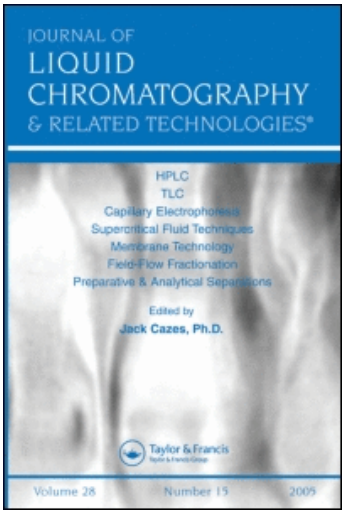

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Effects on Enantioselectivity by the Use of Polysaccharide-Based Columns by Multimodal Elution

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Online publication date: 07 February 2003

To cite this Article Cass, Quezia B. , Degani, Ana Luiza G. and Cassiano, Neila M.(2003) 'Effects on Enantioselectivity by the Use of Polysaccharide-Based Columns by Multimodal Elution', Journal of Liquid Chromatography & Related Technologies, 26: 13, 2083 — 2101
To link to this Article: DOI: 10.1081/JLC-120022395
URL: http://dx.doi.org/10.1081/JLC-120022395

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 26, No. 13, pp. 2083–2101, 2003

Effects on Enantioselectivity by the Use of Polysaccharide-Based Columns by Multimodal Elution

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ABSTRACT

The effects in chiral recognition of polysaccharide-based stationary phases by changing the chromatographic mode and elution conditions were investigated for a series of three congeneric chiral sulfoxides: omeprazole, lanzoprazole and pantoprazole and the hydroxyl compound metyrapol. They were chromatographically analyzed at normal, polar, organic, and reversed-phase elution modes, under the same conditions, with columns of cellulose and amylose *tris*(3,5-dimethylphenylcarbamate), amylose *tris*(3,5-dimethoxy-phenylcarbamate), and amylose *tris*[(*S*)-1-phenylethylcarbamate] phases. This systematic, comparative study of the four chiral phases using a single column on the three different chromatographic modes, has highlighted that changing the chromatographic mode and/or composition of the mobile phase can dramatically affect

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DOI: 10.1081/JLC-120022395
1082-6076 (Print); 1520-572X (Online)

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the enantioselectivity and/or enantioresolution of the polysaccharide-based columns. These differences were due solely to mobile phase effect and not to column batch effect. The quality of the performance of the four columns was maintained during the complete study. Excellent enantioresolutions were obtained for the four chiral compounds in two out of the four chiral phases in at least two of the three chromatographic modes.

Key Words: Enantioseparation; Multimodal elution; Benzimidazoles; Metyrapol.

INTRODUCTION

Among the most useful and versatile chiral columns described for high performance liquid chromatography are polysaccharide-based columns. They are not only effective under normal phase conditions but also under polar and reversed phase modes of elution.[1–12]

It is known, that, in chromatography, the retention factor can be dramatically altered by the mobile phase composition. This is also true in chiral chromatography. In normal phase conditions, the retention factor is decreased by increasing the percentage of the polar modifier. In polysaccharide-based chiral columns, these modifiers are usually alcohols such as ethanol, 2-propanol, and tert-butanol. Under reversed-phase conditions an aqueous mobile phase is used. Methanol and acetonitrile are the most often used organic modifiers and the retention factor is decreased by increasing these modifiers concentrations. For analysis of neutral analytes with the polysaccharide-based columns the use of water is usually the rule, but for ionizable compound buffers, acidic or basic modifiers are employed.[12,13] The polar organic mode,[14] which was first introduced for analysis in cyclodextrin columns, is now widely used. For the polysaccharide type columns, a 100% of acetonitrile or a 100% of alcohols such as ethanol, methanol, and 2-propanol, or different proportions of these polar solvents are used.[13]

The mobile phase in chiral chromatography plays a crucial part in the interaction process. It does not affect only the retention factor but also the enantioselectivity or enantioresolution.[2,12] Thus, in developing chromatographic conditions for enantiomeric separation, the use of multimodal elution is an excellent approach to achieve the desired enantioselectivity with reduction of cost. According to literature, multimodality means the same column can be used in different chromatographic modes.[14]

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Kummer and Werner[15] switched a new Chiralpak AD column from normal to reversed-phase condition to evaluate a series of enantiomeric steroids, but pointed out that that there are contradictory opinions about the question whether or not commercial columns of polysaccharides can be switched between chromatographic modes at multiple times. In their work, they used two separate columns for normal and reversed-phase chromatography. In a recent paper, however, Okamato and collaborators have conceded that some of the polysaccharide derivatives may be used in the three-elution mode.[16] Previously, we have demonstrated that the performance of polysaccharide-based columns can be maintained by conditioning it in the appropriate manner, while switching the elution mode.[4,17]

Thousands of different chiral compounds have been efficiently enantioresolved by polysaccharide-based columns and a number of different mobile phases have been described for achieving enantioselectivity.[1–12] Thus, the selection of an appropriate polysaccharide chiral column and mobile phase for a given separation is a difficult task.

