ELSEVIER

Journal of Chromatography A, 906 (2001) 185–193

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Review

High-performance liquid chromatographic enantioseparation of drugs containing multiple chiral centers on polysaccharide-type chiral stationary phases

Hassan Y. Aboul-Enein*

Pharmaceutical Analysis Laboratory, Biological and Medical Research Department (MBC-03), King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, Riyadh 11211, Saudi Arabia

Abstract

Enantioseparation of drugs with multiple chiral centers is challenging. This article describes resolution of some drugs with multiple chiral centers using polysaccharide-type chiral stationary phases. Also, the use of the column-switching technique is demonstrated to achieve the resolution of this type of compounds.

Keywords: Reviews; Enantiomer separation; Chiral stationary phases, LC; Polysaccharides; Nadolol; Indonolol; Nebivolol; Diltiazem

Contents

1. Introduction

Chirality is a prominent feature of most biological processes, and the enantiomers of a bioactive molecule often possesses different biological effects. The phenomenon of enantioselectvity in biological action is not restricted to pharmaceuticals but is characteristic of all biologically active agents, including insecticides, herbicides, flavors and fragrances, food additives, etc.

Drugs that are derived from natural products are usually obtained in the optically active or pure form of a single isomer. However, the drugs that are produced by chemical synthesis are usually a mixture of equal parts of two, four or more isomers, depending on the number of asymmetric centers. Accordingly, stereoselectivity in chiral drug bioavailability, distribution, interaction with receptor sites, metabolism and elimination produces differences in isomer activity, ranging from unwanted toxicity to no significance to enhanced activity [1].

Over the past three decades, enantioseparations by high-performance liquid chromatography (HPLC)

*Tel.: +966-144-278-59; fax: +966-144-278-58.
E-mail address: enein@kfshrc.edu.sa (H.Y. Aboul-Enein).

0021-9673/01/$ – see front matter
PII: S0021-9673(00)00950-X

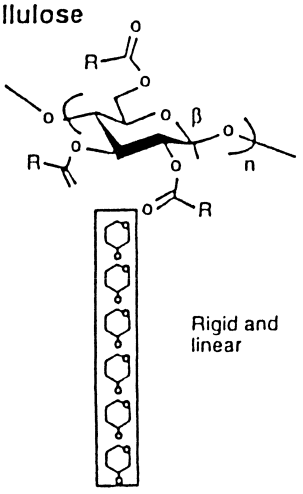
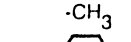
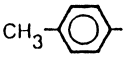
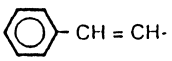
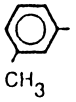
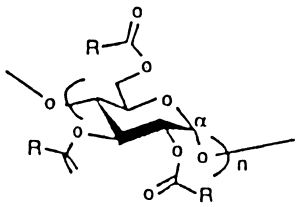
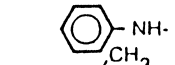
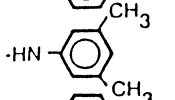
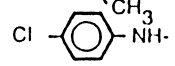
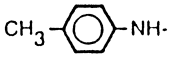
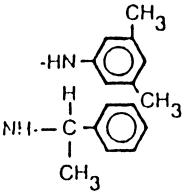
has become increasingly important. This can be achieved through:

(a) indirect methods of chiral separations which involves the synthesis of diastereoisomers by a chiral derivatizing agent followed by chromatography on an achiral column; or

(b) direct methods involving separation of the racemic drugs to their corresponding enantiomers using chiral stationary phases (CSPs).

Direct methods based on CSPs are preferred since they are rapid and suitable to resolution of racemates on both analytical, and preparative scales. There is a wide variety of natural and synthetic CSPs, most of which are commercially available (more than 120 phases). Many of these CSPs are of limited application. Thus, polysaccharide based stationary phases, together with the chemically bonded protein phases, cyclodextrins and their derivatives, Pirkle-type

Table 1
Structure of the cellulose and amylose CSPs

	R	Name
Cellulose 		*Cellulose esters*
		Cellulose triacetate (CTA-1)
		Cellulose tribenzoate (Chiralcel OB)
		Cellulose tri-4-methylbenzoate (Chiralcel OJ, Chiralcel OJ-R)
		Cellulose tricinnamate (Chiralcel OK)
		Cellulose tri-3-methylbenzoate (CMB)
		Cellulose carbamates
Amylose 		Cellulose triphenylcarbamate (Chiralcel OC)
		Cellulose tri-3,5-dimethylphenycarbamate (Chiralcel OD, Chiralcel OD-R)
		Cellulose 4-chlorophenylcarbamate (Chiralcel OF)
		Cellulose tri-4-methylphenylcarbamate (Chiralcel OG)
		Amylose carbamates
		Amylose tri-3,5-dimethylphenylcarbamate (Chiralpak AD)
		Amylose tri-*S*-α-methylphenylcarbamate (Chiralpak AS)

phases and macrocyclic antibiotics, have proved to be the most useful phases for the resolution of chiral drugs by HPLC [2–5].

