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Chiral Separation of β_2-Agonists by Capillary Electrophoresis Using Hydroxypropyl-α-Cyclodextrin as a Chiral Selector

Kyeong Ho Kim[1], Hyun Ju Kim[1], Eun Young Jeun[1], Sang Hun Seo[1], Seon-Pyo Hong[2], Jong-Seong Kang[3], Jeong-Rok Youm[4], and Sang Cheal Lee[1]

[1]College of Pharmacy, Kangwon National University, Kangwon, South Korea, [2]College of Pharmacy, Kyung Hee University, Seoul, South Korea, [3]College of Pharmacy, Chungnam National University, Taejon, South Korea, and [4]College of Pharmacy, Chung Ang University, Seoul, South Korea

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Enantiomers of five racemic β_2-agonists were investigated by capillary electrophoresis employing a hydroxypropyl-β-cyclodextrin (HP-β-CD). The effects of the concentration of HP-β-CD added to the background electrolyte and of the pH of the buffer on the effective mobility and resolution of the studied compounds were examined. Very good resolution was achieved for terbutaline and clenbuterol; salbutamol and bambuterol was able to be partially resolved. Enantioselectivity and resolution were influenced by the concentration of the HP-β-CD, buffer composition and pH.

Key words: Capillary electrophoresis, Chiral separation, Hydroxypropyl-β-cyclodextrin, β_2-agonist

INTRODUCTION

Since the enantiomers of chiral drugs often present different pharmacological and toxicological properties, enantiomeric separations are particulary important in the pharmaceutical field. The determination of individual enantiomers is required for the control of the optical purity of bulk drug substances as well as in pharmacokinetic and clinical studies (Ariens *et al.*, 1971 and Bechet *et al.*, 1994).

For the purpose of chiral separation, various chromatographic techniques, particularly high-performance liquid chromatographic (HPLC) and gas chromatography (GC) can be used: however, GC is limited to volatile compounds and chiral HPLC often shows poor efficiency and is relatively expensive. Recently, capillary electrophoresis (CE), because of its high separation efficiency, relatively simple instrumental set-up and several different separation modes, has shown rapid developments in chiral separations (Wang *et al.*, 1996 and Desiderio *et al.*, 1995). There are several advantage of CE compared with HPLC for chiral separations. Direct chiral separations using CE can be performed easily by adding chiral compounds or chiral surfactants, which interact with enantiomeric solutes to the buffer solution without changing the capillary tube. Because of the low volume of the CE system, the amount of chiral selector consumed during analysis is small, making chiral separations by CE relatively inexpensive. The most commonly used chiral additives in HPCE are those which give rise to inclusion complexes, in particular clodextrins (CDs) and their derivatives (Palmarsdottir *et al.*, 1994). The first chiral separations based on the use of cyclodextrins and derivatives in capillary zone electrophoresis were reported by Fanali and co workers, who studied the influence of the type and the concentration of cyclodextrins on migration times and enantiomeric resolution (Aumatell *et al.*, 1994).

The disparate physiological effects of the different enantiomers of β_2-agonists have stimulated intense research on the chromatographic resolution of the optical isomers of such compounds (Whalgen *et al.*, 1989 and Joyce *et al.*, 1998). Typically, the (R)-forms are often 50 to 10,000-fold more effective than their (S)-enantiomers, and the latter may also be toxic (Ahuja *et al.*, 1990). A technique for the easy and sensitive determination of enantiomeric purity of β_2-agonists can, therefore be very useful.

In this paper, CE with hydroxypropyl-β-cyclodextrin

Correspondence to: Dr. Kyeong Ho Kim, College of Pharmacy, Kangwon National University, Chuncheon 200-701, Korea
E-mail: kyeong@kangwon.ac.kr

was applied to the chiral separation of five drugs belonging to the class of β_2-agonists. The effect of the concentration of the chiral selector added to the background electrolyte (BGE) and the effect of the pH of the BGE on the effective mobility, resolution and selectivity were examined.

