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Direct separation and optimization of timolol enantiomers on a cellulose tris-3,5-dimethylphenylcarbamate high-performance liquid chromatographic chiral stationary phase[a]

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ABSTRACT

A high-performance liquid chromatographic method was developed for the direct resolution and optimization of the separation of timolol enantiomers. The method involves the use of a cellulose tris-3,5-dimethylphenylcarbamate chiral stationary phase (OD-Chiralcel) column. The effects of concentration of 2-propanol, various aliphatic alcohols and diethylamine in the mobile phase and column temperature on the retention and enantioselectivity of timolol enantiomers were studied. The maximum resolution factor obtained was 4.00 when using the solvent system hexane–2-propanol (95:5) containing 0.4% (v/v) diethylamine at 5°C.

INTRODUCTION

Timolol maleate, (*S*)-(−)-1-(*tert.*-butylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanol maleate (Fig. 1), is a β-adrenergic blocking agent and is used for the treatment of essential hypertension, ocular hypertension and chronic, open-angle glaucoma, including aphakia. Timolol eye drops are widely used for the treatment of glaucoma. Although timolol, like all β-adrenoceptor antagonist, is contraindicated in patients with asthma, its inadvertent administration as eye drops to such patients continues to have severe and even fatal consequences[1]. Richards and Tattersfield[2] reported that the (*R*)-(+)-timolol enantiomer, L-714,465 was considerably less potent as a β-adrenoceptor antagonist in animals than the clinically used (*S*)-(−)-timolol enantiomer, but only slightly less potent in reducing intraocular pressure. The (*R*)-(+)-timolol enantiomer is 49 times less potent than (*S*)-(−)-timolol on β_2-adrenoceptor in animals and thirteen times less potent in constricting the airways of normal subjects, yet it is only four times less potent in reducing intraocular pressure in

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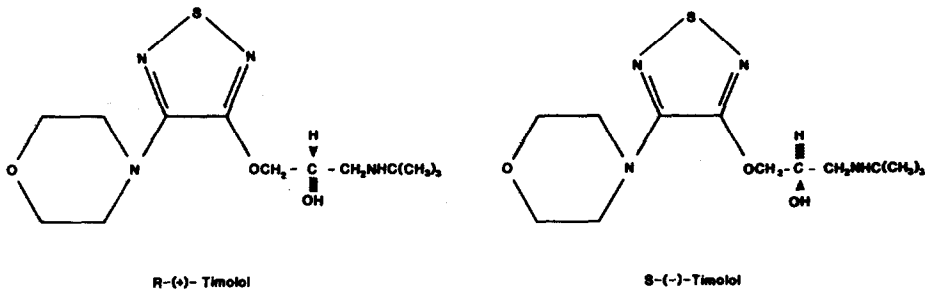

Fig. 1. Absolute configuration of (*R*)-(+)-timolol and (*S*)-(−)-timolol.

man[3]. These findings suggested that (*R*)-(+)-timolol might be a safer alternative for the treatment of glaucoma with less systemic side-effects than the (*S*)-(−)-enantiomer when applied as eye drops (in concentrations ranging between 0.25 and 4.0%), as it has a smaller bronchoconstricting effect.

A number of cellulose-based high-performance liquid chromatographic (HPLC) chiral stationary phases (CSPs) have been developed and are now commercially available[4,5]. This paper reports the direct separations of timolol enantiomers on a cellulose tris-3,5-dimethylphenylcarbamate column (OD-Chiralcel) without any derivatizations (Fig. 2). The effects of 2-propanol, various aliphatic alcohols, temperature and diethylamine concentration on resolution of timolol are discussed.

EXPERIMENTAL

Apparatus

The liquid chromatographic system consisted of a Waters Model M-45 pump, a U6K injector and a Lamda-Max Model 480 LC UV detector operated at 224 nm. A Chiralcel OD analytical column (25 cm × 0.46 cm I.D.) (Daicel Chemical Industries, Tokyo, Japan) containing cellulose tris-3,5-dimethylphenylcarbamate coated on silica gel of particle size 10 μm was used.

