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Chiral Purity Test of Bevantolol by Capillaryelectrophoresis and High Performance Liquid Chromatography

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Two methods for the chiral purity determination of bevantolol were developed, namely capillary electrophoresis (CE) using carboxymethyl-β-cyclodextrin (CM-β-CD) as a chiral selector and high-perfomance liquid chromatography (HPLC) using a chiral stationary phase. In the HPLC method, the separation of bevantolol enantiomers was performed on a Chiralpak AD-H column by isocratic elution with n-hexane-ethanol-diethylamine (10:90:0.1, v/v/v) as mobile phase. In the CE method, bevantolol enantiomers were separated on an uncoated fused silica capillary with 50 mM amonium phosphate dibasic adjusted to a pH 6.5 with phosphoric acid containing 15 mM CM-β-CD as running buffer. Validation data such as linearity, recovery, detection limit, and precision of the two methods are presented. The detection limits of S-(-)-bevantolol were 0.1% and 0.05% for CE and HPLC method, respectively and R-(+)-bevantolol were 0.15% and 0.05% for CE and HPLC method, respectively. There was generally good agreement between the HPLC and CE results.

Key words: Bevantolol, HPLC, CE, Chiral separation, Optical purity

INTRODUCTION

Most often the enantiomers of chiral drugs have different pharmacological and toxicological properties and therefore the quantitative enantiomeric composition of these drugs should be determined (Williams and Lee, 1985; Drayer, 1986; Drayer, 1988). The separation and detemination of enantiomers are required for enantiomeric purity testing, chiral stability testing of pharmaceutical formulations and in pharmacokinetic and clinical studies (Fillet *et al.*, 1995).

Bevantolol is an experimental drug belonging to therapeutic category of adrenergic β-receptor blockers. The compound has a chiral center at the 2-carbon (denoted by * in Fig. 1a). which is responsible for the existence of bevantolol as two enantiomers, denoted (+) and (-) by convention.

There are considerable pharmacologic, pharmacokinetic and metabolic differences between the enantiomers of several β-blockers. The (-)- enantiomer of propranolol, for example, is mainly responsible for antihypertensive and other cardiovascular actions (Barrett *et al.*, 1968) of the drug. Similarly, bevantolol's β-blocking activity has been shown to reside in the (-)-enantiomer. Bevantolol is synthesized, manufactured and dispensed as the racemate. So analytical chirotechnology is needed for the development of enantiomeric pure β-blocker without side effect, for example, lowered heart rate. In the case of single enantiomer drugs, all other stereoisomers should be treated as any other organic impurities and the enantiomeric impurity of such drugs should be controlled.

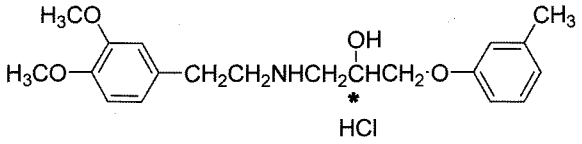

Fig. 1. Structures of bevantolol·HCl

A few papers have been published on the chiral separation of bevantolol enantiomers. HPLC assay for the bevantolol enantiomers in human plasma after derivatization of racemic bevantolol to its diastereomeric thioureas with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl isothiocyanate was reported (Rose *et al.*, 1991). Direct, isocratic and simple chromatographic method for the enantiomeric separation of bevantolol using normal and reverse cellulose

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chiral stationary phases was reported (Aboul-Enein and Serignese., 1994). Capillary electrophoresis has become an established powerful tool for enantiomeric separation of racemic drugs (Fanali *et al.*, 2001; Nishi, 1996; Verleysen and Sandra, 1998) due to its main advantage over HPLC and capillary gas chromatography, such as high separation efficiency, short ananlysis time, instrument simplicity, low consumption of chiral selectors and reduced operation cost. There are many papers published which reported on the application of capillary electrophoresis method to the separation and determination of chiral drugs such as penicillamine (Min *et al.*, 2006), Adrenaline(Sänger-van de Griend *et al.*, 2006), Salbutamol and bupivacaine (Wei *et al.*, 2006), Tamsulosin (Maier *et al.*, 2005). Until now, there is no report on the enantiomeric purity determination of bevantolol.

