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Short communication

Direct enantioseparation of some β-adrenergic blocking agents using impregnated thin-layer chromatography

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Abstract

The resolution of (±)-atenolol, (±)-propranolol and (±)-metoprolol into their enantiomers was achieved by TLC on silica-gel plates impregnated with optically pure L-lysine (0.5%) and L-arginine (0.5%) as the chiral selectors. In all cases, different combinations of acetonitrile–methanol solvent systems were found to be successful in resolving these compounds. Spots were detected using iodine vapour. The detection limit for both (±)-atenolol and (±)-propranolol was 2.6 μg and for (±)-metoprolol, it was 0.26 μg. © 1998 Elsevier Science B.V.

Keywords: Enantiomer separation; Atenolol; Propranolol; Metoprolol

1. Introduction

β-Adrenergic blocking agents are chiral hydroxylamine-containing compounds. They are synthetically produced and most of them exist as racemic mixtures. Pharmacological action of these racemates is largely confined to the levo isomers [1]. Some of the clinical uses of these drugs include treatment of hypertension, angina pectoris, supraventricular and ventricular arrhythmias and they are known to reduce the intensity of migraine headaches among others. However, β-blockers are known to have several side effects such as gastrointestinal disturbances, tiredness, dizziness, depression, paresthiasis, muscle aching, asthmatic wheezing and many others [2].

Most of the β-blockers are marketed as racemic mixtures and given the fact that these drugs have many side effects and that only the levo enantiomer is pharmaceutically active while the other is either inactive or in some cases harmful, no doubt there is great need to develop quick methods for their enantiomeric resolution. Recent experimental studies show that enantiomeric resolution of one or more β-blockers has been successfully achieved using HPLC [3–5]. (±)-Atenolol has been resolved using capillary electrophoresis [6], HPLC [7,8] and HPTLC [9]. (±)-Metoprolol has been resolved using HPLC [10], while various chromatographic methods have been used in resolving (±)-propranolol [11–13]. TLC resolution of (±)-ibuprofen on L-arginine-impregnated plates has already been reported from this laboratory [14]. There is no report on TLC resolution of β-blockers on plates impregnated with basic amino acids. In this work, we describe a direct,

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quick and cheap method for enantiomeric separation and determination of the enantiomeric purity of β-blockers on silica-gel plates impregnated with some basic amino acids.

2. Experimental

(±)-Atenolol was obtained from Dabur India pharmaceutical division (New Delhi, India). (±)-Propranolol was obtained from I.C.I. (India, Madras), India, and (±)-metoprolol was obtained from CIPLA (Bombay, India). The three racemic compounds were purified by recrystallization with MeOH–H_2O before subjecting them to enantiomeric resolution. Silica-gel G with 13% calcium sulphate as binder having chloride, iron and lead impurities up to 0.02% and with pH 7.0 in a 10% aqueous suspension, was from E. Merck (Bombay, India). The other reagents and chemicals were used of analytical reagent grade and were obtained from SISCO Research Laboratory (Bombay, India) and E. Merck (Bombay, India). L-Lysine and L-arginine were taken in their free form.

Impregnated thin-layer plates (10 cm×20 cm×0.5 mm) were prepared by spreading a slurry of silica-gel G (30 g) in distilled water (60 ml containing 0.3 g of L-lysine or L-arginine), with a Stahl-type applicator. In each case, a few drops of ammonia solution were added to maintain the pH above the isoelectric points of the amino acids. The plates were dried overnight at 60°C. The solutions of racemic mixtures of (±)-propranolol (10^{-2} *M*), (±)-metoprolol (10^{-3} *M*), (±)-atenolol (10^{-2} *M*) and their pure isomers were prepared in 70% ethanol in the same concentration and were applied to the plates at 10-μl level. The spots of each racemic compound and their respective (−)-isomer were applied side by side on the same plate.

Chromatograms were developed at 15±2°C for 40 min in the case of (±)-atenolol while for (±)-propranolol and (±)-metoprolol, they were developed at 20±2°C for 40 min. Chromatograms were developed in paper-lined rectangular glass chambers preequilibrated with the solvent system acetonitrile–methanol for 10–15 min. Successful solvent systems worked out in each experiment are given in Table 1. The developed plates were dried at 60°C for 15 min and the spots were located in an iodine chamber.

