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Applicability of new chiral stationary phases in the separation of racemic pharmaceutical compounds by high-performance liquid chromatography

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ABSTRACT

The potential of contemporary chiral liquid chromatographic columns for the enantioseparation of racemates of pharmaceutical compounds was studied. Sixteen Organon compounds were selected, mostly cardiovascular or CNS-active drugs. Seven chiral stationary phases were used, *viz.*, five different cellulose derivatives, an α_1-acid glycoprotein and a polymethacrylate phase, all coated on silica particles. A good enantioseparation, with a resolution higher than 1.0, was achieved for fifteen of the sixteen racemates. The best results were obtained on a Chiralcel OJ column, on which seven enantiomers were separated. With respect to the chromatographic performance, stability and/or selectivity, the cellulose derivatives (Chiralcel columns) were preferred over the protein and polymethacrylate columns.

INTRODUCTION

A large number of drugs have one or more asymmetric centres. Mostly, these drugs are applied as their racemic mixtures in a pharmaceutical formulation. This procedure is justifiable in cases where both enantiomers have similar activity or where they enhance each other's activity. In some instances, however, one of the enantiomers is inactive and can be considered as being redundant. Moreover, enantiomers can have different biological activities and there are cases in which one of the enantiomers shows a more or less pronounced toxic effect [1,2].

Mostly, liquid chromatography (LC) [1–5] is used for the separation of enantiomers. Experimentally, chiral LC can be divided into two groups [1]: (1) a chiral stationary phase (CSP) with a non-chiral mobile phase and (2) a non-chiral stationary phase with a chiral additive in the mobile phase. In practice, chiral additives are less often used than chiral stationary phases because the equilibration times are longer, there are maintenance problems with the equipment and in preparative applications the chiral additives have to be removed after the isolation.

A few years ago, ten commercially available CSPs were tested in our laboratory on ten Organon compounds. The results obtained at that time could be summarized as follows: (i) CSP columns are expensive and suffer from poor stability; (ii) the low

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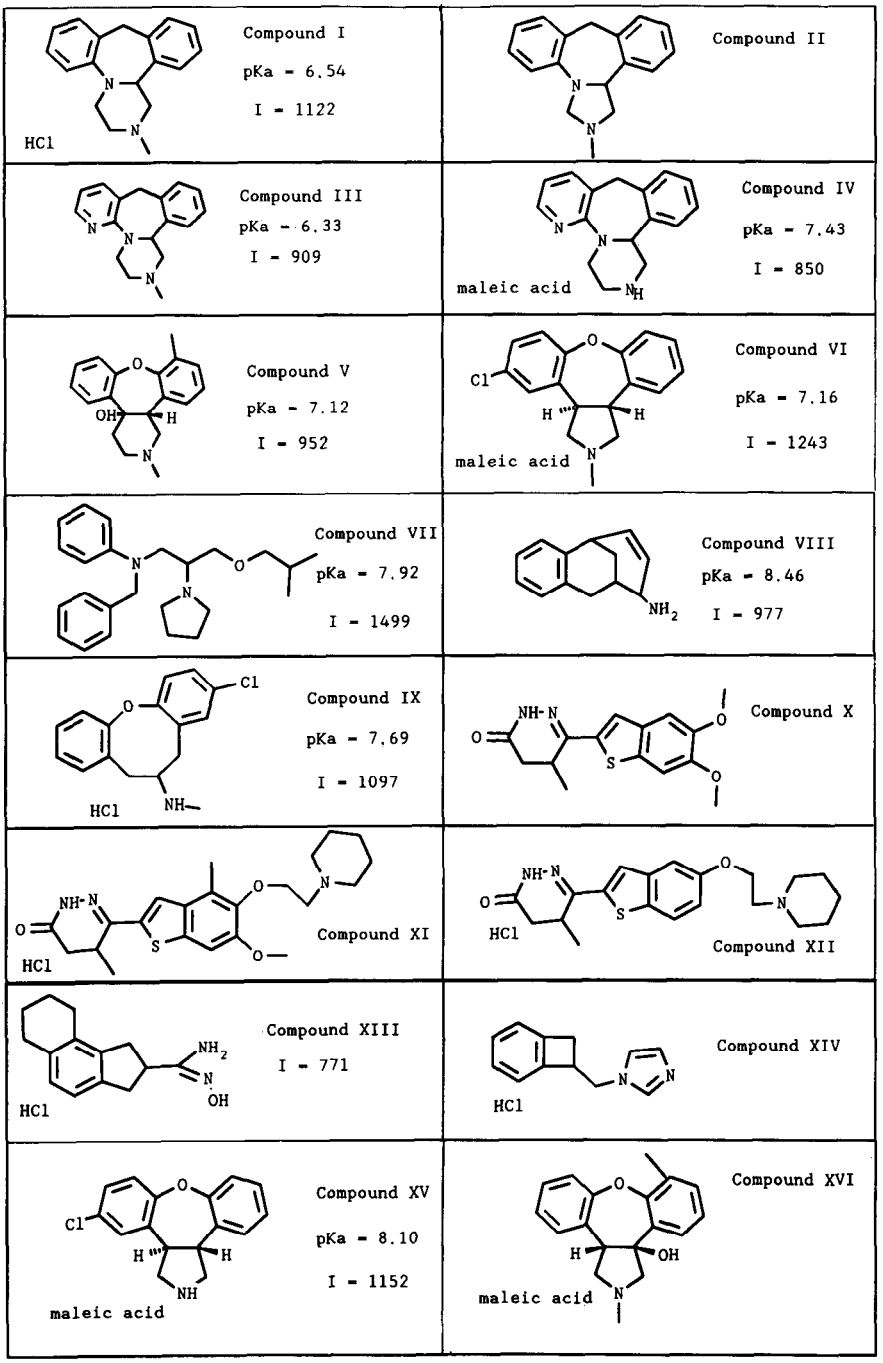


