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Journal of Chromatography A, 1088 (2005) 82–93
JOURNAL OF CHROMATOGRAPHY A
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Chiral separation of beta-adrenergic antagonists, profen non-steroidal anti-inflammatory drugs and chlorophenoxypropionic acid herbicides using teicoplanin as the chiral selector in capillary liquid chromatography

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Available online 25 February 2005

Abstract

Three groups of structurally diverse chiral compounds were used to study the interaction mechanism responsible for stereoselective recognition with teicoplanin as chiral selector in capillary liquid chromatography. Teicoplanin-based chiral stationary phase (CSP) was used. The effect of the variation of mobile phase composition on retention and enantioselective separation was studied. The mobile phase composition suitable for enantioresolution of the various chiral compounds differed according to the interaction forces needed for chiral recognition. Mobile phases with high buffer portion (70–90 vol.%) were preferred for separation of enantiomers of profen non-steroidal anti-inflammatory drugs and chlorophenoxypropionic acid herbicides that require hydrophobic interactions, inclusion and π–π interactions for stereoselective recognition with teicoplanin. Higher concentration triethylamine in the buffer (0.5–1.0%) increased resolution of these acids. On the other hand, H-bonding and electrostatic interactions are important in stereoselective interaction mechanism of β-adrenergic antagonists with teicoplanin. These interaction types predominate in the reversed phase separation mode with high organic modifier content (95% methanol) and in polar organic mobile phases. For this reason β-adrenergic antagonists were best enantioresolved in the polar organic mode. The mobile phase composed of methanol/acetic acid/triethylamine, 100/0.01/0.01 (v/v/v), provided enantioresolution values of all the studied β-adrenergic antagonists in the range 1.1–1.9. Addition of teicoplanin to the mobile phase, which was suitable for enantioseparation of certain compounds on the CSP, was also investigated. This system was used to dispose of nonstereoselective interactions of analytes with silica gel support that often participate in the interaction with CSPs. Very low concentration of teicoplanin in the mobile phase (0.1 mM) resulted in enantioselective separation of 2,2- and 2,4-chlorophenoxypropionic acids.

Keywords: Chiral stationary phases; LC; Enantiomer separation; Teicoplanin; β-Adrenergic antagonists; Profen NSAIDs; Chlorophenoxypropionic acids

1. Introduction

Capillary liquid chromatography (cLC) can be considered a variant of high-performance liquid chromatography (HPLC). Miniaturization of separation columns in LC has some advantages such as small volumes of sample (tens of nanoliters), mobile and stationary phases. The benefits of cLC for enantioselective separations is related with the fact that it allows also using expensive mobile phase additives, such as chiral selectors. The situation is more difficult with availability of CSPs for cLC. While a wide variety of ready-to-use chiral columns are produced for HPLC, chiral capillary columns must be prepared by packing a capillary with chiral stationary phase.

Macrocyclic antibiotics (MA) are a relatively new class of chiral selectors for chromatography and capillary electrophoresis (CE). The macrocyclic antibiotics have been used in HPLC [1,2], CE [3–5], capillary electrochromatography (CEC) [6], as reported in the review papers [1–6]. The most important selectors of this type are teicoplanin, vancomycin, ristocetin A and avoparcin. Teicoplanin (Fig. 1) is probably

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doi:10.1016/j.chroma.2005.02.027

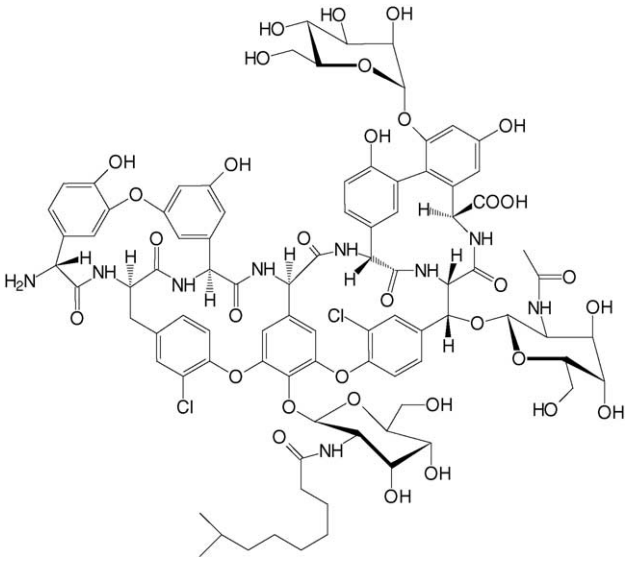

Fig. 1. Structure of teicoplanin used as the chiral selector in this study.

the most effective for enantioseparation of many structurally different compounds as amino acids, proteins and various drugs [1,2].

Three groups of chiral compounds with different chemical structures, namely β-adrenergic antagonists, profen NSAIDs and chlorophenoxypropionic acid (CPPA) herbicides, have been used to study their enantiomeric behavior in cLC with teicoplanin as the chiral selector. β-Adrenergic antagonists, compounds containing hydroxyl and amine groups and possessing at least one aromatic moiety in their molecules, are used in the treatment of some neurological, neuropsychiatric and cardiovascular disorders [7]. It is known that enantiomers of β-adrenergic antagonists have different therapeutic potencies and effects. For example, the *S*-enantiomer of propranolol is 100 times more potent as β-blocking agent than the *R*-enantiomer [8].

Various HPLC methods were proposed for the chiral separation of one or even a set of β-adrenergic antagonists [9–21]. Their enantiomers were separated using α-acid glycoprotein CSP (AGP) [9], polysaccharide [9–15] and cyclodextrin based [9,16] CSPs. Macrocyclic glycopeptides also contain in their structures peptides and carbohydrates. This indicates that all the typical interactions characteristic for cellulose (carbohydrates) and protein types of CSPs can be employed in the separation mechanism on the glycopeptide phases. Therefore, CSPs based on glycopeptides, namely on teicoplanin [17–21], teicoplanin aglycon [20] and vancomycin [17,21] were also tested for enantioseparation of several β-adrenergic antagonists. From the tested analytes atenolol and pindolol could not be baseline resolved on teicoplanin aglycone CSP [20].

Profen NSAIDs (2-arylpropionic acids) represent an important group of non-steroidal anti-inflammatory drugs, characterized by a chiral carbon atom near the carboxylic acid group. Several direct or indirect liquid chromatographic methods involving a variety of CSPs were reported for their enantiomeric analysis [22–26]. Ibuprofen, fenoprofen, carprofen and flurbiprofen have been enantioseparated on cellulose tris-(4-methylbenzoate) CSP [22] and flurbiprofen on tris-(3,5-dimethylphenylcarbamate) of amylose CSP [23]. Native and derivatized β-cyclodextrins were used as chiral mobile phase additives to investigate the enantiomeric separation of ketoprofen, fenoprofen and ibuprofen [24]. Enantiomers of flurbiprofen and ketoprofen were resolved by using vancomycin CS [25]. Flurbiprofen could not be separated in the separation system when vancomycin is covalently bonded to silica gel support (column Chirobiotic V), but successful enantioresolution was obtained by addition of vancomycin to the mobile phase [26].

