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Comparison of vancomycin-based stationary phases with different chiral selector coverage for enantioselective separation of selected drugs in high-performance liquid chromatography

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Abstract

Two vancomycin-based chiral stationary phases (CSPs) with different coverage of the chiral selector vancomycin (Chirobiotic V and Chirobiotic V2) were compared. β-Blockers and profens, as structurally diverse groups of drugs, were chosen as analytes. Retention and enantioseparation of β-blockers were studied in reversed-phase (RP) and polar-organic (PO) separation modes. Higher retention and better enantioresolution were obtained on the CSP with higher coverage of vancomycin in the both separation modes. Baseline separation of four β-blockers (eight enantiomers) in the PO mode was achieved on the Chirobiotic V2 column within 15 min. The enantioseparation of profens did not bring so excellent and easy to interpret results. Higher retention of profens on the Chirobiotic V2 column was not always accompanied by an improvement of their chiral separation in the RP mode. The polar-organic mode was not suitable for these derivatives at all. The most interesting result was obtained with flobufen; its chiral center is further away from the rigid part of the molecule, which mostly causes difficulties in enantioselective recognition. Nevertheless, the enantiomers of flobufen were shown to be much better (baseline) resolved on the CSP with lower coverage of the chiral selector (Chirobiotic V).

Keywords: Vancomycin; Selector coverage; Chiral stationary phases; Enantiomeric separations; Drugs

1. Introduction

Study of enantioselective properties of pharmaceutical substances is an important stage of drug evaluation. Chiral high-performance liquid chromatography is a fast, selective and efficient technique, successfully employed for determination of enantiomers of drugs. In this method, chiral selectors can be used as mobile phase additives or as part of the stationary phase. The most common HPLC approach for resolving of enantiomers involves the use of chiral stationary phases (CSPs) [1]. Many various CSPs have been introduced and searching for new ones continues [2]. One of the resent trends in new chiral stationary phase development is, along with introduction of novel or modified chiral selectors, attempt to prepare supports with higher coverage of chiral selectors in order to get improved stereoselective interaction with analytes [3].

The amount of chiral selector used on the support can affect retention, selectivity, efficiency and enantioresolution. The influence of the amount of chiral selector on enantioseparation behavior of various compounds was studied in capillary electrophoresis [4–8] and electrochromatography [9–11] in details but only few studies addressed this problem on the HPLC stationary phase performance [12–15]. The effect of particle size and pore size of underivatized [12] and aminopropylated silica support [13] on loading amount of cellulose tris(phenylcarbamate) and cellulose tris(3,5-dimethylphenylcarbamate) was studied. The influence of pore size of silica gel, coating solvents, loading amount of cellulose tris(3,5-dimethylphenylcarbamate) and column temperature on chiral discrimination of structurally different racemates were investigated [14]. Silica gel having a large pore size and small surface area showed higher chiral

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recognition. Amount of the adsorbed cellulose tris(3,5-dimethylphenylcarbamate) influenced the enantioselective recognition ability, however, it also depends on the character of examined racemate. It was shown that the enantioresolution of some racemates decreased with the increased coating amount of cellulose tris(3,5-dimethylphenylcarbamate). Three CSPs with different contents of covalently bonded ristocetin A (macrocyclic antibiotic) to silica gel support were prepared and evaluated for enantioresolution of selected amino acids in reversed-phase, polar-organic, and normal-phase separation modes [15]. The comparison of retention parameters (k, α, R_s) obtained in the individual separation systems showed that the coverage of the chiral selector has a profound effect on the chromatographic behavior of the more retained enantiomers.

In the past decade, the macrocyclic antibiotic CSPs became a very important tool for separation of a wide range of structurally different chiral compounds. At present, the most useful and popular of them, based on teicoplanin and vancomycin chiral selectors, are commercially available also in the modified forms—columns Chirobiotic TAG with bonded teicoplanin aglycon, Chirobiotic T2 with higher content of teicoplanin bonded to the silica gel support and Chirobiotic V2 with higher content of vancomycin bonded to the silica gel support [3].

Comparison of enantioresolution capability of Chirobiotic V and V2 columns was performed for racemic mixture of tolperisone (muscle relaxant) in reversed-phase separation mode. The better value of chiral resolution was obtained on the column with higher content of vancomycin [3].

In this study, two groups of structurally different chiral drugs, namely β-blockers and profens, have been selected.

β-Blockers, relatively less polar, basic compounds, contain hydroxyl and amine groups located close to their chiral center and at least one aromatic moiety which together are capable of providing several potential interactions, e.g. hydrogen-bonding or π–π interactions. Some CSPs were successfully tested for enantioseparation of one or a set of β-blockers, among them CSPs based on polysaccharides [16–18], cyclodextrines [19] and macrocyclic antibiotics [16,20–22] dominated. The chiral stationary phase based on vancomycin (Chirobiotic V) was applied for chiral recognition of commercially available [16,22], as well as, newly synthesized analogues [23] of β-blockers. Racemic alprenolol, pindolol, propranolol and metoprolol were baseline-resolved both in polar-organic and in reversed-phase separation modes [16].

More polar profens (2-arylpropionic acid derivatives) are characterized by a stereogenic center adjacent to the carboxylic acid moiety. The most frequently used CSPs for enantioseparation of individual profens are based on cyclodextrin [24–26], polysaccharide [27–30] and macrocyclic antibiotic [16,21,22,31–34] chiral selectors. A set of profens (namely fenoprofen, flurbiprofen, ibuprofen, indoprofen, ketoprofen and suprofen) was baseline-enantioresolved on avoparcin based CSP in the normal separation mode [32] and on the teicoplanin based CSP in the reversed separation mode [31]. The vancomycin based CSP (Chirobiotic V) was shown to be suitable for chiral separation of flurbiprofen and ketoprofen in the reversed-phase separation mode with small amount of organic modifier in the mobile phase [34]. Improved chiral selectivity for flurbiprofen was observed after addition of vancomycin to the mobile phase on the Chirobiotic V column [33].