MATERIALS AND METHODS

Materials

Phosphoric acid (85%), citric acid, acetic acid, ammonium acetate, sodium dihydrogen phosphate, sodium hydroxide and polyethylene glycol 300 (PEG 300) were of analytical grade from Duksan Pure Chemical Co. (Ansan, Kyeonggi, Korea). Methanol from Duksan Pure Chemical Co. was of HPLC grade. Hydroxypropyl-β-cyclodextrin (HP-β-CD) was obtained from Aldrich (Milwaukee, WI, USA). Terbutaline sulfate, salbutamol, clenbuterol hydrochloride, salmeterol hydroxynaphthoate and bambuterol hydrochloride were purchased from Sigma (Dorset, UK). Their structures are shown in Fig. 1. (S)-(+)-terbutaline and (R)-(-)-terbutaline were prepared from racemic terbutaline by semi-preparative chiral high-performance liquid chromatography (Kim *et al.*, 2000).

Apparatus

All experiments were performed on a HP 3D CE instrument (Hewlett Packard, Waldbronn, Germany), comprising a diode-array detector and Chemstation software for data handling. The compounds were separated in an uncoated fused silica capillary (50 μm I.D., Hewlett Packard).

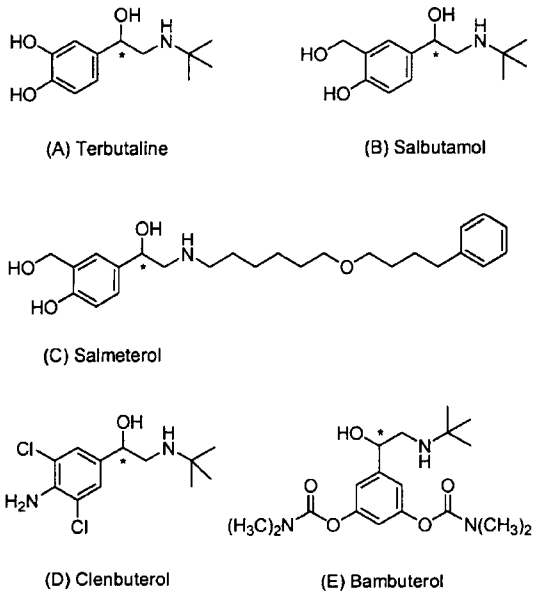

Fig. 1. Structures of β_2-agonist (Chiral centers in these molecules are indicated by asterisks)

The capillary length was 80.5 cm (to the detector 72.0 cm). The applied voltage was 30 kV. The temperature was 20°C. Injection was performed at 30 kV for 5s. Detection was at 210 nm. The capillaries were conditioned according to the following procedure before every run: water for 2 min; 0.1 M NaOH for 2 min; water for 2 min; background electrolyte solution (BGE) for 5 min; and finally with the chiral selector solution for 2 min prior to application of the analyte.

Preparation of buffers

A 0.1 M sodium phosphate running buffer with HPLC grade water as solvent was used for all experiments. Phosphoric acid or NaOH was used to adjust the appropriate buffer salt to the desired pH. For all of the compounds in the study, the pH values examined were 2.5, 3.5, 4.5 and 5.5. The following HP-β-CD concentrations were also added to the above phosphate buffers and studied: 5, 10, 15, 20, 25 and 30 mM. Additionally, 0 - 10% concentrations of polyethylene glycol 300 were utilized in the studies.

The resulting running buffer was degassed by sonication and filtered through a 0.45 μm membrane filter before use.

Sample solution

All test samples were dissolved in methanol at concentration of 0.1 mg/mL.

RESULTS AND DISCUSSION

Effect of buffer type

Preliminary experiments were performed to determine the most suitable buffer composition for the chiral separation of analytes with uncoated fused silica capillaries. The separation was investigated in the pH range 2.5-3.5 using ammonium acetate buffer, NaH_2PO_4 - citric acid buffer, NaH_2PO_4 - H_3PO_4 buffer and NaOH - H_3PO_4 buffer.