Phenoxypropionic acid (PPA) derivatives are widely used in agriculture as selective herbicides. Due to their solubility in water, they can easily move in agriculture ecosystems, causing surface and ground water pollution [27]. *R*-enantiomer of PPAs is known for its herbicidal activity while *S*-isomer is inactive as herbicidal agent [28]. Chiral separations of these herbicides are required in order to assess the enantiopurity and to optimize enantioselective production processes. The HPLC CSPs, which have been used to separate enantiomers of phenoxypropionic acid derivatives, included teicoplanin [20,29], cellulose derivatives [30,31], derivatized cyclodextrin [32] and some brush-type CSPs [33–35].

The aim of this work was to study the interaction of three structurally different groups of chiral compounds, i.e. β-adrenergic antagonists (Fig. 2), profen NSAIDs (Fig. 3) and chlorophenoxypropionic acids (Fig. 4), with teicoplanin-based CSP in capillary liquid chromatography. The influence of two separation modes, organic modifier content, concentration and pH of triethylamine acetate buffer on the chiral separation was investigated. Moreover, enantioseparation system with the chiral selector added to mobile phases was tested. Chromatographic systems with teicoplanin bonded to the silica gel support and free in solution were compared.

2. Experimental

2.1. Chemicals

The mobile phases were prepared from the following compounds and solvents: methanol, LiChrosolv, purity >99.8% (Merck, Darmstadt, Germany); triethylamine, purity >99.5% (Fluka, Buchs, Switzerland) and acetic acid (p.a. 99%) (Lachema, Brno, Czech Republic). The water used for preparation of all the mobile phases was purified with a Milli-Q water purification system (Millipore, USA).

Triethylamine acetate (TEAA) buffers, 0.1–1.0%, pH from 4.0 to 6.6, containing various percentages of methanol were used as mobile phases with a teicoplanin-based chiral stationary phase. Methanol/0.1% TEAA, pH 5.0 eluents

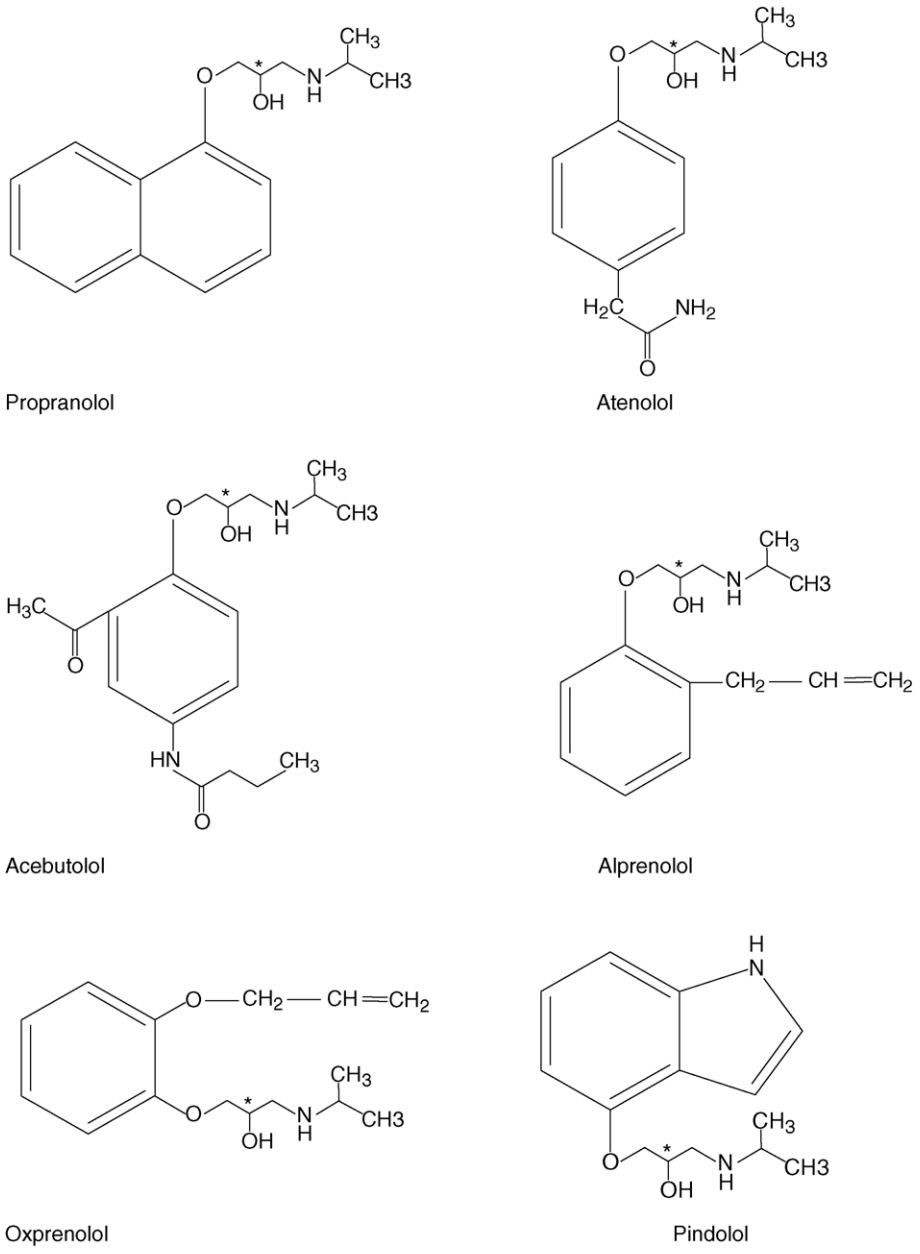

Fig. 2. Chemical structures of studied β-adrenergic antagonists.

containing various concentration of teicoplanin were used as mobile phases if an achiral stationary phase was employed. The eluents were sonicated just before use for at least 10 min.

Racemic profen NSAIDs, i.e. fenoprofen, carprofen, flurbiprofen, indoprofen, ibuprofen, flobufen, ketoprofen and suprofen; β-adrenergic antagonists, oxprenolol, alprenolol, propranolol, atenolol, acebutolol and pindolol; phenoxypropionic acid herbicides, 2-(2-chlorophenoxy) propionic acid (2,2-CPPA), 2-(3-chlorophenoxy) propionic acid (2,3-CPPA) and 2-(4-chlorophenoxy) propionic acid (2,4-CPPA), all p.a. purity, were obtained from (Sigma Aldrich, St. Louis, MO, USA). All the studied derivatives were injected as 0.2 mg ml^{-1} methanolic solutions.

2.2. Equipment

An ISCO syringe pump model 100 DM (Lincoln, NE, USA), a Valco injection valve with a 60 nl internal loop (Schenkon, Switzerland) and a Linear UV–VIS 205 detector equipped with a CE on-column flow cell (San Jose, CA, USA) were applied for the cLC experiments. A fused-silica capillary column of 25 cm × 300 μm I.D. packed with 5 μm Nucleosil 100 C_{18} HD was purchased from GROM (Herrenberg-Kayh, Germany). Flow-rate of mobile phases was 5 μl min^{-1}. Free teicoplanin chiral selector was provided by Astec (Whippany, NY, USA).

