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Enantiomeric resolution of a series of chiral sulfoxides by high-performance liquid chromatography on polysaccharide-based columns with multimodal elution

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Abstract

The enantiomeric resolution of a series of 20 asymmetric sulfoxides was systematically investigated by HPLC using multimodal elution with amylose tris[(*S*)-1-phenylethylcarbamate], amylose tris(3,5-dimethoxyphenylcarbamate) and amylose and cellulose tris(3,5-dimethylphenylcarbamate) phases. The sulfoxide series was composed of aromatic, olefinic and ketosulfoxides, sulfinyl acids and esters. This work has shown that enantioselectivity and enantioresolution of the polysaccharide-based columns can be achieved by changing the type and composition of the mobile phase, widening the applicability of these chiral phases.

Keywords: Enantiomer separation; Stationary phases, LC; Sulfoxides

1. Introduction

Of the many known methods of chiral separation, high-performance liquid chromatography (HPLC) with chiral stationary phases is a suitable approach for enantiomeric separation in analytical or preparative scale for a number of different chiral compounds [1–4].

Chiral sulfoxides constitute a class of highly valuable chiral auxiliaries, reagents in asymmetric synthesis [5,6] and drugs [7]. Methods are required for the rapid, accurate, and sensitive determination of enantiomeric ratios in compounds where the chirality is due only to the presence of an asymmetric sulfoxide function.

A number of publications have described the successful resolution of chiral sulfoxides on different types of chiral stationary phases including derivatives of cellulose and amylose [8–13].

The use of polysaccharide columns in multimodal elution has shown that differences in enantioselectivity can be obtained by alteration of the elution mode [14,15]. This is an important issue to be examined since an enantiomeric separation can be achieved and the cost reduced.

This work reports a systematic investigation on the enantioresolution of a series of asymmetric sulfoxides, including aromatic, olefinic and ketosulfoxides,

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sulfinil acids and esters by HPLC using amylose and cellulose carbamates phases with multimodal elution.

2. Experimental

2.1. General

Solvents were HPLC grade from J.T. Baker (Phillipsburg, USA) except for ethanol which was from Tedia (Fairfield, USA). The ethanol used was HPLC grade and contained 5% (v/v) of 2-propanol and 5% (v/v) of methanol in its composition. Water was purified with a Milli-Q system (Millipore, São Paulo, Brazil).

Racemic sulfoxides were prepared in very high yields by oxidation of corresponding sulfides using oxo diperoxo complexes of molybdenum coated on silica gel [16].

Isocyanates and amylose were purchased from Sigma–Aldrich (St. Louis, MO, USA). The cellulose used was Avicel from Merck (Darmstadt, Germany). HPLC dead times (t_o) were estimated by using 1,3,5-tri-*tert*.-butylbenzene for normal mode of elution and acetonitrile (ACN) for organic and reversed modes of elution.

The chiral columns (150×4.6 mm I.D.) were prepared as described elsewhere [8,9]. The columns used were amylose tris[(*S*)-1-phenylethylcarbamate] (CSP I) and amylose tris(3,5-dimethoxyphenylcarbamate) (CSP II) coated onto APS-Nucleosil (7 μm particle size and 500 Å pore size, 20%, w/w), and amylose (CSP III) and cellulose tris(3,5-dimethylphenylcarbamate) (CSP IV) coated onto APS-Hypersil (5 μm particle size and 120 Å pore size, 20%, w/w).

2.2. Equipment

The HPLC system consisted of a Shimadzu LC-10AD pump (Kyoto, Japan), a SPD-10A variable-wavelength UV–Vis detector, Rheodyne 7125 injector fitted with a 20 μl loop. For data acquisition, CLASS LC 10 software was used.

3. Results and discussion

Analytical methods are necessary for controlling enantiomeric purity of starting material and products and preparative enantiomeric separation is, sometimes, preferred as a method for obtaining single-enantiomer before the protocol of production of a given chiral compound is chosen. The use of multimodal elution is an excellent approach to reduce cost.

