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Journal of Chromatography A, 966 (2002) 119–134

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Screening approach for chiral separation of pharmaceuticals Part II. Reversed-phase liquid chromatography

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Received 25 April 2002; received in revised form 4 June 2002; accepted 4 June 2002

Abstract

A screening strategy for the rapid separation of drug enantiomers by reversed-phase liquid chromatography was developed using three cellulose/amylose stationary phases. The key point to achieve enantioselectivity is the control of the compound ionisation. Only two mobile phases, i.e. an acidic phosphate buffer (pH 2.0) containing a chaotropic salt (KPF_6) and a borate buffer (pH 9.0) mixed with acetonitrile, are used in the proposed strategy. This strategy was successfully applied to a set of 37 diverse chiral pharmaceuticals. Satisfactory enantioselectivity was achieved for 89% of them.

Keywords: Enantiomer separation; Screening; Chiral stationary phases, LC; Polysaccharide stationary phases

1. Introduction

Hundreds of chiral selectors have been developed for the separation of enantiomers in liquid chromatography which makes feasible the separation of almost any pair of enantiomers [1–3]. However, the recognition mechanisms involved in the chiral recognition are complex and not always clearly known and therefore, the selection of the appropriate selector is usually done by a trial and error approach which is very costly and too time-consuming for the industry. Indeed, with the development of automated synthesis techniques such as in combinatorial chemistry, new chiral molecules (drugs, intermediates . . .) are produced every day, for which the enantiomeric purity needs to be checked from the early stages of drug development. The time assigned to the analysis of these compounds is usually very short. Therefore, the need for simple strategies for the rapid screening of chiral molecules is important. The aim of a screening strategy is to analyse quickly large series of very diverse molecules. Thus, the first step in the development of such strategy is the selection of a limited number of chiral selectors with very broad enantiorecognition abilities so that in the end most enantiomers can be resolved with at least one of them. Afterwards, a small set (as reduced as possible) of experimental conditions has to be defined. If

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0021-9673/02/$ – see front matter
PII: S0021-9673(02)00746-X

possible, no prior knowledge about the physicochemical properties of the analytes should be required in order to keep the strategy as simple and as rapid as possible. Short analysis times are clearly needed. At this stage, no optimal conditions are sought, the objective being to determine quickly if an acceptable separation can be achieved with the proposed technique.

Screening strategies for separation of chiral molecules by capillary electrophoresis (CE) and normal phase liquid chromatography (NPLC) have already been developed in our laboratories [4,5]. In this article, a screening strategy for the separation of chiral molecules by reversed-phase liquid chromatography (RPLC) is proposed. The use of chiral stationary phases (CSPs) was preferred to other approaches for the development of the screening strategy due to their easier use and efficiency. Among all the CSPs that have been developed for RPLC, the protein, Pirkle type, cyclodextrin and polysaccharide CSPs have been shown to be the most efficient [6–11]. Polysaccharide CSPs have been primarily used with non-polar mobile phases since the $\pi-\pi$, dipole–dipole and hydrogen bond interactions that are believed to be responsible for the chiral discrimination are known to be more efficient under normal-phase conditions [6]. However, several recent studies report the broad enantiorecognition potential of these CSPs with aqueous mobile phases [6,11–22]. Among these CSPs, the ester and carbamate derivatives of cellulose and amylose are reported to be the most efficient selectors [7,10]. Three of them, cellulose tris(3,5-dimethylphenylcarbamate), amylose tris(3,5-dimethylphenylcarbamate) and cellulose tris(4-methylbenzoate), have been reported to allow the separation of many drug enantiomers [11]. These selectors, commercialised under the names Chiralcel OD, Chiralpak AD and Chiralcel OJ, respectively, were selected for the development of the screening strategy in NPLC in our laboratories [5]. Therefore, these three selectors were evaluated under reversed-phase conditions. Different analytical factors susceptible to have a large influence on the separations were first carefully studied with a test set of 10 chiral drugs. From the results, a screening strategy was developed and applied to a large set of diverse chiral drugs.

2. Experimental

2.1. Instrumentation

Two chromatographic systems were used to perform the experiments: a HP 1050 system (Agilent Technologies, Palo Alto, CA) equipped with an automatic injector and a UV detector, and a HP 1100 system (Agilent Technologies) comprising a quaternary pump, a membrane degasser, a photodiode array UV detector, an automatic injector and a Mistral thermostating oven equipped with a six-column switching valve (Spark Holland, Emmen, The Netherlands).

A Chiralcel OD-RH® column (15 cm×4.6 mm I.D) packed with cellulose tris(3,5-dimethylphenyl carbamate), a Chiralpak AD-RH® column (15 cm×4.6 mm I.D) packed with amylose tris(3,5-dimethylphenyl carbamate) and a Chiralcel OJ-R® column (15 cm×4.6 mm I.D) packed with cellulose tris(4-methylbenzoate) coated on 5 μm silica-gel substrate particles (Daicel, Tokyo, Japan) were used.

2.2. Chemicals

Alprenolol, flurbiprofen, ibuprofen, ketoprofen, metoprolol, praziquantel, sulpiride, suprofen and warfarin were purchased from Sigma (Steinheim, Germany); verapamil hydrochloride from Fluka (Buchs, Switzerland). Oxprenolol, pindolol and acenocoumarol were gifts from Novartis (Basel, Switzerland); acebutolol from Rhone-Poulenc (Vitry Sur Seine, France); propranolol hydrochloride from Certa (Braine-l'Alleud, Belgium); atenolol, bisoprolol, oxprenolol from Ciba-Geigy (Barcelona, Spain); leucovorin from Cyanamid-Benelux (Mont Saint-Guibert, Belgium); nadolol from Bristol-Myers-Squibb (Barcelona, Spain); fluoxetine hydrochloride from Lilly (Mont Saint Guibert, Belgium). Diltiazem, hexobarbital, lorazepam, lormetazepam, mianserin, naproxen, oxazepam, propiomazine, temazepam, sotalol and phenobarbital were gifts from diverse sources.

Sodium perchlorate ($NaClO_4$), sodium dihydrogen phosphate, disodium hydrogen phosphate, methanol, isopropanol and ethanol (Licrosolv, HPLC grade)

were purchased from Merck (Darmstadt, Germany); potassium hexafluorophosphate (KPF_6) from Sigma–Aldrich (Steinheim, Germany); phosphoric acid (85%) from Carlo Erba (Milan, Italy) and acetonitrile (Hypersolv, HPLC grade) from BDH (Poole, UK). Water for the preparation of the mobile phases was produced in-house with the Milli-Q system (Millipore, Milford, MA).

2.3. Chromatographic conditions

Enantioseparations were performed isocratically at room temperature. Mobile phases consisted of aqueous buffer solution and acetonitrile mixtures. The flow-rate was 0.5 ml/min.

The samples were dissolved at a concentration of 500 μg/ml in methanol. The injection volume was 5 μl. Detection of the enantiomers was done by UV absorbance at 220 nm.

2.4. Data processing

Analytical data were acquired and treated with the Hewlett-Packard Chemstation for LC software package (Agilent Technologies).

Resolution values (*Rs*) were calculated according to the United States Pharmacopeia (USP) [23]:

$$Rs = \frac{2(t_R(b) - t_R(a))}{W_B(b) + W_B(a)} \quad \text{(Tangent method)} \qquad (1)$$

where $t_R(b)$ and $t_R(a)$ are the retention times of the last and the first eluting peak, respectively (in min), $W_B(b)$ and $W_B(a)$ are the base widths of the peaks *b* and *a* (in min).

3. Results and discussion

In order to determine suitable screening conditions, the effect of several analytical parameters on the quality of the separations was studied.

A set of 10 chiral drugs was used in this first study. Five of them were basic compounds (beta-blockers), four acidic (non steroidal anti-inflammatory drugs (NSAIDs) and anti-coagulants) and one bifunctional (benzodiazepine) (Fig. 1).