Fig. 1. Structures of the selected Organon compounds and pK_a values and retention indices (I).

efficiency of CSP columns results in broad peaks, which severely affects a reliable determination of the optical purity of an enantiomer; and (iii) owing to the low loadability of CSP columns, chiral chromatography lacks wide applicability on a preparative scale.

Subsequently, many improvements have been made to CSPs, *e.g.*, the coating of cellulose derivatives on 10-μm silica particles (Chiralcel) [6,7] and a new generation of α_1-acid glycoprotein columns (CHIRAL-AGP). Additionally, we tested a recent poly(triphenylmethyl methacrylate) column (Chiralpak OT). In this paper, we report a follow-up study in which sixteen racemic Organon compounds were chromatographed on seven different chiral columns.

EXPERIMENTAL

Chemicals and test compounds

The test compounds (Fig. 1) were synthesized by Organon (Oss, Netherlands). Freshly distilled methanol and Milli-Q purified water were used. Disodium hydrogenphosphate ($Na_2HPO_4 \cdot 12H_2O$) and ethanol were obtained from J. T. Baker (Deventer, Netherlands) and *n*-hexane (extra pure), 2-propanol, diethylamine (for synthesis) and phosphoric acid were obtained from Merck (Darmstadt, Germany).

The pH values of the buffers were measured before dilution with the organic modifiers. The buffers were prepared by dissolving 3.6 g of $Na_2HPO_4 \cdot 12H_2O$ in 1000 ml of water. Concentrated H_3PO_4 was added until the desired pH value was reached.

Apparatus and materials

The chromatographic experiments were carried out on an HP1090M liquid chromatograph, equipped with an HP1040M diode-array detector. Data were collected on an HP7999A HPLC workstation (Hewlett-Packard, Amstelveen, Netherlands).

All CSP materials were obtained as prepacked columns supplied by J. T. Baker. The CSPs used are listed in Table I.

TABLE I

CHIRAL LC COLUMNS USED

Name	Type	Dimensions
Chiralcel OB	Cellulose ester	250 × 4.6 mm I.D.; 10 μm
Chiralcel OC	Cellulose carbamate	250 × 4.6 mm I.D.; 10 μm
Chiralcel OD	Cellulose carbamate	250 × 4.6 mm I.D.; 10 μm
Chiralcel OF	Cellulose carbamate	250 × 4.6 mm I.D.; 10 μm
Chiralcel OJ	Cellulose carbamate	250 × 4.6 mm I.D.; 10 μm
CHIRAL-AGP	α_1-Acid glycoprotein	100 × 4.0 mm I.D.; 5 μm
Chiralpak OT	Poly (triphenylmethyl methacrylate)	250 × 4.6 mm I.D.; 10 μm

TABLE II

RESULTS OF SCREENING EXPERIMENTS

Hex = *n*-hexane; 2-PrOH = 2-propanol; EtOH = ethanol; CH_3OH = methanol; DEA = diethylamine.

