

Evaluation of In Vitro Equivalence for Tablets Containing the Poorly Water-Soluble Compound Atorvastatin

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ABSTRACT

This paper describes the evaluation of the in vitro equivalence of tablets containing a poorly water-soluble compound, atorvastatin, marketed in Bangladesh under biowaiver conditions. Drug release was compared with that of a reference product. The in vitro equivalence test was carried out in three different media (pH 1.2, pH 4.5, and pH 6.8). Test results were subjected to statistical analysis to compare the dissolution profiles. Model-independent approaches of difference factor (f_1), similarity factor (f_2), and dissolution efficiency (%DE) were employed. Dissolution profiles of test and reference (innovator) atorvastatin are equivalent at pH 6.8 without statistical treatment. The test products are equivalent at pH 4.5 ($f_1 < 15$ and $f_2 > 50$) and not equivalent at pH 1.2 ($f_1 > 15$ and $f_2 < 50$). Other general quality parameters of these tablets (e.g., weight variation, crushing strength, friability, and disintegration time) were also determined according to established protocols, and test results were within limit.

INTRODUCTION

Some drugs that have a good clinical therapeutic effect often show low systemic availability because of poor water solubility. Up to 40 percent of new chemical entities discovered by the pharmaceutical industry today are poorly soluble or lipophilic compounds. The solubility issues complicate the delivery of these new drugs and many existing drugs (1). Poorly water-soluble drugs show unpredictable absorption and high intrasubject and intersubject variability (2–4). Therefore, constant surveillance of marketed, poorly water-soluble drugs by the government, manufacturers, 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 (5). The calcium salt of atorvastatin is currently used for the treatment of hypercholesterolemia (6).

The intestinal permeability of atorvastatin is high at the physiologically relevant intestinal pH (7, 8). However, it has been reported that the absolute bioavailability of atorvastatin is only 12% after a 40-mg oral dose (9). The low systemic availability is attributed to low dissolution, presystemic clearance in gastrointestinal mucosa, and hepatic first-pass metabolism (10).

Atorvastatin calcium is a crystalline powder and is insoluble in aqueous solution at pH 4 and below. It is very slightly soluble in water. The solubility in aqueous solu-

tion at pH 2.1 is about 0.0204 mg/mL, while the solubility in pH 6.0 aqueous solution is about 1.23mg/mL (11). The dose/ solubility (D/S) ratio for atorvastatin is greater than 250 mL for the 10-mg dose at pH 1.2, although the drug dissolves in 250 mL of buffer solution at pH 6.8. Therefore, atorvastatin is a low solubility drug according to WHO Guidance (12). Atorvastatin is not listed in the WHO Model list and is not classified according to BCS by WHO (13). Because of its solubility (low) and permeability (high), atorvastatin is assigned to BCS Class 2 according to WHO guidance.

We selected atorvastatin tablets to evaluate the quality of locally available, lipid-lowering drugs with special emphasis on the study of disintegration and dissolution properties of the test samples due to their immense importance in predicting drug bioavailability as well as product quality. We used USP buffer solutions of pH 1.2 (hydrochloric acid solution), pH 4.5 (acetate buffer solution), and pH 6.8 (phosphate buffer solution). Six units were tested for dissolution. Other general quality parameters of these tablets like weight variation, crushing strength, friability, disintegration time were also determined according to established protocols. The test results were subjected to statistical analysis to compare the dissolution profile. Model independent approaches of difference factor (f_1), similarity factor (f_2), and dissolution efficiency (%DE) were employed.

MATERIALS AND METHODS

Chemicals

Standard atorvastatin calcium was a kind gift from Incepta Pharmaceuticals Ltd., Bangladesh. Three brands

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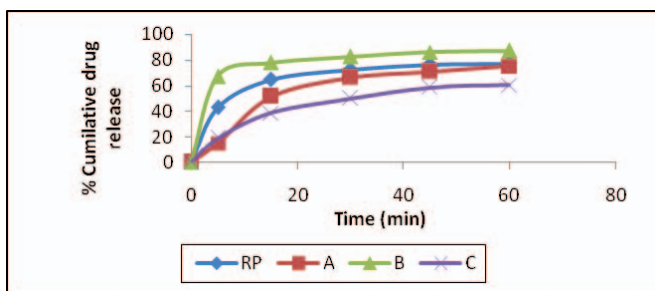


Figure 1. Dissolution profiles of test and reference drugs at pH 1.2.