3.1. Effect of the pH of the mobile phase

The effect of the pH of the aqueous mobile phase on the retention and therefore on the separation of ionisable compounds is fundamental in RPLC. When fully ionised, the analytes partition preferentially in the aqueous mobile phase. Due to the resulting decrease of retention, their separation can be strongly affected. Several studies [5,10,11] have shown the fundamental importance of keeping the chiral analytes neutral when working with polysaccharide stationary phases. Indeed, these CSPs do not possess any ionic site susceptible to interact with the charged analytes. Consequently, the interactions with the CSPs are considerably reduced which can affect the chiral recognition and therefore the separation of the enantiomers.

Mixtures of phosphate electrolyte (50 m*M*) of different pH and acetonitrile were used as mobile phases to study the effect of the pH on the chiral separation of the 10 test compounds (Table 1). The pH of the phosphate electrolyte was varied within the stability range of the columns, i.e. 2.0–9.0.

3.1.1. Basic compounds

As expected an increase of the retention of the basic beta-blockers (alprenolol, metoprolol, oxprenolol, pindolol and propranolol) was observed with increasing pH resulting from a decrease of their ionisation. At pH 2.0, the compounds were almost not retained on any of the columns and consequently no separation of the enantiomers could be observed. At pH 5.5, although the analytes are still fully ionised and little retained on the columns, some initial separation is observed for pindolol on both Chiralcel OD-RH and Chiralpak AD-RH columns and for propranolol on the Chiralcel OD-RH column. At pH 9.0, which is approximately the pK_A value of the beta-blockers, a clear increase of the retention was observed on the three columns. Enantioselectivity was achieved for all analytes on the Chiralcel OD-RH and the Chiralpak AD-RH columns. No separation occurred on the Chiralcel OJ-R column, while the retention times of the analytes were similar to those obtained with the other columns. The absence of separation is probably attributed to a lack

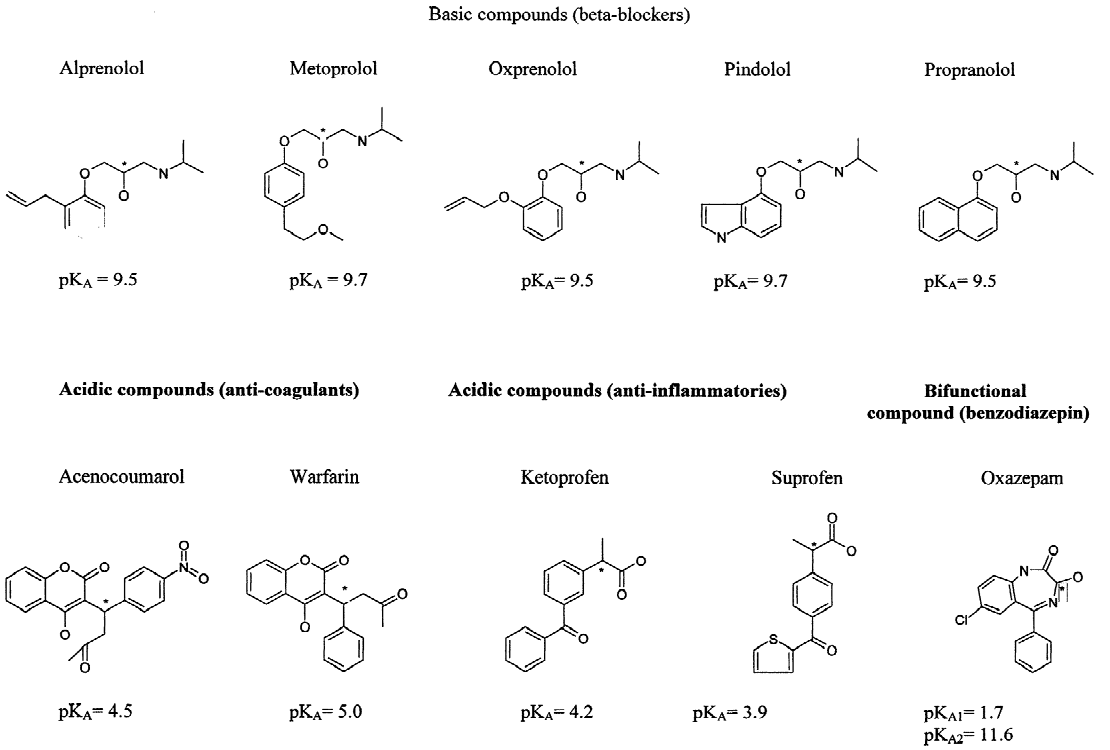

Fig. 1. Structure and pK_A of the chiral drugs used to study the influence of several analytical parameters on the separations.

of enantiorecognition of this selector towards the selected beta-blockers.

3.1.2. Acidic compounds

In contrast to basic compounds, the retention of the acidic analytes (acenocoumarol, warfarin, ketoprofen and suprofen) decreased with increasing pH due to their progressive ionisation. Similarly, a decrease in the separation of anti-coagulant (acenocoumarol and warfarin) enantiomers was observed. Different results were however obtained for the anti-inflammatory drugs. Indeed, the enantiomers of ketoprofen could only be resolved at pH 9.0 despite a very small retention of the enantiomers, and this, for the three columns. On the other hand, enantioselectivity was observed at pH 2.0 and pH 9.0 for suprofen enantiomers but not at pH 5.5 with the Chiralcel OD-RH and the Chiralpak AD-RH columns. These latter results are very interesting and suggest that although, in most cases as observed previously [6,12,13], the ionisation of chiral compounds has a negative effect on their enantioseparation, mainly due to a lack of retention, better separations can sometimes be achieved. The polysaccharide CSPs having no ionic site, the enantioselectivity observed for ketoprofen and suprofen at high pH is probably attributed to changes in the solvatation of these analytes or in the structure of the CSPs which favour the enantiorecognition.

3.1.3. Bifunctional compound

No enantioselectivity was observed for oxazepam with the Chiralpak AD-RH column and very little change in the analysis times were observed on this column. For the other columns, a slightly better separation of the enantiomers was obtained at pH 5.5 where the analyte is in a neutral form. At pH 2.0, where the analyte is still little positively charged, no separation is observed on the Chiralcel OJ-R column. A decrease in resolution is observed on both columns from pH 5.5 to 9.0 where the analyte becomes slightly negatively charged.

Table 1
Resolution (*Rs*) and retention times (t_1, t_2, in min) of the drug enantiomers on the Chiralcel OD-RH, the Chiralcel OJ-R and the Chiralpak AD-RH columns at different pH

