

NEW CHIRAL REVERSE PHASE HPLC METHOD FOR DETERMINATION OF ATENOLOL ENANTIOMERS IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, specific, precise, sensitive and rapid reverse phase-HPLC method was developed for determination of atenolol enantiomers, a beta₁ adrenergic receptor blocker in pharmaceutical formulations. The method was developed on a Chiralcel AGP column (150 x 4.0 mm I.D., particle size 5 µ); the mobile phase was 10 mM sodium phosphate buffer (pH- 7.0)-methanol- (95:5 v/v); ultraviolet detection was at 225 nm; and flow rate was 0.9 ml/min. The linearity range was 10 to 100 µg/ml. The intra-day and inter-day coefficient of variation of the assay method were found to be 2.69 to 3.97 and 7.05 to 9.43 respectively, with high accuracy and precision results. The proposed RP-HPLC method is suitable for analysis of atenolol enantiomers in different pharmaceutical dosage forms.

Keywords: R-atenolol, S-Atenolol, enantiomers, RP-HPLC, Tablets.

INTRODUCTION

Atenolol is a cardioselective beta₁ adrenoceptor blocker devoid of intrinsic sympathomimetic and membrane-stabilizing activity. It is mainly used in angina pectoris, certain arrhythmias and systemic hypertension. They are also efficacious in several other cardiovascular disorders such as atrial fibrillation, flutter, hypertrophic cardiomyopathy, myocardial infarction, and sinus tachycardia, including objective signs of anxiety. It is well known that the enantiomers of chiral drugs can present differences in pharmacological activity or efficacy¹. Chemically it is (RS) - 4-(2-hydroxy-3-isopropylaminopropoxy) phenyl acetamide. It is well known that the enantiomers of chiral drugs can present differences in pharmacological activity or efficacy. Enantioselective analytical techniques have therefore become more and more important in the field of drug analysis. Enantiomeric separations are often performed nowadays by liquid chromatography (LC). The use of chiral stationary phases (CSPs) is by far the most popular way to perform enantioselective LC assays. Among the numerous CSPs currently available, those based on the utilization of a protein as the chiral selector have become increasingly popular due to their wide range of applicability. The Chiral-AGP stationary phase is obtained by immobilizing α₁-acid glycoprotein on silica². This CSP was used for the enantioselective determination of a series of drugs in bulk form or in biological fluids. Beta-adrenoreceptor blocking agents are well known to be strongly affected by chirality. The chiral separation of beta-blocking drugs can be performed by means of different chromatographic techniques, with gas chromatography (GC), thin-layer chromatography (TLC) or LC being the most frequently used techniques. In LC, numerous CSPs were used for the separation of the enantiomers of beta-blocking drugs. Some CSPs consisted of immobilized proteins such as bovine serum albumin (BSA), α₁-acid glycoprotein, cellulobiohydrolase and ovomucoid. Other ones were based on the use of

cyclodextrins or cellulose derivatives, especially cellulose Tris(3,5-dimethylphenylcarbamate), as chiral selectors. Some Pirkle-type columns were also reported to be suitable for achieving chiral separation of beta-blockers. More recently, the enantioseparation of beta-blocking drugs was also achieved in capillary electrophoresis (CE) by adding derivatized cyclodextrins as chiral selectors to the running buffer³⁻²³. The separation of atenolol enantiomers could be achieved on another normal phase Chiral OD column, but this method was very expensive²⁴.

The aim of this study was to develop a simple, specific, sensitive and rapid reverse phase chiral high performance liquid chromatographic method for the determination of atenolol enantiomers in pharmaceutical formulations using the chiral AGP column with UV detection.

MATERIALS AND METHODS

Chemicals and Reagents:

Atenolol enantiomers were a gift sample from M/s Emcure Pharmaceutical Ltd. Pune, India. Methanol used was of HPLC grade (Merck, India). Sodium di-hydrogen phosphate, Sodium hydroxide and Phosphoric acid GR grade was obtained from Merck, India. Commercially available racemic atenolol tablets claimed to contain 50 mg of drug were procured from local market.

Instrumentation:

Quantitative RP-HPLC was performed on gradient high-pressure liquid chromatography (Shimadzu, Japan) auto sampler consisting of a LC-20HT solvent module, SPD-10A, and an UV-Visible Spectrophotometric detector with LC 10 software. The column used was Chiral-AGP column (150 x 4.0 mm I.D., particle size 5 µ, Chrom tech Ltd, Sweden).



HPLC conditions:

The composition of the mobile phase was 10 mM sodium phosphate buffer (pH 7.0) and methanol in the ratio of 95:05 v/v. They were filtered before use through a 0.2 µm membrane filter, degassed in a bath sonicator for 10 min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 0.9ml/min, which yielded a column backpressure of 150- 180 kg/cm². The run time was set at 30.0 min and column temperature was ambient. The volume of injection loop was 20 µl. prior to injection of drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 225 nm and data was acquired, stored and analyzed with the LC 10 software. The sensitivity was set to 0.005 AUFS.

