BIOMEDICAL CHROMATOGRAPHY
Biomed. Chromatogr. **17**: 113–117 (2003)
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/bmc.220

ORIGINAL RESEARCH

Enantioseparation of some clinically used drugs by HPLC using cellulose Tris (3,5-dichlorophenylcarbamate) chiral stationary phase

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Received 8 July 2002; accepted 9 August 2002

ABSTRACT: The chiral resolution of some clinically used drugs namely metoprolol, teratolol, tolamolol, nebivolol (β-adrenergic blockers), econazole, miconazole (anti-fungal agents), cromakalim (anti-hypertensive agent) and etodolac (anti-inflammatory agent) was achieved on cellulose tris (3,5-dichlorophenylcarbamate) chiral stationary phase. The mobile phase used was 2-propanol at 0.5 mL/min with detection at 220 nm. The separation factors (α) of these drugs ranged from 1.24 to 3.90 while the resolution factors were from 1.05 to 5.0. The chiral recognition mechanisms between the racemates and the chiral selector are discussed. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: econazole; etodolac; cromakalim; metoprolol; nebivolol; miconazole; teratolol; tolamolol

INTRODUCTION

The different pharmacological activities of the enantiomers have created an interest in studying the pharmacological and toxicological properties of the enantiomers of drugs and agrochemicals (Stevenson and Wilson, 1988; Zief and Crane, 1988; Krstulovic, 1989a, 1989b; Allenmark, 1991; Subramanian, 1994; Aboul-Enein and Wainer, 1997). Only about 20–25% of the optically active pharmaceuticals are sold and administrated as pure enantiomers. The US Food and Drug Administration has issued certain guidelines to pharmaceutical and agrochemical industries to specify the enantiomeric purity of the optically active compounds (FDA Policy, 1992). Therefore, the enantiomeric resolution of optically active compounds became an urgent need of pharmaceutical, agrochemical and other chemical-based industries. Accordingly, there is an increasing demand for the direct methods of chiral resolution of enantiomers of the optically active compounds. HPLC has been used in the last 15 years as the method of choice for chiral resolution (Zief and Crane, 1988; Krstulovic, 1989a, 1989b; Allenmark, 1991; Subramanian, 1994; Aboul-Enein and Wainer, 1997). The development of the chiral stationary phases (CSPs) in HPLC has proved to be an effective modality in the resolution of racemic compounds. Various chiral columns have been used for the enantio-

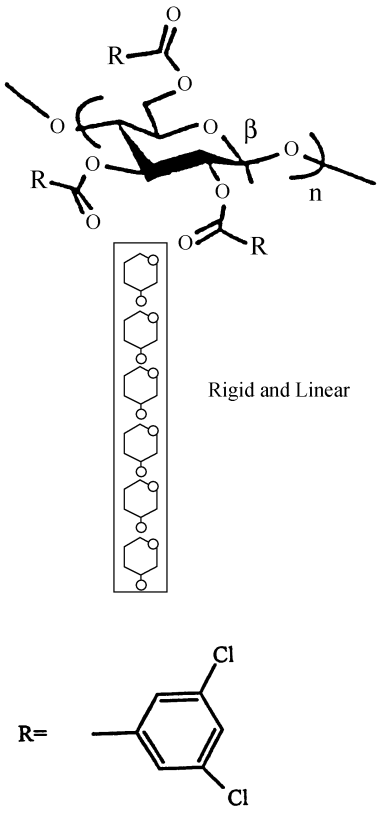

Figure 1. Chemical structure of cellulose Tris (3,5-dichlorolphenylcarbamate) CSP.

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Abbreviations used: CSP, chiral stationary phase; R_S, resolution factors.

