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Short Communication

Determination of ephedrine alkaloids by capillary electrophoresis

Ying-Mei Liu and Shuenn-Jyi Sheu*

Department of Chemistry, National Taiwan Normal University, Taipei (Taiwan)

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ABSTRACT

A simple and rapid method for the simultaneous determination of six ephedrine alkaloids (ephedrine, pseudoephedrine, norephedrine, norpseudoephedrine, methylephedrine and methylpseudoephedrine) in *Ephedrae herba* by capillary electrophoresis was developed. A buffer solution that contained 0.005 *M* barium hydroxide and 0.02 *M* isoleucine and adjusted to pH 10.0 with ammonia solution was found to be the most suitable electrolyte for this separation. The contents of the six alkaloids in the crude drug of *Ephedrae herba* could be easily determined.

INTRODUCTION

Ephedrae herba (Ma-Huang) is a commonly used Chinese herbal drug intended for diaphoretic purposes, and is known to contain (−)-ephedrine (E), (+)-pseudoephedrine (PE), (−)-methylephedrine (ME), (+)-methylpseudoephedrine (MPE), (−)-norephedrine (NE) and (+)-norpseudoephedrine (NPE) as its major bioactive components [1–3].

Several methods have been reported for the determination of some of these six alkaloids, including a copper complex method [4], thin-layer chromatography [5,6], gas–liquid chromatography [7] and ^{13}C NMR spectrometry [8] for E and PE and high-performance liquid chromatography (HPLC) [9–11] for three, four or five alkaloids. Recently, two methods for the simultaneous determination of all these six ephedrine alkaloids have been developed, one by HPLC [12] and the other by isotachophoresis [13]. However, the former requires tedious pretreatment of *Ephedrae herba* extracts before analysis and the latter is not able to separate ME and NPE well in *Ephedrae herba*.

We describe here the development of a simple, rapid and simultaneous method for determining these six alkaloids in crude and processed samples of *Ephedrae herba* by capillary electrophoresis.

EXPERIMENTAL

Reagents and materials

Ephedrine hydrochloride, pseudoephedrine hydrochloride, methylephedrine, methylpseudoephedrine and norephedrine hydrochloride were purchased from Aldrich (Milwaukee, WI, USA) and norpseudoephedrine hydrochloride, isoleucine and benzyltriethylammonium chloride from Merck (Darmstadt, Germany). Barium hydroxide and ammonia solution were of extra-pure grade. *Ephedrae herba* was purchased from the Chinese herbal market in Taipei (Taiwan).

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Preparation of Ephedrae herba extracts

A 1.0-g sample of pulverized *Ephedrae herba* was extracted with 50% ethanol (15 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 adding a 5-ml aliquot of internal standard solution (1 mg of benzyltriethylammonium chloride in 1 ml of water), the *Ephedrae herba* extract was diluted to 50 ml with 50% ethanol. This solution was passed through a 0.45-μm filter and about 1.7 nl (10-s hydrostatic sampling) of the filtrate was injected into the capillary electrophoresis system directly.

Apparatus and conditions

All analyses were carried out on a Waters Quanta 4000 capillary electrophoresis system equipped with a UV detector set at 185 nm and a 60 cm × 75 μm I.D. capillary. The running conditions were as follows: sampling time, 10 s hydrostatic; running time, 10 min; applied voltage, 28 kV; and temperature, 25.0–25.5°C. The electrolyte was a buffer solution consisting of 0.02 *M* isoleucine and 0.005 *M* barium hydroxide, adjusted to pH 10.0 with ammonium solution. The electrolyte was filtered through a 0.45-μm filter before use.

RESULTS AND DISCUSSION

Analytical conditions

We tried to apply HPLC to the constituents of *Ephedrae herba* and found that by connecting μBondapak C_{18} and phenyl columns (both 15 cm × 3.9 mm I.D.) in series, and eluting with ammonium buffer solution (consisting of 0.003 *M* di-*n*-butylamine, 0.002 *M* ammonium dihydrogenphosphate and 0.003 *M* ammonium chloride, adjusted to pH 2.8 with phosphoric acid), we were able to separate the six ephedrine alkaloids well. However, these conditions cannot be applied directly to water or ethanol–water extracts of the herb, owing to interferences from other constituents in the extract.