The polysaccharide based chiral stationary phases were introduced by Okamoto and his group in 1984 and are prepared by coating cellulose and amylose derivatives on a pretreated silica [6–10]. Using the polysaccharide-type CSPs, the structures of which are shown in Table 1, enantiomeric separations can be performed in normal and reversed-phase modes, the latter with Chiralcel OD-R, Chiracel OJ-R and the recently introduced Chiralpack AD-R. Although the mechanism of chiral discrimination with polysaccharide phases has not been satisfactorily elucidated, it is believed that the differential binding of enantiomers is a result of a combination of attractive forces such as hydrogen bonding, hydrophobic interactions, dipole–dipole interactions and charge transfer complex ($\pi-\pi$) formation. The main chiral adsorbing sites in cellulose and amylose derivatives are considered to be the polar ester and carbamate groups. The introduction of substituents in the phenyl group of these derivatives also affects their resolving ability [6]. In addition, chiral recognition also seems to be a function of the fit of the asymmetric portion of the solute in a chiral cavity or channel of the stationary phase [7–9].

A large number of drugs with one stereogenic center have been resolved by these phases [10–20]. It has been found that 84% of the small molecule racemates can be successfully resolved by using the following columns: Chiracel OD, Chiralcel OJ, Chiralpak AD and Chiralpak AS [21]. These four previously mentioned phases have shown high chiral discriminatoratory ability for a wide range of racemates with various chemical structures.

This review summarizes the efficiency of the polysaccharide-type CSPs in the enantioselective separation of some drugs with multiple stereogenic centers. The resolution of these drugs becomes more difficult and represents a challenge in the chiral separation of all possible enantiomers.

2. Examples of enantioseparation of drugs with two stereogenic centers

The following are examples of drugs with two stereogenic centers that were successfully resolved using one polysaccharide-type CSP.

Nadolol (SQ11725) is a β-adrenergic blocker which has two chiral centers. It is of interest to note that nadolol has three chiral centers, yet two of these centers (the hydroxy groups at the 2- and 3-positions of the tetrahydronaphthalene ring) are fixed in the *cis* configuration and thus are considered as one chiral center. The other chiral center is the hydroxy group at the side chain. This is responsible for the presence of two racemates, known as racemate A (SQ12181) and racemate B (SQ12182), and four enantiomers.

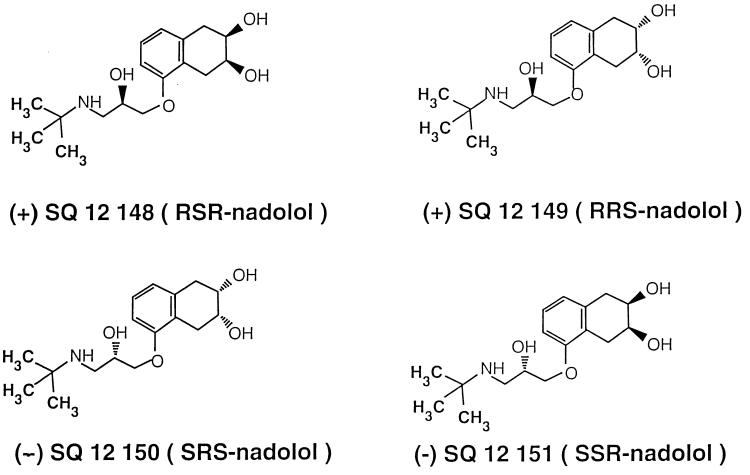


Fig. 1. The stereochemical structure of the four nadolol enantiomers.

The structure of these enantiomers are shown in Fig. 1. A simple isocratic HPLC method was developed by Aboul-Enein and Abou-Basha [22] to separate nadolol racemate A (SQ12181) to its corresponding enantiomers *RSR*-nadolol (SQ12148) and *SRS*-nadolol (SQ12150) and racemate B (SQ12182) to its corresponding enantiomers *RRS*-nadolol (SQ12149) and *SSR*-nadolol (SQ12151) as shown in Figs. 2 and 3, respectively.

