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DIRECT ENANTIOMERIC SEPARATION OF BETAXOLOL WITH APPLICATIONS TO ANALYSIS OF BULK DRUG AND BIOLOGICAL SAMPLES

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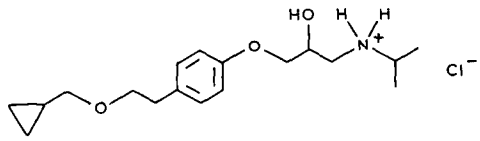
SUMMARY

A direct method is described for the resolution of the enantiomers of betaxolol, a novel cardioselective β-adrenergic blocking agent, using a tris(3,5-dimethylphenylcarbamate)cellulose chiral column. An excellent resolution of the two antipodes is obtained ($R_s > 2$) with high peak symmetries. The method is simple and ideally suited to the routine control of the enantiomeric excess in the bulk drug and the analysis of the enantiomers of betaxolol in hepatocyte suspensions. With modification of the polar modifier in a hexane-based mobile phase, most commercially available β-blockers can be baseline resolved.

INTRODUCTION

Betaxolol, (±)-1-{4-[2-cyclopropylmethoxy)ethyl]phenoxy}-3-isopropylamino-2-propanol hydrochloride, is a cardioselective β-adrenergic blocking agent characterized by a high bioavailability (90%) and a long half-life of about 15 h in man[1]. Its structure is



The drug, marketed as a racemic mixture under the name of Kerlone, is highly efficacious for the treatment of hypertension. The same drug substance, used for the treatment of glaucoma, is marketed under the name of Betoptic.

Most of the frequently prescribed β-blocking agents are developed and marketed as a racemic mixture, although it has been shown for some of them that the principal pharmacological effect is due to the *S*-enantiomer. Further, the hepatic oxidation of drugs such as propanolol[2], metoprolol[3], alprenolol[4] and bufuralol[5] is highly stereospecific. It is therefore important to have a method for the precise and accurate

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determination of the enantiomeric excess of bulk drugs obtained through resolution or enantiospecific synthesis and the determination of the metabolic disposition of racemic drugs.

Chiral stationary phases, which have been recently reviewed by Wainer[6] and Krstulovic[7], are becoming increasingly popular for direct enantiomeric separations. At present, there is no universal chiral bonded phase capable of separating all classes of compounds and analytical chemists are often faced with the problem of choosing the most suitable column for a particular application. The general guidelines for selection have been discussed[6,7].

In spite of their expense and limited efficiency, chiral stationary phases generally offer high selectivity, which results in the need for a wide range of columns when seeking the best separation conditions. The recently available tris(3,5-dimethylphenylcarbamate)cellulose column (Chiralcel OD), developed by Okamoto's group[8], is ideally suited to analytical and preparative separations of β-blockers[6,8]. As the only previously reported method[9] for resolution of enantiomers of betaxolol required a lengthly deriativatization with (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate, we attempted to develop a direct method needed for routine control of the bulk drug. This method was also found suitable for analysing the enantiomers of betaxolol in rat hepatocyte suspensions.

EXPERIMENTAL

Reagents and chemicals

(*R*)- and (*S*)-betaxolol were synthesized by the Chemistry Department of L.E.R.S. — SYNTHELABO. Chromatographic solvents of high-performance liquid chromatographic (HPLC) grade were obtained from Merck (Darmstadt, F.R.G.). Non-stabilized diethyl ether (Carlo Erba, Paris, France) was used for extractions of hepatocyte suspensions. The remaining chemicals were of analytical-reagent grade and were used without further purification.

Apparatus and HPLC conditions

Bulk drug analysis. The HPLC equipment used for the determination of the enantiomeric excess of the bulk drug consisted of an SP 8780XR automatic sampler (Spectra-Physics France, Les Ulis, France), a Jasco BIP-I pump (Prolabo, France) and a SpectroMonitor D variable-wavelength detector (LDC/Milton Roy, Paris, France). The detection wavelength was set at 273 nm. The column was a Chiralcel OD (250 × 4.6 mm I.D., 10 μm average particle size) from Daicel Chemical Industries (Sochibo, Vélizy Villacoublay, France). The column temperature was ambient (22 ± 1°C). The mobile phase were mixtures of hexane, 2-propanol and/or ethanol and the flow-rate was 1.5 ml/min.

Analysis of hepatocyte suspensions. The need for high sensitivity and selectivity in this application necessitated the use of a fluorimeter (Jasco FP-210, Prolabo) with excitation at 270 nm and emission at 310 nm (attenuation 0.4, gain × 1). The analytical column was protected by a 1.5-cm long Brownlee RP-18 guard column (Brownlee, Santa Clara, CA, U.S.A.). The mobile phase was hexane–2-propanol–diethylamine (87:13:0.05, v/v) at a flow-rate of 1.5 ml/min. A WISP 710B autoinjector (Waters, Paris, France) was employed for sample injection; the remaining components and

parameters of the HPLC system were the same as those used for the analysis of the bulk drug.

