

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

Sana Saleh Fara Al-Kubati^{1,*}, Fadhel Mohammed Al- Hariri² and Gobran Kalil Ibraheem¹

¹Department of Pharmaceutics, Faculty of Pharmacy, Aden University, Aden, Yemen

²Supreme board of drugs and medical appliances, Aden Branch, Aden, Yemen

E-mail: drsana2020@gmail.com

DOI: <https://doi.org/10.47372/uajnas.2020.n1.a23>

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 f_1 , similarity factor f_2 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 f_1 , f_2 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.

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 associated 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 f_2 and a difference factor f_1 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-methylethyl) 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 interchangeably 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.

Comparison of in vitro dissolutionSana Al-Kubati, Fadhel Al- Hariri, Gobran Ibraheem

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 20 µl 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 25 µg/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 .

$$f_1 = \left\{ \frac{\sum_{t=1}^n |R_t - T_t|}{\sum_{t=1}^n R_t} \right\} \times 100$$
$$f_2 = 50 \log \left\{ \left(1 + \frac{1}{n} \sum_{t=1}^n (R_t - T_t)^2 \right)^{-0.5} \times 100 \right\}$$

Comparison of in vitro dissolutionSana Al-Kubati, Fadhel Al- Hariri, Gobran Ibraheem

Where (n) is the number of withdrawal points, (R_i) is the reference product dissolved at time t and (T_i) is the percentage of test product dissolved at time t. When the test and reference drug profiles are identical, f₁ is zero and f₂ is 100. f₁ increases and f₂ decreases proportionally as the dissimilarity increases. Two dissolution profiles are considered similar and bioequivalence if f₁ is between 0 and 15 and if f₂ is between 50 and 100 which means an average difference ≤ 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 t₁ and t₂ expressed as a percentage of the curve at maximum dissolution, y₁₀₀, 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:

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

Where t_i is the tth 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 µg/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 µg/ml and 0.05 µg/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 f₁ and similarity factor f₂, and to ensure similarity or equivalence of two curves, f₁ values between 0-15% and f₂ values ranging from 50% to 100%. As shown in Table 3, the values f₁ and f₂ 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 f₁ and f₂ 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 dissolutionSana Al-Kubati, Fadhel Al- Hariri, Gobran Ibraheem efficiencies is within appropriate limit $\pm 10\%$ (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.

Aacknowledgement

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

Description	Value
Retention time (min.)	9.5
Linear range ($\mu\text{g/ml}$)	5-25
Regression line	$y = 12346x + 6238.3$
Correlation coefficient (R^2)	0.996
Limit of detection ($\mu\text{g/ml}$)	0.02
Limit of quantification ($\mu\text{g/ml}$)	0.05
Theoretical plates	962
Tailing factor	1.18

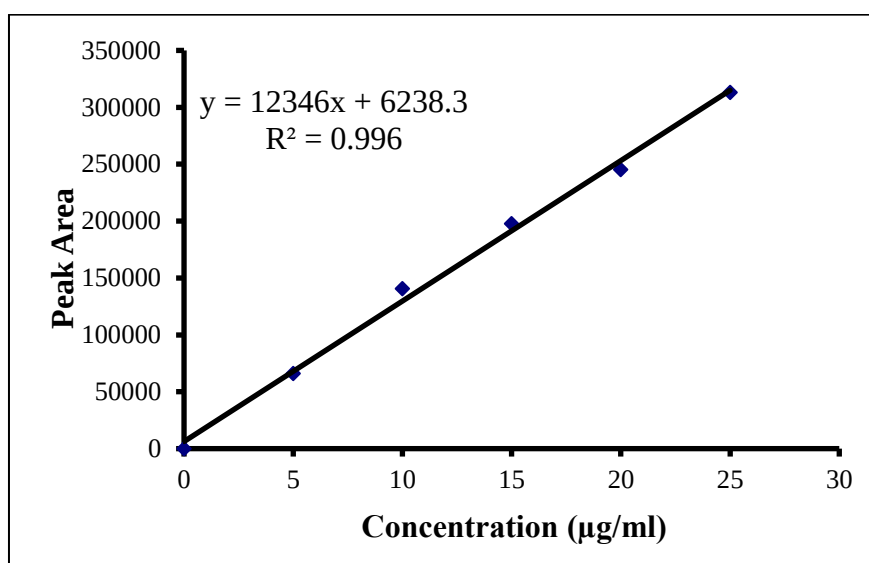


Figure 1: Calibration curve of bisoprolol fumarate

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

Brand code	Assay (%)	Mean % of drug dissolved				
		t ₅ (min.)	t ₁₀ (min.)	t ₁₅ (min.)	t ₃₀ (min.)	t ₆₀ (min.)
A	98.93	54.53	68.41	69.16	99.50	84.12
B	102.43	53.43	75.30	78.60	96.48	83.37
C	97.52	63.12	68.56	69.40	87.35	93.05
D	101.12	55.12	66.87	67.50	93.27	82.92
E	102.29	70.67	72.59	88.55	93.55	89.87

