Comparison of in vitro dissolution of Bisoprolol Fumarate tablets of five Brands marketed in Aden, Yemen
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Abstract

Bisoprolol fumarate is a selective β-1 blocker and is useful in the management of cardiovascular diseases. According to Biopharmaceutical Drug Classification System, it is a class I drug, which has high solubility and permeability. In this study, five brands of bisoprolol fumarate 10 mg tablets that are marketed in Aden, Yemen, have been evaluated using dissolution test with the aim to assess bioequivalence of the generic products B, C, D and E with the innovator product A. A high Performance Liquid Chromatographic method was used for the analysis of bisoprolol fumarate in the tablets. The method was validated for the parameters like system suitability, linearity, limit of detection and limit of quantification. The dissolution test was performed according to the United States Pharmacopoeia-30 (USP-30) for the five brands and the obtained dissolution profiles data of the four generic brands were subjected to comparison with the innovator brand using difference factor f₁, similarity factor f₂ and dissolution efficiency. The results of the method validation revealed its suitability for quantification of bisoprolol in the tested tablets. The five brands contain between 97.52%-102.43% of the labeled amount of bisoprolol fumarate and released more than 80% of drug within 30 minutes, which were within the USP acceptance criterion. The calculated f₁, f₂ and DE indicated that the generic brands, except brand E, were bioequivalent to the innovator and could be used as generic substitutes for the innovator brand.

Keywords: Bisoprolol fumarate tablets, in vitro dissolution, bioequivalence, fit factors, dissolution efficiency.

Introduction

In many countries, generic copies of the innovator drug products containing identical amounts of the same active ingredient in the formulation and same route of administration are made, and these generic drug products have become very popular (21). They are promoted to be used in practice because they are usually less expensive than the innovator products, thereby improving access to life-saving drugs, especially in developing countries (14). However, the marketing of multisource drug products registered by national drug agencies in developing countries, with the view of improving health care delivery through competitive pricing, has its attendant problem of ascertaining their quality and interchangeability (3). Regardless of price, the generic drug quality should be comparable to that of the innovator product and it could be used as a generic substitute only when it is pharmaceutically and therapeutically equivalent with the innovator (12). However, evidences point to the fact that the marketed products with the same amount of active ingredient have exhibited distinct differences in their therapeutic effects. This may be due to the differences in the rate and extent of absorption, mainly, because of the difference in dissolution. Therefore, there are serious concerns that various marketed generic products may have different bioavailability and couldn’t be used interchangeably (2, 9).

The release of active ingredient from the dosage form, its dissolution under physiological conditions and its permeability across the gastrointestinal tract are essential steps in drug absorption. Bioequivalence studies focus on the drug release from the formulation and subsequent absorption into the systemic blood circulation which involve both in vivo and in vitro studies.
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Considering the first two steps in absorption, in vitro dissolution may be applicable to the prediction of in vivo bioequivalence (16). Until recent years, bioequivalence has been determined only by in vivo tests. However, with the introduction of Biopharmaceutical Classification System (BCS), in vivo bioequivalence studies could be waived for immediate released solid oral dosage forms for classes I (high solubility and permeability) and III drugs (high solubility and low permeability). Therefore, in vitro tests may be used solely to determine bioequivalence for highly soluble and highly permeable drugs (20). In addition, the immediate released solid dosage forms are routinely subjected to tests such as content uniformity, weight, hardness, friability and disintegration test, but the test that is most often ciated with the assessment of in vivo performance is the dissolution test because it provides useful information at several stages of the drug development process (7, 13). Dissolution test is used as a surrogate marker for bioequivalence test and is practical and economic and could be utilized effectively in developing countries where technology and resources are limited to conduct in vivo bioequivalence studies (17).

The similarity of drug dissolution profiles could be evaluated using a similarity factor f₂ and a difference factor f₁ that were recommended by FDA Center for Drug Evaluation and Research (CDER) (8). In addition, the European Medicines Agency (EMA) Committee for Medicinal Products for Human Use (CHMP) has adopted the similarity factor (6).

