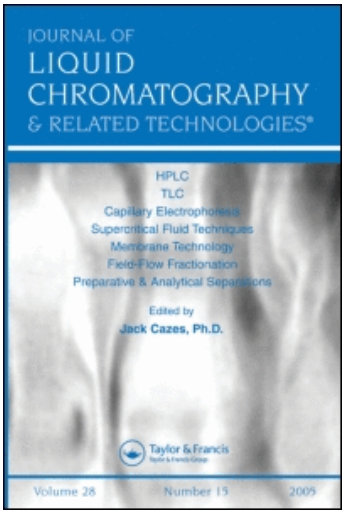

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CHIRAL RESOLUTION OF CROMAKALIM BY HPLC ON TEICOPLANIN AND TEICOPLANIN AGLYCON CHIRAL STATIONARY PHASES

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ABSTRACT

The chiral resolution of cromakalim has been achieved on macrocyclic glycopeptide antibiotics columns, namely teicoplanin and teicoplanin aglycon known as Chirobiotic T and Chirobiotic TAG, respectively. The mobile phases used were hexane–ethanol (95 : 5, v/v) on Chirobiotic T and hexane–ethanol (75 : 25, v/v) on Chirobiotic TAG columns, respectively, at a flow rate of 0.5 mL/min. The detection was carried out at 220 nm. The α values of the resolved enantiomers were 1.13 and 1.12 on Chirobiotic T and Chirobiotic TAG columns, respectively. The values of *Rs* were 1.27 and 1.25 on Chirobiotic T and Chirobiotic TAG columns, respectively. The detection of the enantiomers was better on Chirobiotic T column than on Chirobiotic TAG column.

Key Words: Cromakalim; Stereoselective chromatography; Teicoplanin; Teicoplanin aglycon; Macrocyclic glycopeptide antibiotics

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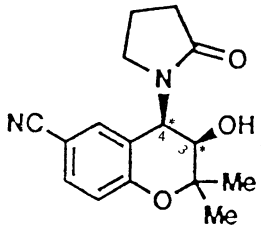
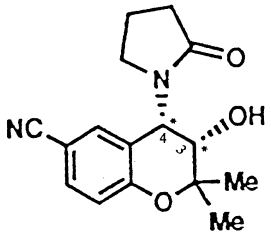
INTRODUCTION

Cromakalim is a potent vasodilator and used as a anti-hypertensive drug. This drug is also known as potassium channel activator, functioning by hyperpolarization of vascular smooth muscle membranes and opening of potassium channel.[1,2] This drug contains two chiral centres (Fig. 1) and, hence, develops into four enantiomers. It is well known that the enantiomers may differ in their pharmacological actions.[3–5] Moreover, one of them may be toxic or inactive. Therefore, the determination of enantiomeric purity is of high importance in pharmaceutical and pharmacological activities. The US Food and Drug Administration has also issued certain guidelines for marketing the drugs containing chiral centres.[6] Various workers have reported the differences in the biological activities of cromakalim drugs.[7–10] The glycopeptide antibiotics chiral stationary phases (CSPs) have a great potential for the resolution of a variety of racemates.[11,12] Therefore, attempts have been made to resolve cromakalim enantiomers namely (−)-(3S, 4S) and (+)-(3R, 4R) enantiomers on glycopeptide antibiotics CSPs. The results of these findings are presented herein.



(+)-(3R,4R)-Enantiomer



(-)-(3S,4S)-Enantiomer

Figure 1. The chemical structure of cromakalim drug.

EXPERIMENTAL

Chemicals and Reagents

The racemic mixtures and the optically pure enantiomers (−)-(3S, 4S) and (+)-(3R, 4R) of cromakalim were obtained from SmithKline Beecham Frythe, Welwyn, UK. Solutions of cromakalim (0.025 mg/mL) were prepared in ethanol. Hexane of HPLC grade was purchased from Fisher Scientific (Fairlawn, New Jersey, USA). Ethanol was obtained from Merck, Darmstadt, Germany.

Chromatographic Conditions

Five micro liter of each of the solutions were injected on to a 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 columns used were Chirobiotic T (25 cm × 0.20 cm id, particle size 5 μm) and Chirobiotic TAG (15 cm × 0.46 cm id, particle size 5 μm) and obtained from Advanced Separation Technologies Inc., Whippaney, USA. The structures of these CSPs are shown in Fig. 2. The mobile phases used were hexane–ethanol (95 : 5, v/v) on Chirobiotic T and hexane–ethanol (75 : 25, v/v) on Chirobiotic TAG columns separately and, respectively. The mobile phases were filtered and degassed before use. The flow rate of the mobile phases was 0.5 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 (k), separation factor (α), and resolution factor (Rs) were calculated.