The aim of our work is comparison of two vancomycin based chiral stationary phases that varied in the chiral selector coverage—Chirobiotic V and Chirobiotic V2. Structurally different drugs, β-blockers and profens, are studied because their different enantioselective behavior can be expected. Effects of mobile phase composition on retention and enantioseparation are studied in two separation modes—reversed-phase and polar-organic ones.

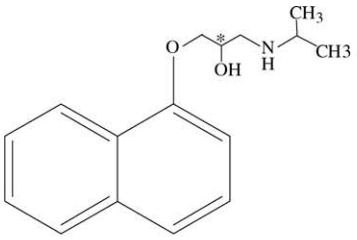
2. Experimental

2.1. Chemicals

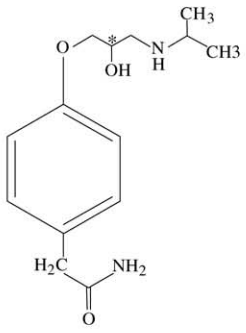
All the tested standards of β-blockers (atenolol, pindolol, acebutolol, propranolol, oxprenolol and alprenolol) and profens (fenoprofen, carprofen, flurbiprofen, ketoprofen, ibuprofen, flobufen, suprofen and indoprofen) (see Fig. 1) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The mobile phases were prepared from the following compounds and solvents: triethylamine (Sigma-Aldrich), purity > 99%; glacial acetic acid (Lachema Brno, Czech Republic), analytical grade purity; methanol (Merck, Darmstadt, Germany), purity for chromatography. Distilled and deionized water was used throughout the experiments (Milli-Q water purification system, Millipore, USA).

2.2. Instrumentation and chromatographic conditions

The HPLC equipment (Dionex Corporation, Sunnyvale, CA, USA) consisted of a P 580 Pump, an UVD 170S detector and Rheodyne injection valve Model 7125 (Cotati, CA, USA) with 10-μL sample loop. Signal acquisition and data handling were performed with the PC Chromeleon PeakNet 6 software. Two commercially available steel columns (250 mm × 4.6 mm i.d.), particle size 5 μm (ASTEC, Whippany, NY, USA) with different contents of vancomycin bonded to silica gel support—Chirobiotic V and Chirobiotic V2 (with higher content of vancomycin) were used in the reversed-phase (RP) and polar-organic (PO) separation modes. Mixtures of different volume ratios of methanol (MeOH) and 0.1–1.0% triethylamine acetate (TEAA) buffer, pH 3.0, 4.0 or 5.0 were employed in the RP separation mode. The buffer pH was adjusted with acetic acid to required values before the addition of methanol. In the PO separation mode methanol with small amount of acetic acid and triethylamine in various volume ratios were used as mobile phases. The detection wavelength was 230 nm (with exception as specified below). The measurements were carried out



Propranolol



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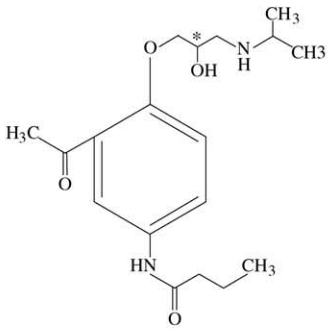
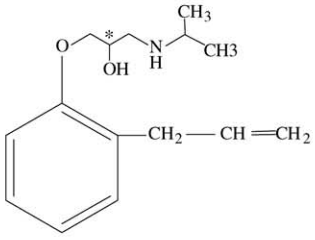
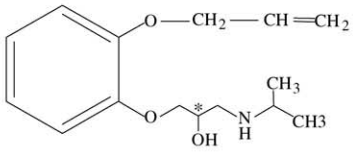