Bisoprolol fumarate is a synthetic selective beta 1-blocker. It is used in the management of cardiovascular diseases such as hypertension, reduced blood flow to the heart (cardiac ischemia) and congestive heart failure. It is also used as preventive treatment before heart attacks and as primary treatment after heart attacks, decreasing the chances of recurrence (15). Bisoprolol is almost completely absorbed and undergoes minimal first-pass metabolism (less than 20%) after oral administration resulting in an oral bioavailability of about 90%. The biological half-life of it is 9-10 hours gives 24-hour effect after dosing once daily (19). Chemically, it is \( \pm 1-[4-[[2-(1-methylethoxy) ethoxy] methyl] phenoxy]-3-[[1- methylthyl] amino]-2-propanol \) (E)-2 butenedioate. Bisoprolol showed a rapid and complete dissolution in water, 0.1 M HCl, pH 4.75 and pH 7.2 buffers. According to Biopharmaceutical Drug Classification System (BCS), it is a class I drug, which has high solubility and high permeability (4).

The presence of various brands of bisoprolol fumarate tablets of different pharmaceutical companies in the local drug market in Aden, Yemen, makes the clinicians, pharmacists and patients in a difficult situation for choosing the suitable brand or the possibility of using and interchangeability of the generic brands with the innovator brand in order to obtain effective therapeutic results. The quality and bioequivalence of these generic brands need to be monitored with the innovator brands even after marketing as they may be introduced without license from the regulatory agencies.

The purpose of this study is to assess the equivalence of four marketed generic brands of bisoprolol fumarate tablets with the innovator brand that are marketed in Aden, Yemen, based on the in vitro dissolution study results and to determine that these product could be used as generic substitutes and interchangeable with the innovator product.

Materials and Methods

Materials

Pure bisoprolol fumarate was donated by the Supreme Board of Drugs and Medical Appliances, Sana’a, Yemen. Methanol of HPLC grade was purchased from Scharlau, Spain. Triethylamine was purchased from BDH-laboratory Suppliers, England, and orthophosphoric acid was purchased from Merck, Germany and these reagents of analytical grade. Deionized double and distilled water was used. Five marketed brands of bisoprolol fumarate tablets (10 mg) were purchased from pharmacies in Aden, Yemen. The innovator brand was denoted as (A) and the generic brands were denoted as (B, C, D and E).

Equipment: the analysis was performed on HPLC equipment (JASCO, Japan) consisted of a LC-2ADC, P4-2089 Quaternary Gradient pump, CO-2065 Intelligent column oven and Intelligent UV\Visible detector. Chromatograms were analyzed by Chrom Nave chromatography data system.
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Chromatographic Condition: The mobile phase consisted of a mixture of methanol: triethylamine: water (340:10:500) adjusted to pH 4.0± 0.1 using orthophosphoric acid and was pumped at a flow rate of 1 ml/minute through the column (C18; 150 mm X 4.6 mm, Jasco, Japan). Before use, the mobile phase was filtered through a 0.45 µm nylon membrane filter and degassed by ultrasonic bath. The injection volume 20µl and the wavelength were set to 227 nm (18).

Preparation of Standard Solution

Stock solution of bisoprolol was prepared by dissolving 25 mg of bisoprolol fumarate with distilled water. Then, the volume was completed to 100 ml with distilled water to obtain a solution of concentration (250 µg/ml). To construct the standard calibration curve of bisoprolol, a standard stock solution was diluted by using the mobile phase to give solutions of concentrations of 5, 10, 15, 20 and 25µg/ml and these solutions were injected into HPLC at 227 nm. The average peak area was plotted against the concentration (µg/ml).

