



RESEARCH ARTICLE

IN VITRO-IN VIVO BIO-EQUIVALENCE CORRELATION STUDY OF ATENOLOL, AND ITS BRANDS OF IMMEDIATE RELEASE TABLET UNDER BIO-WAIVER CONDITIONS

Ahmed EM¹, Ibrahim ME¹, Magbool FF²

¹College of Pharmacy, Department of Pharmaceutical Chemistry, Ribat National University, Khartoum, Sudan.

²Department of Pharmaceutical Chemistry, Omdurman Islamic University, Sudan.

Article Info:

Abstract



Article History:

Received: 5 October 2019
 Reviewed: 7 November 2019
 Accepted: 28 December 2019
 Published: 15 January 2020

Cite this article:

Ahmed EM, Ibrahim ME, Magbool FF. *In vitro-in vivo* bio-equivalence correlation study of atenolol, and its brands of immediate release tablet under bio-waiver conditions. Universal Journal of Pharmaceutical Research 2019; 4(6):25-29.

<https://doi.org/10.22270/ujpr.v4i6.332>

*Address for Correspondence:

Fatehalrahman F. Magbool, Department of Pharmaceutical Chemistry, Omdurman Islamic University, Sudan. Tel: +966-582158608, E-mail: fmagbool@yahoo.com

Objective: The aim of present study was to examine the *in vitro-in vivo* correlation (IVIVC) of immediate release product. Atenolol 100 mg and its brands of immediate release dosage forms. Atenolol is clearly classified into BCS class III, and could be evaluated under bio waiver conditions.

Methods: The *in vitro* parameters employed were hardness, weight uniformity, friability, disintegration time, absolute drug content, dissolution rate (in 0.1 N Hydrochloric acid, phosphate buffer and acetate buffer at 37°C), and dissolution efficiencies were also analyzed. The *in-vitro* dissolution study was performed on the brands, according to FDA, USP dissolution profile in three different PH (1.2), (4.5), and (6.8) at 37°C, using the USP apparatus II. A non linear relation was established which is typical for immediate release formulation, of class III.

Results: All Atenolol brands released about 90% drug in PH (6.8), where about 87% in PH (4.5), reference drug released about 91% and test drug released about 87% in pH (1.2). Dissolution efficiency of the entire brands differed by less than 10% from the innovator brand. According to MINITAM 14 statistical program, there was significant relationship between *in vitro* and *in vivo* data of reference Atenolol product.

Conclusion: By applying level A *in vitro-in vivo* correlation, study concluded that there is no linear correlation between percent of drug released and percent of drug absorbed, this may be due to uncontrollable permeability rate for class three Atenolol.

Keywords: Bioavailability, bioequivalence, biopharmaceutical classification system, Bio-waiver correlation.

INTRODUCTION

Bio-equivalence is defined as the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study. If two medicines are bioequivalent there is no clinically significant difference in their bioavailability¹. Development and optimization of formulation is an integral part of manufacturing and marketing of any therapeutic agent which is indeed a time consuming and costly process. Optimization process may require alteration in formulation composition, manufacturing process, equipment and batch sizes. If these types of changes are applied to a formulation, studies in human healthy volunteers may be required to prove that the new

formulation is bioequivalent with the old one². Certainly, implementation of these requirements not only halts the marketing of the new formulation but also increases the cost of the optimization processes. It would be, desirable, therefore, to develop *in vitro* tests that reflect bioavailability data. A regulatory guidance for both immediate- and modified-release dosage forms has been, therefore, developed by the FDA to minimize the need for bioavailability studies as part of the formulation design and optimization³. IVIVC can be used in the development of new pharmaceuticals to reduce the number of human studies during the formulation development.

Atenolol is a selective β_1 receptor antagonist, a drug belonging to the group of beta blockers (sometimes written β -blockers), a class of drugs used primarily in cardiovascular diseases. Introduced in 1976, atenolol was developed as a replacement for propranolol in the

treatment of hypertension. It works by slowing down the heart and reducing its workload⁴.

MATERIALS AND METHODS

ATN reference standard USP, Mfg. August 2013, Exp. July 2018, and three different brands of ATN tablets 50 mg obtained from local market, DW and Methanol 99.8% (Sharlau, Spain).

