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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.

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## 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, even though 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 based on α-Glycoprotein

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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.

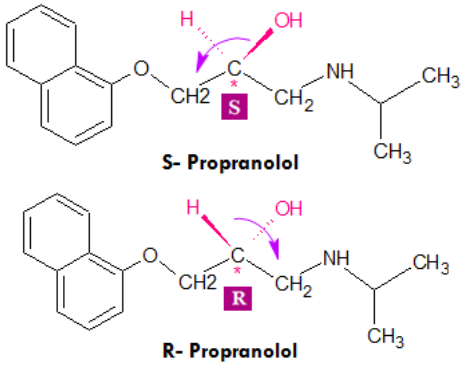


Fig 1. Chemical structures of *Propranolol* enantiomers **[11].**

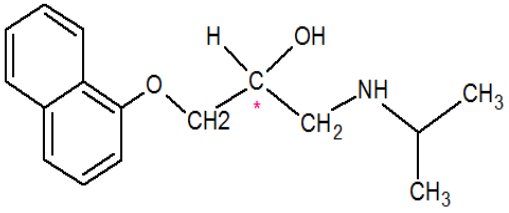


Fig 2. Chemical structure of *(RS)-Propranolol* racemate **[11].**

## 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:

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

**[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:

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

## 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 8$^{th}$ 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 %.

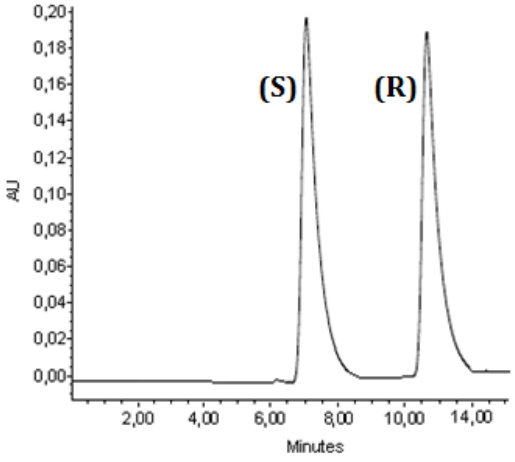


**Fig 3.** Separation chromatogram of (RS)-Propranolol racemate using AGP column.

**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.

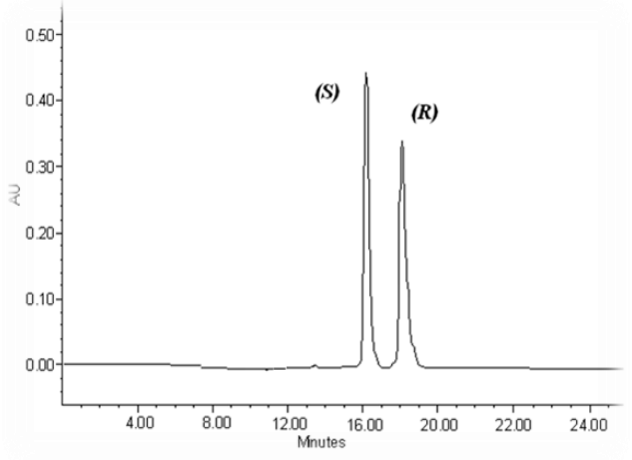


**Fig 4.** Separation chromatogram of (RS)-Propranolol racemate using BCD column.

**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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