Sample preparation for analysing the enantiomers of betaxolol in a rat hepatocyte suspension

Betaxolol (2.5 μg/ml) was added to a suspension of rat hepatocytes ($2 \cdot 10^6$ cells/ml) prepared according to a method adapted from Guillouzo and Gurgen-Guillouzo[10]. Sodium hydroxide (0.1 ml, 1 *M*) and then 7 ml of diethyl ether were added to a 0.5-ml aliquot of hepatocyte suspension. The mixture was agitated for 15 min and then centrifuged at 4°C and 1000 *g* for 5 min. The aqueous phase was discarded and 6 ml of the organic phase were transferred into a clean tube containing 2 ml of 0.2 *M* hydrochloric acid. The compounds were then back-extracted into the acidic aqueous phase by shaking the tubes for 10 min. Following centrifugation, the organic phase was discarded and the aqueous phase was rendered alkaline by the addition of 0.25 ml of 2 *M* sodium hydroxide solution. The enantiomers of betaxolol were finally re-extracted with diethyl ether as described above and 6 ml of the solvent were transferred into a clean tube and evaporated to dryness under a gentle stream of nitrogen. The residue was taken up in 100 μl of hexane–isopropanol (9:1) and 50 μl were injected on to the column. Using exact volumetric transfers, the concentrations of the enantiomers were determined at different incibation times by comparing the peak areas with those obtained from the standards processed is an identical manner.

RESULTS AND DISCUSSION

Analysis of the bulk drug

Stationary phases with cellulose triesters coated on macroporous silica belong to the general category of polysaccharide sorbents. The formation of transient diastereomeric complexes with the solute molecules involves hydrogen bonding and dipole interactions. The interactive sites are located within the cavities rather than on the surface as in the Pirkle-type phases. They are used with mobile phases composed of a non-polar solvent such as hexane, modified with an alcohol or with pure polar eluents (*e.g.*, ethanol).

The use of several mobile phases was investigated for the separation of enantiomers of betaxolol on Chiralcel OD. Fig. 1 shows the separations obtained with hexane-based mobile phases containing different polar modifiers. The evolution of the chromatographic separation reflects the solubilities of the solute in the mobile phases. The best results in terms of resolution, peak symmetry and analysis time were obtained with the mobile phase hexane–2-propanol–diethylamine (87:13:0.05, v/v/v), wih $R_s > 2$, asymmetry factors for the two peaks equal to 1 and a column efficiency (calculated for the *S*-isomer) of 6400 plates/m. The addition of diethylamine improved markedly the efficiency of separation and the peak symmetries (Fig. 1A and B).

In all instances, the *R*-enantiomer was eluted before the *S*-enantiomer, resulting in a high precision of the determination of the enantiomeric excess of (*S*)-betaxolol. These conditions were therefore chosen for the routine measurement of the enantiomeric purity of (*S*)-betaxolol bulk drug. Fig. 2a and b show the HPLC traces for a sample containing 0.5% of (*R*)-betaxolol with respect to (*S*)-betaxolol and a typical HPLC trace for a sample of (*S*)-betaxolol bulk drug. The concentration–response

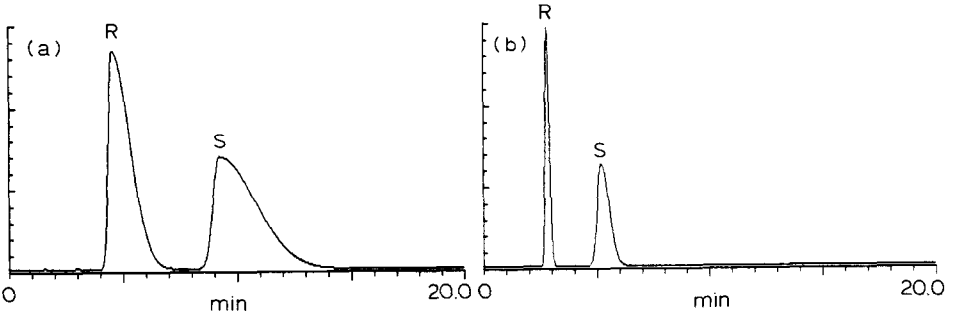


Fig. 1. Chromatograms illustrating the separation of a racemic mixture of enantiomers of betaxolol. Mobile phase: (a) hexane–2-propanol (92:8, v/v), flow-rate 1.5 ml/min, resolution R_s = 1.77; (b) hexane–2-propanol –diethylamine (92:8:0.05, v/v/v), flow-rate 1.5 ml/min, R_s = 3.58.