Chiralcel OD column was used to separate the individual nadolol enantiomers from their corresponding racemates, however, complete separation of all four enantiomers from the bulk nadolol material (SQ11725) was partial since the enantiomers SQ12148 and SQ12149 co-eluted (Fig. 4) [22]. McCarthy [23] reported the resolution of the four nadolol enantiomers on Chiralpak AD under both normal and reversed-phase modes (using ethanol water and diethylamine as the mobile phases). It seems clear in this example that the helical configuration of the amylose derivative did effectively contribute to the resolution of all four nadolol enantiomers, along with the interactive forces, contrary to the cellulose derivative which has a more

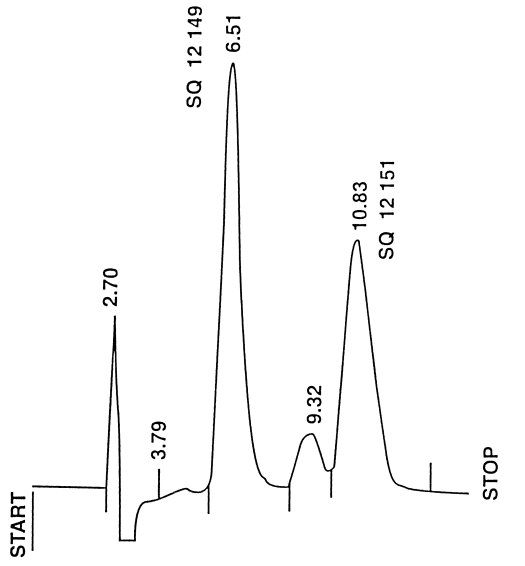


Fig. 3. Chromatogram of nadolol SQ12182. Column: Chiralcel OD (250×4.6 mm I.D.); mobile phase: hexane–ethanol–diethylamine (80:20:0.4 v/v/v); other chromatographic conditions are the same as in Fig. 2.

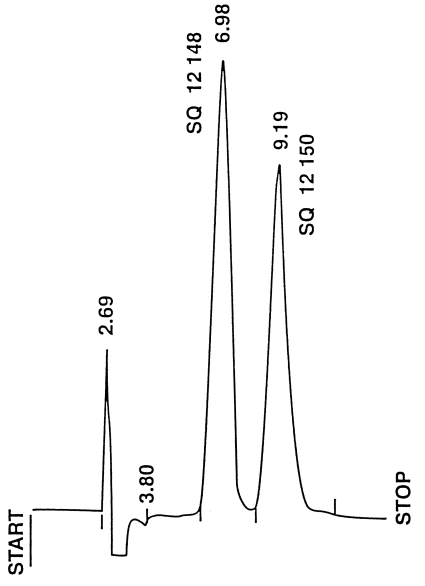


Fig. 2. Chromatogram of nadolol SQ12181. Column: Chiralcel OD (250×4.6 mm I.D.); mobile phase: hexane–ethanol–diethylamine (80:20:0.4 v/v/v); flow-rate: 1 ml/min; temperature: 23°C; detector: UV 254 nm (Ref. [22]).

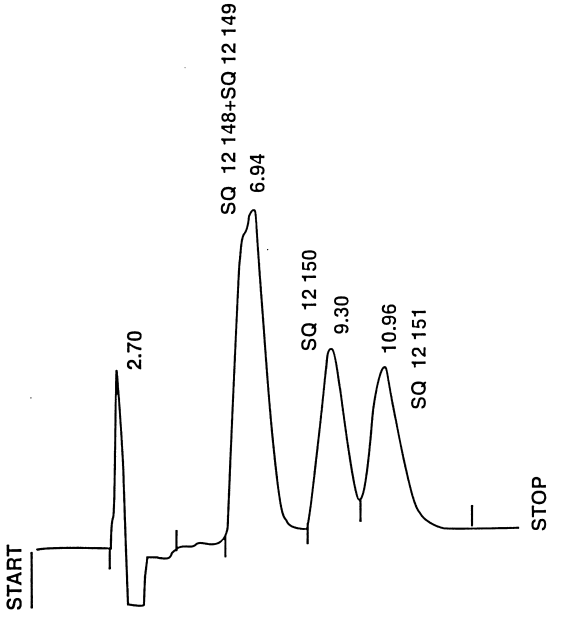


Fig. 4. Chromatogram of nadolol SQ11725. Column: Chiralcel OD (250×4.6 mm I.D.); mobile phase: hexane–ethanol–diethylamine (80:20:0.4 v/v/v); other chromatographic conditions are the same as Fig. 2.

