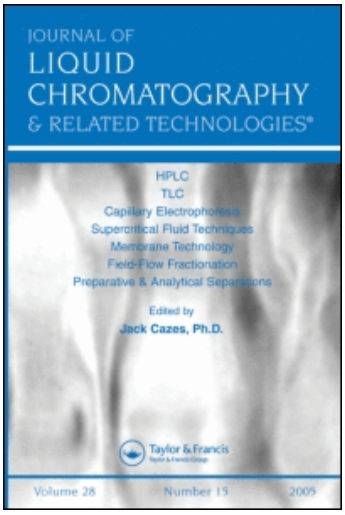

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THE USE OF A POLYSACCHARIDE-BASED COLUMN ON MULTIMODAL ELUTION

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ABSTRACT

Amylose tris (3,5-dimethylphenylcarbamate) coated onto APS-Hypersil (5 μm particle size and 120 Å pore size) was used under normal, reversed-phase and polar-organic conditions for the enantioseparation of six racemates of different classes. The CSP was not altered when going from one mobile phase to another. All compounds were enantioresolved within the elution modes with excellent selectivity factor. The simultaneous analysis of metyrapol enantiomers and metyrapone on reversed-phase conditions and the separation of the enantiomers of omeprazole in human plasma in the polar-organic mode of elution are also described.

INTRODUCTION

In the field of chiral discrimination a preoccupation with development of new methodologies has been valued. Among the most useful and versatile chiral columns described are polysaccharide-based columns. These columns have been used extensively, with success, not only in the analytical mode but also in preparative scale.[1-3]

A limitation to the performance of these columns was, for a long time, the narrow range of solvent that could be used and only on the normal elution

mode.[4] Reversed-phase separations and polar organic were introduced to add more value.[5-7] These columns are, however, commercialized to be used only in one mode of elution. Multimodal operation would expand the applicability of these phases for chiral separation.

This work describes the multimodal operation for this type of phase.

EXPERIMENTAL

General

The column was prepared as described elsewhere[8] and consisted of amylose tris (3,5-dimethylphenyl carbamate) coated onto APS-Hypersil (5 μm particle size and 120 Å pore size) (20% w/w, 15 X 0.46 cm ID). HPLC dead times (t_0) were estimated by using 1,3,5-tri-*tert*-butylbenzene for normal and polar organic modes of elution and acetonitrile for reverse phase mode. Solvents were either HPLC grade from Merck (Darmstadt, Germany) or ChromAR HPLC grade from Mallinckrodt Baker (St. Louis, Missouri, USA) or were purified as usual.[9] In switching the elution mode, the column was conditioned by recycling 100% ethanol for about 6 hours at 0.5 $mL.min^{-1}$.

Equipment

Three HPLC systems were used. System 1 consisted of a Shimadzu (Kyoto, Japan) LC-10AD pump, a Rheodyne 7125 injector fitted with a 20 μL loop, a SPD-6AV UV detector, and a LC-R6A chromatopac recorder. Two Shimadzu LC 10ADVP pumps, a photodiode array SDP-10AVP detector, and an auto sampler SIL 10 AVP with a SCL 10AVP interface composed System 2. The third system was formed by one Shimadzu LC-10AD pump, an auto injector model SIL 10A, and a Chiralyser polarimetric detector with a CBM 10A interface. Data acquisition in system 2 was performed using a CLASS-VP software and at system 3 on a CLASS LC 10 software. The chromatographic parameters were calculated for *Trans*-stilbene oxide(1) at $\lambda = 260$ nm, Troger's base (2) at $\lambda = 290$ nm, *Trans*-2-phenylditiane sulfoxide (3) at $\lambda = 220$ nm, Omeprazole (4) at $\lambda = 302$ nm, Metyrapol (5) at $\lambda = 260$ nm and the Axial chiral amide (6) at $\lambda = 226$ nm.

RESULTS AND DISCUSSION

Polysaccharides-based CSPs are among the most widely used stationary phases for enantiomeric separations and proved to be effective in normal, reversed-phase and polar organic modes of elution.[1-7]

The advantage of using a chiral column on multimodal elution can be noticed by analyzing the marvelous results reported for the macrocyclic antibiotics columns.[10-11]

In developing a method for chiral separation, if it is to be used routinely, one needs to be aware of the cost involved. The limitation imposed by the use of the polysaccharides columns in only one mode of elution makes it unnecessarily expensive.

Previously we demonstrated that a column that was used in the normal elution mode could be efficiently used on reversed-phase mode.[12] This result prompted us to investigate the use of these columns on multimodal elution.