	pH 2.0			pH 5.5			pH 9.0		
	Rs	t_1	t_2	*Rs*	t_1	t_2	*Rs*	t_1	t_2
OD-RH column									
Alprenolol	0.00	4.18	4.18	0.00	4.69	4.69	0.68	10.94	12.04
Metoprolol	0.00	3.95	3.95	0.00	4.08	4.08	0.58	6.20	6.50
Oxprenolol	0.00	3.75	3.75	0.00	4.42	4.42	1.19	8.87	10.11
Pindolol	0.00	4.13	4.13	0.86	4.40	4.97	3.06	7.91	12.91
Propranolol	0.00	4.46	4.46	0.60	5.38	5.82	0.00	23.18	23.18
Acenocoumarol	3.94	32.08	38.33	0.00	5.50	5.50	0.00	4.17	4.17
Warfarin	8.64	26.53	39.60	3.67	6.41	7.82	0.85	3.58	4.06
Ketoprofen	0.00	14.11	14.11	0.00	4.20	4.20	0.78	3.61	4.08
Suprofen	0.49	13.62	13.88	0.00	4.07	4.07	0.77	3.62	4.07
Oxazepam	12.51	14.33	29.25	12.61	14.11	28.73	11.06	13.45	25.34
OJ-R column									
Alprenolol	0.00	4.08	4.08	0.00	9.47	9.47	0.00	14.32	14.32
Metoprolol	0.00	4.19	4.19	0.00	4.35	4.35	0.00	8.77	8.77
Oxprenolol	0.00	4.19	4.19	0.00	3.22	3.22	0.00	9.63	9.63
Pindolol	0.00	4.06	4.06	0.00	5.89	5.89	0.00	8.74	8.74
Propranolol	0.00	4.23	4.23	0.00	7.67	7.67	0.00	14.17	14.17
Acenocoumarol	1.37	18.09	19.13	0.64	12.95	13.50	0.00	4.40	4.40
Warfarin	3.51	28.50	34.07	3.23	19.25	22.98	1.05	3.64	4.28
Ketoprofen	0.00	10.73	10.73	0.00	8.39	8.39	0.96	3.54	4.14
Suprofen	3.33	10.72	12.26	1.00	7.28	7.78	0.93	3.54	4.14
Oxazepam	0.00	6.64	6.64	0.57	10.40	10.73	0.31	6.64	6.75
AD-RH column									
Alprenolol	0.00	4.25	4.25	0.00	4.34	4.34	1.87	8.43	9.40
Metoprolol	0.00	4.06	4.06	0.00	4.16	4.16	0.29	8.99	9.31
Oxprenolol	0.00	4.09	4.09	0.00	4.19	4.19	0.99	6.42	6.84
Pindolol	0.00	4.04	4.04	0.29	4.03	4.21	0.74	5.79	6.26
Propranolol	0.00	4.17	4.17	0.00	4.37	4.37	0.56	9.72	10.19
Acenocoumarol	np	np	np	1.40	8.42	12.78	0.00	4.11	4.11
Warfarin	2.67	18.82	24.89	1.76	8.72	10.71	0.88	3.65	4.06
Ketoprofen	0.00	10.54	10.54	0.00	5.28	5.28	0.66	3.66	3.93
Suprofen	0.74	16.69	17.92	0.00	5.67	5.67	0.63	3.65	3.92
Oxazepam	0.00	12.27	12.27	0.00	12.36	12.36	0.00	11.97	11.97

Chromatographic conditions: mobile phase, (50 m*M* phosphate)–CH_3CN, 60:40 (v/v); flow-rate, 0.5 ml/min; temperature, ~25 °C; injection volume, 5 μl; detection, 220 nm; np, no peak detected.

The above results confirm that the control of the pH of the mobile phase is fundamental to achieve good enantioselectivity. According to these results and previous studies, better enantioselectivity should be achieved when analytes are in a neutral state. Thus, it would be appropriate to analyse basic compounds at high pH (9.0), acidic compounds at low pH and bifunctional compounds at a pH where they are neutral. However, the stability of the column at high pH (i.e. 9.0) is reduced compared to low pH (i.e. 2.0), and for many basic drugs (beta-blockers for instance), pH 9.0 (which is the highest pH that can be used with the CSPs) is not sufficient to suppress ionisation. Furthermore, in the perspective of the development of a screening strategy, the use of a single mobile phase (i.e. low pH aqueous mobile phase) would be preferable. However, as no retention is usually observed at low pH for basic compounds, the addition of an anionic component to the mobile phase to control their charge was investigated.

3.2. Effect of the addition of a chaotropic salt at low pH

Some previous studies have shown that the addition of a chaotropic salt, such as sodium perchlorate ($NaClO_4$), to the mobile phase was very useful to achieve retention of positively charged compounds and consequently enantioselectivity on the Chiralcel OD-RH column [6,13]. It is believed that the perchlorate anion forms an ion pair with the positively charged analyte so that the global charge of the analyte is reduced. Furthermore, chaotropic salts have the property to disrupt the water structure by breaking up hydrogen bonds and hydrophobic interactions, and therefore their addition to the mobile phase probably favours interactions between the chiral analytes and the CSPs.

The effect of the addition of perchlorate to the mobile phase and its concentration were studied for the three columns and different test compounds. Mixtures of phosphate electrolyte (50 m*M*, pH 2.0) containing sodium perchlorate ($NaClO_4$) and acetonitrile were used. The effect of the addition of perchlorate to a low pH (i.e. 2.0) mobile phase on resolution and retention is shown in Table 2.

3.2.1. Basic compounds

An increase in analysis time is observed with increasing concentration of perchlorate on the three columns. However, this increase is much more important with the Chiralcel OD-RH column than with the others. This difference in retention time reflects in the separation of the enantiomers. On the Chiralcel OD-RH, a great improvement in the separation of all enantiomers is observed when perchlorate is added to the mobile phase. Resolution is increased with increasing concentration of perchlorate up to 500 m*M*. On the contrary, no enantioselectivity was observed for any compound on the Chiralpak AD-RH and the Chiralcel OJ-R columns. In contrast to the Chiralcel OD-RH column, the addition of perchlorate to the mobile phase does not significantly increase retention of basic compounds on the Chiralpak AD-RH column. Therefore, interactions between the chiral analyte and the CSPs are still limited and the separations are not improved. As no enantioselectivity was observed at any pH on the Chiralcel OJ-R column (Table 1), it is probable that this selector does not have any enantiorecognition capabilities towards the studied beta-blockers. However, according to the results obtained for the retention, the addition of perchlorate will probably have limited effects on the separations as for the Chiralpak AD-RH column.

When higher concentrations of perchlorate were used (800 m*M* and 1 *M*), a decrease in retention and selectivity were observed for most compounds on the Chiralcel OD-RH column.

3.2.2. Acidic compounds and bifunctional compounds

As expected, retention and selectivity were not significantly affected by the addition of perchlorate in the mobile phase.

According to the above results, the addition of a chaotropic salt is very useful to improve the retention and the separation of basic enantiomers on the Chiralcel OD-RH under acidic conditions. However, its effect on the two other columns is limited and thus experiments at high pH (i.e. 9.0) appear to be necessary in the case of basic compounds.

3.3. Effect of the addition of a chaotropic salt at high pH

Previous results have shown that the presence of a chaotropic salt (i.e. perchlorate) was necessary to achieve the separation of the beta-blockers at low pH on the Chiralcel OD-RH column. An increase of the pH of the mobile phase to pH 9.0 (Table 1) also brought some improvement but to suppress totally the ionisation of these compounds, a pH of 11 or more would be required. However, the current columns cannot stand such a high pH mobile phase. Therefore, it was tested whether the addition of a chaotropic salt could be useful, at least on the Chiralcel OD-RH column, to reduce the ionisation of these analytes at pH 9.0. Mixtures of phosphate electrolyte (50 m*M*, pH 9.0) containing sodium perchlorate ($NaClO_4$) and acetonitrile were used. The effect of the addition of perchlorate on resolution and retention are shown in Table 3.

