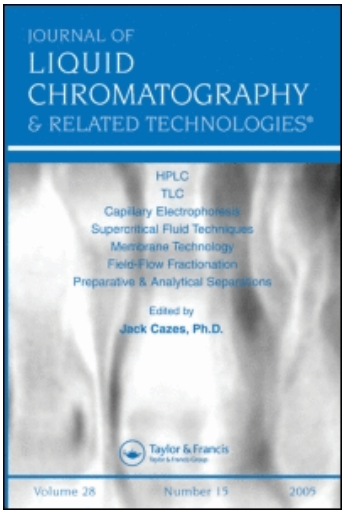

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CHIRAL STATIONARY PHASES BASED ON CELLULOSE AND AMYLOSE *TRIS*-3,5-DIMETHYLPHENYLCARBAMATE DERIVATIVES FOR THE RESOLUTION OF SELECTED CHIRAL DRUGS AND METABOLITES

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ABSTRACT

Chiral stationary phases based on tris-3,5-dimethyl-phenylcarbamate derivatives of cellulose and amylose were used for the resolution of some chiral drugs and their metabolites. The Chiralcel OD-H and Chiralpak AD columns were employed under normal phase conditions using hexane/ethanol or hexane/2-propanol as the mobile phases. The influence of trace amounts of diethylamine in the mobile phase was also evaluated. The Chiralcel OD-R column was employed under reversed phase condition using mainly perchlorate solution supplemented with acetonitrile.

The results showed that the resolution obtained under normal phase conditions in general is better than that obtained using the same kind of stationary phase under reversed phase conditions. The Chiralpak AD provided the best resolution for all drugs studied.

INTRODUCTION

Although chiral stationary phases can be prepared using a broad variety of natural and synthetic chiral compounds, most of them are of limited application. Thus, polysaccharide based stationary phases together with the chemically bound protein phases and cyclodextrin and its derivatives have proved to be the most useful stationary phases for the resolution of chiral drugs by high performance liquid chromatography.[1] The polysaccharide based chiral stationary phases were introduced by Okamoto and his group in 1984 and are prepared by coating cellulose and amylose derivatives on a pretreated silica gel matrix. Although the mechanism of chiral discrimination in polysaccharide phases has not been satisfactorily elucidated, it is believed that the differential binding of enantiomers is a result of a combination of attractive forces such as hydrogen bonding, dipole-dipole interaction, and charge transfer complex (π-π) formation. The main chiral adsorbing sites in cellulose and amylose derivatives are considered to be the polar ester and carbamate groups. The introduction of substituents in the phenyl group of these derivatives also affects their resolution ability. In addition, chiral recognition also seems to be a function of the fit of the asymmetric portion of the solute in a chiral cavity or channel of the chiral stationary phase.[2-4]

Different types of this kind of stationary phases are available on the market (Daicel Company) but the phases prepared with the tris-3,5-dimethylphenylcarbamate derivative of cellulose and amylose have shown high chiral discrimination ability for a wide range of racemates with different chemical structures.[2,5] The commercial columns Chiralcel OD-H and Chiralpak AD are used under normal phase conditions with mobile phases consisting of hexane and alcohol, usually 2-propanol or ethanol. Changing the alcohol modifier may result in different chromatographic behavior. Organic acids or organic bases are also used as additives in the mobile phase to improve the enantioseparation of acidic and basic compounds by reducing the interaction with silanol groups present in the silica support.[6-8]

The Chiralcel OD-R column has the same chiral selector present in the Chiralcel OD-H column but is employed with organic/aqueous mobile phases. In this case, the resolution depends on the composition of the mobile phase, pH, and kind and concentration of the ion pair reagent added to the mobile phase.[9,10]

In the present study, these columns were evaluated for the resolution of some chiral drugs and their metabolites (Figure1). The influence of mobile phase composition on the capacity factors (k') and enantioselectivity (α) in the Chiralcel OD-H and Chiralpak AD column were evaluated by changing the amount and the kind of alcoholic modifier and by adding trace amounts of diethylamine. The Chiralcel OD-R column was evaluated by changing the amount of acetonitrile in the mobile phase, pH and the concentration of a perchlorate solution.