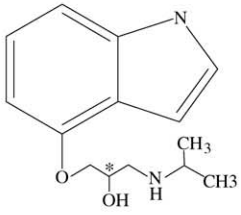
Acebutolol



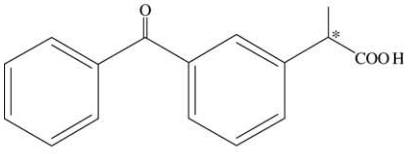
Alprenolol



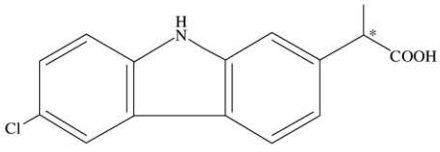
Oxprenolol



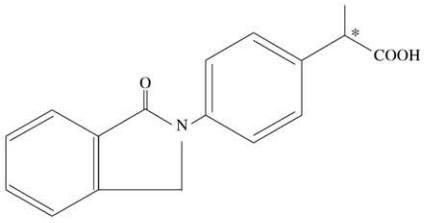
Pindolol



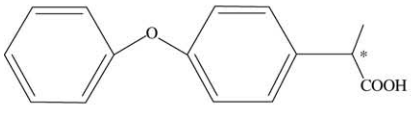
Ketoprofen



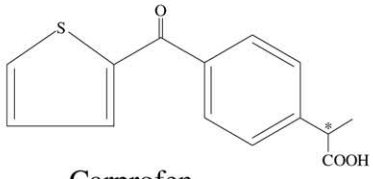
Suprofen



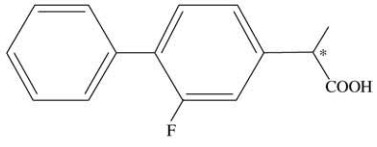
Indoprofen



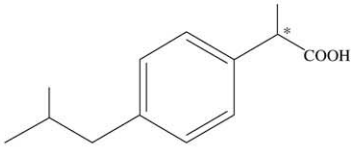
Fenoprofen



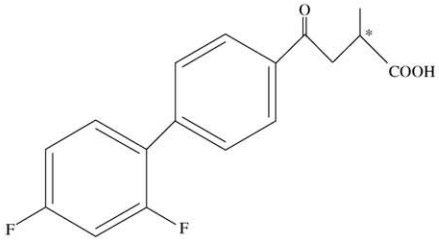
Carprofen



Flurbiprofen



Ibuprofen



Flobufen

Fig. 1. Structures of the studied β-blockers and profens.

Table 1
The effect of the organic modifier content on retention and enantioseparation of β-blockers

MeOH–TEAA (v/v)		Atenolol	Pindolol	Acebutolol	Propranolol	Oxprenolol	Alprenolol
	k_1	2.21	2.44	2.57	3.21	2.21	2.44
50:50	α	1.00	1.00	1.00	1.00	1.00	1.00
	R	0.00	0.00	0.00	0.00	0.00	0.00
	k_1	2.95	2.68	2.80	2.96	2.51	2.41
70:30	α	1.04	1.03	1.05	1.05	1.00	1.05
	R	0.32	0.33	0.12	0.47	0.00	0.53
	k_1	6.82	5.74	5.48	6.02	5.11	5.00
80:20	α	1.04	1.05	1.04	1.06	1.03	1.06
	R	0.25	0.36	0.16	0.51	0.14	0.58
	k_1	8.47	6.73	7.52	7.08	6.00	5.84
85:15	α	1.05	1.05	1.06	1.07	1.04	1.07
	R	0.37	0.25	0.35	0.64	0.13	0.72
	k_1	13.72	10.23	12.01	10.65	8.71	8.80
90:10	α	1.07	1.07	1.08	1.08	1.07	1.08
	R	0.95	0.91	1.17	1.33	0.97	1.19

Stationary phase: Chirobiotic V, mobile phase: MeOH–0.1% TEAA, pH 5.0 (v/v); k_1, retention factor of the first eluting enantiomer; α, separation factor; R, resolution.

at the laboratory temperature ($22 \pm 2\,^{\circ}C$). The stock solutions of individual analytes were dissolved in methanol in the concentration of $1\,mg\,mL^{-1}$. The separation of β-blockers was performed at the mobile phase flow rate $1\,mL\,min^{-1}$ in RP separation mode and $2\,mL\,min^{-1}$ in PO separation mode. Profens were separated at flow rate $0.7\,mL\,min^{-1}$.

3. Results and discussion

3.1. Chiral separation of β-blockers in the reversed-phase separation mode

Based on the literature data [16] methanol was selected as organic modifier of mobile phases used in this work. The aqueous part of the mobile phases was composed of 0.1, 0.5 or 1.0% triethylamine acetate buffers, their pH was varied between 3 and 5. The buffer pH can affect not only dissociation/protonization of analytes but also ionization of functional groups of the chiral selector (or even of the silanol groups of the silica gel support) and in this way vary the possibility of the stereoselective interaction.

The retention of all the β-blockers studied increased with increasing contents of methanol regardless of the buffer pH used (pH 3.0, 4.0, 5.0) on both the chiral stationary phases. Nevertheless, the most distinct effect of the methanol content in the mobile phase on both the retention and enantioresolution was observed at the highest pH value (pH 5.0). Therefore, Tables 1 and 2 show only chromatographic data obtained in the mobile phases composed of MeOH with 0.1% TEAA, pH 5.0, on the columns Chirobiotic V and Chirobiotic V2, respectively. The retention of β-blockers was in identical mobile phases always greater on the CSP with higher vancomycin coverage but the difference was diminished if the

Table 2
The effect of the organic modifier content on retention and enantioseparation of β-blockers

MeOH–TEAA (v/v)		Atenolol	Pindolol	Acebutolol	Propranolol	Oxprenolol	Alprenolol
	k_1	4.60	6.49	5.90	10.06	5.14	6.27
50:50	α	1.06	1.10	1.07	1.10	1.06	1.10
	R	0.70	0.96	0.65	0.70	0.48	0.90
	k_1	6.97	7.08	6.33	8.17	5.38	5.88
70:30	α	1.08	1.10	1.08	1.09	1.06	1.10
	R	0.84	1.13	0.84	1.21	0.67	0.90
	k_1	7.69	7.66	7.84	8.70	6.27	6.62
80:20	α	1.10	1.11	1.06	1.11	1.05	1.09
	R	0.85	1.00	1.13	1.25	0.92	1.26
	k_1	11.54	9.08	9.78	10.17	7.55	7.95
85:15	α	1.11	1.10	1.08	1.10	1.05	1.09
	R	0.78	1.33	1.19	1.28	1.06	1.55
	k_1	14.52	11.73	12.79	11.89	9.03	9.38
90:10	α	1.11	1.07	1.09	1.10	1.06	1.09
	R	1.08	1.52	1.32	1.46	1.20	1.48

Stationary phase: Chirobiotic V2, mobile phase: MeOH–0.1% TEAA, pH 5.0 (v/v); variables as in Table 1.