This difficulty was felt in the course of developing multidimensional HPLC methodologies for the analysis of clinical samples, in the pharmacokinetics studies of a benzimidazoles series of proton pump inhibitors, and, also, for the enantiomers of metyrapol.[8,9] For multidimensional HPLC, the use of reversed-phase or polar organic chromatography is recommended, since it makes the coupling of columns a much easier task.

Although the enantiomers of omeprazole (1), lanzoprazole (2), pantoprazole (3), and metyrapol (4) (Fig. 1) can be separated in a number of different mobile phase conditions in different chiral columns,[4,18–21] they were selected as chiral probes in order to obtain a thorough evaluation of the elution mode effect on the enantioselectivity and enatioresolution of the polysaccharide-based columns on multimodal elution. The reason for the selection of these chiral compounds for this study is justified, because the benzimidazoles are congeneric chiral sulfoxides, which could be used to probe the interaction on the different chromatographic modes. The selection of metyrapol, however, as the member of this series of compounds was done based on our previous observation of great differences in enantioselectivity under multimodal elution condition with a polysaccharide-based chiral phase.[4]

Thus, the retention factor (k), enantioselectivity (α), and enantioresolution (Rs) of these compounds (Fig. 1) were evaluated using cellulose (CSP 1) and amylose *tris*(3,5-dimethylphenylcarbamate) (CSP 2), amylose *tris*(3,5-dimethoxyphenylcarbamate) (CSP 3) and amylose *tris*[(*S*)-1-phenylethylcarbamate] (CSP 4) phases, on multimodal elution. This work reports the results.

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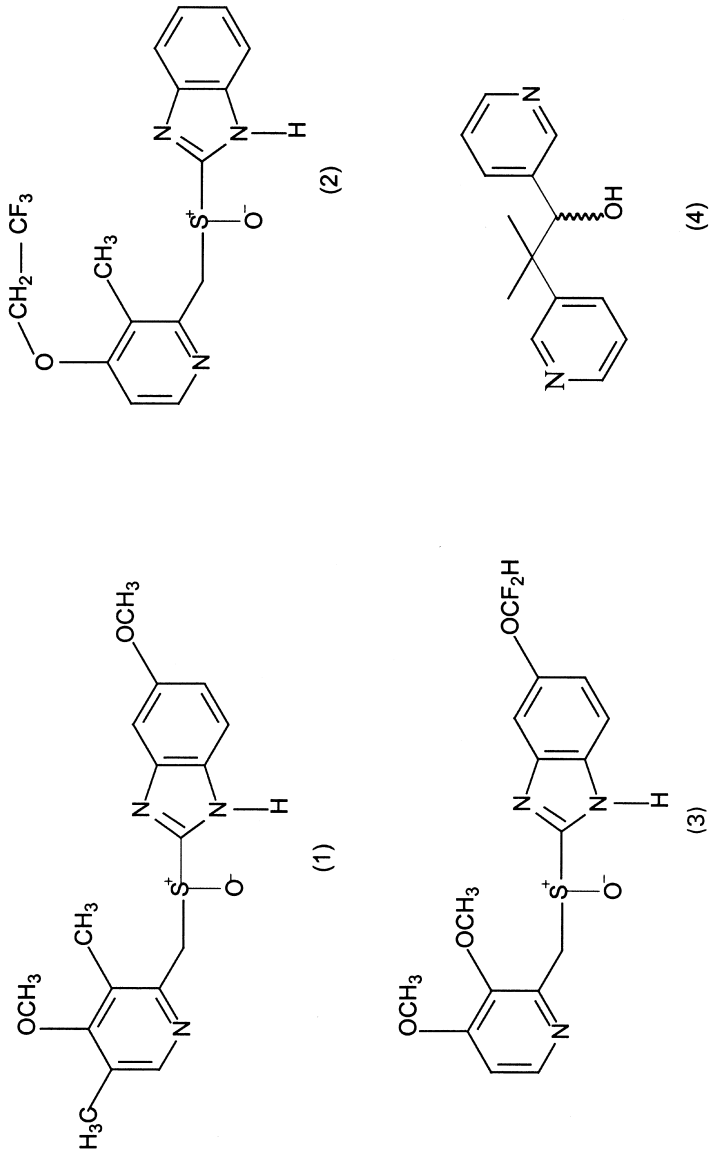

Figure 1. Selected chiral compounds.

