Article

Chinese Science Bulletin

Pharmacy

April 2013 Vol.58 No.11: 1267–1273
doi: 10.1007/s11434-012-5636-7

Separation of four -lol drugs by HPLC with new bonded chiral stationary column

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Received February 24, 2012; accepted June 20, 2012; published online January 15, 2013

Separation of betaxolol, bevantolol, metoprolol, bisoprolol with new bonded chiral stationary (3,5-dimethylcabamate cellulose) Chiralpak IB column was investigated. The factors, such as mobile phase composition and the ratio, column temperature, flow rate and the proportion of chiral additives, were also investigated. The optimal mobile phase compositions of betaxolol, bevantolol, metoprolol, bisoprolol were *n*-hexane/ethanol (95/5, v/v; 0.2% DEA); *n*-hexane/ethanol (90/10, v/v; 0.1% DEA); hexane/ethanol (60/40, v/v; 0.1% DEA); *n*-hexane/ethanol (95/5, v/v; 0.2% DEA), respectively. The optimal temperature was 30°C with a flow rate of 0.8 mL min^{-1}. The detection wavelength, by 1200VWD UV detector, of betaxolol, bevantolol, metoprolol, bisoprolol was 259, 274, 224 and 223 nm, respectively. Experimental results demonstrated that baseline separation (R_s>1.5) of the betaxolol, bevantolol, metoprolol, bisoprolol enantiomers were obtained with new bonded Chiralpak IB column. This method can be used for analysis and detection of these four drugs.

bonded Chiralpak IB column, enantiomeric separation, betaxolol, bevantolol, metoprolol, bisoprolol, HPLC

Citation: Zhou J, Pei W J, Su N, et al. Separation of four -lol drugs by HPLC with new bonded chiral stationary column. Chin Sci Bull, 2013, 58: 1267–1273, doi: 10.1007/s11434-012-5636-7

β-Receptor blockers are widely used in clinical treatment [1] of angina pectoris, arrhythmia, hypertension, myocardial infarction and other heart diseases, as well as hypertrophic heart disease, pheochromocytoma, hyperthyroidism, migraine and glaucoma. The drugs contain 1 or 2 asymmetric carbon atoms. Structure of betaxolol, bevantolol, metoprolol, bisoprolol is shown in Figure 1.

This study on separation methods of -lol drugs enantiomers is of great significance to the stereo selectivity differences between their dynamics and pharmacokinetics. A few of chromatographic methods for the separation of -lol drugs enantiomers have been reported, such as High Performance Capillary Electrophoresis (HPCE) [2,3], Capillary Electrochromatography (CE) [4], High Performance Liquid Chromatography (HPLC) [5–9], etc. According to the literature, by lipase catalyzed β-amino alcohol, Yang and Chen [10] gets S-betaxolol and R-betaxolol enantiomers. By chiral derivatizating RP column, Darmon et al. [11] separated betaxolol successfully. By Chiralcel OD column, Krstulovic et al. [12] separated betaxolol directly and successfully. By Chiralcel OD column, Ekelund et al. [13] successfully separated betaxolol and bevantolol, metoprolol, bisoprolol as well.

The chiral stationary phase of Chiralpak IB column [14] was 3,5-dimethyl carbamate polysaccharide cellulose derivatives bonded silica gel. Compared to the coated stationary phase, bonded ones expanded the use of the mobile phase, and the performance of HPLC was also improved. Besides, it can be used not only in the normal phase column, but also in the RP system. Experiments showed that bonded Chiralpak IB column worked effectively and it had advantages in separating chiral drugs.

