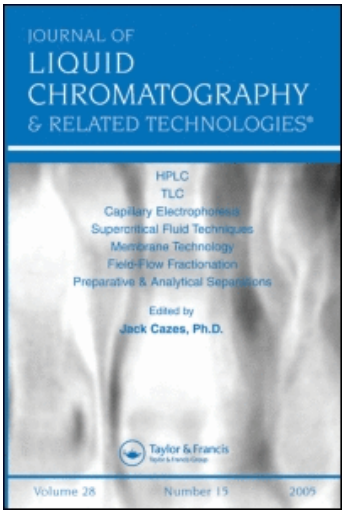

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A NEW APPROACH FOR THE DIRECT RESOLUTION OF RACEMIC BETA ADRENERGIC BLOCKING AGENTS BY HPLC

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ABSTRACT

Although beta-adrenergic blocking agents are a relatively new group of drugs, they have proven to be very useful in medical pharmacology. Since the successful introduction of propranolol (Inderal) a variety of analogous compounds have been developed. Most beta-blockers are racemic modifications and it is known that their enantiomers have different potencies and pharmacological effects. Hence, there has been considerable impetus for the production and study of the pure enantiomers. The chromatographic separation of these compounds has been dominated by the protein-based chiral stationary phases. Although selective, protein columns have limited capacity and stability. An efficient, alternative method has been found that does not suffer from the limitations of the protein phases. By using an unusual mobile phase consisting of a mixture of polar organic solvents in conjunction with the original native cyclodextrin bonded phase, the following compounds were resolved: propranolol, metoprolol, timolol, atenolol, cateolol alprenolol, pindolol, oxprenolol, labetolol and nadolol. These cyclodextrin chiral stationary phases have exceptional stability when used with these mobile phases. In addition, these separations are easily scaled to preparative proportions.

INTRODUCTION

Beta-adrenergic blocking agents (a.k.a. beta-blockers or beta–adrenoreceptor receptor blocking agents) competitively bind to beta-adrenergic receptor sites on the heart (cardiac) and/or nonvascular smooth muscle. In doing so they inhibit the action of adrenergic agents (e.g., stimulants) such as amphetamine, adrenaline, catecholamines and so on. Beta-blockers reduce the force of the heart muscle contraction and tend to reduce the heart rate under many circumstances. They do not seem to produce vasodilatation as in the case of alpha-adrenergic blocking agents. Beta-blockers have several clinical uses, many of which are listed in Table I (1).

It is well known that beta-blockers are chiral and their enantiomers have different potencies and effects. For example, the isomer of propranolol that is most active in correcting ventricular arrhythmias is much less active as a beta-blocker (1). One of the isomers of labetolol is an effective beta-blocker while another is thought to be an alpha-blocker. Because of these differing effects there is a good deal of interest in separating the stereoisomers of the beta-blocking agents.

Beta-blockers are hydroxyl-amine containing compounds. The amine functional group is always secondary and many of these compounds are N-isopropyl amines. Beta-blockers also contain at least one aromatic or substituted aromatic moiety. Typical structures are given in the Results and Discussion section. The beta-blockers are produced synthetically and most exist as racemic modifications. A few, such as labetolol, contain more than one stereogenic center and therefore exist as a mixture of enantiomers and diastereoisomers.

Several HPLC methods have been proposed for the separation of one or more of the beta-blockers (2-5). Probably, the most successful column for separating this entire class of compounds has been the α_1-acid

Table I. Some Clinical Uses for Beta-Blockers[a]

A. Treatment of Hypertension
B. Prevention of Anginal Pain
C. Treatment of Cardiac Arrhythmias
D. Control of Thyrotoxicosis
E. Treatment of Glaucoma
F. Prevention of Migraine Attacks
G. Used after Myocardial Infarctions

