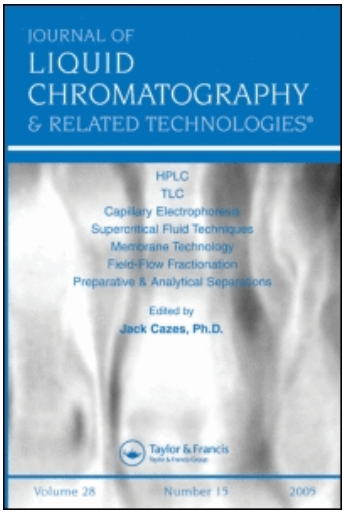

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ENANTIOSEPARATION ON AMYLOSE TRIS(3,5-DIMETHOXYPHENYL CARBAMATE): APPLICATION TO COMMERCIAL PHARMACEUTICAL CHIRAL DRUGS

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ABSTRACT

The enantioselectivity of the amylose tris(3,5-dimethoxyphenyl carbamate) phase towards some β-blockers was investigated. A sensitive HPLC method was developed for the enantiomers of metoprolol and betaxolol. The validated methods were suitable for the analysis of enantiomeric composition in a variety of pharmaceutical formulations of both drugs.

INTRODUCTION

The growing interest of the pharmaceutical industry in the use of single enantiomers has led to an increasing demand for direct methods for analytical separations of enantiomers of pharmaceuticals.

Among the most useful and versatile chiral analytical columns described in recent years are coated carbohydrate carbamate columns, which were originally developed by Okamoto et al.[1]

The amylose tris(3,5-dimethoxyphenyl carbamate) phase which is readily prepared from commercially available materials, has already showed some advantages over the widely used cellulose and amylose tris(3,5-dimethylphenylcarbamate) phases for the resolution of certain chiral sulphoxides.[2]

The enantioselectivity of this phase towards some β-blockers was investigated in this work.

EXPERIMENTAL

General

The columns were prepared as described elsewhere[2] and consisted of amylose tris(3,5-dimethoxyphenyl carbamate) coated onto APS-Nucleosil (7 μm particle size and 500 Å pore size) (20% w/w, 15 X 0.45 cm ID), APS-Hypersil (5 μm particle size and 120 Å pore size) (20% w/w, 15 X 0.45 cm ID) and ODS-Hypersil (5 μm particle size and 120 Å pore size) (15% w/w, 10 X 0.45 cm ID) respectively. The performance of all columns used was first evaluated using Troger's base and *trans*-stilbene oxide. HPLC dead times (t_0) were estimated by using 1,3,5-tri-*tert*-butylbenzene. Solvents were either HPLC grade from Merck or from Carlo Erba or were purified as usual.[3]

The standards propranolol and pindolol were donated by Laboratório de Controle de Qualidade, Faculdade de Farmácia UFMG, Belo Horizonte, Brazil. Metoprolol standard was kindly donated by Biogalência Química Farmacêutica, São Paulo, SP, Brazil and betaxolol standard by Alcon Laboratórios do Brasil, São Paulo, SP, Brazil.

The pharmaceutical formulations used were purchased at a drug store as tablets for metoprolol: Lopressor®, Seloken® (Batches N^0 25758 and PN011, respectively) and for betaxolol as an ophthalmic solution: Betoptic® (Batch No. RF06) and Betoptic S® as an ophthalmic suspension (Batches 4305 and 4535).

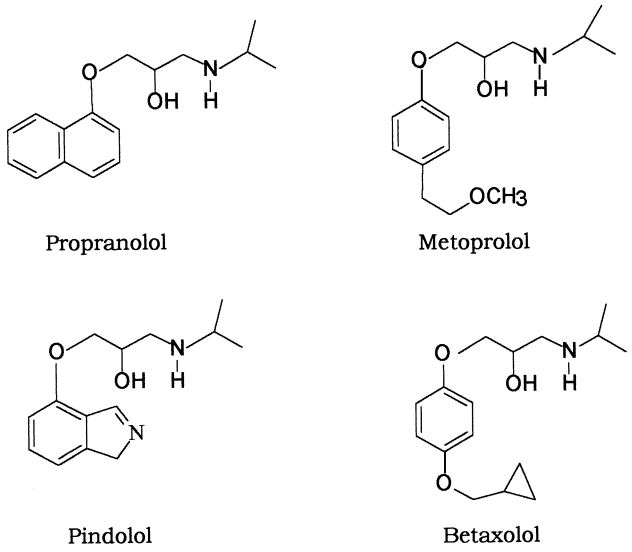

Figure 1. Structures of β-Blockers Analyzed.