A capillary column of 22.5 cm × 320 μm I.D. packed with teicoplanin bonded to silica gel, particle size, 5 μm (CHIRO-

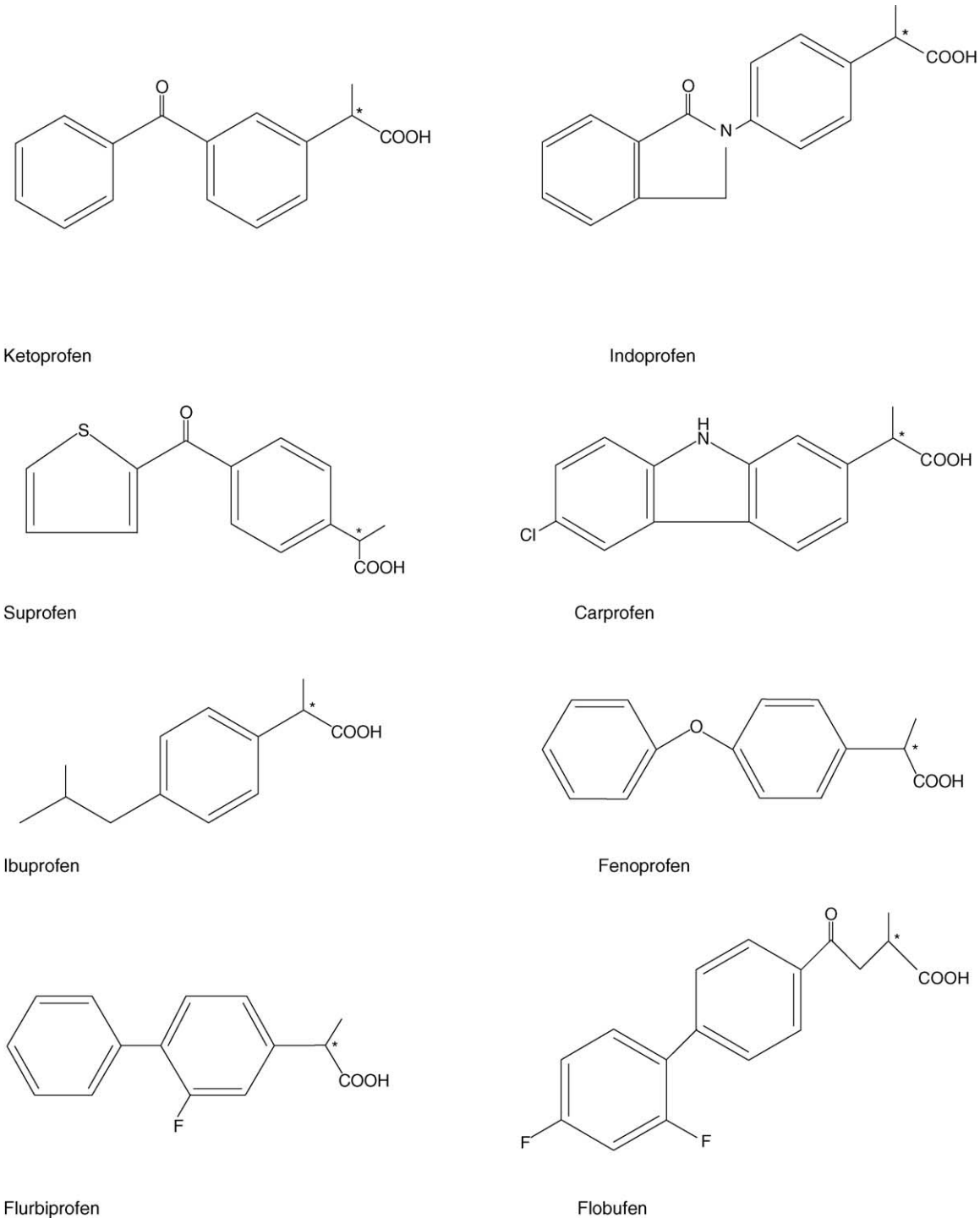

Fig. 3. Chemical structures of studied profen NSAIDs.

BIOTIC T, Astec, Whippany, NY, USA) was prepared by Ing. J. Planeta, Ph.D., from the Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic. Flow-rate of mobile phases was 4 μl min^{-1}.

The column inlet was installed in the injection valve using a 5-cm polyether ether ketone (PEEK) sleeve (500 μm I.D.) and a PEEK finger-tight fitting and the column outlet was connected by PTFE tubing to a 100 μm I.D. fused-silica capillary with detection window located 7 cm from the column outlet.

Individual samples were detected at various wavelengths according to the absorption maxima elicited from their absorption spectra. The detection wavelengths for profen NSAIDs were 230 or 270 nm; β-adrenergic antagonists were detected at 230 or 254 nm and chlorophenoxypropionic acid derivatives also at 230 nm. Chromatograms were recorded

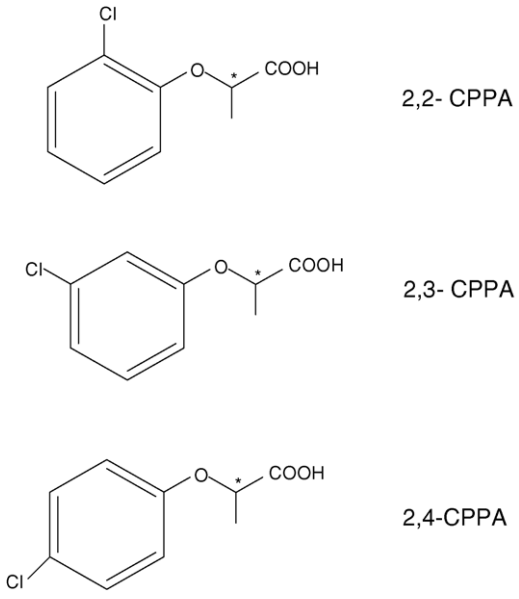

Fig. 4. Chemical structures of studied CPPA herbicides.

and evaluated employing CSW computer software provided by DataApex (Prague, Czech Republic).

3. Results and discussion

Separation systems composed of a teicoplanin-based chiral stationary phase and various mobile phases were studied for enantioseparation of β-adrenergic antagonists, profen NSAIDs and chlorophenoxypropionic acid herbicides in cLC. Reversed phase (RP) separation mode, as well as, polar organic (PO) mode were tested. Also teicoplanin was added to the mobile phase if an achiral capillary column Nucleosil 100 C_{18} HD was used.