Compound No.	Chiralcel																						CHIRAL-AGP: 2-PrOH–0.01 *M* Na_2HPO_4 (1:9) (pH 7.4)			Chiralpak OT					
	OB						OC						OD: Hex–2-PrOH (9:1)			OF: Hex–2-PrOH (9:1)			OJ: Hex–2-PrOH (9:1)						CH_3OH			CH_3OH–0.1% (v/v) DEA		
	Hex–2-PrOH (9:1)			EtOH			Hex–2-PrOH (9:1)			EtOH																					
	k'_1	R	α	k'_1	R	α	k'_1	R	α	k'_1	R	α	k'_1	R	α	k'_1	R	α	k'_1	R	α	k'_1	R	α	k'_1	R	α	k'_1	R	α
I	0.3	0	1	0.2	0	1	0.3	0.57	1.16	0.2	0	1	0.5	0.77	1.10	0.3	0	1	0.3	4.40	2.13	18.9	0.63	1.10	9.2	[c]			–	
II	1.2	0.73	1.56	0.4	2.38	1.73		n.d.			n.d.			n.d.		1.1	4.41	1.57	1.2	0.77	1.09		n.d.		3.2	0.99	1.33	0.9	0.94	1.51
III	0.5	0	1	0.2	0	1	0.9	0.93	1.19	0.3	1.05	1.38	0.8	3.28	1.33	1.2	0.91	1.11	0.4	0	1	8.0	0.53	1.08	5.7	1.18	1.36	0.8	1.24	2.55
IV		–[a]			n.d.[b]			–		1.2	[c]		1.9	0	1	6.0	[c]		1.2	[c]		8.0	1.60	1.18		–		0.8	[c]	
V		–		0.1	0	1	1.5	0	1	0.3	0	1	0.6	1.05	1.13	1.4	1.40	1.18	0.5	1.06	1.19	5.8	0.98	1.11		–			–	
VI	1.0	0	1	0.2	0	1	0.7	0	1	0.5	0	1	0.4	0	1	0.5	1.08	1.18	0.3	1.27	1.28	25.7	0	1		–			–	
VII		–		0.2	0	1		–		0.4	0	1		–			–			–			–			–			–	
VIII				1.4	0	1		–		3.5	0	1	1.2	0	1	1.5	[c]		1.2	6.24	1.72	4.5	0	1	5.2	0	1		–	
IX	0.8	0	1	0.2	0	1	0.6	0	1	0.4	0	1	0.4	0	1	0.5	0	1	0.4	2.22	1.37	12.5	0.66	1.09		–			–	
X		–		1.0	2.12	1.46		–		2.5	2.36	1.30		–			–			–		2.2	1.02	1.20	0.5	0.74	1.20	0.3	1.01	1.31
XI		–		0.4	0	1		–		2.8	2.71	1.66		–			–			–		13.8	[c]			–		0.9	0.88	1.15
XII		–		0.5	[c]			–		3.2	2.03	1.42		–			–			–		10.8	0	1		–		0.6	1.32	1.31
XIII		–		0.1	0	1		–			n.d.			–			–			–		3.1	3.55	2.00	1.7	0	1		–	
XIV		–		0.5	[c]			–			n.d.		2.0	0	1	2.8	1.18	1.18	0.9	1.29	1.24	24.5	0.76	1.09		–			–	
XV		–		0.1	0	1	1.1	0	1		n.d.		0.6	1.47	1.17	0.8	3.59	1.61	0.3	1.56	1.35	10.8	5.11	2.03		–			–	
XVI		–		0.2	0	1		–			n.d.			–			–			n.d.		3.3	0	1	0.9	0	1		–	
$R>1.0$		0			2			0			4			3			5			7			4			1			3	
$R>1.5$		0			2			0			3			1			2			4			3			0			0	

[a] Compounds do not elute within 30 min.
[b] N.d., no data available.
[c] Compounds start to separate.

Standard LC conditions

Chiralpak OT column

A flow-rate of 1.0 ml/min, an injection volume of 10 μl and a column temperature of 15°C were used. Methanol was used as the eluent and UV detection was carried out at 210 nm. Samples of about 1 mg/ml dissolved in methanol were injected.

Chiralcel columns

A flow-rate of 1.0 ml/min, an injection volume of 2–10 μl and a column temperature of 50°C were used. Hexane–2-propanol (9:1), 2-propanol or ethanol was used as the eluent and UV detection was carried out at 210 nm. Samples of about 1 mg/ml dissolved in *n*-hexane or ethanol were injected.

CHIRAL-AGP column

A flow-rate of 1.0 ml/min, an injection volume of 2–10 μl and a column temperature of 30°C were used. 2-Propanol–buffer (pH 7.0 or 3.8) (1:9) was used as the eluent. UV detection was carried out at 210 nm and samples of about 1 mg/ml dissolved in ethanol were injected.

Calculations

The tailing factor (*Tf*), *i.e.*, the peak asymmetry, was calculated at 5% of the peak height using the ratio of the widths of the rear and front sides of a peak.

RESULTS AND DISCUSSION

The aim of this study was to explore the potential of seven chiral LC columns for the separation of the enantiomers of sixteen selected compounds. The strategy for method development was as follows. In first instance a mobile phase composition recommended by the manufacturer was selected. With this mobile phase, all test compounds were screened. Subsequently, for some of the compounds which were not separated or which only started to separate, an optimization of the separation was carried out.