Chemicals

(*R*)-(+)-timolol (Lot No. L-714,465-001E015) and (*S*)-(−)-timolol (Lot No. L-714,503-01T12) were kindly supplied by Merck Sharp & Dohme (NJ, U.S.A.) HPLC-grade hexane was obtained from Fisher Scientific (NJ, U.S.A.), HPLC-grade 2-propanol from Romil (U.K.), diethylamine from BDH (Poole, U.K.) and HPLC-

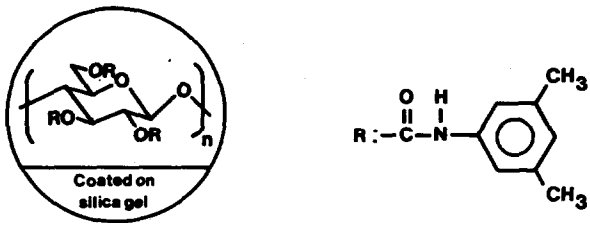

Fig. 2. The structure of the Chiralcel OD chiral stationary phase used.

grade 2-butanol, 1-butanol, 1-pentanol and 1-propanol, from Aldrich (WI, U.S.A.).

RESULTS AND DISCUSSION

To the best of our knowledge, no work has been reported on the direct resolution of timolol using a chiral stationary phase column. We have studied the separation of timolol enantiomers using a Chiralcel OD column. This cellulose-based chiral phase has been used successfully to separate directly several β-adrenergic blockers, *e.g.*, alprenolol, oxyprenolol, propranol, pindolol and atenolol[6]. The mobile phase consists of a mixture of hexane, 2-propanol and diethylamine. A chromatogram of the enantiomeric separation of timolol is shown in Fig. 3. A comparison of the chromatograms and capacity factors of (*R*)-(+)-timolol (Fig. 4a) and (*S*)-(−)-timolol (Fig. 4b) indicated that the peak that eluted with a lower capacity factor was that of the former enantiomer and the peak with a higher capacity factor was that of the latter. The maximum resolution (*R*) obtained was 4.00.

Effects of mobile phase on retention and stereoselectivity

The capacity factors (k'), separation factors (α) and resolutions (R_s) of solutes can be regulated over a wide range by the addition of an alcohol. The influence of the concentration of 2-propanol and other aliphatic alcohols in the mobile phase on the separation of timolol enantiomers was thoroughly studied.