Calibration curves:

Primary stock solutions of 1 mg/ml R-atenolol and S-atenolol were prepared separately by dissolving 25 mg of R-atenolol and 25 mg of S-atenolol in a 25 ml volumetric flasks containing water respectively. Daily working standard solutions of R-atenolol and S-atenolol were prepared by suitable dilution of primary stock solutions with water. Six sets of the drug solutions were prepared in water containing R-atenolol and S-atenolol at a concentration of 10 to 100 µg/ml. Each of these drug solutions (20 µl) was injected six times in to column and the peak area and retention time was recorded. The calibration curves were constructed by plotting the peak areas against the concentration of R-and S-atenolol in µg/ml.

Assay validation:

The intra and inter-run precision and accuracy of the assay (n=6) were determined by percent C.V and percent error values, respectively, based on reported guidelines.²⁵ Samples containing 10.0, 40.0 and 100 µg/ml concentrations were spiked for the determination of precision and accuracy. Six replicates at each concentration were prepared from primary stock solutions on day 1, 3, 5 and 10 to determine intraday and inter day precision and accuracy. The intraday precision of the assay was determined by analyzing six spiked samples at each concentration on the same day and inter day precision was determined by analyzing the samples on four different days. The percent error values were calculated by the following equation.

$$\text{Percent error} = \frac{(\text{Calculated concentration} - \text{Added concentration})}{\text{Added Concentration}} \times 100$$

Procedure for pharmaceutical formulations:

Twenty tablets were weighed to obtain the average tablet weight, and were then powdered. A sample of the powdered tablets, claimed to contain 50 mg of active ingredient of racemic atenolol was mixed in 50 ml of water in 100ml volumetric flasks for 30 min. finally made up to the volume with same solvent. This mixture was shaken well with intermittent sonication for 10 min to ensure complete solubility of the drug and was centrifuged at high speed and supernatant was collected and filtered through a 0.45 µm membrane filter. An aliquot of these solutions were diluted with water and spiked in to column, peak area and retention time was recorded.

Table 1: Intra and Inter-day accuracy and precision of R-atenolol assay in proposed HPLC method

Concentration of R-atenolol (µg/ml)	Observed concentration of R-atenolol					
	Intra day			Inter day		
	Mean ± SD*	% C.V	% Error	Mean ± SD*	% C.V	% Error
10	10.21 ± 0.04	3.97	1.84	09.84 ± 1.04	8.16	4.00
40	39.91 ± 0.22	3.07	1.38	38.98 ± 1.47	6.98	1.03
100	100.35 ± 0.87	3.19	2.53	99.62 ± 2.17	7.05	1.22

*Readings are mean of six observations (n=6)

Table 2: Intra and Inter-day accuracy and precision of S-atenolol assay in proposed HPLC method

Concentration of S-atenolol (µg/ml)	Observed concentration of S-atenolol					
	Intra day			Inter day		
	Mean ± SD*	% C.V	% Error	Mean ± SD*	% C.V	% Error
10	10.06 ± 0.04	3.92	2.00	10.53 ± 0.05	9.43	6.00
40	40.11 ± 0.22	2.69	1.38	39.93 ± 0.56	7.06	-0.88
100	100.76 ± 0.87	2.84	2.53	99.28 ± 2.59	8.57	0.93

*Readings are mean of six observations (n=6)



Table 3: Mean (\pm SD) amount of atenolol enantiomers in tablets estimated by Proposed RP-HPLC method.

Labeled amount of Racemic atenolol (mg)	Observed amount of R-enantiomer (%)	Observed amount of S-enantiomer (%)
50	25.24	24.76