Literatures about the use of bonded Chiralpak IB columns in betaxolol, bevantolol, metoprolol, bisoprolol have not been found. This article was about chiral separation of the above 4 -lol drugs. The factors, such as mobile phase

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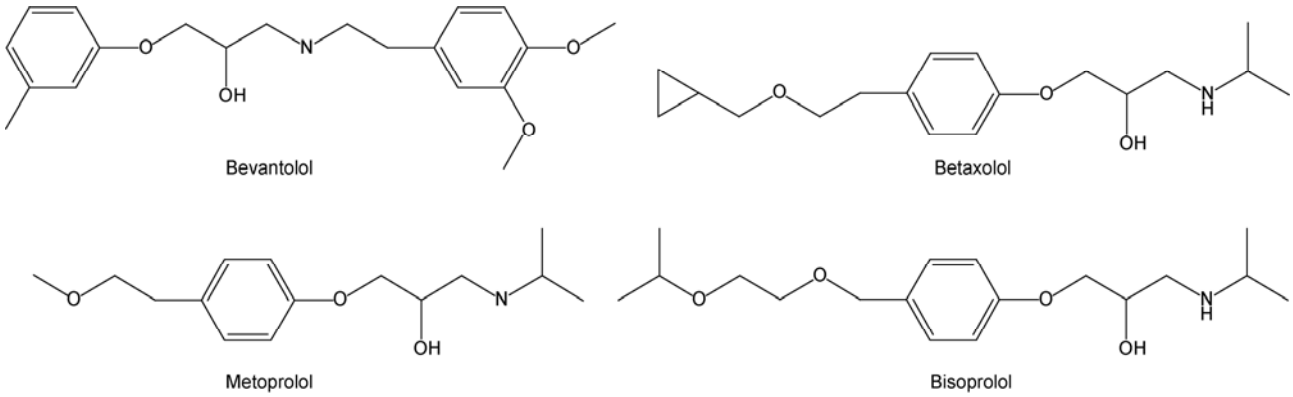

Figure 1 Molecule structure of four β-blockers.

composition and the ratio, column temperature, flow rate and the proportion of chiral modifier diethyl amine, were also investigated.

1 Experimental

1.1 Reagents

Betaxolol hydrochloride was obtained from Zhengzhou University New Drugs Research and Development Center, the content of which was 99.6%, determined by USP24 method. Metoprolol tartrate was obtained from Weikang (China). Bevantolol hydrochloride was obtained from J&N Chuangyi (China). Bisoprolol fumarate was obtained from B&J Fengde (China), the purity of which was 99%. HPLC-grade *n*-hexane, absolute ethyl ethanol, isopropanol and methanol were chromatographic pure, and diethylamine (DEA) was analytical pure (T&J Kermel Reagent).

1.2 Apparatus

The chromatograph system consisted of an Agilent HP1100 HPLC apparatus, a 1200VWD UV detector, a 1200 manual sample injector, HP chemistry workstation. The HPLC column was a Chialpak IB (250 mm×4.6 mm, 5 μm) (Japan). Ultra pure water was obtained by Millipore system (USA). The size of balance was BP211D1/100 thousand (Sartorius, Germany). KQ3200DE numerical control ultrasound cleaning apparatus was from K&S Ultrasound Apparatus Cor. The size of millipore filter (mix cellulose membrane) was 0.25 μm (S&H Xinya, China).

1.3 Chromatographic condition

The base solvent of mobile phase was *n*-hexane, with the polarity modifier was isopropanol or ethanol and the mobile phase additive was DEA. Mobile phase were filtered with 0.45 μm solvent filter and ultrasonically degassed. The UV detecting wavelength of betaxolol, bevantolol, metoprolol, bisoprolol was 259, 274, 224 and 223 nm, respectively. The column temperature was 30°C with a flow rate of 0.8 mL min^{-1}, and the sampling volume was 20 μL.

1.4 Sample preparation

The 1.0 mg of chiral bulk drug was dissolved in 5 mL ethanol and then filtered (0.45 μm).

2 Results and discussion

The type and constitute of mobile phase, the content of alkalinity additive, the column temperature and the flow rate of 4 -lol drugs were investigated, hoping to get optimal retention time and resolution.

2.1 Effect of ethanol content on the enantioselectivity of the 4 -lol drugs

With the flow rate of 0.8 mL min^{-1} and the column temperature at 25°C, the content of ethanol was investigated. Table 1 summarizes their retention time (t_{R1}, t_{R2}) for the first and second eluted enantiomers, retention factors (k'_1, k'_2) for the first and second eluted enantiomers, separation factors (α) and resolution factors (R_s).

