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ORIGINAL RESEARCH

Direct enantiomeric resolution of (±)-atenolol, (±)-metoprolol, and (±)-propranolol by impregnated TLC using L-aspartic acid as chiral selector

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ABSTRACT: Resolution of three commonly used β-blockers, (±)-atenolol, (±)-metoprolol and (±)-propranolol, into their enantiomers has been achieved using normal-phase TLC on silica gel plates impregnated with L-aspartic acid as the chiral selector. Different combinations of acetonitrile–methanol–water as mobile phase were found to be successful in resolving the enantiomers. The spots were detected with iodine and the detection limits were found to be 0.26 μg for atenolol and 0.23 μg for each of metoprolol and propranolol as racemate. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: (±)-atenolol; (±)-metoprolol; (±)-propranolol; enantiomerc resolution; impregnated TLC; L-aspartic acid

INTRODUCTION

The title compounds are commonly referred to as the β-adrenergic blockers and are the agents that block adrenergic stimuli, which are responsible for the stimulation of heart and inhibition of several types of smooth muscles. They are used for the treatment of hypertension, angina pectoris, supraventricular and ventricular arrhythmias and to reduce the frequency and intensity of migraine headache, among other applications. Administration of successive large doses of these drugs might lead to some side effects such as cardiac depression, blockade of cardiac vascular or bronchial β-adrenoreceptor, visual disturbance (Wilson *et al.*, 1977) etc. (−)-Propranolol is the active β-blocking enantiomer as measured by inhibition of isoprenaline-induced tachycardia (Howe and Shanks, 1966). Most of these β-blockers are marketed and therefore administered as racemic mixtures, although it has been demonstrated that their *levo* isomer only has the desired therapeutic activity (Burger, 1970), while some metabolites of D-form show signs of toxicity. The two enantiomers should be considered as different drugs and a clear picture of their pharmacodynamic and pharmacokinetic profile cannot emerge until the fate of each enantiomer is established. There is a great need to develop rapid methods for their enantiomeric resolution.

A literature survey showed enantiomeric resolution of β-blockers using HPLC without derivatization (Schmitthenner *et al.*, 1989; Ikeda *et al.*, 1989; Erlandsson *et al.*, 1990), and via derivatization (Armstrong *et al.*, 1992; Yang *et al.*, 1997). (±)-Atenolol has been resolved using capillary electrophoresis (Wren, 1995), HPLC (Miller and Guertin, 1992; He *et al.*, 1993), and HPTLC (Tivert and Beckman, 1993). (±)-Metoprolol has been resolved using HPLC (Bueschges *et al.*, 1996), and (±)-propranolol has been resolved by different chromatographic methods (Facklam and Modeler, 1994; Aboul-Enein *et al.*, 1996). These three compounds have also been resolved into their enantiomers by impregnated TLC (Bhushan and Thiong'o, 1998) using L-lysine and L-arginine as the impregnating reagents.

TLC provides direct resolution of enantiomers of a variety of compounds (Martens and Bhushan, 1990; Bhushan and Martens, 1996, 1997, 1998, 2000) and is used for its several advantages, which include parallel separation of samples, high-throughput screening, static and sequential detection for identification, and integrity of the total sample, besides being simple and less expensive. Direct resolution of enantiomers by impregnated TLC has been reviewed by Bhushan and Martens (1997, 2000, 2001).

In a quest for new suitable chiral selectors for resolution of commercial enantiomeric pharmaceuticals, the present paper reports a direct approach for the reolution and control of enantiomeric purity of these three compounds by impregnted TLC using L-aspartic acid as the chiral selector. To the best of authors knowledge there is no earlier report on the TLC resolution of these three racemic compounds using L-aspartic acid.

EXPERIMENTAL

(±)-Atenolol, was obtained from Dabur (India) Pharmaceutical

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Table 1. hR_F ($R_F \times 100$) values of enantiomers of (±)-atenolol, (±)-metoprolol and (±)-propranolol on plates impregnated with L-aspartic acid

Compound	Solvent system CH_3CN–MeOH–H_2O	Pure (+)	hR_F from mixture (+)	(−)
(±)-Atenolol	18:4:2	25	25	40
(±)-Metoprolol	10:4:1	32.5	32.5	42.5
(±)-Propranolol	16:5.0.5	25	25	41

Time, 20 min; solvent front, 12 cm; temperature, 17 ± 2°C; detection, iodine vapours.

