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Short communication

Analysis of tetrandrine and fangchinoline in traditional Chinese medicines by capillary electrophoresis

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Abstract

A simple, rapid and accurate capillary electrophoresis method was developed for the determination of tetrandrine and fangchinoline in traditional Chinese medicines. The buffer solution used was a solution composed of 60 m*M* phosphoric acid and 50 m*M* Tween-20 containing 20% methanol with the pH value adjusted to 2.50 with triethylamine. The linear calibration range was 50.6–810 μg/ml (R=0.9999) for tetrandrine and 43.3–692 μg/ml (R=0.9998) for fangchinoline. The contents of these two alkaloids in Radix *Stephaniae tetrandrae* S. Moore and seven Radix *Stephaniae tetrandrae* containing Chinese herbal preparations were easily determined within 23 min.

Keywords: Pharmaceutical analysis; *Stephaniae tetrandrae*; Tetrandrine; Fangchinoline; Alkaloids

1. Introduction

Tetrandrine and fangchinoline are two alkaloids (structural formulae shown in Fig. 1) that exist in Radix *Stephaniae tetrandrae* S. Moore with pain-relieving, blood pressure-depressing, antiphlogistic, antineoplastic, and antibiotic activities. This plant is often used in Chinese herbal preparations [1]. It is necessary to establish a suitable analytical method to evaluate or control the quality of these herbal preparations.

Several methods have been reported for the determination of these two alkaloids, including thin-layer ultraviolet spectrophotometry, high-speed counter-current chromatography, and HPLC [2]. However, some of them suffer from being time-consuming and laborious while some others are not suitable to be used in samples of complex composition. In this study, tetrandrine and fangchinoline were chosen as indicators for analyzing Radix *Stephaniae tetrandrae* and various formulations containing it with capillary electrophoresis.

Capillary electrophoresis (CE) is a developing technique characterized by high efficiency, rapidity, low cost, and easy turnover mode. Recently, several studies dealing with herbal medicines, have been reported and two kinds of medicinal compounds, i.e., alkaloids [3–10] and flavonoids [11–16], have been studied extensively.

2. Experimental

2.1. Reagents and materials

The standard samples of tetrandrine and fangchinoline were provided by National Institute for the Control of Pharmaceuticals and Biological Prod-

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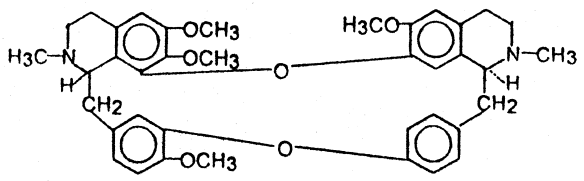
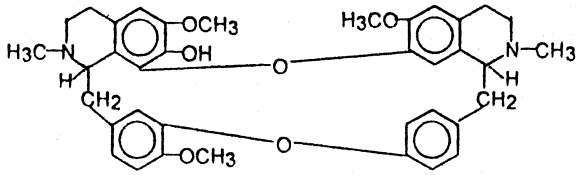


Tetrandrine



Fangchinoline

Fig. 1. Structural formulae of tetrandrine and fangchinoline.

ucts (Beijing, China). The herbal drug and preparations analyzed were purchased from local drugstores. All other chemicals were of analytical grade. The water used was re-distilled.

Herbal medicines analyzed and their compositions and sample amount for each analysis are as follows.

(1) Radix *Stephaniae Tetrandrae*: Radix *Stephaniae tetrandrae* (0.500 g).

(2) Feng shi zhen tong gao: Radix *Aconiti* (*Aconitum carmichaeli* Debx.) (0.670 g), Radix *Stephaniae tetrandrae* (0.400 g), *Camphora* (*Cinnamomum camphora* (L.) Presl) (0.067 g), plant bitumen (10.67 g).

(3) Fang ji guan jie wan: Radix *Stephaniae tetrandrae* (1.80 g), *Poria* (*Poria cocos* (Schw.) Wolf) (1.80 g), *Rhizoma atractylodis macrocephalae* (*Atractylodes macrocephala* Koidz) (1.20 g), Radix *Aconiti* (0.60 g), Cortex *Cinnamomi* (*Cinnamomum cassia* Presl) (0.60 g), Radix *Glycyrrhizae* (*Glycyrrhiza uralensis* Fisch.) (0.60 g), Radix *Codonopsis pilosulae* (*Codonopsis pilosula* (Franch.) Nannf.) (0.60 g).