The results showed that with the reduction of the proportion of ethanol, 4 -lol drugs enantiomers peak time was extended, however the resolution was increased. This showed that with the decreasing of ethanol ratio, the force between enantiomers and mobile phase reduced, leading to the increasing of the retention time, so the peak time extended, and at the same time increasing the opportunity that the enantiomers affected chiral stationary phase.

But with the increasing of hexane ratio, most of the -lol drugs did not reach the baseline separation. At this point, basic regulator DEA can be added to improve the peak shape.

2.2 Effect of isopropanol content on the enantioselectivity of the 4 -lol drugs

With flow rate of 0.8 mL min^{-1} and at 25°C column

Table 1 Effect of content of ethanol on the enantioselectivity of the 4 -lol drugs[a)]

Drugs	*n*-hexane/ethanol	t_{R1} (min)	t_{R2} (min)	k'_1	k'_2	α	R_S
Betaxolol	99:1	44.3	49.2	22.7	25.3	1.1	0.71
	95:5	28.3	31.1	14.2	15.6	1.1	0.63
	90:10	14.5	15.9	6.8	7.5	1.1	0.58
	80:20	9.1	9.8	3.9	4.3	1.1	0.52
	70:30	8.3	8.9	1.4	3.6	2.6	0.41
	60:40	7.3		2.9		–	–
Metoprolol	99:1	40.5	47.2	20.1	24.2	1.2	2.91
	95:5	18.2	20.2	8.7	9.8	1.1	1.66
	90:10	11.5	12.8	5.1	5.8	1.1	1.56
	80:20	7.9	8.6	3.2	3.6	1.1	1.12
	70:30	6.5	7.1	2.5	2.8	1.1	0.82
	60:40	5.3	6.2	1.8	2.3	1.3	0.63
Bevantolol	95/5	>100		–		–	–
	90:10	>100		–		–	–
	80:20	36.3	55.7	18.4	28.8	1.6	8.4
	70:30	18.7	28.7	8.9	14.3	5.0	7.4
	60:40	7.4	13.6	3.0	6.3	2.1	2.6
Bisoprolol	99:1	46.2	68.8	23.7	35.8	1.5	0.87
	95:5	28.5	30.6	14.2	15.4	1.1	0.50
	90:10	19.0	19.9	9.2	9.6	1.0	0.44
	80:20	10.0	10.4	4.3	4.6	1.1	0.41
	70:30	8.4	8.6	3.5	3.6	1.0	0.37
	60:40	7.5		3.0		–	–

a) Chromatographic condition: Column temperature was at 25°C. Flow rate was 0.8 mL min^{-1}. "–" means that retention time was too long or did not observe the separation. ">100" means that retention time was over 100 min. If there is only one numerical value in t_R or k', it means that the enantiomers were not separated at all.

temperature, the content of polarity regulator isopropanol was investigated. The separation data of betaxolol, bevantolol, metoprolol, bisoprolol are shown in Table 2.

The results showed that when the isopropanol ratio was reduced, the peak time extended, this should be conducive to the separation of enantiomers. But except that bevantolol was separated when the ratio of isopropanol was 40%, the other three -lol drugs did not get baseline separation at all. While the peak time extended, eddy diffusion and vertical diffusion will be caused, impacting peak shape at the same time.

In fact, it was not suitable to use isopropanol as mobile phase modifiers to separate the four types of drug enantiomers. In contrast with considering hexane-ethanol as the mobile phase, the hexane-ethanol system was taken.

Table 2 Effect of content of isopropanol on the enantioselectivity of the 4 -lol drugs[a)]

Drugs	*n*-hexane/isopropanol	t_{R1} (min)	t_{R2} (min)	k'_1	k'_2	α	R_S
Betaxolol	80:20	>60		–		–	–
	70:30	>60		–		–	–
	60:40	39.5		20.1		–	–
Metoprolol	80:20	62.5		32.4		–	–
	70:30	35.8		20.7		–	–
	60:40	19.1		10.2		–	–
Bevantolol	80:20	>100		–		–	–
	70:30	>100		–		–	–
	60:40	21.4	54.3	10.4	28.0	2.7	4.6
Bisoprolol	80:20	>100				–	–
	70:30	43.2		22.1		–	–
	60:40	28.8		14.4		–	–

a) Chromatographic condition: Column temperature was at 25°C. Flow rate was 0.8 mL min^{-1}. "–" means that retention time was too long or did not observe the separation. ">100" means that retention time was over 100 min. If there is only one numerical value in t_R or k', it means that the enantiomers were not separated at all.