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EXPERIMENTAL

General

Solvents were either HPLC grade from Merck (Darmstadt, Germany) or Chromar HPLC grade from Mallinckrodt Baker (St. Louis, USA). Water was purified with a Milli-Q system (Millipore, São Paulo, SP, Brazil).

Racemic omeprazole, lansoprazole, and pantoprazole were donated from Libbs Farmacêutica Ltda, Boehringer do Brasil and from Novartis Biociências S. A., respectively.

Metyrapone was purchased from Aldrich (St. Louis, USA). Racemic metyrapol was synthesized by sodium borohydride reduction of metyrapone. The resulting alcohol was characterized by IR and ^{1}H-NMR analysis.

Equipment

Two liquid chromatographic systems were used. One consisted of a Shimadzu LC-10AD pump, a Rheodyne 7125 injector fitted with a 20 or 50 μL loop, a SPD-6AV UV–VIS spectrophotometric detector, and a LC-R6A Chromatopac recorder. The other was a Shimadzu system with two LC-10ADvp pumps, a SDP-10Avp detector, and an auto sampler SIL-10ADvp with a SCL-10Avp interface. In this system, the data acquisition was performed using a Shimadzu CLASS-VP software.

Columns

Chiral columns (150×4.6 mm I.D.) were prepared as described elsewhere[10,11] and consisted of cellulose and amylose *tris*(3,5-dimethylphenylcarbamate), amylose *tris*(3,5-dimethoxyphenylcarbamate), and amylose *tris*[(*S*)-1-phenylethylcarbamate], and coated (20%, w/w) onto APS-Nucleosil (7 μm particle size and 500 Å pore size).

Omeprazole was detected at $\lambda = 302$ nm, lansoprazole and pantoprazole at $\lambda = 285$ nm, and metyrapol at $\lambda = 260$ nm. HPLC dead times (t_0) were determined by using 1,3,5-tri-tert-butylbenzene for normal and polar organic modes of elution and acetonitrile for reversed phase mode. The flow-rate was 0.5 or 1.0 mL min^{-1} and the mobile phase compositions are listed in the appropriate tables and figures.

Sample Preparation

Stock solutions of (±)-omeprazole, (±)-lansoprazole, and (±)-pantoprazole (100 μg mL^{-1}) were prepared in methanol and (±)-metyrapol

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solution ($100\,\mu g\,mL^{-1}$) was prepared in ethanol. All solutions were stored at $-20^{\circ}C$ until analysis.

To prepare the samples, aliquots ($20\,\mu L$) of the stock solutions were placed in a culture tube and the solvent evaporated under a stream of nitrogen. The dried analytes were reconstituted using $200\,\mu L$ of mobile phase and the solutions were vortex-mixed for 15 s. A 20 or $50\,\mu L$ aliquot was then injected onto the chromatographic system.

The analyses were performed, in duplicate, for each selected compound on the four polysaccharide-based chiral column with eight mobile phases. Retention factor, selectivity, and resolution were systematically observed for each selected compound at the three chromatographic modes and no degradation on the performance of each column was observed with the switching in the elution mode.

RESULTS AND DISCUSSION

A number of reports show that the mobile phase plays an important role in the interaction process affecting, not only the retention factor, but, also, the stereoselectivity of the polysaccharide-based chiral columns.[2,12,13] The aim of this work was to investigate the effects on the enantioselectivity and enantioresolution of four selected polysaccharide-based phases, by altering the elution mode and the mobile phase composition using a single column of each type.

The compounds shown in Fig. 1 were selected as chiral probes. Different mobile phase compositions in the three chromatographic modes were examined on each chiral stationary phase to determine its effects on the enantioselectivity and enantioresolution of each chiral compound. The retention (k), selectivity factor (α), and resolution (Rs) were examined for these selected compounds in each mobile phase, under the same chromatographic conditions (Tables 1–4). For some individual compounds, small alterations on the mobile phase strength and/or flow rate were made in order to improve resolution, although, as a rule no optimization for maximizing resolution was performed.