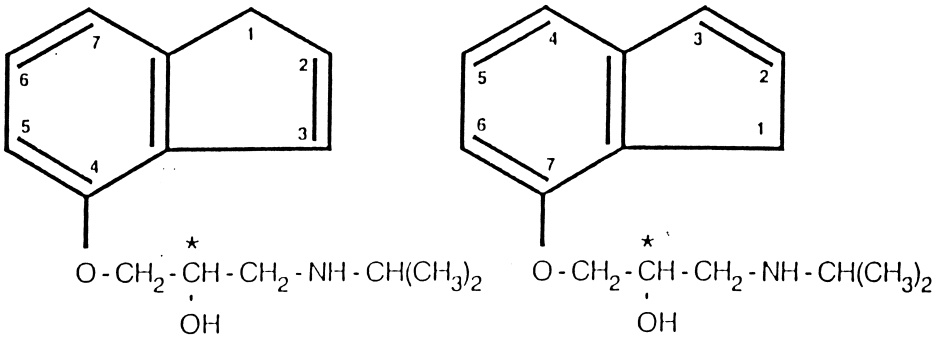


Fig. 5. The tautomeric chemical structures of indenolol: (a) 4-indenyloxy and (b) 7-indenyloxy isomers. Asterisk denotes the position of the chiral carbon.

rigid and linear configuration resulting in its inability to resolve all the four enantiomers.

Aboul-Enein and Serignese [24] reported the resolution of racemic indenolol to its individual enantiomers by HPLC using the Chiralcel OD column. Indenolol contains two positional isomers, giving a total of four enantiomers (Fig. 5). Gradient elution was necessary to separate all four enantiomers as shown in Fig. 6.

Since compounds with two chiral centers are essentially the same as having two independent single stereogenic center compounds in most cases, one center from the compound with two centers will be nicely separated on one CSP and the other center from the same group will more likely be separated on a second CSP. Accordingly, the use of the column switching HPLC system is highly recommended to achieve full resolution of all four enantiomers [25]. The advantages of column switching are: (a) to remove interfering components and related sub-

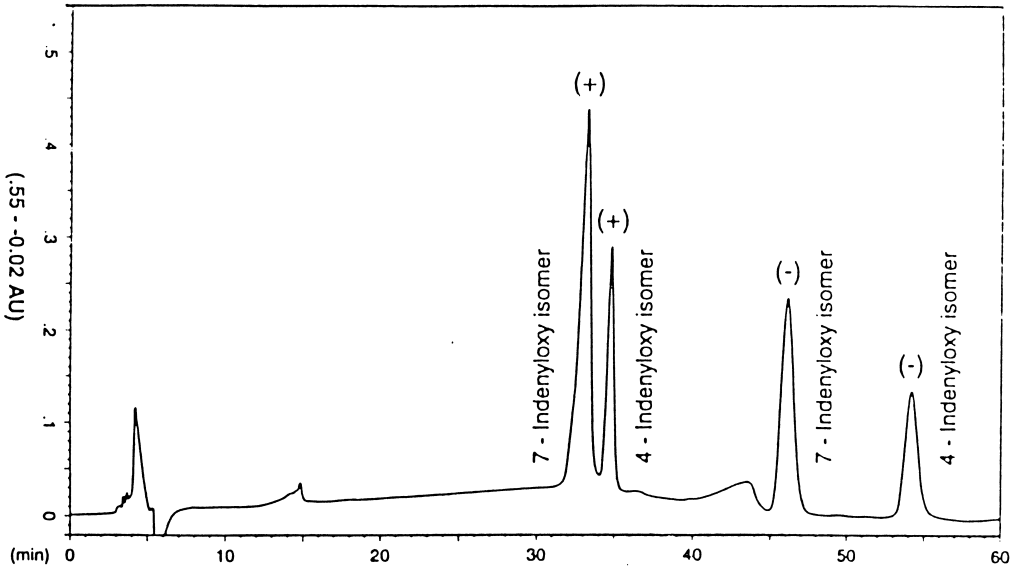


Fig. 6. HPLC enantiomeric separation of racemic indenolol hydrochloride. Column: Chiralcel OD (25 cm×4.6 mm I.D.); mobile phase: Solvent A: hexane–ethanol–diethylamine, 99:1:0.2 (v/v/v); Solvent B: ethanol–diethylamine, 100:0.2 (v/v); linear gradient profile: 0 min, 100% A; 20 min, 100% A; 60 min, 80% A, 20% B; flow-rate: 1 ml/min; temperature: 23°C; chart speed: 0.5 cm/min; detection: UV at 250 nm; sample quantity: 10 nmoles (Ref. [24]).

stances, (b) to pre-concentrate component for sensitivity (through a suitable transfer column) and (c) to ensure peak homogeneity.