Following Zhang *et al.*'s pretreatment method [12], we used 0.5 *M* sulphuric acid for preliminary extraction, and neutralized the extract with 6 *M* sodium hydroxide solution, then extracted with diethyl ether and, after condensation, dissolved the extract in methanol to prepare a test solution that yielded clearly uninterfered peaks. However, this method cannot be used for analyses of herbs containing smaller amounts of constituents, because the NE signal can still be subjected to interference from methanol. Therefore, following Kasahara and Hikino's method [13], we adopted capillary electrophoresis for the analysis, using barium ion as the leading ion and β-alanine as the counter ion, which under a voltage of 28 kV was capable of separating the six alkaloids except for partial overlap of ME and NPE. On using other amino acids in association with barium hydroxide, we found that only 0.03 *M* isoleucine with 0.01 *M* barium hydroxide as electrolyte could completely separate all six peaks. When histidine, valine, glutamine, methionine, leucine, proline, alanine, threonine and lysine were used, ME and NPE completely or partially overlapped, and with the use of arginine and phenylalanine no absorption signal was obtained because of their own high absorbances. Also, in order to overcome the inconveniently low solubility of barium hydroxide, we replaced it with ammonia solution. As a result, the running time was shortened to 3 min and the absorption signals were enhanced, but ME and NPE could not be separated. Finally, we found that we could separate the six peaks completely and also shorten the retention time and enhance the peak intensity by mixing barium hydroxide and ammonia solution in an appropriate ratio.

An electrolyte containing 0.005 *M* barium hydroxide and 0.02 *M* isoleucine adjusted to pH 10.0 with ammonia solution was found to produce the best resolution. Fig. 1 is an electropherogram showing the separation of the six authentic ephedrine alkaloids with retention times of 4.1 min for the internal standard, 5.3 min for MPE, 6.1 min for PE, 6.7 min for E, 7.8 min for ME, 8.0 min for NPE and 8.7 min for NE. The separation of all the constituents can be completed within 10 min. As the ethanol–water extract of *Ephedrae herba* was injected directly and analysed, the results were as good as those obtained with pure chemical samples without interference for each peak and the analysis could be completed within 10 min, as shown in Fig. 2.

Calibration graphs for ephedrine alkaloids

Calibration graphs (peak-area ratio, y, *vs.* concentration, x, in mg/ml) were constructed in the range of 0.02–0.40 mg/ml for E and PE and 0.001–0.040 mg/

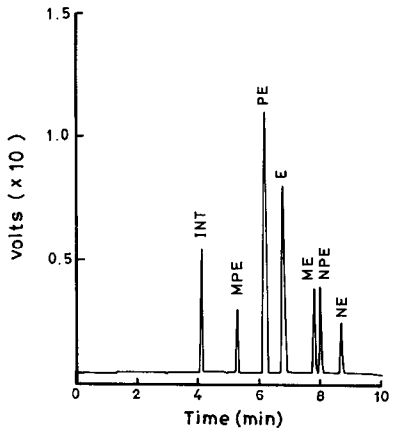

Fig. 1. Capillary electropherogram of a mixture of ephedrine alkaloids. INT = Internal standard (benzyltriethylammmonium chloride); MPE = methylpseudoephedrine; PE = pseudoephedrine; E = ephedrine; ME = methylephedrine; NPE = norpseudoephedrine; NE = norephedrine.

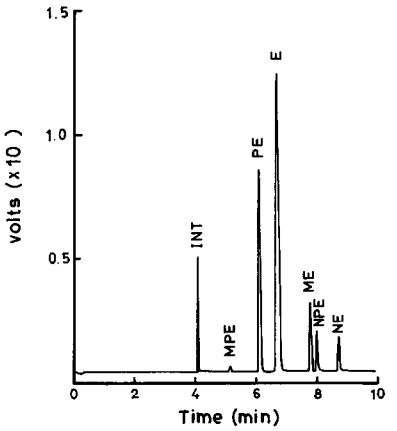

Fig. 2. Capillary electropherogram of the extract of an *Ephedrae herba* sample. Abbreviations as in Fig. 1.

ml for the other four alkaloids. They were linear with a good correlation coefficient of 0.999.

Determination of ephedrine alkaloids in Ephedrae herba

When the test solution of *Ephedrae herba* extract was analysed by capillary electrophoresis under the selected conditions, the graph shown in Fig. 2 was obtained. The calculated contents of the individual ephedrine alkaloids in the *Ephedrae herba* sample ($\pm$S.D.; n = 3) were MPE, <0.005; PE, 0.423 $\pm$ 0.019; E, 0.965 $\pm$ 0.017; ME, 0.133 $\pm$ 0.009; NPE, 0.057 $\pm$ 0.001; and NE, 0.059 $\pm$ 0.006%. Suitable amounts of the six ephedrine alkaloids were added to a sample of *Ephedrae herba* of known alkaloidal content and the mixture was extracted and analysed using the proposed procedure. The recoveries of the alkaloids were 95.5–102.9% with relative standard deviations of 2.2–4.0%.

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