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Separation of β-receptor blockers and analogs by Capillary Liquid Chromatography (CLC) and Pressurized Capillary Electrochromatography (pCEC) using a vancomycin chiral stationary phase column

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Enantiomeric separation of chiral pharmaceuticals was carried out by means of in capillary liquid chromatography (CLC) and pressurized capillary electrochromatography (pCEC) using a vancomycin chiral stationary phase (CSP). A 100 μm I.D. fused-silica capillary was packed with 5 μm diameter silica particles modified with vancomycin. Enantiomeric resolution of fifteen β-receptor blockers and analogs was studied by polar organic CLC mode and reversed-phase pCEC mode using mobile phases containing methanol-isopropanol-acetic acid-triethylamine and TEAA buffer-methanol, respectively. Several factors affecting chiral separation were investigated in both CLC and pCEC mode. Good enantiomeric resolution was achieved by CLC mode for propranolol, celiprolol, esmolol, bisoprolol, atenolol, metoprolol and carteolol using methanol-isopropanol-acetic acid-triethylamine (70 : 30 : 0.05 : 0.05, v/v/v/v) as mobile phase and for clenbuterol, bambuterol, terbutaline, and salbutamol using methanol-isopropanol-acetic acid-triethylamine (50 : 50 : 0.05 : 005 or 50 : 50: 0.025 : 0.05, v/v/v/v) as mobile phase. The baseline was achieved by pCEC mode for the separation of esmolol, bisoprolol, atenolol, metoprolol, carteolol in the mobile phase containing MeOH-0.05%TEAA (pH 7.0) (90 : 10, v/v) (−10 kV), and that of propranolol and celiprolol in the mobile phase containing MeOH-0.025%TEAA (pH 7.0) (90 : 10, v/v)(−10 kV). Comparative enantioseparations performed in polar organic CLC and reversed phase pCEC mode revealed significant difference.

1. Introduction

Enantioseparation in capillary liquid chromatography (CLC) and capillary electrochromatography (CEC) have become popular due to their important advantages, such as a low consumption of organic modifiers, a reduced sample amount, and easier coupling with a mass spectrometer compared to conventional HPLC (Chankvetadze et al. 2001, 2002, 2003; Coufal et al. 2002; Otsuka et al. 2000).
CLC can be considered as the miniaturised version of HPLC. The published data have illustrated that CLC possessed a better sensitivity than conventional HPLC, but less mobile phase will be consumed (Bruns et al. 1992; Meyring et al. 2000). CEC is a separation technique that combines the separation efficiency of capillary electrophoresis (CE) with the selectivity of high performance liquid chromatography (HPLC). Analytes can be separated on the basis of the chromatographic and electromotive principle between the stationary and the mobile phase. Now, as its superexcellent feature, more people focus on chiral separation by using CEC (Fanali et al. 2003; Jinno et al. 2000; Kafkova et al. 2006; Lelievre et al. 1996). However, in practice, when CEC was used without pressure, problems associated with bubbles formation and column dry-out occurred. But these problems can be solved by a pCEC system, in which the mobile phase is driven by electroosmotic flow (EOF), as well as pressurized flow (Steiner and Scherer 2000; Ru et al. 2000; Zhang et al. 2003), therefore the kinds of CLC and pCEC could be carried out in this system.
Various chiral stationary phases (CSP) have been applied to chiral separation using capillary columns (Berthod et al. 2004; Chen et al. 2003; Chankvetadze et al. 2000; Girod et al. 2000; Kartozia et al. 2005; Lloyd et al. 1994; Orazio et al. 2005). Among them, the glycopeptide macrocyclic antibiotic vancomycin exhibited a high enantiorecognition capability. Since Armstrong et al. (1994) firstly reported vancomycin as a chiral selector for CE, numerous studies were published extending the applicability of vancomycin (Ding et al. 2004; Fanali et al. 2001; Karlsson et al. 2000a; Lammerhofer 2005; Orazio et al. 2005).
The molecular structure of vancomycin has a characteristic basket shape with three hydrophobic macrocyclic rings and possesses several stereogenic centres and functional groups, allowing multiple interactions with chiral analytes. These interactions may include hydrophobic inclusion interaction, hydrogen bonding, dipole-dipole, π–π interaction, as well as steric repulsion (Armstrong et al. 1997; Ward et al. 1997). It was reported that dipole-dipole and π–π interactions participate in the process of chiral recog-

Table 1: Effect of different organic modifiers on chiral separation of propranolol and celiprolol by CLC

