Journal of Chromatography, 637 (1993) 219–223
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 958

Short Communication

Determination of ephedrine and pseudoephedrine in Chinese herbal preparations by capillary electrophoresis

Ying-Mei Liu and Shuenn-Jyi Sheu*

Department of Chemistry, National Taiwan Normal University, 88, Sec. 5, Roosevelt Road, Taipei (Taiwan)

(First received November 24th, 1992; revised manuscript received February 5th, 1993)

ABSTRACT

A simple, rapid and accurate capillary electrophoresis method was developed for the assay of ephedrine and pseudoephedrine in Chinese herbal preparations. The buffer solution used was a 0.01 *M* valine solution with the pH adjusted to 10.0 with ammonia solution (7.2 *M*). The linear calibration range was 0.008–0.240 mg/ml ($r = 0.9996$) for both compounds and the recoveries were 98.0–102.3% for ephedrine and 99.0–101.5% for pseudoephedrine. The relative standard deviation was 0.86% for ephedrine and 0.87% for pseudoephedrine ($n = 6$) respectively. The contents of these two alkaloids in Ephedrae Herba-containing Chinese herbal preparations could easily be determined within 3 min.

INTRODUCTION

Ephedrae Herba (Ma-Huang) is a commonly used Chinese herbal drug intended for diaphoretic purposes, and is known to contain mainly (−)-ephedrine and (+)-pseudoephedrine [1]. It may combine with other herbs to form a sudorific, surface-internal acting, vitality-regulating or carminative formula [2]. At present, the best method to evaluate the quality of Ephedrae Herba-containing Chinese herbal preparations is to determine the contents of ephedrine and pseudoephedrine by HPLC [3,4]. However, owing to the complicated components in Chinese herbal formulations, the use of HPLC is restricted owing to the long time required (about 30 min), interference from alkaloidal peaks and contamination of the chromatographic column, which is difficult to clean. Capillary electrophoresis (CE) is a recently developed technique that offers short analysis times and easy thorough cleaning of the capillary, only small amounts of sample are required and autosampling is possible. It has given good results in the analysis of Chinese herbs [5–7]. In this study, CE was applied to various Ephedrae Herba-containing formulations with satisfactory results. Hence CE appears to be a suitable method for analyses of Chinese herbal preparations, especially for large numbers of samples and for quality control in pharmaceutical plants.

EXPERIMENTAL

Reagents and materials

Ephedrine hydrochloride and pseudoephedrine hydrochloride were purchased from Aldrich (Milwaukee, WI, USA) and valine from Merck (Darmstadt, Germany). Methyltriphenylphos-

* Corresponding author.

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phonium iodide, used as an internal standard, was prepared from triphenylphosphine and methyl iodide [8]. Deionized water from a Milli-Q system (Millipore, Bedford, MA, USA) was used to prepare all buffer and sample solutions. Methanol was of HPLC grade. Ammonia solution (7.2 *M*) was of extra-pure grade. Ephedrae Herba-containing Chinese herbal preparations were provided by Sun-Ten Pharmaceutical Manufactory.

Preparation of Chinese herbal preparation extracts

A 0.5-g sample of Chinese herbal preparation was extracted with 70% methanol (3 ml) by stirring at room temperature for 30 min, then centrifuged at 1500 *g* for 10 min. Extraction was repeated three times. The extracts were combined and filtered through a No. 1 filter-paper. After the addition of 1.0 ml of internal standard solution (0.6 mg of methyltriphenylphosphonium iodide in 1 ml of 70% methanol), the Chinese herbal preparation extract was diluted to 10 ml with 70% methanol. This solution was passed through a 0.45-μm filter and *ca.* 0.8 nl (5-s hydrostatic sampling) of the filtrate was injected directly into the capillary electrophoresis system.

Apparatus and conditions

All analyses were carried out on a Waters Quanta 4000 CE system equipped with a UV detector set at 185 nm and a 60 cm × 75 μm I.D. uncoated capillary (Millipore) with the detection window placed at 52.5 cm. The conditions were as follows: sampling time, 5 s, hydrostatic; run time, 3 min; applied voltage, 20 kV (constant voltage, positive to negative polarity); temperature, 26.5–27.0°C. The electrolyte was a buffer solution that contained 0.01 *M* valine and adjusted to pH 10.0 with ammonia solution. The electrolyte was filtered through a 0.45-μm filter before use.

Solution for linearity response

Eight concentrations of ephedrine and pseudoephedrine in the range 0.008–0.240 mg/ml were prepared. Each concentration was analysed three times.

Solution for recovery studies

Different amounts of ephedrine and pseudoephedrine standard were added to two samples of Chinese herbal preparations of known alkaloidal content and the mixtures were extracted and analysed using the proposed procedure.