It has long been recognized, that the chiral recognition ability of the polysaccharide carbamate phases is a result of the combination of attractive forces, such as hydrogen bonding, hydrophobic interaction, dipole–dipole stacking, and π–π interactions of the CSP with the functional groups of the chiral solutes. It is also a function of the steric fit of the solute in the chiral cavity or channel of the stationary phases.[5,13,22]It is assumed, also, that steric interaction seems to be an important feature in the chiral recognition mechanism for chiral sulfoxides.[23–25] Nevertheless, it is always difficult to elucidate the interactions of these complex stationary phases with

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Table 1. Separation performance on a *tris*(3,5-dimethylphenylcarbamate) of cellulose (CSP 1).

	Omeprazole			Lansoprazole			Pantoprazole			Metyrapol		
	k_1	α	*Rs*	k_1	α	*Rs*	k_1	α	*Rs*	k_1	α	*Rs*
Hex : 2-prOH (60 : 40)	2.19	1.00	—	2.67	1.36	PR	6.21	1.00	—	1.41	1.86	2.52
Hex : EtOH (70 : 30)	2.09	1.00	—	2.32	1.28	PR	5.49	1.00	—	1.59	1.82	3.62
EtOH	0.69	1.00	—	0.63	1.00	—	1.07	1.00	—	0.30	1.33	PR
MeOH	0.68	1.00	—	0.48	1.21	PR	0.88	1.00	—	0.24	1.00	—
MeCN	3.85	1.00	—	2.41	1.00	—	4.87	1.00	—	2.47	1.00	—
MeCN : MeOH (95 : 05)	1.25	1.00	—	1.01	1.00	—	1.32	1.00	—	1.24	1.00	—
MeCN : H_2O (50 : 50)	0.61	1.00	—	1.03	1.10	PR	0.70	1.00	—	0.28	1.00	—
MeOH : H_2O (70 : 30)	2.70	1.00	—	2.97	1.23	0.95	2.34	1.00	—	0.65	1.21	PR

Note: PR, partial resolution (<0.80).

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Table 2. Separation performance on a *tris*(3,5-dimethylphenylcarbamate) of amylose (CSP 2).

	Omeprazole			Lansoprazole			Pantoprazole			Metyrapol		
	k_1	α	*Rs*	k_1	α	*Rs*	k_1	α	*Rs*	k_1	α	*Rs*
Hex : 2-prOH (60 : 40)	3.55	1.00	—	3.09	1.00	—	8.06	1.00	—	1.01	1.00	—
Hex : EtOH (70 : 30)	5.13	1.57	3.15	2.97	1.00	—	5.89	1.22	0.81	1.69	1.32	1.33
EtOH	1.54	1.27	0.90	0.24	1.00	—	1.03	1.17	PR	0.34	1.50	0.96
MeOH	1.34	1.88	2.24	0.46	1.20	PR	0.68	1.18	PR	0.24	1.38	PR
MeCN	16.8	1.00	—	5.05	1.00	—	8.06	1.00	—	10.2	1.57	1.19
MeCN : MeOH	4.10	1.24	PR	1.42	1.32	0.97	1.64	1.24	PR	4.12	1.51	2.59
(95 : 05)	3.85[a]	1.70	2.52	1.34[a]	1.34	1.08				4.18[a]	1.75	3.98
MeCN : H_2O (50 : 50)	1.66	1.37	0.88	1.31	1.37	1.11	0.94	1.37	0.94	0.88	1.95	1.48
	1.79[a]	1.62	1.32	1.39[a]	1.43	1.27	1.01[a]	1.37	0.97	0.89[a]	2.07	2.29
MeOH : H_2O (70 : 30)	8.91	1.56	PR	4.73	1.00	—	4.23	1.35	PR	0.93	1.00	—

Note: PR, partial resolution (<0.80).
[a]0.5 mL min^{-1}.

Table 3. Separation performance on a *tris*(3,5-dimethoxyphenylcarbamate) of amylose (CSP 3).

	Omeprazole			Lansoprazole			Pantoprazole			Metyrapol		
	k_1	α	*Rs*	k_1	α	*Rs*	k_1	α	*Rs*	k_1	α	*Rs*
Hex : 2-prOH (60 : 40)	17.0	1.00	—	27.4	1.00	—	R	—	—	1.39	1.00	—
Hex : EtOH (70 : 30)	5.71	1.00	—	2.39	1.09	PR	11.4	1.00	—	1.17	1.00	—
EtOH	1.19	1.00	—	0.62	1.00	—	1.74	1.00	—	0.26	1.00	—
MeOH	2.61	1.92	2.35	1.06[a]	1.22	0.86	1.98[a]	1.30	1.67	0.26	1.00	—
MeCN	4.01	1.31	PR	1.79	1.21	PR	3.09	1.25	PR	2.93	1.53	1.22
MeCN : MeOH (95 : 05)	1.72	1.44	1.02	0.80	1.34	PR	1.29	1.33	PR	1.94	1.34	0.97
MeCN : H_2O (50 : 50)	7.55	1.00	—	2.48	1.72	3.57	2.85	1.58	3.85	0.43	1.26	PR
										0.95[b]	1.72	1.84
MeOH : H_2O (70 : 30)	2.63	1.00	—	0.79	1.32	PR	1.49	1.30	PR	0.43	1.00	—

Note: PR, partial resolution (<0.80); R, retained compound.
[a]0.5 mL min^{-1}.
[b]mobile phase: MeCN : H_2O (30 : 70 v/v).