Screening of chiral LC columns

Chiralpak OT

The results of the screening experiments, using methanol as mobile phase, are summarized in Table II. It was found that most compounds were not eluted within 30 min. Of the seven compounds that were eluted, three were well separated, but none with a resolution larger than 1.5. The Chiralpak OT column showed a reasonable selectivity for the three compounds but a poor efficiency. For example, the chromatogram obtained for compound III had a broad first peak and an even broader second peak. The plate numbers were only about 400. Therefore, a reliable determination of the enantiomeric purity was impossible.

Chiralcel

Five of the eight commercially available Chiralcel columns were selected. This selection was based on the similarity of our compounds with compounds already separated and the number of separations shown in manuals and the literature [6–12].

The Chiralcel columns were initially tested using hexane–2-propanol (9:1) as the mobile phase. As an alternative, ethanol is recommended. The screening results are summarized in Table II.

Chiralcel OB and OC gave poor results with hexane–2-propanol. Most of the compounds were not eluted within 30 min, and only for one compound was a slight separation seen. When ethanol was used the retention decreased drastically. This means that the step from hexane–2-propanol (9:1) to ethanol is large with respect to the optimization of the retention.

The third column tested was Chiralcel OD using only hexane–2-propanol as the mobile phase. In this instance 56% of the compounds eluted with k'_1 values less than 2. Compared with Chiralcel OB and OC, this CSP showed better results. Four enantiomers were separated, three of which with a resolution larger than 1.0.

The Chiralcel OF column separated five of the racemates with a resolution larger than 1.0, for two of them larger than 1.5. Using hexane–2-propanol (9:1) six compounds did not elute within 30 min.

The best results were obtained with the Chiralcel OJ column. Seven of the racemates were separated with a resolution larger than 1.0, for four of them larger than 1.5. The best results were obtained for compounds I and VIII with resolutions of 4.4 and 6.2, respectively. As an illustration, the separation of compound VIII is shown in Fig. 2.

Of the sixteen Organon compounds, all the tetracyclic compounds, except compound IV, as well as compounds X, XI and XII were well separated on at least one of the five Chiralcel columns. Most of the compounds which were not separated at all have clearly deviating structures.

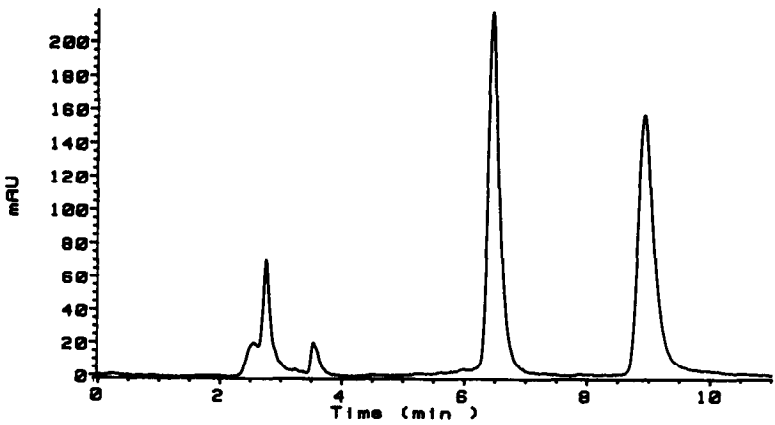


Fig. 2. Chromatogram of the separation of compound VIII on a Chiralcel OJ column. Eluent, hexane–2-propanol (9:1, v/v).

CHIRAL-AGP

The use of an immobilized α_1-acid glycoprotein as CSP is well known. Several applications with this CSP have been published [4,13]. With the CHIRAL-AGP column, we separated four of the compounds with a resolution larger than 1.0 (see Table II).

Although the results are promising, the peak shapes and the tailing factors on the AGP phase were clearly worse than those on the Chiralcel columns. As an example, the separation of compound XIII is shown in Fig. 3.

Optimization studies

When a separation obtained under the screening conditions is not satisfactory or when the compounds have too high retentions, the chromatography can be optimized. One way to do this is by changing the mobile phase composition. Also, diethylamine can be added as a tailing-suppressing agent and finally the column temperature can be varied.

Chiralpak OT

Addition of 0.1% (v/v) of diethylamine to the mobile phase, in order to decrease the silanol activity, resulted in a decrease in the retention for six compounds. This indicates clearly that the Chiralpak OT column has some (residual) silanol activity. However, for four compounds the retention increased. An explanation for this phenomenon has not been found. It was also observed that whenever the retention decreased on adding diethylamine, the separation of the enantiomers improved (Table II).

With compound III, addition of diethylamine did not result in an improvement in the peak shapes but it reduced the retention (see Fig. 4). However, for the separations of compounds X, XI and XII two sharp peaks were obtained using diethylamine. The plate counts were about 2500.