RESULTS AND DISCUSSION

Analytical conditions

In the study of Ephedrae Herba crude drugs [5,9], we found that a buffer solution containing 0.005 *M* barium hydroxide and 0.02 *M* isoleucine and adjusted to pH 10.0 with ammonia solution could give a good resolution of the six ephedrine alkaloids (ephedrine, pseudoephedrine, norephedrine, norpseudoephedrine, methylephedrine and methylpseudoephedrine) and setting the detection wavelength at 185 nm could enhance the alkaloidal signals. However, surveys of a number of commercial Ephedrae Herba-containing Chinese herbal preparations showed that generally only ephedrine and pseudoephedrine were present in the test solutions. These two compounds are now used as marker substances on the evaluation of Chinese herbal preparations by HPLC [3]. Therefore, it would be desirable to have a far simpler method with a shorter analysis time that can be applied to various Ephedrae Herba-containing formulations, though it may only be able to separate ephedrine and pseudoephedrine well.

From the analysis of Ephedrae Herba [5], we knew that amino acids were good counter ions for ephedrine and pseudoephedrine and the use of ammonia solution for pH adjustment could shorten the analysis time and enhance the absorption signals. Hence we tried to prepare the buffer solutions with alanine, valine, isoleucine, leucine separately and adjusted their pH values with ammonia solution, and found that all these amino acids were suitable for this purpose. After a series of experiments, it was found that 0.01 *M* valine solution (pH 10.0, adjusted with ammonia solution) could resolve ephedrine and pseudoephedrine within 3 min. In comparison with buffer solutions of different concentrations, two concentrations (0.02 and 0.005 *M*) of valine solution were prepared. It was found that at the

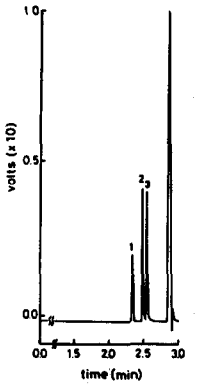

Fig. 1. Capillary electropherogram of a mixture of ephedrine and pseudoephedrine. Peaks: 1 = internal standard (methyltriphenylphosphonium iodide), 0.060 mg/ml; 2 = pseudoephedrine, 0.080 mg/ml; 3 = ephedrine, 0.080 mg/ml.

higher concentration of valine solution, the absorption signals of ephedrine and pseudoephedrine became very weak, whereas at the lower concentration, the peaks of ephedrine and pseudoephedrine were found to be partially overlapped. Comparison was also made with buffer solutions of different pH. Two buffer solutions of pH 10.5 and 9.5 were prepared. It was found that at high pH, the separation was good, but the absorption signals of ephedrine and pseudoephedrine were decreased and the analysis time was prolonged to 4 min, whereas at the lower pH, the separation was not good.

An electrolyte containing 0.01 *M* valine solution and adjusted to pH 10.0 with ammonia solution was found to produce the best resolu-

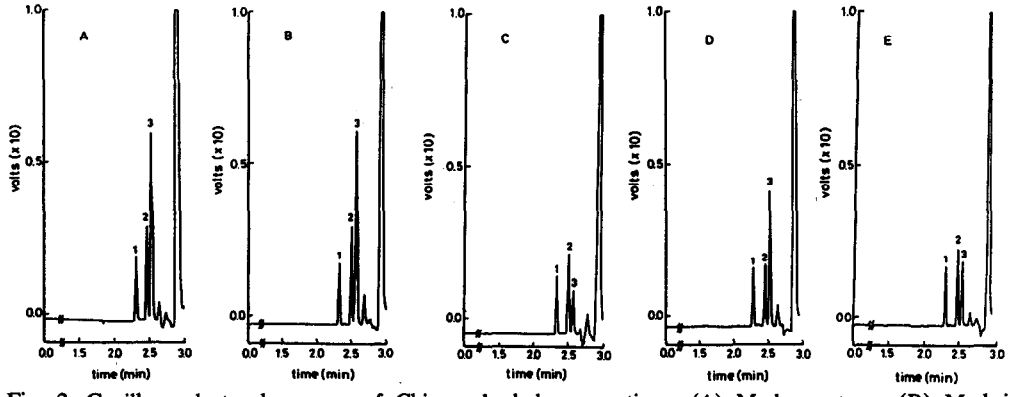

Fig. 2. Capillary electropherogram of Chinese herbal preparations: (A) Ma-huang-tang; (B) Ma-hsing-kan-shih-tang; (C) Ko-ken-tang; (D) Ta-ching-lung-tang; (E) Ma-hsing-i-kan-tang. Peaks as in Fig. 1.