Against our expectations, the addition of perchlorate resulted in a decrease of retention of the compounds on the three columns. The effect on the separation depends on the column. For the Chiralcel

Table 2
Resolution (*Rs*) and retention times (t_1, t_2, in min) of the drug enantiomers on the Chiralcel OD-RH, the Chiralcel OJ-R and the Chiralpak AD-RH columns, after addition of different concentrations of perchlorate to a low pH mobile phase

	0 m*M*			250 m*M*			500 m*M*		
	Rs	t_1	t_2	*Rs*	t_1	t_2	*Rs*	t_1	t_2
OD-RH column									
Alprenolol	0.00	4.18	4.18	0.77	6.30	6.59	1.11	6.83	7.23
Metoprolol	0.00	3.95	3.95	0.63	4.70	4.88	0.87	4.91	5.16
Oxprenolol	0.00	3.75	3.75	1.35	5.66	6.06	1.68	6.05	6.56
Pindolol	0.00	4.13	4.13	4.74	6.00	8.94	4.69	7.33	12.49
Propranolol	0.00	4.46	4.46	2.71	8.48	10.56	2.74	9.76	12.55
Acenocoumarol	3.94	32.08	38.33	3.91	31.04	37.22	3.90	31.34	37.71
Warfarin	8.64	26.53	39.60	8.49	26.44	39.55	8.44	27.73	41.64
Ketoprofen	0.00	14.11	14.11	0.00	14.29	14.29	0.29	14.78	14.94
Suprofen	0.49	13.62	13.88	0.49	13.59	13.85	0.49	13.96	14.24
Oxazepam	12.51	14.33	29.25	11.53	13.60	26.99	10.97	13.12	25.02
OJ-R column									
Alprenolol	0.00	4.08	4.08	0.00	5.73	5.73	0.00	6.04	6.04
Metoprolol	0.00	4.19	4.19	0.00	4.86	4.86	0.00	5.31	5.31
Oxprenolol	0.00	4.19	4.19	0.00	5.24	5.24	0.00	5.67	5.67
Pindolol	0.00	4.06	4.06	0.00	5.24	5.24	0.00	5.24	5.24
Propranolol	0.00	4.23	4.23	0.00	6.35	6.35	0.00	6.06	6.06
Acenocoumarol	1.37	18.09	19.13	1.41	22.24	23.55	1.34	20.23	21.69
Warfarin	3.51	28.50	34.07	3.55	36.37	43.51	3.42	34.57	41.98
Ketoprofen	0.00	10.73	10.73	0.00	12.17	12.17	0.00	11.91	11.91
Suprofen	3.33	10.72	12.26	3.62	12.21	14.12	3.80	11.88	13.91
Oxazepam	0.00	6.64	6.64	0.00	7.02	7.02	0.00	6.54	6.54
AD-RH column									
Alprenolol	0.00	4.25	4.25	0.00	5.06	5.06	0.00	6.62	6.62
Metoprolol	0.00	4.06	4.06	0.00	4.48	4.48	0.00	5.43	5.43
Oxprenolol	0.00	4.09	4.09	0.00	4.74	4.74	0.00	5.37	5.37
Pindolol	0.00	4.04	4.04	0.00	4.33	4.33	0.00	4.67	4.67
Propranolol	0.00	4.17	4.17	0.00	5.09	5.09	0.00	7.16	7.16
Acenocoumarol	np	np	np	np	np	np	np	np	np
Warfarin	2.67	18.82	24.89	2.51	20.22	26.73	2.39	40.12	45.26
Ketoprofen	0.00	10.54	10.54	0.00	11.13	11.13	0.00	11.54	11.54
Suprofen	0.74	16.69	17.92	0.73	16.20	17.46	0.48	16.63	18.01
Oxazepam	0.00	12.27	12.27	0.00	11.74	11.74	0.00	10.75	10.75

Chromatographic conditions: mobile phase, (50 m*M* phosphate, pH 2.0, $NaClO_4$)–CH_3CN, 60:40 (v/v); flow-rate, 0.5 ml/min; temperature, ~25 °C; injection volume, 5 μl; detection, 220 nm; np, no peak detected.

OD-RH, the separation of the beta-blockers increased when 250 m*M* perchlorate is added to the mobile phase. Higher concentrations of perchlorate resulted in a decrease of resolution. For the Chiralpak AD-RH column, a decrease in resolution was observed for all types of compounds when perchlorate was added to the mobile phase. A decrease in resolution was observed for acidic and bifunctional compounds on the Chiralcel OJ-R column. Chromatograms illustrating the effect of sodium perchlorate on the separation of basic enantiomers at different pH are shown in Fig. 2.

The addition of perchlorate resulted in a slight decrease of resolution for all acidic and bifunctional compounds.

3.4. Effect of the type of chaotropic salt ($NaClO_4$ versus KPF_6)

The previous results have shown that the presence

Table 3
Resolution (*Rs*) and retention times (t_1, t_2, in min) of the drug enantiomers on the Chiralcel OD-RH, the Chiralcel OJ-R and the Chiralpak AD-RH columns, after addition of different concentrations of perchlorate to a high pH mobile phase

	0 m*M*			250 m*M*			500 m*M*		
	Rs	t_1	t_2	*Rs*	t_1	t_2	*Rs*	t_1	t_2
OD-RH column									
Alprenolol	0.68	10.94	12.04	1.52	10.78	11.79	1.27	9.84	10.65
Metoprolol	0.58	6.20	6.49	0.65	6.48	6.90	0.58	5.59	6.00
Oxprenolol	1.19	8.87	10.11	2.52	8.90	10.07	1.41	8.04	8.97
Pindolol	3.06	7.91	12.91	4.78	8.27	13.68	3.94	8.09	12.74
Propranolol	0.00	23.18	23.18	0.00	22.18	22.18	0.88	17.77	19.10
Acenocoumarol	0.00	4.17	4.17	0.00	4.10	4.10	0.00	4.10	4.10
Warfarin	0.85	3.58	4.06	0.79	4.06	4.06	0.66	3.69	4.05
Ketoprofen	0.78	3.61	4.08	0.81	3.71	4.13	0.71	3.69	4.06
Suprofen	0.77	3.62	4.07	0.78	3.72	4.12	0.68	3.68	4.02
Oxazepam	11.06	13.45	25.34	10.30	13.28	25.23	10.84	13.50	25.56
OJ-R column									
Alprenolol	0.00	14.32	14.32	0.00	8.73	8.73	0.00	6.78	6.78
Metoprolol	0.00	8.77	8.77	0.00	5.69	5.69	0.00	5.10	5.10
Oxprenolol	0.00	9.63	9.63	0.00	7.06	7.06	0.00	5.51	5.51
Pindolol	0.00	8.74	8.74	0.00	7.03	7.03	0.00	5.31	5.31
Propranolol	0.00	14.17	14.17	0.00	13.05	13.05	0.00	7.87	7.87
Acenocoumarol	0.00	4.40	4.40	0.00	5.16	5.16	0.00	4.03	4.03
Warfarin	1.05	3.64	4.28	0.87	4.81	5.45	0.23	3.48	4.02
Ketoprofen	0.96	3.54	4.13	0.99	4.37	5.02	0.84	3.49	4.04
Suprofen	0.93	3.54	4.14	0.95	4.79	5.44	0.69	3.48	3.95
Oxazepam	0.31	6.64	6.75	0.20	9.00	9.10	0.00	6.34	6.34
AD-RH column									
Alprenolol	1.87	8.43	9.40	1.53	7.86	8.67	0.72	7.26	7.80
Metoprolol	0.49	8.99	9.31	0.00	8.56	8.56	0.00	7.15	7.15
Oxprenolol	0.99	6.42	6.84	0.64	6.23	6.62	0.00	5.66	5.66
Pindolol	0.74	5.79	6.26	0.48	5.85	6.36	0.00	4.92	4.92
Propranolol	0.56	9.72	10.19	0.57	8.92	9.36	0.00	7.95	7.95
Acenocoumarol	0.00	4.11	4.11	0.00	4.08	4.08	0.00	4.09	4.09
Warfarin	0.88	3.65	4.06	0.53	3.68	3.93	0.92	3.59	4.00
Ketoprofen	0.66	3.66	3.93	0.55	3.69	3.96	0.50	3.95	4.28
Suprofen	0.63	3.65	3.92	0.51	3.72	3.96	0.47	3.54	3.92
Oxazepam	0.00	11.97	11.97	0.00	11.61	11.61	0.00	11.72	11.72

Chromatographic conditions: mobile phase, (50 m*M* phosphate, pH 9.0, $NaClO_4$)–CH_3CN, 60:40 (v/v); flow-rate, 0.5 ml/min; temperature, ~25 °C; injection volume, 5 μl; detection, 220 nm.

of a chaotropic salt was necessary to achieve a good separation of basic compounds on the Chiralcel OD-RH column at low pH. However, the use of perchlorate is not recommended due to its explosive properties. Consequently, the use of another chaotropic salt, potassium hexafluorophosphate (KPF_6), which was reported to be as efficient as perchlorate [11], was examined.

