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ORIGINAL RESEARCH

Application of dimethyl-β-cyclodextrin as a chiral selector in capillary electrophoresis for enantiomer separation of ephedrine and related compounds in some drugs

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ABSTRACT: Dimethyl-β-cyclodextrin (DM-β-CD) modified capillary electrophoresis has been developed for chiral separation of ephedrine and related compounds, such as (±)-norephedrine, (±)-*N*-methylephedrine, (±)-ephedrine and (+)-pseudoephedrine. The influence of some crucial parameters such as buffer concentration, pH value, DM-β-CD concentration, applied voltage and separation temperature on the separation was investigated. Under the optimum conditions, i.e. 40 mM DM-β-CD in 75 mM Tris (pH 2.5) as the running electrolyte, separation voltage +25 kV and temperature 25°C, a satisfactory separation of the enantiomers was accomplished. The detection limits (S/N = 3) ranged from 65 to 161 ng/mL and the linear range was 0.15 to 101.0 μg/mL for pressure injection. The present method was successfully applied for the analysis of a series of drugs such as anti-tussive, the drug for rheum, the drug for rhinitis and a Chinese traditional herbal medicine, *Ephedrae herba* (Ma-Huang in Chinese). The recoveries of ephedrine and related compounds in real samples ranged from 97.6 to 103.5%. This method is useful in the simple and rapid analysis of ephedrine derivatives in marketed products. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: capillary electrophoresis; chiral separation; dimethyl-β-CD; ephedrine

INTRODUCTION

Many therapeutic substances bearing chiral centers are clinically administered as racemic mixtures because of difficulties in stereoselective synthesis and purification. In a symmetric environment, the isomers have nearly identical physical and chemical properties; however, in a stereospecific biological environment, such as the human body, differences in pharmacological properties, pharmacokinetic disposition and metabolic rates have often been observed (Peng and Chiou, 1990; Aboul-Enein and Abou-Basha, 1997; Williams and Wainer, 2002). Ephedrine and related compounds are potential central nervous stimulant drugs that are widely used in many pharmaceutical preparations. Each ephedrine exists as pair of enantiomers, which may differ in pharmacological activity as well as in the rate of their metabolism.

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Abbreviations used: CD, cyclodextrin; DM-β-CD, dimethyl-β-cyclodextrin; EOF, electroosmotic flow.

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Drug action is the result of a large number of pharmacological and pharmacokinetic processes that take place in the living systems. Most of these processes present a high degree of stereoselectivity. Pharmacological characteristics often vary between enantiomers. When a racemic mixture of a compound is administered both enantiomers should not have to be equally potent. In fact, very often one of them is the most active isomer while the other may produce side-effects and/or toxicity. For example, the stimulant effect of the (+)-ephedrine enantiomer amounts to 80% of the activity of the (−)-ephedrine. Consequently, the safety and efficiency of many racemic drugs could be improved if they are marketed as the most active enantiomer. For these reasons, the development of chiral methods of analysis for ephedrines is receiving increasing attention in the clinical, toxicological and pharmaceutical fields. Thus, there is a special interest within pharmaceutical laboratories in developing single-enantiomer formulations and consequently a need for analytical methods to determine both separately has arisen (Maier *et al.*, 2001).

Capillary electrophoresis (CE) has become one of the most powerful analytical tools, due to its high separation efficiency and short analysis time (Kuhr and Monning, 1992). The application of CE in the separation of chiral drugs and biological molecules has drawn particular attention. During recent years, it has