Polysaccharide-based chiral phases are among the most widely used stationary phase for enantiomeric separation by HPLC and has been used in the normal, reversed-phase and polar organic modes of elution [17,18]. To use them in multimodal elution would widen the applicability of these phases. In accordance to literature, multimodality means that the same column can be used in different chromatographic modes [19].

Previously we demonstrate that the performance of a polysaccharide-based column can be maintained by conditioning it in the appropriate manner while switching the elution mode [14].

Sulfoxides are key materials not only in asymmetric synthesis but also in the pharmaceutical industry, and methods for producing single-enantiomer sulfoxides, or for measuring their enantiomeric ratios, are of importance and we have worked systematically on this purpose [8,9,20,21]. These facts prompted us to further explore conditions for enantiomeric separation for a series of 20 asymmetric sulfoxides (Fig. 1). The series this time is composed of different functional sufoxides, including olefinic and ketosulfoxides, sulfinil acids and esters, besides the usual aromatic sulfoxides.

The same set of sulfoxides (Fig. 1) was eluted on four different polysaccharide-based columns in the three-elution modes. Retention factors, selectivity and resolution were examined for these sulfoxides in each elution mode at the same established conditions (Tables 1–3).

The use of mesoporous silica (500 Å) and microporous silica (120 Å) as support for the carbohydrate carbamate phases with good analytical performance has already been demonstrated [7–9,21]. It is important to note that although the chiral columns prepared using mesoporous silica (500 Å) as support has always showed somewhat better enantioselectivity than the ones with microporous silica (120 Å) this effect is due mainly to the differences in acidity of these supports [9,21–23].

The amylose tris[(*S*)-1-phenylethylcarbamate]