Mobile phase	Propranolol	Celiprolol
MeOH-TEA-HOAc (100 : 0.1 : 0.1, v/v/v)	0.8	0.9
MeOH-ACN-TEA-HOAc (90 : 10 : 0.1 : 0.1,v/v/v/v)	0.8	0.9
MeOH-EtOH-TEA-HOAc (90 : 10 : 0.1 : 0.1, v/v/v/v)	0.9	1.1
MeOH-isoPrOH-TEA-HOAc (90 : 10 : 0.1 : 0.1, v/v/v/v)	1.0	1.3

Flow rate was 0.02 ml/min.

nition for most of chiral compounds in the organic polar mode, while $\pi-\pi$ and hydrophobic interactions in the reversed-phase mode. Hydrogen bonding contributed only little to chiral separation, although it intensively influenced the material retention (Berthod et al. 1996).
In this study, we developed CLC and pCEC methods using vancomycin modified silica CSP for the separation of β-receptor blockers and analogs of interest. Different experimental factors were investigated to optimize the enantioseparation of the studied drugs both in CLC and pCEC.

2. Investigations, results and discussion

2.1. Polar organic CLC mode

2.1.1. Effect of organic modifier type on chiral separation by CLC mode

Preliminary experiments were carried out according to the method proposed by Yao et al. (2004) and Kang et al. (2002) with the mobile phase consisting of MeOH-TEA-HOAC (100 : 0.1 : 0.1, v/v/v), but the baseline separation between two enantiomers was not achieved. The difference was that the column used in Yao's and Kang's paper was longer than ours. To achieve good enantiomeric resolution the effect of different organic modifiers (ACN, EtOH and iso-PrOH) in mobile phase on enantioseparation was examined. The ratio of methanol to each organic modifier was set at 90 : 10, the content of TEA and HOAC was 0.1% in mobile phase respectively. The results (Table 1) show that the addition of organic modifiers to the mobile phase was helpful in improving resolution. Especially the isopropanol improved separation more than other modifiers. Generally, hydrogen-hydrogen and $\pi-\pi$ interactions were the major forces in the process of chiral recognition using polar organic solvents as mobile phase. Adding of EtOH iso-PrOH and ACN could increase hydrogen interactions in MeOH mobile phase, however, $\pi-\pi$ interactions may decrease when ACN used as organic modifier, with no change of resolution obtained by adding ACN.

2.1.2. Effect of ratio of MeOH and iso-PrOH on chiral separation

Table 2 shows the effect of MeOH to iso-PrOH ratio on separation of β-receptor blockers and its analogs. It was observed that the resolution between two enantiomers of each analyte was generally increasing, when the iso-PrOH concentration was increased in the mobile phase from 0 to 30% with a decrease of theoretical plate exception of bambuterol, terbutaline, isoprenaline and carvedilol. But the resolution was stable when iso-PrOH content was increased from 30% to 50%. As shown in Table 2, the enantioseparation of β-receptor blockers **1–7** was much easier than those of their analogs **8–13** under the same chromatographic conditions.

2.1.3. Effect of the content of HOAc and TEA on chiral separation

The resolution of drugs was better when HOAc and TEA were used together than if each of them was used alone. To find the optimal content of HOAc and TEA, both of their contents were evaluated. To examine the effect of the content of HOAc on enantioseparation of the analytes, the amount of TEA was fixed at 0.1% (v/v) and the ratio of MeOH: iso-PrOH at 70 : 30 (v/v). The content of HOAc was varied from 0.025% to 0.3% and the results are shown in Fig. 1. The lower the HOAc amounts in mobile phase, the greater the resolution was. The resolution was almost the same when the content of HOAc was between 0.05% and 0.025%, both could make good resolution for enantiomers.
To evaluate the effect of the content of TEA on chiral separation, a fixed amount of HOAc (0.05 %) and a ratio

Table 2: Effect of the ratio of methanol and isopropanol on chiral separation of β-receptor blockers and its analogs