EXPERIMENTAL

Chemicals and Drugs

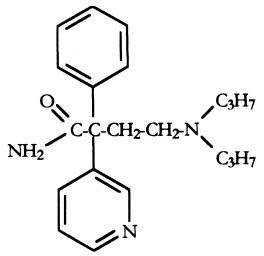
The solvents used in the mobile phases were chromatography grade, purchased from Merck (Darmstadt, Germany) or EM Science (Gibbstown, USA). All other reagents were of analytical grade (Merck, Carlo Erba, Milan, Italy). Standard solutions of *rac*-albendazole sulfoxide (Robert Young & Co. Ltd., Glascow, UK), *rac*-disopyramide, *rac*-mono-N-dealkyldisopyramide (Laboratórios Silva Araujo Roussel S/A, Rio de Janeiro, Brazil), *rac*-praziquantel (Merck SA Indústrias Químicas, Rio de Janeiro, Brazil), *rac*-trans-4-hydroxypraziquantel (Dr. G. Blaschke, Institute of Pharmaceutical Chemistry, University of Munster, Munster, Germany), and *rac*-fluoxetine (Eli Lilly do Brazil, São Paulo, Brazil) were prepared in methanol at the concentration of 100.0 μg/mL. The solutions were stored at -20°C and were stable for at least three months.

Columns

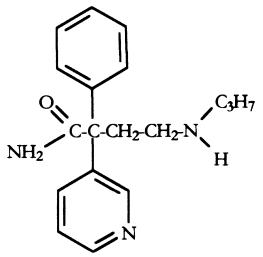
Chiralcel OD-H (150 mm x 4.6 mm i.d., 5 μm particle size), Chiralcel OD-R (250 mm x 4.6 mm i.d., 10 μm particle size), and Chiralpak AD (250 mm x 4.6 mm i.d., 10 μm particle size) were purchased from Chiral Technologies, Exton, USA.

Apparatus

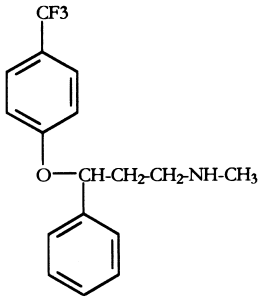
The enantioseparations were performed on a SHIMADZU (Kyoto, Japan) chromatography apparatus consisting of a model LC-10AS solvent pump, a model 7125 Rheodyne injector with a 20 μL loop, a model SPD-10A variable-wavelength UV detector and a model CR6-A integrator.



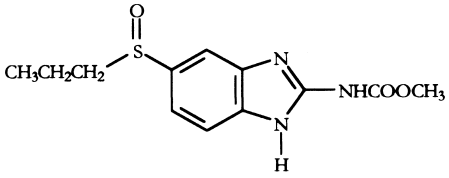
Disopyramide



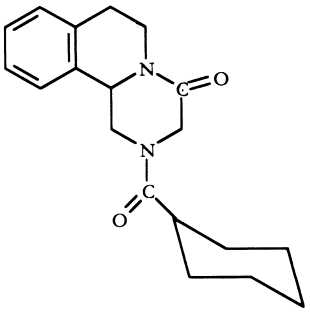
Mono-N-Dealkyldysopyramide



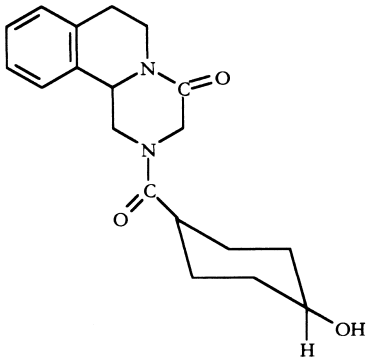
Fluoxetine



Albendazole Sulfoxide



Praziquantel



Trans-4-Hydroxypraziquantel

Figure 1. Structure of the chiral drugs and their metabolites.

Column Evaluation

Aliquots of 25 μL of the standard solutions of the drugs and metabolites were evaporated under an air flow at room temperature and the residues were dissolved in 100 μL of the mobile phase and 20 μL were chromatographed.

The columns were conditioned by eluting approximately 100 mL of the mobile phase. When the columns were not in use, the mobile phase was replaced with the storage solvent for each column (hexane-2-propanol, (9:1) for the Chiralcel OD-H and Chiralpak AD columns and methanol for the Chiralcel OD-R column).