Table 1
hR_F (R_F×100) values of enantiomers of (±)-atenolol, (±)-propranolol and (±)-metoprolol on impregnated plates with L-lysine (0.5%) and L-arginine (0.5%)

Compound	Impregnating reagent	Solvent system acetonitrile–methanol	hR_F values		
			Pure	From racemic mixture	
			(−)	(−)	(+)
(±)-Atenolol	L-lysine	16:4	3	3	10
		16:2	3	3	6
		15:2	4	4	7
	L-arginine	15:5	8	8	12
		14:6	22	22	30
(±)-Propranolol	L-lysine	15:2	4	4	13
		16:2	4	4	15
	L-arginine	15:3	7	7	26
		15:4	14	15	39
(±)-Metoprolol	L-lysine	15:4	11	11	15
		15:5	15	15	25
	L-arginine	15:3	6	6	17
		16:4	12	12	23

Time: 35–40 min; solvent front: 13 cm, (12 cm for atenolol); detection: iodine vapour; temperature: 22±2°C for both (±)-propranolol and (±)-metoprolol; 15±2°C for (±)-atenolol.

3. Results and discussion

A number of solvent mixtures used in enantioseparation of β-blocking drugs using HPLC [3,12,15] were selected. These solvents included acetonitrile, methanol, water, triethylamine, hexane and propan-2-ol. In our studies, solvent mixtures from these reports were systematically worked out. The best separations were obtained using acetonitrile–methanol solvent mixtures. The hR_F ($R_F \times 100$) values for the resolved isomers of the three racemates in various solvent systems are given in Table 1. These are averages of at least five identical runs.

The effect of the concentration of impregnating reagents with silica-gel was investigated. It was observed that the best resolution was at 0.5% of both the impregnating reagents for all the three racemates. As the concentration was decreased to 0.4% and 0.3%, the resolution became poorer in all the solvent combinations. An increase in the concentration of the impregnating reagent to 0.6% resulted in poor resolution of (±)-metoprolol, while there was no resolution for both (±)-atenolol and (±)-propranolol in various combinations of the solvents, as given in the Table 1. Photographs of the chromatograms showing resolution of (±)-atenolol and (±)-metoprolol on L-lysine impregnated plates are shown in Figs. 1 and 2, respectively.

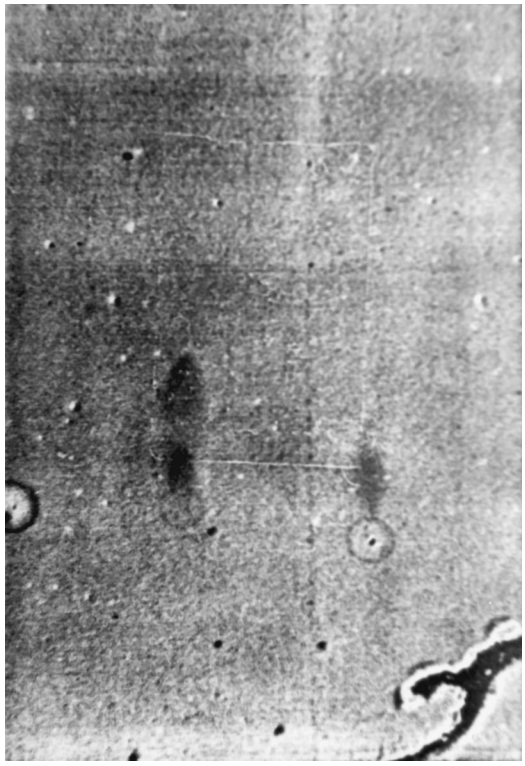

Fig. 1. Photograph of the chromatogram showing resolution of (±)-atenolol on a L-lysine impregnated plate. From L to R: Spot 1: lower spot for (−)-isomer and the upper spot for (+)-isomer resolved from the mixture. Spot 2: pure (−)-isomer.

