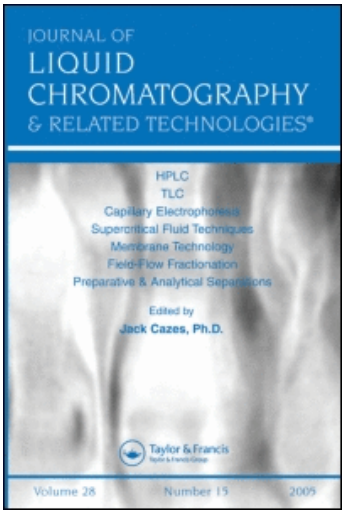

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Aboul-Enein, Hassan Y. and Serignese, Vince(1993) 'Role of Solvents in the Resolution of Some Beta-Adrenergic Blockers on Cellulose 3, 5-Dimethylphenyl Carbamate Chiral Stationary Phase', Journal of Liquid Chromatography & Related Technologies, 16: 1, 197 — 207
To link to this Article: DOI: 10.1080/10826079308020906
URL: http://dx.doi.org/10.1080/10826079308020906

JOURNAL OF LIQUID CHROMATOGRAPHY, 16(1), 197-207 (1993)

ROLE OF SOLVENTS IN THE RESOLUTION OF SOME BETA-ADRENERGIC BLOCKERS ON CELLULOSE 3,5-DIMETHYLPHENYL CARBAMATE CHIRAL STATIONARY PHASE

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ABSTRACT

The stereoselective resolution of racemic mixture of seven beta-adrenergic blockers were achieved on cellulose 3,5-diemethylphenyl carbamate chiral stationary phase (Chiralcel OD). The role of the mobile phase solvents in the resolution of these beta-blockers, namely, tertalolol, oxprenolol, p-oxprenolol, alprenolol, acebutolol, bisoprolol and tolamolol were presented. It was found that it is not essential to add diethylamine as a suppressor in the mobile phase to effect the separation of the racemate to their corresponding enantiomers. However, addition of diethylamine improved the symmetry and sharpness of the peaks. The chiral recognition mechanism(s) of this phase is discussed.

INTRODUCTION

Beta-adrenergic blockers have wide clinical applications. They are used in the treatment of hypertension, angina pectoris, cardiac arrhythmias, glaucoma, and the prevention of migraine attacks (1).

It is well-known that beta-adrenergic blockers are chiral and their enantiomers have different potencies. The beta-blocking activity of these drugs is found only in the levorotatory enantiomers (2). Typically, the (-)-S-enantiomers are 50-500 fold more active than the (+)-R-

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enantiomers (3). Their elicited responses , metabolic pathways, disposition, and pharmacokinetics may also differ (4,5).

However, most of the clinically used beta-blockers are marketed and used as a racemic mixture. Due to the current awareness of chirality and the differing pharmacological effects of the enantiomers, there is great interest in separating the enantiomers and evaluating them for possible therapeutic activity.

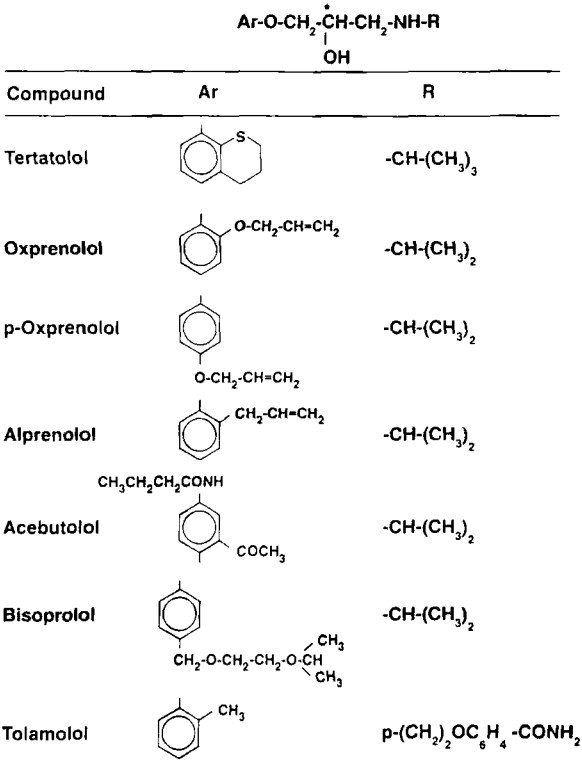
Recently, the enantiomers of several beta-blockers were successfully separated using cellulose tris (3,5-dimethylphenyl carbamate) known as Chiralcel OD as the chiral stationary phase (CSP) (6,7,8,9,10).