Table 1

Investigation of the Influence of the Silica Surface on the Enantioselectivity of the Phase Amylose Tris(3,5-Dimethoxyphenyl Carbamate)

Supports Used	APS-Nucleosil (500 Å, 7μm)			APS-Hypersil (120 Å, 5 μm)			ODS-Hypersil (120, Å, 5 μm)		
Drugs	k'_1	α	Rs	k'_1	α	Rs	k'_1	α	Rs
Propranolol	2.10	1.52	1.80	4.10	1.12	0.66	4.6	1.0	---
Pindolol	33.14	1.34	0.60	43.13	1.15	0.74	39.0	1.0	---
Betaxolol	3.22	1.40	1.54	5.65	1.12	0.70	6.0	1.0	---
Metoprolol	3.99	1.46	1.50	7.29	1.13	0.70	8.0	1.0	

Mobile Phase: Hexane:2-propanol (90:10 v/v).

Preparation of Samples

Standards

The hydrochloride salts of propranolol, pindolol, and betaxolol and the tartarate salt of metoprolol standards (100 mg) were solublized in a saturated solution of sodium hydrogen carbonate (100 mL) and the free bases were

Table 2

Influence of the Mobile Phase on the Enantioselectivity of the Phase Amylose Tris(3,5-Dimethoxyphenyl carbamate) Coated onto APS-Nucleosil

	Betaxolol		Metoprolol	
Mobile Phase	**α**	**Rs**	**α**	**Rs**
Hexane/2-propanol (90:10 v/v)	1.40	1.54	1.46	1.50
	1.50	1.84	1.31	2.03
Hexane/2-propanol/Et_3N (90:10:0.1 v/v)	1.42	2.16	1.49	3.10
Hexane/Ethanol (95:05 v/v)	1.45	1.94	1.41	2.30
Hexane/Ethanol (90:10 v/v)	1.37	3.00	1.41	2.76
Hexane/Ethanol (88:12 v/v)	1.45	2.74	1.46	2.63
Hexane/Ethanol (85:15 v/v)	1.40	2.69	1.41	2.45
Hexane/Ethanol/Et_3N (90:10:0.1 v/v/v)	1.36	2.60	1.40	2.72

extracted with diethyl ether (3x4mL). The organic phase was then washed with water (3x4 mL), dried with sodium sulfate and evaporated *in vacuo* to afford the free bases as a powder, except for the metoprolol which was an oil. They were dissolved in the mobile phase for HPLC analysis.

Finished Pharmaceuticals

A pool of three tablets of Seleken® or Lopressor® (100 mg) was macerated and extracted using the same procedure as for the standards. The ophthalmic solution of Betoptic® (1 mL) was diluted in a saturated solution of sodium hydrogen carbonate (10 mL), extracted with diethyl ether (3 x 3 mL), washed with water, dried with sodium sulfate, and evaporated *in vacuo* and the free base dissolved in the mobile phase.

A saturated solution of sodium hydrogen carbonate (10 mL) was added to the ophthalmic suspension of Betoptic S® which was then extracted with diethyl ether (3 x 3 mL), which was washed with brine (3 x 3 mL), water, dried with sodium sulfate, and evaporated *in vacuo.* The residue was then dissolved in the mobile phase for HPLC analysis.

Equipment

The HPLC system consisted of a Shimadzu LC-10AD pump, a SPD-6AV UV detector operated at 254 nm, and a LC-R6A chromatopac recorder. A Rheodyne 7125 injector fitted with a 20 mL loop was used in both cases. Unless otherwise stated, the flow rate was always 0.5 $mL.min.^{-1}$

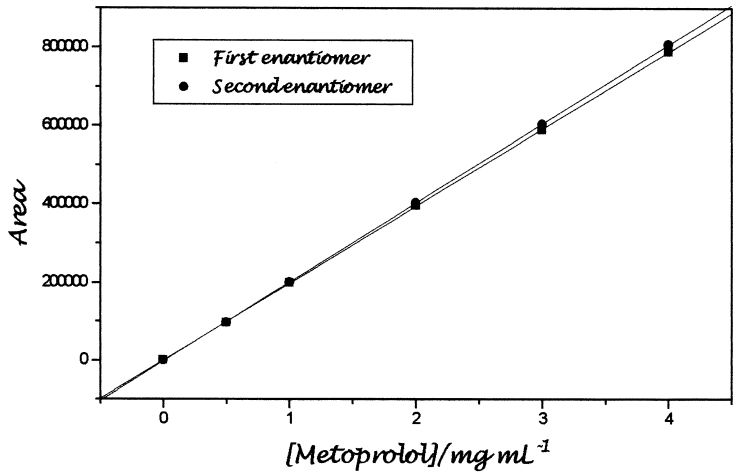

Figure 2. Metoprolol calibration curve.