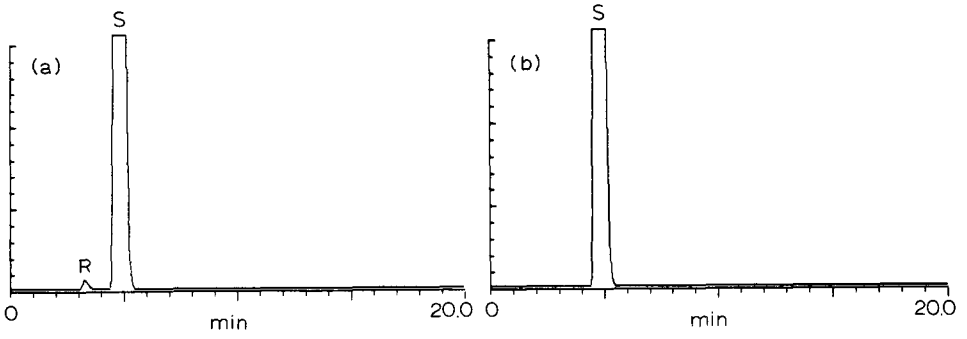
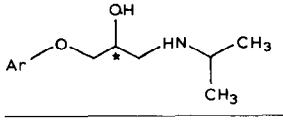
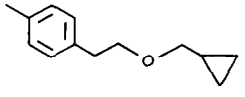
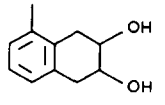
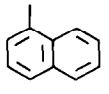
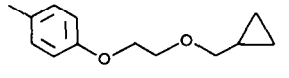
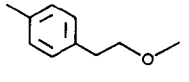
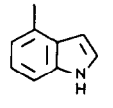


Fig. 2. Chromatograms obtained using the mobile phase hexane–2-propanol–diethylamine (87:13:0.05, v/v/v). (a) 0.5% of (*R*)-betaxolol with respect to (*S*)-betaxolol; (b) (*S*)-betaxolol bulk drug.

TABLE I

STRUCTURES OF COMMERCIALLY AVAILABLE β-BLOCKERS



Compound	*Ar*	*Compound*	*Ar*
Betaxolol		Nadolol	
Propranolol		Cicloprolol	
Metoprolol		Pindolol	

curve was linear for both enantiomers in the concentration range of interest, the average within-day coefficient of variation being 0.3% and the between-day coefficient of variation 0.6%.

This column was also found useful for the analysis of other commercially available β-blockers. Their structures are shown in Table I and examples of chromatograms in Fig. 3.