3.1. Enantioseparation of β-adrenergic antagonists

3.1.1. Reversed separation mode

With respect to the chemical structure of β-adrenergic antagonists (Fig. 2) we studied the influence of mobile phase composition on their enantioseparation in two separation modes, i.e. reversed phase and polar organic ones. The effect of methanol (MeOH) contents in mobile phases on retention factors (k), selectivity factors (α) and enantioresolutions (R) in the reversed separation mode can be seen from Table 1. The retention of all β-adrenergic antagonists first decreased and at higher concentration of methanol (close to 95%) again substantially increased with increasing content of methanol. (At even lower percentages of the organic modifier in the mobile phase, the retention times were too long and no partial separation was observed, so we did not investigate the chromatographic behavior of β-adrenergic antagonists further to this region.) Such dependency is a typical indication for suitability to apply polar organic separation mode. The best enantioresolution and the highest values of selectivity of all the derivatives were obtained in the mobile phase containing 95% methanol. If the MeOH–buffer (v/v) ratio was shifted just to 90/10, the resolution markedly decreased or was even lost.

Effect of the buffer concentration on the chromatographic data of β-adrenergic antagonists is summarized in Table 2. Increasing concentration of TEAA decreased retention of all the derivatives, which was accompanied at 0.5% TEAA by higher resolution values (except of alprenolol). At the highest TEAA concentration tested (1.0%), all interaction types (stereoselective and nonstereoselective) were reduced and as the result enantioresolutions decreased again to almost the same values as at 0.1% buffer concentration. Too low concentration of TEAA (0.1%) cannot eliminate nonstereoselective interactions with the carrier of the CSP while at high concentration of the buffer (1.0%), there is no sufficient difference in the interaction of both enantiomers with the CSP to yield higher resolution values.

Assuming the structure of β-adrenergic antagonists significant influence of buffer pH on change of their dissociation/protonation in the “allowed” pH range could not be expected. On the other hand, pH can affect the polar groups of teicoplanin and in this way the stereoselective (but also nonstereoselective) interactions with the analytes. Enantioseparations of atenolol at three different pH values are compared in Fig. 5. It is obvious that higher pH value of the buffer elongates elution of analytes, which is accompanied by only

Table 1
Effect of methanol content in the mobile phase (0.1% aqueous TEAA, pH 5.0) on chromatographic data of β-adrenergic antagonists using the teicoplanin-based CSP

β-Adrenergic antagonists	Methanol (%)											
	40			50			90			95		
	k_1	α	R	k_1	α	R	k_1	α	R	k_1	α	R
Oxprenolol	6.78	1.00	0.00	5.45	1.00	0.00	2.98	1.00	0.00	8.64	1.04	0.75
Alprenolol	8.95	1.00	0.00	5.43	1.00	0.00	2.97	1.05	0.37	9.05	1.08	1.69
Propranolol	10.32	1.00	0.00	9.15	1.00	0.00	3.47	1.05	0.38	9.87	1.07	1.25
Atenolol	7.70	1.00	0.00	6.65	1.00	0.00	5.39	1.03	0.30	15.67	1.06	1.03
Acebutolol	8.95	1.00	0.00	6.88	1.00	0.00	3.98	1.00	0.00	13.22	1.04	0.59
Pindolol	6.15	1.00	0.00	6.72	1.00	0.00	3.24	1.03	0.21	9.07	1.06	0.79

Note: k_1, retention factor of the first eluting enantiomer; α, selectivity factor; R, resolution.

Table 2
Effect of the TEAA concentration in the mobile phase on chromatographic data of β-adrenergic antagonists using the teicoplanin-based CSP

β-Adrenergic antagonists	TEAA (%)								
	0.1			0.5			1.0		
	k_1	α	R	k_1	α	R	k_1	α	R
Oxprenolol	8.64	1.04	0.75	5.69	1.08	1.04	3.21	1.05	0.74
Alprenolol	9.05	1.08	1.69	4.66	1.12	1.42	2.89	1.08	1.09
Propranolol	9.87	1.07	1.25	6.27	1.10	1.30	3.52	1.08	1.00
Atenolol	15.67	1.06	1.03	10.12	1.09	1.13	5.79	1.07	1.00
Acebutolol	13.22	1.04	0.59	7.93	1.06	0.98	4.07	1.05	0.56
Pindolol	9.07	1.06	0.79	5.97	1.09	1.12	3.17	1.07	0.88

The mobile phase composition: 95/5 (v/v) methanol/TEAA, pH 5.0.

small change of enantioresolution. The higher retention can be attributed to stronger interaction of analytes with the dissociated carboxylic group of teicoplanin. The best chiral separation of β-adrenergic antagonists enantiomers was obtained in the mobile phase composed of methanol and 0.5% TEAA, pH 5.0 in the ratio 95/5. In the reversed phase separation mode, hydrophobic interactions and inclusion are favored but the importance of H-bonding in the interaction mechanism increases with increasing contents of methanol.

Due to solubility problems it was not possible to add teicoplanin to the mobile phase (with high content of methanol), which yielded the enantioseparation of β-adrenergic antagonists, and so to investigate a separation system with an achiral stationary phase.

3.1.2. Polar organic separation mode

Polar organic separation mode was developed for separation of enantiomer pairs possessing two functional groups capable of electrostatic interactions. These groups shall be located near the stereogenic center. Polar organic mobile phase is typically based on methanol (or acetonitrile/methanol) with very small amounts of acetic acid (HAc) and triethylamine (TEA). As enantioseparations of β-adrenergic antagonists were succeeded at high methanol contents in the RP mode, the PO mode was applied in the following experiments. Fig. 6 shows that with increasing concentration of HAc and TEA, the retention and the enantioresolution of alprenolol significantly decreased. The same trend was observed for all β-adrenergic antagonists. From the tested concentrations of acetic acid and triethylamine (their volumes [ml] added to 100 ml MeOH) and their ratios, the maximum resolution was obtained at HAc/TEA = 0.01/0.01. Results summarized in Table 3 depicts that almost all the derivatives were baseline separated. If we compare this optimized mo-

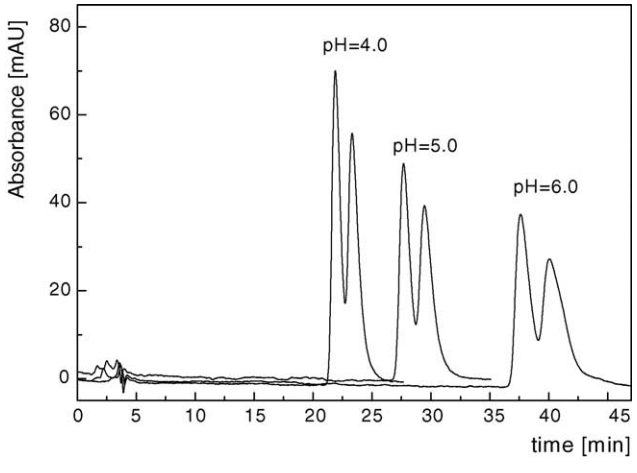

Fig. 5. Effect of TEAA buffer pH on cLC enantioseparation of atenolol using the teicoplanin-based CSP. Mobile phase composition: 95/5 (v/v) methanol/1.0% TEAA, pH 4.0–6.0.