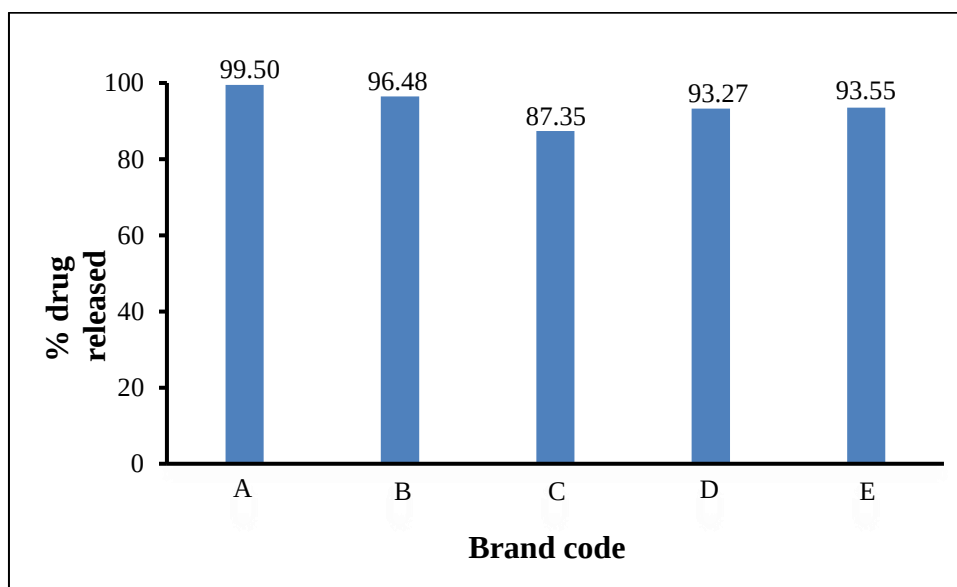


Figure 2: Percentage of biosoprolol released from the five brands within 30 minutes