In this paper, The chiral purity test of enantiomeric bevantolol by CE and HPLC methods were developed and validated.

MATERIALS AND METHODS

Materials

Bevantolol·HCl was obtained from Sigma-Aldrich (U.S.A.) and used as received. The single enantiomers of bevantolol, R-(+)-bevantolol and S-(-)-bevantolol, were prepared in our laboratory. Carboxymethyl-β-cyclodextrin (CM-β-CD) was purchased from Wacker (Burghausen-Germany). n-Hexane, Methanol and Ethanol (HPLC grade) and Diethylamine, Phosphoric acid, amonium phosphate dibasic and Sodium hydroxide (analytical reagent grade) were from Duksan Pure Chemicals Co. (Ansan, Kyeonggi, Korea). Deionized water was prepared in the laboratory.

HPLC Equipment and operating conditions

Chromatographic system was consisted of LC-10ADvp Shimadzu pump, Shimadzu SPD-10Avp UV-VIS detector and CTO-10A Shimadzu column oven. The analytical column was a CHIRALPAK AD-H, 5 μm, 250 × 4.6 mm I.D. (DAICEL CHEMICAL INDUSTRIES, LTD, Japan). The mobile phase used for the chiral purity testing of enantiomeric bevantolol was a mixture of n-hexane-ethanol-diethylamine (10:90:0.1, v/v/v). The column was thermostated at 35°C. The mobile phase flow rate was at 0.4 mL/min and samples was injected by SIL-10A Shimadzu auto injector. Detection were at 276 nm. The data were acquired and processed using Shimadzu CLASS-LC10 software *via* Shimadzu CBM-10A Communication Bus Module.

Capillary electrophoresis equipment and operating conditions

All experiments were performed on a Model 3^{D}CE instrument (Hewlett Packard, Waldbronn, Germany), comprising a diode-array detector and Chemstation softwave for data handling. The capillary (Hewlett Packard) was uncoated fused silica, 56 cm long (47.5 cm effective length) with a 50 μm internal diameter. The applied voltage was at 25 kV. The capillary was thermostated at 20°C. Injections were made using the hydrodynamic mode (injection pressure at 50 mbar) for 10 sec. UV detection was at 220 nm. The capillaries were conditioned according to the following procedure before every run : water for 2 min; 0.1 M NaOH for 5 min; water for 2 min; background electrolyte solution (BGE) for 5 min and running buffers for 5 min prior to application of the ananlyte. The BGE solution was a solution of 50 mM amonium phosphate dibasic adjusted to pH 6.5 with phosphoric acid. The running buffer solution was the BGE solution containing 15 mM CM-β-CD. The pH of the buffers was adjusted by means of a Suntex pH Meter 2000A (Taiwan).

Preparation of standard and sample solutions

All stock solutions were prepared weekly. Amounts equivalent to 10 mg of bevantolol (using Bevantolol hydrochloride standard powder) were dissolved 10 mL of methanol to produce a concentration of 1 mg/mL and stored at -4°C. 5 mg of each enantiomer of bevantolol, R-(+)-bevantolol and S-(-)-bevantolol, were dissolved in 2 mL of methanol to produce a concentration of 2.5 mg/mL and stored at -4°C.

In Chiral purity determination, 100 μg/mL solutions of R-(+)-bevantolol in methanol were spiked with proportional amounts of S-(-)-bevantolol to obtain the sample solutions with concentrations of S-(-)-bevantolol ranged from 0.5% to 5% in R-(+)-bevantolol and 100 μg/mL solutions of S-(-)-bevantolol in methanol were spiked with proportional amounts of R-(+)-bevantolol to obtain the sample solutions with concentrations of R-(+)-bevantolol ranged from 0.5% to 5% in S-(-)-bevantolol. For the HPLC analysis, prepared sample solutions of mixture of enantiomers were dried under a nitrogen stream at room temperature and the residues were redissolved in mobile phase. For CE analysis, prepared sample solutions of mixture of enantiomers were dried under a nitrogen stream at room temperature and the residues were redissolved in the BGE solution. All solutions were filtered with 0.45 μm syringe filters (Microfiltration Systems, CA, U.S.A.).