RESULTS AND DISCUSSION

The retention (k), separation (α) and resolution (Rs) factors for the resolved enantiomers of cromakalim drugs are given in Table 1. The values of α of the resolved enantiomers were 1.13 and 1.12 while the values of Rs were 1.27 and 1.25, respectively on Chirobiotic T and Chirobiotic TAG columns. It is interesting to note that no resolution for cromakalim enantiomers was achieved on Chirobiotic V and Chirobiotic R columns. These values indicate successful resolution of the enantiomers of cromakalim on both the Chirobiotic T and Chirobiotic TAG columns. These results indicate that the resolution of cromakalim enantiomers on Chirobiotic T columns is better than that obtained on Chirobiotic TAG columns. Typical chromatograms of the

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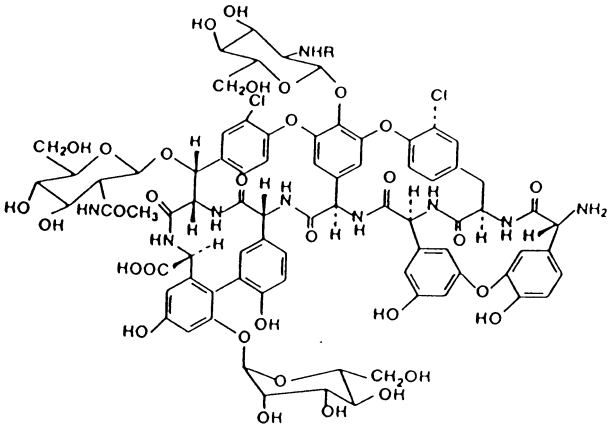
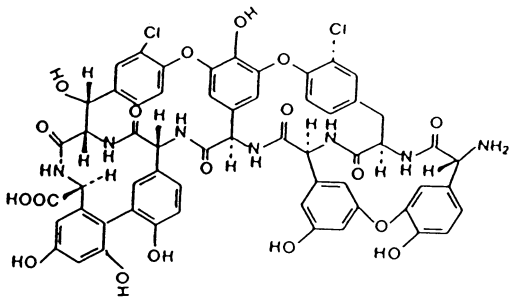


Teicoplanin



Teicoplanin Aglycon

Figure 2. The chemical structure of teicoplanin and teicoplanin aglycon CSPs.

Table 1. The Retention (k), Separation (α), and Resolution (Rs) Factors for the Enantiomeric Resolution of Cromakalim on Chirobiotic T and Chirobiotic TAG Columns

k_1 (−)-(3S, 4S)	k_2 (+)-(3R, 4R)	α	Rs
Chirobiotic T with hexane–ethanol (95 : 5, v/v)			
15.67	17.71	1.13	1.27
Chirobiotic TAG with hexane–ethanol (75 : 25, v/v)			
7.10	7.96	1.12	1.25

For details see experimental section.

resolved enantiomers of cromakalim on Chirobiotic T are shown in Fig. 3. The order of the elution was confirmed by running the chromatograms of the optically pure enantiomers under the identical chromatographic conditions. It has been observed that the (−)-(3S, 4S) enantiomer eluted first, followed by the (+)-(3R, 4R) enantiomer on both chiral columns. A variation in the chromatographic parameters was carried out to obtain the best resolution. To optimize the chromatographic conditions, various ratios of alcohols, hexane, diethylamine were tested, but no good resolution could be achieved. Besides, attempts were also made to resolve the enantiomers of cromakalim under the reversed phase mode by using different ratios of water and acetonitrile, but again, no resolution could be achieved. As a result of extensive experiments, the optimized chromatographic conditions were developed and reported, herein.

Recently, we have reviewed[11] the possible bondings between the enantiomers and these CSPs. The most important bondings involved are π-π complexation, hydrogen bonding, inclusion complexation, dipole interactions, steric interactions, and anionic and cationic bindings. These bondings are a result of the complex structures of the macrocyclic glycopeptide antibiotics CSPs which consists of sugar moieties, phenyl rings, quinoline, and thiazole rings, along with several chiral centres, inclusion baskets, hydrogen donor, and acceptor sites (Fig. 2). It has been reported that these bonding sites are responsible for the surprising chiral selectivities of these antibiotics.[11–14] The studied enantiomers of cromakalim (Fig. 1) contain nitrogen and oxygen atoms, along with one phenyl ring, which interact with the complimentary groups on the chiral selectors (antibiotics). The inclusion baskets and the sugar moieties provide the chiral sites in which the enantiomers fit in different fashion stereogenically, which result in the chiral discrimination between the cromakalim enantiomers. Besides, the steric effect is also playing an important role for the chiral resolution of the studied drug on these CSPs.

