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RESEARCH PAPER

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

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ABSTRACT

This work reports an application of chiral high-performance liquid chromatography (HPLC) in the separation and quantitative determination of propranolol isomers in tablets. The isomers were separated using a Chiralcel OD® column (250 × 4.6 mm, 10 μm) with a mobile phase of hexane : ethanol (75 : 25 v/v) at a flow rate of 0.7 ml/min and ultraviolet detection at 280 nm. The coefficient of variation and average recovery of (R)-isomer for samples A, B, C, and D were 0.72% and 100.30%, 0.67% and 99.40%, 0.62% and 99.76%, and 0.70% and 99.68%, respectively. The coefficient of variation and average recovery of (S)-isomer for samples A, B, C, and D were 0.74% and 99.62%, 0.64% and 100.27%, 0.71% and 99.99%, and 0.70% and 99.72%, respectively.

Key Words: *Chiral HPLC; Enantiomeric separation; Pharmaceutical preparations; Propranolol*

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INTRODUCTION

Currently, many of the pharmaceuticals used in therapeutics are chiral drugs. Propranolol is a beta blocker widely used to control hypertension, migraine headaches, and angina pectoris (1–3). The propranolol molecule has a chiral center, and it is used in its racemic form as a majority of the beta blockers, although it is a well-known fact that the (S)-isomers are more potent than the (R)-isomers. It has been reported in the scientific literature that some (R)-isomers are toxic and present undesirable side effects (4).

Among the chiral columns being used for beta blocker enantiomeric separation, Chiralcel OD® is one of the most efficient (4–6). This column can be considered as a normal-phase column for traditional chromatography, and the enantiomeric resolution is based on the formation of an inclusion complex between the drug and the helicoidal cavity of the modified cellulose polymer (7,8).

The aim of this work was to develop and to standardize a chiral high-performance liquid chromatographic (HPLC) method that enables the separation and quantitative determination of propranolol 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 ultraviolet (UV) detector set at 280 nm connected to a CG integrator (model CG-200) (Instrumentos Científicos CG Ltda, São Paulo, Brasil). The system was equipped with a Rheodyne® 7125 injection valve fitted with a 20-μl loop.

Reagents and Solutions

All reagents and solvents were analytical grade. Hexane (Omnisolv) and ethanol (Merck) used in the mobile phase were HPLC 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. Propranolol isomers, (R)- and (S)-propranolol hydrochloride, were provided by Aldrich and were used as standards.

Samples

Commercially available tablets from three different manufacturers containing 40.0 mg of propranolol hydrochloride (samples A, B, and C) and another produced by a fourth manufacturer containing 80.0 mg of propranolol hydrochloride (sample D) were used in this research.

Preliminary Tests

A careful study of the wavelength detection was accomplished. The aim of this selection was to determine the actual substance in the presence of other sample components. Thus, it was experimentally observed that, at 280 nm, the propranolol had a response that enabled detection without excipient interference, which was proved by injecting the placebos of the studied samples in the same experimental conditions.

Chromatographic Conditions

The mobile phase used was hexane : ethanol (75 : 25 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 at a flow rate of 0.7 ml/min.

Calibration Curves

Solutions of (R)- and (S)-propranolol isomers ranging from 20.0 to 100.0 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)-propranolol hydrochloride in micrograms per milliliter.

Sample Preparation

An amount of sample A, B, C, or D equivalent to 12.5 mg of (R)-propranolol hydrochloride and 12.5 mg of (S)-propranolol hydrochloride was accurately weighed and transferred to a 50-ml volumetric flask. About 40 ml of absolute ethanol was added; the flask was placed in an ultrasonicator for 5 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 contain

50.0 μg of (R)-propranolol hydrochloride and 50.0 μg of (S)-propranolol hydrochloride per milliliter. Standard solutions of propranolol isomers were prepared with the same solvents and in the same concentration. 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).

Recovery Tests

Recovery tests were made to determine the accuracy of the method. These were performed by adding a known concentration of standard solution to samples, followed by analysis using the proposed method. In brief, 25.0 mg of (R)-propranolol and 25.0 mg of (S)-propranolol were weighed and transferred to a 50-ml volumetric flask. Both flasks were brought to volume with ethanol. Aliquots of 5.0 ml of each solution were transferred to 25-ml volumetric flasks, and the volumes were brought to completion with the mobile phase. These solutions contained 100.0 μg of (R)-propranolol hydrochloride per milliliter and 100.0 μg of (S)-propranolol hydrochloride per milliliter. Appropriate amounts of these solutions were added to the sample solution according to the schematic representation in Table 1.

RESULTS AND DISCUSSION

The chromatogram of propranolol enantiomers can be observed in Fig. 1. The isomers were separated using a mixture of hexane : ethanol (75 : 25 v/v) as the mobile phase. It was observed that ethanol decreased the retention time of propranolol isomers more than isopropranol.

System suitability tests are an integral part of the liquid chromatographic method. Precision and accuracy of the proposed method were determined to prove the adequacy of the system for analysis. A method is accepted as precise if the coefficient of variation is less than 2.0%. Limit of detection and limit of quantitation were calculated based on standard deviation and inclination of the standard curve, as indicated by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (9).

It is well documented that columns like Chiralcel OD lose efficiency when a pressure greater than 700 psi is used in the analysis (10). 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, the detection wavelength was set at 280 nm, which prevented interference from excipients and solvents and the use of unnecessarily high concentrations of analytes during HPLC analysis (8).