Literature reveals that chiral recognition decreases with an increase in temperature [16]. Therefore, the effect of temperature on enantioseparation of these compounds was also investigated. It was observed that resolution occurred only at 15±2°C for (±)-atenolol and there was no resolution at 30°C, 25°C or 20°C; it was interesting to observe that (±)-atenolol did not resolve at a temperature of 8°C or 0°C also. The same studies with both (±)-propranolol and (±)-metoprolol showed best resolution at 22±2°C; an increase of temperature to 32°C resulted in tailing of the resolved spots of enantiomer of the compounds while a decrease in temperature upto 6°C had little or no effect on resolution. It was interesting to note that (±)-propranolol gave a good resolution with MeCN–MeOH mixture 15:3 at 6°C.

Enantioseparation may be possible via π–π complexation, hydrogen bonding, inclusion in an hydrophobic pocket, dipole stacking, steric interactions or combinations thereof. Bhushan and Parshad [14] considered formation of diastereomers via ion-pair formation leading to enantiomeric resolution of (±)-ibuprofen on plates impregnated with L-arginine. Simmons [4] using HPLC proposed that for enantioseparation of β-blockers, ion-pairing is the primary interactive force between the stationary phase and the analyte. According to Armstrong et al. [3], hydrogen bonding and steric interactions were responsible for the resolution of certain β-blockers. Further, Matchett et al. [15] using HPLC and nonaqueous mobile phases proposed that enantiomeric resolution of the same compounds was also effected by hydrogen bonding, hydrophobic and steric interactions. In the present study, the thin-layer plates used were impregnated with L-lysine or L-arginine as chiral selectors for enantiomeric resolution of β-adrenergic blocking agents. Both L-lysine and L-arginine have a pI of 9.8 and 10.8

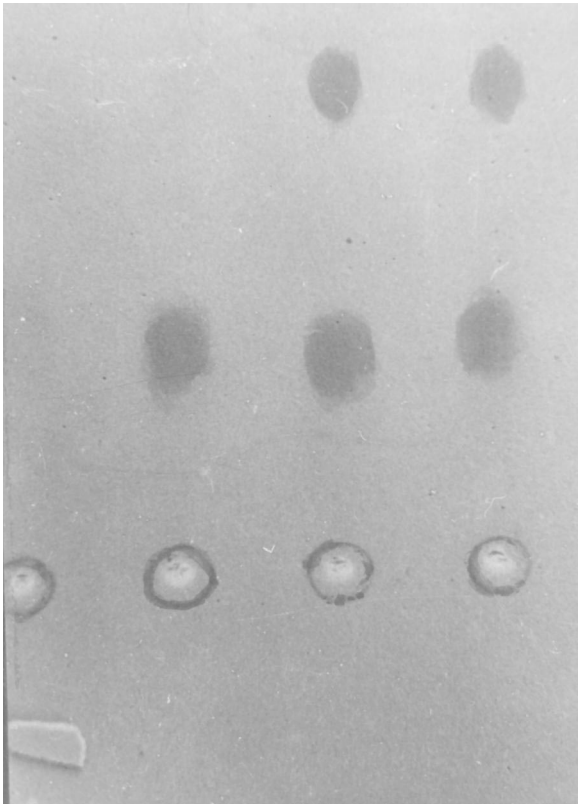

Fig. 2. Photograph of the chromatogram showing resolution of (±)-metroprolol on L-lysine impregnated plates. From left to right; spot 1, pure (−)-isomer; spot 2, 15 μl of 10^{-3} *M* solution of racemic mixture; and spot 3, 10 μl of the same solution; in each case the lower spot corresponds to the (−)-isomer and the upper spot corresponds to the (+)-isomer resolved from the mixture.

