Comparative *in vitro* Bioequivalence Analysis of Some Generic Tablets of Atorvastatin, a BCS Class II Compound

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Abstract

This study was aimed to assess the bioequivalence of ten generic atorvastatin tablets from different manufacturers using *in vitro* dissolution and membrane permeability studies. Other general quality parameters of these tablets like weight variation, hardness, friability, disintegration time were also determined according to established protocols. The active ingredients were assayed by a validated HPLC method. All brands complied with the official specification for friability and disintegration time but two brands did not comply official specification for uniformity of weight. Assay of atorvastatin tablets revealed that all samples contained over 98% (w/w) of labeled potency. The dissolution profiles showed inter brand and intra brand variability. Only four samples attained 70% dissolution within 45 min. Membrane permeability rate of selected brands were found to be proportional to the *in vitro* dissolution rate. The test results were subjected to statistical analysis to compare the dissolution profile. A model independent approach of difference factor (f1), similarity factor (f2) and dissolution efficiency (%DE) were employed and the data indicated that only 4 brands may be used interchangeably.

Keywords: Atorvastatin, Bioequivalence, Generic tablets

Introduction

The therapeutic efficacy of a drug depends on rate and extent of drug absorption in the systemic circulation. The dissolution rate of poorly water-soluble drugs is often a rate-limiting step in their absorption from the GI tract (Chiba *et al*, 1991). Such drugs suffer limited oral bioavailability and are often associated with high intra subject and inter subject variability. So, constant surveillance on the marketed poorly water soluble drugs by the government, manufactures and independent research groups is essential to ensure availability of quality medicines.

Atorvastatin, a synthetic lipid-lowering agent, is an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMGCoA) reductase which catalyzes the conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol biosynthesis (Lennernas, 2003). Atorvastatin is currently used as calcium salt for the treatment of hypercholesterolemia (Colhoun *et al.*, 2004). It is a class II compound (low soluble and highly permeable) according to the biopharmaceutical classification system (Amidon *et al.*, 1995). It is insoluble in aqueous solution at pH 4 and below; but it is slightly soluble in water. The intestinal permeability of atorvastatin is high at the physiologically relevant intestinal pH (Lennernas, 1997; Wu *et al.*, 2000). However, it has been reported that the absolute bioavailability of atorvastatin is only 12% after a 40 mg oral dose (Corsini *et al.*, 1999). The low systemic availability is attributed to low dissolution, pre-systemic clearance in gastrointestinal mucosa and hepatic first-pass metabolism (Cilla *et al.*, 1996; Lennernas, 2003).

Bioavailability assessment of various categories of commercial tablets in different countries has been published (Nikolie *et al.*, 1992; Romero *et al.*, 1988; Wood *et al.*, 1990). But no such information is available on marketed BCS class II compound, atorvastatin. Thus, atorvastatin tablets were selected to evaluate the quality of locally available lipid lowering drugs with special emphasis on the study of disintegration and dissolution

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properties of the test samples due to their immense importance in predicting drug bioavailability as well as product quality.

Materials and Methods

Drug: Standard of atorvastatin calcium was a kind gift from Incepta Pharmaceuticals Ltd, Bangladesh.

Dosage form: Ten brands of atorvastatin tablets (10 mg) were purchased from local drug store in Dhaka city. The samples were properly checked for their manufacturing license numbers, batch numbers, production and expiry dates. They were randomly coded as A-J and stored properly.

Solvents and reagents: Acetonitrile and methanol were of HPLC grade. Ammonium acetate, acetic acid and other reagents were of analytical-reagent grade and obtained from Germany. Water was deionised and double distilled.

Determination of uniformity of weight: 20 tablets from each of the 10 brands were weighed individually with an analytical balance (AY-200, Shimadzu, Japan). The average weights for each brand as well as the percentage deviation from the mean value were calculated.

Hardness test: The crushing strength was determined with an Automatic Tablet Hardness Tester (8M, Dr Schleuniger, Switzerland). 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 were weighed and subjected to abrasion by employing a Veego friabilator (VFT-2, India) at 25 rev/min for 4 min. The tablets were then weighed and compared with their initial weights and percentage friability was calculated.