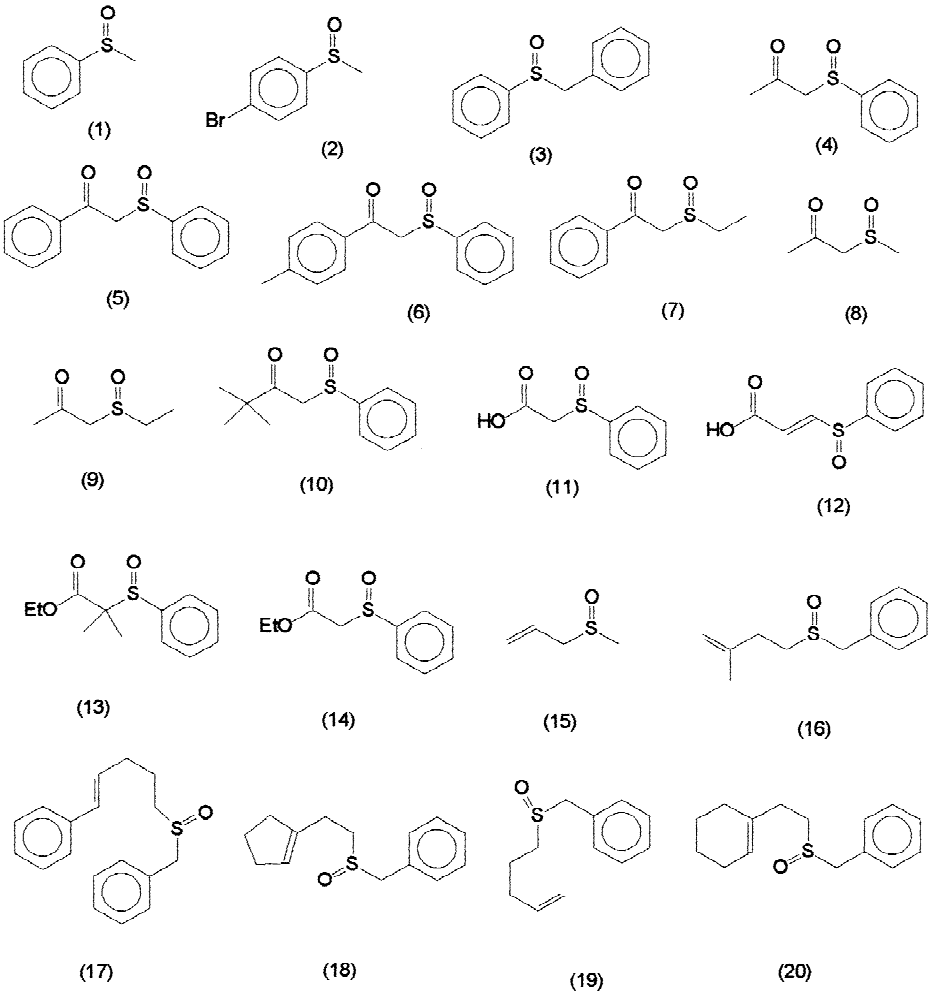

Fig. 1. Chemical structures of selected racemic sulfoxides.

phase showed the highest enantioselectivity for the selected sulfoxide series (Fig. 1) on the three-elution modes (Tables 1–3). Fifteen out of 20 sulfoxides were enantioresolved with excellent selectivity and resolution in the normal elution mode (α 1.19–3.40; R_s 1.14–7.46) while 11 were resolved on the polar organic mode (α 1.16–2.65; R_s 0.65–4.11). Enantioselectivity and enantioresolution differences were observed with this chiral phase by altering the polar solvent. The olefinic sulfoxide (18), which was not enantioresolved when ACN–MeOH (98:2, v/v) was used, had excellent resolution with methanol and very poor one with ethanol as mobile phases. It is interested to note that the analogous olefinic sulfoxide (20) was not enantioresolved using methanol as mobile phase but was with ethanol and with ACN–MeOH (98:2, v/v) (Table 2). The chiral recognition ability of the polysaccharide carbamates phases is a result not only of combination of attractive forces of the CSP with the functional groups at the chiral solutes but it is also a function of steric fit of the solute in the chiral cavity or channel of the stationary phases [4,17,24].

In the reversed-phase mode great differences were observed in enantioresolution in changing the modifier from acetonitrile to methanol. At reversed-phase conditions the CSP I behaved as a typical reversed stationary phase with methanol as a weaker modifier compared to acetonitrile. The opposite behavior is also described in the literature [18,24] and it was

Table 1
Resolution of the chiral sulfoxides series in amylose tris[(*S*)-1-phenylethylcarbamate] (CSP I) and tris(3,5-dimethylphenylcarbamate) (CSP III) and cellulose tris(3,5-dimethylphenylcarbamate) (CSP IV) phases using normal mode of elution; hexane–EtOH (85:15, v/v)

Sulfoxide	Column								
	CSP I			CSP III			CSP IV		
	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s
1	3.98	1.34	1.86	3.86	1.00	–	1.68	1.00	–
2	4.38	1.28	2.27	5.05	1.00	–	2.08	1.00	–
4	8.00	1.34	2.85	3.87	1.00	–	3.25	1.00	–
5	12.37	1.28	1.91	3.60	1.00	–	3.56	1.27	0.65
6	2.43	2.22	2.42	3.18	1.00	–	3.29	1.50	1.27
7	4.95	1.27	2.11	2.27	1.00	–	3.92	1.00	–
8	10.66	1.19	1.22	2.45	1.00	–	3.24	1.49	1.29
10	4.22	1.75	2.97	5.08	1.00	–	3.48	1.00	–
11	6.23	1.43	3.00	0.50	3.64	4.48	3.37	1.00	–
12	3.92	1.31	1.14	2.45	1.00	–	2.55	1.00	–
14	6.13	1.00	–	6.94	1.29	2.13	2.88	1.00	–
16	3.90	2.30	5.69	2.77	1.27	2.32	2.43	1.00	–
17	7.19	1.34	1.92	4.81	1.16	1.22	3.91	1.00	–
18	5.22	3.40	7.46	4.79	1.16	1.25	3.58	1.12	PR
19	4.89	1.67	3.71	2.84	1.22	1.35	2.58	1.00	–
20	4.00	3.09	6.86	2.72	2.15	5.40	2.60	1.00	–

Flow rate: 1.0 ml/min; λ=254 nm. PR, partial resolution <0.6.

observed with CSP III for some of the sulfoxides (Table 3).