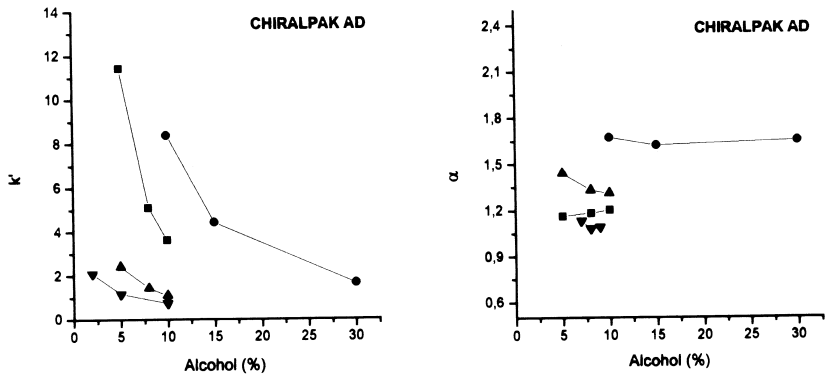
Flow rate was kept at 1.0 mL/min unless the back pressure exceeded the maximum pressure established by the manufacturer. The wavelengths used for the detection were chosen based on the λ_{max} for each compound analyzed. The separations were performed in a climatized room (22 ± 2°C). The retention of the enantiomers was characterized by the capacity factor (k'), estimated as $(t_R - t_o)/t_o$, where t_R is the retention time of each enantiomer and t_o is the retention time of a non retained solute. The separations were characterized by the enantioselective factor (α) estimated as k'_2/k'_1.

RESULTS AND DISCUSSION

Chiral columns prepared with stationary phases obtained by coating macroporous silica with cellulose or amylose derivatives have proved to be quite effective in the resolution of chiral drug enantiomers. On this basis, we selected three chiral stationary phases based on tris-3,5-dimethylphenylcarbamate derivatives of cellulose and amylose to study the influence of mobile phase composition on the retention and separation of the enantiomers of some chiral drugs and their metabolites. The Chiralcel OD-H (or the similar one based on 10 μm particle size) and Chiralpak AD columns have already been used for the resolution of praziquantel,[11-13] trans-4-hydroxypraziquantel,[11] and albendazole sulfoxide[14,15] enantiomers, whereas the Chiralcel OD-R column has been used for the resolution of fluoxetine enantiomers.[16] To our knowledge, there is no study in literature describing a complete evaluation of these columns for the resolution of the drugs selected for the present investigation.

Figure 2a shows the k' values for the first eluted enantiomer of some of the drugs studied and the enantioselective factors obtained in the evaluation of the Chiralpak AD column with mobile phases consisting of hexane-2-propanol (albendazole sulfoxide and fluoxetine) and hexane-ethanol (disopyramide and its metabolite). The mobile phases used for the evaluation of the basic compounds contain diethylamine in order to avoid secondary interaction with the silanol

a)



b)

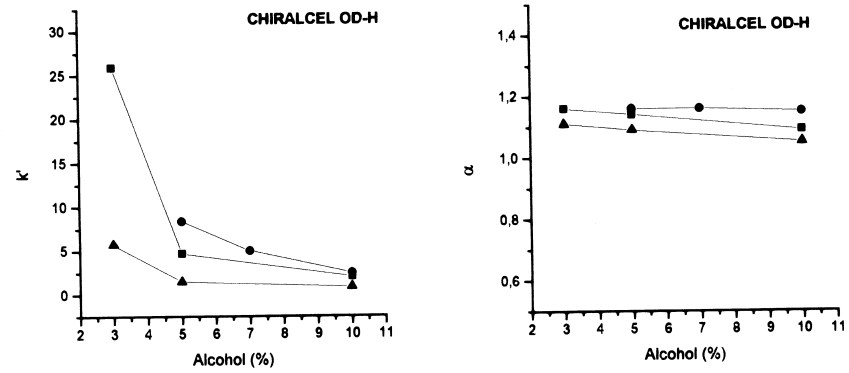


Figure 2. Effect of polar modifier on retention (k') for the first eluted enantiomer and enantioselectivity (α) in the Chiralpak AD and Chiralcel OD-H columns. a) Chiralpak AD: Albendazole sulfoxide: hexane-2-propanol; Disopyramide and mono-N-dealkyldisopyramide: hexane-ethanol + 0.1 % diethylamine; Fluoxetine: hexane-2-propanol + 0.1 % diethylamine. b). Chiralcel OD-H: Albendazole sulfoxide: hexane-ethanol; Disopyramide and mono-N-dealkyldisopyramide: hexane-ethanol + 0.1 % diethylamine.