2.3 Effect of DEA on the enantioselectivity of the 4 -lol drugs

When selected polarity regulator, neither ethanol nor isopropanol could well separate the 4 -lol drugs. In view of this, DEA was taken as the suppressing reagent, hoping to improve the shape of peak. Experiments were carried out to investigate the effect of the content of additive DEA on the enantioselectivity of the 4 -lol drugs. The separation data of betaxolol, metoprolol, bevantolol, bisoprolol in mobile phase with different proportion of DEA are listed in Table 3.

The results showed that four drugs were baseline separated and peak shapes were fine. With the increase of the content of DEA, 4 -lol drug enantiomers were separated earlier and its resolution also increased. But when the DEA concentration increased to a certain value, the resolution will appear extreme and then reduce. This showed that by adding DEA to the mobile phase, the separation appeared better. This may be due to the fact that the 4 drugs all contain kinds of drugs polar groups such as $-NH_2$ and –OH. Without DEA, they can form hydrogen bonding with –CO or $-NH_2$ in the chiral stationary phase. This increased the retention time that alcohol amine drug enantiomers stay in the column so that even if ethanol or isopropanol was added to the mobile phase, they cannot be washed off. When adding DEA, the polarity increased, helping drug molecules elute from the stationary phase. What's more, with DEA, alkaline environment was obtained, and drugs existenced as molecular form. Thus the selectivity of stationary phase was improved. With the increase of DEA content, the separation capacity improved. When it continued to increase, due to the decrease of retention, the resolution decreased as well.

2.4 Effect of column temperature on the enantioselectivity of the 4 -lol drugs

On the basis of baseline separation, we selected the best separation conditions except the column temperature. By changing column temperature, we studied the impact of column temperature on separation of drugs. The separation data of betaxolol, bevantolol, metoprolol, bisoprolol in different column temperatures are listed in Table 4.

The experimental results showed that in this mobile phase system, separation factor and resolution of the enantiomers

Table 3 Effect of content of DEA on the enantioselectivity of the 4 -lol drugs[a)]

Drugs	DEA (%)	t_{R1} (min)	t_{R2} (min)	k'_1	k'_2	α	R_S
Betaxolol	0.1	11.5	14.7	5.2	6.8	1.3	4.39
	0.2	10.4	13.5	4.6	6.2	1.4	5.19
	0.3	9.6	12.6	4.1	5.7	1.4	4.88
	0.4	9.5	12.6	4.1	5.7	1.4	5.00
	0.5	43.2	12.3	3.9	5.6	1.4	1.43
Metoprolol	0.1	8.7	10.3	3.7	4.5	1.2	2.95
	0.2	8.0	9.3	3.3	4.0	1.2	2.97
	0.3	7.6	9.2	3.1	3.9	1.3	2.99
	0.4	7.2	8.7	2.9	3.7	1.3	2.46
	0.5	7.1	8.6	2.8	3.6	1.3	2.39
Bevantolol	0.1	6.5	13.0	2.5	6.0	2.4	13.4
	0.2	6.5	12.8	2.5	5.8	2.3	13.3
	0.3	6.3	12.4	2.4	5.6	2.3	13.3
	0.4	6.3	12.2	2.4	5.5	2.3	13.3
	0.5	6.1	11.2	2.3	5.0	2.2	11.9
Bisoprolol	0.1	12.8	14.7	5.8	6.9	1.19	2.38
	0.2	12.3	14.3	5.6	6.6	1.18	2.49
	0.3	11.4	13.5	5.1	6.2	1.22	2.96
	0.4	11.4	13.4	5.1	6.2	1.22	2.55
	0.5	11.1	13.2	5.0	6.1	1.22	2.42

a) Chromatographic condition: Column temperature was at 25°C. Flow rate was 0.8 mL min^{-1}. Mobile phase: betaxolol: *n*-hexane/absolute ethanol (95/5, v/v), metoprolol: *n*-hexane/absolute ethanol (95/5, v/v), bevantolol: *n*-hexane/absolute ethanol (60/40, v/v), bisoprolol: *n*-hexane/absolute ethanol (95/5, v/v).