Calculation of (S)-(-)-bevantolol and (R)-(+)-bevantolol

The content of (S)-(-)-bevantolol in (R)-(+)-bevantolol and (R)-(+)-bevantolol in (S)-(-)-bevantolol were calculated by the following equation:

$$\text{(S)-(-)-bevantolol (\%)} = \frac{A_S}{A_S + A_R} \times 100$$

$$\text{(R)-(+)-bevantolol (\%)} = \frac{A_R}{A_S + A_R} \times 100$$

Where A_S, is the peak area of (S)-(-)-bevantolol and A_R is the peak area of (R)-(+)-bevantolol.

Resolution factor was used in calculating the peak resolution as a function of separation selectivity:

$$R_S = \frac{2(t_{2migr} - t_{1migr})}{w_1 - w_2}$$

Where w_1 and w_2 are widths at the peak base, t_{1migr} and t_{2migr} the apparent migration times (min) of both enantiomers.

RESULTS AND DICUSSION

HPLC

Bevantolol enantiomers was separated on a CHIRALPAK AD-H column (5 μm, 250 × 4.6 mm.ID) using mixture of n-hexane, ethanol and diethylamine (10:90:0.1,v/v/v) as mobile phase. A typical chromatogram of racemic bevantolol is shown in Fig. 2, along with a chromatogram of 0.5% of S-(-)-bevantolol in R-(+)-bevantolol (Fig. 2B) and chromatogram of 0.5% of R-(+)-bevantolol in S-(-)-bevantolol (Fig. 2C). The first eluted peak was identified as R-(+)-bevantolol and the second peak was identified as S-(-)-bevantolol.

The linearity, accuracy and precision (CV) of the method for deterimantion of the S-(-)-bevantolol in R-(+)-bevantolol and R-(+)-bevantolol in S-(-)-bevantolol were examined at concentrations of 0.5, 1.0, 2.0, 3.0 and 5.0%. The correlation coefficients (r^2) of curves of percentage concentrations of S-(-)-bevantolol in R-(+)-bevantolol and R-(+)-bevantolol in S-(-)-bevantolol versus peak area responses were 0.9997 and 0.9993, respectively. Accuracy of the method for determination of S-(-)-bevantolol in R-(+)-bevantolol ranged from 97.34 to 107.13% (Table I) while the CV ranged from 0.25 to 3.72% (Table I). Accuracy of the method for determination of R-(+)-bevantolol in S-(-)-bevantolol ranged from 92.17 to 108.32% (Table II) while the CV ranged from 0.36 to 3.60%.

The detection limit of both enantiomers at a signal to noise ratio of 3 was 0.05%.

The precision of peak areas for six replicate injections of 1.0% solutions of S-(-)-bevantolol and R-(+)-bevantolol were determined. The coefficient of variation (C.V) were 7.13% and 5.11% for S-(-)-bevantolol and R-(+)-bevantolol, respectively .

Precisions (CV) of retention time, resolution, efficiency and peak area (by replicate analyzing a sample containing 5% of each enantiomer in the other antipode present as impurity) are shown in Table III and Table IV.