been clearly shown that CE is an excellent method for the enantioseparation of a large variety of molecules (Chankvetadze, 1997a,b, 1999, 2001; Chankvetadze and Blaschke, 2001; Blaschke and Chankvetadze, 2000; Rizzi, 2001; Amini, 2001). Enantiomeric separations by capillary electrophoresis have been achieved by supplying the background electrolyte with a chiral selector capable of discriminating between the enantiomers concerned. Native cyclodextrins (CDs) and modified CDs have been most widely used as additives in the background electrolyte. CDs are non-ionic cyclopolysaccharides of glucose with the shape of an atoroid or hollow truncated cone. The cavity is relatively hydrophobic while the external faces are hydrophilic, with the edge of the pores of the larger circumference containing chiral secondary hydroxyl groups (Lin *et al.*, 1996). So far, a variety of CD derivatives have been synthesized and are now commercially available. The modification of the native CDs leads to significant changes in their physicochemical properties and chiral recognition ability. It can be said that the separation is based on the three-point interaction, i.e. the formation of diastereoisomeric complexes between enantiomers and the complexing chiral agent. Here, selective or differential complexation of ephedrine or related compounds results from the size of the hydrophobic group with respect to the ability of the solute to penetrate into the cavity.

The purpose of the present work was to optimize a simple method for the chiral separation of ephedrine and related compounds (norephedrine, *N*-methylephedrine and pseudoephedrine) by dimethyl-β-CD-modified capillary electrophoresis and apply it for the analysis of some drugs such as antitussive, the drug for rheum, the drug for rhinitis and *Ephedrae herba* (Ma-Huang in Chinese).

EXPERIMENTAL

Instrumentation

All experiments were carried out on a Beckman P/ACE™ MDQ capillary electrophoresis system (Beckman, Fullerton, CA, USA) equipped with a photodiode array detection system. The electropherograms were recorded and integrated by an IBM personal computer with 32 Karat software (version 4.0, Beckman). The pH of running buffers was measured by a model 828 pH meter (Orion, USA).

Chemicals

Racemic norephedrine, *N*-methylephedrine and ephedrine were all purchased from Sigma (St Louis, MO, USA). (+)-Pseudoephedrine was obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). (2,6-di-*O*-methyl)-β-cyclodextrin (dimethyl-β-cyclodextrin, DM-β-CD) was from Nakarai Chemicals (Kyoto, Japan). Figure 1 illustrates the chemical structures of the model compounds used in the present investigation.

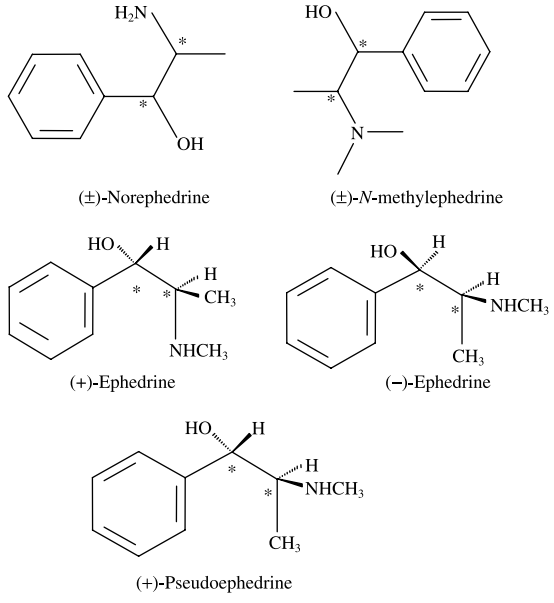

Figure 1. Molecular structures of norephedrine, *N*-methylephedrine, ephedrine and pseudoephedrine.

Phosphoric acid (85%) and hydroxymethyl aminomethane (Tris) were of analytical reagent grade and were obtained from Beijing Chemical Factory (China). Water used to prepare sample and buffer solutions was freshly deionized using an EASYpure water purification system with a 0.2 μm fiber filter (Barnstead, CA, USA). Stock solutions of the standard samples were prepared by dissolving each compound in deionized water and stored at 4°C. The concentration of all stock solutions was 50 mM. Standard solutions were prepared by dilution of stock solutions.