Table 4 Effect of temperature on the enantioselectivity of the 4 -lol drugs[a)]

Drugs	T(°C)	t_{R1} (min)	t_{R2} (min)	k'_1	k'_2	α	R_S
Betaxolol	20	10.6	14.0	4.7	6.5	1.4	5.01
	25	10.4	13.5	4.6	6.2	1.4	5.19
	30	10.1	13.1	4.4	6.0	1.4	5.26
Metoprolol	20	9.0	10.7	3.8	4.7	1.2	3.07
	25	8.7	10.3	3.7	4.5	1.2	3.08
	30	8.6	10.2	3.6	4.4	1.2	3.17
Bevantolol	20	6.6	13.3	2.6	6.1	2.4	12.2
	25	6.5	13.0	2.5	6.0	2.4	13.2
	30	6.5	12.6	2.5	5.8	2.3	13.3
Bisoprolol	20	12.7	14.8	5.8	7.0	1.2	2.46
	25	12.3	14.3	5.6	6.6	1.2	2.49
	30	12.2	14.0	5.5	6.5	1.2	2.50

a) Chromatographic condition: Flow rate was at 0.8 mL min^{-1}. Mobile phase: betaxolol: *n*-hexane/absolute ethanol (95/5, v/v; 0.1% DEA); metoprolol: *n*-hexane/absolute ethanol (95/5, v/v; 0.1% DEA); bevantolol: *n*-hexane/absolute ethanol (60/40, v/v; 0.1% DEA); bisoprolol: *n*-hexane/absolute ethanol (95/5, v/v; 0.1% DEA).

reduced with the increase of column temperature.

The relationship between solute capacity factor and the temperature can be expressed by the following equation:

$$\ln \alpha = -\Delta_{R,S} \Delta H^0 / RT + \Delta_{R,S} \Delta S^0 / R, \quad (1)$$

where $\Delta_{R,S}\Delta H^0$, $\Delta_{R,S}\Delta S^0$ were the difference of enthalpy and entropy of two enantiomers from the mobile phase to the stationary phase during the distribution process, respectively. With lnα for the vertical axis Y, $1/T$ abscissa X, Y on X mapping, as shown in Figure 2. The results showed that the linear of the regression equations was perfect. Over the temperature ranges of 293–303 K, $|\Delta_{R,S}\Delta H^0| > |T \cdot \Delta_{R,S}\Delta S^0|$, as shown in Table 5. So the conclusion that the chiral separation process is controlled by enthalpy can be obtained.