Division (New Delhi), while (±)-metoprolol and (±)-propranolol were from CIPLA (Bombay, India). These were recrystallized with methanol–water before subjecting them to enantiomeric separation. The three recrystallized samples were examined for their optical rotation, to ensure their racemic nature, before subjecting them to resolution. Silica gel G, with 13% calcium sulphate as binder having chloride, iron and lead impurities up to 0.02% and pH 7.0 in a 10% aqueous suspension, was from E. Merck (Bombay, India). The optically pure enantiomers, (+)-atenolol, and (+)-propranolol were from Aldrich while the pure (+)-metoprolol was from Sigma (St Louis, MO, USA). The other reagents were obtained from SISCO Research Laboratory (Bombay, India) and E. Merck (Bombay, India). A polarimeter from Krüs Optronics, Germany, model R-3002, was used.

Impregnated thin layer plates (20 × 10 cm × 0.5 mm) were prepared by spreading a slurry of silica gel G (50 g) in distilled water (100 mL), containing L-aspartic acid (0.5 g), with a Stahl-type applicator. A few drops of ammonia were added to slurry to maintain the pH above the isoelectric point of the amino acid. The plates were activated overnight at 60°C. The solutions of racemic and optically pure atenolol, metoprolol and propranolol (10^{-2} M) were prepared in 70% ethanol and the spots were applied side-by-side to the plates at 10 μL level.

Chromatograms were developed at 17 ± 2°C for 20 min in each case. Paper-lined rectangular glass chambers pre-equilibrated with the solvent system for 20–25 minutes were used. Different combinations of acetonitrile, methanol and water were used and the successful systems were worked out and are given in Table 1. The developed chromatograms were dried in air and the spots were located in an iodine chamber.

RESULTS AND DISCUSSION

Different solvent mixtures used for separation of β-blockers by HPLC (Armstrong *et al.*, 1992; Aboul-Enein *et al.*, 1996; Matchett *et al.*, 1996) and TLC (Bhushan and Thiong'o, 1998) were initially selected. These solvents included combinations of acetonitrile, methanol, water, triethyl amine, hexane, propan-2-ol, etc. In the present studies, solvent mixtures from these reports were systematically modified to work out successful ones. The best separations were obtained using mixtures of acetonitrile–methanol–water. The hR_F ($R_F \times 100$) values for the resolved isomers in various solvent systems, along with those of the pure isomers in identical conditions, are given in Table 1. The results are average from at least five runs on different plates developed under identical conditions. Relative standard deviations varied between ±0.017 and ±0.020. Photograph of the chromatograms showing resolution of the three racemates on L-aspartic acid impregnated plates, are shown in Figures 1, 2 and 3, respectively, for (±)-atenolol, (±)-metoprolol and (±)-propranolol. The structures of title compounds are shown in Fig. 4.

Studies on effect of concentration of the impregnating reagent on resolution of the three analytes showed that the best resolution for all the three into their enantiomers was at 0.5% of the impregnating reagent. As the concentration was decreased to 0.4% the resolution became poor and at 0.3% no resolution was observed for any of

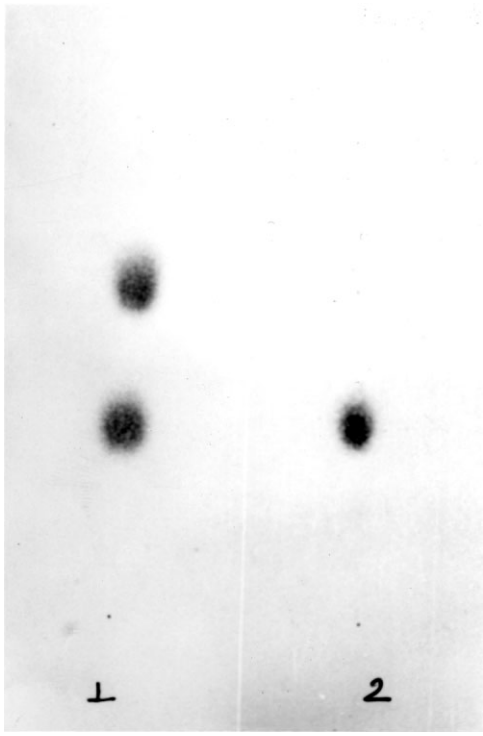

Figure 1. Photograph of an actual chromatogram showing resolution of (±)-atenolol on L-aspartic acid impregnated plate. From left to right: Spot 1: lower spot for (−)-isomer and the upper spot for (+)-isomer resolved from the mixture. Spot 2: pure (−)-isomer; Development time, 20 min; Temperature, 17 ± 2 °C; Detection, Iodine vapours.