In this work, we describe the separation of seven beta-blockers (tertatolol, oxprenolol, p-oxprenolol, alprenolol, acebutolol, bisoprolol and tolamolol). The structures of these drugs are shown in Table 1. All these beta-blockers studied belong to the N-substituted-3-aryloxy-2-hydroxypropylamine type, in an attempt to study the role of the mobile phase solvents in optimizing the resolution of these agents. Also, the effect of diethylamine as a suppressor on the cellulose tris(3,5-dimethylphenyl carbamate) CSP, and the chiral recognition ability of this cellulose phase is discussed.

EXPERIMENTAL

Chemicals. Racemic tertatolol (Ref. No. 30657), (+)-R-and (-)-S-tertatolol (Lot No. DF902 and Lot No. DF971, respectively) were kindly supplied by Les Laboratoires Servier, Gidy, France as hydrochloride salts, racemic oxprenolol hydrochloride (Batch No. 47920A) were kindly supplied by Ciba-Geigy, Basle, Switzerland. Racemic acebutolol (M&B2637), (+)-R-acebutolol (M&B26373) and (-)-S-acebutolol (M&B26720) were kindly supplied by Rhone-Poulenc, Rorer, Dagenheim, Essex, United Kingdom as hydrochloride salts. Racemic bisoprolol (EMD33512), (+)-R-bisoprolol (EMD38341) and (-)-S-bisoprolol (EMD38342) were kindly supplied as hemifumarate salts by E. Merck, Darmstadt, Germany. Racemic alprenolol hydrochloride (Batch No. 769) was kindly supplied by Hassle, Mondal, Sweden. Racemic tolamolol (Lot No. R17-R103) was kindly supplied by Pfizer, Groton, CT, U.S.A.

TABLE 1 - Chemical Structures of β -Adrenergic Blockers Studied



** Asterisk Denotes Chiral Carbon*

HPLC grade hexane and 2-propanol were obtained from Fisher Scientific, Fairlawn, N.J., U.S.A. Absolute ethanol was purchased from E. Merck, Darmstadt, Germany and diethylamine was obtained from BDH, Chemicals, Poole, England.

Apparatus

The HPLC analysis was performed at room temperature with a Bio-Rad 1350 solvent delivery pump, a Rheodyne model 7125 injector, a Waters Lambda Max 481 variable wavelength detector

Table 2 - Chromatographic Conditions, Capacity (k), Separation (α) and Resolution Factors for the Beta-Adrenergic Blockers Studied

Compound	Mobile Phase	FlowRate (ml./min.)	Wavelength (nm.)	k_1	k_2	α	R_s
Tertatolol-HCl	A	0.8	254	0.377,(+)-R	2.23,(-)-S	5.92	4.64
Oxprenolol-HCl	B	0.4	273	0.455	1.46	3.20	4.09
p-Oxprenolol-HCl	B	0.4	273	0.285	0.82	2.89	2.50
Alprenolol-HCl	B	0.4	273	0.204	0.47	2.31	1.55
Tolamolol	C	0.4	254	0.498	0.88	1.76	1.66
Acebutolol-HCl	D	0.8	265	2.93,(-)-S	3.34,(+)-R	1.14	0.86
Bisoprolol hemifumarate	E	0.38	273	0.25,(+)-R	0.45,(-)-S	1.80	1.00

Mobile phase A = Hexane:2-propanol:diethylamine (50:50: 0.4)
B = Hexane:2-propanol:diethylamine (20:80: 0.4)
C = Hexane:ethanol: diethylamine (20:80: 0.4)
D = Hexane:2-propanol: diethylamine (15:85: 0.4)
E = Hexane:ethanol: diethylamine (90:10: 0.4)

k_1 = capacity factor of first eluted enantiomer
k_2 = capacity factor of second eluted enantiomer
α = stereo chemical separation factor
R_s = stereochemical resolution factor

set at the appropriate wavelength (see Table 2), and a Hewlett-Packard 3394 A integrator. The cellulose tris (3,5-dimethylphenyl carbamate) Chiralcel OD column (25 cm x 4.6mm I.D. coated on silica gel of 10μm particle size) was obtained from Daicel Chemical Industries, Tokyo, Japan.