Compound pseudoephedrine hydrochloride tablets (Topsun Science and Technology Qidong Gaitianli Pharmaceutical Stock Co., catalogue no. CK19181), compound *Fritillaria* extract tablets (Handan Pharmaceutical Co., catalogue no. 1020), Ditong nasal solution (Guangzhou Weicai Pharmaceutical Co., catalogue no. 20031203) and *Ephedrae herba* (Ma-Huang) were all purchased from the drugstore.

Electrophoretic technique

Enantioseparation was performed in a 57 cm × 75 μm i.d. uncoated fused-silica capillary (Yongnian Optical Fiber Factory, Hebei, China) with an effective length of 50 cm. The capillary temperature was maintained at 25°C by the cooling system of the CE instrument. Samples were injected hydrodynamically at 0.5 p.s.i. for 5 s and separated at +25 kV. The eluent was monitored at 195 nm. To clean and activate the inner surface, new capillaries were rinsed with methanol for 10 min, followed by deionized water for 5 min, 1 M HCl for 10 min, deionized water for 5 min, 1 M NaOH for 10 min and deionized water for 5 min. Daily before use, the capillary was rinsed for 5 min with 1 M NaOH, 3 min with deionized

water and 3 min with running electrolyte. Between analyses, the capillaries were rinsed for 3 min with running electrolyte. For the electrophoretic experiments, a buffer of 75 mM Tris adjusted to pH 2.5 with phosphoric acid was used.

Calculation

Resolution of each enantiomer of chiral drugs was calculated by measuring the R_s value, which is defined by the following equation:

$$R_s = 2[(t_2 - t_1)/(w_2 + w_1)]$$

where t_1 and t_2 are the migration times and w_1, w_2 are the peak widths at baseline of the first and second enantiomers, respectively.

Sample preparation

Compound pseudoephedrine hydrochloride tablets. Tablets were ground into a fine powder and approximately 9 mg were dissolved in 25 mL deionized water. Before injection into the CE system, the samples were filtered through a 0.2 μm syringe filter.

Compound *Fritillaria* extract tablets. After taking away the sugar coating, the tablets were ground into a fine powder and approximately 20 mg were dissolved in 25 mL deionized water. Prior to injection into the CE system, the samples were filtered through a 0.2 μm syringe filter.

Ditong nasal solution. The solution were diluted 10 times and filtered through a 0.2 μm syringe filter before injection.

Ephedrae herba* (Ma-Huang).** 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 0.45 μm filter, then diluted before injection (Liu and Sheu, 1993).

RESULTS AND DISCUSSION

Selection of DM-β-CD as chiral selector

Although the cooperation interaction between DM-β-CD and β-CD was expected to be better for the resolution, chiral separation of norephedrine, *N*-methylephedrine and ephedrine enantiomers using DM-β-CD and β-CD as binary selectors was not satisfactory. The experiments showed that DM-β-CD did not show a cooperative effect with β-CD, while β-CD competed with DM-β-CD in the host–guest interaction with ephedrine and related compounds. The presence of β-CD made the separation worse (the electropherograms are not shown). Therefore, only DM-β-CD was selected as a chiral selector in the present study.

Effect of running buffer pH

The pH of the running electrolyte is one of the most important factors in chiral recognition. It must be carefully controlled as it not only influence the electroosmotic flow (EOF), but also the protonation degree of ephedrine and its related compounds, ultimately affecting chiral resolution. The influence of the pH value of the running electrolyte was investigated in this work. The dependence of resolution on the pH value of the running electrolyte was thus studied from 2.0 to 5.0 at an increment of 0.5 with 75 mM Tris containing 13 mM DM-β-CD. The pH value of buffer was adjusted by phosphoric acid.

As shown in Fig. 2, from pH 2.0 to 3.0, the resolution of norephedrine and ephedrine had small variation while decreased for *N*-methylephedrine. Beyond pH 3.0, the resolution decreased for all three analytes. This phenomenon indicated that the EOF was not good for the separation because the presence of EOF (higher than pH 3.0) could make the analytes migrate more quickly to the detection window, which resulted in inadequate interaction between the analytes and DM-β-CD. On the other hand, at lower pH value, the protonation degree of the analytes was greater, which led to better separation. Considering the resolution of the three analytes, pH 2.5 of running electrolyte buffer was selected for the separation.