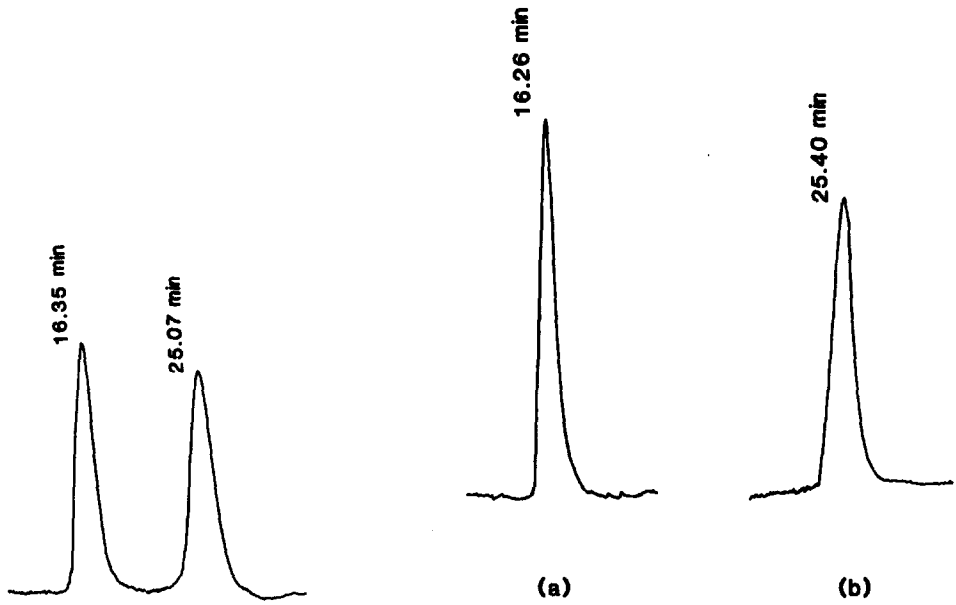

Fig. 3. HPLC separation of timolol maleate enantiomers. Column: Chiralcel OD (250 mm × 4.6 mm I.D.); mobile phase, hexane–2-propanol–diethylamine (95:5:0.4); flow-rate, 0.7 ml/min; chart-speed, 0.5 cm/min; temperature, 5°C; pressure, 250 p.s.i.; sample amount, 10 nmol; detector: UV (224 nm); sensitivity, 0.01 a.u.f.s.

Fig. 4. Chromatograms of (a) (*R*)-(+)- and (b) (*S*)-(−)-timolol maleate. Sample amount, 5 nmol. Other conditions as in Fig. 3.

TABLE I

EFFECT OF THE 2-PROPANOL CONTENT IN MOBILE PHASE ON CAPACITY FACTOR, SEPARATION FACTOR AND RESOLUTION IN THE SEPARATION OF TIMOLOL ENANTIOMERS

Conditions: column, Chiralcel OD (250 × 4.6 mm I.D.); mobile phase, hexane–2-propanol with 0.4% (v/v) diethylamine; temperature, 5°C; flow-rate, 0.7 ml/min.

Parameter[a]	*2-Propanol concentration (%, v/v)*				
	0	*5*	*10*	*15*	*20*
k'_1	No elution	4.58	2.32	1.78	1.51
k'_2	No elution	7.56	3.59	2.48	1.98
α	No elution	1.65	1.55	1.39	1.31
R_s	No elution	4.00	3.05	1.98	1.44

[a] k'_1 = capacity factor of 1st-eluted compound; k'_2 = capacity factor of 2nd-eluted compound; α = separation factor; R_s = resolution.

Effects of 2-propanol concentration

Concentrations of 0–20% 2-propanol in the mobile phase were investigated (Table I). Timolol enantiomers did not elute from the column when a mobile phase without any modifier was used. An increase in 2-propanol concentration resulted in a corresponding decrease in retention. The change in α is significant (1.3–1.6), providing almost twice the contribution to R_s at 5% 2-propanol, as compared to addition of 20% 2-propanol.

Effects of straight- and branched-chain aliphatic alcohols

The effects of the structure of the polar mobile phase modifier were investigated using a series of primary and secondary alcohols. The changes in k' and R_s observed with a series of primary and secondary alcohols (1-propanol, 2-propanol, 1-butanol, 2-butanol, 1-pentanol and 2-pentanol) are presented in Table II. An increase in the chain length of the alkyl group of the primary alcohol increased k', but the effect on α was not significant. Thus, a better resolution was obtained using 1-butanol as a modifier in the mobile phase. On the other hand, the use of the branched-chain

TABLE II

EFFECT OF DIFFERENT PRIMARY AND SECONDARY ALIPHATIC ALCOHOLS IN THE MOBILE PHASE ON CAPACITY FACTOR, SEPARATION FACTOR AND RESOLUTION IN THE SEPARATION OF TIMOLOL ENANTIOMERS

Conditions as in Table I and mobile phase [hexane–2-propanol (95:5, v/v) containing 0.4% (v/v) diethylamine].