[a]There are several known side-effects associated with beta-blockers including gastrointestinal disturbances, tiredness, dizziness, depression, paresthesias, muscle aching and asthmatic wheezing. Some allergic reactions such as skin rash have been reported. Overdosage toxicity can occur particularly if these compounds are administrated intravenously rather than orally (1).

glycoprotein (AGP) chiral stationary phase (2). In general, the recommended mobile phase for this column consists of phosphate buffer containing a few percent of an organic modifier such as isopropanol. Early versions of the α_1-acid glycoprotein column suffered from severe stability and efficiency problems. Later versions were significantly improved. However, the AGP column, like all protein-based CSPs, has a very low capacity and is easily overloaded. This makes preparative separations difficult or impossible. In addition, the overall stability of these CSPs is still less than desired and their cost is high.

In this work we describe another approach for the efficient enantiomeric separation of beta-adrenergic blocking agents. It involves the

use of a new mobile phase and the original native cyclodextrin bonded phase columns. Under these conditions, column stability, column capacity and separation efficiency greatly exceed that of the protein CSPs.

EXPERIMENTAL

Chemicals. (L)- and (DL)-alprenolol, atenolol, labetolol, (±)-metoprolol, nadolol, oxprenolol, pindolol, and (S)-timolol were purchased from Sigma Chemical Company (St. Louis, MO). (R)- and (S)-propranolol were bought from Aldrich Chemical Company, Inc. (Milwaukee, WI). dl-Timolol were obtained from Merck Sharp & Dohme Research Lab, (Rahway, NJ). The structures of these compounds used are given with each chromatogram. All solvents (acetonitrile, methanol, triethylamine and acetic acid) were obtained from Fisher Scientific (Pittsburgh, PA).

Methods. The HPLC was performed at room temperature with a Shimadzu model LC-6A solvent delivery module, SPD-6A UV detector, and CR2AX Chromatopac recorder. The β-CD bonded phase column was a Cyclobond I column (250 x 4.6 mm i.d., 5 μm particle diameter) which was obtained from Advance Separation Technologies (Whippany, NJ). The analogous Γ-CD column (Cyclobond II) was used as well.

RESULTS AND DISCUSSION

Figure 1 (A through J) shows the separation of ten beta-blockers on native cyclodextrin bonded phase columns. The first eight compounds (Fig. A through H) are racemic mixtures. Labetolol (Fig. 1-I) has two stereogenic centers and exists as two pairs of enantiomers. Nadolol (Fig. 1-J) has three stereogenic centers, but only isomers of the *cis*-diol are present. These separations cannot be obtained in the traditional reversed phase mode (hydro-organic solvents) where inclusion complexation is prevalent. These