Coupling of two chiral columns together is an alternative approach to column switching. However, the following are some disadvantages to this approach: (a) all peaks eluted have an average retention time from both columns, (b) mobile phase will not be optimized for each column and (c) back pressure is normally too high.

Fig. 7 shows the results of the resolution of a compound with two stereogenic centers completely resolved to the four individual enantiomers by using the column-switching technique while only partial resolution was achieved by using any of the coupled columns separately. The columns used were Chiralpack AD and Chiralcel OA columns.

Other interesting examples where polysaccharide-based CSPs were used successfully in separating drugs with two stereogenic centers includes:

(i) the separation of the four enantiomeric forms of α-hydroxymetoprolol, a urinary human metabolite of the β-adrenoreceptor blocking drug metoprolol on Chiralcel OD [26].

(ii) the separation of the four enantiomers of the benzothiazepine-type calcium antagonist diltiazem on Chiralcel OD using sub- and/or supercritical fluid chromatography [27]. It is of interest to mention that

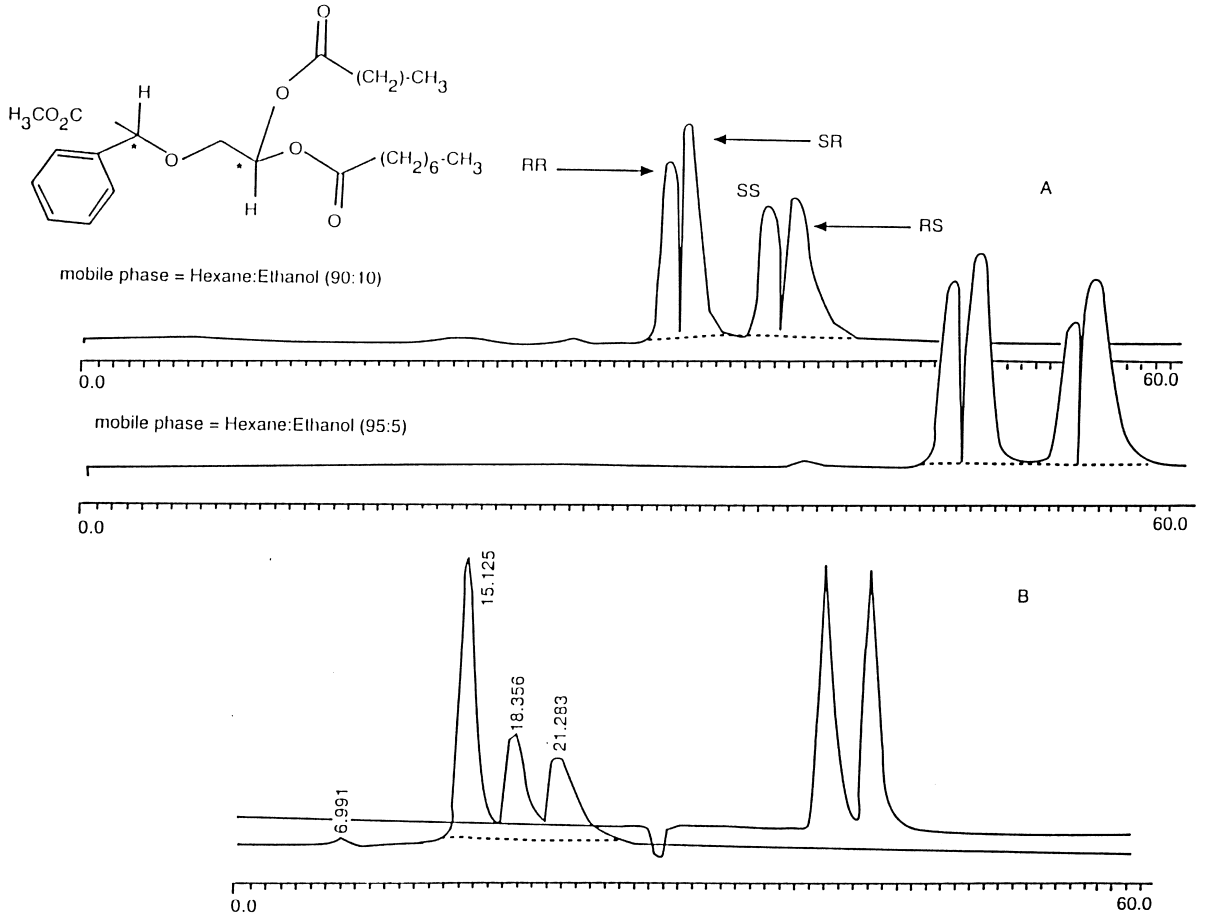


Fig. 7. Separation of a compound with two stereogenic centers. Asterisk indicates the position of the chiral center (Ref. [25]). (A) Partial separation of the four enantiomers on coupled Chiralpak AD and Chiralcel OA columns. (B) Full separation of the four enantiomers using column-switching on Chiralcel OA and Chiralpak AD. Mobile phase: hexane–methyl-*tert*.-butylether–ethanol (76:22:2, v/v/v) for Chiralcel OA and hexane–ethanol (90:10, v/v) for Chiralpak AD column. Transfer column: Chiralcel OK (5 cm×4.6 mm I.D.).

