Chiral Separation of Ephedrine Isomers by Capillary Electrophoresis Using Bovine Serum Albumin as a Buffer Additive

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Abstract

A method utilizing bovine serum albumin (BSA) as buffer additive for chiral separation by means of capillary electrophoresis is described. Parameters that affect chiral separation, such as buffer pH, buffer concentration, BSA concentration, and organic modifier, are investigated. Baseline resolution of ephedrine–pseudoephedrine and norephedrine–norpseudoephedrine isomers are achieved in an uncoated capillary with a 20 mmol/L phosphate buffer at pH 9.0 in the presence of 10 µmol/L BSA and 15% (v/v) 2-propanol at 25°C. The developed method can be applied for the analysis of ephedra plant extracts that contain the four test drugs.

Introduction

Ephedrine (E) and pseudoephedrine (PE) are the main active medical ingredients in *ephedra* (Chinese name, Ma-Huang). Norephedrine (NE) and norpseudoephedrine (NPE) can also be found in *ephedra*, and these four drugs are two groups of isomers. Ephedrine alkaloids are amphetamine-like compounds used in over-the-counter and prescription drugs with potentially lethal stimulant effects on the central nervous system and heart. There are some reports on the separation of ephedrine and its derivatives by capillary electrophoresis (CE) (1–4), high-performance liquid chromatography (HPLC) (5,6), molecularly imprinted polymers (7,8), gas chromatography (9), and supercritical fluid chromatography (SFC) (10).

CE has been demonstrated to be a highly efficient separation technique suitable for the separation of isomers (11–15). The chiral additives employed in CE include cyclodextrins (16–20), crown ether (21), proteins (22,23), and amino acid (24). Among the chiral selectors, proteins have drawn great attention for chiral separations because of its high nature of stereo selectivity. On the other hand, it is interesting to note the interaction of drugs and proteins. Proteins, such as bovine serum albumin (BSA) and human serum albumin (HSA), have been used as the chiral selectors for the chiral separation of many isomers (25,26). However, there were few publications for the separation of E–PE and NE–NPE isomers by CE with albumin as buffer additive.

In this study, baseline-separation for E–PE and NE–NPE isomers were obtained by adding BSA into the running buffer. The effects of buffer pH, buffer concentration, BSA concentration, and organic modifier in background electrolytes on the resolution of the test drugs were investigated.

Materials and Methods

Instrument

Experiments were performed on a Bio-Rad 3000 capillary electrophoresis system (Bio-Rad Laboratories, Hercules, CA) equipped with a variable-wavelength UV detector. An uncoated fused-silica capillary (Yongnian Optical Fiber Factory, Hebei, China) was used as the separation column. The total length was 43.0 cm (50-µm i.d.), and the length to the detection window was 38.2 cm. Separations were carried out in the constant voltage mode, and the applied voltage was 15.0 kV. The system temperature was set at 25°C. Samples were introduced into the capillary by pressure at 5 psi for 4 s (1 psi = 6894.76 Pa). On-column detection was performed at 190 nm, and data were recorded by a Biofocus 3000 integration system.

Chemicals

1R,2S-Ephedrine hydrochloride was purchased from the National Institute for the Control of Pharmaceuticals and Biological Products (Beijing, China). 1S,2S-Pseudoephedrine hydrochloride was supplied from the National Laboratory of Narcotic Drug (Beijing, China). 1R,2S-Norephedrine hydrochloride and 1S,2S-norpseudoephedrine hydrochloride were obtained from Sigma (St. Louis, MO). BSA was purchased from Beijing Chemical Factory (Beijing, China). All other chemicals were of analytical grade. Double-distilled water was used to pre-

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pare the buffer and the standard sample solutions. The phosphate buffer was prepared by mixing sodium dihydrogenphosphate solution and disodium hydrogenphosphate solution in such proportions that the desired pH was obtained. The protein solution was prepared by dissolving BSA at a given concentration in the phosphate buffer. The two solutions in the two electrode reservoirs were changed after a certain period to avoid changes in pH because of electrolysis of the water.

Maintenance of the capillary

The method using protein as a chiral selector for the CE method has a disadvantage of adsorption of protein onto the capillary wall, which causes capillary blockage and limits the usage of high concentration solutions of protein. In order to minimize the absorption of BSA on the capillary wall, the capillary was regenerated by the following steps. Before the first injection, the capillary was flushed daily with 0.1 mol/L NaOH solution for 10 min, then rinsed with double-distilled water for 10 min, followed by the running buffer for another 10 min. During runs, the capillary was washed with 0.1 mol/L NaOH, double-distilled water, and phosphate buffer for 2 min each, respectively. After a day of work, the capillary was washed by 0.1 mol/L NaOH solution for 10 min and, subsequently, for 10 min with double-distilled water, and 0.45-µm filters were used to filter the background electrolytes and the standard sample solutions before injections.