Effect of DM-β-CD concentration

The concentration of DM-β-CD plays a crucial role in the separation. The influence of the concentration of DM-β-CD on the resolution of three analytes was

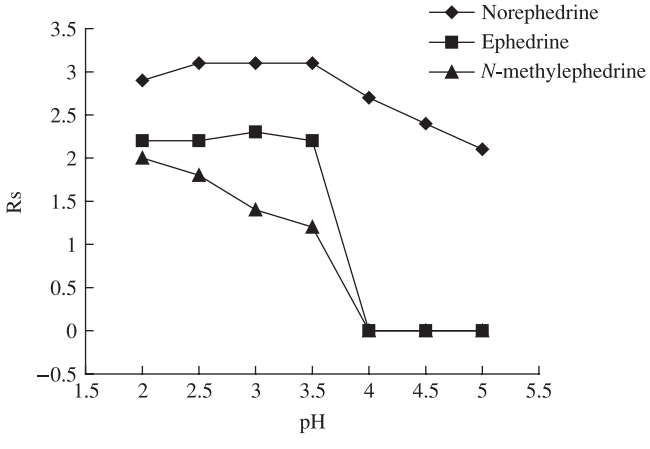

Figure 2. Influence of pH on the resolution of norephedrine, ephedrine and *N*-methylephedrine. Conditions: capillary, 57 cm × 75 μm i.d. uncoated fused-silica capillary with an effective length of 50 cm; applied voltage, +25 kV; temperature, 25°C; detection wavelength, 195 nm; sample injection, 0.5 psi for 5 s; electrolyte, 75 mM Tris running electrolyte containing 13 mM DM-β-CD (pH 2.0–5.0).

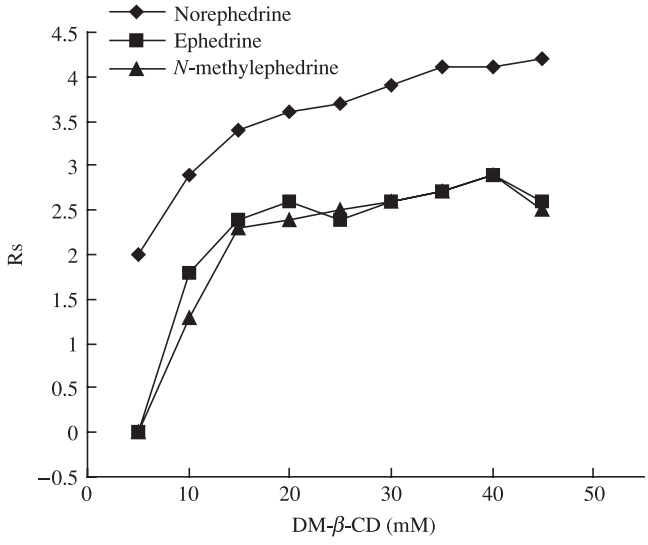

Figure 3. Influence of DM-β-CD concentration on chiral separation in the presence of 75 mM Tris running electrolyte at pH 2.5. Other conditions are the same as in Fig. 2.