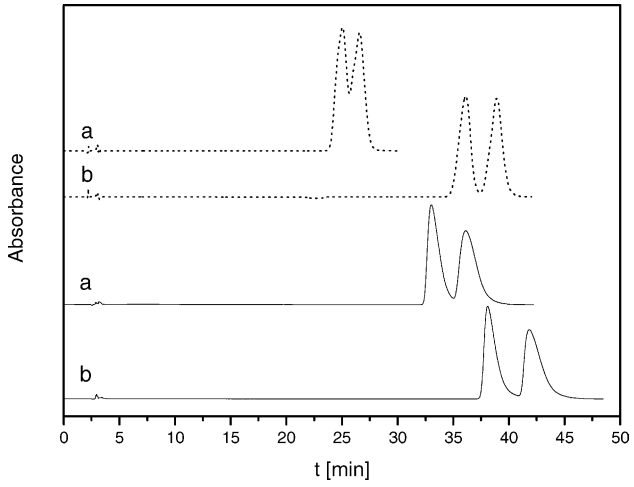


Fig. 2. Comparison of the retention behavior of propranolol on Chirobiotic V (dotted lines) and Chirobiotic V2 (solid lines); mobile phase composition: MeOH/0.1% TEAA, pH 5, (a) 85/15 (v/v), (b) 90/10 (v/v); flow rate 1.0 mL min^{-1}; detection wavelength 230 nm.

methanol content in the mobile phases increased. This observation can be clearly related to weakening of hydrophobic interactions in mobile phases with higher organic modifier content. The hydrophobic interactions, which play an important role in the reversed-phase separation mechanism, predominate in mobile phases with high buffer to organic modifier ratio. Higher vancomycin coverage resulted in increased enantioresolution values of all the studied β-blockers in all the compared mobile phases. Comparison of the separation of propranolol enantiomers on the both columns at two mobile phase compositions is shown in Fig. 2 for illustration. Table 3 summarizes the effect of TEAA concentration on retention and separation of β-blockers on both the tested columns. It is obvious that retention of all β-blockers substantially decreased with increasing TEAA concentration in the buffer on both the CSPs and the retention on the Chirobiotic V2 column was always higher. Consequently, greater improvement of enantioresolution was observed on the CSP with higher coverage of vancomycin if buffer concentration increased from 0.1 to 0.5%. Worse enantioseparation was obtained on the both columns at the highest studied TEAA content (1.0%) because the time available for interaction of the analytes with the chiral selector was too short. The differences in the enantioselective behavior of β-blockers on both columns at various contents of TEAA in the buffer can be explained as follows: TEA can reduce the effect of free silanol groups on the surface of silica gel and in this way reduces the non-stereoselective interactions. This holds mainly for the CSP with lower vancomycin coverage. However, TEA can also provide some ion-pairing interaction possibilities either with the analyte or the chiral selector. This effect is similar on the both columns.

The best enantioseparation of β-blockers in the reversed-phase mode was obtained on the both columns in the mobile phase composed of MeOH–0.5% TEAA, pH 5.0 (90:10, v/v). Selectivity was quite similar on both the CSPs, so that higher enantioresolution values on Chirobiotic V2 can be related to better peak symmetry. Illustrative chromatograms of the enantioseparation of three β-blockers on Chirobiotic V and Chirobiotic V2 columns are shown in Fig. 3A and B, respectively. Under the compared conditions (lower flow-rate on the former column that was expected to improve the enantioresolution) the analysis time was the same but better resolution of

Table 3
The effect of the concentration of TEAA buffer on retention and enantioseparation of β-blockers in the mobile phase MeOH–TEAA, pH 5.0 (90:10, v/v); variables as in Table 1

MeOH–TEAA (v/v)		Atenolol	Pindolol	Acebutolol	Propranolol	Oxprenolol	Alprenolol
Chirobiotic V							
0.1% TEAA	k_1	13.72	10.23	12.01	10.65	8.71	8.80
	α	1.07	1.07	1.08	1.08	1.07	1.08
	R	0.95	0.91	1.17	1.33	0.97	1.19
0.5% TEAA	k_1	4.31	3.11	3.42	3.29	2.71	2.66
	α	1.07	1.07	1.07	1.08	1.06	1.08
	R	1.19	1.20	1.22	1.44	0.90	1.29
1.0% TEAA	k_1	3.00	2.15	2.51	2.20	1.83	1.79
	α	1.08	1.07	1.08	1.09	1.06	1.16
	R	1.10	1.09	1.11	1.10	0.89	1.16
Chirobiotic V2							
0.1% TEAA	k_1	14.52	11.73	12.79	11.89	9.03	9.38
	α	1.11	1.07	1.09	1.10	1.06	1.09
	R	1.08	1.52	1.32	1.46	1.20	1.48
0.5% TEAA	k_1	6.83	4.54	5.36	5.32	3.82	3.93
	α	1.09	1.12	1.11	1.12	1.07	1.11
	R	1.45	1.79	1.68	2.00	1.25	1.80
1.0% TEAA	k_1	3.89	2.75	3.02	3.03	2.13	2.19
	α	1.10	1.13	1.11	1.20	1.08	1.12
	R	1.31	1.71	1.47	1.79	1.13	1.47