Uniformity of weight test

Twenty randomly selected tablets were weighed. The average weights were determined. The tablets were weighed individually and the percentage of deviation of its weight from the average weight was determined for each tablet⁴.

Hardness test

The hardness of 10 tablets randomly selected from each batch was determined on an automatic tablet hardness tester. The crushing strength of uncoated tablets is accepted within 4-8 kg/cm².

Friability test

Total 20 tablets previously freed of dust were weighed together before transferring to a friabilator set to run for 4 min at 25 rpm. Thereafter they were removed, dusted and reweighed:

$$\% \text{ Friability} = \frac{W_i - W_f}{W_i} \times 100$$

Where, W_i is the initial weight and

W_f is the final weight of the tablets.

Disintegration time test

According to official monograph determination of disintegration time for uncoated tablets was adopted using a disintegrating apparatus and the medium was distilled water at 37±1°C. Six tablets were used for the determination. Accepted range for the uncoated tablet up to 30 minutes⁵.

Absolute drug content

Five pre-weighed tablets were crushed; the equivalent weight of a tablet was weighed out and dissolved in 100 ml volumetric flask and filtered. The absorbance reading was determined using UV-visible spectrophotometer at 273 nm⁴.

In vitro dissolution test

Volume of 900 ml of each buffer was employed. Dissolution testing was performed using Tablet Dissolution Tester (USP Apparatus 2) at 75 rpm for class III test and reference products, temperature will be adjusted to 37±0.5°C. Twelve dosage units of each product test and reference were evaluated in the three media. Sample aliquots of 10 ml were taken manually with syringes. Samples were withdrawn at specified time intervals (10, 15, 30, 45, and 60 min) and replaced with 10 ml of appropriate medium. Withdrawn samples were filtered using 0.45 µm Millipore Filters, then 5 ml taken after filtration by volumetric pipette (3 ml taken when use HCL buffer solution, and 1 ml taken in case of acetate and phosphate buffer, and diluted to 50 ml). A UV-visible spectrophotometer was used to analyze dissolved drug in dissolution testing. Scanning of wavelength done in each buffer, and spectrum recorded between 200-800 nm, and percentage of drug dissolved calculated⁶.

Buffers preparation

Simulated gastric fluid (SGF), simulated intestinal fluid (SIF), and acetate buffer PH (4.5) were prepared according to instructions in USP test solution. All media were prepared without enzymes, as follows:

Simulated Gastric Fluid (SGF) PH (1.2)

To prepare hydrochloric acid 0.1N, 8.5 ml was taken from concentrated HCL (37%) and volume completed to 1000 ml by distilled water⁷.

Simulated Intestinal Fluid (SIF) pH (6.8)

Potassium phosphate monobasic KH₂PO₄ 0.2 M was prepared by dissolving 27.22 g in water, and volume diluted to 1000 ml by distilled water. Then sodium hydroxide 0.2 M prepared by dissolving 8 g in water and volume diluted to 1000 ml by distilled water. 250 ml from Potassium phosphate monobasic KH₂PO₄ 0.2 M was placed into 200 ml volumetric flask, also 112 ml taken from sodium hydroxide 0.2 M and volume completed to 1000 ml with distilled water⁸.

Acetate buffer pH (4.5)

Firstly acetic acid 0.2 N was prepared from concentrated acetic acid 99.93%. Measured amount of 116 ml was taken and diluted with distilled water. Then 2.99 g of sodium acetate (NaC₂H₃O₂) taken, and placed in 1000 ml volumetric flask, 14 ml from acetic acid was added and volume completed to 1000 ml by distilled water⁸.

Preparation of standard stock solutions

Standard stock solutions of Atenolol in HCL, phosphate and acetate buffers were prepared by dissolving 100 mg of standard in 100 ml volumetric flask using acetate and phosphate buffers as solvents to give concentration of 1000 µg/ml, 5 ml diluted to 100 ml volumetric flask (50 µg/ml), using 50 ml volumetric flask to give serial concentration of standard curve⁸.

Statistical analysis

All dissolution data evaluated using Excel spread sheet, and the results will be plotted for each brand. Average of % content of active pharmaceutical ingredient (API) dissolved in each media of 12 tablets will be taken and a plot of % of (API) dissolved against time will be drawn to represent the dissolution profile. The dissolution profile for local brand will be compared to that of the reference drug⁹.