Different concentrations of KPF_6 were tested at low pH (i.e. pH 2.0). Comparable or even better separations were achieved when 100 m*M* KPF_6 was added to the mobile phase instead of 500 m*M* $NaClO_4$ (Table 4). Consequently, KPF_6 should be used preferably instead of $NaClO_4$.

Several studies have reported that the effect of the cationic salt was rather small. However, it should be noticed that in the case of PF_6^-, the potassium salt has to be used and not the sodium salt for solubility reasons. Indeed, it was found that precipitation of the sodium salt occurred when its concentration was

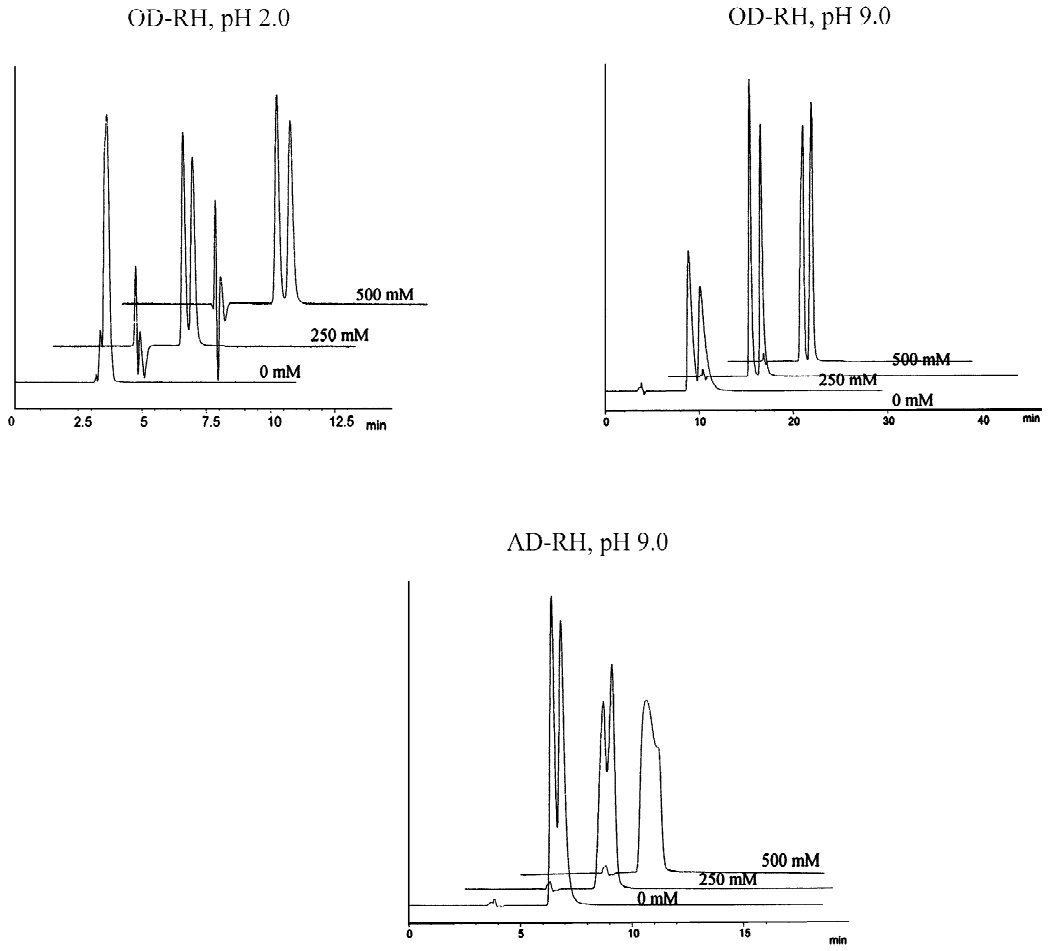

Fig. 2. Effect of the addition of sodium perchlorate on the separation of oxprenolol enantiomers.

above 50 m*M*. At 50 m*M*, the retention and consequently the separation of the basic species was considerably reduced compared to 100 m*M*. Therefore, the use of the potassium salt is required.

3.5. Effect of the type of buffer

The pH being a crucial factor for the separations, the effect of the type of acid/base used to set the pH of the mobile phase was also studied.

3.5.1. Acidic mobile phase

Trifluoroacetic acid (TFA) and perchloric acid were used instead of phosphoric acid. Against our expectations, the addition of perchloric acid did not improve the separations. The use of TFA also did not result in any significant changes of the separations. Therefore, the nature of the acid used to adjust the pH of the mobile phase did not appear to have any influence on the separation of the different species.

3.5.2. Basic mobile phase (i.e. pH 9.0)

The use of borate was compared to phosphate. In contrast with the results obtained at low pH, the nature of the electrolyte was found to have an important effect on the retention and thus on the separation of the compounds.

The use of 20 m*M* borate buffer leads to a significant improvement of the separation compared to 50 m*M* phosphate buffer (Table 5). The improve-

Table 4
Comparison of separation of the drug enantiomers with either $NaClO_4$ or KPF_6 added to a low pH mobile phase on the Chiralcel OD-RH, the Chiralcel OJ-R and the Chiralpak AD-RH (t_1, t_2, in min)

	$NaClO_4$			KPF_6		
	Rs	t_1	t_2	*Rs*	t_1	t_2
OD-RH column						
Alprenolol	1.11	6.83	7.23	0.63	7.70	8.04
Metoprolol	0.87	4.91	5.16	1.11	5.50	5.81
Oxprenolol	1.68	6.05	6.56	1.53	6.79	7.35
Pindolol	4.69	7.33	12.49	4.85	7.28	11.03
Propranolol	2.74	9.76	12.55	2.58	10.93	13.26
Acenocoumarol	3.90	31.34	37.71	3.87	33.28	39.78
Warfarin	8.44	27.73	41.64	8.28	26.41	39.15
Ketoprofen	0.29	14.78	14.94	0.00	14.01	14.01
Suprofen	0.49	13.96	14.24	0.46	13.64	13.89
Oxazepam	10.97	13.12	25.02	11.65	14.34	28.15
OJ-R column						
Alprenolol	0.00	6.04	6.04	0.00	6.15	6.15
Metoprolol	0.00	5.31	5.31	0.00	4.95	4.95
Oxprenolol	0.00	5.67	5.67	0.00	5.40	5.40
Pindolol	0.00	5.24	5.24	0.00	5.04	5.04
Propranolol	0.00	6.06	6.06	0.00	6.52	6.52
Acenocoumarol	1.34	20.23	21.69	1.35	18.48	19.54
Warfarin	3.42	34.57	41.98	3.29	29.86	35.82
Ketoprofen	0.00	11.91	11.91	0.00	10.97	10.97
Suprofen	3.80	11.88	13.91	3.53	11.04	12.74
Oxazepam	0.00	6.54	6.54	3.74	12.64	14.72
AD-RH column						
Alprenolol	0.00	6.62	6.62	0.00	5.69	5.69
Metoprolol	0.00	5.43	5.43	0.00	5.02	5.02
Oxprenolol	0.00	5.37	5.37	0.00	5.19	5.19
Pindolol	0.00	4.67	4.67	0.00	4.71	4.71
Propranolol	0.00	7.016	7.16	0.00	5.75	5.75
Acenocoumarol	np	np	np	np	np	np
Warfarin	2.39	40.12	45.26	2.29	19.86	26.64
Ketoprofen	0.00	11.54	11.54	0.00	11.16	11.16
Suprofen	0.48	16.63	18.01	0.62	16.45	17.64
Oxazepam	0.00	10.75	10.75	0.00	12.24	12.24

Chromatographic conditions: mobile phase, (50 m*M* phosphate, pH 2.0, 500 m*M* $NaClO_4$ or 100 m*M* KPF_6)–CH_3CN, 60:40 (v/v); flow-rate, 0.5 ml/min; temperature, ~25 °C; injection volume, 5 μl; detection, 220 nm; np, no peak observed.

ment is observed for all types of compounds (except for beta-blockers on the Chiralcel OJ-R column where no separation occurred) and is mainly attributed to an important improvement of the peak shapes. This improvement may be attributed to the good buffering capacity of borate in this range and also to a difference in ionic strength between the two electrolytes. On the Chiralcel OD-RH column, similar separations were obtained as to when 250 m*M* perchlorate was added to the mobile phase, which led to the best results.