RESULTS AND DISCUSSION

The enantioselectivity of cellulose tris(3,5-dimethylphenyl carbamate) towards β-adrenergic blockers belonging to the aryloxyamino-2-ol class, which includes propanolol among others, has been extensively evaluated.[4,5] The previous result obtained with the amylose tris (3,5-dimethoxyphenyl carbamate)[2] phase prompted us to further investigate its enantioselectivity and for this purpose the well-studied β-adrenergic blockers drugs propranolol, pindolol, betaxolol, and metoprolol were chosen (Figure 1).

Since it has been shown that different supports can be used efficiently[6] for this class of chiral phase and that the acidity of the support can play an important role in the enantioselectivity of these phases,[7] the influence of the support surface was initially investigated.

When APS-Hypersil was used as support, a marked decreased in resolution was observed when compared with the APS-Nucleosil. However, when ODS-Hypersil was used as the support, no enantioselectivity was observed for the β-blockers examined, although *trans*-stilbene oxide and Troger's base had given a good performance, with capacity ratios α of 1.96 and 1.32 and resolutions R_s of 2.10 and 1.53 respectively.

The best enantioselectivity was found when APS-Nucleosil was used as the support and was, therefore, selected for development of the analytical method for pharmaceutical formulations containing metoprolol and betaxolol (Table 1).

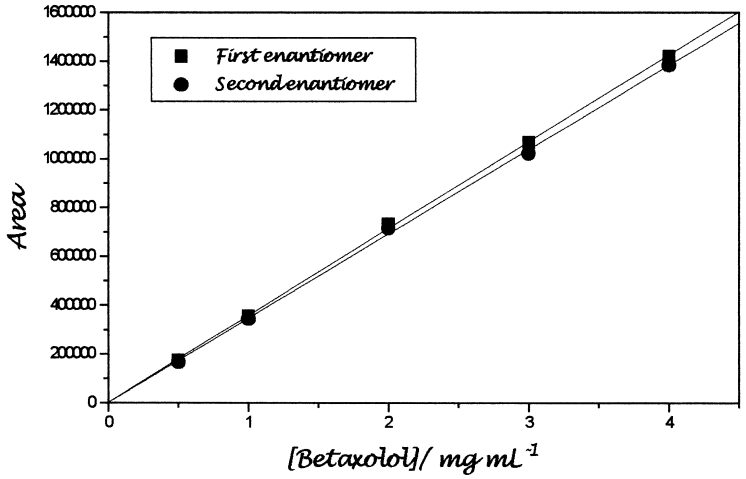

Figure 3. Betaxolol calibration curve.

Table 3

Precision Data for the Determination of the Racemic Drugs Betaxolol and Metoprolol

	Repeatability*		Reproducibility*	
Drugs	**1st Enantiomer**	**2nd Enantiomer**	**1st Enantiomer**	**2nd Enantiomer**
Betaxolol	1.00	1.43	1.30	2.00
Metoprolol	0.51	1.12	1.46	1.40

* Expressed as C.V. %

The influence of the mobile phase and addition of a silanol suppressor, such as triethylamine, was investigated. When ethanol was used as the organic modifier, good resolutions were obtained but better peak shape was obtained with ethanol and triethylamine and, for this reason, the later mobile phase was selected.

Capacity ratios α 1.36 for betaxolol and 1.40 for metoprolol and good resolutions Rs 2.60 and 2.72 respectively, were obtained using hexane:ethanol:triethylamine (90:10:0.1v/v) as the mobile phase. (See Table 2.)