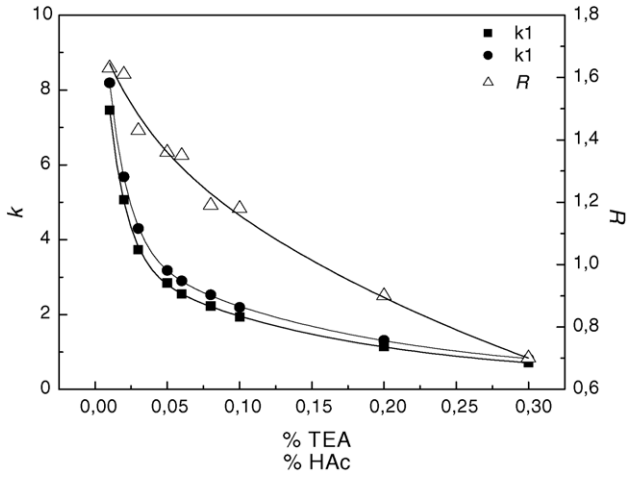

Fig. 6. Effect of the concentration of TEA and HAc in the mobile phase on the retention factors of the first (k_1) and second (k_2) enantiomer and the enantioresolution (R) of β-adrenergic antagonist (alprenolol) on the teicoplanin-based CSP. Mobile phase composition: methanol/HAc/TEA from 100/0.01/0.01 to 100/0.3/0.3 (v/v/v).

Table 3
Enantioresolution of β-adrenergic antagonists in the polar organic separation mode on the teicoplanin-based CSP

β-Adrenergic antagonists	k_1	α	R
Oxprenolol	4.30	1.07	1.08
Alprenolol	4.11	1.10	1.63
Propranolol	5.34	1.10	1.85
Atenolol	10.02	1.09	1.58
Acebutolol	6.12	1.07	1.10
Pindolol	4.60	1.09	1.50

The mobile phase composition: methanol/HAc/TEA, 100/0.01/0.01 (v/v/v).

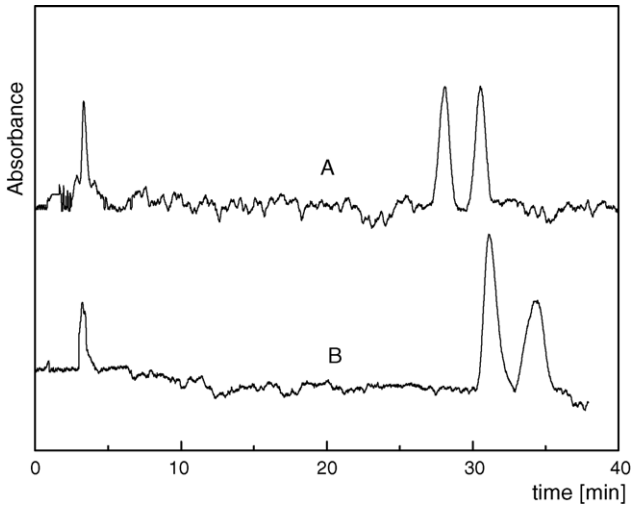

Fig. 7. Chromatograms showing the enantioseparation of alprenolol on the teicoplanin-based CSP. Mobile phases composition: (A) methanol/HAc/TEA, 100/0.01/0.01 (v/v/v) and (B) 95/5 (v/v) methanol/0.5% TEAA, pH 5.0.

bile phase composition for enantioseparation of β-adrenergic antagonists with that published previously [36], i.e. acetonitrile/methanol/HAc/TEA = 55/45/0.3/0.2, it is obvious that the addition of acetonitrile, as a proton acceptor, requires an increase of the HAc/TEA ratio and concentration. This shows on the importance of a proton-donating agent in the mobile phase to ensure protonation of the amino groups of the analytes. Protonated β-adrenergic antagonists provide interaction possibilities for H-bonding with teicoplanin-based CSP.

3.1.3. Comparison of enantioseparation of β-adrenergic antagonists in PO and RP modes

Fig. 7 allows comparison of the enantioseparations of alprenolol on teicoplanin-based capillary column in PO and RP separation modes. The respective separation parameters of alprenolol in the polar organic and the reversed phase separation modes are: resolution 1.63 and 1.42, selectivity factor 1.12 and 1.10, efficiency of the first eluted peak 36530 and 23660 theoretical plates per meter of column, and asymmetry of the second peak 1.00 and 0.70. Some β-adrenergic antagonists show much better enantioseparation parameters in the PO mode (the example of alprenolol, but also pindolol, propranolol and atenolol) while some others give similar results in both separation modes (for example acebutolol, and also oxprenolol). Generally said, selectivity is similar in both separation modes. The main contributors to the improved enantioresolution of β-adrenergic antagonists in the polar organic mobile phase are higher efficiency and better peak symmetry. These parameters also make the PO mode advantageous for practical purposes. The polar organic separation mode prefers electrostatic interactions and hydrogen bonding. The better enantioseparation in the polar organic mode shows the importance of the H-bonding and/or electrostatic interactions in the chiral recognition mechanism of β-adrenergic antagonists with teicoplanin. This is also the reason why no chiral resolution appears if mobile phases with lower amount of methanol are applied in the RP mode (see Table 1). In such separation systems, these electrostatic/H-bonding types of interactions are suppressed while hydrophobic and π–π interaction predominate. Due to the fact that the aromatic moiety is far away from the chiral center the latter interaction have no stereoselective significance.

3.2. Enantioseparation of profen NSAIDs

3.2.1. Reversed separation mode

Teicoplanin stationary phase shows affinity to compounds with a carboxylic group. It is important for enantioselective separations of amino acids and proteins [37,38]. The primary interaction of acids with teicoplanin is realized between their carboxyl group and amino group of the chiral selector [20]. Profen NSAIDs, as compounds with significant hydrophobic moiety close to the chiral carbon (Fig. 3), require for the enantioselective recognition also hydrophobic interaction or inclusion. These interaction types are favored if mobile phases with great aqueous portion are used. For this reason we examined the influence of methanol content in the mobile phase down to much lower values (in the range 10–30% of methanol) than in the case of β-adrenergic an-

Table 4
Effect of the TEAA in the mobile phase on chromatographic data of profen NSAIDs using the teicoplanin-based CSP; the mobile phase (aqueous TEAA, pH 5.0) and methanol

Profen NSAIDs	10% Methanol						20% Methanol						30% Methanol					
	0.1[a]		0.5[a]		1.0[a]		0.1[a]		0.5[a]		1.0[a]		0.1[a]		0.5[a]		1.0[a]	
	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R
Fenoprofen	1.29	0.00	2.57	0.00	2.87	0.00	0.59	0.00	1.90	0.00	1.96	0.00	0.00	0.00	0.94	0.00	1.19	0.00
Carprofen	5.56	0.00	9.37	0.00	9.45	0.00	3.22	0.00	6.75	0.00	6.89	0.00	0.41	0.46	2.34	0.26	3.22	0.26
Flurbiprofen	1.98	0.15	3.84	0.61	3.86	0.60	1.03	0.00	2.90	0.67	3.05	0.70	0.00	0.00	1.30	0.55	1.65	0.69
Flobufen	5.48	0.00	6.44	0.00	7.39	0.00	2.48	0.00	5.06	0.00	5.08	0.00	0.29	0.00	1.92	0.00	2.75	0.00
Ketoprofen	2.16	0.82	3.47	0.98	3.73	1.05	0.91	0.00	2.54	0.80	2.71	0.84	0.00	0.00	1.21	0.18	1.83	0.64
Suprofen	2.45	0.37	3.86	0.58	5.16	0.59	1.08	0.00	3.03	0.58	3.38	0.70	0.26	0.00	1.41	0.31	2.34	0.68
Indoprofen	4.26	0.50	6.46	0.70	7.39	0.72	2.10	0.65	4.95	0.64	4.98	0.64	0.51	0.00	2.18	0.51	2.82	0.59
Ibuprofen	1.17	0.00	1.82	0.00	2.06	0.00	0.53	0.00	1.61	0.00	1.66	0.00	0.10	0.00	0.84	0.00	0.98	0.00

[a] TEAA (%).