Compd.	MeOH : iso-PrOH											
	100 : 0		90 : 10		80 : 20		70 : 30		60 : 40		50 : 50	
	Rs	N_1	Rs	N_1	Rs	N_1	Rs	N_1	Rs	N_1	Rs	N_1
1 Propranolol	0.8	3952	1.0	3652	1.1	3502	1.4	3357	1.4	3005		
2 Celiprolol	0.9	3339	1.3	2712	1.4	2700	1.6	2600	1.6	2547		
3 Esmolol	0.9	3282	1.0	3148	1.1	2902	1.2	2743	1.2	1599		
4 Bisoprolol	0.6	2665	1.0	3244	1.1	3049	1.3	2516	1.3	2255		
5 Atenolol	0.8	2342	0.9	2669	1.0	2469	1.0	2178	1.2	1096		
6 Metoprolol	1.1	2356	1.2	2204			1.4	2189			1.4	2047
7 Carteolol	1.4	2451	1.5	2377			1.6	1729			1.6	1154
8 Clenbuterol							0.8	3188	0.9	2800	0.9	1712
9 Bambuterol	–	931	0.5	5008			0.7	3298	0.7	2881	0.9	2249
10 Terbutaline	–	1087	–	628			0.8	4940	0.8	2529	0.9	1119
11 Isoprenaline	–	755	–	731			0.3	5947	0.2	940	0.3	799
12 Salbutamol	0.8	4316	0.8	3935			0.9	2652	0.9	1990	1.0	1608
13 Carvedilol	–	895	0.3	4675			0.6	3725	0.6	3404	0.6	2771
14 Phenylpropanolamine	–	958	–	763			–	667	–	633	–	435
15 Methylephedrine	–	3599	–	3596			–	3572	–	3541	–	3209

The content of TEA and HOAc was 0.1 % in mobile phase, respectively; flow rate was 0.02 ml/min; without the voltage; N_1, theoretical plate of the first eluting enantiomer; the white spaces mean the samples not analyzed

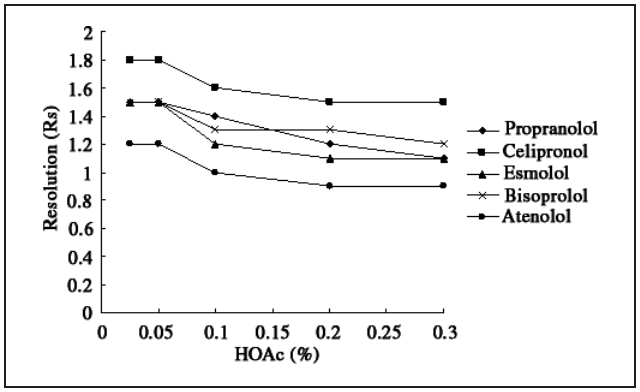

Fig. 1: Effect of the content of HOAc on chiral separation of β-receptor blockers by CLC
Mobile phase: MeOH-iso-PrOH-TEA (70 : 30 : 0.1) with different amounts of HOAc; flow rate was 0.02 ml/min

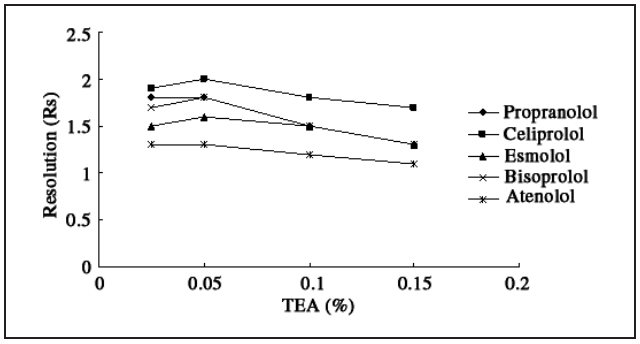

Fig. 2: Effect of the content of TEA on chiral separation of β-receptor blockers
Mobile phase : MeOH : i-PrOH : HOAC (70 : 30 : 0.05) with different amounts of TEA; flow rate was 0.02 ml/min

of MeOH-iso-PrOH 70 : 30 were used. The content of TEA was varied from 0.025%-0.15% (Fig. 2). The enantioresolution was better under lower concentration of TEA. The best resolution was achieved when the content of TEA was 0.05%. The TEA is a strong base and can compete with solutes for hydrogen bonding sites and sites, which decreased the interactions between solutes and CSP. In general, lower TEA content was helpful for the separation of the studied drugs. The results were similar to previous data achieved in the CEC mode (Karlsson et al. 2000b; Wikstrom et al. 2000).

2.1.4. Separation of compounds

Most of the studied drugs were separated successfully or partly separated at the optimal conditions except for phenylpropanolamine and methylephedrine by polar organic CLC mode, as shown in Table 3 and Fig. 3.

2.2. Reversed-phase pCEC mode

Compared to CLC, pCEC can bring some changes: the electrical field allows fine-tuning of the retention of solutes and improves selectivity (Yao et al. 2004; Zhang et al. 2003). In this work, a reversed-phase pCEC mode was applied. Based on the literature (Bosakova et al. 2005) MeOH was selected as organic modifier of the mobile phase in this study. The aqueous part of the mobile phase was composed of TEAA buffer, with different pH. As shown in Table 4 and Fig. 4, most of the studied drugs were successfully or partly enantioseparated by reversed-phase pCEC, except for terbutaline, isoprenaline, phenylpropanolamine and methylephedrine.