Assay: A simple, selective and rapid reversed phase High Performance Liquid Chromatographic (RP-HPLC) method was used to determine the potency of related tablets. Standard and sample solutions were prepared by dissolving 5 mg standard atorvastatin and powdered tablets equivalent to 5 mg of the drug in 10 ml methanol separately. Then the solutions were diluted with the mobile phase. The chromatographic system consisted of a LC-20 AT pump and SPD-20 UV/visible detector (Shimadzu, Japan). The separation was achieved from C_{18} column (5µ, 4.6 X 150 mm, Waters, USA) at 25 ºC temperature with a mobile phase comprising of acetonitrile and buffer (solution of ammonium acetate, ratio 55:45 v/v, pH=4.00 adjusted with acetic acid) at a flow rate of 1.5 ml/min. The data were acquired and processed using LC solution (Version 1.2, Shimadzu, Japan) software running under Windows XP on a Pentium PC. The retention time was about 7.685 minutes both for standard and sample solution (Figure 1). Potency was calculated for each brand by comparing the standard and sample peak area.

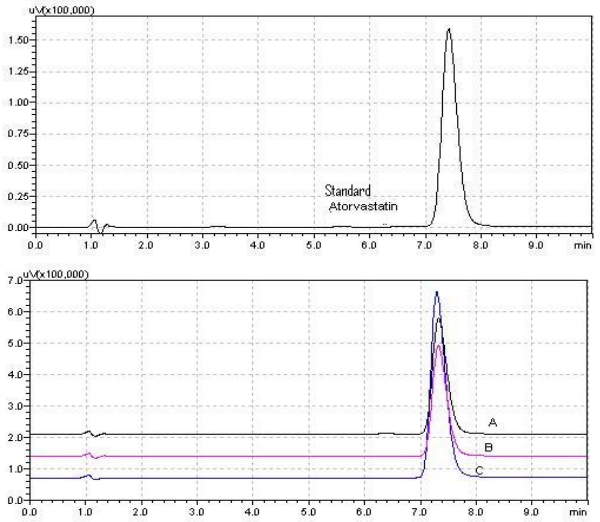

Fig.1. Chromatogram of standard atorvastatin and brand A, B, C

Disintegration test: Six tablets from each brand were employed for the test in distilled water at 37 °C using a Tablet Disintigration Tester (Model: VDT-2, Veego, India). The disintegration time was taken as the time when no particle remained on the basket of the system.

Dissolution test: The dissolution test was undertaken using Tablet Dissolution Tester (TDT-08L, Electrolab, India) in 6 replicates for each brand. The dissolution medium was 900 ml of 0.1N HCl which was maintained at 37 ± 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 (UV-1700, Shimadzu, Japan) at 241 nm. The concentration of each sample was determined from a ten point calibration curve obtained from standard samples of atorvastatin.

In vitro drug diffusion studies: In vitro diffusion studies were carried out for brand A and D. Atorvastatin solution in methanol and atorvastatin dispersed in water

were also included in this study for comparison. One end of pretreated cellulose dialysis tube (7 cm in length) was tied with thread and then tablet dispersed in 10 ml water was placed in it and the other end of the tube was also tied with thread and was allowed to rotate freely in the dissolution vessel of a USP 24 type II dissolution test apparatus (Electrolab TDT-06P, India) that contained 900 ml purified water maintained at 37 ± 0.5 °C and stirred at 100 rpm. Aliquots were collected periodically and replaced with fresh dissolution medium. Aliquots, after filtration through Whatman filter paper (No. 41), were analyzed by using UV-VIS spectrophotometer (UV-1700, Shimadzu, Japan) at 241 nm for atorvastatin content.

Data analysis: The uniformity of weight was analyzed with simple statistics while the dissolution profiles were analyzed by difference factor (f1), similarity factor (f2) and dissolution efficiency (%DE).