If they are similar the similarity factor, f_2 equal to or more than 50. This means that they are equivalent, if it's less than 50 they are not equivalent.

$$f_1 = \{ [3t = 1n / Rt - Tt] / [3t = 1n Rt] \} C$$

$$f_2 = 50 C \log \{ [1 + (1/n) 3t = 1n (Rt - Tt)^2] - 0.5C 100 \}$$

Similarity factor f_2 has been adopted by FDA and the European Agency for the Evaluation of Medicinal Products (EMA) by the Committee for Proprietary Medicinal Products (CPMP) as a criterion to compare the similarity of two or more dissolution profiles. Similarity factor f_2 is included by the Centre for Drug Evaluation and Research (CDER) in their guidelines such as guidance on dissolution testing of immediate release solid oral dosage forms (FDA, 1997) and guidance on waiver of *in-vivo* bioavailability and bioequivalence studies for immediate release solid oral dosage forms based on a biopharmaceutics classification system (FDA, 2000).

The area under the dissolution-time curve method was used in calculating the dissolution efficiency (DE), and this was calculated at 30 min. The higher the dissolution efficiency (DE) is, the better the release efficiency of the tablets' active ingredient, according to equation:

$$DE = \left\{ \left[\int_0^t \% D_t \cdot dt \right] / [\% D_{max} \cdot (t_2 - t_1)] \right\} \cdot 100 = [AUC_{0-T} / \% D_{max} \cdot T] \cdot 100$$

Where %D is the percentage dissolved at time t, % D (max) is the maximum dissolved at the final time T, and AUC (0-T) is the area under the curve from zero to time T^{10,11}. Correlation calculation was carried out using MINITAB14 specific statistical program.

In vitro-In vivo relationship determination of level A correlation.

In vivo percent absorbed of reference product was calculated by following equation:

$$\frac{A_t}{A_0} = \frac{C_t + K_{el} \cdot AUC_0^t}{K_{el} \cdot AUC_0^\infty}$$

Where, $\frac{A_t}{A_0}$ denotes the fraction of drug absorbed at time t, C_t is the plasma drug concentration at time t, K_{el} is elimination rate constant, AUC_0-t and $AUC_0-\infty$ are the area under the plasma concentration– time profile curve at time t and ∞ respectively. Then the values of percent of drug released were plotted against the percent of drug absorbed for reference products of Atenolol using MINITAB14 analysis program to find out the relationship between data (correlation).

Table 2: Quality control test results of atenolol brands.

Brands	Hardness (kg/cm)	Weight variation (RSD)	Disintegration Time(min)	Friability %	Assay %
Sample (A)	6.7	0.027	8:7	0.01158	99.88
Sample (B)	5.9	0.185	6:6	0.0184	103

Friability for all brands of Atenolol was below 1%. Disintegration is the process of breaking of tablets in the liquid. Disintegration is a crucial step for immediate release dosage forms because the rate of disintegration affects the dissolution and subsequently the therapeutic efficacy of the medicine. A drug will be released rapidly as the dosage forms disintegrate.

Table 3: F1 and F2 values for Atenolol.

Samples	1.2		4.5		6.8	
	F1	F2	F1	F2	F1	F2
Sample (B)	3	71	1	92	2	87

British Pharmacopoeia specifies that uncoated tablets should disintegrate within 15 min and film coated tablet disintegrate within 30 min while USP specification for disintegration is 30 min for both uncoated and film coated tablets. All the brands were complied with both BP and USP specifications for disintegration as maximum disintegration time. Potency is the average amount of the active ingredient present per tablet. All the brands complied both BP and USP specification, as USP specification is that the content of active ingredient should not be less than 90% and not more than 110% while BP specifies that

RESULTS AND DISCUSSION

A summary of the results of weight uniformity, hardness, friability, disintegration and assay are shown in Table 2. Weight uniformity may serve as a pointer to amount of the active pharmaceutical ingredient (API) contained in the formulation.

Table 1: Weight uniformity of atenolol tablets.