Chromatographic Characteristics

The separation factor (α), which represents a measure of relative peak separation is expressed as follows:

α = $'k_2/'k_1$ where $'k_1$ and $'k_2$ are the capacity factor for the first and second eluting enantiomer, respectively.

The capacity factor ('k) is calculated as follows: $'k_1 = (t_{R1}-t_{R0})$ and $'k_2 = (t_{R2}-t_{R0})$ where t_{R0},t_{R1} and t_{R2} refer to the retention time in seconds for the solvent peak and first and second eluting enantiomers, respectively.

The stereochemical resolution factor (R_S) is calculated by the following formula:

$Rs = 2(t_{R2}-t_{R1})/(W_1+W_2)$

where W_1 and W_2 are the peak width for the first and second eluting enantiomer peaks, respectively.

RESULTS AND DISCUSSION

Figure 1 (A through G) shows the separation of seven beta-blockers on cellulose tris (3,5-dimethylphenyl carbamate) chiral stationary phase. In the first instance, a mobile phase composition recommended by the manufacturer was selected which consisted of hexane: 2-propanol or ethanol:diethylamine (90:10:0.4). With this mobile phase, only acebutolol could be separated with baseline separation while the other beta-blockers are, at most, partially separated. An optimization of the separation was carried out by increasing the alcohol content which improved the separation of the racemic mixture to its corresponding enantiomers, as was the case for tertatolol, oxprenolol, p-oxprenolol, alprenolol, tolamolol, and bisoprolol. The optimum chromatographic conditions are shown in Table 2.

These separations could be achieved with the same mobile phase described in Table 2 but without the addition of diethylamine. Yet, diethylamine improves the peak sharpness and symmetry and to some extent the stereochemical resolution factor (R_S). This can be explained by the fact that there are residual silanol groups which can interact with the solutes on these cellulose-based CSP's since the silica particles are not completely covered during the coating with the stationary phase. All beta-blockers contain a basic secondary amino group which can interact with free silanol groups and cause peak broadening. Thus, addition of 0.1-0.4% diethylamine as a silanol suppressor and competitor with the secondary amino group of the beta-blockers leads to sharper, symmetrical peaks, and also slightly decreases the retention