Preparation of *ephedra* plant extracts

The powder of *ephedra* was extracted in 10 mL of 80% (v/v) ethanol with ultrasonication for 30 min, then centrifuged at 1000 × *g* for 5 min. The extraction procedure was repeated twice more, and the extracts were combined and diluted with 80% (v/v) ethanol in a 25-mL volumetric flask and then filtered by 0.45-µm filters before injecting into the CE system.

Results and Discussion

Effect of the buffer pH

The pH of the background electrolytes has a strong influence on the chiral separation of the basic drug. Sodium dihydrogenphosphate and disodium hydrogenphosphate were mixed to obtain 40 mmol/L running buffer in the pH range of 6.0–10.0, and the concentration of BSA was maintained at 20 µmol/L, and the concentration of 2-propanol was 10% (v/v). For NE–NPE isomers, the resolution increased with the increasing of pH in the range of 6.0–10.0, and baseline-separation was achieved at pH 9.0 (see Figure 1). For E–PE isomers, the optimal resolution was obtain at pH 9.0. However, it can be seen in Figure 1 that at this pH, the E–PE isomers were partly separated. In this study, phosphate buffer at pH 9.0 was chosen for further experiments.

Effect of the buffer concentration

The concentration of phosphate buffer in the range of 10–80 mmol/L does not have an obvious influence on the separation of E–PE isomers. For the four test drugs, the satisfied resolution was achieved in 20 mmol/L phosphate buffer (pH 9.0), as shown in Figure 2. The E–PE isomers weren't baseline-separated under this condition.

Effect of the BSA concentration

The effect of increasing BSA concentration from 10 to 60 µmol/L in 20 mmol/L phosphate buffer (pH 9.0) containing 10% (v/v) 2-propanol was examined on the chiral separation of E–PE and NE–NPE isomers. The results are shown in Figure 3. In the future experiments, 10 µmol/L BSA in the phosphate buffer was chosen for the chiral separation.

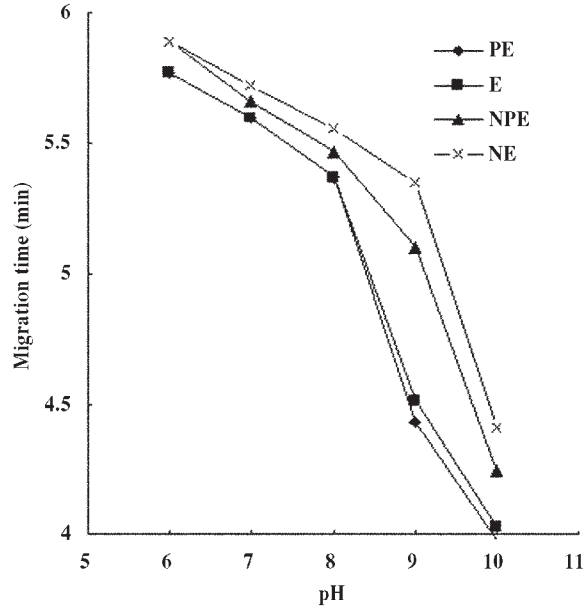

Figure 1. Effect of buffer pH on the separation of ephedrine isomers. Conditions: 20 µmol/L BSA; 40 mmol/L phosphate buffer; 10% (v/v) 2-propanol. The applied voltage is 15 kV. The detection wavelength is 190 nm, the capillary is 43.0 cm (38.2 cm to the detector) × 50-µm i.d., and the system temperature is 25°C. Sample is injected by pressure at 5 psi for 4 s.