2.2.1. Effect of ratio of TEAA buffer and MeOH on chiral separation

Earlier studies (Steiner and Scherer 2000) illustrated that higher resolution values were obtained at higher MeOH content. Similar results were attained in our work, namely the resolution of studied drugs increased with increasing contents of MeOH, as shown in Table 5. However the value of k1 varied in an unpredicable manner, it might be affected by the voltage and the variation of TEAA buffer to MeOH ratio.

2.2.2. Effect of pH value of TEAA buffer on chiral separation

The pH of the buffer was another very important experimental parameter, so the effect of pH on chiral separation of studied drugs was investigated, as shown in Table 6. A decrease in pH of TEAA buffer caused a decrease in resolution of the studied drugs. The resolution of the studied drugs was much higher at pH 6.0 than at pH 4.0, but almost the same at pH 6.0, pH 7.0 and pH 8.0. It was disappointing that terbutaline, methylephedrine, phenylpropanolamine and isoprenaline could not be separated under

Table 3: The results ofchiral separation of 15 β-receptor blockers and analogs

Compo.	Mobile phase	k_1	k_2	α	N_1	N_2	Rs
Propranolol	A	3.57	4.26	1.19	2709	2590	1.8
Celiprolol	A	2.83	3.56	1.26	2159	2048	2.0
Esmolol	A	3.10	3.67	1.18	2331	2744	1.6
Bisoprolol	A	2.52	3.09	1.23	2171	2675	1.8
Atenolol	A	6.33	7.33	1.16	1976	1757	1.3
Metoprolol	A	2.37	2.84	1.20	2285	2505	1.6
Carteolol	A	4.45	5.57	1.25	1742	1498	2.7
Carvedilol	A	2.88	3.12	1.08	2465	934	0.6
Clenbuterol	B	2.79	3.27	1.17	1991	2343	1.4
Bambuterol	B	4.99	5.64	1.13	2075	1372	1.0
Terbutaline	C	3.60	4.19	1.16	1771	1707	1.2
Isoprenaline	A	2.70	2.82	1.04	9237	2419	0.5
Salbutamol	B	3.06	3.67	1.20	1477	1472	1.3
Phenylprop-anolamine	B	3.65	3.65	1.00	2560	2560	–
Methylephe-drine	B	4.86	4.86	1.00	3306	3306	–

Mobile phase compositions (MeOH-iso-PrOH-TEA-HOAc, v/v/v/v) : A (70 : 30 : 0.05 : 0.05); B (50 : 50 : 0.05 : 0.05); C (50 : 50 : 0.05 : 0.025); flow rate was 0.02 ml/min; k_1, retention factor of the first eluting enantiomer; k_2, retention factor of the second eluting enantiomer; α, separation factor; Rs, resolution