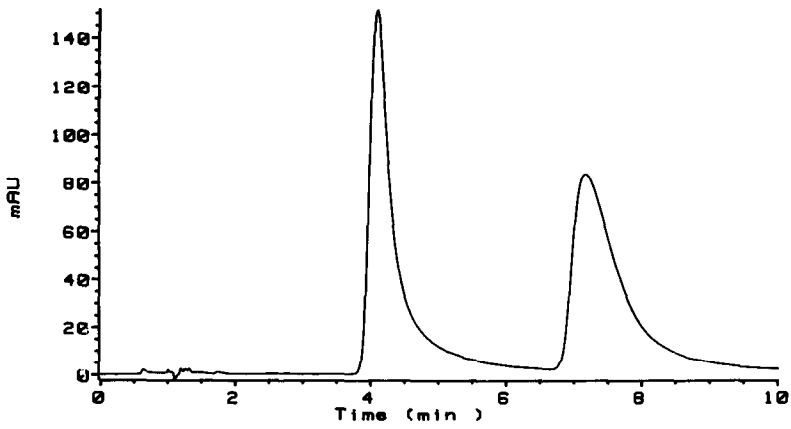


Fig. 3. Chromatogram of the separation of compound XIII on a CHIRAL-AGP column. Eluent, 2-propanol–0.01 *M* Na_2HPO_4 (pH 7.0) (1:9, v/v).

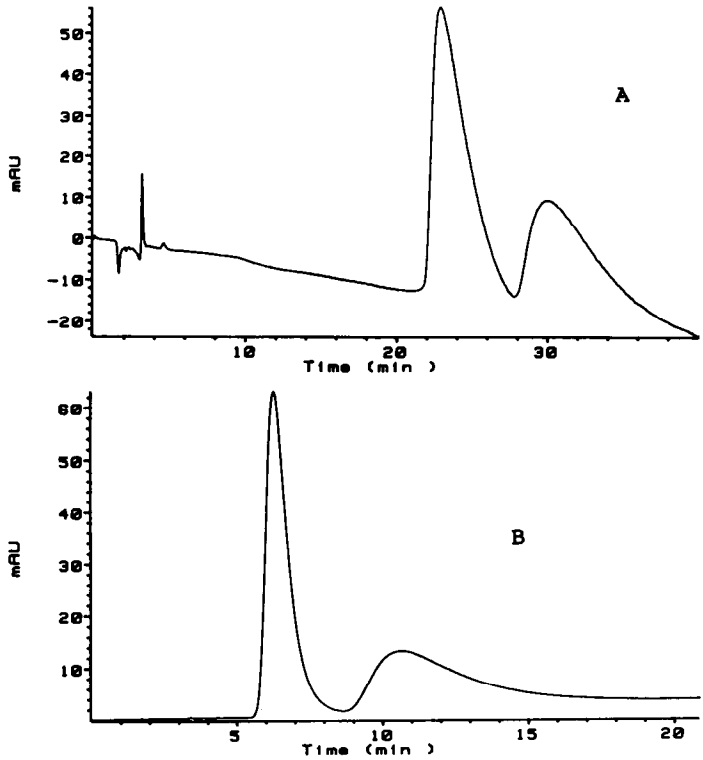


Fig. 4. Chromatogram of the separation of compound III on a Chiralpak OT column (A) without and (B) with the addition of 0.1% (v/v) of diethylamine. Eluent, methanol.

In order to reduce the retention of the test compounds, 4% of dichloromethane was added to the methanol. Although acceptable separations ($R \approx 0.7$) were obtained for the apolar compounds VI and VII, this approach was not used further. It turned out that dichloromethane drastically reduced the column lifetime.

Chiralcel

Influence of column temperature. Contrary to findings in the literature [14–16], we observed on several occasions with ethanol and also hexane–2-propanol mobile

TABLE III

INFLUENCE OF COLUMN TEMPERATURE ON RESOLUTION (R), SELECTIVITY (α), CAPACITY FACTOR (k'_1) OF THE FIRST-ELUTING ENANTIOMER, PLATE NUMBER (N) AND TAILING FACTOR (Tf) FOR THE SEPARATION OF COMPOUND X ON A CHIRALCEL OC COLUMN

Temperature (°C)	R	α	k'_1	N	Tf
30	1.94	1.20	5.1	2540	1.21
40	2.54	1.24	4.2	3380	1.18
50	3.29	1.29	3.3	4439	1.23

phases that the resolution improved when the column temperature was raised. A typical example is given in Table III. A temperature of 50°C was considered optimum, especially as at that point the stationary phase starts to strip off slowly when ethanol is used. Because of the good chromatographic effects 50°C was maintained throughout our screening experiments.