The best separations with respect to selectivity were achieved using NaH_2PO_4 - H_3PO_4 buffer (pH 2.5) and NaOH - H_3PO_4 buffer (pH 3.3) in the terbutaline and clenbuterol, respectively. And we found that when ammonium acetate buffer (pH 3.5) containing 10 mM HP-β-CD was used, the enantiomeric resolution of terbutaline and clenbuterol declined to zero. The resolution difference between Fig. 2 and Fig. 3 may further indicate selectivity differences in chiral separation between the two buffer systems, viz., the NaH_2PO_4 - H_3PO_4 system and the ammonium acetate buffer system. Bambuterol was only partly resolved and no resolution was recorded in the composition of buffer studied for salbutamol and salmeterol.

The enantiomeric elution order of terbutaline was deter-

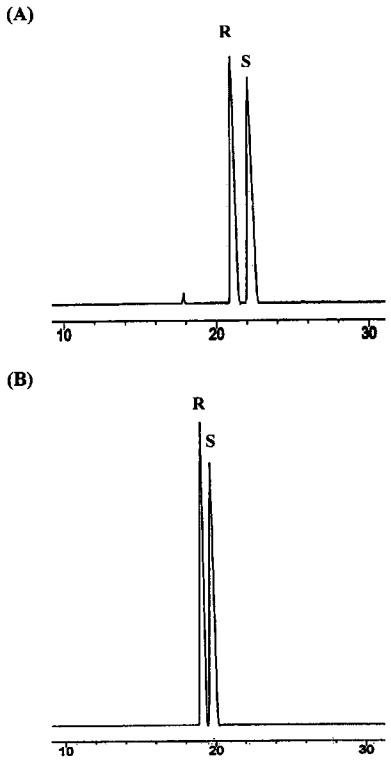

Fig. 2. Electropherograms of the separation of (A) racemic terbutaline and (B) clenbuterol. Condition; capillary, 50 m × I.D. 80.5 cm (effective length 72 cm); applied voltage, 30 kV; detection, 210 nm; temperature, 20°C, BGE, 0.1 M NaH_2PO_4-H_3PO_4 (pH 2.5); chiral selector solution, 0.1 M phosphate buffer containing 10 mM HP-β-CD

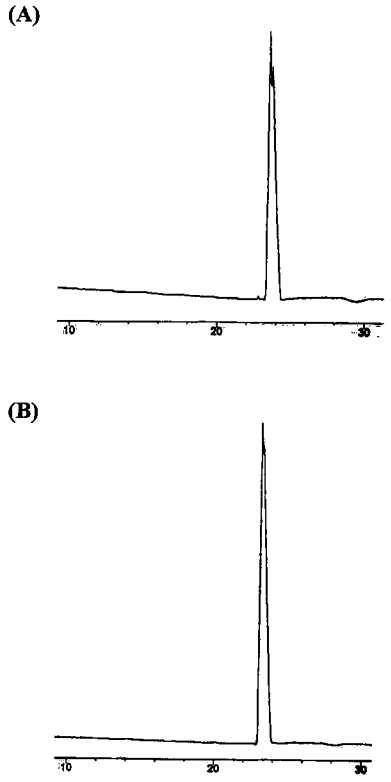

Fig. 3. Electropherograms of the separation of (A) racemic terbutaline and (B) clenbuterol. Condition: BGE, 0.1 M ammonium acetate buffer (pH 3.5); chiral selector solution, 0.1M ammonium acetate buffer containing 10 mM HP-β-CD. Other experimental conditions as shown in Fig. 2

mined by injected each enantiomer separately under the same CE conditions. And the elution order of the clenbuterol enantiomers was determined by Gausepohl *et al.*, 1998. For terbutaline and clenbuterol, (R)-form was eluted before the (S)-form.