(4) Feng shi zhi tong wan: Radix *Aconiti* (10.0 g), Radix *Ledebouriellae* (*Saposhnikoria divaricata* (Turcz.) Schischk.) (6.00 g), Radix *Angelicae sinensis* (*Angelica sinensis* (Oliv.) Diels) (6.00 g), Radix *Stephaniae tetrandrae* (2.00 g), Radix *Ginseng* (*Panax Ginseng* C.A. Mey.) (1.00 g), Radix *Glycyrrhizae* (1.00 g).

(5) Ling long gan mao jiao nang: *Rhizoma seu radix notopterygium* (*Notopterygium incisum* Ting) (2.50 g), Radix *Gentianae* (*Gentiana scabra* Bge.) (2.50 g), Radix *Gentianae macrophyllae* (*Gentiana macrophylla* Pall.) (2.50 g), Caulis *Aristolochiae manshuriensis* (*Aristolochia manshuriensis* Kom.) (2.50 g), Radix *Stephaniae tetrandrae* (2.50 g), Radix *Clematidis* (*Clematis chinensis* Osbeck) (2.50 g), Radix *Glycyrrhizae* (1.25 g).

(6) Xi xian feng shi wan: *Herba Siegesbeckiae* (*Siegesbeckia orientalis* L.) (5.00 g), Radix *Clematidis* (3.75 g), *Ramulus mori* (*Morus alba* L.) (3.75 g), *Ramulus taxilli* (*Taxillus chinensis* (Dc.) Danser) (3.75 g), Radix *Stephaniae tetrandrae* (2.50 g).

(7) Qu feng gu tong lu: Caulis *Piperis futokadsurae* (*Piper futokadsura* Sinb. et Zucc.) (3.40 g), Radix *Clematidis* (2.80 g), Caulis *spatholobi* (*Spatholobus suberectus* Dunn) (2.80 g), *Lignum sappan* (*Caesalpinia sappan* L.) (2.00 g), Radix *Stephaniae tetrandrae* (2.00 g), Cortex *Periplocae radicis* (*Periploca sepium* Bge.) (2.00 g), *Herba Siegesbeckiae* (2.00 g), Radix *Aconiti kusnezoffii* (*Aconitum kusnezoffii* Rchb.) (1.40 g).

(8) Shen jin dan jiao nang: *Lumbricus* (*Pheretima aspergillum* Perrier) (8.33 g), *Flos Carthami* (*Carthamus tinctorius* L.) (5.83 g), *Olibanum* (*Boswellia carterii* Birdw.) (2.50 g), *Myrrha* (*Commiphora myrrha* Engl.) (2.50 g), Radix *Stephaniae tetrandrae* (2.50 g), Cortex *Periplocae radicis* (2.50 g), *Rhizoma drynariae* (*Drynaria fortunei* (Kunze) J. Sm.) (2.50 g).

2.2. Sample preparation

Pulverized herbal drug or preparations of suitable amounts (see the above paragraph) were extracted with the appropriate amount of 50% aqueous ethanol (being capable of immersing the solid sample) by stirring at room temperature for 30 min, then centrifugation (Shanghai 10th Operation Machine Factory) at a speed of 4000 rpm for 10 min (at ca. 1600

g). Extraction was repeated three times. The extracts were combined and filtered through a 0.45-μm cellulose acetate membrane filter. The final combined extracts amounted to about 20 ml. The exact amount of the aqueous ethanol solution used at each time depended upon the contents of analytes in the sample. For example, for samples 1 and 8 in Table 1, 0.5 and 27 g were taken, respectively. The combined extract solution was diluted to 25 ml with 50% aqueous ethanol solution, with the final solution containing 40 m*M* sodium chloride, and was passed through a 0.45-μm membrane filter again. The filtrate was injected into the capillary electrophoresis system directly.

2.3. Apparatus and CE conditions

Experiments were carried out on a laboratory-assembled CE system. An uncoated fused-silica capillary of 64 cm length (42 cm effective length from the anode to the detector)×50 μm I.D.×375 μm O.D. (Yongnian Optical Fiber Factory, Hebei, China) was used as a separation tube. A laboratory-made high-voltage power supply that can provide voltage from 0 to 30 kV was used to drive the separation. On-column detection was performed at the cathode on a CV^4 UV detector (ISCO, Lincoln, NE, USA) at 214 nm with a rise time of 0.4 s. Electropherograms were recorded on an HP 3394 integrator (Hewlett-Packard, USA). A small fan was used to dissipate the Joule heating generated by power. A pHs-3C pH meter with an E-201-C combination electrode (Rex Instrument Factory, Shanghai, China) was used for pH measurements. A Sartorius 2004 MP electronic balance (Sartorius, Germany) was used to weigh all the standards.