Number of tablets	Deviation (%)	Average weight of tablets
Minimum 18	±10.0	Less than 80 mg
Maximum 20	±20.0	
Minimum 18	±7.5	80 mg to 250 mg
Maximum 20	±15.0	
Minimum 18	±5.0	More than 250 mg
Maximum 20	±10.0	

All the brands complied with the compendial specification for weight uniformity. Hardness is referred to as non-compendial test. The hardness or crushing strength assesses the ability of dosage form to withstand handling without fracturing or chipping, It can also influence other parameters such as friability and disintegration. Hence, the dosage forms of all brands were satisfactory for hardness⁷. Friability test is used to evaluate the tablets resistance to abrasion. Friability is now included in the United States Pharmacopoeia (USP, 1995) as a compendia test. The compendial specification for friability is less or equal to 1%.

the content should not be less than 95% and not more than 105%.

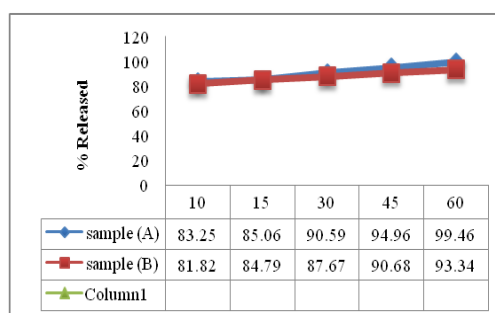


Figure 1: Dissolution profile of Atenolol in pH (1.2).

The results of dissolution studies are graphically represented in the Figure 1, Figure 2 and Figure 3. All dissolution data are based on the actual drug content of the test dosage form as calculated from the assay results. All Atenolol brands released about 90% drug in PH (6.8), where about 87% in PH (4.5), reference drug released about 91% and test drug released about 87% in pH (1.2). To compare the dissolution profiles of the brands, a model independent approach of difference factor f1 and similarity factor f2 were employed.

Difference factor f1 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 (f2) 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 two curves.

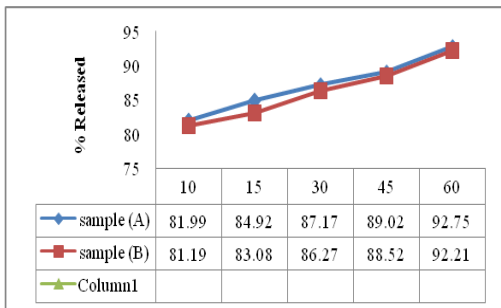


Figure 2: Dissolution profile of Atenolol in pH (4.5).

Two dissolution profiles to be considered similar and bioequivalent, f1 should be between 0 and 15 while f2 should be between 50 and 100 (FDA, 1997). All the

values for f2 and f1 shown in Table 3 for atenolol, all brands f2 values were more than 50 and f1 values were less than 15. It means that all brands were equivalent with the innovator brand. *In-vitro* AUC in three PH (1.2), (4.5), (6.8) for class III product, there was large difference between *in-vitro* AUC and *in-vivo* AUC, the *in-vivo* AUC is too small due to the lower permeability for this class of drug products, which will affect their AUC. The reference and the test product can said to be equivalent if the difference between their dissolution efficiencies is within appropriate limits ($\pm 10\%$, which is often used). Dissolution efficiency of the entire brands differed by less than 10% from the innovator brand. So, we can say that all the brands are pharmaceutically equivalent with the innovator brand⁶. As IVIVC is a predictive mathematical model describing the relationship between variables (an *in vitro* property of a dosage form and a relevant *in vivo* response). According to MINITAM 14 statistical program, there was significant relationship between *in vitro* and *in vivo* data of reference Atenolol product.

Table 4: Dissolution efficiency for Atenolol brands.

Brands	1.2		4.5		6.8	
	AUC	Difference with reference	AUC	Difference with reference	AUC	Difference with reference
Brand (A)	348.83	-	357.68	-	364.69	-
Brand (B)	358.05	-9.22	355.93	1.75	359.43	-5.26

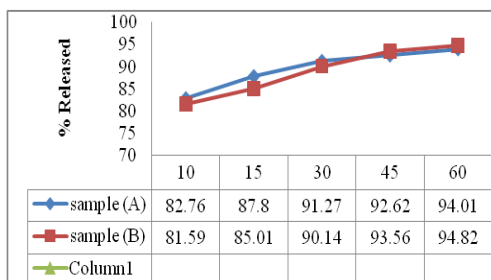


Figure 3: Dissolution profile of Atenolol in pH (6.8).