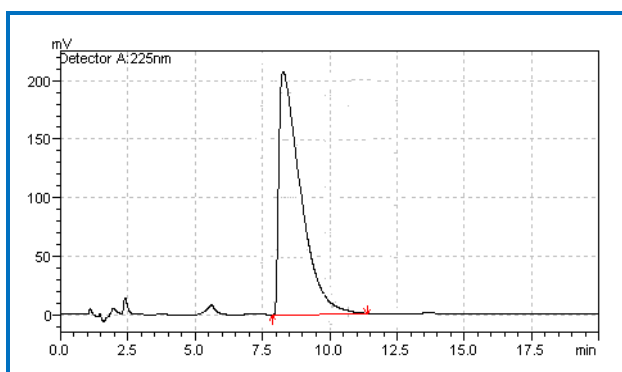
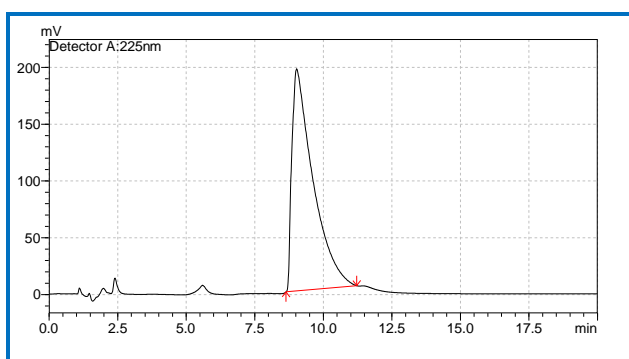
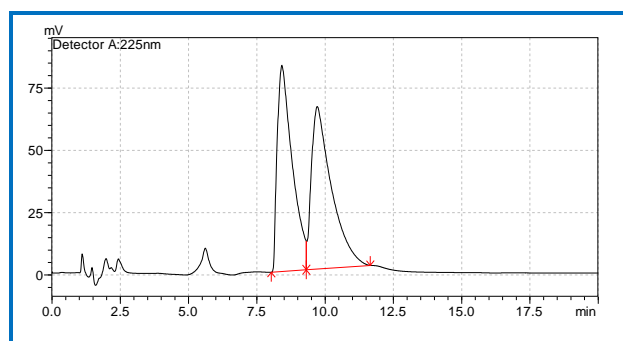
Table 4: Experimental values obtained in a recovery test for atenolol enantiomers in tablets by HPLC method (mean \pm SD)

Amount of atenolol enantiomers added (μ g) to powdered tablet formulations	Recovery from powdered tablet formulation			
	Amount of R-atenolol found (μ g)*	% Recovery*	Amount of R-atenolol found (μ g)*	% Recovery*
10.0	9.98 \pm 0.06	99.85 \pm 1.02	9.87 \pm 0.08	98.27 \pm 1.02
30.0	30.24 \pm 0.15	100.8 \pm 4.25	30.08 \pm 0.24	102.66 \pm 4.25
50.0	49.76 \pm 1.22	99.52 \pm 2.22	50.64 \pm 0.83	101.28 \pm 2.22

*Readings are mean of six observations (n=6)

RESULTS AND DISCUSSION

The development of RP-HPLC method for the determination of enantiomers has received a considerable attention in recent past because of its importance in the quality control of drugs and drug products. The assay of atenolol enantiomers was resolved with good accuracy. The retention time (t_R) of R-atenolol and S-atenolol were found to be 7.58 and 9.14 min, respectively. A typical chromatogram of R-atenolol and S-atenolol is shown in Fig. 1. Tailing factor was less than 0.6 for both R-atenolol and S-atenolol. The calibration curve was constructed by plotting the peak areas against the concentration of R- and S-atenolol in μ g/ml.

**Figure 1a:** (R)-atenolol standard (7.58 min)**Figure 1b:** (S)-atenolol standard (9.14 min)**Figure 1c:** (R)-atenolol (8.19 min) and S-atenolol (9.41min) form racemic tablet.

It was found to be linear with a correlation coefficient of 0.9992 for R-atenolol and 0.9999 for S-atenolol, the representative linear regression equation being $y = 41598x + 35094$ and $y = 40291x + 33120$ for both the enantiomers respectively. In the range of 10 - 100 μ g/ml, the relative standard deviations based on the peak area for six triplicate injections were found to be between 0.6% and 3%. The intraday and inter day coefficient of variation (%CV) and error (%) values were shown in Table 1 & 2. These values were within the limits (<15%) specified for inter and intraday precision^{26, 27}.

The chiral RP-HPLC method developed in the present investigation was used to quantify atenolol enantiomers in tablet dosage forms. The obtained results are given in Table 3. The average drug content was found to be 25.24 mg for R-atenolol and 24.76 mg for S-atenolol of the labeled amount in 50mg of racemic atenolol, respectively. No interfering peaks were found in chromatogram, indicating that the tablet excipients did not interfere with the estimation of drug thereby confirming the specificity of the proposed HPLC method. To determine the percent recovery the known amount of enantiomeric solutions were added to a powdered sample of tablet dosage form and estimated by the proposed method.

The amount of atenolol enantiomers in the matrix was calculated using following formula.

$$\% \text{ Recovery} = \frac{T - A}{S} \times 100$$

T–total amount of drug estimated, A-initial amount of drug in the tablet powder and S- amount of pure drug added. The results revealed (Table 4), high recovery of atenolol enantiomers, indicating that the proposed method for the determination of atenolol enantiomers in the tablet is highly accurate.

CONCLUSION

The experimental results of the present study showed that the proposed RP-HPLC method is simple, specific, precise, sensitive, rapid and accurate and is useful for estimation of atenolol enantiomers in its pharmaceutical formulations.

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