Parameter[a]	*1-Propanol*	*2-Propanol*	*1-Butanol*	*2-Butanol*	*1-Pentanol*	*2-Pentanol*
k'_1	1.66	2.34	1.78	2.86	3.40	4.42
k'_2	1.83	3.32	2.27	4.06	3.60	6.99
α	1.17	1.42	1.27	1.42	1.07	1.58
R_s	0.449	2.47	1.20	2.37	0.30	2.92

[a] See Table I

TABLE III

EFFECT OF DIETHYLAMINE CONTENT IN MOBILE PHASE ON CAPACITY FACTOR SEPARATION FACTOR AND RESOLUTION IN THE SEPARATION OF TIMOLOL ENANTIOMER

Conditions as in Table I, except the mobile phase [hexane–2-propanol (95:5, v/v)] contained different concentrations of diethylamine.

Parameter[a]	*Diethylamine concentration (%, v/v)*				
	0	*0.1*	*0.4*	*0.7*	*1.0*
k'_1	No separation	4.41	4.58	4.24	4.24
k'_2	No separation	7.14	7.56	7.01	7.09
α	No separation	1.62	1.65	1.65	1.67
R_s	No separation	2.95	4.00	3.85	4.32

[a] See Table I.

alcohols as modifiers in the mobile phase gave a significantly better resolution than straight-chain alcohols. Both primary and secondary alcohols yield largers k' values for higher-molecular-weight alcohols, whereas the α values do not change mucht with the larger alcohols. However, a better resolution was obtained using 2-propanol as a modifier in the mobile phase. There is a single point of interaction between the hydroxyl hydrogen on the alcohol and the carbonyl oxygen of the CSP, while the solute competes with the modifier for hydrogen-bonding sites on the CSP. This competition takes place at both chiral and achiral sites on the CSP[7]. This does not preclude interactions between the modifier and the solute, which appears to play a lesser role in the determination of the chromatographic parameters.

Effects of diethylamine concentration in the mobile phase

The effects of diethylamine concentration are shown in Table III. No separation was obtained without diethylamine present. The resolution factor ($R_s = 4.32$) did improve in the presence of 1% (v/v) diethylamine as suggested by the manufacturer[7]. In order to prevent the detrimental effect of the basic mobile phase on the stationary phase, as contained ester groups were present in the packing material, only

TABLE IV

EFFECT OF TEMPERATURE ON CAPACITY FACTOR, SEPARATION FACTOR AND RESOLUTION IN THE SEPARATION OF TIMOLOL ENANTIOMERS

Conditions as in Table I, except temperature and mobile phase [hexane–2-propanol (95:5, v/v) containing 0.4% (v/v) diethylamine].

Parameter[a]	*Temperature (°C)*				
	5	*10*	*15*	*23*	*30*
k'_1	4.58	4.35	4.08	3.96	3.77
k'_2	7.56	6.97	6.23	5.72	5.35
α	1.65	1.60	1.53	1.44	1.42
R_s	4.00	3.46	2.94	2.88	2.55

[a] See Table I.

0.4% (v/v) diethylamine was used, and gave a reasonably improved resolution factor ($R_s = 4.0$).

Effect of temperature

Optimization of the separation at different temperatures was studied (Table IV). It was found that chiralcel OD is sensitive to any small changes in temperature. The resolution increased about 1.57-fold when the temperature was decreased from 30 to 5°C. The existence of both simultaneous and stepwise binding is supported by the effect of temperature on stereochemical resolution. An increase in temperature resulted in a corresponding increase in the conformational mobility of the solute, which destabilized the solute–CSP complex and reduced the stereoselectivity[8].

CONCLUSION

Direct separation of timolol enantiomers was achieved using a Chiralcel OD column with hexane–2-propanol (95:5) containing 0.4% (v/v) diethylamine at 5°C. The maximum R_s obtained could be due to the chiral recognition mechanism explained by Wainer and Stiffen[8]. As the maximum R_s was obtained using this column, it could possibly be used for the preparative separation and optical purity determination of the drug. This technique can also be used for the determination of the (*R*)-(+)- and (*S*)-(−)-enantiomers of timolol in biological fluids, which is currently under study.

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