Selection of buffer pH

Buffer pH is an important parameter in CE, since alterations in pH can affect the solute charge, depending on the solute properties, and change the electroosmotic flow (EOF), which generally increase as the pH is increased, thus influencing the resolution (Wang *et al.*, 1996). It has been shown that generally, successful chiral separation of basic drugs is achieved under acidic buffer conditions when using neutral CDs as chiral selector (Terabe *et al.*, 1994).

The effect of the BGE pH on the resolution factor (Rs) was investigated using for the NaH_2PO_4 - H_3PO_4 buffer at pH 2.5, 3.5, 4.5 and 5.5 and containing 10 mM HP-β-CD. Fig. 4 shows the effect of the pH of the buffer on the resolution of terbutaline enantiomers and clenbuterol enantiomers. At pH 3.5, maximum of Rs and migration time was showed. This result was similar to the findings

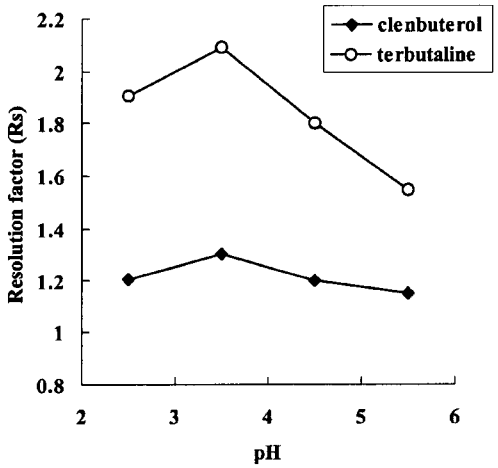

Fig. 4. Effect of the pH on chiral resolution of terbutaline and clenbuterol enantiomers. Experimental conditions as shown in Fig. 2

previously reported (Ingelse *et al.*, 1995).

Effect of concentration of HP-β-CD

The analytes were injected using a BGE at pH 3.5 sup-

plemented with different amount of HP-β-CD in the range 0-30 mM. It was found that the chiral resolution was strongly influenced by the HP-β-CD concentration. Resolution of the racemic mixtures into their enantiomers was obtained for terbutaline and clenbuterol (R≥1). Salbutamol was only partly resolved (α=1.012, at 30 mM of chiral additive) and no resolution was recorded in the concentration range of HP-β-CD studied for salmeterol and bambuterol.

Table I shows the effect of the concentration of HP-β-CD on the resolution factor (Rs) for the five compounds. Obviously, there is an optimum HP-β-CD concentration at which chiral resolution reaches a maximum value for a particular compound. Such a relationship between resolution and cyclodextrin concentration has also been reported by other authors (Wren *et al.*, 1993). An increase in the concentration of HP-β-CD added to the BGE at pH 3.5 led to a general increase in resolution and migration time. Among the β_2-agonists studied, terbutaline and clenbuterol were to be resolved into its enantiomers even at a relatively low concentration of chiral additive (5 mM of HP-β-CD). Maximum of resolution was obtained when 15 mM of HP-β-CD for the terbutaline and 25 mM of HP-β-CD for the clenbuterol were used.

Table I. Effect of the concentration of HP-β-CD on resolution

Compounds	HP-β-CD (mM)					
	5	10	15	20	25	30
Bambuterol	0	0	0	0	0	0
Clenbuterol	0.98	1.06	1.34	1.46	1.52	1.38
Salbutamol	0	0	<0.1	<0.1	0.20	0.39
Salmeterol	0	0	0	0	0	0
Terbutaline	1.37	1.44	2.60	2.31	2.22	1.94

Capillary, 80.5 (72.0) cm × 0.05 mm I.D. (uncoated); BGE, 0.1M phosphate buffer (pH 3.5); applied voltage, 30 kV; injection, 30 kV, 5s of 0.1 mg/mL racemic compounds; chiral selector solution, BEG containing indicated amount of HP-β-CD.