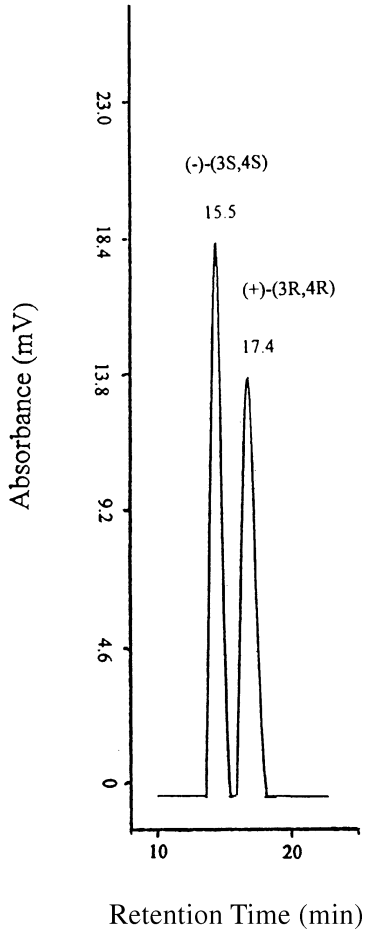


Figure 3. The chromatograms showing the enantiomeric resolution of cromakalim enantiomers on Chirobiotic T columns using hexane–ethanol (95 : 5, v/v) at 0.5 mL/min flow rate.

No chiral resolution was achieved for cromakalim enantiomers on Chirobiotic V and Chirobiotic R columns, which could be due to the steric effect. Of course, Chirobiotic V and Chirobiotic R CSPs have sufficient chiral centres, inclusion baskets, and different sites for bondings and, hence, are capable of resolving the enantiomers of a variety of racemates;[11] however, the steric effect in the present study may be responsible for not achieving chiral discrimination between the chromakalim enantiomers on Chirobiotic V and Chirobiotic R

columns. Furthermore, it may be assumed that the strength of the bondings between the enantiomers and these CSPs is poor. The presence of weak bondings may be due to the structure of cromakalim (Fig. 1), which contains only three oxygen, two nitrogen atoms, and only one phenyl ring. Chirobiotic T CSP contains more bonding sites than the Chirobiotic TAG (teicoplanin aglycon) column (Fig. 2). Therefore, the retention times of the enantiomers on Chirobiotic T columns should be higher than the retention times on Chirobiotic TAG columns. However, on the contrary, it has been observed that the retention times of the enantiomers were greater on Chirobiotic TAG columns in comparison to the retention times of the enantiomers on Chirobiotic T columns. This observation could be due to the higher steric effect in the case of Chirobiotic T columns, which has more complex structure than Chirobiotic TAG columns, which lacks the three sugar moieties. Accordingly, steric effect is playing a dominant role in the chiral resolution of cromakalim.

The mobile phases used in this study were hexane–ethanol (95 : 5, v/v and 75 : 25, v/v). Both the mobile phases were tested with Chirobiotic V, Chirobiotic R, Chirobiotic T, and Chirobiotic TAG columns, separately, but the maximum resolution obtained is reported herein. It is very interesting to note that the detection of the enantiomers decreases with increase of hexane ratio in the mobile phase. No detection was obtained on the reported CSPs, except with Chirobiotic T, using hexane–ethanol (95 : 5, v/v) as the mobile phase. Of course, the detection was obtained at higher ratios of ethanol, but no resolution was achieved on all the CSPs except on Chirobiotic TAG columns (with hexane–ethanol, 75 : 25, v/v only). Attempts have been made to determine the limit of detection (LOD) of the enantiomers on Chirobiotic T and Chirobiotic TAG columns. The LODs determined were 0.5 and 1.0 mg/L on Chirobiotic T and Chirobiotic TAG columns, respectively.

CONCLUSION

In this study the chiral resolution of cromakalim enantiomers on Chirobiotic T and Chirobiotic TAG CSPs was achieved. The resolution was better on Chirobiotic T columns than on Chirobiotic TAG columns. The better resolution on Chirobiotic T may be due the presence of sugar moieties in Chirobiotic T columns. Therefore, the presence of sugar moieties in Chirobiotic T columns provides the extra chiral binding sites in comparison to Chirobiotic TAG columns, which enhance the resolution and also the detection of cromakalim enantiomers. Taking into the consideration the results obtained, one can conclude that the enantiomeric resolution of cromakalim on Chirobiotic T and Chirobiotic TAG columns is governed by a complex mechanism involving various types of bondings as discussed above. The reported HPLC system is simple, fast, and reproducible, and can be used for the resolution of cromakalim enantiomers on a

semi-preparative scale for further pharmacological investigations of the individual enantiomers of this drug.

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