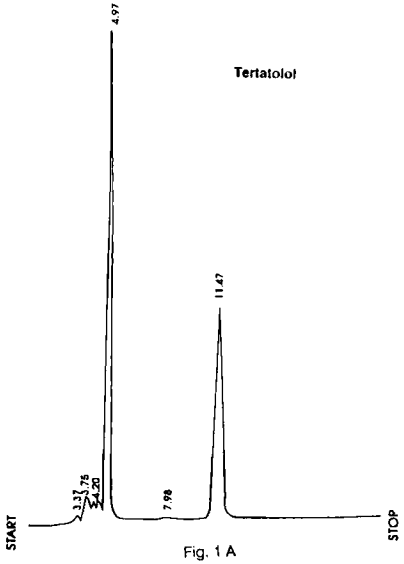


Fig. 1 A

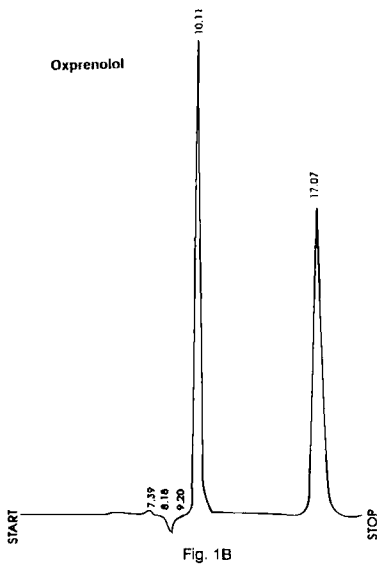


Fig. 1B

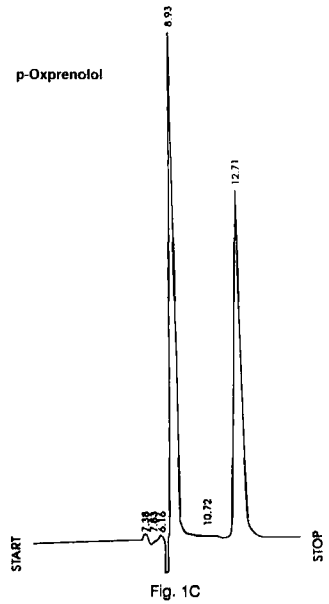


Fig. 1C

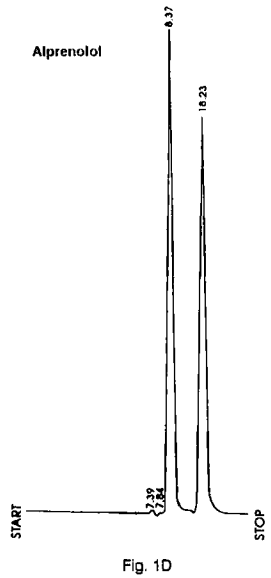


Fig. 1D

Fig. 1. Chromatograms of Enantioselective Separation of Beta-Adrenergic Blockers Studied.

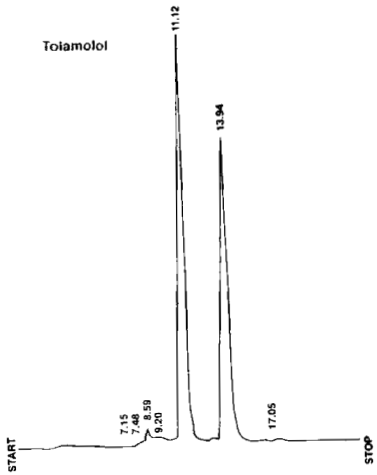


Fig. 1E

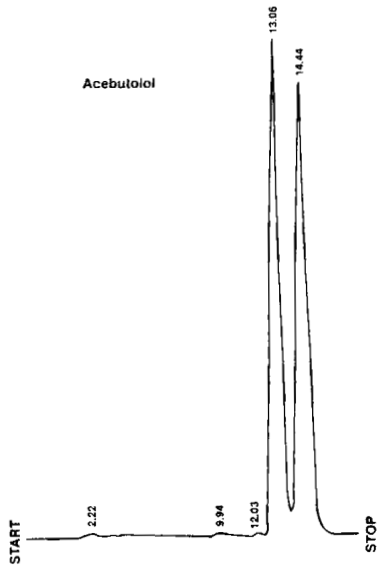


Fig. 1F

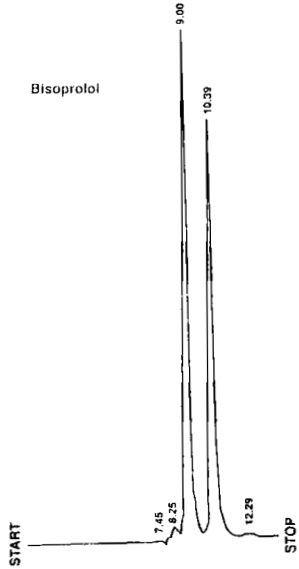


Fig. 1G

FIG. 1 (continued)

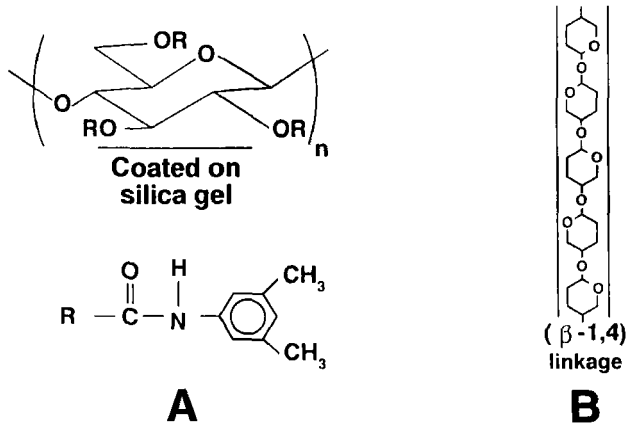


Fig. 2 A. The structure of chiral stationary phase Chiralcel OD.
B. The tertiary structure of the cellulose-based CSP.

times. It has also been noticed that when the mobile phase contains a high percentage of ethanol or 2-propanol the (+)-R enantiomer elutes before the (-)-S-enantiomer. This was verified by chromatographing the individual enantiomers of the beta-blocker, if available, as in the case of tertatolol and bisoprolol under the same chromatographic conditions. For oxprenolol, p-oxprenolol, alprenolol and tolamolol, the optical sign for the eluting enantiomer was determined by using a Jasco Chiralyzer polarimetric detector (Jasco-Gross-Zimmem, Germany) under the same chromatographic conditions described in Table 2. However, the order of stereoselectivity is reversed when the alcohol content is low, e.g. in the case of acebutolol, the mobile phase contained 10% ethanol and the (-)S-enantiomer eluted first.