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Table 4. Separation performance on a *tris*[(*S*)-1-phenylethylcarbamate] of amylose (CSP 4).

	Omeprazole			Lansoprazole			Pantoprazole			Metyrapol		
	k_1	α	*Rs*	k_1	α	*Rs*	k_1	α	*Rs*	k_1	α	*Rs*
Hex : 2-prOH (60 : 40)	5.96	1.26	0.80	0.37	1.54	PR	R	—	—	1.16	1.00	—
Hex : EtOH (70 : 30)	2.06	1.48	1.71	1.81	2.70	2.89	5.78	1.83	2.15	0.80	1.00	—
EtOH	0.74	1.39	0.91	0.43	1.88	1.17	6.16	1.18	0.90	0.25	1.00	—
MeOH	1.05	1.00	—	0.91	1.00	—	0.48	1.48	0.82	0.13	1.00	—
MeCN	11.1	1.00	—	2.69	1.00	—	7.17	1.00	—	3.80	1.00	—
MeCN : MeOH (95 : 05)	1.39	1.20	PR	3.72	1.00	—	16.1	1.00	—	1.54	1.00	—
MeCN : H_2O (50 : 50)	0.57	1.18	PR	0.73	1.18	PR	0.59	1.00	—	0.29	1.00	—
MeOH : H_2O (70 : 30)	2.31	1.00	—	2.89	1.10	—	3.75	1.09	—	0.45	1.00	—

Note: PR, partial resolution (<0.80); R, retained compound.

the enantiomeric mixture and, thus, to predict which organic modifier will give the highest enantioselectivity with higher resolution.

The Effect of the CSP

Although the mobile phase compositions and or flow rate were not optimized for maximizing resolution, all four compounds were enantioseparated with excellent resolution in at least two out of the four CSPs.

Metyrapol was the sole compound of the examined series that was adequately enantioresolved by the cellulose CSP 1. Excellent resolution ($Rs = 3.62$) and selectivity ($\alpha = 1.82$) were obtained using hexane : EtOH (70 : 30, v/v) as mobile phase (Fig. 2). Although the selectivity was maintained ($\alpha = 1.86$) when ethanol was changed to 2-propanol, the resolution decreased to $Rs = 2.52$. Partial resolutions, with median selectivity were observed in the polar organic mode with ethanol as mobile phase ($\alpha = 1.33$), and in the reversed phase mode ($\alpha = 1.21$) using MeOH : H_2O (70 : 30, v/v). Metyrapol was not enantioresolved in any other condition evaluated with this chiral phase. Lansoprazole, however, had a selectivity

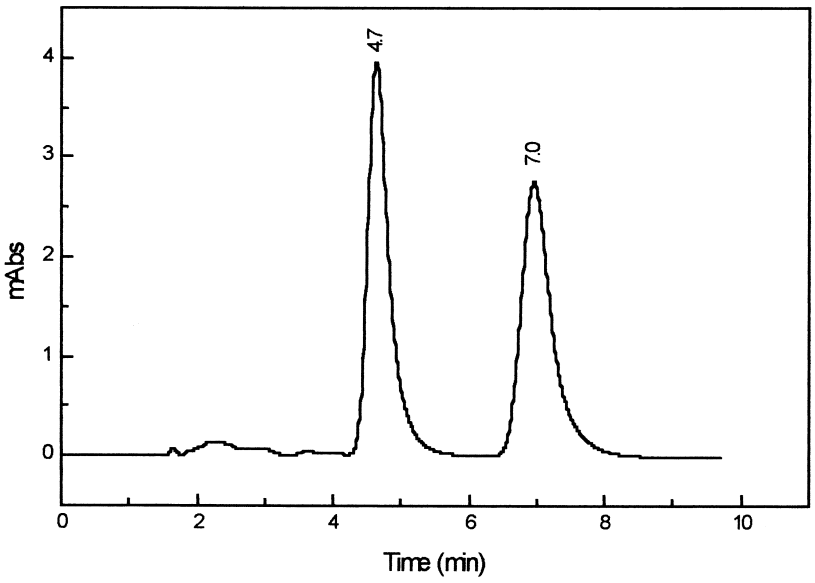

Figure 2. Chromatograms of the separation of metyrapol enantiomers at CSP 1 with Hexane : EtOH (70 : 30, v/v) at a flow rate of 1 mL min^{-1}.