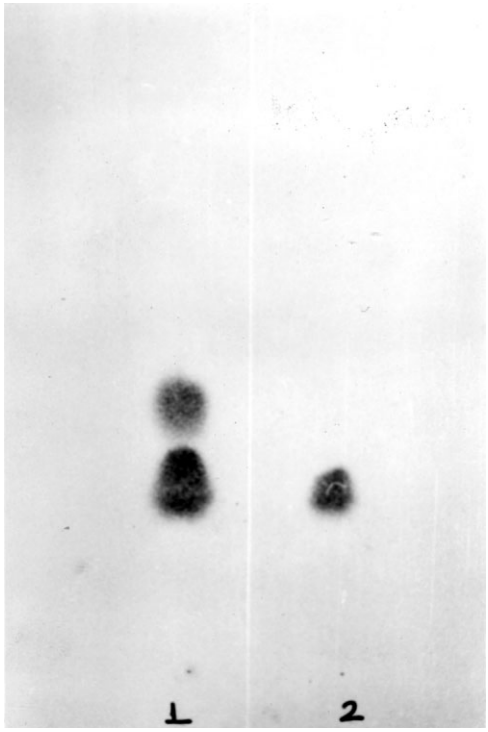

Figure 2. Photograph of an actual chromatogram showing resolution of (±)-metoprolol on L-aspartic acid impregnated plate. From left to right: Spot 1: lower spot for (−)-isomer and upper spot for (+)-isomer resolved from the mixture. Spot 2: pure (−)-isomer; Development time, 20 min; Temperature, 17 ± 2 °C; Detection, Iodine vapours.

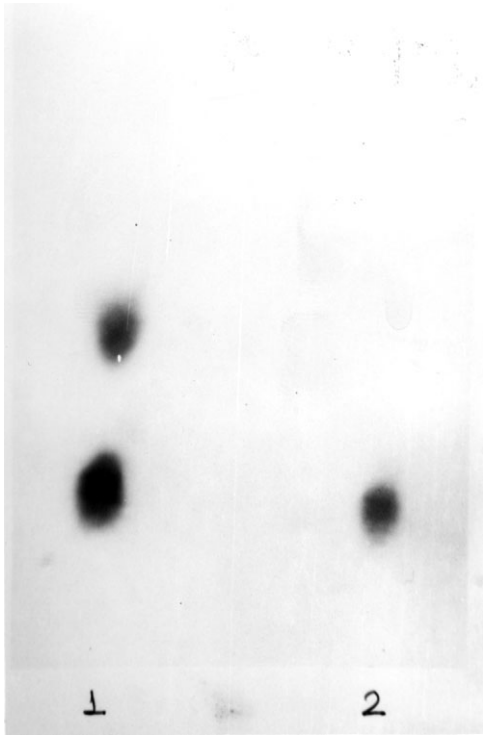

Figure 3. Photograph of an actual chromatogram showing resolution of (±)-propranolol on L-aspartic acid impregnated plate. From left to right: Spot 1: lower spot for (−)-isomer and the upper spot for (+)-isomer resolved from the mixture. Spot 2: pure (−)-isomer; Development time, 20 min; Temperature, 17 ± 2 °C; Detection, Iodine vapours.

the three β-blockers. An increase in the concentration of the chiral selector to 0.6% resulted in poor resolution while there was no resolution at 0.7% in case of all the three analytes.

Earlier studies on resolution of enantiomers (Bhushan and Thiong'o, 1998, 1999, 2000; Bhushan *et al.*, 2000; Armstrong *et al.* 1994) have shown that chiral interactions between chiral selector and analyte are affected by temperature. The investigations carried out to study the effect of temperature in present case revealed that (±)-atenolol, (±)-metoprolol and (±)-propranolol did not resolve into enantiomers at 5, 10, 15 and 20°C; while there was a tendency to resolve at 15 and at 20°C, there was poor resolution with elongated spots. Thus 17°C was found to be the right temperature for resolution in all the three cases.

Of the various factors influencing enantiomerc resolution, the effect of pH is very prominent (Bhushan and Thiong'o, 1998, 1999, 2000; Bhushan and Martens, 1996; Bhushan *et al.*, 2000; Armstrong *et al.*, 1994). Thin layer plates were prepared at pH between 4–5, 6–7 and 9–10. Resolution of enantiomers was observed in the pH range 6–7, while there was poor resolution with tailing of spots in the pH range 4–5, and there was no resolution in the pH range 9–10.