We believe that structural effects can give, at least partly, an explanation of the temperature effect. The rigid part of the molecules studied have 2–4 coupled ring structures which acquire more flexibility at higher temperatures and, therefore, fit better in the cavities. On the other hand, the molecules reported in the literature [14–16] have flexible cyclic groups which are not connected together. It is thought that the fit of these independent groups is optimum at low(er) temperatures.

Varying the content of 2-propanol. In chromatography a capacity factor of 5 is considered to give optimum resolution in a reasonable time. The effect of varying the 2-propanol content in order to obtain better k' values is well illustrated in Fig. 5. With respect to resolution the optimum situation for compound V is attained with hexane–2-propanol (96:4). Further, the peaks of compound V are sharp and show almost no tailing. Surprisingly, with pure hexane as mobile phase the peaks of compound V were clearly broader. A large tailing factor of about 5 and only 500 plates were obtained.

Sometimes hexane–2-propanol (1:1) or even 100% 2-propanol had to be used in order to decrease the retention. This is illustrated in Table IV for compounds XI and XII. A high selectivity was obtained with symmetrical peaks but the use of pure 2-propanol resulted in very broad peaks. For these compounds the results on Chiralcel with ethanol as mobile phase were slightly better (Table II).

Influence of mobile phase additives. As the silica particles are not completely covered during the coating with the stationary phase, residual silanol groups can interact with the solutes. An example is compound IV, where the interaction of the

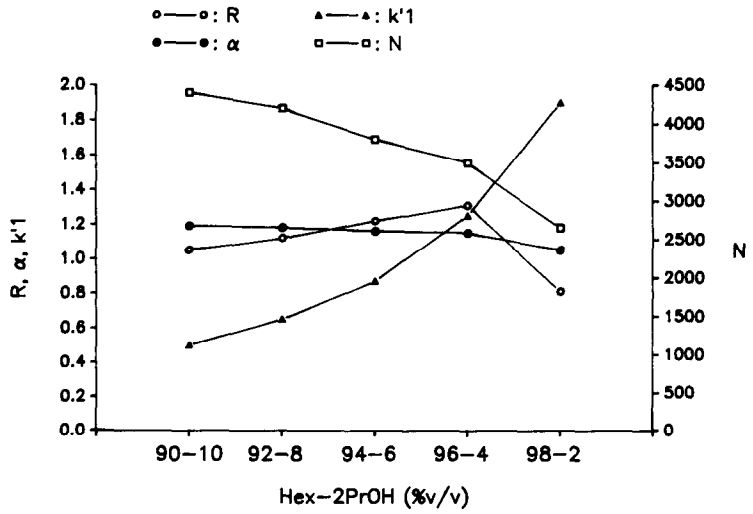


Fig. 5. Influence of the percentage of 2-propanol on (○) resolution (R), (●) selectivity (α), (△) capacity factor (k'_1) and (□) plate numbers (N) for the separation of compound V on a Chiralcel OJ column.

TABLE IV

CHROMATOGRAPHIC DATA FOR COMPOUNDS XI AND XII ON CHIRALCEL OF USING 2-PROPANOL AS MOBILE PHASE

Compound	R	α	k'_1	N	Tf
XI	2.68	1.81	1.6	854	1.18
XII	1.70	1.37	2.1	1006	1.14

secondary N atom in the non-aromatic ring with free silanols results in broad tailing peaks (Fig. 6A). Addition of 0.1% of diethylamine (DEA) as a silanol suppressor leads to sharper, symmetrical peaks and a decrease in the retention time. A further increase in DEA concentration resulted in only marginal improvements.

Viscosity. It was concluded that the plate numbers varied widely with the mobile phase composition. With respect to the plate numbers, we considered that it is desirable to use a viscosity as low as possible. This is also of advantage for the column back-pressure. For these reasons it is preferred to add ethanol instead of 2-propanol to the mobile phase in order to reduce the retention. It is interesting that reducing the content of 2-propanol in hexane–2-propanol mixtures below 2% or using pure etha-