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factor of 1.36 in the normal elution mode, with 2-propanol as the modifier, and of 1.28 with ethanol, it was only partially resolved in this elution mode. When methanol was the eluent in the polar organic mode or was the modifier in the reversed-phase mode, the resolutions obtained were below 1, though the selectivity factors were good ($\alpha = 1.23$). Recently, Nakano and collaborators. reported[19] the enantioseparation of lanzoprazole in a Chiracel® OD R column using methanol : water (75 : 25, v/v) at a flow rate of 0.5 mL at 30°C. In spite of this knowledge, no optimization of the chromatographic conditions was performed to increase the resolution of lanzoprazole. Omeprazole and pantoprazole were not enantioseparated in any of the elution conditions examined (Table 1).

The analogous amylose phase (CSP 2) (Table 2) showed excellent selectivity for omeprazole enantiomers when ethanol was the modifier in normal elution mode. Good enantioseparations with very good resolution were obtained in the polar organic mode when methanol was used as the eluent or as the modifier. Omeprazole was also enantioseparated on the reversed phase mode. The chromatograms at Fig. 3 exemplify the differences in enantioresolution obtained for omeprazole in two different chromatographic conditions. No resolutions, or poor ones, were obtained for lansoprazole and pantoprazole with the CSP 2 under the mobile phases examined. Metyrapol was enantioresolved in this chiral phase on the three chromatographic modes. Excellent resolutions were observed with MeCN : MeOH (95 : 05, v/v) and also with MeCN : H_2O (50 : 50, v/v). A large increase in resolution for some of the compounds was obtained by decreasing the flow rate from 1.0 mL min^{-1} to 0.5 mL min^{-1}. No enantioresolutions were observed when 2-propanol was used as the modifier, in the normal mode, for any of the four compounds evaluated with this chiral phase (Table 2).

The amylose *tris*(3,5-dimethoxyphenylcarbamate) phase (CSP 3) showed the highest enantioselectivity for the series of drugs examined (Table 3). The four chiral compounds were enantioresolved in this CSP with *Rs* in the range of 1.84–3.85, when polar organic or reversed-phase chromatographic conditions were used. However, under the examined conditions, none of the compounds could be enantioresolved by CSP 3 in the normal elution mode.

Lansoprazole and pantoprazole were enantioresolved with excellent selectivity and resolution on reversed phase mode using MeCN : H_2O (50 : 50, v/v) as mobile phase, while metyrapol had partial resolution. When the solvent strength was decreased to 30 : 70 (v/v) an increase in selectivity was observed and good resolution was obtained for the enantiomers of metyrapol ($Rs = 1.84$) (Fig. 4). Omeprazole could not be resolved on this mode of elution, even when methanol was used as the modifier. However, when methanol was used as the eluent, in the polar organic mode, excellent enantioselectivity and resolution were obtained. With this mobile phase,