TABLE I

RECOVERY OF EPHEDRINE AND PSEUDOEPHEDRINE FROM CHINESE HERBAL PREPARATIONS ($n = 3$)

Sample[a]	Alkaloid	Added (μg)	Found (μg)	Recovery (%)
Ma-huang-tang	Ephedrine	200	196	98.0
		400	395	98.8
	Pseudoephedrine	200	198	99.0
		400	396	99.0
Ma-hsing-kan-shih-tang	Ephedrine	200	204	102.0
		400	409	102.3
	Pseudoephedrine	200	202	101.0
		400	406	101.5

[a] See footnote to Table II.

TABLE II

CONTENTS OF EPHEDRINE AND PSEUDOEPHEDRINE IN CHINESE HERBAL PREPARATIONS ($n = 3$)

Sample[a]	Ephedrine (mg/g)	Pseudoephedrine (mg/g)	Total (mg/g)
1	0.49	0.43	0.92
2	0.59	0.21	0.80
3	0.23	1.57	1.80
4	1.31	0.44	1.75
5	0.45	0.36	0.81
6	1.77	0.87	2.64
7	0.81	0.36	1.17
8	0.89	1.68	2.57
9	3.45	0.62	4.07
10	0.86	1.04	1.90
11	2.92	1.29	4.21
12	4.30	1.71	6.01
13	2.85	1.30	4.15
14	4.54	1.66	6.20
15	0.49	0.67	1.16
16	2.14	0.89	3.03
17	1.27	0.60	1.87
18	0.98	0.45	1.43
19	1.03	0.48	1.51

[a] Names and compositions of the Ephedrae Herba-containing Chinese herbal formulations: **1** = *Ching-pi-tang:* Pueriae Radix, Cinnamomi Cortex, Zingiberis Rhizoma, Ligustici Rhizoma, Glycyrrhizae Radix, Platycodi Radix, Ephedrae Herba, Paeoniae Radix, Gypsum Fibrosum, Rhei Rhizoma, Coicis Semen, Magnoliae Flos, Zizyphi Fructus; **2** = *Fang-feng-tung-sheng-san:* Angelicae Radix, Paeoniae Radix, Gardeniae Fructus, Ligustici Rhizoma, Forsythiae Fructus, Schizonepetae Herba, Ledebouriellae Radix, Natrium Sulfuricum, Ephedrae Herba, Zingiberis Rhizoma, Rhei Rhizoma, Atractylodis Rhizoma, Menthae Herba, Platycodi Radix, Scutellariae Radix, Glycyrrhizae Radix, Talcum, Gypsum Fibrosum; **3** = *Hsiao-ching-lung-tang:* Ephedrae Herba, Cinnamomi Ramulus, Glycyrrhizae Radix, Paeoniae Radix, Zingiberis Siccatum Rhizoma, Asari Herba Cum Radice, Pinelliae Tuber, Schizandrae Fructus; **4** = *Hsiao-hsu-ming-tang:* Ledebouriellae Radix, Cinnamomi Ramulus, Ephedrae Herba, Armeniacae Semen, Ligustici Rhizoma, Paeoniae Radix, Ginseng Radix, Glycyrrhizae Radix, Scutellariae Radix, Aristolochiae Fangchi Radix, Aconiti Tuber, Zingiberis Rhizoma; **5** = *Hsu-ming-tang:* Ephedrae Herba, Cinnamomi Ramulus, Ginseng Radix, Angelicae Radix, Ligustici Rhizoma, Zingiberis Siccatum Rhizoma, Glycyrrhizae Radix, Armeniacae Semen, Gypsum Fibrosum; **6** = *Hua-kai-san:* Ephedrae Herba, Mori Cortex, Perillae Semen, Poria, Armeniacae Semen, Citri Leiocarpae Exocarpium, Glycyrrhizae Radix; **7** = *I-yi-jen-tang:* Ephedrae Herba, Cinnamomi Ramulus, Angelicae Radix, Paeoniae Radix, Atractylodix Rhizoma, Glycyrrhize Radix, Coicis Semen; **8** = *Ko-ken-tang:* Paeoniae Radix, Cinnamomi Ramulus, Zingiberis Rhizoma, Glycyrrhizae Radix, Zizyphi Fructus, Puerariae Radix, Ephedrae Herba; **9** = *Kuei-chih-shao-yao-chih-mu-tang:* Cinnamomi Ramulus, Paeoniae Radix, Glycyrrhize Radix, Ephedrae Herba, Zingiberis Rhizoma, Atractylodis Rhizoma, Anemarrhenae Rhizoma, Ledebouriellae Radix, Aconiti Tuber; **10** = *Ma-hsing-i-kan-tang:* Ephedrae Herba, Armeniacae Semen, Glycyrrhizae Radix, Coicis Semen; **11** = *Ma-hsing-kan-shih-tang:* Ephedrae Herba, Armeniacae Semen, Glycyrrhizae Radix, Gypsum Fibrosum; **12** = *Ma-huang-fu-tzu-hsi-hsin-tang:* Ephedrae Herba, Aconiti Tuber, Asari Herba Cum Radice; **13** = *Ma-huang-tang:* Ephedrae Herba, Cinnamomi Ramulus, Armeniacae Semen, Glycyrrhizae Radix; **14** = *Shen-mi-tang:* Ephedrae Herba, Glycyrrhizae Radix, Armeniacae Semen, Perillae Folium, Magnoliae Cortex, Bupleuri Radix, Citri Leiocarpae Exocarpium; **15** = *Shih-shen-tang:* Ephedrae Herba, Paeoniae Radix, Glycyrrhizae Radix, Puerariae Radix, Angelicae Dahuricae Radix, Cimicifugae Rhizoma, Ligustici Rhizoma, Perillae Folium, Citri Leiocarpae Exocarpium, Cyperi Rhizoma; **16** = *Ta-ching-lung-tang:* Ephedrae Herba, Armeniacae Semen, Cinnamomi Ramulus, Zingiberis Rhizoma, Zizyphi Fructus, Glycyrrhize Radix, Gypsum Fibrosum; **17** = *Ting-chuan-tang:* Pinelliae Tuber, Zingiberis Rhizoma, Ginkgo Semen, Ephedrae Herba, Glycyrrhizae Radix, Fararae Flos, Mori Cortex, Perillae Semen, Armeniacae Semen, Scutellariae Radix; **18** = *Wu-chi-san:* Glycyrrhizae Radix, Angelicae Dahuricae Radix, Ligustici Rhizoma, Atratylodis Lanceae Rhizoma, Aurantii Fructus, Platycodi Radix, Ephedrae Herba, Zingiberis Rhizoma, Pinelliae Tuber, Zingiberis Siccatum Rhizoma, Paeoniae Radix, Citri Leiocarpae Exocarpium, Magnoliae Cortex, Angelicae Radix, Cinnamomi Cortex, Poria, Allii Fistulosi Bulbus; **19** = *Wu-yao-shun-chi-san:* Ephedrae Herba, Glycyrrhizae Radix, Angelicae Dahuricae Radix, Ligustici Rhizoma, Linderae Radix, Citri Leiocarpae Exocarpium, Platycodi Radix, Aurantii Fructus, Bombyx Batryticatus, Zingiberis Siccatum Rhizoma.