The olefinic sulfoxides (16), (17), (18) and (19) were resolved with excellent selectivity and resolution using acetonitrile as modifier at CSP I. Five other sulfoxides were enantioresolved with very low resolution under this condition. The use of methanol as modifier increased the resolution for sulfoxide (2) and decreased for sulfoxide (16) with no resolution for any other sulfoxide in the series (Table 3).

The chromatogram given in Fig. 2 exemplifies the excellent enantioresolution (α=3.40 and R_s=7.46) obtained for the chiral sulfoxide (18) using amylose tris[(*S*)-1-phenylethylcarbamate] as stationary phase and hexane–EtOH (85:15, v/v) as mobile phase. 2-Propanol was not used as modifier in the normal elution mode due to the higher retention factor usually obtained for chiral sulfoxides with the cellulose and amylose-based chiral phases [8,9].

The amylose tris(3,5-dimethoxyphenylcarbamate) phase (CSP II) was not able to enantioresolve any of the 20 sulfoxides of the series in any of the evaluated conditions. Considering the excellent resolutions observed with this chiral phase for some other previously examined chiral sulfoxides [8,15], this was an unexpected result.

In a recent paper, Okamoto and collaborators concede that some of the polysaccharide derivatives may be used in the three-elution mode and that hydrogen-bonding interactions were the prevailing effect for the separation of the five aromatic sulfoxides evaluated in polar organic mode with polysaccharides phenylcarbamates derivatives [25].

The amylose tris(3,5-dimethylphenylcarbamate) phase showed excellent selectivity and resolution to five olefinic sulfoxides of the series (16) to (20) using ACN–MeOH (98:2, v/v) as mobile phase (α 1.65–11.40; R_s 3.20–5.35) (Fig. 3). The use of acetonitrile in high percentage, favoring hydrogen-bonding between the sulfoxides and the chiral stationary phases, probably was the main factor in enhancing enantioselectivity However, with CSP I, better enantioselectivity and resolution were obtained, with methanol or ethanol as the eluent. The alteration on the mobile phase composition, in going to 100% ethanol or methanol, with CSP III, affected drastically the resolution of the five olefinic sulfoxides.

Table 2
Resolution of the chiral sulfoxides series in amylose tris[(*S*)-1-phenylethylcarbamate] (CSP I) and tris(3,5-dimethylphenylcarbamate) (CSP III) and cellulose tris(3,5-dimethylphenylcarbamate) (CSP IV) phases using polar organic mode