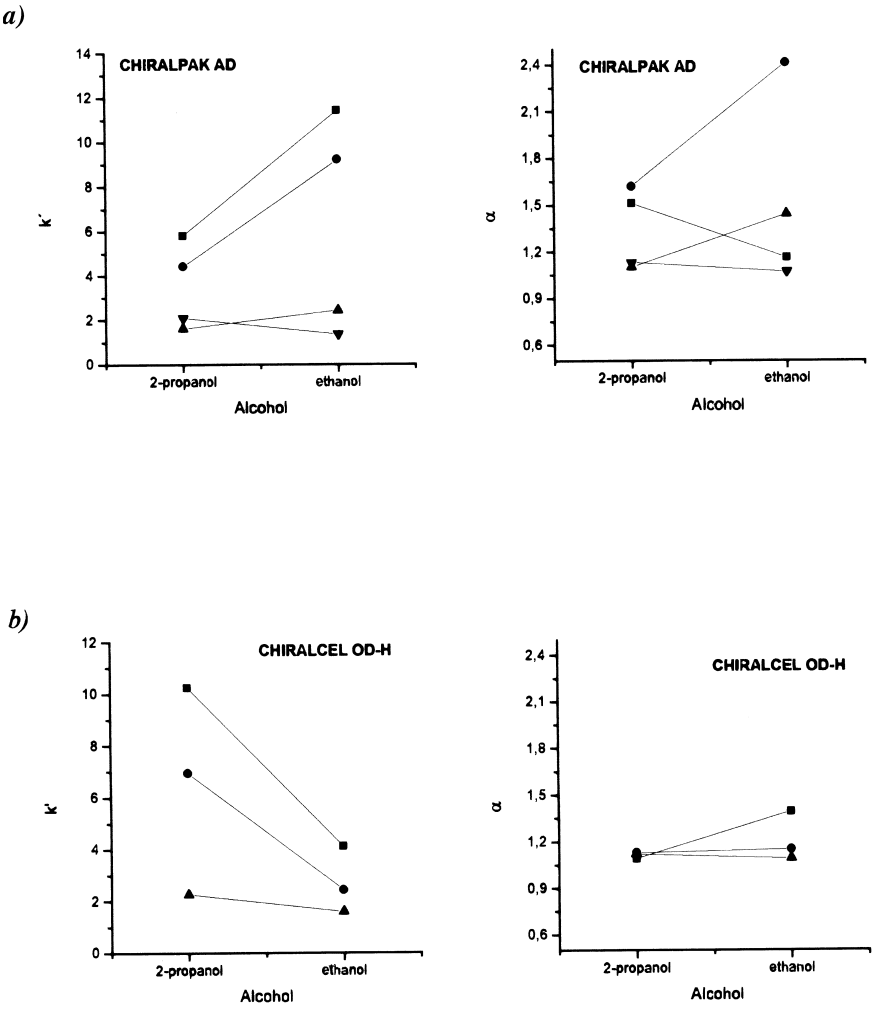


Figure 3. Effect of the kind of polar modifier on retention (k') for the first eluted enantiomer and enantioselectivity (α) in the Chiralpak AD and Chiralcel OD-H columns. Chiralpak AD: Albendazole sulfoxide: hexane-alcohol (85:15); Disopyramide and mono-N-dealkyldisopyramide: hexane-alcohol (95:5) + 0.1 % diethylamine; Fluoxetine: hexane-alcohol (98:2) + 0.1 % diethylamine. b. Chiralcel OD-H: Albendazole sulfoxide: hexane-alcohol (90:10); Disopyramide and mono-N-dealkyldisopyramide: hexane-alcohol (95:5)+ 0.1 % diethylamine.