Method Validation

The method was validated for system suitability of the chromatographic system by injecting of five replicates of standard solution. The linearity was determined from the least-square linear regression analysis of the calibration curve data. Limit of detection (LOD), and limit of quantification were also calculated.

Assay

Ten tablets from each of the five brands were weighed and powdered, and then an amount equivalent to 10 mg of bisoprolol fumarate were weighed and dissolved in methanol and filtered. Then the filtrates were brought to 100 ml, using the mobile phase to obtain 0.1 mg/ml. The resulting solutions were analyzed for the content of bisoprolol using HPLC at 227 nm. The percentage drug content was calculated for each brand.

Dissolution Test

The in vitro dissolution tests were carried out according to USP specifications (18) using dissolution testing apparatus II (paddle) (Pharmatest, D-6, Germany). The dissolution medium, consisted of 900 ml distilled water, maintained at 37± 0.5 °C and stirred at 75 rpm. At appropriate time intervals, samples of 5 ml of the solution were withdrawn at 5, 10, 15, 30 and 60 minutes and were replaced with equal volumes of fresh dissolution medium at the same temperature. The samples were filtered and assayed by HPLC. The results were expressed as mean percentage of drug dissolved as a function of time.

Dissolution Data Analysis

Fit factors: The dissolution profiles of the five brands were compared using two model independent parameters; the difference factor $f_1$ and similarity factor $f_2$ were determined from the data collected in the drug dissolution studies. Difference factor $f_1$ is the percentage difference between two curves at each point and is a measurement of the relative error between the two curves. The similarity factor $f_2$ is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution between the two curves. The following equations were used to calculate the difference factor $f_1$ and similarity factor $f_2$.

$$f_1 = \frac{\sum |R_i - T_i| \times 100}{\sum R_i}$$

$$f_2 = 50 \log \left[1 + \frac{1}{n} \sum_{i=1}^{n} (R_i - T_i)^2 \right]^{-0.5} \times 100$$
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Where (n) is the number of withdrawal points, \((R)\) is the reference product dissolved at time \(t\) and \((T)\) is the percentage of test product dissolved at time \(t\). When the test and reference drug profiles are identical, \(f_1\) is zero and \(f_2\) is 100. \(f_1\) increases and \(f_2\) decreases proportionally as the dissimilarity increases. Two dissolution profiles are considered similar and bioequivalence if \(f_1\) is between 0 and 15 and if \(f_2\) is between 50 and 100 which means an average difference \(\leq 10\%\) at each withdrawal time (11).

Dissolution Efficiency (DE): it was also employed to compare the drug release from various brands. Dissolution efficiency is as follows:

\[
DE = \frac{\int_{t_1}^{t_2} y \cdot dt}{y_{100} \times (t_2 - t_1)} \times 100
\]

Where, \(y\) is the percentage of dissolved product. \(D.E\) is the area under the dissolution curve between time points \(t_1\) and \(t_2\) expressed as a percentage of the curve at maximum dissolution, \(y_{100}\), over the same time period. The integral of the numerator, i.e. the area under the curve, is calculated by a model independent method, the trapezoidal one. The area under the curve is the sum of all the trapeziums defined by:

\[
AUC = \sum_{i=1}^{n} \frac{(t_i - t_{i-1}) (y_{i-1} + y_i)}{2}
\]

Where \(t_i\) is the \(i^{th}\) time point, \(y_i\) is the percentage of dissolved product at time \(t_i\) (11).

Results and Discussion

Validation results of HPLC method for bisoprolol analysis were summarized in Table 1. For the system suitability, it was found that the system was suitable in respect of retention time, mean theoretical plates and tailing factor. The calibration curve was linear in the concentration range of 5-25 \(\mu g/ml\) and giving a correlation coefficient of 0.996 as depicted in Figure 1. The limit of detection (LOD) and the limit of quantification were 0.02 \(\mu g/ml\) and 0.05 \(\mu g/ml\), respectively.