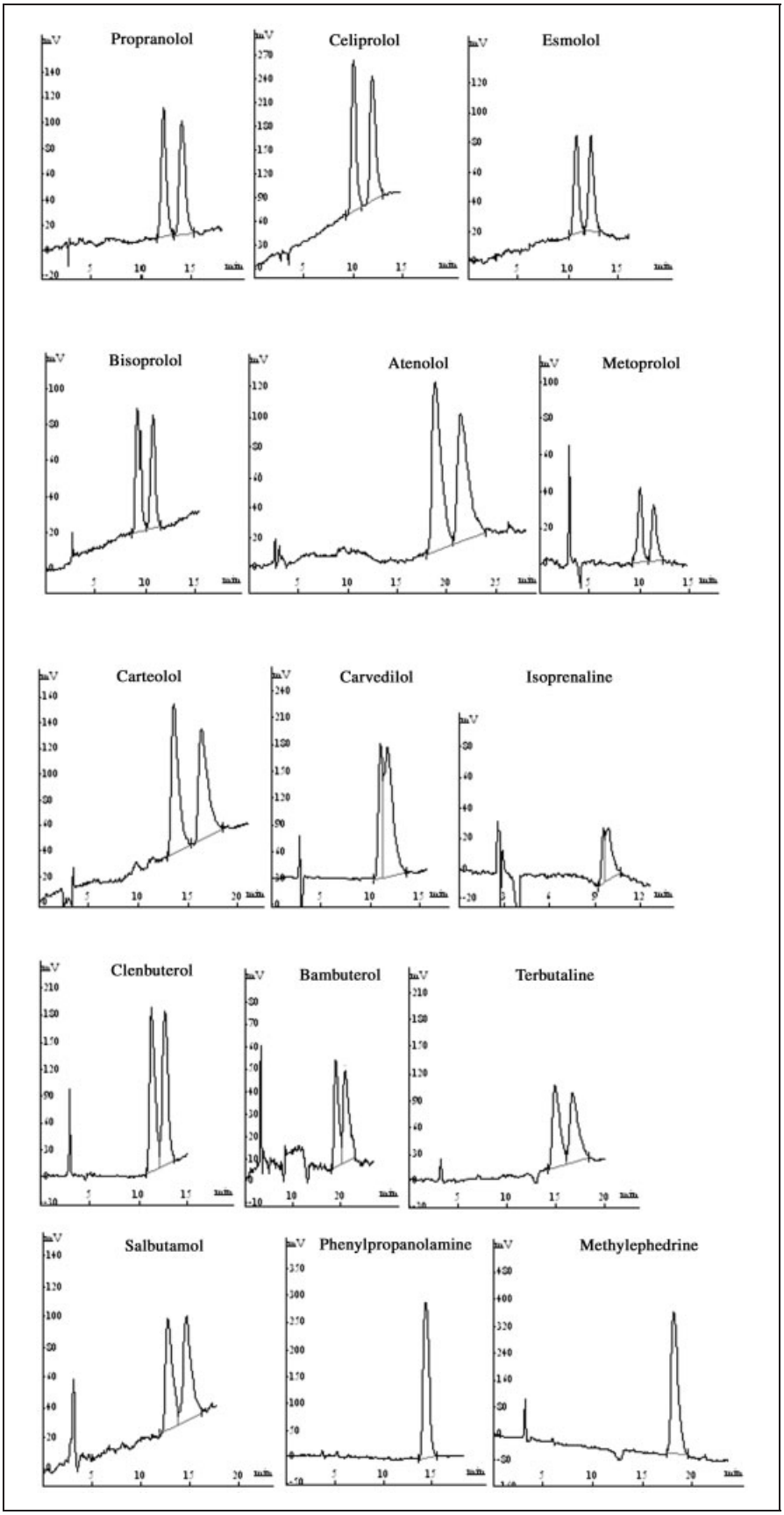

Fig. 3:
Typical chromatograms for the enantiomeric separation of the studied β-receptor blockers and analogs on vancomycin CSP by polar organic CLC mode. Chromatographic condition of each chiral analyte as described in Table 3

any conditions. Apparently, the interactions between the test solutes and vancomycin were enhanced, when high pH buffers were used by reversed-phase pCEC. The tendency about pH variation was similar to the previous results for the chiral separation of basic compounds on vancomycin CSP by HPLC (Bosakova et al. 2005). These basic drugs were ionized in acid mobile phase, resulting in the fact that the retention time shorted and the resolution between enantiomers decreased. In fact changing pH could influence the interaction stability of vancomycin