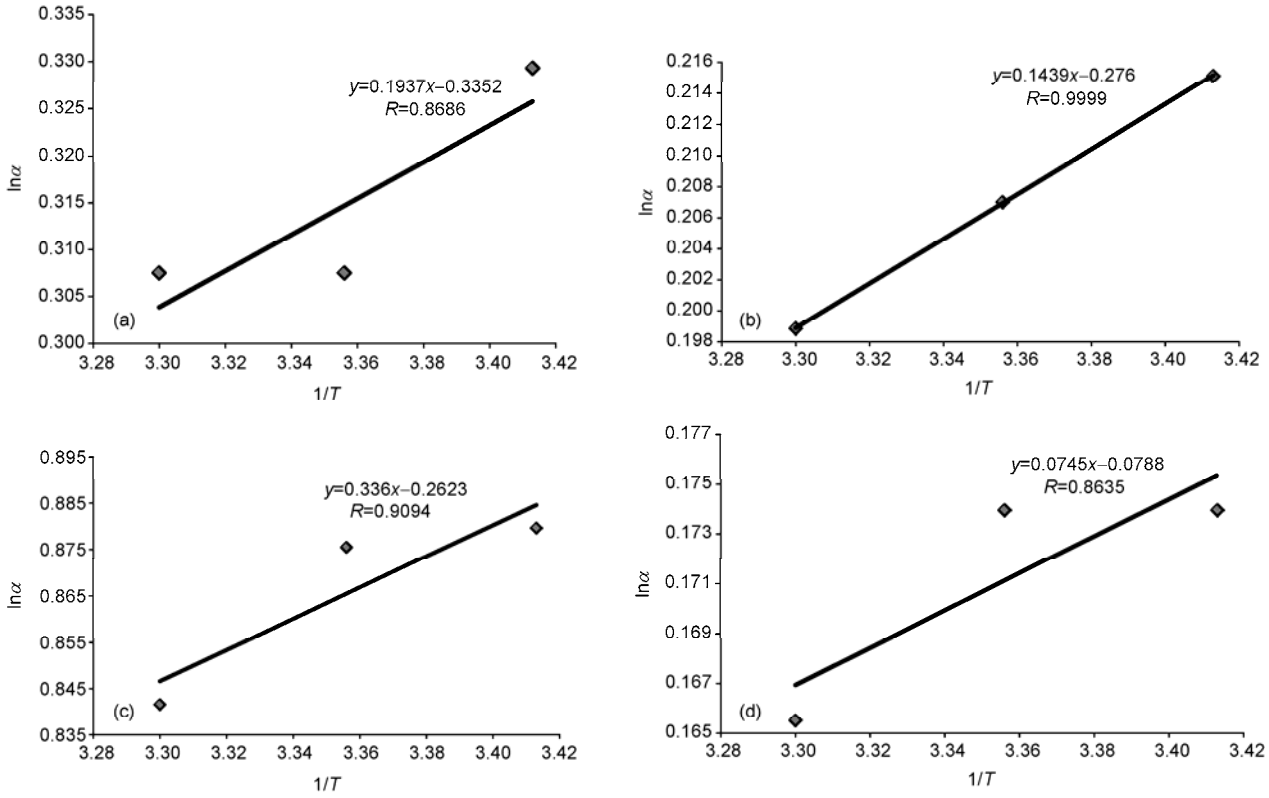

Figure 2 lnα-1/T scatters of 4 -lol drugs.

Table 5 Thermodynamic parameters of the 4 drugs

Drugs	$\Delta_{R,S}\Delta H^0$	$\Delta_{R,S}\Delta S^0$	R
Betaxolol	–168.2	–0.2912	0.8686
Metoprolol	–143.2	–0.2746	0.9999
Bevantolol	–305.6	–0.2385	0.9094
Bisoprolol	–5.867	–0.0680	0.8635

The results showed that with increasing of temperature, the enantiomeric separation increased, peak time appeared slightly earlier. When temperature is 30°C, the drugs were best separated. So the temperature 30°C was taken.

2.5 Effect of flow rate on the enantioselectivity of the 4 -lol drugs

The separation data of betaxolol, bevantolol, metoprolol, bisoprolol in different flow rate are listed in Table 6.

The Van Deemter equation is

$$H = A + B/u + C \cdot u, \tag{2}$$

where H is height of theoretical plate (mm), and u is the flow rate (cm s^{-1}).

By the equation, the H_{min} and the best flow rate were plotted and calculated. The range of best flow rate was 551.9–1003.4 cm s^{-1}, that was 0.55–1.00 mL min^{-1}. At 0.8 mL min^{-1}, baseline separation can be obtained and the shapes were fine, so the rate of 0.8 mL min^{-1} was taken.

The results showed that at different flow rates, the resolution of drugs was similar. By the Van Deemter chromatography theory, the vertical proliferation plays a major role at low flow, and the mass transfer resistance plays a major role at a high rate. Chromatograms of the 4 -lol drugs under the best chromatographic conditions are shown in Figure 3.