The results shown in Table 2 indicate that all the tested brands A, B, C, D and E were within the limits for the drug assay as USP specified that the drug content should not be less than 90% and not more than 105% of the labeled amount (18). The innovator and generic brands have exhibited drug release between 87.35%-99.5% within 30 minutes, which was expected for highly water soluble drug, bisoprolol (Figure 2). So, that all the five brands passed the USP acceptance criterion for immediate release dosage forms (18).

Dissolution test is an important tool to evaluate formulation development and finished products for batch quality control and as an in vitro surrogate for in vivo performance (10). Additionally, it could be used for establishing the similarity between a generic and innovator products by comparing the dissolution profiles (1, 5). Table 2 illustrates the mean percentages of bisoprolol dissolved from each of the five brand tablets and Figure 3 shows the drug dissolution profiles. It is obvious that these profiles were not superimposable. The observed variation in the dissolution test results of the five brands could be attributed to many factors that influence the manufacturing of oral solid dosage form such as types of excipients and proportion of them, as well as the manufacturing variables such as mixing method, granulation procedures and coating parameters (5). Therefore, the dissolution profiles data of generic products were compared with that of innovator, using the fit factors; difference factor \(f_1\) and similarity factor \(f_2\), and to ensure similarity or equivalence of two curves, \(f_1\) values between 0-15% and \(f_2\) values ranging from 50% to 100%. As shown in Table 3, the values \(f_1\) and \(f_2\) were within the recommended values for brands B, C and D which indicated that the release of bisoprolol from these products similar to the innovator brand A. On the other hand, brand E was not similar with innovator as the values of \(f_1\) and \(f_2\) were 16 and 45.93, respectively, which were not within the established limits. Moreover; the dissolution profiles data of the tested brands were assessed, using the dissolution efficiency (% DE). The innovator and generic products could be said to be equivalent if the difference between their dissolution
Comparison of in vitro dissolution .......... Sana Al-Kubati, Fadhel Al- Hariri, Gobran Ibraheem efficiencies is within appropriate limit ±10% (12). Accordingly, the dissolution efficiencies also follow the same trend with brands B, C and D as the difference of the dissolution efficiencies were 5.64, 7.85 and 2.16, respectively, but it was greater than 10% in case of brand E (13.34). In summary, generic brands B, C and D could be interchangeable with innovator brand, while brand E could not used as a generic substitute to the innovator.

Conclusion

In conclusion, brands B, C and D could be considered bioequivalent and interchangeable with the innovator brand A, while brand E showed dissimilarity and could not used as a drug substitute to the innovator. Based on our results, fit factors and dissolution efficiency are useful and reliable methods for assessing bioequivalence of two drug products containing the same amount of active ingredients.

Acknowledgement

The authors acknowledge Supreme Board of Drugs and Medical Appliances, Aden, Yemen, in which this work was performed.

Table 1: Results from regression analysis and system suitability of bisoprolol fumarate

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Retention time (min.)</td>
<td>9.5</td>
</tr>
<tr>
<td>Linear range (µg/ml)</td>
<td>5-25</td>
</tr>
<tr>
<td>Regression line</td>
<td>y = 12346x + 6238.3</td>
</tr>
<tr>
<td>Correlation coefficient (R²)</td>
<td>0.996</td>
</tr>
<tr>
<td>Limit of detection (µg/ml)</td>
<td>0.02</td>
</tr>
<tr>
<td>Limit of quantification (µg/ml)</td>
<td>0.05</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>962</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.18</td>
</tr>
</tbody>
</table>
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Figure 1: Calibration curve of bisoprolol fumarate

\[ y = 12346x + 6238.3 \]
\[ R^2 = 0.996 \]

Figure 2: Percentage of bisoprolol released from the five brands within 30 minutes