An amilose tris (3,5-dimethylphenylcarbamate) phase coated onto APS-Hypersil (120 Å of pore size and 5 μm of particle size) and a series of six racemates of different classes (Figure 1), known to be resolved in this phase on normal[8, 13-15] elution, were selected to perform this investigation.

The column was prepared as usual[8] and then the six racemates were analyzed first on normal mode and then on reversed-phase.

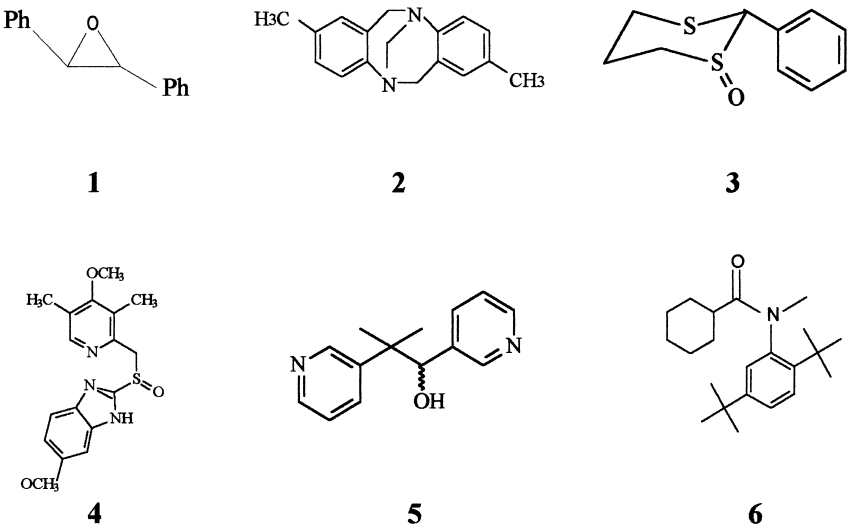

Figure 1. Selected racemates.

Table 1

Separation Performance of Some Compounds on a Tris (3,5-Dimethylphenylcarbamate of Amylose) on APS-Hypersil Column at Normal Mode of Elution[a]

	0.5 mL.Min^{-1}			1.0 mL.Min^{-1}		
	k_1	α	Rs	k_1	α	Rs
1[b]	0.75	1.87	3.91	0.73	1.81	3.14
[c]	0.78	2.46	6.27			
2[b]	0.75	2.12	4.72	0.73	2.15	2.79
[c]	1.02	1.55	2.84			
3[b]	3.13	2.74	9.79	3.17	2.77	8.64
4[b]	8.65	1.43	3.09	9.03	1.39	2.28
5[b]	1.98	1.27	1.62	1.95	1.27	1.27
6[b]	0.13	1.00	---			
[c]	0.38	1.43	1.31			

[a] (120 Å, 5 μm), (20% w/w). [b] Eluent: Hexane/Ethanol (70:30). [c] Eluent: Hexane/2-propanol (90:10).

The separation factor and the resolution obtained were excellent for all compounds except for the axial chiral amide (6) which was not resolved when hexane:ethanol (70:30 v/v) was used as mobile phase with a flow rate of 0.5 mL. min^{-1} (Table 1). When the solvent strength was decreased to hexane:2-propanol (90:10 v/v) the axial chiral amide (6) showed a good separation factor. The enantioresolution of *trans*-stilbene oxide (1) was also improved while for the Troger's base it was decreased. The sulfoxide (3), omeprazole (4) and metyrapol (5) had large capacity factors on this mobile phase and were not analyzed.[8,14-15]

The column was then conditioned for about 6 hours by recycling 100% ethanol at 0.5 mL. min^{-1} and then with acetonitrile before conditioning with the reversed-phase solvent. The separations obtained for five out of the six compounds analyzed were excellent. Resolution was slightly decreased for compounds 3 and 6 when the retention was altered by increasing the amount of acetonitrile in the mobile phase but the best results were achieved with a slower flow rate (Table 2).

The enantioresolution observed on normal and reversed-phase was excellent. The CSP was not altered when going from one mobile phase to another. Retention factors, selectivity, and resolution were examined for these selected

Table 2

Separation Performance of Some Compounds on a Tris (3,5-Dimethylphenylcarbamate) of Amylose on APS-Hypersil Column at Reversed Mode of Elution

	CH_3CN/H_20 (50:50) 1.0 mL·Min^{-1}			CH_3CN/H_20 (70:30) 1.0 mL·Min^{-1}			CH_3CN/H_20 (70:30) 0.5 mL·Min^{-1}		
	k_1	α	Rs	k_1	α	Rs	k_1	α	Rs
1	10.49	1.00	---	1.86	1.00	---			
2	3.71	1.54	3.00						
3	1.67	3.13	5.87	0.92	2.91	5.48	0.72	3.18	6.82
4	2.30	1.42	1.07	1.28	1.41	1.05	1.06	1.46	1.38
5	1.22	1.93	2.09	0.93	1.83	2.09	0.74	1.97	2.65
6	14.26	1.32	1.65	2.35	1.31	1.09	2.04	1.33	1.30

compounds in each mode and no degradation on the column performance was observed with the switching in the elution mode. Table 3 shows the chromatographic parameters obtained after going back to normal elution.

