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Original Article

Comparative Chiral Separation of (RS)-Propranolol Racemate by HPLC Using α-Glycoprotein and β-Cyclodextrin Stationary Phases.

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ABSTRACT

The most of the β-blockers are still clinically being sold as a racemic mixture despite the fact that their enantiomers show significant differences in the pharmacological effects and activities. This paper describes a comparative study of tow chiral separations of *(RS)-Propranolol* racemate by HPLC using α-Glycoprotein (AGP) and β-Cyclodextrin (BCD) Stationary Phases. For the AGP separation, the column size was (150 mm X4 mm X 5 µm), the mobile phase composed of Propanol-2 and Ammonium acetate (0.5:99.5 v/v), at a flow rate of 0.9 mL/min and the detection by ultraviolet absorption at 225 nm. For the BCD separation, the column size was (200 mm X4 mm X 5 µm), the mobile phase composed of Acetonitrile: Ethanol: Acetic acid: Triethylamine (960: 33: 4: 3 v/v/v/v), at a flow rate of 1 mL/min and the detection by ultraviolet absorption at 225 nm. The retention time of *S-Propranolol* and *R-Propranolol* with AGP separation was respectively: 7.25 min and 11.82 min while with the BCD *separation* 16.18 min and 18.50 min respectively. The racemate contains 50.46 % of *S-Propranolol* and 49.53 % of *R-Propranolol* with AGP separation while with BCD *separation, it contains* 50.43 % of S- enantiomer and 49.57 % of R-enantiomer. There is a similarity between the enantiomeric purity values and the enantiomeric excess values of tow separations, but the separation with AGP stationary phase is faster than with the BCD stationary phase. For a selective β-blocking use, it could be very interesting to encourage its production in its form enantiomerically pure wich is the S-enantiomer.

1. Introduction

Nowadays, single enantiomer drugs make up a large and growing portion of over-the-counter and prescription drug products [1]. Unfortunately, most of the β-blockers are still clinically being sold as a racemic mixture except for a few of them, e.g., Timolol, despite the fact that their enantiomers show significant differences in the pharmacological effects and activities [2]. In some cardiac diseases, the β-blocking activity of β-blockers resides generally in their S (-) enantiomer [3–4], and the reported S:R activity ratio ranges from 33 to 530 [5] due to the diverse degree of binding affinity to the β-receptor. For example, S-propranolol is 100 times more potent than R-propranolol (Fig. 1) [6]. Therefore, the separation of racemates of β-blockers is essential both in the laboratory and industry. In the last 30 years, HPLC has obtained a great reputation in the field of enantioseparation, owing to its rapidness, reproducibility, sensitivity, mild operating temperature and availability of a tremendous number of chiral selectors [7–8]. The enantiomeric resolution can be obtained using a direct method on chiral stationary phase

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based on α-Glycoprotein or β-Cyclodextrin. The α-Glycoprotein is a protein wich has enantioselective properties and β-Cyclodextrin is an oligosaccharide with a cyclic structure, it is able to differentiate molecules of similar chemical structure, such as enantiomers [9-10].

The objective of this study is to separate the enantiomers of a β-blocker which is marketed in racemic form and to compare tow Chiral Separations of (RS)-Propranolol (Fig. 2) Racemate by High-Performance-Liquid-Chromatography (HPLC) Using α-Glycoprotein and β-Cyclodextrin Stationary Phases.