The applied voltage was 20 kV. The CE operation was performed at ambient temperature (19±1°C). Samples were injected with the electromigration mode at the anode with 4 kV for 21 s.

The capillary was rinsed with 0.1 *M* NaOH for 5 min, followed with water and the background electrolyte solution of interest for 10 min, respectively, every morning. Between injections, it was washed with the background electrolyte (BGE) for 5 min.

2.4. Solutions for constructing the calibration curve

Seven different concentrations of tetrandrine ranging from 50.6 to 810 μg/ml, and six different concentrations of fangchinoline ranging from 43.3 to 692 μg/ml, were prepared by dissolving the standard in 50% aqueous ethanol containing 40 m*M* NaCl. Each standard solution was analyzed three times.

2.5. Recovery test

Different amounts of tetrandrine and fangchinoline standards were weighed and added to two samples of known tetrandrine and fangchinoline contents, and

Table 1
Determined values (mg/g) of tetrandrine and fangchinoline in eight traditional Chinese medicines ($n=3$)

Sample no.	Name of medicine	Tetrandrine (mg/g)	R.S.D.$_a$ (%)	R.S.D.$_t$ (%)	Fangchinoline (mg/g)	R.S.D.$_a$ (%)	R.S.D.$_t$ (%)
1	Radix *Stephaniae tetrandrae*	5.15	1.5	2.4	5.06	0.7	2.7
2	Feng shi zhen tong gao	0.13	1.7	0.4	0.13	2.4	0.3
3	Fang ji guan jie wan	0.21	0.8	0.5	0.22	2.4	0.5
4	Feng shi zhi tong wan	0.096	2.9	0.9	0.088	1.5	0.4
5	Ling long gan mao jiao nang	0.13	1.5	0.1	0.13	3.1	0.1
6	Xi xian feng shi wan	0.11	0.9	1.8	0.10	3.1	1.7
7	Qu feng gu tong lu	0.081	1.3	1.2	0.081	0.2	1.1
8	Shen jin dan jiao nang	0.10	2.5	0.3	0.096	0.6	0.4

Note: *n* denotes the number of injections of one and the same sample; *a* and *t* denote area and migration time, respectively.

the mixtures were extracted and analyzed with the proposed procedure.

3. Results and discussion

3.1. Optimization of separation conditions

For the analysis of alkaloids, it is convenient to choose a suitable pH to protonate basic analytes into cations and then analyze them by CE. At pH 2.50 tetrandrine and fangchinoline being nearly completely protonated elute before the electroosmotic flow (EOF), while the neutral or anionic species, if present, elute at or after the EOF. Thus, a pH value of 2.50 was chosen.

For optimization of the separation, the following attempts were made. At the very beginning, the buffer solution employed was a solution containing 60 m*M* phosphoric acid with its pH value adjusted to 2.5 with sodium hydroxide. With this buffer the two alkaloids were not separated at all (figure not shown). As we know, tetrandrine and fangchinoline are similar in their structures, at pH 2.5 they eluted early before EOF.

In order to improve the resolution, triethylamine was used instead of sodium hydroxide to neutralize phosphoric acid during buffer preparation, leading to a considerable decrease of EOF and increase of resolution, as evidenced by the appearance of two partially resolved peaks (Fig. 2a). This might presumably be due to a more effective shielding of the residual negatively charged silanol groups on the inner capillary surface by the protonated triethylamine cations.

For further elevating the resolution, 50 m*M* Tween-20 was added to the solution which led to a complete separation of the two peaks; however, the column efficiency was not very good (see Fig. 2b), with the efficiency of fangchinoline being only 6.9×10^3 plates/m.

Finally, it was found that with the addition of 20% methanol into the background electrolyte the separation could be further improved resulting in two well-shaped peaks (see Fig. 2c), and the column efficiency for fangchinoline increased to 1.6×10^5 plates/m. Under this optimized condition the electro-