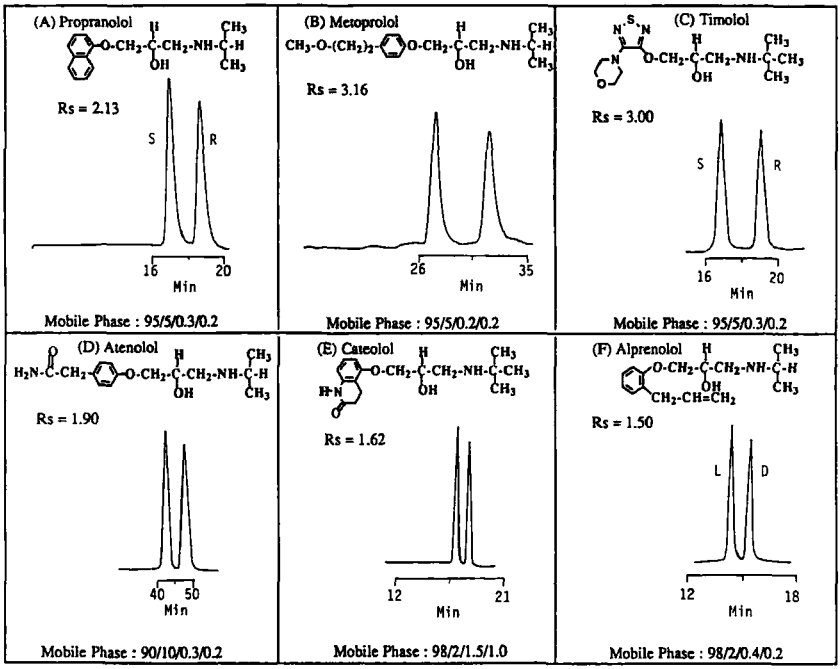

Figure 1(A)-(J). Chromatograms showing the resolution, retention and structure of the beta-adrenergic blocking agents. Conditions: Mobile phase conditions are given as volume ratios of acetonitrile/methanol/acetic acid/triethylamine just below each chromatograms. The UV detector wavelength was 254 nm and the flow rate was 1.0 ml/min. (A)-(F) One 25-cm β-cyclodextrin was used. (G) One 25-cm Γ-cyclodextrin column was used. (H) Two 25-cm β-cyclodextrins were used in series. (I)-(J) One 25-cm β-cyclodextrin columns was used. All separations were done at room temperature (23^{0}C).

(continued)

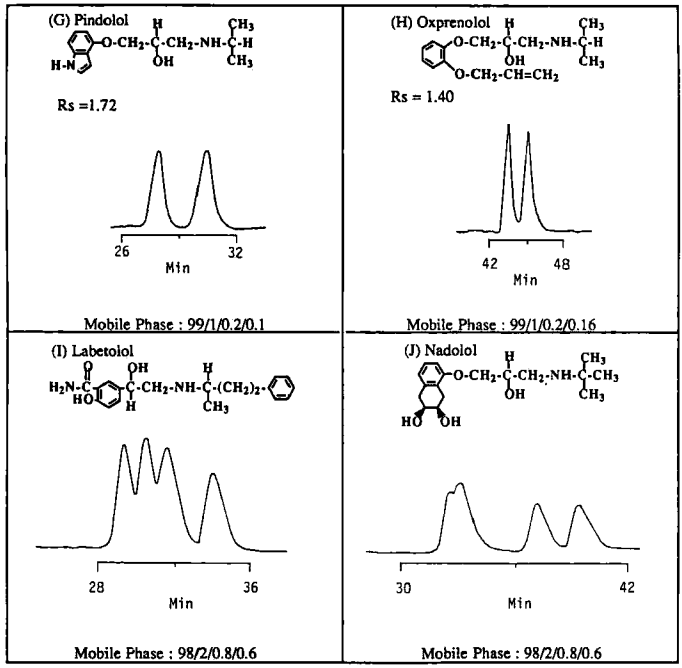

Figure 1 (continued)

separations require mobile phases consisting of polar organic solvents. The main solvent is acetonitrile (*i.e.*, 90 to 99%, by volume). However, these separations do not occur with neat acetonitrile. Small amounts of methanol are needed (*i.e.*, 1 to 10% by volume) to bring retention times down to practical levels. In addition, very small amounts of glacial acetic acid and an organic amine (*i.e.*, 0.2 to 1.2%, by volume) are needed to optimize enantioselectivity.

Propranolol (Fig. 1-A) is a typical case. The optimum mobile phase consists of 95/5/0.3/0.2, by volume of acetonitrile/methanol/acetic acid/triethylamine. Figure 2 shows that by increasing the methanol concentration relative to the other three components of the mobile phase, the