Table 5
Effect of the buffer pH on cLC enantioseparation of profen NSAIDs using the teicoplanin-based CSP

Profen NSAIDs	k_1				α				R			
	4.0[a]	5.0[a]	6.0[a]	6.6[a]	4.0[a]	5.0[a]	6.0[a]	6.6[a]	4.0[a]	5.0[a]	6.0[a]	6.6[a]
Fenoprofen	2.15	1.96	1.89	1.22	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00
Carprofen	6.19	6.89	5.46	3.20	1.00	1.00	1.04	1.05	0.00	0.00	0.15	0.18
Flurbiprofen	3.03	3.05	2.70	1.89	1.00	1.11	1.12	1.15	0.00	0.70	0.66	0.79
Flobufen	4.77	5.08	3.89	3.25	1.00	1.00	1.08	1.16	0.00	0.00	0.41	0.90
Ketoprofen	2.47	2.71	2.41	1.66	1.03	1.13	1.15	1.17	0.10	0.84	0.84	0.85
Suprofen	3.05	3.38	2.90	2.09	1.07	1.09	1.10	1.11	0.36	0.70	0.71	0.73
Indoprofen	4.96	4.98	5.02	2.98	1.06	1.08	1.08	1.08	0.47	0.64	0.52	0.64
Ibuprofen	1.68	1.66	1.35	0.86	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00

The mobile phase composition: 20/80 (v/v) methanol/1.0% TEAA, pH 4.0–6.6.
[a] pH values.

tagonists. The effect of the methanol content on retention factors and enantioresolution of profen NSAIDs was studied in the mobile phases composed of methanol and (0.1–1.0%) TEAA buffer, pH 5.0. Results obtained in the range 10–30% of methanol and different concentrations of TEAA are shown in Table 4. (In the range 30–90% of methanol, almost all profen NSAIDs were eluted at the death time.) Lower methanol contents resulted in higher retention and partial enantioresolution. In general, increasing concentration of TEAA in the mobile phase resulted in increased retention and improved enantioresolution of the profen NSAIDs. However, the difference between resolution values obtained using 0.5% and 1.0% TEAA buffer was not significant. Also the retention factors were almost not affected with this change of the buffer concentration. On contrary, increase of retention of profen NSAIDs was observed if TEAA concentration was raised from 0.1% to 0.5%. These results indicate that TEA forms ion-pairs with the analytes and in this way increase their hydrophobicity. Stronger hydrophobic interactions, which can be both enantioselective and nonenantioselective, between the analytes and CSP in higher polarity mobile phases are then responsible for higher retention and improved enantioseparation at 0.5% (or 1.0%) TEAA.

Based on the fact that 1.0% TEAA yielded the best results, the effect of the buffer pH was studied at this TEAA concentration. The results are shown in Table 5. The retention remained almost unchanged at pH 4.0 and 5.0, while it decreased more significantly towards pH 6.6. Selectivity and enantioresolution was markedly improved with increasing buffer pH only for flobufen. Considering the pK_a values of profen NSAIDs (3.9–5.0), chiral recognition interactions are favored if their carboxyl group is dissociated. The highest enantiomeric resolution was achieved for flurbiprofen, flobufen, ketoprofen, suprofen and indoprofen in the mobile phase composed of methanol/1.0% TEAA, pH 6.6, in the ratio 20/80. Fig. 8 shows enantioseparation of flobufen at different pH values of the TEAA buffer. The structure of flobufen differs from the other profen NSAIDs derivatives because the aromatic part is not attached directly to the asymmetric center. This is the reason why separation of flobufen enantiomers is more difficult to achieve. It is obvious from Fig. 8 that the best enantioresolution of flobufen can be reached in the

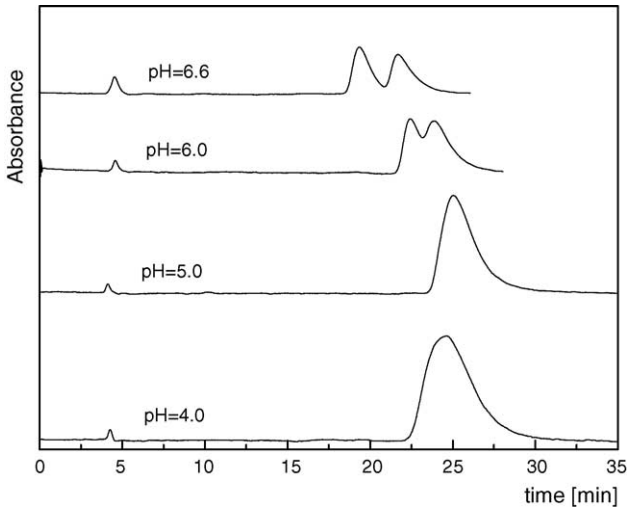

Fig. 8. Effect of the buffer pH on cLC enantioseparation of flobufen using the teicoplanin-based CSP. Mobile phase composition: 20/80 (v/v) methanol/1.0% TEAA, pH 4.0–6.6.

mobile phase composed of methanol/1.0% TEAA, pH 6.6, in the ratio 20/80 on the teicoplanin-based column in cLC.

Results obtained in the reversed phase mode in the mobile phases with higher methanol contents indicated that there is no need to investigate profen NSAIDs in the polar organic separation mode because they are not retained on the column under these conditions.

An attempt was made to use an achiral capillary column Nucleosil 100 C_{18} HD and mobile phase composed of methanol and 0.1% TEAA, pH 5.0, in the range 10–30% of methanol. However, retention of profen NSAIDs in these mobile phases was too long (without the addition of teicoplanin). Higher concentration of methanol in the mobile phase did not allow the addition of teicoplanin for the solubility reasons. As a result, enantioseparation of profen NSAIDs with teicoplanin chiral additive to the mobile phase was not realized.