the four enantiomers of diltiazem were resolved on a Chiralcel OF column which is coated with cellulose tris(4-chlorophenylcarbamate), although the separation showed poor efficiency [28]. Furthermore, Yaku et al. [29] recently studied the thermodynamics and separation mechanism of diltiazem enantiomers on Chiralcel OF [29]. The study indicated that the separation of the *cis*-diltiazem enantiomers was enthalpy-controlled, whereas the separation of *trans*-diltiazem enantiomers was entropy-controlled in packed-column supercritical fluid chromatography in the temperature range examined. However, in HPLC both *cis*- and *trans*-enantiomers showed enthalpy-controlled separation.

3. Example of enantioseparation of a drug with multiple (more than two) stereogenic centers

Nebivolol, chemically known as α,α′-(iminodimethylene)bis[6-fluoro-2-chromanmethanol], is a β-adrenergic blocker with four chiral centers (2^4= 16 stereoisomers). Owing to the presence of a plane of symmetry, some of these isomers are identical and exist in the *meso* form. Accordingly, only ten stereoisomers are present.

This drug represents quite a challenging case for chiral separation which was successfully achieved using Chiralpack AD. [30]. All the ten stereoisomers of nebivolol were separated under isocratic conditions as shown in Fig. 8. The effect of the column temperature is crucial for full resolution of all possible stereoisomers. At room temperature, the total analysis time was approximately 120 min. The effects of the temperature on the different k'-values is shown in Fig. 9. Within the studied temperature range, a decrease of the k' values of about 45% is observed for some of the isomers, while for the other isomers the retention time remains practically constant over the entire temperature range (25–45°C). It is interesting to mention that between the *RSRR* and *RRRR* isomers, which were generally baseline separated, co-eluted at these temperatures. Based on this data, one can conclude that for nebivolol full en-