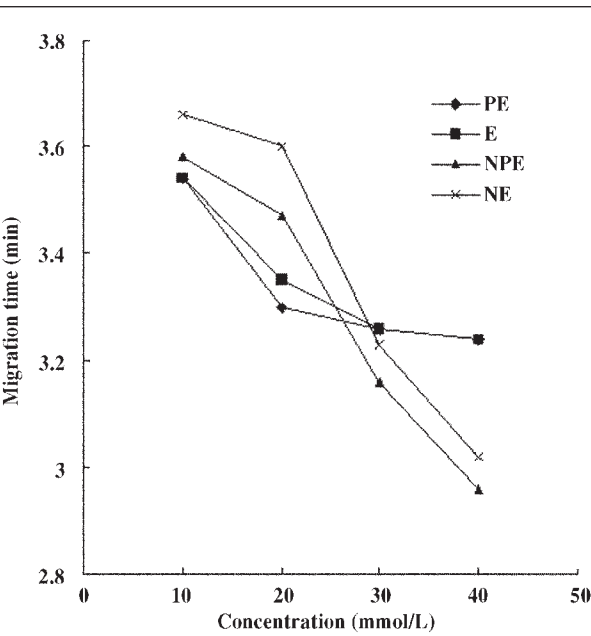

Figure 2. Effect of buffer concentration on the separation of ephedrine isomers. Conditions: buffer, pH 9.0, 20 µmol/L BSA, 10% (v/v) 2-propanol. Other conditions were the same as in Figure 1.

Effect of organic modifier

After the described optimal experiments, the E–PE isomers can't be baseline-separated. In the chiral separation CE method, organic solvents are introduced into the running buffer to improve the separation. The influences of methanol, ethanol, tetrahydrofuran, 1-propanol, and 2-propanol as organic modifiers on the resolution of E–PE and NE–NPE isomers were tested. When 2-propanol was used as the organic modifier, the separation of E–PE isomers was improved. Figure 4 showed the effect of 2-propanol concentration on the separation of E–PE and NE–NPE isomers. When phosphate buffer containing 15% (v/v) 2-propanol was used, both E–PE and NE–NPE isomers were baseline-separated, as seen in Figure 5.

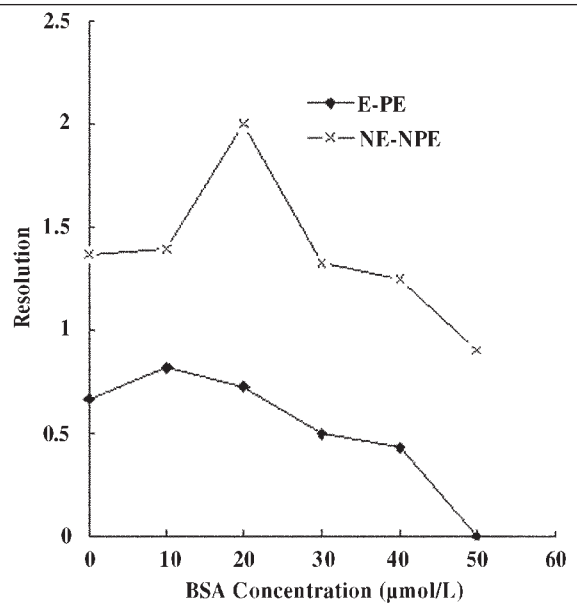

Figure 3. Effect of BSA concentration on the separation of ephedrine isomers. Conditions: 20 mmol/L phosphate buffer, pH 9.0, 10% (v/v) 2-propanol. Other conditions were the same as in Figure 1.

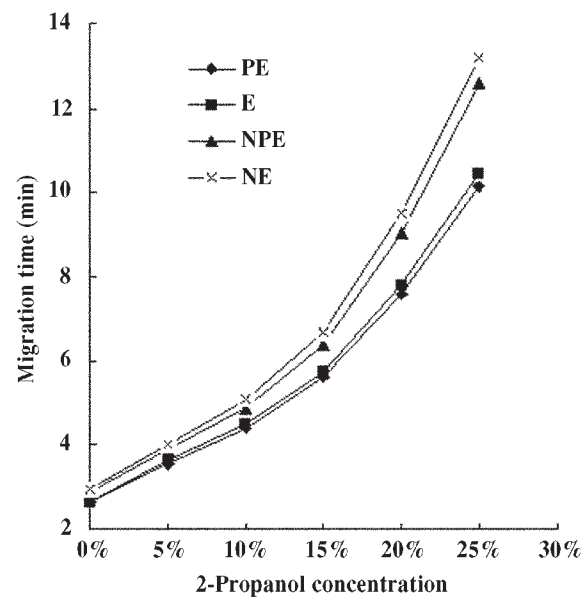

Figure 4. Effect of 2-propanol concentration on the separation of ephedrine isomers. Conditions: 10 µmol/L BSA; 20 mmol/L phosphate, pH 9.0. Other conditions were the same as in Figure 1.

Chiral separation of E–PE and NE–NPE isomers

According to the described optimal experiments, E–PE and NE–NPE isomers could be separated by the CE method employing 20 mmol/L phosphate buffer (pH 9.0) containing 10 µmol/L BSA and 15% (v/v) 2-propanol as the running buffer (see Figure 5).