The chromatograms shown in Figure 2 are illustrative of the performance of the column in this study.

Table 3

Separation Performance of Some Compounds on a Tris (3,5-Dimethylphenylcarbamate) of Amylose on APS-Hypersil Column at Reversed Mode of Elution[a]

	k_1	α	Rs
1[b]	0.74	1.80	3.63
[c]	0.84	2.46	6.10
2[b]	0.76	2.03	4.53
[c]	1.14	1.50	2.48
3[b]	3.28	2.69	9.31
4[b]	7.98	1.47	3.18
5[b]	2.18	1.31	1.46
6[b]	0.15	1.00	---
[c]	0.44	1.44	1.53

[a] (120 Å, 5 μm), (20% w/w). [b] Eluent: Hexane/Ethanol (70:30). [c] Eluent: Hexane/2-propanol (90:10).

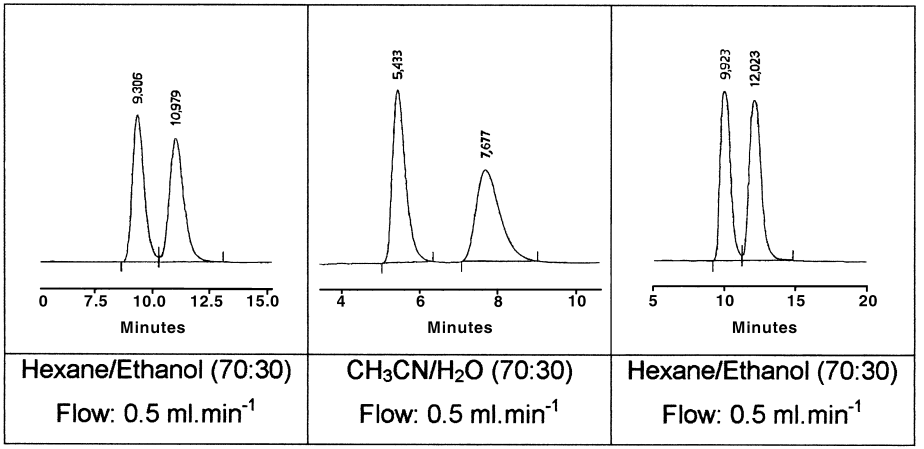

Figure 2. Obtained resolution of Metyrapol on normal and reversed elution mode.

Lately, attention has been focused on the use of mobile phase with high amounts of organic modifier: the so called polar-organic.[10,11] The polar-organic is similar to normal-phase mode but without hexane, which is an advantage when one needs to improve solute solubility.

Thus, the use of ethanol:methanol and acetonitrile:methanol as mobile phases were also evaluated. Excellent selectivity factor and resolution were obtained for all compounds except for the axial chiral amide (6) when ethanol:methanol (90:10 v/v) was used as solvent. Decreasing the content of methanol to 5% had little effect on the capacity factors and also on selectivity for the compounds analyzed (Table 4). The enantioresolution of the majority of the compounds decreased when ethanol:methanol (10:90 v/v) was used as mobile phase though separation factor and resolution of Omeprazole were enormously increased (Table 4).

Changing, however, to acetonitrile:methanol (98:02 v/v) the effect on selectivity and resolution was impressive for the sulfoxide (3) (Figure 3). *Trans*-stilbene oxide (1) was not resolved while the axial chiral amide (6) had a separation factor of $\alpha = 1.91$ although, with poor resolution Rs = 0.87. The same case was observed with metyrapol which with an $\alpha = 4.86$ had a very bad resolution (< 0.70). The reason for this is probably the very small capacity factors obtained for the enantiomers. (Table 5).