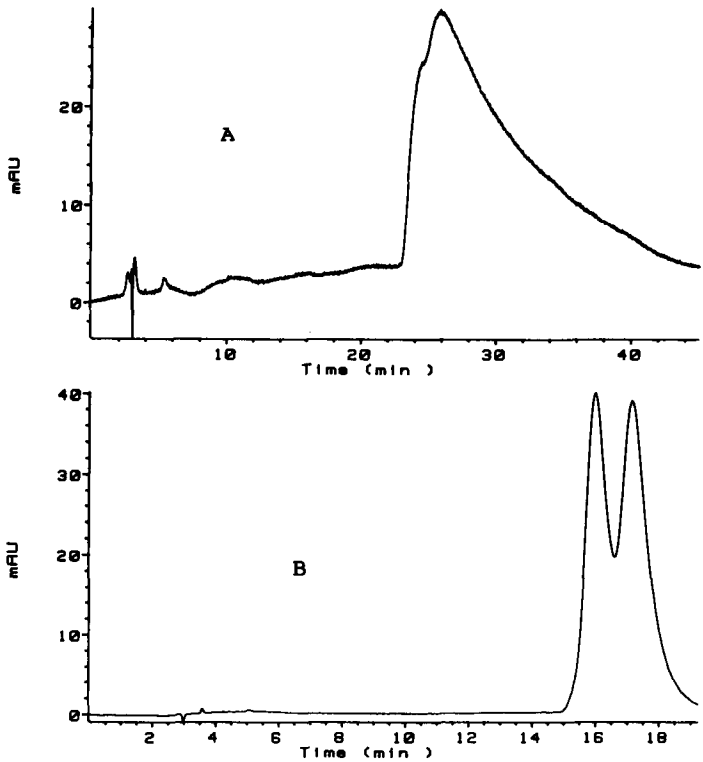


Fig. 6. Chromatograms of compound IV on a Chiralcel OC column (A) without and (B) with the addition of 0.1% (v/v) of diethylamine. Eluent, hexane–2-propanol (9:1, v/v).

nol as the mobile phase results in more tailing. Therefore, it seems advisable always to use between 2 and 10% of 2-propanol.

Stability of the stationary phase. It was found that with ethanol at 50°C the stationary phase is slowly stripped off. This was established from elevation of the background UV signal. The effect is not very serious, but after a few weeks of continuous use a slight increase in silanol activity, measured by an increase in peak tailing, could be observed. However, in comparison with our experiences with other chiral stationary phases, the Chiralcel columns are very stable.

CHIRAL-AGP

Influence of type of modifier and pH of buffer. To determine the influence of the type of modifier at a pH of 7.0, three different modifiers (2-propanol, ethanol and methanol) were selected. The results are summarized in Table V. The mobile phase composition was modifier–0.01 *M* Na_2HPO_4 (pH 7.0) (1:9, v/v). It is obvious that 2-propanol is the strongest eluent and methanol the weakest. The best separations were obtained with 2-propanol.

Under standard conditions compound VII was still retained and did not elute within 30 min. Lowering the pH to 3.8 and thus making the molecule more polar decreased the retention drastically. As is seen in Table VI, this is a general and expected effect. However, it might also result in a complete loss of resolution, as demonstrated for compound V. The effect of the amount of modifier at pH 3.8 is shown in Table VII for 2-propanol. The expected decrease in retention is seen when the amount of modifier is increased. As a result, the resolution is also decreased (almost lost).

Finally, the column temperature can be varied. In Table VIII this is shown for the mobile phase 2-propanol–0.01 *M* Na_2HPO_4 (pH 3.8) (3:97, v/v). The temperature influences the retention and separation in such a way that a low temperature increases the retention and improves the separation. The latter aspect suggests that the separation mechanism for this series of compounds is completely different from that of Chiralcel columns.

Summarizing the results on a CHIRAL-AGP column, we obtained a separation of ten racemates with a resolution larger than 0.5. Two compounds (VII and XIII) were separated on the AGP column and not on the Chiralcel columns. Further, for

TABLE V

EFFECT OF THE TYPE OF MODIFIER ON THE CHROMATOGRAPHIC PERFORMANCE OF A CHIRAL-AGP COLUMN WITH MODIFIER–0.01 *M* Na_2HPO_4 (pH 7.0)/(1:9, v/v) AS ELUENT

Compound	2-Propanol			Ethanol			Methanol		
	k'_1	R	α	k'_1	R	α	k'_1	R	α
V	5.8	0.98	1.11	11.1	0.68	1.08	–		
VII	–[a]			–			–		
XI	13.8	[b]		22.6	0	1	–		
XII	10.8	0	1	16.1	0	1	–		

[a] Compounds do not elute within 30 min.
[b] Compounds start to separate.

TABLE VI

INFLUENCE OF THE TYPE OF MODIFIER AT pH 3.8 USING A CHIRAL-AGP COLUMN WITH MODIFIER–0.01 *M* Na_2HPO_4 (pH 3.8) (1:9, v/v) AS ELUENT

Compound	2-Propanol			Ethanol		
	k'_1	R	α	k'_1	R	α
V	0.8	0	1	0.5	0	1
VII	1.4	[a]		2.9	1.10	1.29
XI	0.9	[a]		1.3	[a]	
XII	0.7	0	1	0.9	[a]	

[a] Compounds start to separate.

compounds IV and XVI a better separation was obtained on the AGP than on a Chiralcel column.