3.6. Effect of the type of organic solvent

Different types of organic solvents, ethanol, methanol and isopropanol were used instead of acetonitrile. The concentration of each solvent was

Table 5
Comparison of the separation of the drug enantiomers on the Chiralcel OD-RH, the Chiralcel OJ-R and the Chiralpak AD-RH columns at pH 9.0 with either phosphate (50 m*M*) or borate electrolyte (20 m*M*) (t_1, t_2, in min)

	Phosphate			Borate		
	Rs	t_1	t_2	*Rs*	t_1	t_2
OD-RH column						
Alprenolol	0.68	10.94	12.04	1.50	16.58	19.58
Metoprolol	0.58	6.20	6.50	0.54	7.14	8.57
Oxprenolol	1.19	8.87	10.11	1.93	7.43	11.85
Pindolol	3.06	7.91	12.91	4.35	6.43	11.49
Propranolol	0.00	23.18	23.18	0.00	22.45	22.45
Acenocoumarol	0.00	4.16	4.16	0.00	3.98	3.98
Warfarin	0.85	3.58	4.06	0.91	3.54	3.89
Ketoprofen	0.78	3.61	4.08	1.05	3.54	3.82
Suprofen	0.77	3.62	4.07	0.82	3.58	4.01
Oxazepam	11.06	13.45	25.34	10.94	11.76	23.43
OJ-R column						
Alprenolol	0.00	14.32	14.32	0.00	9.69	9.69
Metoprolol	0.00	8.77	8.77	0.00	8.26	8.26
Oxprenolol	0.00	9.63	9.63	0.00	8.76	8.76
Pindolol	0.00	8.74	8.74	0.00	8.69	8.69
Propranolol	0.00	14.17	14.17	0.00	13.78	13.78
Acenocoumarol	0.00	4.40	4.40	0.00	3.86	3.86
Warfarin	1.05	3.64	4.28	1.16	3.87	4.36
Ketoprofen	0.96	3.54	4.13	1.15	3.46	3.74
Suprofen	0.93	3.54	4.14	1.21	3.61	4.23
Oxazepam	0.31	6.64	6.75	0.35	6.58	6.90
AD-RH column						
Alprenolol	1.87	8.43	9.40	2.11	13.47	15.83
Metoprolol	0.49	8.99	9.31	0.58	15.69	16.50
Oxprenolol	0.99	6.42	6.84	1.32	9.12	10.16
Pindolol	0.74	5.79	6.26	1.65	8.00	9.11
Propranolol	0.56	9.72	10.19	0.68	16.30	17.39
Acenocoumarol	0.00	4.11	4.11	0.00	4.46	4.46
Warfarin	0.88	3.65	4.06	0.95	3.82	4.17
Ketoprofen	0.66	3.66	3.93	0.73	3.75	4.15
Suprofen	0.63	3.65	3.92	0.75	3.78	4.21
Oxazepam	0.00	11.97	11.97	0.00	13.06	13.06

Chromatographic conditions: mobile phase, (50 m*M* phosphate or 20 m*M* borate, pH 9.0)–CH_3CN, 60:40 (v/v); flow-rate, 0.5 ml/min; temperature, ~25 °C; injection volume, 5 μl; detection, 220 nm.

adapted so that similar retention times were obtained. However, extremely long retention times were obtained with acidic compounds using either ethanol or isopropanol. The use of methanol resulted in an important increase of the pressure.

In general, the separation of basic compounds was inferior to those obtained with acetonitrile due to an important decrease of the peak efficiencies. Therefore, acetonitrile should be preferred as the organic modifier for the chromatographic system and the substances considered.

The effect of the type of organic modifier on the separation of metoprolol enantiomers on the Chiralcel OD-RH column is shown in Fig. 3.

3.7. Determination of screening conditions

The initial study has shown that the ionisation of

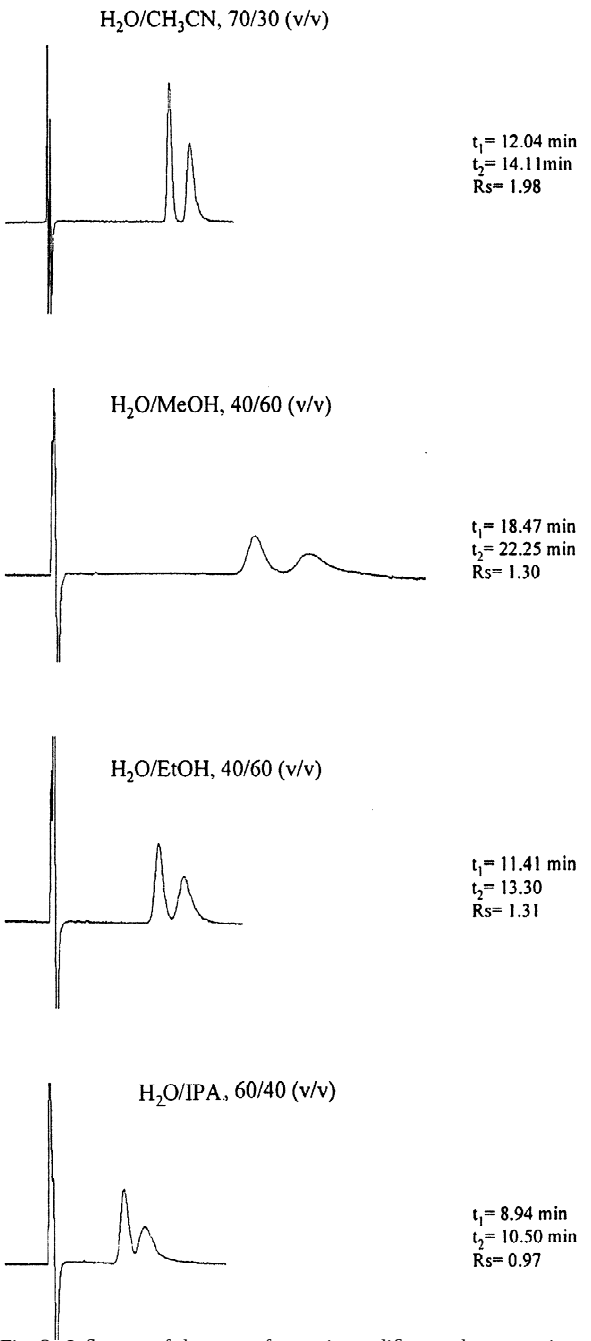

Fig. 3. Influence of the type of organic modifier on the separation of metoprolol enantiomers (mobile phase, 50 m*M* phosphate buffer pH 2.0, 500 m*M* $NaClO_4$/organic modifier).

the chiral compounds was the most important parameter to control in order to achieve good separation of the enantiomers with polysaccharide CSPs. In general, enantiomers should be better separated when they are uncharged. Therefore, most acidic drugs should be better enantiomerically resolved with an acidic mobile phase (i.e. pH 2.0) and most basic enantiomers should be better separated under basic conditions. Neutral compounds should be quite insensitive to pH changes. However, it was also shown that the separation of basic compounds was possible under acidic conditions on the Chiralcel OD-RH column when a chaotropic salt such as KPF_6 or $NaClO_4$ was added to the mobile phase. Therefore, this approach should be preferred when possible as the life time of the column is severely reduced when basic mobile phases are used. Unfortunately, this latter approach was shown to be not very efficient for the Chiralpak AD-RH and the Chiralcel OJ-R columns and therefore, basic mobile phases were necessary to achieve the separation of basic compounds. The presence of a chaotropic salt in an acidic mobile phase did not have any influence on the separation of acidic compounds. Therefore, an acidic mobile phase containing KPF_6 can be used to screen any type of molecule. A basic mobile phase should only be tested when no separation was achieved at low pH on any column. A simple borate mobile phase is then needed. Acidic compounds should also be screened at basic pH when they cannot be resolved at low pH. Indeed, in this study, ketoprofen enantiomers, for instance, were better resolved at pH 9.0.

