International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

COMPARATIVE *IN VITRO* PHARMACEUTICAL EQUIVALENCE STUDIES OF DIFFERENT BRANDS OF ATORVASTATIN CALCIUM TABLETS MARKETED IN BANGLADESH

Md. Abir Khan[1], Abu Asad Chowdhury[2], Nur Jaharat Lubna[1*]

[1]Laboratory of Pharmaceutics, Department of Pharmacy, Primeasia University, Banani, Dhaka 1213, Bangladesh
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh

***Corresponding author e-mail:** nurjaharatlubna@yahoo.com

ABSTRACT

The main target of the present study was to evaluate the pharmaceutical equivalence of atorvastatin calcium tablets of five brands marketed in Bangladesh using *in vitro* dissolution study. The dissolution was carried out using the apparatus II according to USP guidelines. Evaluation of physicochemical parameters such as content uniformity test, weight variation analysis, and hardness, friability and disintegration test was carried out according to United States of Pharmacopoeia. All brands complied with the official specification for content uniformity test, weight variation, hardness, and friability and disintegration time. Despite the inter brand and intra brand variability of dissolution profiles of the atorvastatin tablets, three out of five brands attained more than 75% dissolution within 45 minutes. For comparative analysis of dissolution, a model independent approach of similarity factor (f2) was used. The results showed that three brands of atorvastatin calcium tablets out of five passed all the pharmacopoeia tests for satisfactory quality. Thus, only these can be interchanged in clinical practice.

Key words: Atorvastatin calcium, pharmaceutical equivalence, *in vitro* dissolution.

INTRODUCTION

The therapeutic efficacy of a drug depends on rate and extent of drug absorption from the site of administration to the systemic circulation. The dissolution rate of poorly water-soluble drugs is often a rate-limiting step in their absorption from the GI tract.[1] Poorly soluble drugs suffer limited oral bioavailability and are often associated with high intra subject and inter subject variability. For this, constant surveillance on the marketed poorly water soluble drugs by the government, manufactures and independent research groups is essential to ensure the quality of medicines.

Atorvastatin is an inhibitor of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMGCoA) reductase enzyme which catalyzes the conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol biosynthesis.[2] Recently it is used as calcium salt for the treatment of hypercholesterolemia.[3] According to the biopharmaceutical classification system, it is a low soluble and highly permeable drug.[4] It is slightly soluble in water and its intestinal permeability is high but the absolute bioavailability of atorvastatin is only 12% after a 40 mg oral dose.[5] The reduced bioavailability of the drug might be due to low dissolution, degradation in gastrointestinal tract and hepatic first-pass metabolism.[2,6]

The bioavailability assessment of various categories of commercial tablets in Bangladesh has been published.[7,8] But no such information is available on marketed atorvastatin tablets. So, the purpose of the study is to determine dissolution profiles of locally manufactured atorvastatin calcium tablets and to

compare those profiles statistically with drugs from innovator company (as reference) using similarity factor (f2) to confirm the quality, safety and efficacy of the marketed atorvastatin tablets.

MATERIALS AND METHODS

Drugs and chemicals: Standard atorvastatin calcium was a kind gift from Eskayef Pharmaceuticals Bangladesh Ltd., Bangladesh. Five brands of atorvastatin calcium (10 mg) were purchased from local drug store in Dhaka city, Bangladesh. The samples were properly checked for their manufacturing license numbers, batch numbers, production and expiry dates. They were randomly designated as B1, B2, B3, B4 and B5. Innovator brand (Lipitor® 20 mg) was used as the reference sample (IB). Chemicals and all other reagents were of analytical grade and were purchased from local suppliers.

Determination of uniformity of weight: 20 tablets from each of the brands were weighed individually with an analytical balance (AY-200, Shimadzu, Japan). The average weights and the percentage deviation from the mean value for each brand were calculated.

Preparation of stock solutions and calibration curve of atorvastatin calcium: Hundred milliliter stock solution of 50 µg/mL was prepared by dissolving 0.05 g of atorvastatin in suffifient amount of 0.1 N hydrochloric acid. From this stock, different concentration solution 0, 2.5, 5.0, 7.5, 10.0, 15, 20 µg/mL were prepared using the same solvent. Absorbance of each solution was taken at 241 nm using a UV-Visible spectrophotometer (Model UV-800 Shimadzu, Japan). A plot of absorbance versus concentration of atorvastatin calcium was made from which the regression equation was calculated.