Results and Discussion

The *in vitro* bioequivalence of 10 generic atorvastatin tablets was evaluated by dissolution and membrane permeability study. The results of uniformity of weight, hardness, friability, disintegration time and assay are shown in Table 1. Uniformity of weight serves as a monitor to good manufacturing practices (GMP) as well as amount of the active pharmaceutical ingredient (API) contained in the formulation. Out of ten brands examined eight brands complied with the compendial specification for uniformity of weight which states that for tablets having 80-250 mg weight, not more than 2 tablets should differ from the average weight by more than 7.5% and none will deviate by 15% of average weight. Brand C did not comply uniformity of weight test as 4 tablets crossed the limit of 7.5%. On the other hand brand E did not comply uniformity of weight test as 1 tablet crossed the limit of 15%.

Table 1. A summary of the quality control tests undertaken on different brands of atorvastatin tablets

Brand Code	Average Wt	% Deviation from average weight	Hardness (N) (Ave ± SD)	Friability (%)	DT (min)	Potency (%)
A	163.16	8.37	61.9 ± 7.19	0.32	3	101.06
B	179.72	8.84	56.3 ± 11.10	0.45	0.5	105.78
C	173.35	12.32	127.1 ± 33.57	0.12	17	103.68
D	152.88	5.42	89.9 ± 25.92	0.34	1	105.35
E	183.99	16.46	99.4 ± 20.00	0.24	5	109.45
F	93.15	6.28	57.3 ± 8.00	0.24	8	103.51
G	128.38	6.10	87.9 ± 10.61	0.17	4	98.30
H	156.18	3.22	101.1 ± 8.14	0.31	4	98.24
I	180.38	3.42	102 ± 4.57	0.15	10	108.53
J	161.83	8.82	88.6 ± 16.47	0.22	2	102.98

Hardness is referred to as non-compendial test. It can also influence other parameters such as friability and disintegration. Tablet hardness was found 56.3 - 127.1 N. A force of about 40 N is the minimum requirement for a satisfactory tablet (Allen *et al.*, 2004). Hence the tablets of all brands were satisfactory for hardness.

Friability test is now included in the United States Pharmacopeia (USP, 1995) as a compendial test. The compendial specification for friability is 1%. Friability for all the brands was below 1%.

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 17.00 min in case of brand C.

Potency of all the brands was found within 98.24%-109.45%. Atorvastatin is an INN drug, no official specification is available. But by comparing with the USP specification of another brand, simvastatin (potency limit: 90%-110%) we can say that potency was within limit.

The results of dissolution studies are graphically represented in Figures 2 and 3. Both inter-brand (brand to brand) and intra-brand (within a brand) variations in dissolution profiles were observed. Brands A, E, F and G

released less than 40% drug within 15 minutes. Brands C and J released about 60% atorvastatin within 15 minutes. On the other hand brands H, B, D and I released more than 60% drug within 15 minutes. From these data it is clear that although potency and DT were almost similar within different brands but the brands differ in case of drug release.

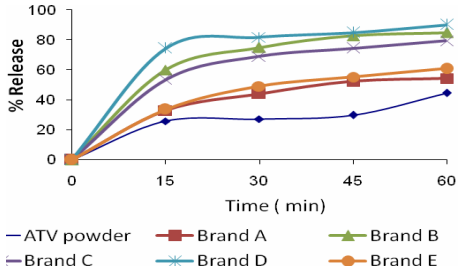

Figure 2. Dissolution profiles of different brands (A-E) of atorvastatin tablets with pure drug.

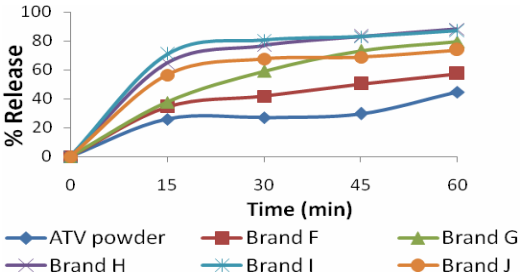

Figure 3. Dissolution profiles of different brands (F-J) of atorvastatin tablets with pure drug.

The results of *in vitro* drug diffusion studies are graphically represented in Figure 4. Drug solution in methanol passed the cellulose membrane quickly. This clearly indicates that the absorption of atorvastatin is completely dissolution rate limited. On the other hand, drug dispersed in water diffused slowly.