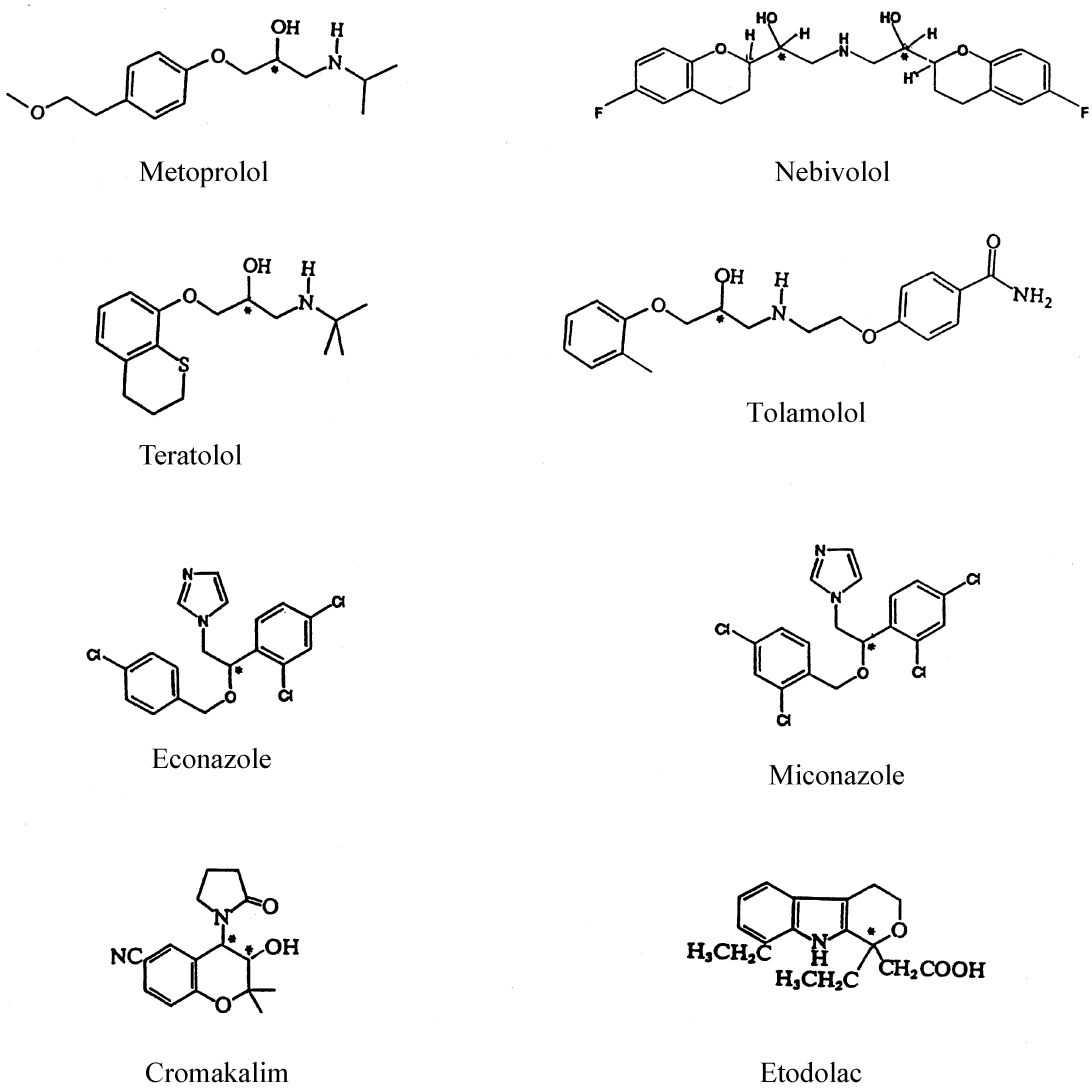

Figure 2. Chemical structures of some of the clinically used drugs resolved in this study. The asterisk denotes the position of chiral carbon.

meric resolution of a wide variety of racemates (Krstulovic, 1989a, 1989b; Allenmark, 1991; Subramanian, 1994; Aboul-Enein and Wainer, 1997). Among these, polysaccharide-based derivatives are currently the most useful chiral stationary phases in HPLC enantioseparation because of their wide range of applications (Shibata *et al.*, 1989; Subramanian, 1994; Aboul-Enein and Wainer, 1997; Beesley and Scott, 1998; Okamoto and Yashima, 1997). Recently, a new polysaccharide CSP namely cellulose Tris (3,5-dichlorophenylcarbamate) (Fig. 1) was developed and coated on silica surface (Chankvetadze *et al.*, 2000). The authors have also advocated the good chiral resolution capacity of this CSP. Furthermore, they explained the good chiral recognition ability because of its intact rigid linear and helical structure as it is in the coated form on silica gel. In view of this, we have tried the chiral resolution of some clinically used drugs (Fig. 2), namely metoprolol, teratolol, tolamolol, nebivolol (β-adrenergic blockers), econazole, miconazole (anti-fungal agents), cromakalim (anti-hypertensive agent) and etodolac (anti-inflammatory agent) on this CSP. The results of this study are presented herein.