Chemical Assay: Each brand of atorvastatin tablet was crushed into powder and dissolved in 100 ml of 0.1 N HCl. After firltration, the sample was diluted to 10-fold and subjected to measuring the absorbance at 241 nm. From the absorbance value and regression equation of calibration curve, the amount of atorvastatin in each tablet was calculated.

Hardness test: The hardness was determined with an automatic tablet hardness tester (Model HDT–300F, Logan Instrument Corp.). Ten tablets were randomly selected from each brand and the pressure at which each tablet crushed was recorded.

Friability test: Twenty tablets of each brand was weighed and subjected to abrasion using a friability tester (Model FIB-2S Logan Instrument Corp.) at 25 rotation/minutes for 4 minutes. The tablets were then weighed and percentage friability was calculated.

Disintegration test: Six tablets of each brand were taken for the test in distilled water at 37°C with an automatic disintegration tester (Model DST-3, Logan Instrument Corp.) employing plastic discs. The disintegration time was taken as the time when no particles remained in the basket of the tester.

Dissolution test: The dissolution test was carried out using a dissolution tester (Model UDT-804, Logan Instrument Corp.) according to USP guidelines in 6 replicates for each brand.[9] The dissolution medium was 900 mL of 0.1 N HCl which was maintained at 37.0±0.5°C. In all the experiments, 5 ml of dissolution sample was withdrawn at 0, 15, 30, 45 and 60 min and replaced with equal volume to maintain sink condition. Samples were filtered and assayed by UV-VIS spectrophotometer (Model UV-800, Shimadzu, Japan) at 241 nm. The concentration of each sample was determined from a calibration curve obtained from standard samples of atorvastatin. The percent dissolutions were computed. The data were tailed and computed the means. The percent dissolutions of the samples and reference innovator were graphed against time. The values for T50% and T90% were determined as they are used as guides for dissolution.[10]

A**nalysis of similarity factor:** The uniformity of weight was analyzed with simple statistics while the dissolution profiles were analyzed by a mathematical model, similarity factor (f2). Mean dissolution values were employed to estimate the similarity factor (f2). A factor value of 50 or greater (50-100) ensures sameness or equivalence of the two products. The following equations were used to calculate similarity factor (f2).[11]

$$f_2 = 50 \text{ x } \log \left[\frac{100}{\sqrt{1+\frac{\sum[Rt-Tt]^2}{n}}} \right]$$

Where n is the number of time points, Rt is the dissolution value of reference product at time 't' and Tt is the dissolution value for the test product at time.

RESULTS AND DISCUSSION

The *in vitro* bioequivalence of five generic atorvastatin tablets in Bangladesh was evaluated by dissolution study. The results of uniformity of weight, hardness, friability, disintegration time and assay are shown in Table 1. Weight variation test is

an important test for the evaluation of tablet because its variation lead to variation of content uniformity which ultimately results in sub-therapeutic dose or over dose of the tablet. The average weight of A, B, C, D, E and IB was 155.0±1.66, 159.93±2.89, 168.22±2.36, 183.96±1.5, 165.77±2.45 and 147.0±0.73 respectively. According to USP, the not more than two tablets having weight 120-300 mg should fall ±7.5% weight variation. In this experiment, all the tablets of each brand show weight variation within the range and no one show outside the range indicate that they comply the USP specification.

The percent potency of the drug is important to maintain the therapeutic efficacy. According to USP specification the potency of the drugs must be with 95-105%. The calibration curve as shown in Figure 1 has good correlation (r^2=0.9901). The results indicate that all the brand of atorvastatin comply the USP specification. The low deviation of the value might be due to the lower weight variation of the tablets.

Hardness is referred to as non-compendial test. It can also influence other parameters such as friability and disintegration. According to USP, the tablet hardness should be 40-80 N. All the barnds of atorvastatin show hardness within the range specified in USP.

Friability test is now included in the United States Pharmacopeia (USP, 1997) as a compendial test. The USP specification for friability is less than 0.5%. All the brands of atorvastatin comply with the USP specification.