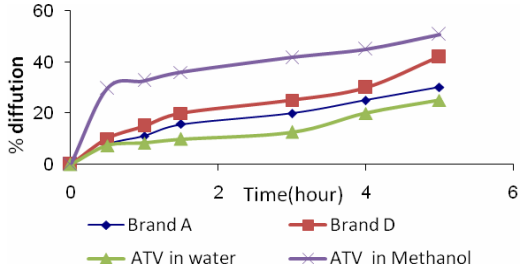

Figure 4. Diffusion profiles of selected brands (A&D) of atorvastatin tablets with atorvastatin powder.

Brand D whose *in vitro* dissolution rate was higher passed the membrane quickly. So membrane permeability rate was found proportional to the *in vitro* dissolution rate.

Comparison of dissolution data: Difference factor (f1), similarity factor (f2) and dissolution efficiency (%DE) were calculated to compare the dissolution profile. 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 the two curves. The following equations were used to calculate difference factor (f1) and similarity factor (f2).

$$f_1 = \left\{ \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \right\} X100$$

$$f_2 = 50 \log \left\{ \left(1 + \frac{1}{n} \sum_{i=1}^{n} (R_t - T_t)^2 \right)^{-0.5} X100 \right\}$$

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 t.

Similarity factor (f2) has been adopted by FDA (1997) and the European Agency for the Evaluation of Medicinal Products (EMEA, 2001) by the Committee for Proprietary Medicinal Products (CPMP) to compare dissolution profile. Two dissolution profiles are considered similar and bioequivalent, if f1 is between 0 and 15 and f2 is between 50 and 100 (FDA, 1997).

Table 2. f1 and f2 of ten brands tested

Pair Comparison	f2	f1
A vs D	21.60	44.49
B vs D	53.59	8.57
C vs D	42.18	16.48
E vs D	23.83	39.90
F vs D	21.62	44.45
G vs D	31.93	24.71
H vs D	62.22	5.51
I vs D	75.14	3.38
J vs D	39.46	19.53

Table 2 shows the f1, f2 values of different brands in respect of brand D as a reference brand. For brands B, H and I, f2 value were more than 50 and f1 were less than

15. So they are similar with brand D and can be used interchangeably.

Dissolution efficiency (DE) was also employed to compare the drug release from various brands. Dissolution efficiency is the area under the dissolution curve within a time range (t1 - t2). DE was calculated by using the following equation:

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

where *y* is the percentage dissolved at time *t.*

Table 3. Dissolution efficiencies (% D.E) of the ten brands.

Brand Code	%DE	Difference of % DF (test product - reference product)
Brand A	46.73	36.27
Brand B	76.78	6.22
Brand C	70.02	12.98
Brand D	83.00	0.00
Brand E	50.59	32.41
Brand F	46.03	36.97
Brand G	63.62	19.38
Brand H	78.83	4.16
Brand I	80.79	2.21
Brand J	67.14	15.86
Powder Drug	30.76	52.23

Table 3 shows the dissolution efficiency of different brands along with the differences with brand D. The reference and the test product can be said to be equivalent if the difference between their dissolution efficiencies is within appropriate limits (± 10%, which is often used) (Anderson *et al*, 1998). Higher dissolution efficiency was found in case of brand D. The dissolution efficiencies of brand B, C, H and I were more than 70% and may be considered as quality products. Brand B, H and I are equivalent to brand D as difference of % DF (test product – reference product) is less than 10. However, the rest of the brands were very much away from the limit (± 10%). So, they are not similar with brand D and can not be considered as interchangeable.

Conclusion

The oral delivery of poorly soluble drugs is frequently associated with low bioavailability and high intra- and inter subject variability. The present study proved that atorvastatin is such a drug that is associated with low bioavailability and high intra- and inter subject variability. This study has also emphasized that chemical equivalence does not indicate bioequivalence and one brand substituted on assumption of chemical equivalence with another brand may not give the desired onset of action and subsequent therapeutic effectiveness. However, *in vitro* dissolution test in three pH levels and probably *in vivo* test may be required for final comments regarding the quality of marketed brands of atorvastatin.

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