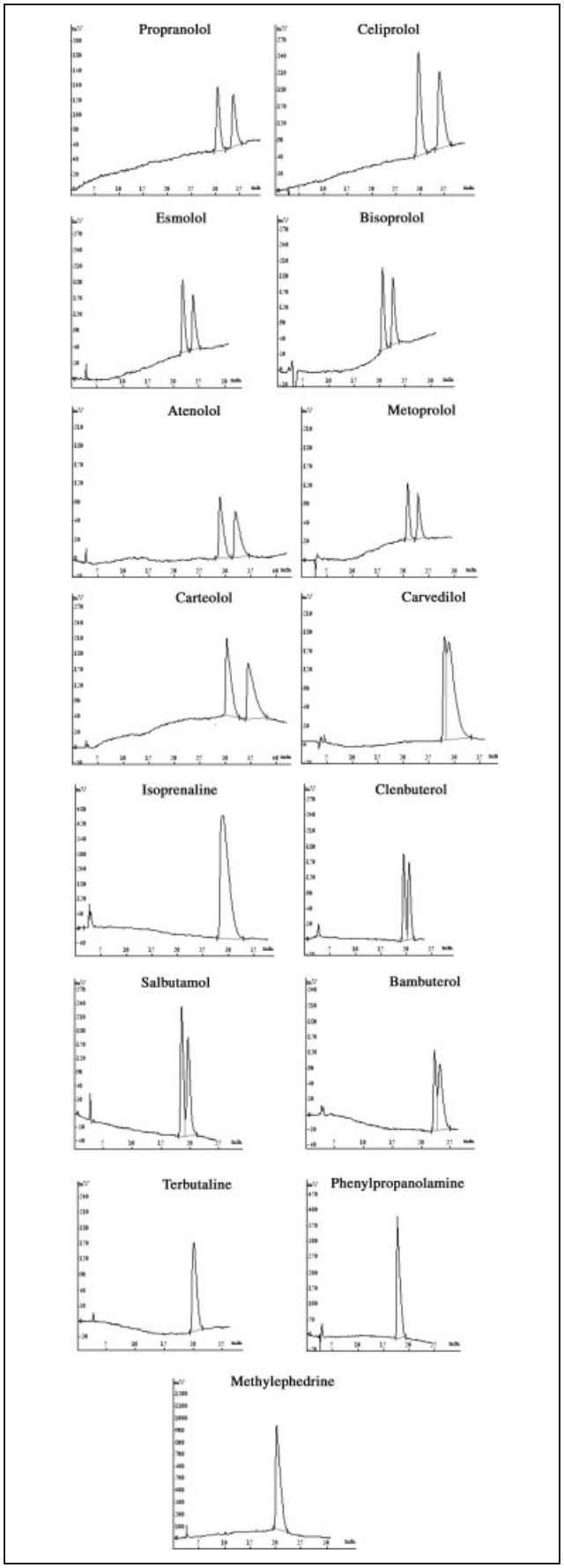

Fig. 4: Typical chromatograms for the enantiomeric separation of the studied β-receptor blockers and analogs on vancomycin CSP by reversed-phase pCEC mode
Chromatographic condition of each chiral analyte as described in Table 4

CSP with analytes, thus a suitable pH should be selected to attain a good resolution of the analytes.

2.2.3. Effect of voltage on chiral separation

In this pCEC system, the inlet is cathode(−) and the outlet is anode(+), therefore the negative(−) voltage was used, that means the EOF is going from cathode to anode. The EOF generates a flat profile, which improves the separation efficiencies generated in pCEC when compared to the laminar flow obtained in pumped LC separations (Altria 1999).

To evaluate the effect of the voltage on chiral separation, $-15 \sim 0$ kV voltage was used. According to the results, the best resolution of studied drugs was achieved when the voltage was at −10 kV. But the resolution with voltage ($-5 \sim -15$ kV) was better than that without voltage (0 kV). It was to say that the resolution between enantiomorphs could be tuned by adjusting the electrical field on the column. Obviously, electrophoretic mobility of the studied drugs contributed to the resolution.

2.2.4. Effect of TEAA concentration on chiral separation

Fig. 5 shows the resolution at different TEAA concentration in mobile phase. When the TEAA concentration at pH 7.0 was increased from 0.05% to 0.2%, resolution of the compounds was reduced slightly. Best resolution of the most studied drugs was obtained at the concentration of 0.05%, but that of celiprolol and propranolol was at 0.025%. In order to get a higher resolution and efficiency, 0.05% or 0.025% of TEAA content was used. From Fig. 5, it could be concluded that the concentration of TEAA influenced the chiral separation of some drugs. And lower concentration of TEAA may improve the interaction between enantiomers and CSP, thus it would be advantageous for the enantiomeric separation of chiral drugs.

Chiral separation of drugs had been studied in reversed-phase mode by CEC, but the results were surprisingly disappointing, only thalidomide was successfully separated (Wikstrom et al. 2000). However, a reversed-phase pCEC mode was successfully established in this study, which was a good compensation to the reversed-phase CEC mode.

According to the results, resolutions of aryloxypropanolamines (such as propranolol and celiprolol) were better than those of phenylethanolamines (such as bambuterol and clenbuterol) both in polar organic CLC mode and reversed phase pCEC mode, the similar result was reported by Wikstrom et al. (2000) in vancomycin CSP by CEC. Comparing to phenylethanolamines, aryloxypropanolamines drugs have a $-OCH_2$-group between the aryl ring and the β-carbon atom near the chiral center, which may be advantageous for side-chain of aryloxypropanolamines to enter into hydrophobic rings of vancomycin. Thus it would improve the chiral recognition for vancomycin CSP to these drugs. No enantiorecognition was observed for phenylpropanolamine and methylephedrine, it would be assumed that the electron donor groups on phenyl ring may play a great role for these compounds.