Disintegration time of all the brands was within limit. The BP specifies that uncoated tablets should disintegrate within 15 min and film coated tablets in 30 min, while the USP specifies that both uncoated and film coated tablets should disintegrate within 30 min. All atorvastatin tablets were film coated and maximum time for disintegration was found 27.08 min in case of brand B5.

The dissolution mean values of the generic and reference innovator in 0.1 N HCl were shown in Table 2.The results of dissolution studies were presented in Figure 2. Both inter- and intra-brand variations in dissolution profiles were observed. Marketed Brands B1, B2 and B3 had release equal to or greater than 75% of label claim of atorvastatin calcium within 45 min, as specified in the B.P for conventional tablets. In contrast, B4 and B5 released less than 75% drug within 45 min. The relatively slow ability of these two brands to release the active compound gives cause for therapeutic concern as this can have negative impact on the pharmacokinetics and therapeutic efficacy of this formulation. Interestingly, all the brands was shown to contain adequate level of pure atorvastatin calcium in the tablets during the chemical assay experiments.

Similarity factor (f2) has been adopted by FDA and the European Agency for the Evaluation of Medicinal Products to compare dissolution profiles.[12,13] Two dissolution profiles are considered similar and bioequivalent, if f2 is between 50 and 100.[12] A T90% of 30 minutes is satisfactory and is an excellent goal.[10] In this study, parameters like T50%, T90% and f2 were derived from the dissolution profiles of the different brands. Table 3 showed the f2 values of different brands in respect of brand IB. For brand B1, B2 and B3, f2 values were more than 50. So they are similar with brand IB and can be used interchangeably. For brand B4 and B5, f2 value was less than 50. So it is not similar with brand IB and cannot be used interchangeably.

CONCLUSION

Of the five immediate release formulations of atorvastatin calcium tablets that were available in Bangladesh, only three brands met all the pharmacopoeial standards for satisfactory tablets. However, *in vivo* test may be required for final comments regarding the quality of marketed brands of atorvastatin.

Table 1: Summary of the quality control tests undertaken on different brands of atorvastatin tablets

Brand Code	Average Weight±SD (mg)	Weight variation(%)	Potency (%)	Hardness ± SD (N)	Friability (%)	Disintegration time ± SD (min)
B1	155±1.66	1.73	97.11±1.62	74.50± 3.17	0.41	1.58± 0.23
B2	159.93±2.89	3.71	97.55±1.74	61.90±7.02	0.43	2.49± 0.11
B3	168.22±2.36	2.92	97.02±1.80	69.70±6.25	0.38	3.32± 0.34
B4	183.96±1.50	1.44	97.78±2.12	75.00±2.58	0.39	24.40± 3.52
B5	165.77±2.45	2.26	98.94±4.18	70.30±5.58	0.05	27.08± 4.48
IB	147.73±1.36	1.27	99.23±1.57	63.04±2.34	0.06	1.28± 0.27

Table 2: Mean percent dissolution of atorvastatin tablet.

Time (min)	B1±SD	B2±SD	B3±SD	B4±SD	B5±SD	IB±SD
0	0	0	0	0	0	0
15	56.14±2.14	55.52±3.01	58.27±3.11	38.17±2.43	34.74±3.27	66.23±2.31
30	70.53±3.75	72.73±2.74	76.28±2.84	45.51±2.86	47.37±3.86	83.52±3.86
45	82.35±3.64	84.74±2.43	82.39±3.14	60.73±3.26	57.37±4.23	90.34±2.05
60	85.27±4.29	88.38±1.81	91.04±3.61	64.21±3.22	60.43±4.07	93.12±1.87

Table 3: $T_{50\%}$, $T_{75\%}$, $T_{90\%}$ and f2 of three brands of atorvastatin tablet.