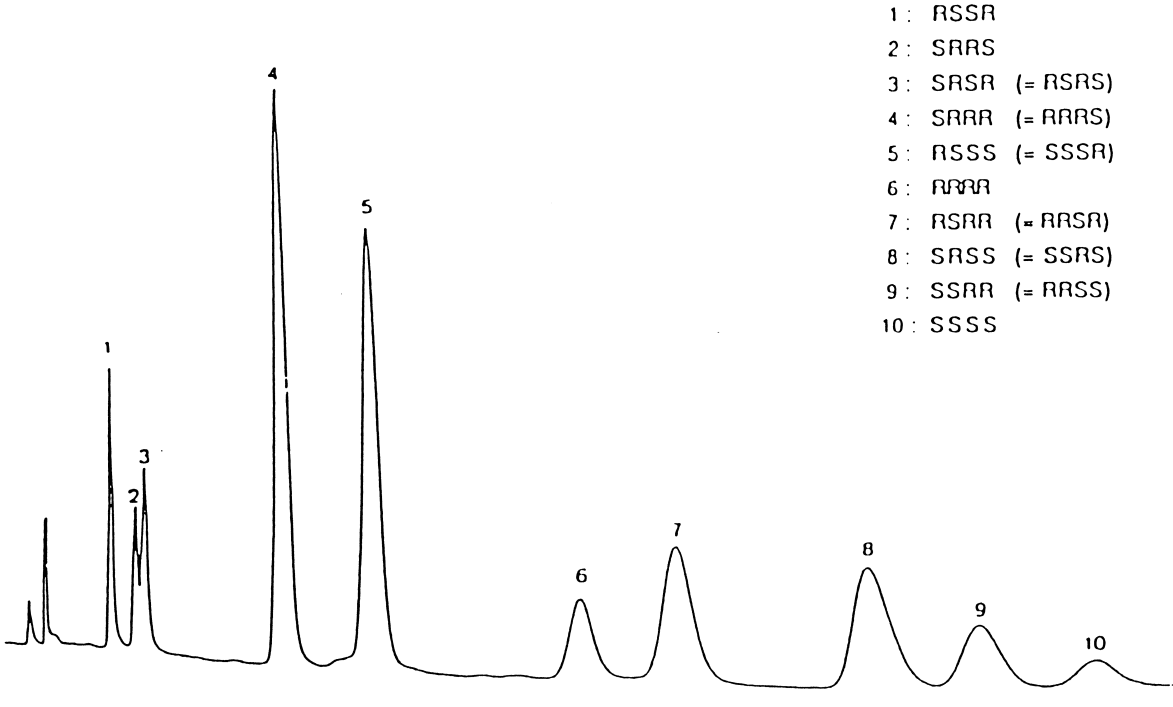


Fig. 8. HPLC analysis of nebivolol. Column: Chiralpak AD (250×4.6 mm I.D.); mobile phase: ethanol; flow-rate: 0.5 ml/min; temperature: 23°C; detector UV: 220 nm (Ref. [30]).

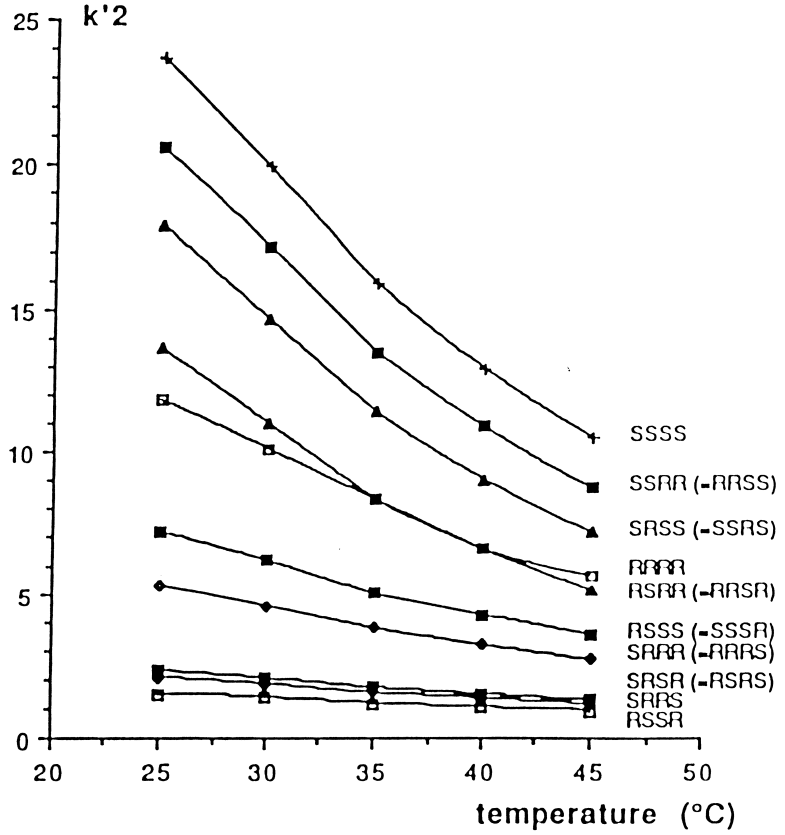


Fig. 9. Effect of temperature on the capacity factor of nebivolol isomers (Ref. [30]).

antiomeric resolution, working at a column temperature of 45°C is recommended because: (a) this will reduce substantially the analysis time and (b) it will allow better quantification of the enantiomers which elute at the highest k' values.

4. Conclusions

Polysaccharide-type chiral stationary phases, namely cellulose and amylose derivatives, have demonstrated efficient capabilities in resolving drugs with two or more stereogenic centers. Separation of stereoisomers of nadolol, indenolol, nebivolol, among other molecules are achieved using various cellulose and amylose derivatives. The use of the column-switching HPLC system is highly recommended and should be considered to be a useful approach in order to achieve full resolution of all the possible enantiomers.