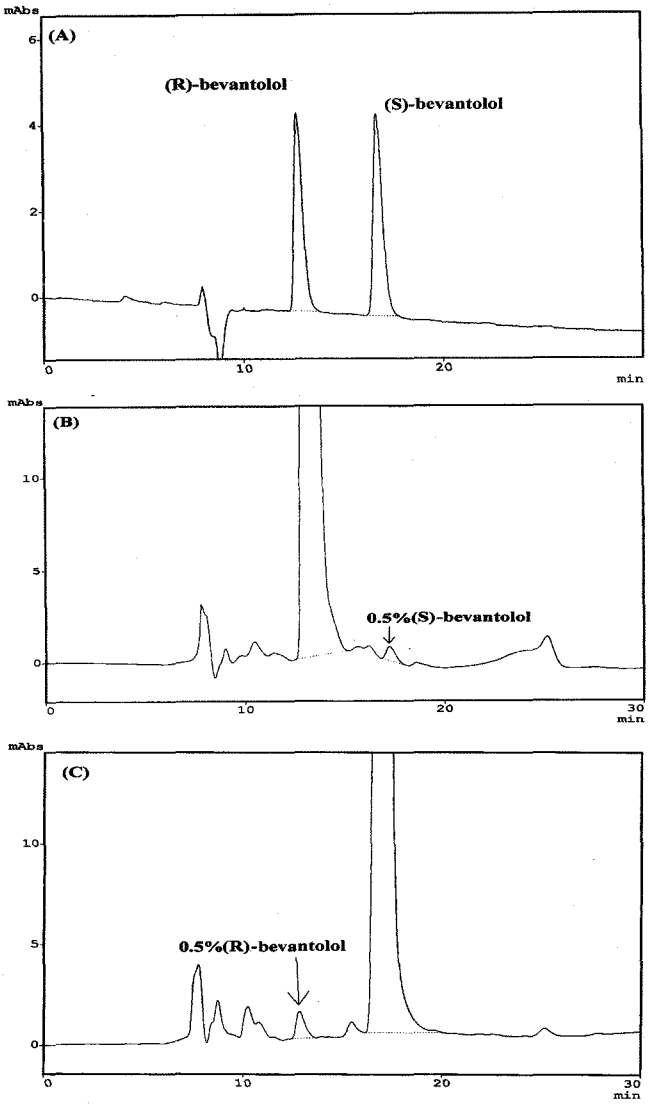

Fig. 2. Typical chiral HPLC chromatograms of (A) a racemic bevantolol (10 μg/mL), (B) R-(+)-bevantolol standard solution containing 0.5% of S-(-)-bevantolol, (C) S-(-)-bevantolol standard solution containing 0.5% of R-(+)-bevantolol. Column: CHIRALPAK AD-H (5 μm, 250 × 4.6 mm I.D.); mobile phase : n-hexane-ethanol-diethylamine (10:90:0.1,v/v/v); UV detector at 276 nm; flow rate at 0.4 mL/min.

Capillary electrophoresis

The effect of the CM-β-CD concentrations on chiral resolution and capacity factor investigated at 10, 12 and 15 mM of CM-β-CD in 50 mM $(NH_4)_2HPO_4$ buffer (adjusted to pH 6.5 by phosphoric acid) is shown in Fig. 3. The highest resolution was obtained with 15 mM CM-β-CD in 50 mM $(NH_4)_2HPO_4$ buffer which was chosen as running buffer for chiral purity testing of bevantolol by capillary electrophoresis in this method.

A typical electropherogram of racemic bevantolol was shown in Fig. 4, along with a electropherogram of 0.5% of S-(-)-bevantolol in R-(+)-bevantolol (Fig. 4B) and electropherogram of 0.5% of R-(+)-bevantolol in S-(-)-bevantolol

Table I. The accuracy and precision of the method for S-(-)-bevantolol by HPLC and CE

Theory Content (%)	Found Content (%)					
	HPLC (n=6)			CE (n=5)		
	Mean measured Content (%)	Accuracy (%)	C.V.[a] (%)	Mean measured Content (%)	Accuracy (%)	C.V.[a] (%)
0.5	0.51	101.09	3.72	0.46	93.67	12.22
1.0	1.07	107.13	0.78	1.18	118.96	6.33
2.0	1.95	97.49	0.46	1.99	99.51	10.37
3.0	2.92	97.34	0.61	2.84	94.82	3.29
5.0	5.05	101.06	0.25	5.06	101.39	1.49

[a] Coefficient of Variation

Table II. The accuracy and precision of the method for R-(+)-bevantolol by HPLC and CE

Theory Content (%)	Found Content (%)					
	HPLC (n=6)			CE (n=5)		
	Mean measured Content (%)	Accuracy (%)	C.V.[a] (%)	Mean measured Content (%)	Accuracy (%)	C.V.[a] (%)
0.5	0.54	108.32	3.60	0.63	127.74	9.69
1.0	1.05	105.03	0.96	0.96	96.31	12.20
2.0	2.03	101.34	1.85	1.84	91.50	7.49
3.0	2.77	92.17	1.33	3.01	100.54	6.65
5.0	5.12	102.31	0.36	5.05	101.03	1.78