Sulfoxide	Mobile phase	Column								
		CSP I			CSP III			CSP IV		
		k_1	α	R_s	k_1	α	R_s	k_1	α	R_s
1	EtOH (100%)	0.96	1.18	PR	0.57	1.00	–	0.47	1.00	–
	MeOH (100%)	0.30	1.00	–	0.62	1.00	–	0.16	1.00	–
	ACN–MeOH (98:2, v/v)	1.08	1.42	1.30	0.56	1.00	–	0.13	1.00	–
4	EtOH (100%)	1.18	1.23	0.65	0.78	1.00	–	0.65	1.00	–
	MeOH (100%)	0.28	1.00	–	0.54	1.00	–	0.25	1.00	–
	ACN–MeOH (98:2, v/v)	0.65	1.00	–	0.40	1.00	–	0.34	1.00	–
5	EtOH (100%)	1.76	1.47	1.83	1.20	1.00	–	0.87	1.00	–
	MeOH (100%)	0.50	1.60	1.28	3.07	1.10	0.89	0.50	1.00	–
	ACN–MeOH (98:2, v/v)	0.82	1.21	PR	0.53	1.00	–	0.13	1.23	PR
6	EtOH (100%)	0.79	1.00	–	1.25	1.00	–	1.11	1.00	–
	MeOH (100%)	0.48	1.52	1.29	1.27	1.00	–	0.34	1.35	0.99
	ACN–MeOH (98:2, v/v)	0.97	1.27	0.73	0.18	1.00	–	0.10	1.30	PR
7	EtOH (100%)	1.85	1.31	1.22	0.86	1.00	–	0.41	1.00	–
	MeOH (100%)	0.44	1.52	1.28	0.68	1.00	–	0.18	1.44	0.67
	ACN–MeOH (98:2, v/v)	1.33	1.13	PR	0.24	1.00	–	0.12	1.00	–
8	EtOH (100%)	1.97	1.53	1.93	1.48	1.00	–	0.90	1.20	PR
	MeOH (100%)	0.60	1.62	2.01	1.42	1.00	–	0.51	1.00	–
	ACN–MeOH (98:2, v/v)	1.02	1.00	–	0.83	1.00	–	0.10	2.40	0.77
16	EtOH (100%)	1.15	1.84	1.27	1.70	1.22	0.98	0.56	1.00	–
	MeOH (100%)	0.37	1.70	1.01	0.52	1.27	PR	0.26	1.00	–
	ACN–MeOH (98:2, v/v)	1.07	2.63	1.51	1.29	2.62	5.13	0.22	1.00	–
17	EtOH (100%)	2.59	1.20	0.94	1.00	1.00	–	1.34	1.00	–
	MeOH (100%)	0.82	1.21	PR	1.47	1.00	–	1.10	1.00	–
	ACN–MeOH (98:2, v/v)	1.70	1.70	1.31	2.52	1.65	3.20	0.97	1.00	–
18	EtOH (100%)	5.26	1.16	0.65	0.67	1.57	1.21	0.92	1.00	–
	MeOH (100%)	2.04	2.37	4.11	0.78	1.15	0.80	0.34	1.33	1.49
	ACN–MeOH (98:2, v/v)	1.69	1.00	–	0.11	11.40	5.35	0.64	1.00	–
19	EtOH (100%)	3.62	1.18	1.12	0.54	1.39	PR	0.56	1.00	–
	MeOH (100%)	1.67	1.76	2.67	1.94	1.00	–	0.26	1.00	–
	ACN–MeOH (98:2, v/v)	1.10	2.55	1.54	3.95	1.79	3.55	0.79	1.00	–
20	EtOH (100%)	1.59	2.65	3.26	0.57	1.00	–	0.69	1.00	–
	MeOH (100%)	0.12	1.00	–	0.85	1.16	0.88	0.45	1.00	–
	ACN–MeOH (98:2, v/v)	1.66	1.71	1.25	0.13	10.40	5.32	1.78	1.00	–

Flow rate: 1.0 ml/min; λ=254 nm. PR, partial resolution <0.6.

Besides the olefinic sulfoxides (16) to (20), two other sulfoxides (11) and (14) were enantioresolved with CSP III when normal condition was used (Table 1). The decrease in the enantioselectivity factor and also in the resolution in going from polar organic mode, using ACN–MeOH (98:2, v/v), to normal elution with hexane–EtOH (85:15, v/v) for the olefinic sulfoxides (16) to (20) at CSP III is worth noticing. It might be a result of differences in solvation of the CSPs and/or solutes with this two mobile phases [24]. The analogous phase of cellulose showed only minor chiral resolution for the twenty sulfoxides of the series in all of the established conditions examined (Tables 1–3). Nonetheless, the sulfoxides (6) and (8) which were not resolved in the corresponding amylose phase had minor resolution on the cellulose phase at normal elution.

The elution mode significantly affected the en-

Table 3
Resolution of the chiral sulfoxides series in amylose tris[(*S*)-1-phenylethylcarbamate] (CSP I) and tris(3,5-dimethylphenylcarbamate) (CSP III) and cellulose tris(3,5-dimethylphenylcarbamate) (CSP IV) phases using reversed-phase conditions