Table 6 Effect of flow rate on the enantioselectivity of the 4 -lol drugs[a)]

Drugs	Flow rate (mL min^{-1})	k'_1	k'_2	α	R_s	HETP$_1$ (m)	HETP$_2$ (m)
Betaxolol	0.5	4.3	5.8	1.35	5.41	3.853	3.813
	0.8	4.4	6.0	1.36	5.26	3.424	3.781
	1.0	3.3	4.4	1.33	4.74	4.679	4.766
Metoprolol	0.5	3.5	4.3	1.23	3.14	4.964	4.738
	0.8	3.6	4.4	1.22	3.17	3.553	2.951
	1.0	3.5	4.3	1.23	2.99	5.950	5.720
Bevantolol	0.5	2.4	5.1	2.13	12.55	4.689	4.207
	0.8	2.5	6.0	2.40	13.38	3.828	3.203
	1.0	2.4	5.2	2.17	11.53	3.689	3.138
Bisoprolol	0.5	5.3	6.3	1.19	2.92	4.884	4.524
	0.8	5.5	6.5	1.18	2.50	5.686	5.069
	1.0	5.4	6.3	1.17	2.48	7.196	6.438

a) Chromatographic condition: Column temperature was 30°C. Flow rate was at 0.8 mL min^{-1}. Mobile phase: betaxolol: *n*-hexane/absolute ethanol (95/5, v/v; 0.1% DEA); metoprolol: *n*-hexane/absolute ethanol 95/5, v/v; 0.1% DEA); bevantolol: *n*-hexane/absolute ethanol (60/40 v/v; 0.1% DEA); bisoprolol: *n*-hexane/absolute ethanol (95/5, v/v; 0.1% DEA).

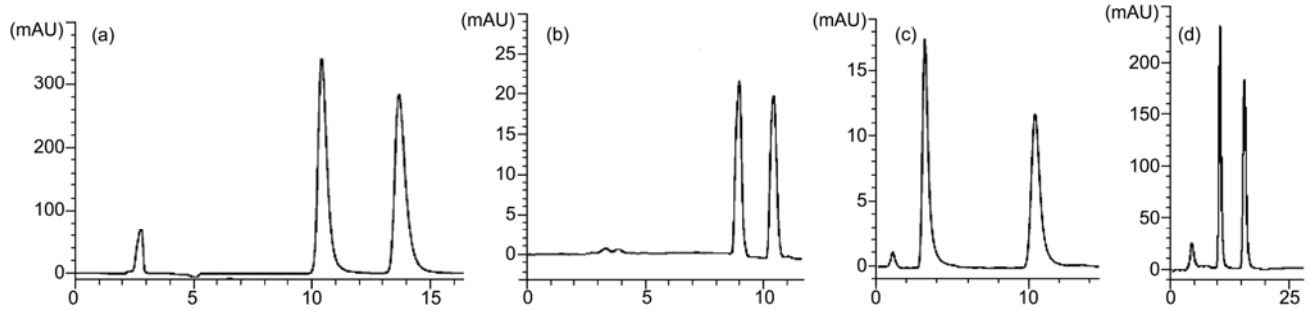

Figure 3 HPLC chromatograms of 4 drugs on bonded Chiralpak IB column. Chromatographic condition: Column temperature was at 30°C. Flow rate was 0.8 mL min^{-1}. Mobile phase: betaxolol: hexane/ethanol (95/5, v/v; 0.1% DEA), metoprolol (95/5, v/v; 0.1% DEA), bevantolol (60/40, v/v; 0.1% DEA), bisoprolol (95/5, v/v; 0.1% DEA). (a) Betaxolol hydrochloride mobile phase: hexane/ethanol (95/5, v/v; 0.2% DEA), temperature: 30°C, flow rate: 0.8 mL min^{-1}; (b) metoprolol tartrate mobile phase: hexane/ethanol (90/10, v/v; 0.1% DEA), temperature: 30°C, flow rate: 0.8 mL min^{-1}; (c) bevantolol hydrochloride mobile phase: hexane/ethanol (60/40, v/v; 0.1% DEA), temperature: 30°C, flow rate: 0.8 mL min^{-1}; (d) bisoprolol fumarate mobile phase: hexane/ethanol (95/5, v/v; 0.2% DEA), temperature: 30°C, flow rate: 0.8 mL min^{-1}.

3 Conclusions

The enantiomers of betaxolol, metoprolol, bevantolol, bisoprolol were separated on column Chiralpak IB successfully. The experiment showed that the Chiralpak IB column worked effectively and had certain advantage in separating chiral drugs. In summary, a specific and sensitive HPLC method was developed for the separation of the enantiomers of betaxolol, metoprolol, bevantolol, bisoprolol.

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