology and Applications of Chinese Materia Medica, vol. 1, World Scientific Publishing Co. Ltd, Singapore, 1986, pp. 108–116.
[2] A.D. Kinghorn, M.F. Balandrin, in: S.W. Pelletier (Ed.), Alkaloids: Chemical and Biological Perspectives, vol. 2, Wiley, New York, 1984, p. 105.
[3] Y.-S. Chang, Y.-R. Ku, J.-H. Lin, K.-L. Lu, L.-K. Ho, J. Pharm. Biomed. Anal. 26 (2001) 849.

[4] J. Cui, G. Zhang, M. Wang, Yaoxue Xuebao 20 (1985) 59.
[5] K. Yamamoto, K. Arimoto, Y. Iwata, K. Ujita, K. Sakurai, Y. Shimaoka, Y. Shimada, A. Takagi, T. Taniyama, Nat. Med. 48 (1984) 181.
[6] M. Luo, P. He, M. Wu, L. Li, Y. Guo, Zhongcaoyao 30 (1999) 261.
[7] J.-Z. Song, H.-X. Xu, S.-J. Tian, P.P.-H. But, J. Chromatogr. A 857 (1999) 303.
[8] K.-L. Lu, Y.-R. Ku, K.-C. Wen, L.-K. Ho, Y.-S. Chang, J. Liq. Chromatogr. Rel. Technol. 23 (2000) 2573.
[9] Y.-R. Ku, Y.-T. Lin, J.-H. Lin, K.-C. Wen, C.-H. Liao, J. Chromatogr. A 805 (1998) 301.
[10] Y.-R. Ku, Y.-T. Lin, K.-C. Wen, J.-H. Lin, C.-H. Liao, J. Chromatogr. A 805 (1998) 330.
[11] S.Z. Bai, J.H. He, Z.Q. Yang, E.C. Rao, Chin. Trad. Herb. Drugs 13 (1982) 8.