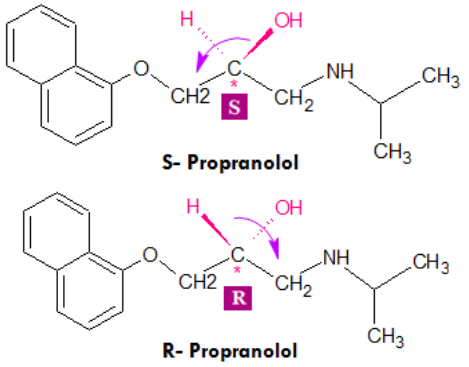

Fig 1. Chemical structures of *Propranolol* enantiomers **[11].**

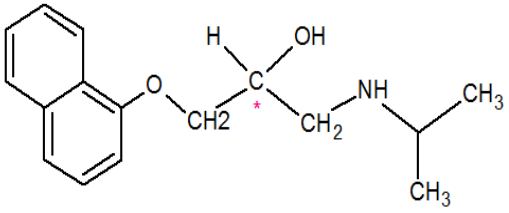

Fig 2. Chemical structure of *(RS)-Propranolol* racemate **[11].**

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.

2.2. Materials

All reagents used (Propanol-2, Ammonium acetate, 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 phases used are based on α-Glycoprotein (AGP) and β-Cyclodextrin (BCD) [12].

2.3. Chromatographic conditions

2.3.1. Separation of (RS)-Propranolol racemate using AGP stationary phase

The size of the AGP chiral analytical column was (150 mm X 4 mm X 5 µm). The mobile phase is composed of Propanol-2 and Ammonium acetate (0.5:99.5 v/v) and it was filtered and degassed in an ultrasonic bath before use. The column temperature was ambient temperature and the flow rate was 0.9 mL/min. The detection by ultraviolet absorption wavelength was 225 nm. The *Propranolol hydrochloride* solution was prepared by dissolving of 10 mg in 10 mL of methanol and filtered before use [13].

2.3.2. Separation of (RS)-Propranolol racemate using BCD stationary phase

The size of the BCD chiral analytical column was (200 mm X 4 mm X 5 µm). The mobile phase is composed of Acetonitrile, Ethanol, Acetic acid and Triethylamine (960: 33: 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 of 10 mg in 10 mL of methanol and filtered before use [13].

2.4. Enantiomeric Purity and Enantiomeric Excess

The Enantiomeric Purity (EP) represents the percentage of the majority enantiomer in a mixture of enantiomers [14,15]. It is expressed by the following formula:

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

[S]: Percentage of S-enantiomer.
[R]: Percentage of R-enantiomer.

The Enantiomeric Excess (EE) expresses the excess of one enantiomer compared to the other [14,15]. It is expressed by the following formula:

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

3. Results and Discussion

The separation chromatogram of *(RS)-Propranolol racemate* by Chiral HPLC using AGP stationary phase is showed in Fig. 3 and Table 1. According to the chromatogram, the resolution between *S-Propranolol* peak and *R-Propranolol* peak is 5, 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: 0.8 and 0.9, values in accordance with the standards (from 0.8 to 1.5), therefore, the system

conformity is validated (Fig. 3). The retention time of *S-Propranolol* is 7.25 min and that of *R-Propranolol* is 11.82 min. The *Propranolol racemate* contains 50.46 % of *S-Propranolol* and 49.53 % of *R-Propranolol* (Table 1).

After calculation, the Enantiomeric Purity equals to 50.46 % and the Enantiomeric Excess equals to 0.93 % (Table 2). The (RS)-*Propranolol racemate* is no-equimolar mixture 50/50 but rather a 49.53/50.46 mixture whose enantiomeric excess is 0.93 %.

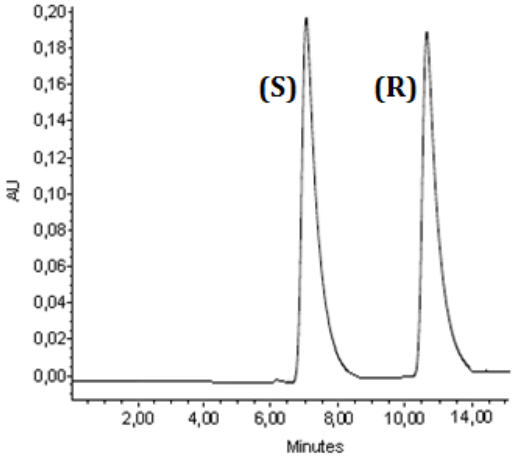

Fig 3. Separation chromatogram of (RS)-Propranolol racemate using AGP column.

