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Research Article

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Enantioseparation of (RS)-Propranolol by Chiral High-Performance-Liquid-Chromatography to Optimize its Therapeutic Profile

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Abstract *Propranolol* is one of the most important beta-blocker drugs, it is an optically active compound that is marketed as a racemate, but its desired activity resides in the S-enantiomer which is about 100 times more potent than *R-Propranolol*. This paper describes an enantioseparation of *(RS)-Propranolol* by HPLC Chiral using β-Cyclodextrin Chiral stationary phase (250 mm X 4 mm X 5 µm) with mobile phase composed of Acetonitrile: Ethanol: Acetic acid: Triethylamine (960: 40: 4: 3 v/v/v/v), at a flow rate of 1 mL/min. Detection was made by ultraviolet absorption at 225 nm. The retention time of *S-Propranolol* and *R-Propranolol* was respectively: 16.18 min and 18.50 min. The *racemic Propranolol* contains 50.43 % of *S-Propranolol* and 49.57 % of *R-Propranolol.* The enantiomeric purity equals to 50.43 % and the enantiomeric excess equals to 0.86 %. The specific rotary power of *(RS)-Propranolol racemic* solution was +17 $deg \cdot dm^{-1} \cdot g^{-1} \cdot mL$ and the optical rotation angle α was between [-1.0 ° and +1.0 °] which shows that the racemic mixture has deflected the polarized light on the right, which deduces that (RS)-*Propranolol* racemic isn't an equimolar mixture. So, for a selective β-blocking use, it could be very interesting to encourage the production of *Propranolol* in its form enantiomerically pure which is the S-enantiomer by chiral separation to find an optimal treatment and a right therapeutic control for the patient.

Keywords Enantioseparation, Propranolol, β-blocker, HPLC Chiral, β-Cyclodextrin, Racemate

1. Introduction

The chirality of drugs is an important issue from pharmacological, pharmacokinetic, toxicological, and regulatory points of view [1,2]. Nowadays, most research efforts concentrate on the production of optically pure products because of the increasing demand that such drugs be administered in optically pure form [3]. *Propranolol (figure 1)* [4] is one of the most important beta-blocker drugs because a variety of analogous compounds have been developed based on it [5]. It is mainly used in the treatment of hypertension and cardiac arrhythmias. *Propranolol* is a chiral compound that is marketed as a racemate, but it has been reported that its desired activity resides in the S-enantiomer *(figure 2)* which is about 100 times more potent than *R-Propranolol (figure 2)* [6,7]. Therefore, chiral separation of *Propranolol* is considered to be an essential issue and it is important to promote the chiral separation in pharmaceutical industry as well as in clinic in order to eliminate the unwanted isomer from the preparation and to find an optimal treatment and a right therapeutic control for the patient [8,9].

The main objective of this study is to separate the racemic mixture of (*RS)-Propranolol* by Chiral High-Performance-Liquid-Chromatography (Chiral HPLC) using β-Cyclodextrin Chiral Stationary Phase.

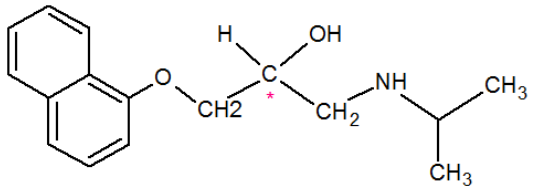

Figure 1: Chemical structure of (RS)-Propranolol racemic [4]

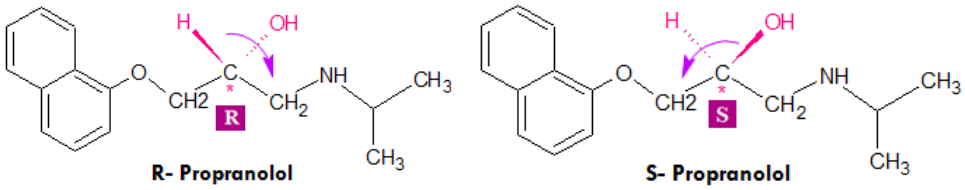

Figure 2: Chemical structures of Propranolol enantiomers

2. Materials and Methods

2.1. Instrumentation

The analytical HPLC system consisted of a Jasco PU-980 HPLC pump, a Waters 2487 detector and a 7725 syringe loading sample injector (Rheodyne, Rohnert Park, CA) equipped with 50 µL loop. The chromatographic data were acquired and processed by MILLENIUM 32 chromatography manager software model. A Zuzi Automatic Polarimeter, Model N° 412 was used for specific rotary power measurement.

2.2. Materials

All reagents used (Acetonitrile, Ethanol, Triethylamine, Acetic acid and Methanol) were of analytical grade from Sigma- Aldrich. The *Propranolol Hydrochloride* was purchased from Osmopharm SA and its batch number is Q0421303RD. The stationary chiral phase used is based on β-cyclodextrin [10].

2.3. Chromatographic conditions

The size of the chiral analytical column was (250 mm X 4 mm X 5 µm). The mobile phase is composed of Acetonitrile, Ethanol, Acetic acid and Triethylamine (960: 40: 4: 3 v/v/v/v) and it was filtered and degassed in an ultrasonic bat before use. The column temperature was ambient temperature and the flow rate was 1 mL/min. The detection wavelength was 225 nm. The *Propranolol hydrochloride* was prepared by dissolving it in methanol and filtered before use [11].