The Chiralcel OD-RH column has been shown to have the broadest enantiorecognition capabilities for the tested compounds. Enantiomers of basic drugs could be resolved at high pH as well as at low pH when a chaotropic salt was present in the mobile phase (Table 6). Acidic compounds, except ketoprofen, were also enantiomerically resolved at both pHs. However, better enantioselectivity was achieved at pH 2.0 for the anti-coagulant drugs while pH 9.0 was necessary for the chiral separation of NAIDS. Oxazepam could be separated at any pH without important changes in resolution and analysis time.

Enantioselectivity was observed for all compounds except oxazepam with the Chiralpak AD-RH column (Table 6), but, as explained previously, enantiomers of basic compounds could only be separated at high

Table 6
Summary of the separations obtained at low (pH 2.0) and high pH (pH 9.0) on the three columns

	OD-RH				OJ-R				AD-RH			
	pH 2.0		pH 9.0		pH 2.0		pH 9.0		pH 2.0		pH 9.0	
	Rs	AT	*Rs*	AT	*Rs*	AT	*Rs*	AT	*Rs*	AT	*Rs*	AT
Alprenolol	0.63	8.04	1.50	19.58	0.00	6.15	0.00	9.69	0.00	5.69	2.11	15.83
Metoprolol	1.11	5.81	0.54	8.57	0.00	4.95	0.00	8.26	0.00	5.02	0.58	16.50
Oxprenolol	1.53	7.35	1.93	11.85	0.00	5.40	0.00	8.76	0.00	5.19	1.32	10.16
Pindolol	4.85	11.03	4.35	11.49	0.00	5.04	0.00	8.69	0.00	4.71	1.65	9.11
Propranolol	2.58	13.26	0.00	22.45	0.00	6.52	0.00	13.78	0.00	5.75	0.68	17.39
Acenocoumarol	3.87	39.78	0.00	3.98	1.35	19.54	0.00	3.86	np	np	0.00	4.46
Warfarin	8.28	39.15	0.91	3.89	3.29	35.82	1.16	4.36	2.29	26.64	0.95	4.17
Ketoprofen	0.00	14.01	1.05	3.82	0.00	10.97	1.15	3.74	0.00	11.16	0.73	4.15
Suprofen	0.46	13.89	0.82	4.01	3.53	12.74	1.21	4.23	0.62	17.64	0.75	4.21
Oxazepam	11.65	28.15	10.94	23.43	3.74	14.72	0.35	6.90	0.00	12.24	0.00	13.06

Chromatographic conditions: mobile phase 1, (50 m*M* phosphate buffer + 100 m*M* KPF_6, pH 2.0)–CH_3CN, 60:40 (v/v); mobile phase 2, (20 m*M* borate buffer, pH 9.0)–CH_3CN, 60:40 (v/v); flow-rate, 0.5 ml/min; temperature, ~25 °C; injection volume, 5 μl; detection, 220 nm; AT, analysis time, in min.

pH. Warfarin was better separated at low pH. Although too long retention times were obtained for acenocoumarol at pH 2.0, the same result is expected. Similar separations were achieved at low and high pHs for suprofen enantiomers but with different analysis times. Ketoprofen enantiomers were only separated at high pH.

The Chiralcel OJ-R column did not show any enantioselectivity towards the beta-blockers. Better separation of acenocoumarol, warfarin, suprofen and oxazepam enantiomers was achieved at low pH (Table 6). Enantiomers of ketoprofen were again only resolved at pH 9.0.

According to the results of this study, the Chiralcel OD-RH appears to perform better than the two other columns in the sense that it seems to have broader enantiorecognition capabilities. However, only 10 molecules were analysed which does not allow one to define final conclusions.

According to the results obtained, the following screening strategy was proposed and tested. The compounds are first analysed with an acidic phosphate mobile phase (50 m*M*, pH 2.0) containing 100 m*M* KPF_6–acetonitrile mobile phase. Several water–acetonitrile ratios were tested. A 60:40 (v/v) ratio was found to be the most appropriate to achieve sufficient and reasonable retention for a wide range of compounds. At the screening stage, only acetonitrile is used as organic modifier. Some studies [15] have shown that in certain specific cases, other modifiers such as ethanol would perform better, but in a screening strategy, only the most widely applicable one is preferred. Indeed, to be efficient, only a very reduced set of analytical conditions can be tested in a screening approach. These conditions should allow the separation of most chiral drugs but it has to be accepted that in a number of cases, some possible separations will be missed.

When very little or no separation is achieved at low pH, then the chiral drug is analysed on the three columns at high pH with a borate buffer (20 m*M*, pH 9.0)–acetonitrile mobile phase. The water–acetonitrile ratio is still 60:40 (v/v). The flow-rate is fixed at 0.5 ml/min.

3.8. Rapid screening of chiral drugs

The screening strategy was applied to 37 chiral drugs with diverse chemical/physical properties. Results are shown in Table 7. When very little or no enantioselectivity was achieved at pH 2.0, results are given at pH 9.0. Some examples of separations obtained on the three columns are shown in Fig. 4.

Results are generally considered satisfactory in the sense that 33 drugs out of 37 were enantioresolved. Some enantioselectivity was observed for 18 out of the 22 basic drugs analysed. Twelve could be resolved at low pH, mainly on the Chiralcel OD-RH

Table 7
Results of the screening of chiral drugs by RPLC according to the proposed strategy