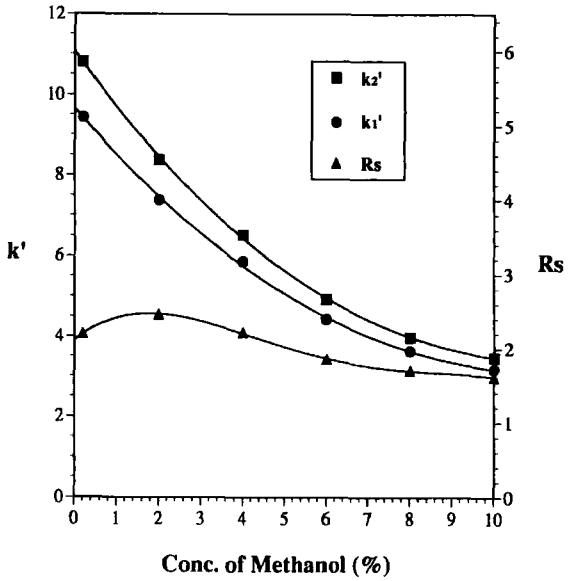

Figure 2. Effect of increasing concentrations of methanol in the mobile phase on the retention (k') and resolution (Rs) of propranolol. Conditions: One 25-cm β-cyclodextrin column was used. The mobile phase consisted of the indicated volume percent of methanol in acetonitrile to which 0.3 ml acetic acid and 0.2 ml triethylamine per 100 ml solvent were added. The flow rate was 1.0 ml/min and the UV detector wavelength was 254 nm.

retention of propranol can be significantly decreased while enantioselectivity and resolution (Rs) change only slightly. Likewise, the retention time can be decreased by increasing the total amount of acid and amine modifier without significantly affecting resolution (Figure 3). However, if the acid and amine modifiers are eliminated, enantioselectivity is greatly diminished or lost (Figure 3). The relative amount of acetic acid and triethylamine added to the mobile phase controls enantioselectivity. As seen in Figure 4, when only the

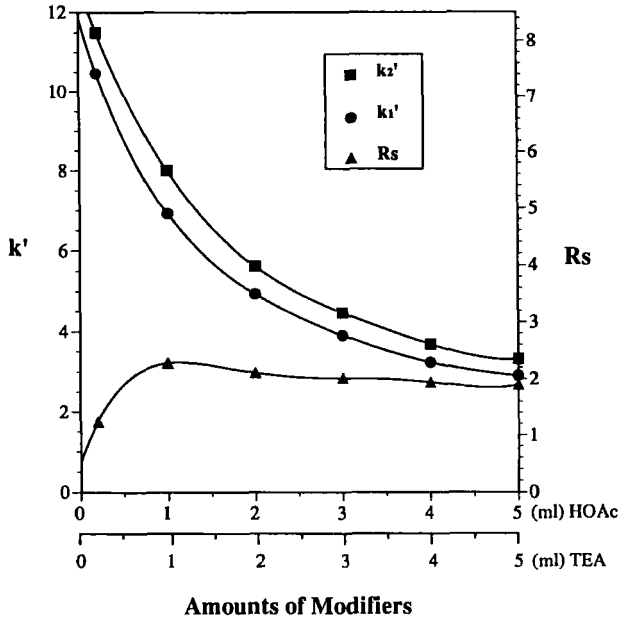

Figure 3. Effect of relative amounts of acid and amine modifiers (acetic acid and triethylamine) in the mobile phase on the resolution (Rs) and retention (k') of propranolol. There are two x-axis scales in this plot which means two kinds of additives were added at the same time. The upper axis represents the amount of acetic acid added and the bottom one represents amount of triethylamine added. Conditions: One 25-cm β-cyclodextrin column was used. The acid and amine modifiers were added to a mobile phase consisting of 490 ml acetonitrile and 10 ml methanol. The flow rate was 1.0 ml/min.and the UV detector wavelength was 290 nm.