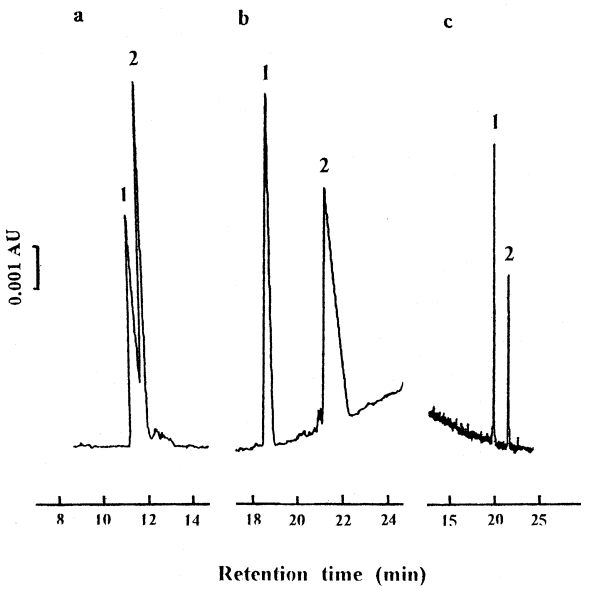


Fig. 2. Optimization of separation conditions for tetrandrine and fangchinoline. Conditions: field strength, 313 V/cm; capillary length, 64 cm (42 cm to detector), capillary diameter 50 μm; detection, 214 nm; injection, electromigration, 4 kV/21 s. pH 2.50 buffer: (a) 60 m*M* triethylamine–phosphate; (b) 60 m*M* triethylamine–phosphate + 50 m*M* Tween-20; (c) 60 m*M* triethylamine–phosphate + 50 m*M* Tween-20 + 20% methanol. Peaks 1 and 2 are tetrandrine and fangchinoline, respectively. Sample: a mixture of the two standards.

osmotic flow of the system became tremendously decreased with the migration time of formamide as a marker exceeding 2 h.

As to the separation mechanism, it is necessary to know the critical micelle concentration (CMC) of Tween-20 in 20% aqueous methanol. However, this value cannot be found in the literature. By referring to the critical micellar concentration (CMC) value of Tween-80 in aqueous solution (10^{-2} to 10^{-3} m*M*) it can be reasonably estimated that 50 m*M* Tween-20 is far exceeding its CMC, so Tween-20 micelles exist predominantly in the BGE solution, leading to the separation mode most likely to be MEKC. Incidentally, the coating of the non-ionic surfactant Tween-20 on the inner capillary wall is suppressed due to the presence of protonated triethylamine cations at pH 2.5 which are capable of interacting more

strongly with the capillary wall by electrostatic attraction.

3.2. Determination of tetrandrine and fangchinoline in Chinese medicines

With the above procedure, tetradrine and fangchinoline in Radix *Stephaniae tetrandrae* and the seven Radix *Stephaniae tetrandrae*-containing Chinese herbal preparations were all successfully separated. A typical electropherogram is shown in Fig. 3 with the two compounds being baseline resolved. All eight herbal medicine samples gave tetrandrine and fangchinoline peaks without any other accompanying peaks within 28 min.

The electropherograms of the real samples were quite simple; however, this does not exclude the possibility for the existence of other compounds with absorbance at a wavelength other than 214 nm. For quantitation, the internal standard method was first tried with failure due to lack of suitable internal standard, therefore the external standard method was employed. The calibration curve for tetrandrine was constructed in the concentration range of 50.6–810 μg/ml with the linear regression equation and correlation coefficient being as follows:

$$y = 6971.7x - 12\,511 \ (R = 0.9999)$$

where y is the integrated peak area (integration units) and x is the concentration (μg/ml) of tetrandrine in the sample.

Similarly, for fangchinoline the calibration curve was constructed in the concentration range of 43.3–692 μg/ml with the linear regression equation and correlation coefficient being as follows:

$$y = 5553.2x - 75\,065 \ (R = 0.9998)$$

where y is the integrated peak area (integration units) and x is the concentration (μg/ml) of fangchinoline in the sample. Thus, the amounts of tetrandrine and fangchinoline in Radix *Stephaniae tetrandrae* and the commercial Chinese herbal preparations containing it can be quantified according to the above regression lines or equations. Table 1 lists the determined values of tetrandrine and fangchinoline in Radix *Stephaniae tetrandrae* and the seven commercial Chinese herbal preparations containing it.

