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COMMUNICATION

Enantiomeric Separation and Quantitative Determination of Atenolol in Tablets by Chiral High-Performance Liquid Chromatography

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ABSTRACT

The separation and quantitative determination of atenolol isomers by chiral high-performance liquid chromatography (HPLC) are described. Atenolol isomers were separated using a Chiralcel OD® column (250 × 4.6 mm, 10 μm); the mobile phase was hexane-ethanol-diethylamine (75:25:0.1 v/v/v); ultraviolet detection was at 276 nm; and flow rate was 0.7 ml/min. The coefficient of variation and average recovery of (R)-isomer were 0.60% and 100.37%, respectively, for sample A and 0.69% and 100.63%, respectively, for sample B. The coefficient of variation and average recovery of (S)-isomer were 0.59% and 100.33%, respectively, for sample A and 0.63% and 99.78%, respectively, for sample B.

INTRODUCTION

Beta-blockers are mainly used in angina pectoris, certain arrhythmias, and systemic hypertension. They are also efficacious in several other cardiovascular disorders, such as arterial fibrillation, flutter, hypertrophic cardiomyopathy, myocardial infarction, and sinus tachycardia, including objective signs of anxiety (1). Currently, besides beta-blockers, many therapeutic drugs possess one or more chiral centers. There are many papers in the scientific literature that describe the use of chiral high-performance liquid chromatography (HPLC) for the de-

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termination of optical purity and isomeric composition of chemical substances, pharmaceuticals, and biological materials and in pharmacokinetics and drug metabolic studies (2–6), but seldom in quantitative analysis of enantiomers in pharmaceutical preparations. The atenolol molecule has a chiral center, and it is used therapeutically in its racemic form, as are the majority of the beta-blockers. Among the chiral columns used for beta-blocker enantiomeric separation, Chiralcel OD® is one of the most efficient (6–8). The aim of this work was to develop and to standardize a chiral HPLC method that enables the separation and quantitative determination of atenolol isomers contained in commercially available tablets.

EXPERIMENTAL

Apparatus

The HPLC separations were made on a system consisting of a CG solvent delivery pump (model 480-C) and a CG variable UV detector set at 276 nm connected to a CG integrator (model CG-200) (Instrumentos Científicos CG Ltda, São Paulo, Brazil). The system was equipped with a Rheodyne 7125 injection valve equipped with a 20-μl loop.

Reagents and Solutions

All reagents and solvents were analytical grade. Hexane (Omnisolv) and ethanol (Merck) were HPLC grade. Diethylamine (Aldrich) was analytical grade. Solutions and mobile phases were prepared on the same day, and all solvents and solutions for HPLC analysis were filtered through a membrane filter (Millipore® Durapore hydrophobic filtration membrane, 0.22-μm pore size) and vacuum degassed before use. Atenolol isomers were provided by Aldrich and were used as standards.

Samples

Commercially available tablets containing 50.0 mg of racemic atenolol (samples A and B) were used in this research.

Chromatographic Conditions

The mobile phase used was hexane:ethanol:diethylamine (75:25:0.1 v/v/v). The analytical column was a Chiralcel OD (250 × 4.6 mm, 10 μm) column. All analyses were done at room temperature under isocratic conditions with a flow rate of 0.7 ml/min.

Calibration Curves

Solutions of (R)- and (S)-atenolol isomers ranging from 10.0 to 190.0 μg/ml were prepared in the mobile phase. The calibration curves were constructed by plotting the peak areas against the concentration of (R)- and (S)-atenolol in μg/ml.

Sample Preparation

An amount of sample A or B equivalent to 25.0 mg of (R)-atenolol and 25.0 mg of (S)-atenolol was accurately weighed and transferred to a 50-ml volumetric flash. About 40 ml of absolute ethanol was added, the flask was placed in an ultrasonicator for 7 min, and the volume was made up with the same solvent. After filtration through a Whatman no. 1 paper filter, a 5.0-ml aliquot was transferred to a 25-ml volumetric flask and diluted to volume with the mobile phase. The obtained solution contained 100.0 μg of (R)-atenolol and 100.0 μg of (S)-atenolol/ml. After filtration, the solutions were injected (20 μl) into the HPLC system (10 injections of each sample solution and 3 of each standard isomer solution).