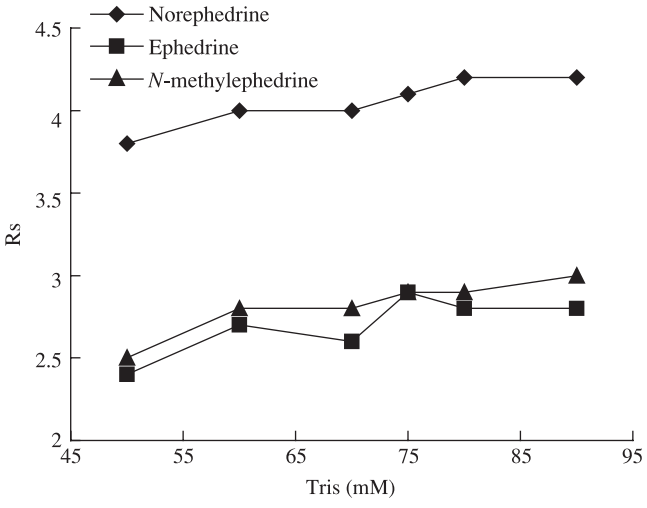

Figure 4. Influence of buffer concentration on chiral separation. Buffer: different concentrations of Tris running electrolyte at pH 2.5 containing 40 mM DM-β-CD. Other conditions are the same as in Fig. 2.

studied in the range from 5 to 45 mM. Different concentrations of DM-β-CD were added to 75 mM Tris buffer at pH 2.5. The results are shown in Fig. 3. As expected, addition of DM-β-CD resulted in a significant improvement in resolution. The separation gradually became better when concentration of DM-β-CD increased and the resolution increased up to a concentration of 40 mM (except ephedrine at 25 mM). Beyond 40 mM, the resolution of ephedrine and *N*-methylephedrine both decreased. As a result, 40 mM DM-β-CD was suitable for the separation.

Effect of running buffer concentration

The effect of concentration of Tris (pH 2.5) from 50 to 90 mM on separation was investigated in present study. The results of resolution are shown in Fig. 4. For ephedrine, 75 mM Tris was the optimal concentration for its separation and this concentration was also good for the separation of the other two analytes. The migration time of the three analytes was prolonged when the buffer concentration increased (data not shown here). Thus, considering the two factors, i.e. migration time and resolution, 75 mM Tris was adopted to yield better resolution for the three analytes.

Effect of applied voltage and separation temperature

Applied voltages from 15 to 25 kV were studied. The applied voltage 20 kV gave the best resolution, but required a longer migration time than 25 kV. Moreover, 25 kV also gave a good resolution. Taking into consideration of resolution and migration time simultaneously, 25 kV was adopted as the applied voltage.

Capillary temperatures ranging from 15 to 25°C were then examined. At 15°C, the peak resolution was slightly better, but the run time was longer. For use as a routine analytical method, the method should have a short analytical time. At 25°C, the resolution and the run time were both acceptable. Therefore, a temperature of 25°C was selected.

Detection limit and calibration curves for standard mixture

For evaluation of the quantitative applicability of the method, six standard solutions of all the enantiomers in the linear range (shown in Table 1) were analysed under the optimum separation conditions. The linearity between the peak-area (y) and the concentration (x, ng/mL) were investigated. The linear regression equations are illustrated in Table 1. The limits of detection (LOD) of all enantiomers dissolved in water, defined as the concentration that produced a signal equal to three times the background noise level, were obtained and are listed in Table 1. The relative standard deviations (RSD) of the migration time and peak area were calculated based on five duplicate injections of a standard sample. The high reproducibility and low LOD indicated that the method was reliable for analysing some real samples.

Application to the real samples

The method was used to determine the ephedrine and its related compounds in some drugs such as

Table 1. Regression data, linear ranges and detection limits of all the enantiomers ($n = 5$)

Compound	Regression equation	r	Linear range (μg/mL)	LOD (ng/mL)	RSD (%)	
					Time	Area
(−)-norephedrine	$y = 3.6234x + 4359.7$	0.9994	0.15–75.50	70	0.56	3.97
(+)-norephedrine	$y = 3.6014x + 4169.4$	0.9993	0.15–75.50	70	0.96	2.11
(−)-ephedrine	$y = 3.4745x + 7151.1$	0.9979	0.20–101.00	65	0.68	3.94
(−)-*N*-methylephedrine	$y = 3.466x + 5294.6$	0.9980	0.18–89.50	143	0.71	4.16
(+)-ephedrine	$y = 3.6039x + 4028.2$	0.9993	0.20–101.00	161	0.72	3.39
(+)-*N*-methylephedrine	$y = 3.6259x + 2663.4$	0.9992	0.18–89.50	143	0.72	2.74
(+)-pseudoephedrine	$y = 3.3261x + 5860.4$	0.9984	0.20–101.00	85	0.79	4.01

y and x are the peak area and the concentration (ng/mL) of the enantiomers, respectively. The correlation coefficients are expressed as r. RSD is relative standard deviation. LOD is limit of detection at 3:1 signal-to-noise ratio.