tion. Fig. 1 is an electropherogram showing the separation of authentic ephedrine and pseudoephedrine with migration times of 2.3 min for the internal standard, 2.5 min for pseudoephedrine and 2.6 min for ephedrine. The separation of these two constituents can be completed within 3 min. As the methanol–water extracts of Chinese herbal preparations were injected directly, the results were as good as those obtained with pure chemical samples without interference with each peak and the analysis could also be completed within 3 min, as shown in Fig. 2.

Calibration graphs for ephedrine and pseudoephedrine

Calibration graphs (peak-area ratio, y, *vs.* concentration, x, mg/ml) were constructed in the range 0.008–0.240 mg/ml. The regression equations of these curves and their correlation coefficients were calculated as follows: pseudoephedrine, $y = 26.35x + 0.03$ ($r = 0.9996$); ephedrine, $y = 26.61x + 0.05$ ($r = 0.9996$).

System suitability test

The reproducibility (relative standard deviation) of the proposed method, on the basis of peak-area ratios for six replicate injections, was 0.86% (intra-day) and 2.1% (inter-day) for ephedrine and 0.87% (intra-day) and 1.7% (inter-day) for pseudoephedrine.

The results of standard addition recovery studies of ephedrine and pseudoephedrine from sample composites of Chinese herbal preparations are given in Table I. The recoveries were 98.0–102.3% for ephedrine and 99.0–101.5% for pseudoephedrine. All the tailing factors of the three peaks (internal standard, pseudoephedrine and ephedrine) are very close to unity.

Determination of ephedrine and pseudoephedrine in Chinese herbal preparations

When the test solutions of extracts of Chinese herbal preparations were analysed by CE under the selected conditions, the calculated contents of ephedrine and pseudoephedrine given in Table II were obtained. Each peak of the extracts in various Chinese herbal preparations showed no interference. These results indicate that the proposed CE method is suitable for the determination of ephedrine and pseudoephedrine in Chinese herbal preparations. Moreover, this method not only uses the methanol–water extract directly, but also offers autosampling. In addition to its rapid and accurate performance, consecutive injections can be made within 5 min with a thoroughly cleaned capillary. Therefore, it should be especially useful for large numbers of samples and for quality control in pharmaceutical plants.

ACKNOWLEDGEMENT

Financial support from the National Science Council, Republic of China, is gratefully acknowledged.

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