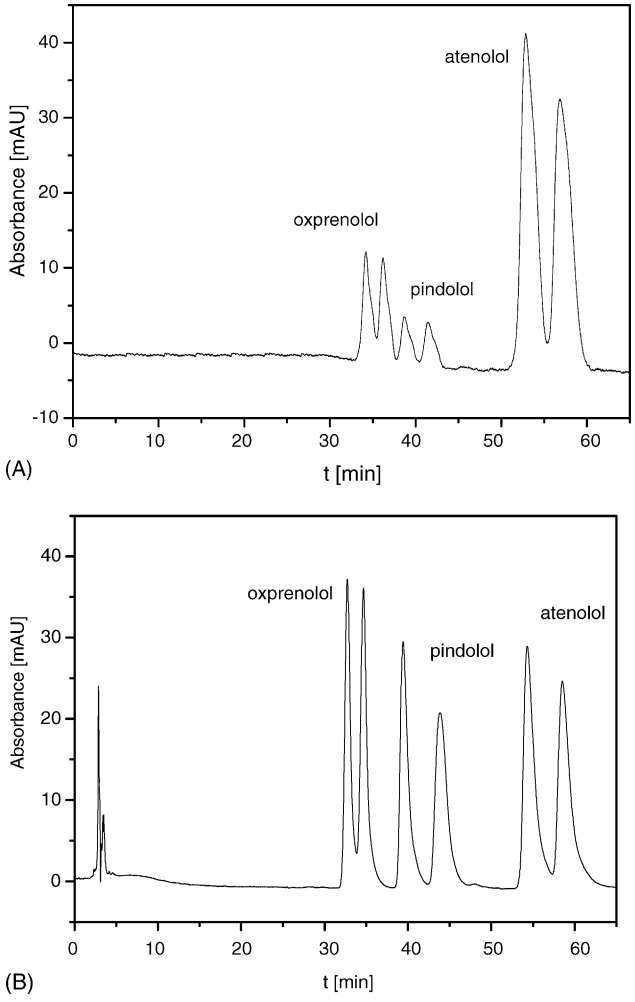


Fig. 3. Enantioseparation of three β-blockers in the reversed-phase separation mode on Chirobiotic V (A) and Chirobiotic V2 (B); mobile phase composition: MeOH/0.1% TEAA, pH 5, 90/10 (v/v); flow rates (A) 0.7 mL min^{-1}, (B) 1.0 mL min^{-1}, detection wavelength 230 nm.

the enantiomeric pairs was obtained on the CSP with higher vancomycin coverage.

3.2. Chiral separation of β-blockers in the polar-organic separation mode

The results obtained with β-blockers in the reversed-phase mode in mobile phases with high organic modifier content have already indicated that the PO mode would be a good choice for enantioseparation of these drugs. Easy-to-prepare mobile phases composed of methanol with small amounts of acetic acid (HAc) and triethylamine (TEA) were used. Although in a pure methanolic mobile phase none of the derivatives eluted by 70 min, just a small addition of HAc and TEA was sufficient to elute the enantiomers in an acceptable time. Results are given in Table 4 (for Chirobiotic V) and Table 5 (for Chirobiotic V2). It is obvious that with an increase of the acid and base concentrations retention decreased, which was mostly accompanied by a decrease of enantioresolution values on the both columns. Nevertheless, the enantioresolution values on the Chirobiotic V2 column were sufficient for baseline separation even at higher HAc and TEA concentrations. Comparison of the retention and enantioseparation behavior on those columns in the PO mode is well demonstrated on oxprenolol in Fig. 4. At lower HAc and TEA amounts in the mobile phase (Fig. 4, chromatograms a) the retention on the Chirobiotic V2 column is greater and enantioresolution much better than on Chirobiotic V CSP. Frontening causes peak asymmetry, which is more pronounced on the column with lower coverage of the CS. If the acid and base contents increase retention on the both columns becomes similar and also both the resolution values seem to be closer to each other (Fig. 4, chromatograms b). The undesirable effects are diminished and the stereoselective character of the interaction predominates.

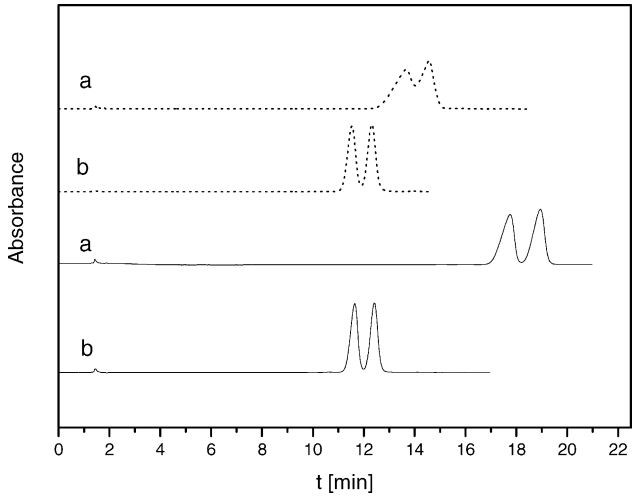


Fig. 4. Comparison of the retention behavior of oxprenolol on Chirobiotic V (dotted lines) and Chirobiotic V2 (solid lines) in the mobile phases MeOH/TEA/HAc (v/v/v) (a) 100/0.005/0.005, (b) 100/0.01/0.01; flow rate 2.0 mL min^{-1}; detection wavelength 230 nm.

High separation efficiency of the Chirobiotic V2 column can be documented by chiral separation of a mixture of four β-blockers in the polar-organic separation mode (Fig. 5B). (See also the separation in the reversed-phase mode on Fig. 3B for comparison.) Fig. 5 shows the separation of the β-blockers mixture on both the tested columns under the same mobile phase composition. On Chirobiotic V2 baseline separation of all eight enantiomers can be achieved within 15 min.