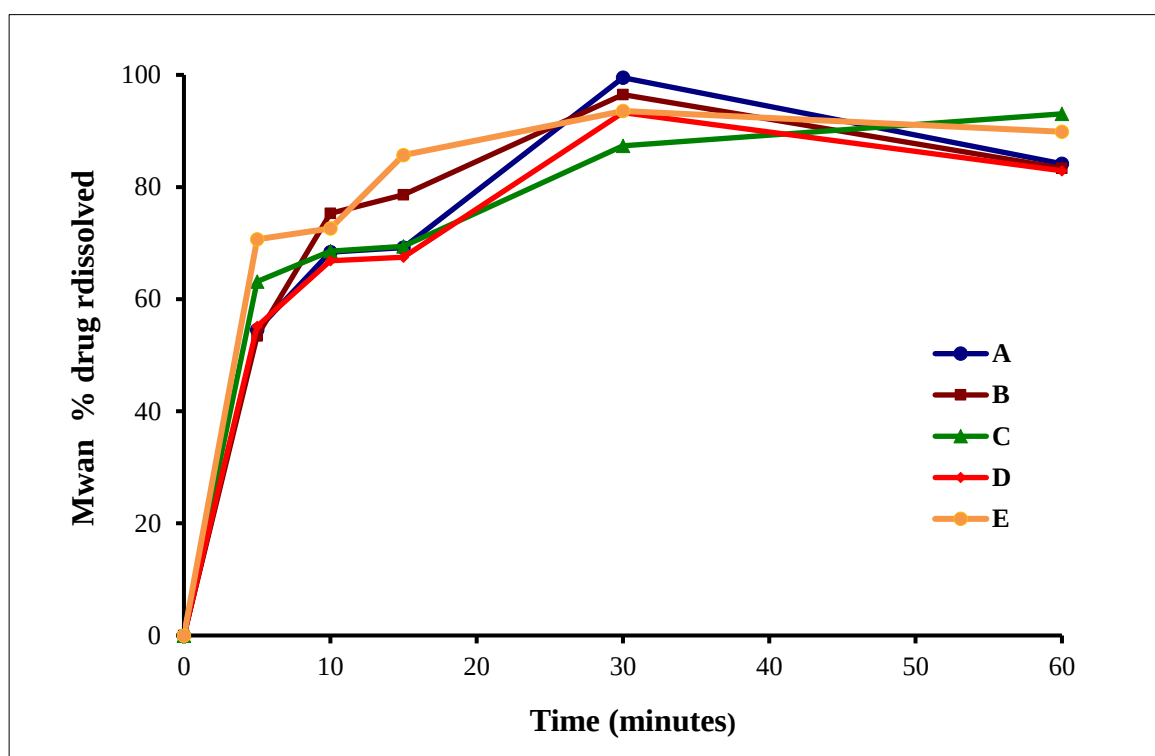


Figure 3: Comparative dissolution profiles of the five brands of bisoprolol fumarate tablets

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

Brand code	f_1	f_2	DE (%)	*DDE
A	-	-	68.76	0
B	6.60	62.89	74.40	5.64
C	9.36	55.31	76.61	7.85
D	3.50	74.83	70.92	2.16
E	16.00	45.93	82.10	13.34

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

References

1. Amidon GL, Lennernas H, Shah VP, Crison JA. (1995). theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm Res*; 12 (3): 413-420.
2. Ashraful Islam SM, Islam S, Shahriar M, Dewan I. (2011). Comparative in vitro dissolution study aceclofenac marketed tablets in two different dissolution media by validated analytical method. *J App Pharm Sci*; 1 (9): 87-92.
3. Awofisayo SO, Awofisayo OA, Eyem N, Udoh IE. (2010). Comparative assessment of the quality control measurements of multisource ofloxacin tablets marketed in Nigeria. *Dissolution Tech*; 17: 20-25.
4. Charoo NA, Shamsher AA, Lian LY, Abrahamsson B, Cristofolletti R, Groot DW, Kopp S, Langguth P, Polli J, Shah VP, Dressman J. (2014). Biowaiver monograph for immediate-release solid oral dosage form: bisoprolol fumarate. *J Pharm Sci*; 103: 378-391.

Comparison of in vitro dissolutionSana Al-Kubati, Fadhel Al- Hariri, Gobran Ibraheem