EXPERIMENTAL

Chemicals and reagents. The racemic mixture of teratolol was supplied by Les Laboratories Servier, Gidy, France while tolamolol was obtained from Pfizer, Groton, CT, USA. The racemic [(+)-*RRRS* and (−)-*SSSR*] nebivolol (product no. R67555) was kindly supplied as gifts by Janssen Research Foundation, Beerse, Belgium. Racemic mixture [(+)-3*R*,4*R* and (−)-3*S*,4*S*] of cromakalim was obtained from SmithKline

Table 1. The chromatographic parameters, retention factor (k), separation factor (α) and resolution factor (Rs) for enantiomeric resolution of some some clinically used drugs on cellulose Tris (3,5-dichlorophenylcarbamte) CSP using 2-propranol as mobile phase at 0.5 mL/min

	k_1	k_2	α	Rs
Metoprolol	0.33	0.59	1.78	1.10
Nebivolol	0.46	0.79	1.72	1.40
Teratolol	0.60	1.46	2.43	1.80
Tolamolol	1.38	1.76	1.28	1.05
Econazole	2.78	3.46	1.25	1.72
Miconazole	4.05	5.00	1.24	2.40
Cromakalim	0.83	2.05	2.47	5.00
Etodolac	0.11	0.43	3.90	1.30

For details see Experimental section.

Beecham (Frythe, Welwyn, UK). Econazole, miconazole, metoprolol and metoprolol were purchased from Sigma Chemical Co. (St Louis, MO, USA). Etodolac was supplied by Wyeth-Ayerst, Maidenhead, Berks, UK. The solutions of the individual drugs (0.1 mg/mL) were prepared in ethanol. 2-Propanol of HPLC grade was purchased from Fisher Scientific (Fairlawn, NJ, USA). The absolute ethanol was obtained from E. Merck (Darmstadt, Germany).

Chromatographic conditions. Aliquots of 20 µL of each of the solutions were injected onto an HPLC system consisting of Waters solvent delivery pump (model 510), Waters injector (model WISP 710B), Waters tunable absorbance detector (model 484) and Waters integrator (model 740). The column used was cellulose Tris (3,5-dichlorolphenylcarbamate) (25 cm × 0.46 cm; Fig. 1) coated on Daisogel SP-2000 (particle size 10 µm) and was kindly donated by Professor B. Chankvetadze. The mobile phase used in this study was 2-propanol, which was filtered and degassed before use. The flow rate of the mobile phase was 0.50 mL/min. The chart speed was kept constant at 0.1 cm/min. All the experiments were carried out at 23 ± 1°C. The detection was carried out at 220 nm. The chromatographic parameters such as retention factor, separation factor and resolution factor were calculated.

RESULTS AND DISCUSSION

The chromatographic parameters, retention factor (k), separation factor (α) and resolution factor (Rs) for the resolved enantiomers of the reported drugs are given in Table 1. It may be observed from Table 1 that the best resolution was achieved for cromakalim. A typical chromatogram of cromakalim enantiomers on this new CSP is presented in Fig. 3. A variation in the chromatographic parameters was carried out to obtain the best resolution. To optimize the chromatographic conditions, methanol and ethanol were tried as the mobile phases. The mixtures of alcohols and alcohol–water were also tested but no good resolution could be achieved.

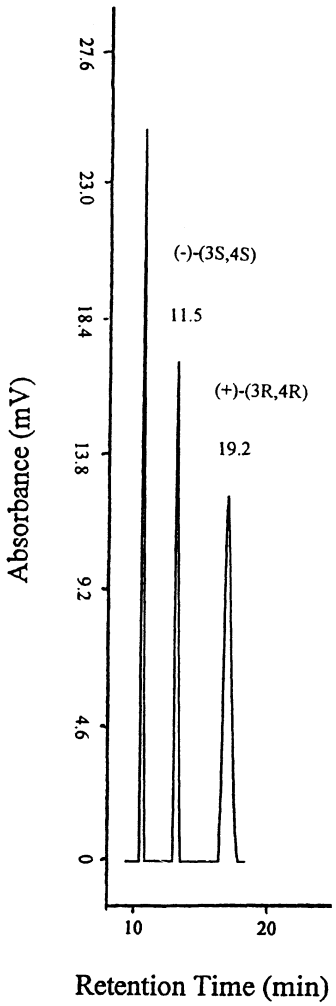

Figure 3. Chromatogram showing the enantiomeric resolution of cromakalim on cellulose Tris (3,5-dichlorophenylcarbamate) column using 2-propanol as the mobile phase at 0.5 mL/min flow rate.

After experimentation the best chromatographic conditions were developed and are reported herein.

