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

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ARTICLE INFO

Article history:
Received 11 July 2021
Revised 20 August 2021
Accepted 02 September 2021

Keywords:
Chiral Separation;
β-blocker;
HPLC Chiral;
α-Glycoprotein;
β-Cyclodextrin.

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 which 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, 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
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 Propanolol 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:

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

[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:

\[
Enantiomeric Excess \ (S) \ (%)=\frac{\%S-\%R}{\%R}\times 100
\]
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.](image)

<table>
<thead>
<tr>
<th>Enantiomer name</th>
<th>Retention time (min)</th>
<th>Area (mAU.min)</th>
<th>Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-Propranolol</td>
<td>7.252</td>
<td>4.99013</td>
<td>50.46</td>
</tr>
<tr>
<td>R-Propranolol</td>
<td>11.820</td>
<td>4.89765</td>
<td>49.53</td>
</tr>
</tbody>
</table>

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

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 racemate 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 Enantioomer 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.](image)

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<td>R-Propranolol</td>
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Table 3. Separation results of (RS)-Propranolol racemate using BCD column.

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).

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Table 4. Enantiomeric Purity and Enantiomeric Excess results.
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 which is the S-enantiomer.

Conflict of Interest
The authors declare that they have no conflict of interest.

References


Recommended Citation