References

[1] H.Y. Aboul-Enein, L.I. Abou-Basha, Chirality and drug hazards, in: H.Y. Aboul-Enein, I.W. Wainer (Eds.), The Impact of Stereochemistry On Drug Development and Use, Wiley, New York, 1997, p. 1, Chapter 1.
[2] G. Subramanian (Ed.), A Practical Approach To Chiral Separations By Liquid Chromatography, VCH Publishers, Weinheim, Germany, 1994.
[3] H.Y. Aboul-Enein, I.W. Wainer (Eds.), The Impact of Stereochemistry in Drug Development and Use, Wiley, New York, 1997.
[4] S. Allenmark, Chromatographic Enantioseparation: Methods and Applications, 2nd Ed., Ellis Horwood, New York, 1991.
[5] T.E. Beesley, R.P.W. Scott, Chiral Chromatography, Wiley, Chichester, UK, 1998.

[6] E. Yashima, Y. Okamoto, Chiral recognition mechanism of polysaccharides chiral stationary phases, in: H.Y. Aboul-Enein, I.W. Wainer (Eds.), The Impact of Stereochemistry in Drug Development and Use, Wiley, New York, 1997, p. 345, Chapter 12.
[7] Y. Okamoto, Y. Kaida, J. Chromatogr. A 666 (1994) 403.
[8] K. Oguni, H. Oda, A. Ichida, J. Chromatogr. A 694 (1995) 91.
[9] E. Yashima, T. Okamoto, Bull. Chem. Soc. Jpn. 68 (1995) 68.
[10] Y. Okamoto, Chemtech (1987) 176.
[11] E. Yashima, P. Sahavattanapong, Y. Okamoto, Chirality 8 (1996) 446.
[12] Y. Okamoto, E. Yashima, Chiral recognition by optically active polymers, Ch. 41, in: K. Hatada, T. Kitayama, O. Vogel (Eds.), Macromolecular Design of Polymeric Materials, Marcel Dekker, New York, 1997, p. 737, and references cited therein.
[13] E. Yashima, C. Yamamoto, Y. Okamoto, J. Am. Chem. Soc. 118 (1996) 4036.
[14] E. Yashima, Y. Okamoto, Bull. Chem. Soc. Jpn. 68 (1995) 3289.
[15] B. Chankvetadze, E. Yashima, Y. Okamoto, J. Chromatogr. A 694 (1995) 101.
[16] E. Yashima, H. Fukaya, Y. Okamoto, J. Chromatogr. A 677 (1994) 11.
[17] Y. Okamoto, M. Kawashima, K. Hatada, J. Chromatogr. 363 (1986) 173.
[18] H.Y. Aboul-Enein, S.A. Bakr, Enantiomer 1 (1996) 223.
[19] A. Van Overbeke, W. Baeyens, H. Oda, H.Y. Aboul-Enein, Chromatographia 43 (1996) 599.
[20] H.Y. Aboul-Enein, S.A. Bakr, Chirality 9 (1997) 10.
[21] F. Geiser, R.J. Bopp, K. Tachibana, in: Pittsburgh Conference and Exposition, Chicago, IL, USA, March 3–8, 1996, Abstract 037P.
[22] H.Y. Aboul-Enein, L.I. Abou-Basha, J. Liq. Chrom. Rel. Technol. 19 (1996) 383.
[23] J.P. McCarthy, J. Chromatogr. 685 (1994) 349.
[24] H.Y. Aboul-Enein, V. Serignese, J. Liq. Chrom. Rel. Technol. 19 (1996) 933.
[25] R.J. Bopp, K. Tachibana, F. Geiser, in: Pittsburgh Conference and Exposition, Chicago, IL, USA, March 3–8, 1996, Abstract 448P.
[26] K. Balmér, A. Persson, P.-O. Lagerström, B.-A. Persson, G. Schill, J. Chromatorgr. 553 (1991) 391.
[27] K. Yaku, K. Aoe, N. Nishimura, T. Sato, F. Morishita, J. Chromatogr. A 785 (1997) 185.
[28] K. Ishii, K. Minato, N. Nishimura, T. Miyamoto, T. Sato, J. Chromatogr. A 686 (1994) 93.
[29] K. Yaku, K. Aoe, N. Nishimura, F. Morishita, J. Chromatogr. A 848 (1999) 337.
[30] J. Dingenen, Polysaccharide phases in enatioseparation, in: G. Subramanian (Ed.), A Practical Approach To Chiral Separations By Liquid Chromatography, VCH, Weinheim, Germany, 1994, pp. 147–150, Chapter 6.