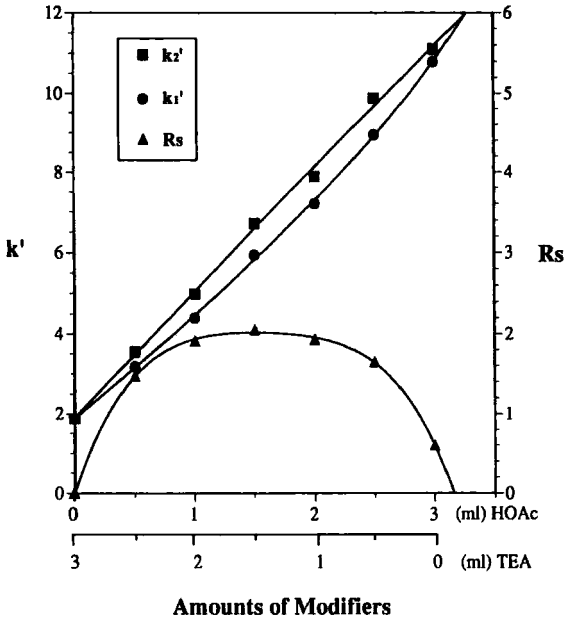

Figure 4. Effect of total amount of modifier (acetic acid and triethylamine) in the mobile phase on the resolution (Rs) and retention (k') of propranolol. There are two x-axis scales in this plot which means two kinds of additives were added at the same time; the upper one represents the amount of acetic acid added and the bottom one represents amount of triethylamine added. Conditions: One 25-cm β-cyclodextrin column was used. The indicated amount of acid and base modifiers were added to a solvent mixture consisting of 490 ml acetonitrile and 10 ml methanol. The flow rate was 1.0 ml/min and the UV detector wavelength was 290 nm.

acid or amine is present, no separation occurs. Both are necessary in order to obtain an enantioselective separation. Each beta-blocker has an optimum ratio of acid to amine where maximum enantiomeric resolution occurs. Also, Figure 4 shows that an excess of acetic acid in the mobile phase dramatically increases the retention time. This is because the acetate salt of the beta-blockers is formed upon protonation of the secondary amine. Most salts tend to elute later than their corresponding free bases when the mobile phase consists of organic solvents.

The separation mechanism for these compounds is not yet fully understood. It is doubtful that a conventional inclusion complex is formed in the absence of water and in the presence of high concentrations of polar organic solvents. In the presence of nonpolar or nonhydrogen bonding organic solvents, retention on cyclodextrin columns is thought to be due mainly to hydrogen bonding with the primary and secondary hydroxyl groups at the top and bottom of the cyclodextrin torous (6,7). Interestingly, none of the beta-blockers separations could be obtained with traditional normal phase solvents such as hexane/isopropanol mixtures. Equally relevant was the observation that the use of different size cyclodextrins (*i.e.*, α-CD or Γ-CD) under optimized mobile phase conditions (Figure 1) frequently resulted in the reduction or loss of enantioselectivity. However pindolol separated better on the Γ-CD bonded phase than the β-CD one. It appears that this "mode" of separation is different from that observed for traditional reversed phase conditions (where inclusion complexation dominates) as well as that for typical normal phase conditions (hexane/isopropanol solvents). Clearly, size selectivity is important in this mode just as it is in the reversed phase mode, even though most of these separations do not occur in the reversed-phase mode.

At the present time, several retention hypothesis are possible. Clearly hydrogen bonding is necessary but not sufficient to obtain enantioselective retention (otherwise separation would have occurred with hexane/isopropanol solvents). It is likely that both the aromatic and polar portions of the β-blockers are closely associated with the cyclodextrin. One way to accomplish this without forming a conventional inclusion complex is if the analyte sits atop of the cyclodextrin cavity like a lid. However, a good deal more mechanistic work must be done before any definitive conclusions can be drawn.

It is likely that a variety of other enantiomeric molecules, other than beta-blockers, can be separated in this mode with cyclodextrin bonded phases. These stationary phases have already been shown to be useful for preparative scale separations (8-10). As might be expected, these stationary phases exhibit long-term stability in the presence of polar organic mobile phases. They also seem to be unaffected by high flow rates (up to 4.0 ml/min) and a wide range of temperatures (0 to 70°C). Currently we are resolving a number of other classes of chiral compounds using this approach.

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