Sulfoxide	Mobile phase	Column								
		CSP I			CSP III			CSP IV		
		k_1	α	R_s	k_1	α	R_s	k_1	α	R_s
1	ACN–water (1:1)	0.44	1.34	0.65	0.37	1.00	–	0.67	1.00	–
	MeOH–water (1:1)	1.27	1.00	–	1.92	1.00	–	0.80	1.00	–
2	ACN–water (1:1)	1.12	1.19	0.63	1.08	1.13	0.65	0.51	1.00	–
	MeOH–water (1:1)	5.23	1.23	1.39	1.70	1.00	–	2.87	1.00	–
3	ACN–water (1:1)	1.92	1.00	–	2.00	1.00	–	1.36	1.00	–
	MeOH–water (1:1)	14.83	1.00	–	2.97	1.58	1.64	0.36	1.00	–
5	ACN–water (1:1)	1.81	1.29	0.64	2.03	1.00	–	1.31	1.00	–
	MeOH–water (1:1)	1.63	1.00	–	1.52	1.00	–	1.44	1.00	–
8	ACN–water (1:1)	2.56	1.22	PR	0.54	1.00	–	1.26	1.00	–
	MeOH–water (1:1)	3.53	1.00	–	2.89	1.00	–	2.76	1.00	–
9	ACN–water (1:1)	0.42	1.36	0.68	0.86	1.00	–	0.25	1.00	–
	MeOH–water (1:1)	1.88	1.00	–	3.22	1.00	–	1.54	1.00	–
10	ACN–water (1:1)	1.17	1.18	0.64	0.84	1.00	–	0.56	1.00	–
	MeOH–water (1:1)	3.17	1.00	–	1.36	1.00	–	3.28	1.00	–
16	ACN–water (1:1)	1.52	2.14	4.00	1.56	2.20	3.77	0.84	1.00	–
	MeOH–water (1:1)	12.38	1.80	2.46	1.20	1.34	0.70	16.78	1.00	–
17	ACN–water (1:1)	6.35	1.45	2.35	8.10	1.48	2.41	0.57	1.00	–
	MeOH–water (1:1)	11.23	1.00	–	1.79	1.47	2.12	14.04	1.17	1.24
18	ACN–water (1:1)	3.64	2.69	6.32	4.39	1.00	–	1.81	1.00	–
	MeOH–water (1:1)	12.05	1.00	–	3.41	2.31	3.10	11.13	1.10	0.67
19	ACN–water (1:1)	1.84	1.69	2.22	1.76	1.70	2.00	0.79	1.00	–
	MeOH–water (1:1)	10.80	1.00	–	5.14	1.80	3.86	5.16	1.15	1.05
20	ACN–water (1:1)	5.13	1.00	–	6.10	1.00	–	1.36	1.00	–
	MeOH–water (1:1)	11.76	1.00	–	1.76	1.00	–	4.62	1.11	0.69

Flow rate: 1.0 ml/min; λ=254 nm. PR, partial resolution <0.6.

antioseparation and enantioresolution of the sulfoxides evaluated. The result reported here is based in the use of a single column for normal, reversed and polar organic chromatography. Therefore, the differences observed in enantioselectivity and enantioresolution for each chiral phase is due solely to the mobiles phase effects and not to column batch effects. This is a very important issue to considerer since works in the literature reports the effects of mobile phase on the enantioselectivity of these CSPs for a number of compounds, however, they are based in the use of separated columns for reversed and normal elution [26,27]. Considering the two amylose phases that had the highest enantioselectivity power for the sulfoxide series, great differences in enantioselectivity were observed by altering the elution mode. For instance, in reversed-phase or polar organic condition these two chiral phases were not able to enantioresolve the chiral sulfoxide (11) but at normal elution mode excellent enantioselectivity were achieved with both chiral phases.

In a previous work it was demonstrated the excellent chiral discrimination ability of the phase amylose tris[(*S*)-1-phenylethylcarbamate] towards a series of 36 chiral sulfoxides [9]. This superior enantioselectivity ability was again demonstrated for this new series of different functional sulfoxides.