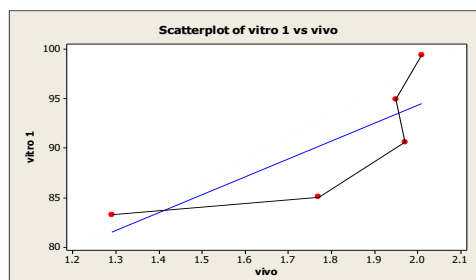


Figure 4: Atenolol correlation at pH (1.2).

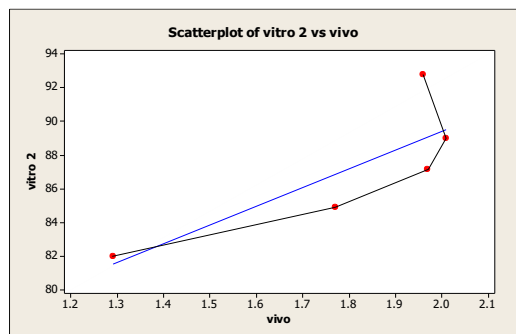


Figure 5: Atenolol correlation at pH (4.5).

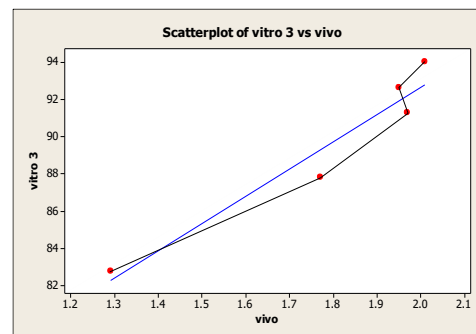


Figure 6: Atenolol correlation at pH (6.8).

Correlation and distribution of data with correlation coefficient ($r=0.798, 0.815, 0.967$), non linear relationship with p -value (>0.05)=(0.106, 0.93, 0.009), there is no out lines, no lake of fits at p -value=0.106, 0.040, 0.056 (>0.05) for the three pH (1.2,4.5,6.8) respectively^{10,11}. Estimating the uncertainty in predicted correlation between *vitro* and *vivo* data. The interval is represented by the curved lines on either side

of the regression line and gives an indication of the range within which the ‘true’ line might lie.

Table 5: Relative dissolution efficiency of sample (B).

PH	1.2	4.5	6.8
%	102.64	99.51	98.56

Note that the confidence interval is narrowest near the center (the point x, y) and less certain near the extremes. By applying analysis of variance (ANOVA) for the dissolution data using MINITAB 14 we concluded that the test products are bioequivalent to reference products of Atenolol and could be interchangeable.

CONCLUSIONS

The bio waiver study has emphasized that pharmaceutical equivalence indicate that product have same drug molecule with approximately same pattern of dissolution release profile. By making fine turning in bioequivalent study we can reduce the time, cost, avoid ethical consideration by unnecessary exposure of healthy subjects to medicines and finally to market the quality generic drug product. By applying level A *in vitro-in vivo* correlation, study concluded that there is no linear correlation between percent of drug released and percent of drug absorbed, this may be due to uncontrollable permeability rate for class three Atenolol. Atenolol are immediate release formulations. As dissolution is not a rate-limiting step in IR products, the fraction of drug absorbed against the fraction of drug released profile would be non-linear type which was obtained in present study. So it may be concluded that the *in vitro-in vivo* correlation is well established and justified for both reference formulations by level A correlation. By applying analysis of variance (ANOVA) for the dissolution data using MINITAB 14 we concluded that the test products are bioequivalent to reference products of Atenolol and could be interchangeable.

ACKNOWLEDGEMENT

The authors are thankful to wish to Azal Industries, Khartoum, Sudan, for providing the gift sample of Atenolol (standard).

AUTHOR'S CONTRIBUTION

Ahmed EM: writing original draft, methodology, investigation. **Ibrahim ME:** formal analysis, data curation, conceptualization. **Magbool FF:** writing, review and editing. All the authors approved the finished version of the manuscript.

DATA AVAILABILITY

The data and material are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

None to declare.

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