5. Esimonea CO, Okoyeb FBC, Onaha BU, Nworuc CS, Omejeb EO. (2008). In vitro bioequivalence study of nine brands of artesunate tablets marketed in Nigeria. J Vector Borne Dis; 45 (1): 60-65.
6. European Medicines Agency. (2010). Guideline on the investigation of bioequivalence. Committee for Medicinal Products for Human Use (CHMP), London UK.
7. Farid Hasan SM, Hassan F, Jabeen S. (2007). Studies on the quantification and comparison of dissolution profiles of two brands of meloxicam tablets. Pakistan J Pharmacol; 24 (1): 43-51.
8. Food and Drug Administration (FDA), Center for Drug Evaluation and Research. (1997). Guidance for industry: Dissolution testing of immediate release solid oral dosage forms, Available at <http://www.fda.gov/cder/Guidance/1713bp1.pdf>.
9. Fujii A, Yasui-Furukori N, Nakagami T, Niioka T, Saito M, Sato Y, Kaneko S. (2009). Comparative in vivo bioequivalence and in vitro dissolution of two valproic acid sustained release formulations. Drug Des Dev Ther; 2: 139-144.
10. Junior ASJ, Barbosa IS, Santos VL, Silva RL, Junior EC. (2014). Test of dissolution and comparison of *in vitro* dissolution profiles of coated ranitidine tablets marketed in Bahia, Brazil. Braz J Pharm Sci; 50 (1): 83-89.
11. Kassaye, L, Genete, G. (2013) Evaluation and comparison of in-vitro dissolution profiles for different brands of amoxicillin capsules. Afri. Heal. Sci; 13 (2): 369-375.
12. Ngwuluka NC, Lawal K, Olorunfemi PO, Ocheke NA. (2009). Post market *in vitro* bioequivalence study of six brands of ciprofloxacin tablets/caplets in Jos, Nigeria. Scientific Res Essay; 4 (4): 298-305.
13. Ocheke NA, Ngwuluka NC, Owolayo H, Fashedemi T. (2006) Dissolution profiles of three brands of Lamivudine and Zidovudine combinations in the Nigerian market. Dissolution Tech; 13 (4): 12-17.
14. Oyetunde OO, Tayo F, Akinleye MO, Aina BA. (2012). In vitro equivalence studies of generic metformin hydrochloride tablets and propranolol hydrochloride tablets under biowaiver conditions in Lagos State, Nigeria. Dissolution Tech; 19: 51-55.
15. Panainte A, Agoroaei L, Bibire N, Tantarug, Apostu M, Vieriu M, Spac FA. (2015). A HPLC method for the determination of bisoprolol in tablets and its application to a bioequivalence study. Rev Chim; 66 (11): 1791-1795.
16. Polli JE. (2008). In vitro studies are sometimes better than conventional human pharmacokinetic in vivo studies in assessing bioequivalence of immediate-release solid oral dosage forms. AAPS J; 10 (2): 289- 299.
17. Shah V. (2001). Dissolution: A quality control test vs a bioequivalent test. Dissolution Tech; 8 (4): 1-2.
18. The United States Pharmacopeia 30: The National Formulary 25. (2007) Rockville, the United States Pharmacopeial Convention, Inc.
19. Tjandrawinata RR, Setiawati E, Yunaidi DA, Santoso ID, Setiawati A, Susanto LW. (2012). Bioequivalence study of two formulations of bisoprolol fumarate film-coated tablets in healthy subjects. Drug Des Dev Ther; 6: 311-316.
20. Yu LX, Amidon GL, Poli JE, Zhao H, Mehta MU, Conner DP, Shah VP, Lesko LJ, Chen M, Lee VHL, Hussain AS. (2002). Biopharmaceutics Classification System: the scientific basis for biowaiver extensions. Pharm Res; 19 (7): 921-925.
21. Zakeri-Milani P, Nayyeri-Maleki P, Ghanbarzadeh S, Nemati M, Valizadeh H. (2012). In-vitro bioequivalence study of 8 brands of metformin tablets in Iran market. J App Pharma Sci; 2 (8): 194-197.

مقارنة الذائبية لأقراص البيسوبرولول فيومارات لخمس أصناف دوائية متداولة في

عدن – اليمن

سناء صالح فارح القباطي¹، فضل محمد الحريري² وجبران خليل إبراهيم¹

¹قسم الصيدلانيات، كلية الصيدلة، جامعة عدن

²الهيئة العليا للأدوية، فرع عدن، عدن

DOI: <https://doi.org/10.47372/uajnas.2020.n1.a23>

الملخص

يُعد عقار البيسوبرولول فيومارات حاصراً انتقائياً لمستقبلات البيتا-1 ويستعمل في علاج أمراض القلب والأوعية الدموية وله ذائبية عالية في الماء ونفاذية عالية عبر الأغشية الحيوية. في هذا البحث دُرِس التكافؤ المقارن لأربعة منتجات جنيسة لأقراص البيسوبرولول فيومارات (10 ملجم) مع المنتج المرجعي من خلال تقييم اختبار ذائبية الدواء من هذه الأقراص في الماء. وقد حُلَّت كمية الدواء في هذه الأقراص عن طريق استعمال جهاز HPLC حيث وجد أنَّ كمية الدواء في جميع الأقراص المختبرة ما بين 97.52% - 102.43%، وكذلك وجد أن كمية الدواء الذائبة خلال 30 دقيقة أكثر من 80% وبذلك تتوافق مع دستور الأدوية الأمريكي. دُرِسَت الذائبية للدواء من الأقراص للمنتجات المختبرة باستعمال الماء كوسط للذائبية وفُورِنَت منحنيات ذائبية وانطلاق الدواء من الأقراص للمنتجات الجنيسة مع المنتج المرجعي من خلال استخدام عامل الاختلاف f_1 وعامل التشابه f_2 وكفاءة الذائبية DE، وبينت النتائج أن ثلاثة من هذه المنتجات الجنيسة B, C, D متكافئة مع المنتج المرجعي A بينما المنتج الجنيس E غير متكافئ مع المنتج المرجعي. وبذلك نستنتج أن الثلاثة المنتجات الجنيسة يمكن استخدامها كبديل للمنتج المرجعي بينما المنتج غير المتكافئ لا يمكن استعمالها كبديل للدواء المرجعي.

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