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

Application Note

Pharmaceutical

Authors

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Abstract

The analysis of plant extracts is problematic due to the very complex nature of the sample matrix which makes identification of individual constituents a challenge. The task is made even more difficult since some separation techniques, for example, HPLC, cannot easily deal with such matrices and injection can lead to column fouling. CE on the other hand is capable of high resolution analysis of such samples and indeed can be characterized by its ability to handle complex and crude samples with a minimum of sample preparation. CE is therefore ideal for the analysis of these natural products. Fangchinoline and tetrandine are two alkaloids of pharmaceutical interest which are present in Radix *Stephaniae tetradrae S. Moore*. The plant is often used in various Chinese herbal preparations. Here we describe the application of a CE method[1] for quantitative analysis of these alkaloids in some Chinese traditional medicines.

Experimental

All analyses were performed using an Agilent CE system equipped with diode array detection and controlled via a PC running the Agilent ChemStation software. Traditional medicine samples and standards were the kind gift of Professor H. Liu, Peking University, Beijing, PR China. Other reagents were supplied by Sigma.

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Name of medicine	Tetrandrine [mg/L]	Tetrandrine % RSD area	Tetrandrine % RSD time	Tetrandrine [mg/g]	Fanchinoline [mg/L]	Fanchinoline % RSD area	Fanchinoline % RSD time	Fanchinoline [mg/g]
Fang ji guan jie wan	28.41	2.44	0.32	0.41	17.03	3.45	0.36	0.25
Qu feng gu tong lu	6.96	1.89	0.08	0.07	3.88	2.15	0.07	0.04
Ling long gan mao jiao nang	16.52	1.08	0.23	0.17	9.79	1.16	0.22	0.10
Xi xian feng shi wan	7.47	5.58	0.28	0.07	3.54	2.11	0.29	0.04
Feng shi zhi ton gao	7.14	0.05	0.15	0.08	3.93	1.16	0.15	0.04
Shen jin dan jiao nang	10.94	3.33	0.21	0.12	5.81	3.97	0.25	0.07
Radix Stephaniae tetrandrae	233.26	1.09	0.21	5.52	185.04	1.06	0.22	4.38

Table 1
Migration time and peak area reproducibility (n=3) and quanitation of tetrandrine and fangchinoline in various traditional Chinese medicines.

Extraction: 2 g of each pulverised herbal drug were extracted with 7 mL 50 % ethanol by stirring for 30 minutes, followed by centrifugation (4000 rpm, 10 minutes). The extraction was repeated two more times and the combined extracts were filtered through 0.45-µm pore. For electrokinetic injection, a volume of 200 mM NaCl solution, equivalent to one fifth of the sample volume was added to the sample to equalize the sample conductivity. The analytical conditions were: buffer: 60 mM phosphoric acid\TAE, 50 mM Tween-20, 20 % methanol, pH 2.5; capillary: 64.5 cm (56 cm) x 50 µm; detection: 214.10; injection: 4 kV · 23 sec; voltage: 20.2 kV; temperature: 19 °C.

Results

The medicines were separated using a MEKC system with Tween-20 as the surfactant. This resulted in a very clean electropherogram where two peaks could easily be seen (figure 1). Due to their similar structure (figure 2) tetrandrine and fangchinoline have very similar spectra and therefore were identified by spiking experiments with pure standards. After identification (figure 1) the two alkaloids were quantified in a number of traditional Chinese medicines. Linearity was determined for both compounds over the range of 5 to 250 µg /mL. Linearity was greater than 0.9999 for both analytes. Reproducibility of migration times was very good (<0.4 %). For quantitation, the reproducibility of peak areas was acceptable (<4 %) but depended on the medicine and therefore the sample matrix (table1).

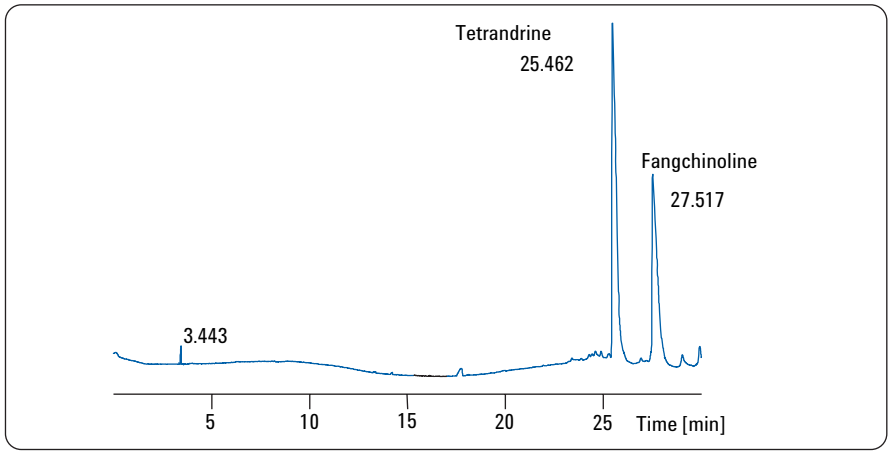

Figure 1
Separation of fangchinoline and tetradrine in a traditional medicine.

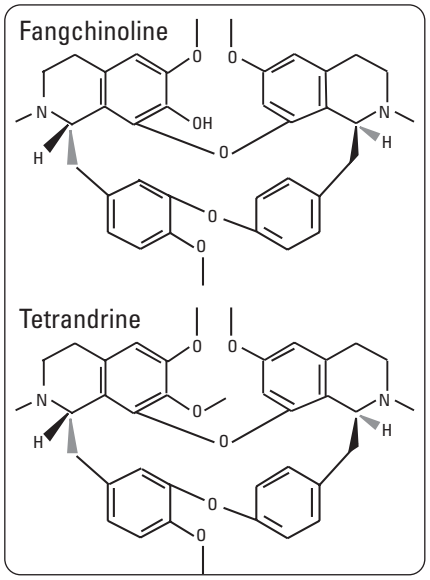

Figure 2
Structure of fangchinoline and tetradrine.

Conclusion

CE is very well suited to the analysis of components of traditional Chinese medicines due to its robust capability of handling complex sample matrices. Two endogenous alkaloids, tetrandine and fangchinoline could be identified and quantified in a number of different traditional medicines.

Reference

1.
Yang, J., Long, H., Liu H and Sun, Y., *J. Chromatogr A. 811, 274-279.* **1998.**

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