RESULTS AND DISCUSSION

The chromatograms of atenolol enantiomers can be observed in Fig. 1. The isomers were separated using a

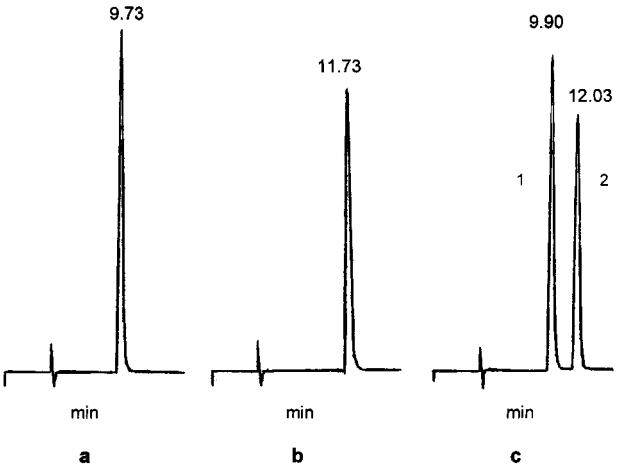

Figure 1. Chromatogram of atenolol using a Chiralcel OD column, hexane:ethanol:diethylamine (75:25:0.1 v/v/v) mobile phase, 0.7-ml/min flow rate, 276 nm UV detection, at ambient temperature: (a) (R)-atenolol standard (100.0 μg/ml); (b) (S)-atenolol standard (100.0 μg/ml); and (c) (R)-atenolol (1) 100.0 μg/ml and S-atenolol (2) 100.0 μg/ml.

Table 1

Statistical Representation of the Data Obtained in the Analysis of Commericially Available Samples (A and B) Using Chiral High-Performance Liquid Chromatography

Sample	Standard Deviation	Coefficient Variation (%)	Confidence Limit (%)
(R)-isomer			
A	0.55	0.60	92.41 ± 0.39
B	0.68	0.69	98.93 ± 0.40
(S)-isomer			
A	0.55	0.59	92.70 ± 0.39
B	0.62	0.63	99.15 ± 0.45

mobile phase consisting of a mixture of hexane:ethanol:diethylamine (75:25:0.1 v/v/v). It is well documented that columns like Chiralcel OD lose efficiency when a pressure greater than 700 psi is used in an analysis (9). Keeping in mind the pressure limitation of the column, a flow rate of 0.7 ml/min was chosen to maintain column pressure below 400 psi. Based on the UV spectral scanning, detection wavelength was set at 276 nm to prevent interference from excipients and solvents during HPLC analysis.

Calibration curves of (R)- and (S)-atenolol isomers were obtained in a concentration range from 10.0 to 190.0 μg/ml with a correlation coefficient of 0.9999. The precision of an analytical method can be obtained by the coefficient of variation (CV), and to be considered precise, a CV should be less than 2.00% (10). The results of statistical data obtained in the analysis of commercially available samples are shown in Table 1. The percentages of recovery results are presented in Table 2. The recovery tests and the percentage of recovery were performed according to the recommendations of the Association of Official Analytical Chemists (AOAC) International (11). The results obtained confirm the accuracy of the method. Table 3 presents the results of the relation between atenolol isomers calculated according to the USP 23 (12).

Table 2

Recovery of (R)- and (S)-Atenolol Isomers Standard Solutions Added to Commericially Available Samples (A and B) Using Chiral High-Performance Liquid Chromatography

Sample	Amount Added (μg)	Amount Found[a] (μg)	Recovery (%)
(R)-isomer			
A	20.00	20.17	100.83
	60.00	60.02	100.04
	100.00	100.26	100.26
B	20.00	20.02	100.11
	60.00	61.09	101.82
	100.00	99.98	99.98
(S)-isomer			
A	20.00	20.14	100.71
	60.00	60.12	100.20
	100.00	100.10	100.10
B	20.00	19.84	99.18
	60.00	60.72	101.20
	100.00	98.97	98.97

[a] Average of two determinations.

Table 3

Relation Between Atenolol Isomers Determined in Commercially Available Samples (A and B) Using Chiral High-Performance Liquid Chromatography P 23

Sample	(R)-Isomer (%)	(S)-Isomer (%)
A	50.30	49.70
B	50.50	49.50

CONCLUSIONS

The proposed chiral HPLC enabled the separation and quantitative determination of (R)- and (S)-isomers of atenolol in commercially available tablets. UV detection at 276 nm was found suitable without any interference from tablet excipients and solvents. The calibration curves of (R)- and (S)- isomers obtained in a concentration range from 10.0 to 190.0 μg/ml were linear with a correlation coefficient of 0.9999 for both (R)- and (S)-isomers. The coefficients of variation of (R)- and (S)-isomers were 0.60% and 0.59%, respectively, for sample A and 0.69% and 0.63%, respectively, for sample B. Recovery tests confirmed the accuracy of the method. The preparation of samples was easy and efficient. There was no excipient interference in the method. The proposed chiral HPLC method is fast, precise, accurate, sensitive, and efficient.

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