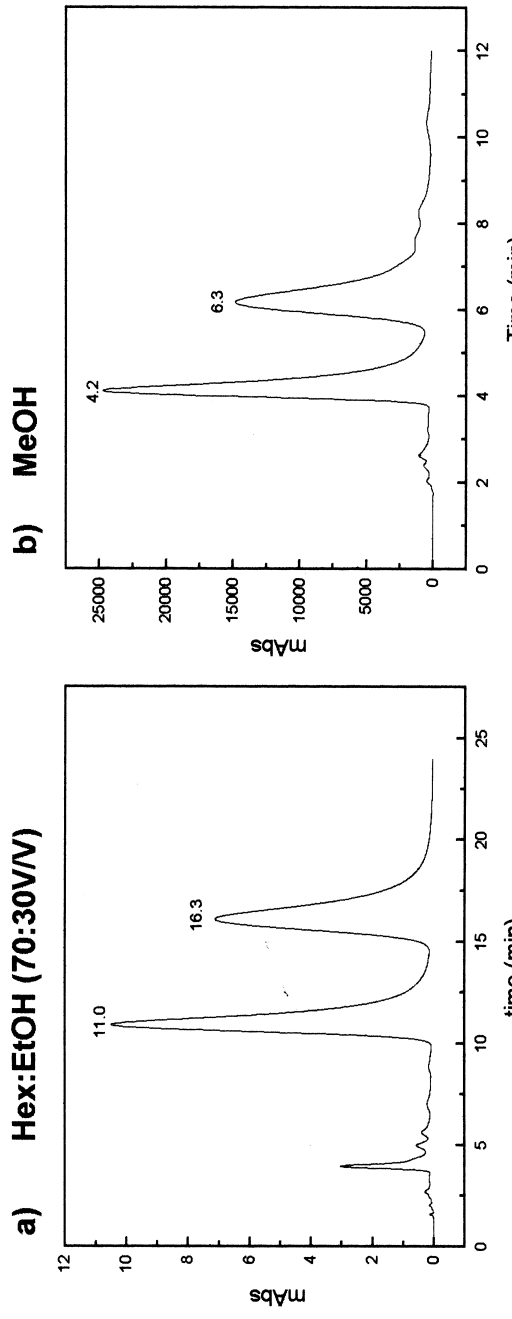

Figure 3. Chromatograms of the separation of omeprazole enantiomers at CSP 2 with multimodal elution at a flow rate of 1.0 mL min^{-1}.

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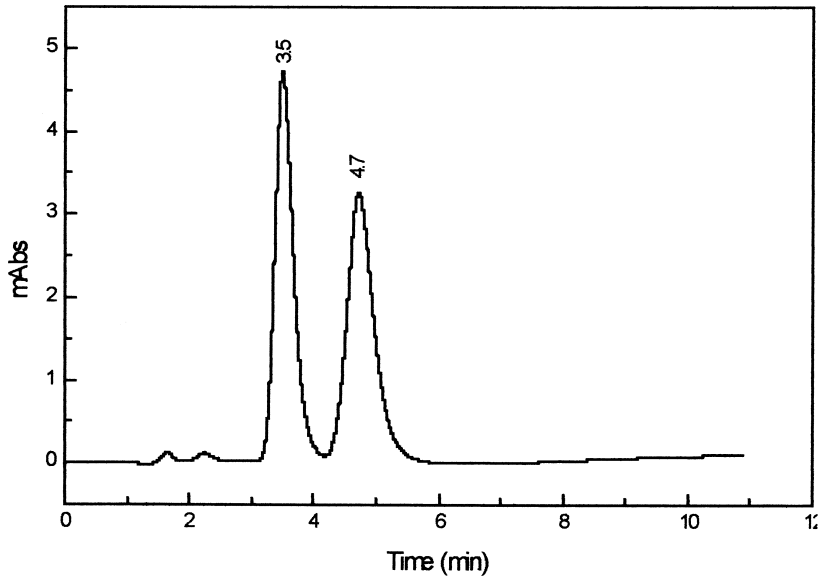

Figure 4. Chromatograms of the separation of metyrapol enantiomers at CSP 3 with MeCN : H_2O (30 : 70, v/v) at a flow rate of 0.5 mL min^{-1}.

good selectivity but poor resolution was observed for lanzoprazole, while for the enantiomers of pantoprazole, at a slow flow rate, good selectivity and resolution were obtained. With acetonitrile as mobile phase, in the polar organic mode, even though the enantioselectivity factors observed were in the range of $\alpha = 1.31–1.53$ for the four compounds, they were not base-line resolved. The use of methanol as modifier had a minor increase in the resolution of omeprazole.

In a previous work, the excellent chiral discrimination ability of the amylose *tris*[(*S*)-1-phenylethylcarbamate] phase for a series of 36 chiral sulfoxides[11] in which 22 were enantioseparated with good resolutions and six with partial resolutions under normal chromatographic conditions, was demonstrated. This ability was again demonstrated. The separation factor and resolution obtained for omeprazole, lansoprazole, and pantoprazole in this CSP were excellent, but only on normal phase conditions using ethanol as modifier. Partial resolutions were obtained in the polar organic mode for the three benzimidazoles and on the reversed-phase mode only for omeprazole, while metyrapol was not resolved in any of the conditions examined with this chiral phase (Table 4).

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Effect of the Chromatographic Mode

As in achiral chromatography, methanol is a weaker modifier than acetonitrile in reversed-phase mode, and 2-propanol than ethanol in normal elution mode. However, all four compounds had shorter retention factors with methanol than with acetonitrile under reversed phase conditions with CSP 3. In normal chromatographic conditions, omeprazole and lansoprazol had a shorter retention factor when 2-propanol was used as modifier instead of ethanol in CSP 2 and CSP 4, respectively. This behavior sometimes happens and has already been described in the literature.[13,23]

Acetonitrile proved to be the weakest eluent in the polar organic mode in all four chiral phases evaluated. The retention factors were adjusted by small percentages of methanol. A decrease in the retention factors when changing from acetonitrile to ethanol or methanol was also observed. This probably is due to a decrease in hydrogen bonding interactions of the solutes with the chiral stationary phases.