The optimization of the separation can be achieved by two major factors: the specificity of the polysaccharide derivative and the mobile phase type and composition. The enantioresolution of sulfoxide (3) was achieved only by the amylose tris(3,5-dimethylphenylcarbamate) phase using methanol as modifier at reversed-phase condition. The amylose tris[(*S*)-1-phenylethylcarbamate] phase, which separated the highest number of chiral sulfoxides of the series, was not able to enantioresolve sulfoxide (3) in any elution condition examined. By this approach the

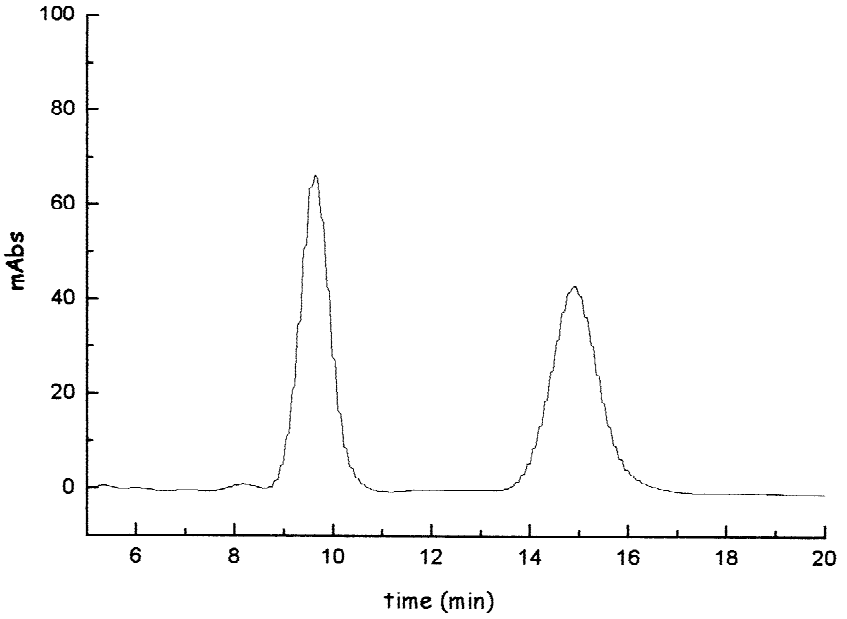

Fig. 2. Enantioresolution of chiral sulfoxide (18) on amylose tris[(*S*)-1-phenylethylcarbamate] coated onto APS-Nucleosil (7 μm particle size and 500 Å pore size, 20%, w/w) using hexane–EtOH (85:15, v/v) as mobile phase. Flow rate: 1.0 ml/min; λ=254 nm.

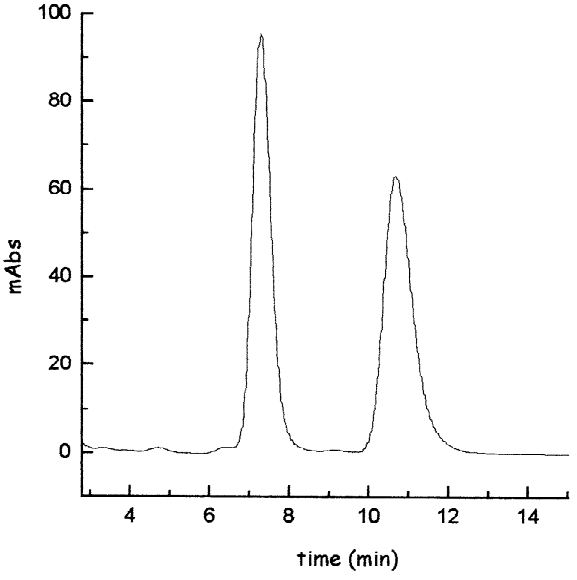

Fig. 3. Enantioresolution of chiral sulfoxide (17) on amylose tris(3,5-dimethylphenylcarbamate) coated onto APS-Hypersil (5 μm particle size and 120 Å pore size, 20%, w/w) using ACN–MeOH (98:2, v/v) as mobile phases. Flow rate: 1.0 ml/min; λ=254 nm.

applicability of these polysaccharide-based phases to enantiomeric separation can be largely broadened.

4. Conclusion

The amylose tris[(*S*)-1-phenylethylcarbamate] and tris(3,5-dimethylphenylcarbamate) phases showed complementary enantioselectivity and resolution for this wide range of chiral sulfoxides.

The use of multimodal elution increases the applicability of these chiral stationary phases by optimization of the resolution gained at different mobile phases composition.

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