Table 2: Assay and mean percentage of bisoprolol dissolved from the five brands

<table>
<thead>
<tr>
<th>Brand code</th>
<th>Assay (%)</th>
<th>( t_5(\text{min.}) )</th>
<th>( t_{10}(\text{min.}) )</th>
<th>( t_{15}(\text{min.}) )</th>
<th>( t_{30}(\text{min.}) )</th>
<th>( t_{60}(\text{min.}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>98.93</td>
<td>54.53</td>
<td>68.41</td>
<td>69.16</td>
<td>99.50</td>
<td>84.12</td>
</tr>
<tr>
<td>B</td>
<td>102.43</td>
<td>53.43</td>
<td>75.30</td>
<td>78.60</td>
<td>96.48</td>
<td>83.37</td>
</tr>
<tr>
<td>C</td>
<td>97.52</td>
<td>63.12</td>
<td>68.56</td>
<td>69.40</td>
<td>87.35</td>
<td>93.05</td>
</tr>
<tr>
<td>D</td>
<td>101.12</td>
<td>55.12</td>
<td>66.87</td>
<td>67.50</td>
<td>93.27</td>
<td>82.92</td>
</tr>
<tr>
<td>E</td>
<td>102.29</td>
<td>70.67</td>
<td>72.59</td>
<td>88.55</td>
<td>93.55</td>
<td>89.87</td>
</tr>
</tbody>
</table>
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Figure 3: Comparative dissolution profiles of the five brands of bisoprolol fumarate tablets

Table 3: The difference factor $f_1$, similarity factor $f_2$ and dissolution efficiency of the five brands of bisoprolol fumarate tablets

<table>
<thead>
<tr>
<th>Brand code</th>
<th>$f_1$</th>
<th>$f_2$</th>
<th>DE (%)</th>
<th>*DDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-</td>
<td>-</td>
<td>68.76</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>6.60</td>
<td>62.89</td>
<td>74.40</td>
<td>5.64</td>
</tr>
<tr>
<td>C</td>
<td>9.36</td>
<td>55.31</td>
<td>76.61</td>
<td>7.85</td>
</tr>
<tr>
<td>D</td>
<td>3.50</td>
<td>74.83</td>
<td>70.92</td>
<td>2.16</td>
</tr>
<tr>
<td>E</td>
<td>16.00</td>
<td>45.93</td>
<td>82.10</td>
<td>13.34</td>
</tr>
</tbody>
</table>

*DDE: Difference of % Dissolution Efficiency (Innovator product – generic product)

References

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مقارنة الذائبية لأقراص البيسوبرولول فيومارات لخمس أصناف دولية متداولة في عدن - اليمن

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المخصر

يُعتبر عقار البيسوبرولول فيومارات حاصراً انتقائياً لمستقبلات البيتا-1 ويستعمل في علاج أمراض القلب والأوعية الدموية وله ذاتية عالية في الماء ونافذية عالية عبر الأغشية الحيوية. في هذا البحث درس التكافؤ المقارن لأربعة منتجات جينية لأقراص البيسوبرولول فيومارات (10 ملجم) مع المنتج المرجعي من خلال تقييم اختبار ذائبية الدواء من هذه الأقراص في الماء. وقد خلقت كمية الدواء في هذه الأقراص عن طريق استخدام جهاز HPLC حيث وجد أن كمية الدواء في جميع الأقراص المختبرة مابين 97.52% - 102.43%، وكذلك وجد أن كمية الدواء الذائبة خلال 30 دقيقة أكثر من 80% وذلك تتوافق مع دستور الأدوية الأمريكي. درست الذائبية للدواء من الأقراص المصغرة باستخدام HPLC حيث وجد أن كمية الدواء في جميع الأقراص مابين 97.52% - 102.43%، وكذلك وجد أن كمية الدواء الذائبة خلال 30 دقيقة أكثر من 80%.

الكلمات المفتاحية: أقراص بيسوبرولول فيومارات، اختبار الذائبية، التكافؤ الحيوي، عوامل تناسب، كفاءة.