Relationship between structure and performance on Chiralcel columns

Various attempts have been made to elucidate the mechanism of chiral recognition for CSPs [11,17–19]. The aim here is not to develop a recognition model, but to show how sensitive a separation is towards changes in a molecule. As an illustration, we selected four structurally related tetracyclic drugs from the sixteen compounds studied measured under the screening conditions (Table IX). Examination of the table in either a horizontal or a vertical way shows the differences in separation. On the one hand, it implies that the separation of new compounds is still based on trial and error. On the other, it can be concluded, also from Table II, that for almost all the compounds studied a suitable CSP can be found. In our case it was most profitable to start with Chiralcel OJ. This finding is supported by the number of applications published in the literature [6,8]. Also in the application notes from Daicel [9,10] the Chiralcel OJ and OD columns are most often successful. Further, we studied the relationship between pK_a values and peak tailing, and between the retention index,

TABLE VII

INFLUENCE OF THE AMOUNT OF 2-PROPANOL AT pH 3.8 USING A CHIRAL-AGP COLUMN

Compound	Amount of 2-propanol (%)								
	3			6			10		
	k'_1	R	α	k'_1	R	α	k'_1	R	α
V	0.6	0	1	0.4	0	1	0.8	0	1
VII	7.0	1.53	1.40	2.4	1.06	1.27	1.4	[a]	
XI	2.1	0.57	1.18	1.2	0.37	1.18	0.9	[a]	
XII	1.5	0	1	0.9	0	1	0.7	0	1

[a] Compounds start to separate.

TABLE VIII

INFLUENCE OF COLUMN TEMPERATURE USING A CHIRAL-AGP COLUMN WITH 2-PROPANOL–0.01 *M* Na_2HPO_4 (pH 3.8) (3:97, v/v) AS ELUENT

Compound	30°C			40°C			50°C		
	k'_1	R	α	k'_1	R	α	k'_1	R	α
V	0.6	0	1	0.4	0	1	0.5	0	1
VII	7.0	1.53	1.40	5.1	1.53	1.35	3.8	1.36	1.29
XI	2.1	0.57	1.18	1.5	[a]		1.2	0	1
XII	1.5	0	1	1.1	0	1	0.9	0	1

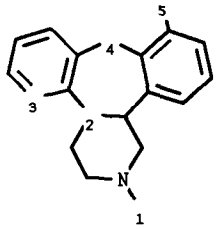
[a] Compounds start to separate.

which is a measure of the polarity [20], and retention on the Chiralcel columns. Also in these cases there are no clear correlations. For example, the pK_a value of compound IV is clearly lower than that of compound VIII (see Fig. 1). However, the tailing of compound IV is much larger. The retention mechanism is also interesting. Increasing the polarity of the mobile phase reduces the retention times, which is comparable to effects in normal-phase chromatography. However, a non-polar compound such as VII does not elute at all. It is clear that more study is needed to clarify the retention and separation mechanism.

TABLE IX

RELATIONSHIP BETWEEN STRUCTURE AND SEPARATION

Basic structure of the compounds selected:



Compound	Structural elements					Chiralcel CSP[a]				
	1	2	3	4	5	OB	OC	OD	OF	OJ
I	CH_3	N	CH	CH_2	H	−	+/−	+/−	−	+
III	CH_3	N	N	CH_2	H	−	+/−	+	+/−	−
IV	H	N	N	CH_2	H	−	+/−	−	+/−	+/−
V	CH_3	C–OH	CH	O	CH_3	−	−	+	+	+

[a] −, resolution is 0; +/−, resolution between 0 and 1; +, resolution larger than 1.

CONCLUSIONS

The enantioselectivity of the Chiralcel columns is excellent for most test compounds, particularly for the tetracyclic compounds. The columns showed good separation efficiency, acceptable plate numbers and symmetrical peaks. The results with these columns were much better than those obtained so far. Although some stripping of the mobile phase was observed, the performance of the columns was not reduced markedly over a long period.

The enantioselectivity of the α_1-acid glycoprotein column is also very good, probably even better than that of a Chiralcel OJ column. The stability of the column is better than that of the first-generation AGP columns. During the experiments we did not observe a decrease in column performance. However, the separation efficiency is still low and tailing peaks are often obtained. This hampers a reliable determination of the optical purity of an enantiomeric compound. The results obtained in this study are also not much better than the results obtained earlier.

The new Chiralpak OT column did not show an improvement in enantioseparation compared with previous columns. Most compounds were not eluted with methanol. For those compounds which were eluted, the separation was not as good as on the Chiralcel columns. The peak shapes were poor and, compared with the Chiralcel columns, the plate numbers were low.

Comparing Chiralcel, CHIRAL-AGP and Chiralpak columns, the Chiralcel columns showed the best results. With respect to selectivity all three types of columns are very suitable. The best results were obtained on a Chiralcel OJ and a CHIRAL-AGP column. Overall it can be concluded that for most compounds, *viz.* fifteen of the sixteen test compounds, a good separation method is available.

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