Table 2. Results of recovery study

Sample	Label claim	Assay results	Alkaloid	Amount added (ng/mL)	Amount found (ng/mL)	Recovery (%)
Pseudoephedrine hydrochloride tablets	30.00 (mg/tablet)	28.60 (mg/tablet)	(+)-Pseudoephedrine	4,034	4,069	100.9
				8,068	8,053	99.8
				12,102	12,153	100.4
Fritillaria extract tablets	1.40 (mg/tablet)	1.29 (mg/tablet)	(−)-Ephedrine	6,051	6,248	103.2
				12,102	12,114	100.1
				18,153	17,991	99.1
Ditong nasal solution	Not given	0.16 (mg/mL)	(−)-Ephedrine	5,043	5,019	99.5
				10,086	10,239	101.5
				15,129	15,208	100.5
	Not given	0.06 (mg/mL)	(+)-Pseudoephedrine	5,043	5,002	99.2
				10,086	10,124	100.4
				15,129	14,914	98.6
Ephedrae herba (Ma-Huang)	>8.00 (mg/g)	11.13 (mg/g)	(−)-Ephedrine	3,362	3,288	97.8
				6,724	6,560	97.6
				10,086	10,419	103.3
	Not given	2.39 (mg/g)	(+)-Pseudoephedrine	3,362	3,479	103.5
				6,724	6,868	102.1
				10,086	10,149	100.6

compound pseudoephedrine hydrochloride tablets, compound *Fritillaria* extract tablets, Ditong nasal solution and *Ephedrae herba* (Ma-Huang). Ephedrine herb, which is known to contain mainly ephedrine and pseudoephedrine as the major bioactive components, is a commonly used Chinese traditional medicine used for diaphoretic purposes. In Chinese herbal therapy it is widely utilized in combination with other crude drugs for the treatment of many diseases. Typical electropherograms are shown in Fig. 5. The results for ephedrine and pseudoephedrine determinations were summarized in Table 2. The recoveries of ephedrine and pseudoephedrine in real samples were 97.6–103.5%.

CONCLUSION

A simple method for the chiral separation of ephedrine and related compounds using DM-β-CD as chiral selector was developed. The procedure has been optimized in terms of the running buffer concentration, pH value, DM-β-CD concentration, applied voltage and separation temperature. Under the optimum conditions, enantioseparation of ephedrine and its related compounds was achieved. Moreover, this method was successfully applied in the determination of ephedrine and related compounds in some drugs such as compound pseudoephedrine hydrochloride tablets, compound *Fritillaria* extract tablets, Ditong nasal solution and *Ephedrae herba*. The recovery results proved the method to be useful for simple and rapid analysis of ephedrine derivatives in real samples.