Calibration curves of (R)- and (S)-propranolol isomers were obtained in a concentration range

Table 1

Volume of Sample and the Standard Solution Used to Perform Recovery Test

	Added Volume	
Volumetric Flask	Sample (A, B, C, D)	Standard (R)- or (S)-(100.0 μg/ml)
1	2.0 ml	—
2	2.0 ml	1.0 ml
3	2.0 ml	2.0 ml
4	2.0 ml	3.0 ml
5	—	2.0 ml

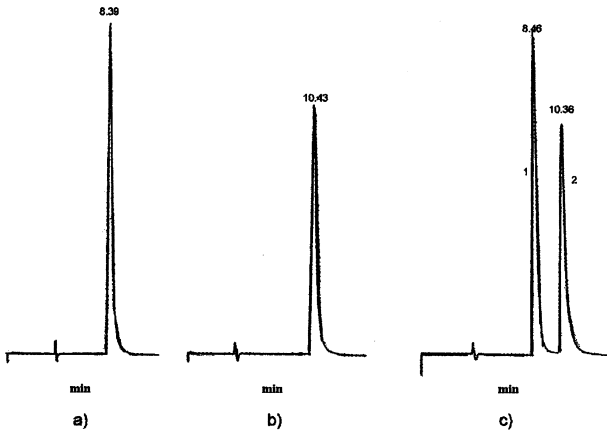

Figure 1. Chromatograms of standard propranolol hydrochloride isomers. Conditions: Chiralcel OD column, mobile phase of hexane : ethanol (75 : 25 v/v), flow rate 0.7 ml/min, UV detection 280 nm, ambient temperature: (a) (R)-propranolol hydrochloride (50.0 μg/ml); (b) (S)-propranolol hydrochloride (50.0 μg/ml); and (c) (R)-propranolol hydrochloride (1) 50.0 μg/ml and (S)-propranolol hydrochloride (2) 50.0 μg/ml.

from 20.0 to 100.0 μg/ml with a correlation coefficient of 0.9999.

The precision of an analytical method can be obtained by the coefficient of variation. To be considered precise, the coefficient of variation should be less than 2.0% (8,10,11). The statistical data obtained in the analysis of commercially available samples are shown in Table 2. The results for percentage of recovery are presented in Tables 3 and 4. The recovery tests and the percentage of recovery tests were performed according to the recommendations of AOAC International (12). The results obtained confirmed the accuracy of the method. In Table 5 are presented the results of the relation between propranolol isomers determined in com-

Table 2

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

Sample	Standard Deviation	Coefficient of Variation (%)	Confidence Limit (%)
(R)-Isomer			
A	0.72	0.72	99.83 ± 0.52
B	0.66	0.67	98.16 ± 0.47
C	0.62	0.62	100.57 ± 0.45
D	0.68	0.70	96.86 ± 0.49
(S)-Isomer			
A	0.74	0.74	99.99 ± 0.53
B	0.63	0.64	98.02 ± 0.45
C	0.71	0.71	99.24 ± 0.51
D	0.67	0.70	95.75 ± 0.48

Table 3

Recovery of (R)-Propranolol Isomer Standard Solution Added to Commercially Available Samples (A, B, C, and D) Using Chiral High-Performance Liquid Chromatography

Sample	Amount Added (μg)	Amount Found[a] (μg)	Recovery (%)
A	10.00	10.10	101.00
	20.00	19.88	99.40
	30.00	30.15	100.50
B	10.00	9.97	99.70
	20.00	19.90	99.50
	30.00	29.70	99.00
C	10.00	9.92	99.20
	20.00	19.89	99.45
	30.00	30.19	100.63
D	10.00	10.05	100.50
	20.00	20.01	100.05
	30.00	29.55	98.50

[a]Average of two determinations.

Table 4

Recovery of (S)-Propranolol Isomer Standard Solution Added to Commercially Available Samples (A, B, C, and D) Using Chiral High-Performance Liquid Chromatography

Sample	Amount Added (μg)	Amount Found[a] (μg)	Recovery (%)
A	10.00	9.91	99.10
	20.00	19.92	99.60
	30.00	30.05	100.17
B	10.00	10.17	101.70
	20.00	19.90	99.50
	30.00	29.89	99.63
C	10.00	9.86	98.60
	20.00	19.91	99.55
	30.00	30.55	101.83
D	10.00	9.90	99.00
	20.00	20.24	101.20
	30.00	29.69	98.97

[a]Average of two determinations.

Table 5

Relation Between Propranolol Isomers Determined in Commercially Available Samples (A, B, C, and D) Using Chiral High-Performance Liquid Chromatography Calculated Comparing the Slope (Response vs. Concentration) of the Calibration Curve for the (R)- and (S)-Isomers[a]

Sample	Declared (mg)	Found (mg)	
		(R)-Propranolol	(S)-Propranolol
A	40.00	19.96	19.99
B	40.00	19.63	19.60
C	40.00	20.11	19.84
D	80.00	38.74	38.30

[a](R)-Propranolol hydrochloride $y = 1701.5557 + 8464.1200x$; (S)-Propranolol hydrochloride $y = 2087.4444 + 8575.2667x$.

mercially available samples calculated by comparing the slope (response vs. concentration) of the calibration curve for the (R)- and (S)-propranolol.

CONCLUSION

The proposed chiral HPLC enables the separation and quantitative determination of (R)- and (S)-propranolol isomers in tablets. UV detection at 280 nm was found to be suitable without any interference from tablet excipients and solvents. The calibration curves of (R)- and (S)-isomers obtained in a concentration range from 20.0 to 100.0 μg/ml was found to be linear with a correlation coefficient of 0.9999 for both (R)- and (S)-isomers. The coefficients of variation of (R)- and (S)-isomers for samples A, B, C, and D were, respectively, 0.64% and 0.63%, 0.63% and 0.59%, 0.60% and 0.79%, and 0.70% and 0.77%. 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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