Various reports have been made to elucidate the mechanism of chiral recognition of these cellulose-based CSP's (11). Cellulose tris (3,5-dimethylphenyl carbamate, Figure 2A) is a synthetic polymer which exists as a β-polymeric chain of derivatized D-(+)-glucose residues in β-1,4-linkage (Figure 2B). These chains lie side by side and possess a certain degree of rigidity and assume extended helical structure into which the enantiomers interact stereospecifically and cause the stereochemical recognition (13).

Francotte, et al (14) postulated that these chiral cavities of the stationary phase have a high affinity for aromatic groups and consequently, for aromatic compounds. For separation to be

achieved, these aromatic groups should fit into the cavity while at least one group on the chiral center should interact with some of the groups on the substituted derivatized cellulose, in this case, the 3,5-dimethylphenyl carbamate group which is located outside the cavity.

Okamoto, et al (6) reported that the hydrogen bonding between the hydroxyl group of the beta-blockers and the carbonyl group of the carbamate function of the CSP seems to play an important role in the chiral recognition which leads to effective stereoselective separation. From the k and αvalues (Table 2), the following qualitative observations could be drawn:

1. The presence of the heterocyclic moiety in the case of tertatolol caused a marked increase in the separation factor and consequently, the enantiomeric separation, contrary to other beta-blockers studied which have a benzene ring system.

2. The presence of a ketonic group CH_3CO in acebutolol increases the interaction with the CSP through hydrogen bonding of the ketonic group with the NH- of the carbamate function. Furthermore, this ketonic function may cause steric hindrance for the aromatic phenyl group to fit the cavity of the CSP. For those reasons, the separation value of acebutolol is relatively low as compared to other beta-blockers studied.

3. The presence of a non-polar group such as an allyl group in the case of alprenolol assists in the separation of the enantiomers through π-π bonding interaction. However, the separation factor is increased and improved where there is an ether linkage in this side chain as is the case for oxprenolol and p-oxprenolol.

4. The separation factor for ortho substituted oxprenolol is larger than its corresponding para substituted isomer p-oxprenolol. This could be rationalized by steric hindrance caused by the allyloxy group in the ortho position as the aromatic group fits the cavity of the CSP.

5. The presence of the amido function in acebutolol and tolamolol increases the retention time of both enantiomers regardless of the alcohol content of the mobile phase. However, it cannot be concluded if this affects the separation factor, although acebutolol and tolamolol have one of the lowest separation factors among the beta-blockers studied.

CONCLUSION

The enantioselectivity of Chiralcel OD-CSP was excellent for most enantiomeric separations achieved in this study. The column showed good separation efficiency and produced symmetrical peaks. Although the CSP, namely, cellulose tris (3,5-dimethyl phenyl carbamate), is coated on the silica gel particles, the performance of this column was not reduced markedly over a long period of time. Chiralcel OD proved to be a useful phase for enantiomeric separation of beta-blockers which could be applied for analytical assays of these agents in biological fluids or enantiomeric purity determination. This phase can also be used for preparative scale chromatography. The addition of diethylamine to the mobile phase improved peak resolution but it did not play a major role in separating the enantiomers of the beta-blockers studied in this paper.

Currently, we are resolving different classes of chiral drugs to further study the mechanism of chiral recognition of this phase.

ACKNOWLEDGMENTS

The authors wish to thank the Administration of King Faisal Specialist Hospital and Research Centre for their continuous support to the Drug Development research program.

The authors also thank Dr. V. Teetz of Hoechst AG, Frankfurt, Germany, for his technical assistance in assigning the optical sign of the eluting enantiomers.

Thanks are also extended to the pharmaceutical companies which provided us with the drug samples in this study and to Ms. Tess Miranda for typing the manuscript.

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Received: July 25, 1992
Accepted: August 11, 1992