groups of the support. Although the k' values substantially changed with the amount of polar modifier in the mobile phase the enantioselective factor was not affected significantly. This behaviour is an indication that most of the specific interactions are not chiral in nature, affecting both enantiomers in a similar way. The same behavior was observed for the Chiralcel OD-H column (Figure 2b), but in general, lower values were observed for the enantioselective factors. In addition, it can be observed that the effect of decreasing k' values with the amount of the alcohol in the mobile phase was more pronounced for lower concentrations of the polar modifier. According to Wainer et al.,[17] the decreasing effect (expressed as a percentage value) on k' upon increasing polar modifier content, indicates that the competition for the binding sites in the chiral stationary phase is a saturable process. The influence of the kind of alcoholic modifier in the mobile phase has been reported to be significantly higher for many chiral compounds.[7,8,18] Literature data have shown that changing the alcohol in the mobile phase may alter k' and α values as well as the elution order.[19] In the present study, the retention and selectivity were significantly changed for all compounds studied. Figure 3a shows our results for albendazole sulfoxide analyzed on the Chiralpak AD column. Although the mobile phase consisting of hexane-ethanol is more polar than hexane-2-propanol, leading to weaker interactions between the chiral stationary phase and the solute, increased capacity factor and enantioseparation factors were obtained.

The analysis of fluoxetine, disopyramide and mono-N-dealkyl-disopyramide with different kinds of alcohol resulted in different behaviours. The retention and enantioselectivity for disopyramide increased by using ethanol in the mobile phase while an inverse behaviour was observed for its metabolite, i.e., the enantioselective factor decreased despite the increasing effect observed on retention. Thus, similar compounds can have different behaviours, which means that different polar modifier should be evaluated when using these columns. Higher values for k' and α were observed for fluoxetine by using 2-propanol. The neutral compounds praziquantel and trans-4hydroxypraziquantel could only be resolved by using 2-propanol in the mobile phase. These findings can be explained by alterations in the size or geometry of the chiral cavity of the stationary phase, caused by the kind of alcoholic modifier.[7] The influence of the kind of alcoholic modifier was also evident in the Chiralcel OD-H column, but the behaviour was different as a result of the different conformation of the cellulose derivative (left-handed threefold (3/2) helix) and the amylose derivative (left-handed fourfold (4/1) helix).[7]

As already pointed out, these chiral stationary phases are prepared in a silica matrix and the secondary interactions of the silanol groups with basic compounds can lead to greater retention and peak asymmetry. The influence of diethylamine was studied for disopyramide and its metabolite on the Chiralpak AD and Chiralcel OD-H columns (Figure 4). Although the peaks were more