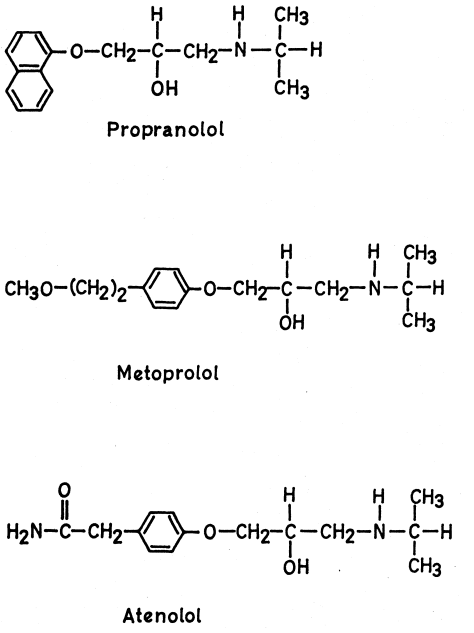

Fig. 3. Chemical structures of β-blocking drugs investigated.

respectively. At a pH greater than their p*I*, these amino acids are anionic. The β-blocking agents can exist as protonated ammonium cations [1,17] and consequently, these compounds can form diastereomers with the anionic amino acid resulting into resolution of their respective enantiomers. Fig. 3 shows structures of the compounds investigated. 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 interations.

Besides locating the spots in an iodine chamber, a separate set of the chromatograms was treated with ninhydrin. It gave a characteristic colour indicating the presence of lysine or arginine in both the spots obtained due to resolution. The experiment with ninhydrin clearly detected the presence of the chiral selector (lysine or arginine) in both the spots, confirming formation of diastereomers in situ. Although the whole plate acquires a light pink background, the resolved spots are visible with a greater characteristic colour intensity and sharpness of the spots. Nevertheless, the detection is satisfactory with iodine.

The method was successful in resolving as little as 2.6 μg for both (±)-atenolol, and (±)-propranolol while for (±)-metoprolol it was 0.26 μg. It is a direct, simple, rapid and economical method and has advantage over other general indirect methods that involve chiral derivatization prior to chromatography or other expensive experimental set up.

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References

[1] A. Burger, Medicinal Chemistry Part II, 3rd ed., Wiley-Interscience, New York, 1970, p. 103, 1246.
[2] F.H. Meyers, E. Jawetz, A. Goldfien, A Review of Medical Pharmacology, 7th ed., Lange Medical Publications, Los Altos, 1980, p. 95.
[3] D.W. Armstrong, S. Chen, C. Sang, S. Chang, J. Liq. Chromatogr. 15 (1992) 545.
[4] B.R. Simmons, T.J. Stewart, J. Liq. Chromatogr. 17 (1994) 2675.
[5] C.J. Welch, S.R. Perrin, J. Chromatogr. A 690 (1995) 218.
[6] S.A.C. Wren, Electrophoresis 16 (1995) 2127.
[7] R.B. Miller, Y. Guertin, J. Liq. Chromatogr. 15 (1992) 1289.
[8] J. He, A. Shibukawa, T. Nakagawa, H. Wada, H. Fujima, E. Imai, Y. Go-Oh, Chem. Pharm. Bull. 41 (1993) 544.
[9] A.-M. Tivert, A. Backman, J. Planar Chromatogr.-Mod. TLC 6 (1993) 216.
[10] R. Bueschges, R. Devant, E. Mutschiler, H. Spahn-Langguth, J. Pharm. Biomed. Anal. 15 (1996) 201.
[11] C. Facklam, A. Modler, J. Chromatogr. A. 664 (1994) 203.
[12] H.Y. Aboul-Enein, I. Laila, S.A. Bakr, Chirality 8 (1996) 153.
[13] Y. Abe, T. Shoji, S. Fakui, M. Sasamoto, H. Nishizawa, Chem. Pharm. Bull. 44 (1996) 1521.
[14] R. Bhushan, V. Parshad, J. Chromatogr. A 721 (1996) 369.
[15] M.W. Matchett, S.K. Branch, T.M. Jefferies, Chirality 8 (1996) 126.
[16] G.D.Y. Sogah, D.J. Cram, J. Am. Chem. Soc. 98 (1976) 3038.
[17] H. He, G. Uray, O.S. Wolfbeis, Proceedings of SPIE-The International Society for Optical Engineering 1368 (1991) 175.