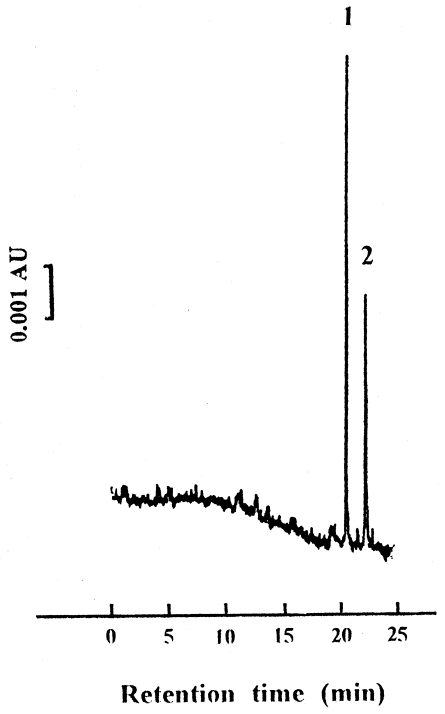


Fig. 3. Typical electropherogram of the Chinese herbal medicines studied. Conditions: field strength, 315 V/cm; buffer, 20% methanol, 60 m*M* phosphate–triethylamine+50 m*M* Tween-20, pH 2.50; capillary length, 64 cm (42 cm to detector); capillary diameter, 50 μm; detection, 214 nm; injection, electromigration, 4 kV/21 s. Sample 5, for its composition refer to Section 2.1.

3.3. System suitability test

Preliminary experiments indicated that, when the standard solutions were prepared in 50% aqueous ethanol, the repeatability was poor because of the large difference of ionic strength between the sample solution and the separation buffer. To overcome this difficulty, a 50% aqueous ethanol aqueous solution containing 40 m*M* NaCl was used to maintain a nearly equal ionic strength between the sample solution and the separation buffer. By this means the R.S.D.s of the determinations were improved.

The recoveries were determined with the standard addition method for tetrandrine and fangchinoline in Radix *Stephaniae Tetrandrae* (sample 1) and Fang ji guan jie wan (sample 3), with results of 99.3 and 99.0%, respectively, for tetrandrine, and 101.0 and 102.5%, respectively, for fangchinoline.

The lower limits of detection (LOD) for tetrandrine and fangchinoline are calculated to be 8.3 and 14.4 pg, respectively.

4. Conclusion

A buffer solution consisting of 60 m*M* phosphoric acid, 50 m*M* Tween-20 and 20% methanol, with the pH value adjusted to 2.5 with triethylamine, was proposed for the determination of tetrandrine and fangchinoline in many traditional Chinese medicines containing Radix *Stephaniae Tetrandrae*. The proposed method is simple, economic, rapid, and robust, and is especially suitable for quality control in pharmaceutical plants.

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References

[1] J. Yin, L.G. Guo, Modern Research and Clinic Application of Traditional Chinese Medicine, vol. 1, Xue Yuan Press, Beijing, 1995 (in Chinese).
[2] P. Zhen, P.R. Chen, H.Q. Wang, Fen Xi Hua Xue Jin Zhan, (Progress in Analytical Chemistry), Analysis of tetrandrine and di-methyltetrandrine in Chinese medicinal herbs *Stephania tetrandra* S. Moore by HPLC, Nanjing University Press, p. 858, 1994 (in Chinese).
[3] H. Stuppner, M. Ganzera, J. Chromatogr. A 717 (1995) 271.
[4] S.S. Yang, I. Smetena, Chromatographia 40 (1995) 375.
[5] Y.M. Liu, S.J. Sheu, J. Chromatogr. 623 (1992) 196.
[6] Y.M. Liu, S.J. Sheu, J. Chromatogr. 639 (1993) 323.
[7] Y.M. Liu, S.J. Sheu, J. Chromatogr. 634 (1993) 329.
[8] H. Stuppner, S. Sturm, G. Konwalinka, J. Chromatogr. 609 (1992) 375.
[9] V.C. Trenerry, R.J. Wells, J. Robertson, J. Chromatogr. A 718 (1995) 217.
[10] H. Stuppner, S. Sturm, N. Mulinacci, F. Vincieri, Chromatographia 37 (1993) 579.
[11] P. Pietta, P. Mauri, A. Rava, G. Sabbatini, J. Chromatogr. 549 (1991) 367.
[12] U. Seitz, G. Bonn, P. Oefner, M. Popp, J. Chromatogr. 559 (1991) 499.
[13] P. Pietta, A. Bruno, P. Mauri, A. Rava, J. Chromatogr. 593 (1992) 165.
[14] T.K. McGhie, J. Chromatogr. 634 (1993) 107.
[15] P. Pietta, P. Mauri, A. Bruno, L. Zini, J. Chromatogr. 638 (1993) 357.
[16] Z.K. Shihabi, T. Kute, L.L. Garcia, M. Hinsdale, J. Chromatogr. A 680 (1994) 181.