It was interesting that the resolution of aryloxypropanolamines except for carteolol increased while phenylethanolamines decreased in the reversed phase pCEC mode compared to the polar organic CLC mode according to Tables 3 and 4. The $-OCH_2$-group of aryloxypropanolamines might participate in the process of chiral recogni-

Table 4: The results of chiral separation of 15 β-receptor blockers and analogs in reversed phase mode by pCEC

Compd.	Mobile phase	k_1	k_2	α	N_1	N_2	Rs
Propranolol	A	10.30	11.46	1.11	9259	8419	2.3
Celiprolol	A	9.97	11.58	1.16	6221	4806	4.3
Esmolol	B	6.67	7.34	1.10	8818	6528	1.9
Bisoprolol	B	5.56	6.20	1.11	8946	6759	2.0
Atenolol	B	9.83	10.99	1.12	5349	3957	1.7
Metoprolol	B	6.66	7.40	1.11	8255	7356	2.0
Carteolol	B	10.16	11.72	1.15	4739	2758	2.0
Carvedilol	B	9.18	9.51	1.04	9681	1693	0.4
Clenbuterol	B	6.09	6.48	1.06	9455	8319	1.2
Bambuterol	B	7.24	7.57	1.04	9765	4183	0.7
Terbutaline	B	6.56	6.56	1.00	3247	3247	–
Isoprenaline	B	9.63	9.63	1.00	1439	1439	–
Salbutamol	B	5.93	6.34	1.07	7435	5438	1.1
Phenylpropanolamine	B	5.87	5.87	1.00	3905	3905	–
Methylephedrine	B	6.98	6.98	1.00	2425	2425	–

Mobile Phase was A: MeOH – 0.025% TEAA (pH 7.0) (90 : 10, v/v); B: MeOH – 0.05% TEAA (pH 7.0) (90 : 10, v/v);
Voltage was −10 kV; flow rate was 0.02 ml/min; N_1, theoretical plate of the first eluting enantiomer; N_2, theoretical plate of the second eluting enantiomer

Table 5: Effect of ratio of TEAA buffer and MeOH on chiral separation

Compd.	0.1%TEAA (pH 8.0)-MeOH					
	5 : 95		10 : 90	20 : 80	30 : 70	40 : 60
	k_1	4.77	5.71	4.00	3.04	4.27
Propanol	α	1.13	1.13	1.10	1.08	1.07
	Rs	1.9	2.0	1.50	1.0	0.8
	k_1	4.82	5.74	3.71	2.82	3.23
Celiprolol	α	1.17	1.17	1.14	1.12	1.10
	Rs	2.2	2.3	1.7	1.5	1.2
	k_1	4.09	4.80	3.12	2.26	2.83
Esmolol	α	1.12	1.11	1.10	1.08	1.05
	Rs	1.8	1.8	1.5	1.0	0.7
	k_1	3.67	4.34	2.38	2.02	2.77
Bisoprolol	α	1.12	1.12	1.10	1.08	1.06
	Rs	1.6	1.7	1.2	1.0	0.8
	k_1	6.00	7.19	3.98	2.48	2.77
Atenolol	α	1.12	1.12	1.09	1.08	1.06
k1 6.00	Rs	1.7	1.7	1.35	1.0	0.9
	k_1	3.98	4.35	2.99	2.08	2.68
Metoprolol	α	1.12	1.12	1.10	1.08	1.06
	Rs	1.8	1.9	1.3	1.0	0.9
	k_1	6.37	6.92	4.56	3.16	3.94
Carteolol	α	1.16	1.16	1.12	1.10	1.08
	Rs	2.0	2.0	1.6	1.3	1.1
	k_1	4.74	4.85	4.36	4.31	7.60
Carvedilol	α	1.04	1.04	1.00	1.00	1.00
	Rs	0.4	0.4	–	–	–
	k_1	4.07	4.26	3.04	2.38	3.37
Clenbuterol	α	1.06	1.07	1.04	1.00	1.00
	Rs	1.0	1.0	0.6	–	–
	k_1	4.24	5.26	3.37	2.44	3.44
Bambuterol	α	1.05	1.05	1.00	1.00	1.00
	Rs	0.7	0.7	–	–	–
	k_1	4.23	4.61	3.05	1.82	2.10
Terbutaline	α	1.00	1.00	1.00	1.00	1.00
	Rs	–	–	–	–	–
	k_1		4.33	2.98	2.06	2.15
Salbutamol	α		1.07	1.04	1.03	1.00
	Rs		1.0	0.6	0.3	–
	k_1		5.10		2.24	4.69
Isoprenaline	α		1.00		1.00	1.00
	R		–		–	–
	k_1	3.95	5.09		1.21	2.39
Methylephedrine	α	1.00	1.00		1.00	1.00
	Rs	–	–		–	–
Phenylpropanolamine	k_1		3.94		1.90	3.01
	α		1.00		1.00	1.00
	Rs		–		–	–