Literature revealed that, for enantioseparation of β-blockers, using HPLC, hydrogen bonding and steric interactions was effective (Armstrong *et al.*, 1992), or the resolution was effected by hydrophobic interactions in addition to hydrogen bonding and steric interactions, particularly using non-aqueous mobile phases (Matchett *et al.*, 1996), while ion pairing has been regarded as the primary interactive force between the stationary phase and the β-blockers as the analytes (Simmons and Stewart, 1994). In the present studies, the thin layer plates were impregnated with L-aspartic acid as chiral selector for enantiomeric resolution of β-blocking agents. L-Aspartic acid has a pI of 3.0 and therefore exists as anion at a pH greater than this. The β-blocking agents can exist as protonated cations (Burger, 1970; He *et al.*, 1991) and consequently these compounds can form diastereomeric ion pairs with the anionic amino acid resulting into resolution of respective enantiomers. Thus, enantioselective interactions may be due to charge-charge interaction, hydrogen bonding between -OH of β-blockers and -NH_2 of chiral selector, and steric interactions. Since enantiomeric resolutions were observed at 17°C and at other tempeatures either no resolution or poor resolution was observed, it can be suggested that only an optimum temperature provides the desired mobility to the diastereomeric ion pair and therefore any change in temperature adversely affects the resolution.

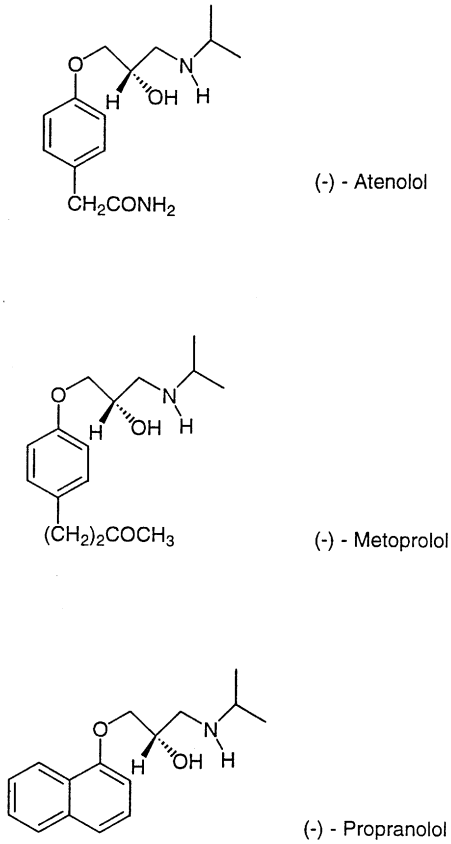

Figure 4. Structures of Atenolol, Metoprolol and Propranolol.

It was interesting to observe that a separate set of chromatogram on treatment with ninhydrin gave characteristic colored spots of aspartic acid in both the spots obtained due to resolution of enantiomers. This clearly detected the presence of chiral selector in both the resolved spots, confirming formation of diastereomers *in situ*. The detection, however, has been satisfactory with iodine. The two sopots were marked and iodine was allowed to evaporate off. The spots representing the two enantiomers of atenolol were cut (from several plates, nearly 40) and eluted with ethanol separately. The solutions were filtered and concentrated *in vaccuo*, and the concentration was determinated using calibration plots. These solutions were examined by polarimeter. Each was found to be optically pure. Therefore, diastereomers of the type (+)-atenolol-L-aspartic acid and (−)-atenolol-L-aspartic acid formed *in situ* and were separated. Since L-aspartic acid is insoluble in ethanol, only (+)- or (−)-atenolol, from the two cut spots, went into solution. Similar experiments were carried out with respect to (±)-propranolol and (±)-metoprolol to confirm enantiomeric resolution. The specific rotation values are summarized in Table 2.

The method was successful in detecting 0.26 μg of atenolol and 0.23 μg of each of metoprolol and propranolol as racemate. Thus, the method reported provides a direct approach for the resolution of enantiomers and control of enantiomeric purity of three β-blocking agents in a simple and economical manner with very low detection limits.

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Table 2. Specific rotation of β-blockers as calculated from the observed rotation and given in the literature

Sample no.	Compound	Specific rotation $(\alpha)^{25}$ D values[a]	Specific rotation $(\alpha)^{25}$ D values given in literature (Aldrich Catalog, 1999)
1	(+)-Atenolol	+16° (C = 1, 1N HCl)	+16° (C = 1, 1N HCl)
		+32° (C = 1, C_2H_5OH)	NA
	(−)-Atenolol	−16° (C = 1, 1N HCl)	−16° (C = 1, 1N HCl)
		−32° (C = 1, C_2H_5OH)	NA
2	(+)-Metoprolol	+30° (C = 1, C_2H_5OH)	NA
	(−)-Metoprolol	−30° (C = 1, C_2H_5OH)	NA
3	(+)-Propranolol	+26° (C = 1, C_2H_5OH)	+26° (C = 1, C_2H_5OH)
	(−)-Propranolol	−26° (C = 1, C_2H_5OH)	−26° (C = 1, C_2H_5OH)

NA, data not available in literature.
[a] From the present experiments.

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