Table II. Effect of the concentration of Polyrthylene glycol 300 on resolution

Compounds	Polyethylene glycol 300 (%)						
	0	1	2	4	6	8	9
Bambuterol	0.52	0.57	0.55	0.49	0.44	0.36	0.25
Clenbuterol	1.66	1.84	1.92	1.58	1.42	1.25	1.25
Salbutamol	0	0	0	0	0	0	0
Salmeterol	0	0	0	0	0	0	0
Terbutaline	3.01	3.28	3.45	3.22	2.72	2.42	2.27

Capillary, 80.5 (72.0) cm × 0.05 mm I.D. (uncoated); BGE, 0.1 M phosphate buffer (pH 2.5); applied voltage, 30 kV; injection, 30 kV, 5s of 0.1 mg/mL racemic compounds; chiral selector solution, BEG containing 10 mM HP-β-CD and indicated percent of PEG 300.

Effect of concentration of polyethylene glycol

To explore the influence of the removable gel, HP-β-CD was used in a concentration of 10 mM. It was dissolved in a removable polyethylene glycol gel and injected hydrodynamically into the capillary until it was fully filled.

The effect of the concentration of PEG 300 on the resolution factor (Rs) is shown in Table II. In general, on increase in the concentration of PEG 300 added to the BGE at pH 2.5 led to increase in migration time. For the terbutaline and clenbuterol, maximum of resolution was obtained when 2% of PEG 300 were used. Fig. 5 illustrates that when 0.1 M NaH_2PO_4 - H_3PO_4 containing 10 mM HP-β-CD and 4% PEG 300 was used, the resolution of terbutaline was further improved. From Fig. 5, it also can be seen that under these conditions, there is an anomalous baseline.

It seems that addition of PEG 300 to the cyclodextrin solution has not a positive effect on the resolution. Beyond a certain concentration of PEG 300, the baseline tends to fluctuate and make it imposible to determine the resolution. Practically, the capillary sometimes was broken

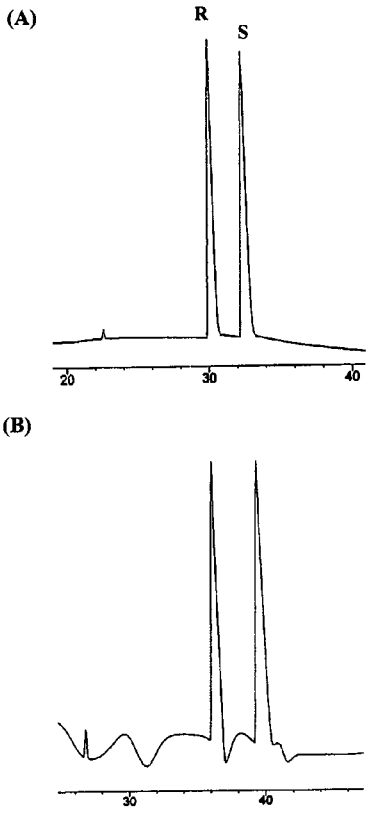

Fig. 5. Electropherograms of the separation of racemic terbutalne. Condition: chiral selector solution, 0.1 M NaH_2PO_4 - H_3PO_4 buffer containing (A) 10 mM HP-β-CD; (B) 10 mM HP-β-CD and 4% PEG 300 (pH 3.5). Other experimental conditions as shown in Fig. 2

at the detection window because a high concentration of the PEG causes a high viscosity and excessive Joule heating inside the capillary.

CONCLUSION

Chiral separation of the enantiomers of five β_2-agonists were investigated in CE in phosphate buffers, pH 2.5 - 5.5, containing HP-β-CD as a chiral selector and uncoated fused silica capillaries thermostated at 20°C. HP-β-CD concentration, buffer composition and pH influence the separation. The complete enantioseparation of terbutaline and clenbuterol can be obtained easily, slbutamol, salmeterol and bambuterol could be resolved under any of the experimental conditions examined. The addition of PEG 300 resulted in an increase in resolution. However at higher concentrations the baseline started to fluctuate impairing quantitation of the individual enantiomers. This work demonstrates the importance of the experimental conditions, which can be used to improve the chiral separation.

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