3.3. Enantioseparation of chlorophenoxypropionic acid derivatives

In the final part of this work, we wanted to compare the effects of separation conditions on the chromatographic

Table 6
Effect of the TEAA in the mobile phase on chromatographic data of CPPAs using the teicoplanin-based CSP; the mobile phase (aqueous TEAA, pH 5.0) and methanol

CPPA	10% Methanol						20% Methanol						30% Methanol					
	0.1[a]		0.5[a]		1.0[a]		0.1[a]		0.5[a]		1.0[a]		0.1[a]		0.5[a]		1.0[a]	
	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R
2,2-CPPA	0.00	0.00	0.43	1.10	0.66	1.30	0.00	0.00	0.36	0.80	0.52	1.10	0.00	0.00	0.14	0.60	0.35	1.08
2,3-CPPA	0.00	0.00	0.60	0.00	0.78	0.62	0.00	0.00	0.48	0.15	0.62	0.56	0.00	0.00	0.29	0.00	0.44	0.54
2,4-CPPA	0.00	0.00	0.54	1.10	0.75	1.35	0.00	0.00	0.43	1.18	0.60	1.56	0.18	0.27	0.25	1.20	0.41	1.70

[a] TEAA (%).

behavior of three racemates, position isomers of chlorophenoxypropionic acid herbicides (Fig. 4) in capillary separation systems with teicoplanin as a chiral selector. Table 6 summarizes the data measured in mobile phases composed of different concentrations of TEAA (0.1%, 0.5% and 1.0%), pH 5.0, and various contents of methanol in the range 10–30% (v/v). (At higher methanol contents the analytes eluted at the dead time.) Retention of all CPPAs decreased with increasing contents of methanol in the mobile phases at any concentration of TEAA. The data displayed in Table 6 show that the retention and enantioresolution values of CPPA derivatives had the same trend as had been observed with profen NSAIDs, i.e. *k*- and *R*-values increased with increasing concentration of TEAA. These stronger interactions between the analytes and CSP are clearly enantioselective. For 2,3-CPPA and 2,4-CPPA, similar retention factors were obtained in the mobile phase methanol/1.0% TEAA, pH 5.0 (in the range 10–30% of methanol). Lower retention of 2,2-CPPA in these mobile phases can be attributed to intramolecular interaction between chlorine and oxypropionic acid moiety in *ortho* position. Nevertheless, even this low retention was sufficient for the chiral separation of 2,2-CPPA. Enantioresolution values of 2,2-CPPA and 2,3-CPPA decreased with increasing content of methanol in the mobile phases composed of 1.0% or 0.5% aqueous TEAA, pH 5.0, while the resolution of 2,4-CPPA had the opposite trend. These results show on the impact of steric factors on stereoselective interaction.

The influence of the buffer pH in mobile phases composed of methanol and 1.0% TEAA in the ratio 20/80 on enantioresolution of CPPAs is shown in Fig. 9. The observed trends are rather similar for all the derivatives. The least effect of buffer pH on the *R*-values of 2,2-CPPA can be again attributed to the intramolecular interaction of

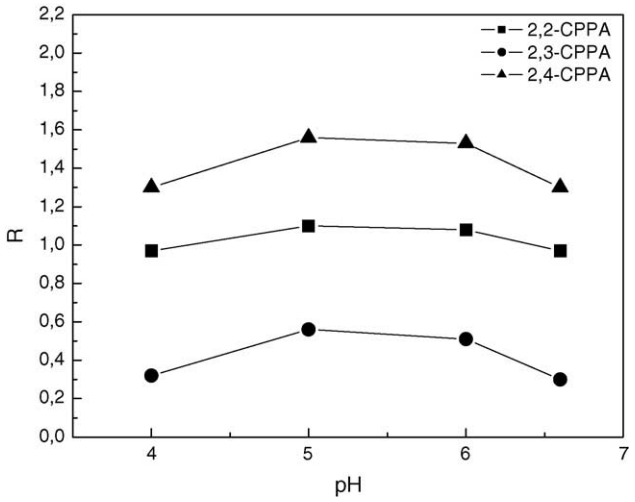

Fig. 9. Effect of the buffer pH on cLC enantioseparation of CPPAs using the teicoplanin-based CSP. Mobile phase composition: 20/80 (v/v) methanol/1.0% TEAA, pH 4.0–6.6.

both substituents on the aromatic ring. This is in accord with the lowest interaction (retention) of 2,2-CPPA in any mobile phase composition. Considering the pK_a value of CPPAs (pK_a(2,4-CPPA) = 4.32 in DMSO/H_2O [39]) chiral recognition interactions are favored if the carboxyl group is dissociated. The same trend was observed for profen NSAIDs.

Through a series of TEAA concentration and buffer pH variations an optimized buffer composition of 1.0% TEAA, pH 5.0 was found for enantiomeric separation of CPPAs on the teicoplanin-based capillary column. This buffer should offer the basis for addition of teicoplanin if a separation system with an achiral capillary column, Nucleosil 100 C_{18}

Table 7
Effect of the concentration of teicoplanin in mobile phase on chromatographic data of CPPA herbicides using Nucleosil 100 C_{18} HD

CPPA	Concentration of teicoplanin (mM)								
	0.00			0.05			0.10		
	k_1	α	R	k_1	α	R	k_1	α	R
2,2-CPPA	4.06	1.00	0.00	4.85	1.19	1.38	7.85	1.36	1.58
2,3-CPPA	5.09	1.00	0.00	6.97	1.00	0.00	10.51	1.00	0.00
2,4-CPPA	5.12	1.00	0.00	6.03	1.00	0.00	11.89	1.32	1.40

The mobile phase composition: 30/70, methanol/0.1% TEAA; pH 5.0.

HD, is used for enantioseparation of CPPAs. The idea was to add the chiral selector to the mobile phase of the same composition, which yielded good enantioresolution with the teicoplanin-based CSP, it means 10/90 (v/v) methanol/1.0% TEAA, pH 5.0, for derivatives 2,2-CPPA and 2,3-CPPA and 30/70 (v/v) methanol/1.0% TEAA, pH 5.0, for 2,4-CPPA (Table 6). Using the achiral column retention of all derivatives of CPPA dramatically increased (to 150 min) even without addition of teicoplanin to the mobile phase composed of 10/90 (v/v), methanol/1.0% TEAA; pH 5.0. Moreover, solubility of teicoplanin in solutions with higher buffer concentration substantially decreased. For this reason, the mobile phase composed of methanol and 0.1% TEAA, pH 5.0, in the ratio 30/70, yielding retention of CPPAs about 30 min, was chosen as the basis. Table 7 shows the effect of concentration of teicoplanin in the mobile phase on the chromatographic data of CPPAs. The concentration of teicoplanin could not be increased above 0.1 mM because CPPAs did not then elute from the column. The teicoplanin concentration in the mobile phase is a very important factor to control the chiral recognition. The retention factors of all derivatives increased with increasing teicoplanin concentration. Enantiomeric separations of 2,2-CPPA and 2,4-CPPA were achieved already at 0.05 and 0.1 mM concentrations of teicoplanin, respectively. Enantiomers of 2,3-CPPA could not be separated under the studied conditions.