Table 1. Separation results of (RS)-Propranolol racemate using AGP column.

Enantiomer name	Retention time (mn)	Area (mAU.min)	Area (%)
S-Propranolol	7.252	4.99013	50.46
R-Propranolol	11.820	4.89765	49.53

Table 2. Enantiomeric Purity and Enantiomeric Excess results.

Name	Value (%)
S-Propranolol	50.46
R-Propranolol	49.53
Enantiomeric Purity	**50.46**
Enantiomeric Excess	**0.93**

The separation chromatogram of *(RS)-Propranolol racemate* by Chiral HPLC using BCD stationary phase is showed in Fig. 4 and Table 3. 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) [11] 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 (Fig. 4). The retention time of *S-Propranolol* is 16.18 min and that of *R-Propranolol* is 18.50 min. The *Propranolol racemate* contains 50.43 % of *S-Propranolol* and 49.57 % of *R-Propranolol* (Table 3).

After calculation, the Enantiomeric Purity equals to 50.43 % and the Enantiomeric Excess equals to 0.86 % (Table 4). The (RS)-*Propranolol racemic* is no-equimolar mixture 50/50 but rather a 49.57/50.43 mixture whose enantiomeric excess is 0.86 %. In the study realized by Limei C and al, 2008 on Semipreparative Enantiomer Separation of Propranolol Hydrochloride by High-Performance Liquid Chromatography Using Cellulose tris (3,5- Dimethyl-phenylcarbamate) Chiral Stationary Phase, at semi-preparative scale, approximately 19 mg/h enantiomers are isolated. The first fraction [(*R*)-(+)-propranolol hydrochloride] is isolated with a purity of > 99.6% (e.e.) and > 97.0% yield, and the second [(*S*)-(–)-propranolol hydrochloride] is isolated with a purity of > 99.3% (e.e.) and > 95.0% yield [16]. Unfortunately, we didn't find any other similar studies to be able to discuss and compare our results.

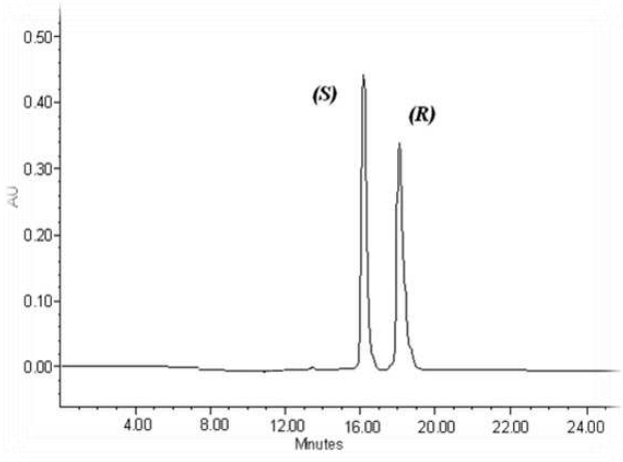

Fig 4. Separation chromatogram of (RS)-Propranolol racemate using BCD column.

Table 3. Separation results of (RS)-Propranolol racemate using BCD column.

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**

Table 4. Enantiomeric Purity and Enantiomeric Excess results.

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

4. Conclusion

In this paper, a comparative study of tow chiral separations of (RS)-Propranolol racemate by HPLC Using AGP and BCD Stationary Phases was realized. We note that there is a similarity between the enantiomeric purity values and the enantiomeric excess values, but the separation with AGP stationary phase is faster than with the BCD stationary phase. 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 wich is the S-enantiomer.

Conflict of Interest

The authors declare that they have no conflict of interest.

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