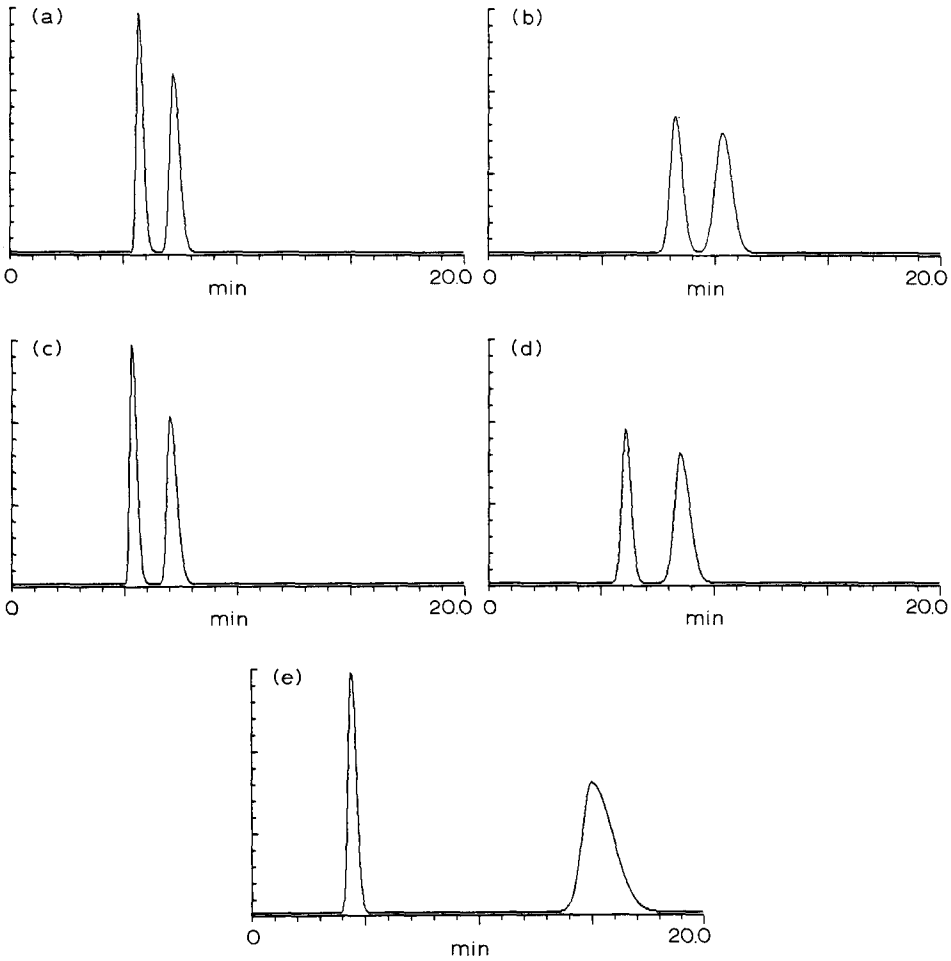


Fig. 3. Chromatograms of some commercially available β-blockers, marketed as racemates. (a) Propranolol. Mobile phase, hexane–2-propanol–ethanol–diethylamine (80:5:15:0.05, v/v/v); flow-rate, 1.0 ml/min; resolution, R_s = 2.15. (b) Nadolol. Conditions as in (A); R_s = 1.68. (c) Cicloprolol. Conditions as in (A); R_s = 2.38. (d) Metoprolol. Mobile phase, hexane–ethanol–diethylamine (90:10:0.05, v/v/v); flow-rate, 1.0 ml/min; R_s = 2.26. (e) Pindolol. Mobile phase, hexane–2-propanol–diethylamine (70:30:0.05, v/v/v); flow-rate, 1.5 ml/min; R_s = 6.48. Individual enantioners were not available for assignment as the peaks. Column temperature, 22 ± 1°C in all instances.

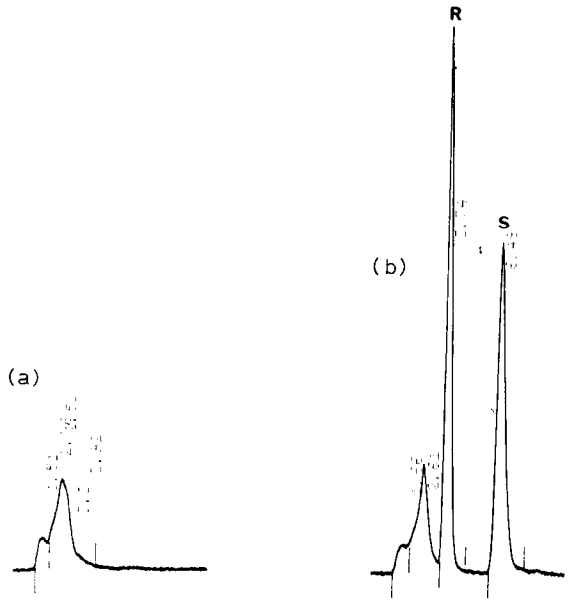


Fig. 4. Analysis of rat hepatocyte suspension; (b) suspension containing 2.5 mg/ml of betaxolol. Retention times are given in minutes.

Analysis of rat hepatocyte suspensions

The described method was applied to the determination of betaxolol in rat hepatocytes. The relative recovery of both enantiomers was essentially 100% and the absolute recovery, which depends on the volumetric transfers, was 37%. The limit of detection (signal-to-noise ratio of 3) calculated from the amount injected on to the column was approximately 5 ng for both enantiomers. The calibration graph was linear for both enantiomers in the concentration range 25–1000 mg in the tube; the mean relative residue was less than 5%. The within-day coefficient of variation was less than 2% at a concentration of 125 mg. Chromatograms of a control rat hepatocyte suspension and one, at time zero, containing equal amounts of both enantiomers (2.5 μg/ml of betaxolol) are shown in Fig. 4a and b; no interfering endogenous peaks were detected. This chromatographic technique therefore permits the relative and absolute concentrations of the enantiomers to be followed over the incubation time (<2 h) necessary to metabolize the compounds completely. The resulting concentration–time data allows a comparison of the relative rates of hepatic metabolism of the two enantiomers; these results will be published in a subsequent paper.

In conclusion, we have developed a direct method for assessing the enantiomeric composition of betaxolol. The method is simple and precise. The column equilibration times are short and the analysis time is approximately 7 min. By changing the nature or the concentration of the polar modifier, enantiomeric separations of other β-adrenergic blocking agents can be obtained. This method has been applied to the routine determination of the enantiomeric excess of (*S*)-betaxolol and we have demonstrated its applicability to the measurement of betaxolol in rat hepatocytes. For the latter analysis it is possible to use atenolol as an internal standard as it is eluted between the

two enantiomers and is well resolved from both. Work is in progress in this area and will be reported in a forthcoming publication.

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