Table 4

Separation Performance of Some Compounds on a Tris (3,5-Dimethylphenylcarbamate) of Amylose on APS-Hypersil Column on Polar Mode of Elution*

	Ethanol/Methanol (90:10)			Ethanol/Methanol (95:05)			Ethanol/Methanol (10:90)		
	k_1	α	Rs	k_1	α	Rs	k_1	α	Rs
1	0.59	2.24	4.74	0.57	2.26	4.02	0.82	1.88	2.43
2	0.69	2.21	4.35	0.68	2.16	3.66	0.8	2.31	3.90
3	0.72	1.81	3.32	0.74	1.83	3.01	0.55	2.07	2.72
4	1.78	1.43	1.90				1.44	2.01	4.18
5	0.38	1.47	1.27				0.28	1.32	0.74
6	0.09	1.00	---	0.09	1.00	---	0.09	1.00	---

* (120 Å, 5 μm), (20% w/w). Flow: 0.5 mL.min^{-1}.

Table 5 shows the order of elution of the six racemates on the polar-organic mode. No reversal in the elution order was observed when the elution mode was changed to normal or reversed-phase.

The amylose tris (3,5-dimethylphenylcarbamate) phase was able to enantioresolve in the three modes of elution five out of the six compounds selected. The *trans*-stilbene oxide (1) was the exception that was not resolved on the reversed-phase conditions examined. The principle of complementary separation with in different modes of elution can thus be used with a same column to extend their applicability.

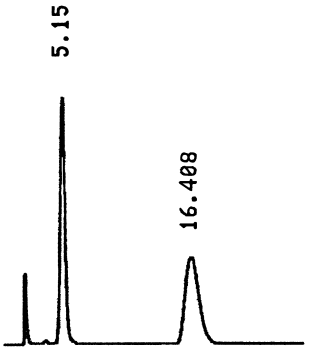

Figure 3. Enantioresolution of the sulfoxide in acetonitrile:methanol (98:02) at 0.5 mL.min^{-1}.

Table 5

Separation Performance of Some Compounds and Order of Elution of the Six Racemates on a Tris (3,5-Dimethylphenylcarbamate of Amylose on a APS-Hypersil Column on Polar Mode of Elution*

	k_1	α	Rs	First Eluted Enantiomer	Second Eluted Enantiomer
1	0.24	1.00	---	(+)	(-)
2	0.95	1.36	1.41	(+)	(-)
3	0.51	7.45	7.00	(+)	(-)
4	3.15	1.28	0.86	(-)	(+)
5	0.07	4.86	<0.70	(-)	(+)
6	0.45	1.91	0.87	(+)	(-)

* (120 Å, 5 μm), (20% w/w). Eluent: Acetonitrile/Methanol (98:02); Flow: 0.5 mL.min^{-1}.

To demonstrate further the use of the column on multimodal elution and based on the excellent enantioseparation obtained for metyrapol on reversed-phase conditions, the analysis of the enantiomers of metyrapol in presence of the parent drug metyrapone was examined.

An excellent enantioseparation, for metyrapol enantiomers with a selectivity factor of 1.63 while being able to analyze metyrapone in the same run, was obtained. $CH_3CN/H_2O/Et_3N/HOAc$ (35:65:0.01:0.01 v/v/v/v) was used as mobile phase at a flow rate of 0.4 mL.min^{-1} and λ=256 nm (Figure 4).

Although a large number of liquid chromatographic methods have been reported for the determination of metyrapone and its metabolites, only recently, enantioselective assays for the determination of the enantiomers of metyrapol have been described.[15-18] To our knowledge this is the first report, in reversed-phase conditions, of the simultaneous analysis of metyrapol enantiomers and metyrapone. These conditions are now being validated for use in metabolic studies and the results will be published elsewhere.

The versatility of the use of the column was further evaluated with success by the analysis of the enantiomers of omeprazole in human plasma using the polar-organic mode of elution.

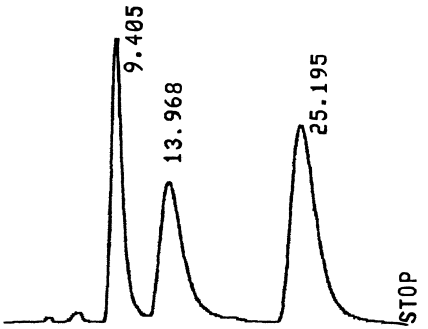

Figure 4. Separation of metyrapol enantiomers and the parent drug, metyrapone, in the same run. Eluent: $CH_3CN/H_2O/Et_3N/HOAc$ (35:65:0.01:0.01). Flow: 0.4 $mL.min^{-1}$. λ=256 nm.

CONCLUSION

Among the commercially available chiral stationary phases the polysaccharide-based columns are some of the most popular ones. The wide variety of chiral compounds that can be efficiently resolved on these columns in analytical and preparative scale makes them a first choice in a method development. This work showed that the applicability of these columns could be widened by the multimodal elution. The performance of the column can be maintained by conditioning it in the appropriate manner while switching the elution mode.

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