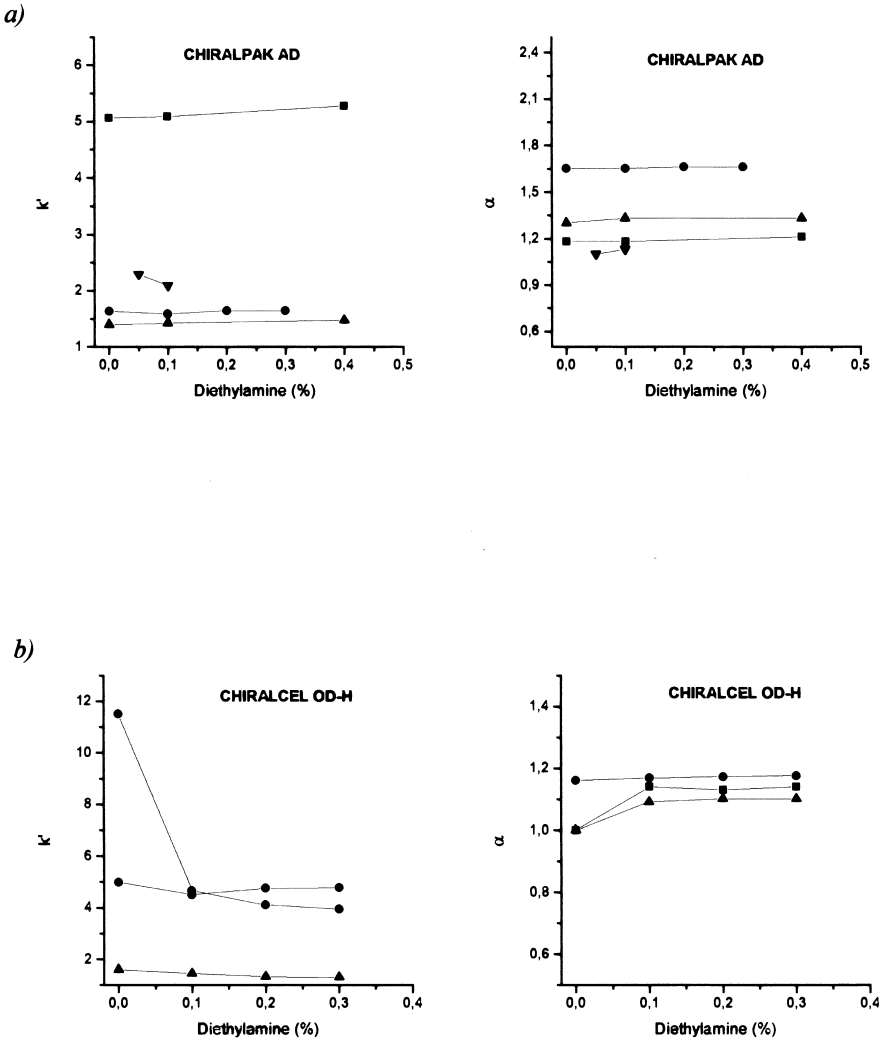


Figure 4. Effect of diethylamine on retention (k') for the first eluted enantiomer and enantioselectivity (α) in the Chiralpak AD and Chiralcel OD-H columns. Chiralpak AD: Albendazole sulfoxide: hexane-2-propanol (70:30); Disopyramide and mono-N-dealkyldisopyramide: hexane-ethanol (92:8); Fluoxetine: hexane-2-propanol (98:2). b. Chiralcel OD-H: Albendazole sulfoxide: hexane-ethanol (93:7); Disopyramide and mono-N-dealkyldisopyramide: hexane-ethanol (95:5).