[a] Coefficient of Variation

Table III. Comparision of chromatographic parameters between HPLC and CE method for the chiral purity test of S-(-)-bevantolol[a]

		Retention time (min)		Peak area (%)		Peak efficiency (plates/m)		Resolution (R_S)	
		Average	C.V.(%)	Average	C.V.(%)	Average	C.V.(%)	Average	C.V.(%)
HPLC (n=6)	S-(-)-bevantolol	17.24	0.085	4.59	0.120	2225	10.40	1.76	1.71
	R-(+)-bevantolol	13.14	0.160	95.49	0.004	392	9.96		
CE (n=5)	S-(-)-bevantolol	22.54[b]	9.830	5.02	3.690	569346	7.70	2.50	10.85
	R-(+)-bevantolol	23.12[b]	9.980	94.96	0.190	63071	10.15		

[a] using a mixture containing 5% of S-(-)-bevantolol in R-(+)-bevantolol
[b] Migration time.

Table IV. Comparision of chromatographic parameters between HPLC and CE method for the chiral purity test of R-(+)-bevantolol[a]

		Retention time (min)		Peak area (%)		Peak efficiency (plates/m)		Resolution (R_S)	
		Average	C.V.(%)	Average	C.V.(%)	Average	C.V.(%)	Average	C.V.(%)
HPLC (n=6)	S-(-)-bevantolol	16.78	0.12	94.65	0.061	543	7.34	1.88	6.05
	R-(+)-bevantolol	12.77	0.21	5.33	1.092	1453	11.59		
CE (n=5)	S-(-)-bevantolol	20.52[b]	3.96	95.70	0.094	38828	8.29	4.35	12.98
	R-(+)-bevantolol	21.94[b]	4.19	4.28	2.760	90096	12.28		

[a] using a mixture containing 5% of R-(+)-bevantolol in S-(-)-bevantolol.
[b] Migration time.

(Fig. 4C). The first eluting peak was identified as S-(-)-bevantolol and the second eluting peak was identified as R-(+)-bevantolol.

The linearity, the accuracy and precision of the method

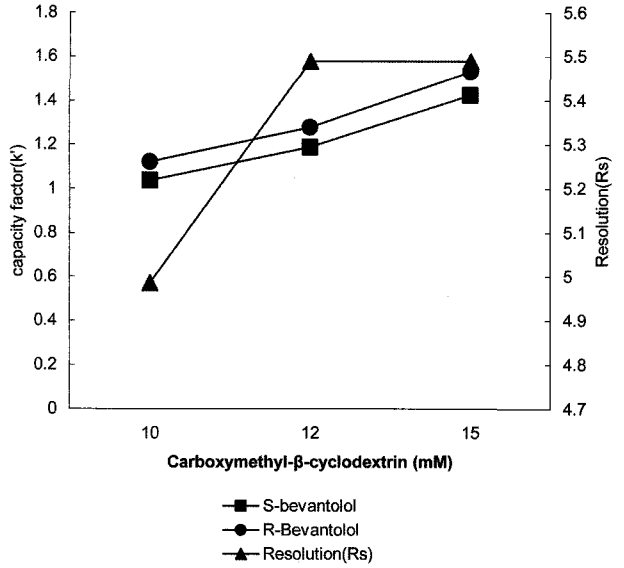

Fig. 3. Effect of the concentration of CM-β-CD on chiral resolution (R_S) and capacity factor (k') of bevantolol enantiomers. Conditions: BGE solution: 50 mM $(NH_4)_2HPO_4$ adjusted to pH 6.5 with phosphoric acid; capillary:uncoated fused silica, 50 μm I.D × 56 cm (effective length 47.5 cm); applied voltage at 25 kV; UV detector at 220 nm; temperature at 20°C; sample concentration : 100 μg/mL of racemic bevantolol.