Atorvastatin Brand	$T_{50\%}$ (minutes)	$T_{75\%}$ (minutes)	$T_{90\%}$ (minutes)	Similarity factor (f2)
B1	<15 min	<45 min	<60 min	79.83
B2	<15 min	<45 min	>60 min	80.79
B3	<15 min	<45 min	<60 min	83.56
B4	>15 min	>45 min	>60 min	47.35
B5	>15 min	>45 min	>60 min	45.41
IB	<15 min	<45 min	<45 min	-

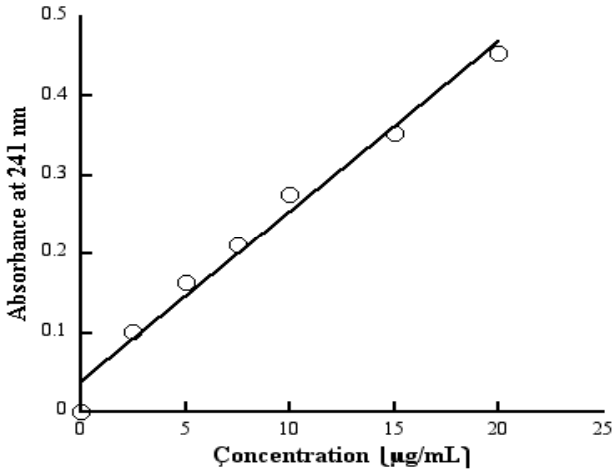

Figure 1: Calibration curve of atorvastatin for measurement of dissolution profiles.

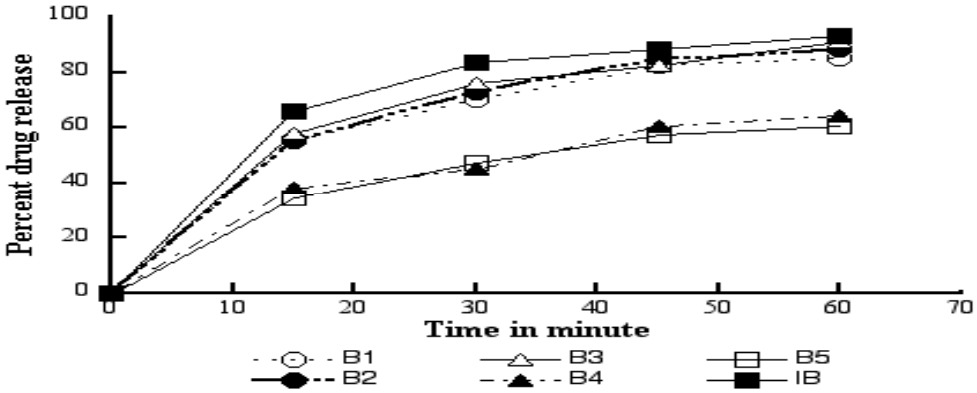

Figure 2: Comparison of dissolution profiles of different brands (A-E) of atorvastatin tablet with innovator product (IB).

REFERENCES

1. Chiba Y, Kohri N, Iseki K, Miyazaki K. Chem Pharm Bull, 1991; 39: 2158-60.
2. Lennernas H. J Pharm Pharmacol, 1997; 49: 627–38.
3. Colhoun HM, Betteridge DJ, Durrington PN. Lancet, 2004; 364: 685.
4. Amidon GL, Lennernas H, Shah VP, Crison JRA. Pharm Res, 1995; 12, 413-20
5. Corsini A, Bellosta S, Baetta R, Fumagalli R, Paoletti R, Bernini F. Pharmacol Ther, 1999;84: 413-28.
6. Cilla DD, Whitfield JLR, Gibson DM, Sedman AJ, Posvar EL. Clin Pharmacol Ther, 1996;60: 687-95.
7. Nikolic L, Jovanovic M. J Pharm Sci, 1992; 81: 386-91.
8. Romero AJ, Grady ET, Rhodes CT. Drug Dev Ind Pharm, 1988; 14: 1549-86.
9. US Pharmacopoeia 30, Sixth Supplement. US Pharmacopeial Convention, Rockville, MD, 2007.
10. Lachman L, Lieberman HA, Kanig JL. The Theory and Practice of Industrial Pharmacy, 2nd ed., Philadelphia, Lea and Febiger: 1976.
11. Shah VP, Tsong Y, Sathe P, Liu JP. Pharm. Res. 1998; 15: 889–96.
12. Guidance for industry: Dissolution testing of immediate release solid oral dosage forms US Food and Drug Administration, Center for Drug Evaluation and Research. August, 1997. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070237.pdf
13. 13. Note for guidance on the investigation of bioavailability and bioequivalence, the European agency for the evaluation of medicinal products (EMEA). http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003519.pdf .