3.3. Chiral separation of profens in the reversed-phase separation mode

Retention and separation behavior of higher polarity acidic drugs—profens was rather different from the behavior of β-blockers discussed above. On the Chirobiotic V column all the studied profen derivatives eluted in a very short time (shorter than 6 min) in all mobile phases with higher methanol contents (90–70%) and at any measured buffer pH (pH 3.0, 4.0, 5.0). Decrease of the organic modifier content resulted

Table 4
The effect of the contents of HAc and TEA in the polar organic mobile phases on retention and enantioseparation of β-blockers

MeOH–HAc–TEA (v/v/v)		Atenolol	Pindolol	Acebutolol	Propranolol	Oxprenolol	Alprenolol
	k_1	14.36	9.50	12.55	10.28	8.30	8.30
100:0.005:0.005	α	1.08	1.09	1.10	1.10	1.07	1.10
	R	1.11	1.17	1.31	1.10	0.63	0.90
	k_1	12.63	8.13	10.73	9.30	6.76	6.70
100:0.01:0.01	α	1.09	1.09	1.11	1.11	1.08	1.10
	R	1.30	1.50	1.64	2.10	1.17	1.66
	k_1	6.93	4.44	6.04	4.85	3.93	3.88
100:0.02:0.02	α	1.09	1.10	1.10	1.16	1.08	1.11
	R	1.13	1.09	1.17	1.49	0.93	1.16
	k_1	2.35	1.35	1.94	1.46	1.15	1.11
100:0.05:0.05	α	1.10	1.12	1.12	1.23	1.10	1.14
	R	1.17	1.07	1.18	1.12	0.87	1.06
	k_1	1.21	0.86	1.12	0.87	0.70	0.67
100:0.1:0.1	α	1.13	1.22	1.11	1.15	1.11	1.18
	R	0.93	0.87	0.93	0.91	0.78	0.94

Stationary phase: Chirobiotic V, mobile phase: MeOH–HAc–TEA, variables as in Table 1.

in an increase of retention that had stereoselective character only in case of indoprofen, fenoprofen and flobufen. For this reason Table 6 summarizes only the separation parameters of those derivatives in mobile phases in which at least partial enantioseparation was obtained. The buffer pH effect was the same for all these three compounds; the highest retention factors were obtained at pH 4.0 and the lowest at pH 5.0. Similar retention behavior of profens had been observed on cyclodextrin bonded CSPs [26]. The pH values at which the maximum enantioresolution was achieved differed probably with respect to pK_a of the profen derivatives. The pK_a values found in water are: pK_a (indoprofen) = 4.60, pK_a (fenoprofen) = 6.86. Of course, a shift of these values must be expected in buffer–methanolic mobile phases [35]. It was obvious that non-dissociated carboxyl group of profens was advantageous for chiral recognition but the influence of the buffer pH on ionization of the chiral selector must be also taken into account. The effect of TEAA concentration at different pH values of the buffer on the CSP with lower vancomycin coverage is shown in Table 7. The increase of the concentration of TEAA resulted in lower retention and worse enantioresolution.

On the column with higher vancomycin coverage a substantial increase of retention of profens was observed in comparable mobile phases. In mobile phases in which the methanol content dropped to 30% and below these analytes did not elute by 70 min, so higher MeOH content was required on the Chirobiotic V2 CSP than on the Chirobiotic V CSP. Therefore, there are shown only separation parameters of indoprofen and flobufen (their partial separation was achieved) in mobile phases with methanol contents in the range 40–90% (v), at two buffer pH values, in Table 8. Again the retention

Table 5
The effect of the contents of HAc and TEA in the polar organic mobile phases on retention and enantioseparation of β-blockers

MeOH–HAc–TEA (v/v/v)		Atenolol	Pindolol	Acebutolol	Propranolol	Oxprenolol	Alprenolol
	k_1	20.35	13.27	14.22	14.62	10.45	11.14
100:0.005:0.005	α	1.10	1.12	1.18	1.16	1.07	1.11
	R	1.48	2.00	2.05	2.21	1.40	2.12
	k_1	12.73	8.61	10.75	9.47	6.77	6.97
100:0.01:0.01	α	1.11	1.13	1.13	1.15	1.08	1.12
	R	1.47	2.14	1.89	2.03	1.25	2.06
	k_1	8.33	5.24	6.66	5.83	4.31	4.45
100:0.02:0.02	α	1.11	1.13	1.13	1.14	1.08	1.12
	R	1.38	1.98	1.76	2.04	1.12	1.74
	k_1	4.60	2.79	3.58	3.11	2.22	2.27
100:0.05:0.05	α	1.11	1.14	1.14	1.16	1.09	1.14
	R	1.11	1.60	1.48	1.79	1.07	1.62
	k_1	2.58	1.72	2.22	1.91	1.33	1.35
100:0.1:0.1	α	1.12	1.15	1.14	1.17	1.10	1.16
	R	0.93	1.47	1.35	1.46	0.94	1.39

Stationary phase: Chirobiotic V2, mobile phase: MeOH–HAc–TEA, variables as in Table 1.