In order to evaluate the CE method for the separation of E–PE and NE–NPE isomers, calibration graphs [peak area of isomers (y) vs concentration of corresponding isomers (x) µg/mL] were constructed in the range of 3.33–20.0 µg/mL for E–PE isomers and 1.33–8.0 µg/mL for NE–NPE isomers, the regression equations were as followings:

$y = 387 + 37239x$, ($r = 0.9987$, $n = 5$)

for E;

$y = -5346 + 47244x$, ($r = 0.9990$, $n = 5$)

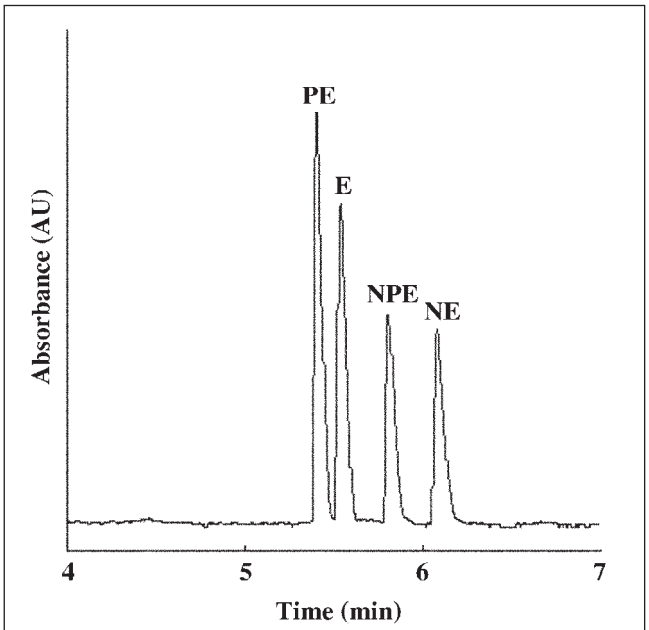

Figure 5. Electropherogram of separation of mixture of E, PE, NE, and NPE. Conditions: 10 µmol/L BSA; 15% (v/v) 2-propanol; 20 mmol/L phosphate, pH 9.0. Other conditions were the same as in Figure 1.

Table I. The Intra- and Inter-Day Reproducibility of E–PE Isomers and NE–NPE Isomers

	RSD%			
	Intraday (n = 5)		Interday (n = 6)	
Isomers	Peak area	Migration time	Peak area	Migration time
E	2.13	1.61	2.65	1.90
PE	1.58	1.60	2.73	1.95
NE	1.45	1.92	2.44	2.12
NPE	3.80	1.76	3.52	1.22

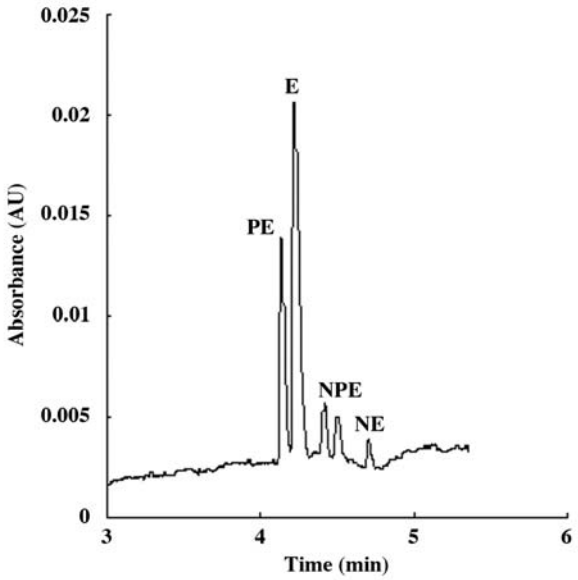

Figure 6. Electropherogram of extract of *ephedra* plant. Conditions were the same as in Figure 5.

for PE;

$y = -4990 + 73050x$, ($r = 0.9995$, $n = 5$)

for NE;

$y = -13954 + 69902x$, ($r = 0.9994$, $n = 5$)

for NPE.

Standard solutions containing 10.0 µg/mL E–PE isomers and 5.33 µg/mL NE–NPE isomers were analyzed for precision data. The results are listed in Table I.

The developed method was applied to the analysis of *ephedra* plant sample extracts. A typical electropherogram of plant sample is shown in Figure 6.

Conclusion

From the preliminary experiments, it can be concluded that BSA as the chiral selector, could be used for the chiral separation of E–PE and NE–NPE isomers. The developed method could be applied to the analysis of herbs containing E–PE and NE–NPE isomers.

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