The results obtained in the comparable mobile phase (MeOH/0.1% TEAA, pH 5.0; 30/70, v/v) with teicoplanin bonded on CSP and free in the solution (Table 6 versus Table 7) show that better enantioseparation of 2,4-CPPA than of 2,2-CPPA was observed on the teicoplanin bonded CSP while the opposite result was obtained with teicoplanin added to the mobile phase. Significantly lower affinity of 2,3-CPPA to teicoplanin in both systems is obvious. Steric effects (both intra- and intermolecular) should be responsible for the different interaction possibilities of these position isomers with the chiral selector. Stabilization of the π-electron system (2,2- and 2,4-derivatives) may also play a role if π–π interactions are involved in the enantioresolution mechanism. Increased rigidity of these derivatives favors chiral recognition ability. Fig. 10 shows the separation of 2,4-CPPA enantiomers on teicoplanin-based CSP with two mobile phases differing in the TEAA concentration (Fig. 10A and B) and in the separation system composed of the achiral column and the mobile phase containing 0.1 mM teicoplanin (Fig. 10C). The best enantioresolution in a short analysis time was obtained with the teicoplanin CSP and the mobile phase containing 1.0% buffer (Fig. 10B), as was already discussed above. Comparison of the two separation systems, with the chiral and achiral column, both using the 0.1% buffer in the mobile phase, shows that baseline separation can be achieved only in the system composed of C-18 column and the chiral selector-contained mobile phase. However, such separation conditions have no practical use because the elution time is too long and bad peak shape makes the separation not suitable for quantitative analysis. The chromatogram evidenced that

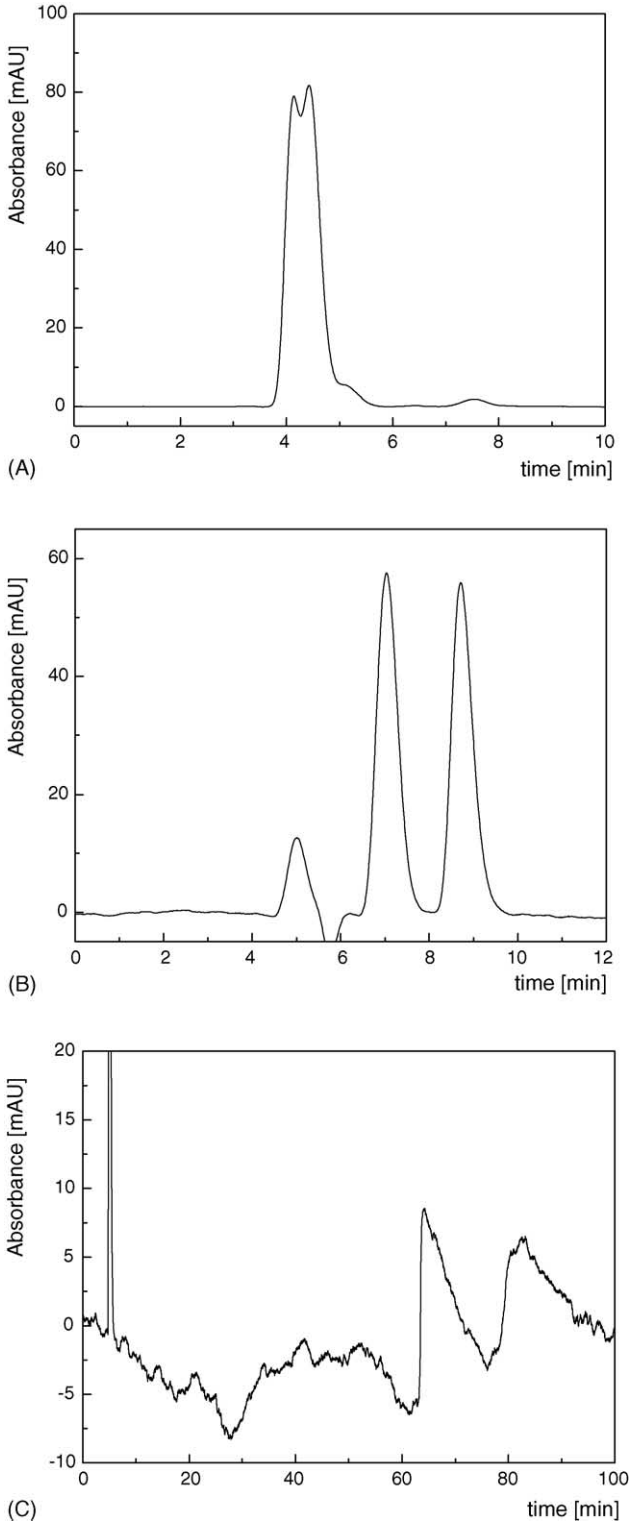

Fig. 10. Comparison of enantioseparation of 2,4-CPPA in mobile phase composition: (A) 30/70 (v/v) methanol/0.1% TEAA, pH 5.0; (B) 30/70 (v/v) methanol/1.0% TEAA, pH 5.0 on teicoplanin-based CSP; (C) 30/70 (v/v) methanol/0.1% TEAA, pH 5.0 with addition 0.1 mM teicoplanin on Nucleosil 100 C_{18} HD.

a competiting interaction of the analyte(s) with teicoplanin sorbed on the achiral stationary phase surface and that present in the mobile phase takes place in the separation system. This competition is responsible for the observed peak tailing.

4. Conclusion

Teicoplanin-based capillary column proved to be suitable for enantioseparation of some β-adrenergic antagonists, profen NSAIDs and chlorophenoxypropionic acid derivatives. Higher methanol content in the mobile phase was essential for enantioselective interactions of β-adrenergic antagonists with the CSP while profen NSAIDs and CPPAs were preferently enantioresolved in mobile phases with low organic modifier content. In the reversed phase mode an increase of the TEAA buffer concentration (as the aqueous part of the mobile phase) resulted in a decrease of the retention of β-adrenergic antagonists but did not influence their chiral resolution so far. On the contrary, retention of profen NSAIDs and CPPAs was increased and their enantioresolution improved at higher buffer concentrations. Chiral separations of the various classes of enantiomers were found to be pH dependent. Regarding the structure of β-adrenergic antagonists significant influence of pH on their dissociation or protonization could not be expected. However, the effect of buffer pH on the CSP (the chiral selector, as well as, the silica gel support) resulted in better enantioresolution of β-adrenergic antagonists at higher pH value (5.0). Despite the different structures of profen NSAIDs and CPPAs (both possessing a carboxyl group next to the chiral carbon atom) compared to β-adrenergic antagonists similar effect of pH on their enantioresolution was found. It can be postulated that the effect of pH on ionization of the functional groups of teicoplanin plays an important role in the stereoselective interaction mechanism. Polar organic separation mode was advantageous for chiral separation of β-adrenergic antagonists. Teicoplanin added to the mobile phase in the separation system with an achiral capillary column yielded chiral separation of 2,2-CPPA and 2,4-CPPA. A very low teicoplanin concentration (0.1 mM) was sufficient for their baseline enantioresolution. Due to the combined interaction of the analytes with teicoplanin partly sorbed on the stationary phase and partly remained in the mobile phase the last separation system did not have sufficient separation efficiency.

Acknowledgements

The authors acknowledge the financial support of this work from the Ministry of Education, Youth and Physical Training of the Czech Republic, grant no. 1893/2004 and from the Grant Agency of the Czech Republic, grant no. 203/03/0161. Special thanks must be expressed to Ing. J. Planeta from the Institute of Analytical Chemistry, Czech Academy of Sciences, Brno, Czech Republic, for packing the teicoplanin-based capillary column.

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