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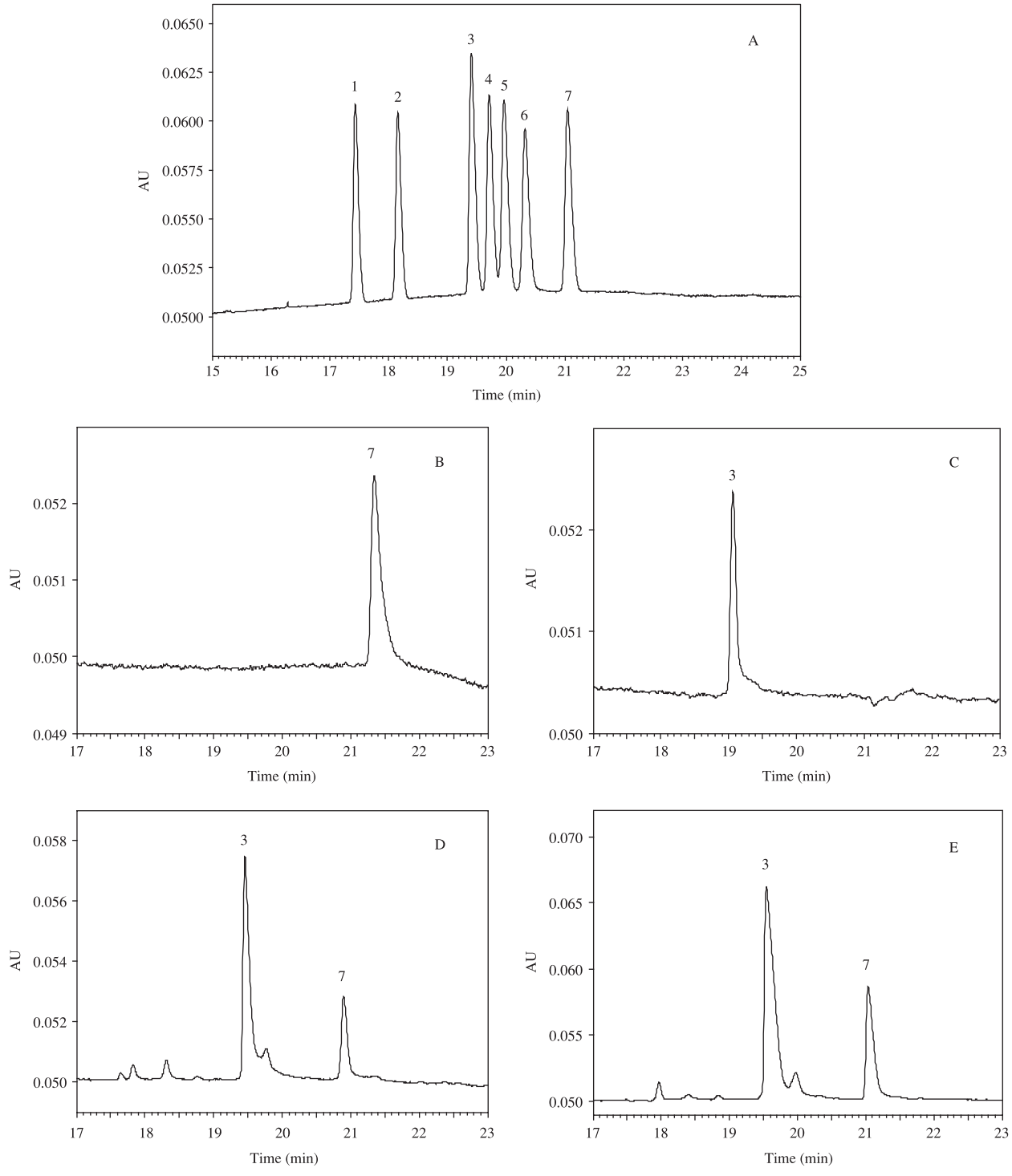

Figure 5. Electropherograms of seven enantiomers. Electrolyte, 75 mM Tris (pH 2.5) containing 40 mM DM-β-CD. Other conditions are the same as in Fig. 2. (A) Electropherogram of standard samples (the concentrations of the samples are all 5×10^{-5} M). (B)–(E) Electropherograms of the analysis of compound pseudoephedrine hydrochloride tablets, compound *Fritillaria* extract tablets, Ditong nasal solution and *Ephedrae herba*. Peaks identification: **1**, (–)-norephedrine; **2**, (+)-norephedrine; **3**, (–)-ephedrine; 4, (–)-N-methylephedrine; **5**, (+)-ephedrine; **6**, (+)-N-methylephedrine; **7**, (+)-pseudoephedrine.

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