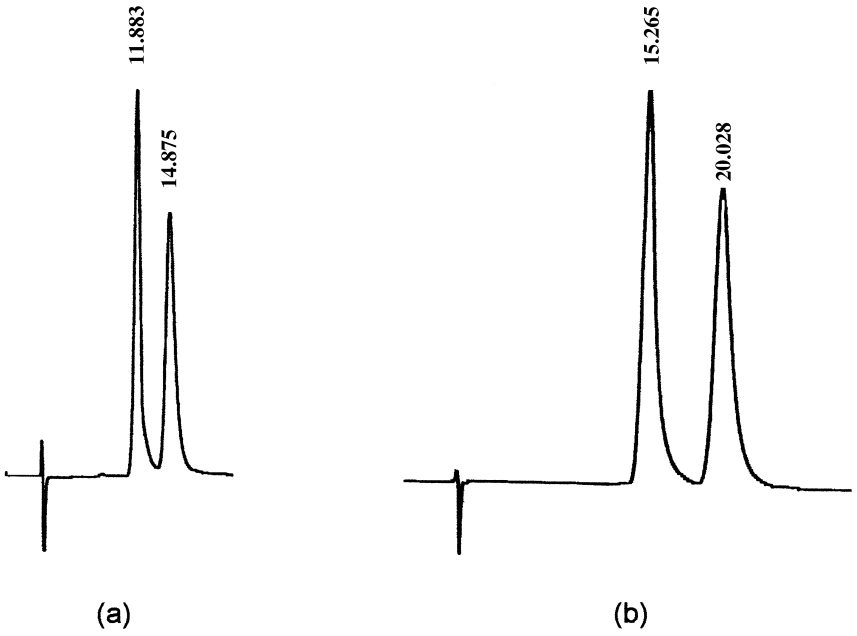

Figure 4. Chromatograms of (a) betaxolol and (b) metoprolol.

The response of the UV detector at 254 nm was linear from 0.5 to 4.0 $mg.mL^{-1}$ for 20 µL injection for the racemic drugs or 0.25 to 2.0 mg. mL^{-1} for each enantiomer and the correlation coefficient was superior to 0.999 for both drugs (Figure 2 and 3).

The reproducibility (expressed as CV n = 15, measured at three different days, n = 5 for each day) and the repeatability (n=10) of the method were excellent for both drugs (Table 3).

The quantification limit was determined as the lowest concentration that could be measured with precision of 3% (CV)[8] for five consecutive injections. The values found were 10 $\mu g.mL^{-1}$ for betaxolol and 25 µg. mL^{-1} for metoprolol.

Typical chromatograms of metoprolol and betaxolol obtained during the validation study are shown in Figure 4. The validated methods were suitable for the quantitative analysis of enantiomeric composition of a variety of pharmaceutical formulations of both drugs.

Table 4

Enantiomeric Composition of Ophthalmic Solution Betoptic® and Suspension Betoptic S® as Analyzed on the Phase Amylose Tris(3,5-Dimethoxyphenyl Carbamate) Coated onto APS-Nucleosil*

Finished Pharmaceutical	Batches	Enantiomeric Composition (%) 1st Enantiomer	2nd Enantiomer	ee
Betopic®	Rf 06	49.40 ± 0.30	49.53 ± 0.30	---
		50.60 ± 0.30	50.63 ± 0.30	
Betoptic S®	4305	49.75 ±0.12	50.25 ± 0.12	---
		49.15 ± 0.93	50.84 ±0.95	
Betoptic S®	4535	50.80 ± 1.11	49.19 ± 1.10	---
		50.93 ± 2.60	49.07 ± 2.70	

* n = 3, confidence level, 95%; mobile phase: hexane: ethanol: triethylamine (90:10:0.1 v/v/v).

Table 5

Enantiomeric Composition of the Tablets Lopressor® and Seloken® as Analyzed on the Phase Amylose Tris (3,5-Dimethoxyphenyl Carbamate Coated onto APS Nucleosil

Finished Pharmaceutical	Enantiomeric Composition (%) 1st Enantiomer	2nd Enantiomer
	52.24 ± 0.08	47.76 ± 0.08
Lopressor®*	51.90 ± 0.71	58.12 ± 0.80
	52.58 ± 0.60	47.42 ± 0.60
Seloken®**	49.12 ± 0.42	50.81 ± 0.44
	49.30 ± 0.07	50.70 ± 0.07

* n = 3 confidence level, 95%; ** n = 4 confidence level, 95%.

The free base forms of betaxolol and metoprolol were prepared in duplicate from the commercial samples and run in triplicate using the external standard calibration method. The area percent ratio was used to determine the enantiomeric composition. All medicines had the claimed enantiomeric composition (Tables 4 and 5), as expected.

CONCLUSION

The enantioselectivity of the amylose tris(3,5-dimethoxyphenyl carbamate) phase was demonstrated for the β-blockers analyzed. The method proved to be suitable for the analysis of finished pharmaceuticals.

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