The α values of these drugs ranged from 1.24 to 3.90 while the Rs values were from 1.05 to 5.0. The resolution of these drugs was in the order cromakalim > miconazole > teratolol > econazole > nebivolol > etodolac > metoprolol > tolamolol. This sort of resolution may be explained on the basis of the different magnitudes of the different types of bonds between racemates of these drugs and the CSP. The chiral recognition mechanism at a molecular level on the cellulose-based CSPs is still unclear, although it has been reported that the chiral resolution by these CSPs is achieved through hydrogen bonding, π–π and dipole–dipole induced interactions between the chiral stationary phase and the enantiomers of the analytes (Wainer and Alembic, 1986; Wainer *et al.*, 1987; Yamamoto *et al.*, 1999). The cellulose-based chiral stationary phases are the semi synthetic polymers, which

contain the polymeric chains of derivatized D-(+) glucose residues in β-1,4 linkage and these chains lie side by side in a linear fashion. The structure of the reported drugs (Fig. 2) contains several electronegative atoms, namely nitrogen, oxygen, sulfur and chlorine along with aromatic rings. Therefore, resolution of the enantiomers of these drugs occurred due to the different hydrogen bonding and dipole–dipole induced interactions of different magnitudes between the electronegative atoms of the racemates and the reported cellulose chiral stationary phase (Fig. 1). It has also been reported (Wainer and Alembic, 1986; Wainer *et al.*, 1987) that the π–π interactions between the substituted phenyl moieties of cellulose-based chiral stationary phase and the aromatic rings of the analytes play an important role in the chiral resolution mechanisms. The steric effect has also been observed to play a crucial role in chiral resolution (Aboul-Enein and Ali, 2001). Finally, the enantiomers of these drugs fit stereogenically in a different fashion into the chiral grooves of the reported stationary phase, which is stabilized by the different types of bonds mentioned above which result in the resolution of enantiomers.

Basically, the chiral resolution of the racemates is controlled by the overall effect of bonds (as discussed above), steric effect and the pattern of fitting of the enantiomers in the chiral grooves. However, we have tried to explain the chiral resolution behavior of these drugs by considering the structures of the reported drugs and the possible bonds involved. The best resolution of cromakalim may be due to its small molecular size which experiences less steric force and hence has the maximum fitting in the chiral groove of the CSP. The better resolution of miconazole in comparison to econazole may be attributed to the stronger hydrogen bond in miconazole. The stronger hydrogen bonding in miconazole may be due to one additional chlorine atom in miconazole. The retention times of the β-blockers were in the order tolamolol > teratolol > nebivolol > metoprolol. This order of retention times could be due to the increase of hydrogen and π–π bondings in the same order as the number of the electronegative atoms and phenyl rings increased in the same order except in nebivolol where the steric effect may be dominant.

Furthermore, to ascertain the mechanisms of the chiral resolution, attempts have been made to resolve these drugs on the cellulose Tris (3,5-dimethylphenylcarbamate) (Chiralcel OD), cellulose Tris (4-methylphenylcarbamate) (Chiralcel OG), cellulose triphenylcarbamate (Chiralcel OC) and cellulose 4-chlorophenylcarbamate (Chiralcel OF) CSPs using the same experimental chromatographic conditions as described in the experimental section. No resolution or partial resolution was observed on these CSPs. Of course the above-mentioned CSPs contain various sites for hydrogen and π–π bondings but they showed no or poor chiral capability in comparison to the cellulose Tris (3,5-dichlorolphenylcarbamate) CSP. This could be due to their poor bonding capacities in comparison to the cellulose Tris (3,5-dichlorophenylcarbamate) column. Cellulose Tris (3,5-dichlorophenylcarbamate) CSP contains six chlorine atoms per unit of cellulose, and hence provide stronger hydrogen bonding. Therefore, it may be concluded that the hydrogen bonding is the major contributor for the chiral resolution on this polysaccharide-based CSP.

CONCLUSION

This study indicates the good chiral resolution capacity of cellulose Tris (3,5-dichlorophenylcarbamate) CSP for several chemically used drugs. The baseline chiral resolution of metoprolol, teratolol, tolamolol, nebivolol (β-adrenergic blockers), econazole, miconazole (antifungal agents), cromakalim (anti-hypertensive agent) and etodolac (anti-inflammatory agent) has been achieved on the reported CSP. Taking into consideration the results obtained, one can conclude that the enantiomeric resolution of these drugs on this chiral stationary phase is governed by hydrogen bondings. Besides, π–π, dipole–dipole induced interactions and steric effect also contribute towards chiral resolution. However, it is essential to mention here that the reported CSP has certain limitations as a mixture of hexane and alcohol (supposed to be suitable mobile phases in polysaccharides CSPs) cannot be used due to the solubility of this CSP in these mixtures. Therefore, to increase the application of the reported CSP some modifications are still required. Finally, the developed stereoselective HPLC method can be used for the resolution of the reported drugs on a semi preparative scale for further pharmacological investigations of the individual enantiomers.

Acknowledgments

The authors (I.A. and H.Y.A-E.) would like to thank the King Faisal Specialist Hospital and Research Center, Riyadh administration for their support for the Pharmaceutical Analysis Laboratory Research Program. The authors are also thankful to Professor B. Chankvetadze, Institute of Pharmaceutical Chemistry, Münster, Germany for providing the chiral column.

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