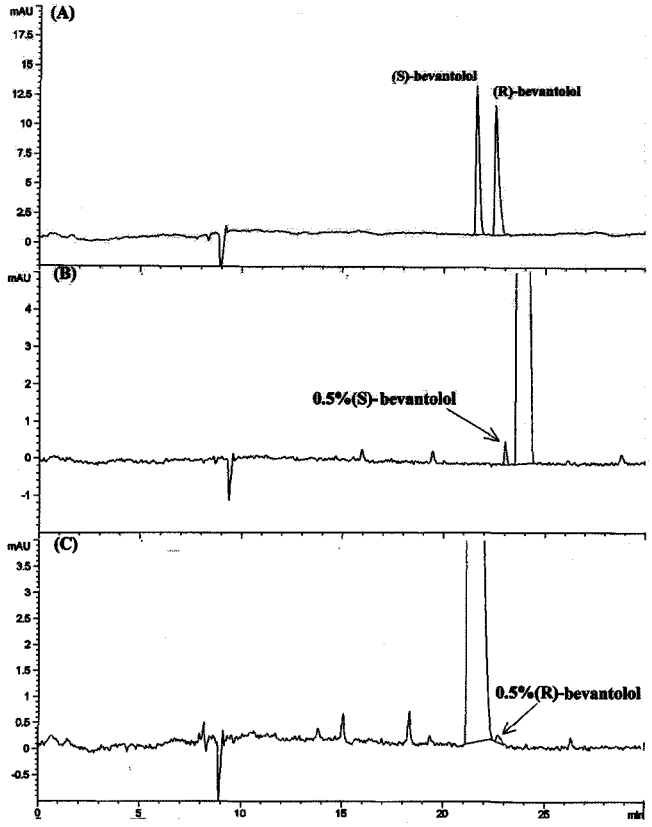

Fig. 4. Typical electropherogram of (A) a racemic bevantolol, (B) R-(+)-bevantolol standard solution containing 0.5% of S-(-)-bevantolol, (C) S-(-)-bevantolol standard solution containing 0.5% of R-(+)-bevantolol. Conditions: BGE solution: 50 mM $(NH_4)_2HPO_4$ (adjusted to pH 6.5 with phosphoric acid); uncoated capillary : 50 μm I.D × 56 cm (effective length 47.5 cm); applied voltage at 25 kV; detection at 220 nm; temperature at 20°C.

for determination of the S-(-)-bevantolol in R-(+)-bevantolol and R-(+)-bevantolol in S-(-)-bevantolol were examined at concentrations of 0.5, 1.0, 2.0, 3.0 and 5.0%. The correlation coefficients (r^2) of curves of percentage concentrations of S-(-)-bevantolol in R-(+)-bevantolol and R-(+)-bevantolol in S-(-)-bevantolol versus peak areas were 0.9968 and 0.9962, respectively. Accuracy of the method for determination of S-(-)-bevantolol in R-(+)-bevantolol ranged from 93.67 to 118.96% (Table I) with the mean value was 101.67% and those of R-(+)-bevantolol in S-(-)-bevantolol ranged from 91.50 to 127.74% (Table II) with the mean value was 103.42%. Whereas The CV of the method for determination of (S)-(-)-bevantolol in R-(+)-bevantolol and R-(+)-bevantolol in S-(-)-bevantolol ranged from 1.49 to 12.22% and from1.78 to 12.20%, respectively. The detection limit of S-(-)-bevantolol and R-(+)-bevantolol at a signal to noise ratio of 3 were 0.1% and 0.15%, respectively.

Chromatographic parameters: migration time (for CE), retention time (for HPLC), resolution, peak efficiency and peak area percentage obtained in the two methods are compared and shown in Table III and Table IV. Some general advantages of CE over HPLC discussed elsewhere and applicable here include lower consumption of chemical reagents and analytes, less waste and lower cost of operation. And the CE method showed higher resolution and peak efficiency than those of HPLC. However, the CE method showed poorer precision and accuracy than those of HPLC due to the difficulties involved in reproducibly injecting nanoliter volumes of sample into the capillary.

The enantiomeric separation of bevantolol was achieved by both the HPLC and CE methods. The optical purity test by two methods was developed and validated and can be useful as a practical quality control method for bevantolol enantiomers in pharmaceutical field.

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