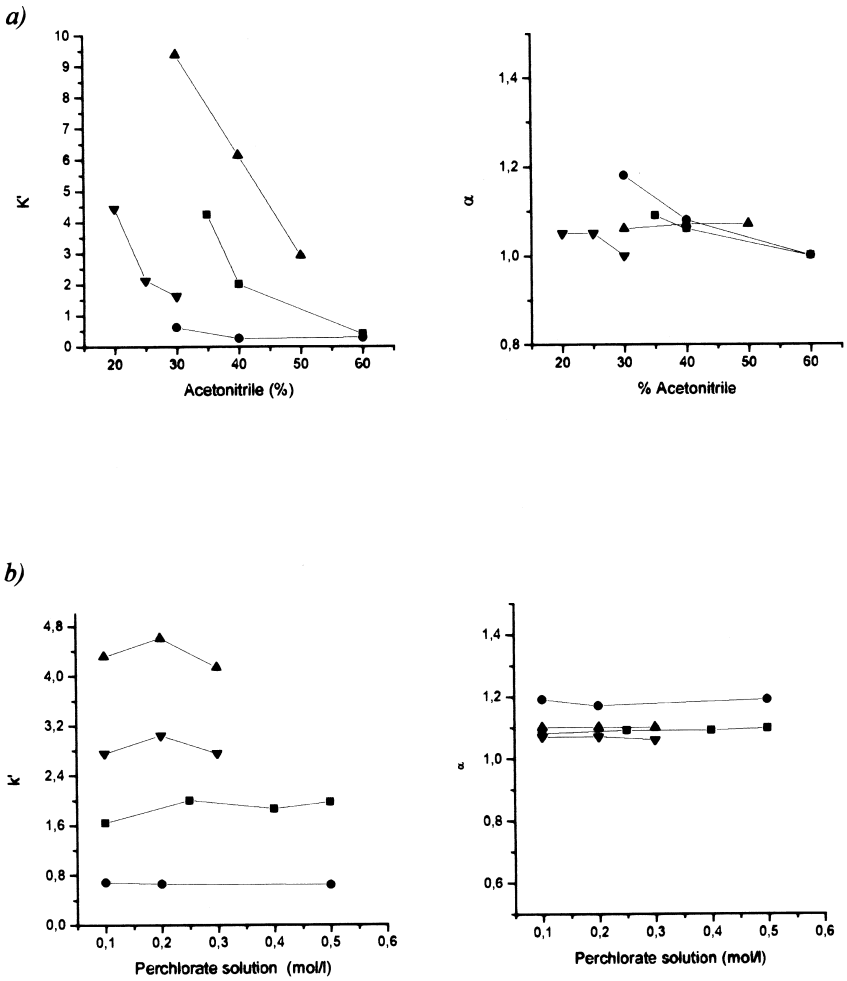


Figure 5. Effect of mobile phase composition on retention (k') for the first eluted enantiomer and enantioselectivity (α) on Chiralcel OD-R column. Influence of acetonitrile: Albendazole sulfoxide: acetonitrile-H_2O; Praziquantel and trans-4-hydroxypraziquantel: acetonitrile-H_2O; Fluoxetine: acetonitrile-$NaClO_4$ 0.25 mol/l, pH 6.0. Influence of perchlorate solution: Albendazole sulfoxide: acetonitrile-$NaClO_4$, pH 6.0 (30:70); Praziquantel: acetonitrile-$NaClO_4$, pH 6.0 (40:60) and trans-4-hydroxypraziquantel: acetonitrile-$NaClO_4$, pH 6.0 (20:80); Fluoxetine: acetonitrile-$NaClO_4$, pH 6.0 (40:60).

symmetrical with mobile phases containing diethylamine, the retention and enantioselectivity were not significantly affected in the amylose derived chiral stationary phase (Figure 4a). On the other hand, separation of these compounds on the Chiralcel OD-H column was only possible by the addition of 0.1 % diethylamine to the mobile phase (Figure 4b). Further increments of this organic base did not affect retention or enantioselectivity. Fluoxetine, another basic compound, was similarly affected by diethylamine. In contrast, the chromatographic parameters for the neutral compound albendazole sulfoxide were not affected by the addition of diethylamine to the mobile phase.

The resolution of albendazole sulfoxide, fluoxetine, praziquantel, and trans-4-hydroypraziquantel was also investigated under reversed phase conditions, using the Chiralcel OD-R column. The major factor affecting the capacity factor of all compounds studied was the amount of organic solvent in the mobile phase (Figure 5a). Although the resolution of fluoxetine depends on the addition of perchlorate to the mobile phase (0.1 mol/L), further increments did not affect its retention significantly. According to Ishikawa and Shibata,[9] the mechanism that governs the retention of basic compounds in the Chiralcel OD-R column could be explained as a result of the formation of ion pair with counter anions.

For the other drugs studied (neutral compounds) the presence of perchlorate in the mobile phase did not affect retention (Figure 5b). Enantioselectivity was not significantly affected by the amount of acetonitrile in the mobile phase. The influence of the pH of the mobile phase was evaluated in the 3-6 range. The results indicated that retention and enantioselectivity were not affected by changing the pH (results not shown). On this basis it may be concluded that the alterations in the mobile phase affect both enantiomers in a similar way, i.e., they are not chiral in nature.

The results indicated that the Chiralcel OD-H column is suitable for the resolution of albendazole sulfoxide, praziquantel, trans-4-hydroxypraziquantel, disopyramide and mono-N-dealkyldisopyramide enantiomers. Although better resolution can be obtained in the Chiralpak AD column for all drugs studied, it was observed that this column is less stable when using shorter wavelength for the detection, such as that required for the analysis of praziquantel and its metabolite. After prolonged use, the Chiralpak AD column gave better results in terms of baseline stability. Fluoxetine can also be resolved in the Chiralpak AD column but not in the Chiralcel OD-H column. The Chiralcel OD-R column is suitable for the resolution of fluoxetine, praziquantel, and albendazole sulfoxide. Figure 6 and 7 show the resolution of the compounds studied using the Chiralpak AD column.