Compound	OD-RH		OJ-R		AD-RH	
	Rs	AT	*Rs*	AT	*Rs*	AT
Basic						
Acebutolol[b]	0.00	9.15	0.00	5.33	0.98	8.84
Alprenolol[b]	1.50	19.69	0.00	9.69	2.11	15.83
Atropine[a]	0.31	4.93	0.00	4.53	0.00	4.39
Clenbuterol[b]	0.55	17.49	0.00	8.60	0.63	31.89
Dilthiazem[b]	0.00	18.89	0.00	10.66	3.60	30.71
Ephedrine[b]	0.00	6.17	0.00	5.50	0.00	6.86
Fluoxetine	1.34[a]	15.29	0.00	9.23	0.51[b]	4.17
Ketamine[a]	2.06	6.78	0.00	5.00	0.00	4.69
Leucovorin[b]	0.00	3.83	0.00	3.51	0.00	3.76
Methadone	2.01[a]	25.32	0.00	11.62[a]	0.49[b]	56.58
Metoprolol	1.11[a]	5.81	0.00	4.95[a]	0.58[b]	16.50
Mianserin	3.07[a]	27.12	0.47[a]	8.34	3.38[b]	35.65
Morphine[b]	0.64	4.33	1.19	4.01	0.00	10.52
Nadolol[b]	0.00	5.25	0.00	4.15	0.00	4.65
Oxprenolol	1.55[a]	7.35	0.00	5.40[a]	1.32[b]	10.16
Pindolol	4.85[a]	11.03	0.00	5.04[a]	1.65[b]	9.11
Promethazine	0.43[a]	14.92	2.47[b]	52.16	0.00[a]	7.84
Propiomazine	0.00[a]	19.29	2.11[b]	41.82	1.67[b]	113.58
Propranolol	2.58[a]	13.26	0.00	6.52[a]	0.68[b]	17.39
Sulpiride[b]	0.00	5.75	0.00	4.33	0.00	5.98
Tetramisol[a]	6.56	57.50	3.30	31.75	1.64	32.75
Verapamil[a]	3.40	20.12	0.00	9.32	0.00	27.16
Acidic						
Acenocoumarol[a]	3.87	39.78	1.35	19.54	0.00	41.45
Fenoprofen[a]	0.60	28.71	1.48	30.05	0.20	14.99
Flurbiprofen[a]	0.61	26.53	1.43	5.83	5.04	26.58
Hexobarbital[a]	0.51	11.81	0.87	9.76	7.30	19.70
Ibuprofen[a]	0.67	24.08	1.84	25.40	1.74	20.53
Ketoprofen[b]	0.68	3.97	0.49	3.84	0.57	8.68
Mandelic acid[b]	0.00	3.36	0.68	3.80	0.00	8.87
Naproxen[a]	0.00	18.09	0.00	22.74	1.68	15.00
Suprofen[a]	0.46	13.89	3.53	12.74	0.62	17.64
Warfarin[a]	8.28	39.15	3.29	35.82	2.29	26.64
Bifunctional, neutral						
Cyclothiazide[a]	2.08	11.97	0.00	9.14	0.00	8.38
Oxazepam[a]	11.65	28.15	3.74	14.72	0.00	12.24
Praziquantel[a]	1.50	22.25	2.64	9.17	0.00	105.91
Thiopental[a]	0.00	20.00	1.10	15.96	0.00	25.03
Trans-stilbene oxide[b]	2.12	21.42	0.00	5.09	5.05	48.18

[a] Mobile phase, (50 m*M* phosphate buffer + 100 m*M* KPF_6, pH 2.0)–CH_3CN, 60:40 (v/v).
[b] Mobile phase, (20 m*M* borate buffer, pH 9.0)–CH_3CN, 60:40 (v/v). Flow-rate, 0.5 ml/min; temperature, ~25 °C; injection volume, 5 μl; detection, 220 nm. AT, analysis time, in min.

column. However, it has to be noted that the enantiomers of tetramisol could also be resolved at low pH on the Chiralcel OJ-R and the Chiralpak AD-RH columns and mianserin enantiomers on the Chiralpak AD-RH column. Several basic drugs were resolved at high pH on the Chiralpak AD-RH and the Chiralcel OJ-R columns. As observed in the preliminary study, the Chiralcel OJ-R column seems to

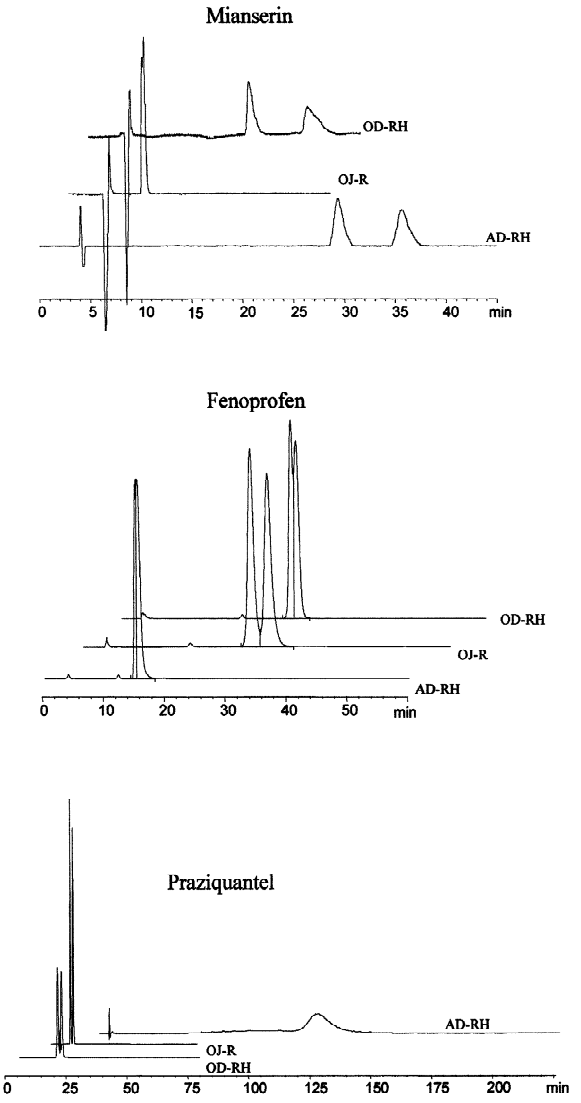

Fig. 4. Chromatograms of the separation of some drug enantiomers obtained in the screening (chromatographic conditions as described in Table 7).

be less efficient than the two others for the chiral separation of basic compounds. However, the best separation of morphine, promethazine and propiomazine enantiomers were achieved on this latter column.

All acidic and neutral/bifunctional drugs were enantioresolved on at least one of the columns. Separations could be achieved at low pH except for ketoprofen, mandelic acid and *trans*-stilbene oxide. Overall, the three columns appeared to perform equally well in a quite complementary way. For instance, the enantiomers of thiopental were only resolved on the Chiralcel OJ-R column, while naproxen enantiomers could only be separated on the Chiralpak AD-RH column.

The three selected columns have been shown to be efficient and complementary selectors as 89% of the screened drugs could be enantiomerically resolved on at least one of them. The use of an acidic and a basic mobile phase was sufficient to achieve the resolution of most chiral molecules tested. The Chiralcel OD-RH appeared to have the broadest enantiorecognition capabilities especially at low pH and for basic compounds. The Chiralpak AD-RH was shown to have complementary capabilities especially for basic compounds at high pH and the Chiralcel OJ-R column appeared to be efficient for acidic/neutral drugs. However, a systematic screening with the three columns is still recommended as good separations can be obtained on two or three columns but with different analysis times or peak efficiencies. These criteria, especially the analysis time, have to be considered in a second instance if further method development is performed. For instance, in the case of tetramisol enantiomers, although the best resolution was achieved on the Chiralcel OD-RH column, the analysis time is very long. Consequently, the separation obtained on the Chiralcel OJ-R column ($Rs = 3.30$, analysis time: 31.75 min) is probably a better choice for further method development for this substance.

Overall, the defined strategy appears to be efficient as 89% of the compounds could be separated and most analysis times were less than 30 min which is required for a screening strategy.

4. Conclusion

The Chiralcel OD-RH, Chiralcel OJ-R and the Chiralpak AD-RH columns have been shown to be efficient chiral selectors with broad applications for the chiral separation of various drugs. A simple screening strategy independent of the chemical nature of the chiral drug could be defined. It was demonstrated that only two mobile phases were needed to screen the molecules in an efficient way.

The first mobile phase consists in an aqueous phosphate buffer pH 2.0 containing 100 m*M* of KPF_6 mixed with acetonitrile. The chaotropic agent PF_6^- is added to the mobile phase to achieve the separation of basic analytes on the Chiralcel OD-RH column. According to the results of this study, most enantiomers should be separated at this stage. If no or very little enantioselectivity is achieved for some components, they should be analysed on the three columns with a basic mobile phase consisting of an aqueous borate buffer and with acetonitrile.

Eventually, this study confirms that cellulose/amylose-based CSPs are not only very useful under normal-phase conditions but also for reversed-phase conditions, and that their use in this mode should be more intensively developed.

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