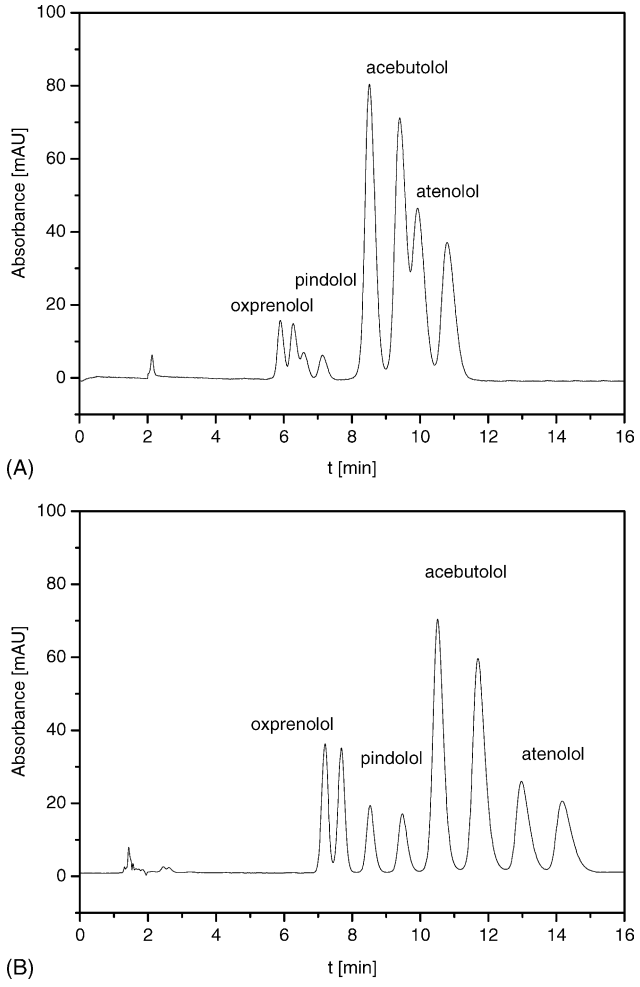


Fig. 5. Enantioseparation of four β-blockers in the polar-organic separation mode on Chirobiotic V (A) and Chirobiotic V2 (B); mobile phase composition: MeOH/TEA/HAc 100/0.02/0.02 (v/v/v); flow rate 2.0 mL min^{-1}; detection wavelength 230 nm.

and enantioresolution decreased with increasing organic modifier content and better chiral separation of indoprofen was obtained at pH 3.0 while higher resolution values of flobufen enantiomers were reached at pH 4.0. Table 9 shows the effect of TEAA concentration on the chromatographic data of these two derivatives in MeOH–buffer (50:50, v/v) mobile phase. While a small increase of enantioresolution of flobufen was observed if concentration of TEAA increased from 0.1 to 0.5% for indoprofen the decrease of retention and resolution could be clearly indicated in the whole studied range of TEAA concentrations. The decrease of retention factors was much more pronounced at lower buffer pH for both derivatives. That means that again the non-dissociated state of these acids is favored in the retention mechanism.

The vancomycin-based columns were undoubtedly less effective for separation of profens than for β-blockers. Polar-organic separation mode could not be used at all as the results in the RP mode indicated. All studied profens eluted from both the CSPs compared in very short retention times in methanol as the solvent and neither the modification of the mobile phase with acetic acid and triethylamine could positively influence the retention and so the enantioselective interaction of profens.

The most interesting result was obtained with flobufen. Enantiomers of flobufen could be baseline resolved on the Chirobiotic V CSP (in the mobile phase composed of 30% (or 25%) MeOH in 0.1% TEAA buffer, pH 4.0) while much worse, only partial separation was obtained on Chirobiotic V2 (see Table 6 versus Table 8). Fig. 6 shows the comparison of enantioseparation of flobufen on the both columns under the same mobile phase composition. It depicts substantial reduction of the analysis time and better resolution of the enantiomers on the column with lower content of vancomycin. Flobufen, due to its structural diversity from the other profens, seems to favor the CSP with lower chiral selector coverage (greater distance of the CS molecules on the silica support) on which the spatial arrangement seems to better fit its requirement for enantioselective interaction. The stereogenic center

Table 6
The effect of the organic modifier content and buffer pH on retention and enantioseparation of selected profens

MeOH–0.1% TEAA (v/v)	pH	Flobufen			Indoprofen			Fenoprofen		
		k_1	α	R	k_1	α	R	k_1	α	R
	3.0	3.67	1.17	1.18	4.59	1.17	1.60	2.39	1.00	0.00
25:75	4.0	4.42	1.15	1.61	6.60	1.12	0.87	3.74	1.00	0.00
	5.0	3.21	1.07	0.58	3.07	1.00	0.00	1.66	1.06	0.23
	3.0	2.21	1.17	0.86	2.74	1.15	1.16	1.58	1.00	0.00
30:70	4.0	3.28	1.16	1.73	3.73	1.10	0.61	2.17	1.00	0.00
	5.0	1.95	1.00	0.00	2.71	1.00	0.00	1.13	1.08	0.48
	3.0	1.59	1.14	0.75	2.23	1.11	0.58	1.27	1.00	0.00
40:60	4.0	1.64	1.13	1.04	2.29	1.07	0.11	1.29	1.00	0.00
	5.0	1.20	1.00	0.00	1.48	1.00	0.00	0.83	1.06	0.15
	3.0	0.36	1.00	0.00	0.65	1.00	0.00	0.30	1.00	0.00
50:50	4.0	0.46	1.17	0.99	0.80	1.00	0.00	0.39	1.00	0.00
	5.0	0.36	1.00	0.00	0.56	1.00	0.00	0.30	1.00	0.00

Stationary phase: Chirobiotic V, mobile phase: MeOH–0.1% TEAA (v/v); variables as in Table 1.

Table 7
The effect of the concentration of TEAA buffer on retention and enantioseparation of selected profens on the Chirobiotic V column in the mobile phase MeOH–TEAA (25:75, v/v); variables as in Table 1

MeOH–TEAA (v/v)	Flobufen			Indoprofen			Fenoprofen		
	k_1	α	R	k_1	α	R	k_1	α	R
0.1% TEAA									
pH 3.0	3.67	1.17	1.18	4.59	1.17	1.60	2.39	1.00	0.00
pH 4.0	4.42	1.15	1.61	6.60	1.12	0.87	3.74	1.00	0.00
pH 5.0	3.21	1.07	0.58	3.07	1.00	0.00	1.66	1.06	0.23
0.5% TEAA									
pH3.0	2.53	1.14	0.38	3.40	1.14	1.22	1.86	1.00	0.00
pH 4.0	4.21	1.15	1.21	5.37	1.10	0.63	2.76	1.00	0.00
pH 5.0	4.88	1.08	0.36	5.44	1.00	0.00	3.07	1.00	0.00
1.0% TEAA									
pH 3.0[a]	1.24	1.00	0.00	1.60	1.10	0.37	1.24	1.00	0.00
pH 4.0	3.54	1.00	0.00	4.46	1.00	0.00	1.92	1.00	0.00
pH 5.0	4.76	1.00	0.00	5.10	1.00	0.00	2.95	1.00	0.00

[a] Data measured at $\lambda = 270$ nm.