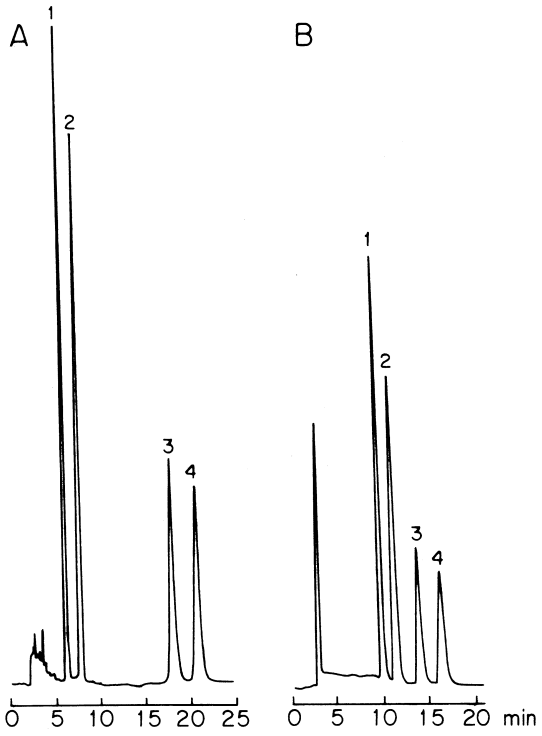


Figure 6. Chromatographic resolution of disopyramide, praziquantel and their metabolites on Chiralpak AD column. A - Disopyramide and mono-N-dealkyldisopyramide, mobile phase: hexane-ethanol (92:8,v/v) + 0.1 % diethylamine, detection: 254 nm, flow rate: 1.2 mL/min; B- Praziquantel and trans-4-hydroxypraziquantel, mobile phase: hexane-2-propanol (70:30, v/v), detection: 220 nm, flow rate: 0.9 mL/min.

The elution order (Table 1) was established by injecting a solution of the pure enantiomers previously obtained by injecting the racemate solution under the conditions (column and mobile phase) described in the literature.[11,14,16,20] The elution order of albendazole sulfoxide, fluoxetine, mono-N-dealkyldisopyramide, disopyramide and trans-4-hydroxypraziquantel enantiomers remained constant regardless of the column employed. Thus the interaction of the enantiomers with the phenylcarbamate group seems to be the most important factor for the chiral resolution of these drugs and metabolites. In contrast, the elution order observed for praziquantel was inverted in the Chiralpak AD column, indicating that chiral discrimination also depends on the conformation of the polysaccharide.

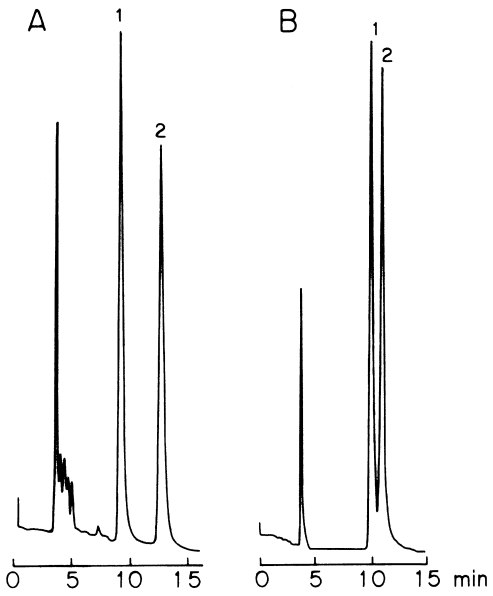


Figure 7. Chromatographic resolution of albendazole sulfoxide and fluoxetine on Chiralpak AD column. A - Albendazole sulfoxide: mobile phase: hexane-2-propanol (70:30, v/v), detection: 290 nm, flow rate: 1.0 mL/min; B- Fluoxetine, mobile phase: hexane-2-propanol (98:2, v/v) + 0.1 % diethylamine, detection: 220 nm; flow rate: 1.0 mL/min.

Table 1

Elution Order for the Chiral Drugs and Their Metabolites

Drugs	Column		
	Chiralcel OD-H	Chiralpak AD	Chiralcel OD-R
Disopyramide	(S)	(S)	---
Mono-N-dealkyldisopyramide	(S)	(S)	---
Albendazole sulfoxide	(+)	(+)	(+)
Praziquantel	(R)	(S)	(R)
Trans-4-hydroxypraziquantel	(R)	(R)	(R)
Fluoxetine	---	(S)	(S)

CONCLUSIONS

The Chiralpak AD column proved to be highly efficient in the resolution of all drugs and metabolites studied. Nevertheless, the instability observed when using shorter wavelength for the detection may be a problem when high sensitivity is required in situations such as the analysis of these compounds in biological fluids. The influence of mobile phase composition in each column has a different pattern for different compounds. On this basis, we recommend a complete evaluation of each column when developing a new methodology for the analysis of chiral compounds in these polysaccharide based stationary phases.

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