Voltage was −10 kV; flow rate was 0.02 ml/min

Table 6: Effect of pH value of TEAA buffer on chiral separation

Compd.	Resolution (Rs)			
	pH 4.0	pH 6.0	pH 7.0	pH 8.0
Propranolol	0.3	1.8	2.0	2.0
Celiprolol	0.8	2.1	2.5	2.3
Esmolol	0.0	1.5	1.9	1.8
Bisoprolol	0.5	1.5	2.0	1.7
Atenolol	0.4	1.4	1.7	1.7
Metoprolol	0.5	1.6	2.0	1.9
Carteolol	0.6	1.5	2.0	2.0
Carvedilol	0.0	0.4	0.4	0.4
Clenbuterol	0.0	1.0	1.0	1.0
Bambuterol	0.0	0.7	0.7	0.7
Terbutaline	0.0	0.0	0.0	0.0
Isoprenaline	0.0	0.0	0.0	0.0
Salbutamol	0.0	0.8	1.0	1.0
Phenylpropanolamine	0.0	0.0	0.0	0.0
Methylephedrine	0.0	0.0	0.0	0.0

TEAA buffer-MeOH (10 : 90, v/v); Voltage was −10 kV; flow rate was 0.02 ml/min

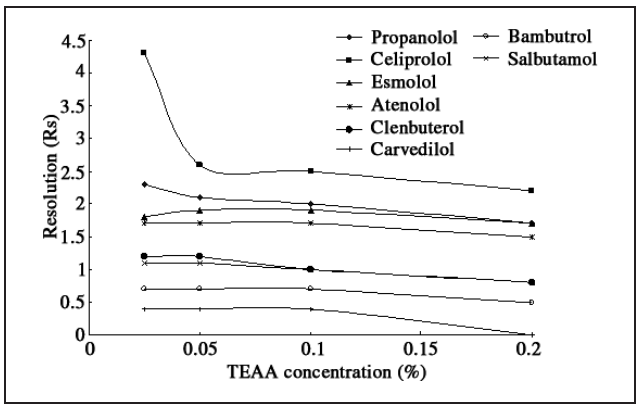

Fig. 5: Effect of TEAA concentration on chiral separation by pCEC. Mobile phase was TEAA (pH 7.0)-MeOH (10 : 90,v/v); voltage was −10 kV; flow rate was 0.02 ml/min

tion in reversed phase through hydrogen bonding interactions. It would be sufficient to say that different chiral recognition was performed applying polar organic CLC and reversed phase pCEC mode.

3. Experimental

3.1. Chemicals and reagents

Acetonitrile (ACN), isopropanol (iso-PrOH), ethanol (EtOH), HPLC grade, were purchased from Tedia company, Inc(USA). Methanol (MeOH), HPLC grade, was purchased from Shanghai Ludu chemical reagents company (Shanghai, China). Triethylamine (TEA), analytical grade, was purchased from Shanghai chemical reagents company (Shanghai, China). Glacial acetic acid (HOAc), analytical grade, was purchased from Hangzhou chemical reagents company (Hangzhou,China). The studied β-receptor blockers and analogs (see Fig. 3) were obtained from different commercial sources. Distilled and deionized water was used.

3.2. Apparatus

pCEC was carried out on a TriSep™–2010GV CEC system (Unimicro Technologies, Inc., Pleasanton, CA, USA) which comprised two pumps, one high voltage power supply (+30 kv and −30 kv), one variable-wavelength UV-Vis detector, one micro fluid manipulation module (manual injector with a small injection volume of 1 μl, a backpressure regulator to maintain a constant pressure on the column, and a splitting cross) and one data acquisition module. The column obtained from Shanghai Unimicro Technologies, Inc, (Shanghai, China), was packed with 5 μm vancomycin CSP (effective length 18.5 cm × 100 μm I.D). DU 640 UV spectrophotometer was purchased from Beckman (USA).

3.3. Methods

Analyte stock solutions were prepared in MeOH at a concentration of 1.0 mg/ml. Sample solutions were prepared by dilution of each stock solution with mobile phase. The composition of mobile phase was MeOH-iso-PrOH-TEA-HOAc in polar organic CLC mode and triethylamine acetate (TEAA)buffer-MeOH in reversed-phase pCEC mode. The flow-rate was set at 0.02 ml/min.The measurements were carried out at the room temperature. The detection wavelength for propranolol, celiprolol, esmolol, bisoprolol, atenolol, metoprolol, carteolol, carvedilol was 230 nm, for clenbuterol, bambuterol, terbutaline 220 nm, for phenylpropanolamine 215 nm, and for isoprenaline, salbutamol, methylephedrine 210 nm, determined by UV spectrophotometer scanning.

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