Table 8
The effect of the organic modifier content and buffer pH on retention and enantioseparation of selected profens

MeOH–0.1% TEAA (v/v)	pH	Flobufen			Indoprofen		
		k_1	α	R	k_1	α	R
40:60	3.0	5.21	1.07	0.27	6.67	2.10	1.57
	4.0	7.07	1.08	0.42	7.66	1.13	0.80
50:50	3.0	1.99	1.08	0.18	2.75	1.17	1.34
	4.0	2.13	1.07	0.52	2.79	1.10	0.90
60:40	3.0	0.75	1.05	0.09	1.24	1.14	0.91
	4.0	0.92	1.05	0.31	1.48	1.09	0.50
70:30	3.0	0.33	1.00	0.00	0.64	1.13	0.84
	4.0	0.36	1.00	0.00	0.77	1.06	0.19
80:20	3.0	0.14	1.00	0.00	0.40	1.12	0.56
	4.0	0.20	1.00	0.00	0.53	1.00	0.00
90:10	3.0	0.10	1.00	0.00	0.35	1.08	0.16
	4.0	0.12	1.00	0.00	0.45	1.00	0.00

Stationary phase: Chirobiotic V2, mobile phase: MeOH–0.1% TEAA (v/v), variables as in Table 1.

Table 9
The effect of the concentration of TEAA buffer on retention and enantioseparation of flobufen and indoprofen on the Chirobiotic V2 CSP in the mobile phase MeOH–TEAA (50:50, v/v); variables as in Table 1

MeOH–TEAA (v/v)	Flobufen			Indoprofen		
	k_1	α	R	k_1	α	R
0.1% TEAA						
pH3.0	1.99	1.08	0.18	2.75	1.17	1.34
pH 4.0	2.13	1.07	0.52	2.79	1.10	0.90
0.5% TEAA						
pH3.0	1.17	1.09	0.65	1.67	1.16	1.08
pH 4.0	1.96	1.08	0.64	2.60	1.13	0.83
1.0% TEAA						
pH3.0[a]	0.95	1.07	0.30	1.13	1.16	1.02
pH 4.0	1.72	1.08	0.45	2.32	1.13	0.75

[a] Data measured at $\lambda = 270$ nm.

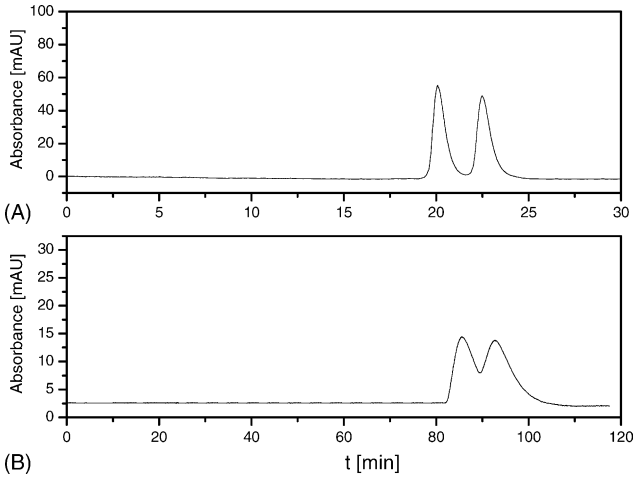


Fig. 6. Comparison of enantioseparation of flobufen on Chirobiotic V (A) and Chirobiotic V2 (B). Separation conditions: mobile phase: MeOH/0.1% TEAA, pH 4.0, 30/70 (v/v); flow rate 0.7 mL min^{-1}; detection wavelength 230 nm. (Note different ranges of the time-axes.)

of flobufen is further from the aromatic moiety and a carbonyl group is incorporated between them. This carbonyl moiety offers an additive hydrogen-bonding possibility. The crystallographic measurements of flobufen have evidenced that (i) the both phenyl rings are almost coplanar and (ii) the presence of two fluorine atoms causes shorter "aromatic" C–C bonds in the difluorophenyl ring than in a non-substituted phenyl ring [36]. These facts indicate a change of the π–π interaction possibilities. Furter investigation will be needed to properly explain the interaction mechanism responsible for the chiral resolution of flobufen on the both vancomycin bonded chiral stationary phases.

4. Conclusion

The comparison of the two vancomycin-based CSPs with different surface coverage of the chiral selector can be

generalized as follows: Higher retention of both the studied groups of structurally diverse drugs on the Chirobiotic V2 column was mostly accompanied by better enantioresolution. Comparing the two separation modes the polar-organic mobile phase gives better enantioresolution of β-blockers in shorter analysis time on both the columns. Mainly baseline enantioseparation of a mixture of four β-blockers in the polar-organic separation mode within 15 min is a nice example of the usefulness of the CSP with higher vancomycin coverage. Nevertheless, this general conclusion has certain exceptions. One of them could be the separation of enantiomers of flobufen. This profen derivative was much better (baseline) resolved on the Chirobiotic V CSP than on the Chirobiotic V2 column.

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