2.4. Enantiomeric Purity and Enantiomeric Excess

The Enantiomeric Purity (EP) represents the percentage of the majority enantiomer in a mixture of enantiomers [12,13]. It is expressed by the following formula:

$$\textbf{Enantiomeric Purity (S)}(\%) = \frac{[S]}{[R]+[S]} \times 100$$

The Enantiomeric Excess (EE) expresses the excess of one enantiomer compared to the other [12,13]. It is expressed by the following formula:

$$\textbf{Enantiomeric Excess (S)}(\%) = \frac{[S]-[R]}{[R]+[S]} \times 100$$

2.5. Measurement of specific rotary power

A stock solution at 10 mg/mL was prepared by dissolving of 0.25 g of *(RS)-Propranolol racemic* in 25 mL of water. A series of four dilutions was made to establish the calibration curve [14].

3. Results and Discussion

The separation chromatogram of *(RS)-Propranolol racemic* by Chiral HPLC using β-Cyclodextrin column is showed in Figure 3 and Table 1.

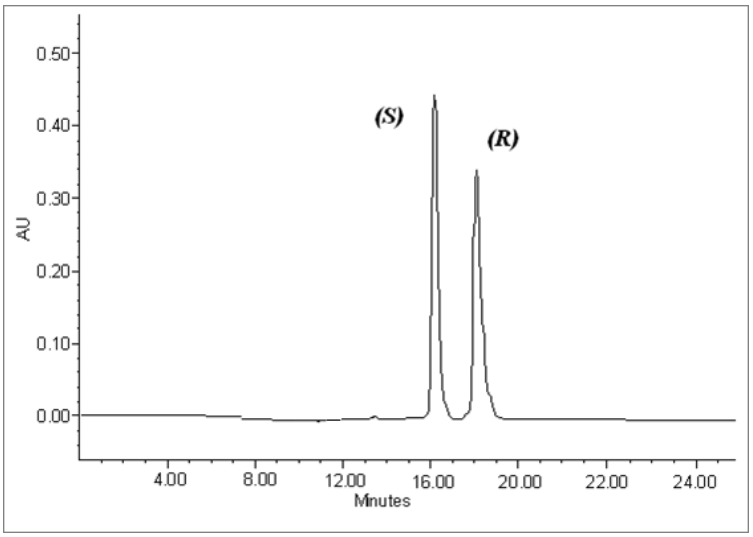

Figure 3: Separation chromatogram of (RS)-Propranolol racemic by Chiral HPLC using β-Cyclodextrin column

Table 1: Separation results of (RS)-Propranolol racemic by Chiral HPLC

Enantiomer name	Retention time (mn)	Area (mAU.min)	Area (%)
S-Propranolol	16.180	5.06703	50.43
R-Propranolol	18.501	4.98068	49.57

According to the chromatogram, the resolution between *S-Propranolol* peak and *R-Propranolol* peak is 3, value in accordance with the standard required by the 8th European Pharmacopoeia (at least 1.3) and the symmetry factor of these peaks are respectively: 0.9 and 1.1, values in accordance with the standards (from 0.8 to 1.5), therefore, the system conformity is validated (figure 3). The retention time of *S-Propranolol* is 16.18 min and that of *R-Propranolol* is 18.50 min. The *racemic Propranolol* contains 50.43 % of *S-Propranolol* and 49.57 % of *R-Propranolol (table 1)*.

After calculation, the Enantiomeric Purity equals to 50.43 % and the Enantiomeric Excess equals to 0.86 % (table 2). The (RS)-*Propranolol racemic* is no-equimolar mixture 50/50 but rather a 49.57/50.43 mixture whose enantiomeric excess is 0.86%.

Table 2: Enantiomeric Purity and Enantiomeric Excess results

Name	Value (%)
S-Propranolol	50.43
R-Propranolol	49.57
Enantiomeric Purity	**50.43**
Enantiomeric Excess	**0.86**

The measurement of the specific rotary power of *(RS)-Propranolol racemic* solution is +17 $deg \cdot dm^{-1} \cdot g^{-1} \cdot mL$ and the optical rotation angle α is between [-1.0 ° and +1.0 °] which shows that the racemic mixture has deflected the polarized light on the right, which deduces that this (*RS)-Propranolol racemic* isn't equimolar mixture 50/50.

The proportions obtained by chiral separation in our study show a proportion of 50.38 % of the S-enantiomer and 49.62 % of the R-enantiomer, the literature reports that for β-blocker activity, the S-enantiomer is 100 times more active than the R-enantiomer which has a contraceptive activity. Should-we be worried about the efficacy and the safety of this treatment?

4. Conclusion

In this paper, a study of enantiomer separation of *(RS)-Propranolol Hydrochloride racemate* by HPLC on β-Cyclodextrin Chiral stationary phase was realized. The purpose of this separation was to quantify the R and the S enantiomers percentages. Knowing that *Propranolol Hydrochloride* is marketed in its racemic form, for a selective β-blocking use, it could be very interesting to encourage its production in its form enantiomerically pure which is the S-enantiomer either by chiral separation or by asymmetric synthesis. As a perspective, it would be interesting to develop the contraceptive activity of the *R-Propranolol* enantiomer on a pharmacological model.

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