The influence on the enantioselectivity of going from a non hydrogen bonding mobile phase to one with this capability can be noticed in changing from 100% of MeCN to ethanol or methanol, or by the use of 5% of methanol as modifier in acetonitrile in the polar organic mode. A similar effect was noticed in going from 100% MeCN (polar organic) to a 50 : 50 (v/v) aqueous solution of acetonitrile (reversed-phase mode). This shows that the ability of the eluent to hydrogen bonding plays an important role in the enantioselectivity process.[23]

A dramatic decrease in the retention factors for all four compounds, in going from neat acetonitrile to aqueous acetonitrile was observed, except at CSP 3 for the three benzimidazoles (Tables 1–4). As expected, the increase in the water content in the reversed-phase mode increased the retention factors. The graph at Fig. 5 shows the retention factors obtained for omeprazole at CSP 2. This retention behavior of the solutes, under reversed phase conditions, identifies that hydrophobic interactions play a dominant part in the retention process. At 100% of acetonitrile (polar organic mode), however, hydrogen bonding between the solute and the CSP is probably the main factor in the retention process. This retention performance is in agreement with the results described by Kummer and Werner[15] and it is similar to the one described for cyclodextrin chiral columns.[26]

In the reversed phase mode, relatively great differences were observed in enantioresolution in changing the modifier from acetonitrile to methanol, as observed for all four compound in CSP 2 and for compounds (2)–(4) in CSP 3.

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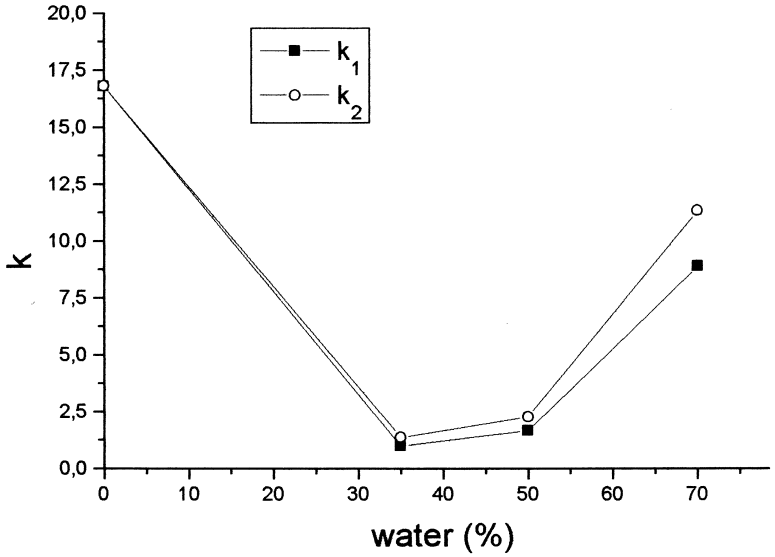

Figure 5. Graphic showing the retention factors (k_1 and k_2) of omeprazole enantiomers at CSP 2 in going from neat MeCN to aqueous MeCN solutions as mobile phases.

CONCLUSION

This comparative study of the four chiral phases on the three different modes of elution for the compounds examined, has highlighted that changing in the chromatographic mode and/or the composition of the mobile phase can dramatically vary the enantioselectivity and/or enantioresolution of the polysaccharide-based columns.

Works in the literature reports the effects of mobile phase on the enantioselectivity of these CSPs for a number of compounds.[5,15] However, they are based on the use of separated columns for reversed and normal elution. The results reported here, in a different manner, are based on the use of a single column for normal, reversed, and polar organic chromatography. We have already demonstrated that the performance of these chiral columns can be maintained under multimodal elution.[4,17] Thus, the differences observed in this work for enantioselectivity and enantioresolution at each chiral phase, is due solely to the mobile phase effects and not to column batch effects. The use of polysaccharide-based CSP under multimodal elution enhances the applicability of this important class of column, since it enables conditions to optimize resolution by modifying the mobile phase composition.

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All four drugs were enantioresolved in reversed chromatographic conditions with resolutions in the range of 1.32–3.98, enabling them, by simple optimization of the chromatographic conditions, to be easily analyzed in human plasma by direct-injection, using a restricted access column coupled to the chiral column.[8,9]

ACKNOWLEDGMENTS

The authors acknowledge the financial support and grants from Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP). A grant from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) is also acknowledged.

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Received October 18, 2002
Accepted February 13, 2003
Manuscript 5995

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