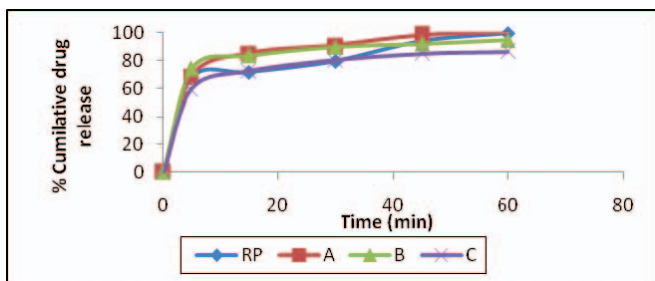


Figure 2. Dissolution profiles of test and reference drugs at pH 4.5.

of generic atorvastatin tablets (10 mg) along with the innovator brand (reference product) were purchased from local drug stores in Dhaka city. The samples were properly checked for their manufacturing license numbers, batch numbers, and production and expiry dates. They were randomly coded as A–C and stored properly. Acetonitrile was of HPLC grade. Orthophosphoric acid and other reagents were of analytical reagent grade. Water was deionized and double distilled.

Test of Physicochemical Parameters

The average weights for each brand as well as the percentage deviation from the mean value were calculated by weighing 20 tablets of each brand with an analytical balance (AY–200, Shimadzu, Japan). The crushing strength was determined with an Automatic Tablet Hardness Tester (8M, Dr. Schleuniger, Switzerland). Twenty tablets of each brand were weighed and subjected to abrasion by employing a Veego friabilator (VFT–2, India) at 25 rev/

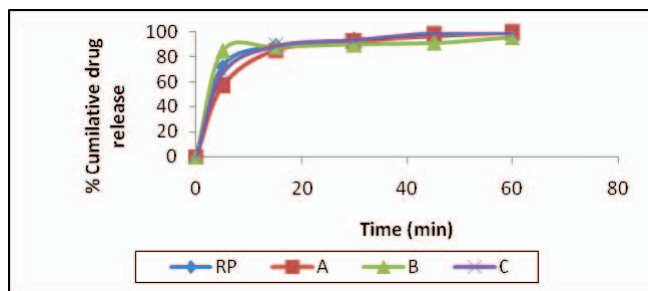


Figure 3. Dissolution profiles of test and reference drugs at pH 6.8.

min for 4 min. The tablets were then weighed, the weights were compared with the initial weights, and the percentage friability was calculated. Six tablets from each brand were employed for the disintegration test using a Tablet Disintegration Tester (Model: VDT–2, Veego, India). The disintegration time was taken as the time when no particle remained on the basket of the system.

HPLC Analysis

A Shimadzu HPLC system consisting of two LC–20 AT pumps was used for the quantification of atorvastatin in tablets. The drug analysis data were acquired and processed using LC Solution software (Version 1.2, Shimadzu, Japan) running under Windows XP on a Pentium PC. Here, 0.025 M phosphoric acid (pH 2.8)/acetonitrile (30:70) was used as the mobile phase. The flow rate was 1 mL/min, injection volume was 20 μ L, and UV detection was at 245 nm. The temperature was maintained at ambient (30 $^{\circ}$ C), and the sensitivity was 0.0005. The retention time of atorvastatin was 2.75 min.

Dissolution Test

The dissolution test was undertaken using USP Apparatus 2 (TDT–08L, Electrolab, India) at 75 rpm. Dissolution media were USP buffer solutions of pH 1.2 (hydrochloric acid solution), pH 4.5 (acetate buffer solution), and pH 6.8 (phosphate buffer solution). The medium was maintained at 37 ± 0.5 $^{\circ}$ C. In all experiments, 5 mL of dissolution sample was withdrawn at 0, 5, 10, 15, 30, and 45 min and replaced with an equal volume to maintain sink conditions. Samples were filtered and assayed by a validated HPLC method. The concentration of each sample was

Table 1. Values of f_1 , f_2 , and %DE for Test and Reference Drugs at pH 1.2, 4.5, and 6.8

Brand	pH 1.2			pH 4.5			pH 6.8		
	f_2	f_1	%DE	f_2	f_1	%DE	f_2	f_1	%DE
Reference Product			67.16			79.62			71.88
A	39.64	20.61	56.19	52.40	8.28	88.27	54.23	8.36	68.08
B	40.58	22.17	79.86	52.40	10.72	86.73	59.20	6.68	71.71
C	32.24	34.93	44.39	53.95	7.40	76.36	77.54	2.21	71.08

determined from a calibration curve obtained from pure samples of atorvastatin.

Data Analysis

The uniformity of weight was analyzed with simple statistics while the dissolution profiles were analyzed by difference factor (f_1), similarity factor (f_2), and dissolution efficiency (%DE).

RESULTS AND DISCUSSION

The tablet weight variation for all the brands was low (<3.5% deviation). Crushing strength of the tablets was in the range 40–75 N, and the percentage weight loss in the friability test was $\leq 0.5\%$ for all batches. Drug contents of the tablets in all batches were within specification (98–102%). Overall, the tablets were of good quality with regard to crushing strength, friability, weight uniformity, and drug content.

The results of the dissolution studies are graphically represented in Figures 1–3. Values for f_1 and f_2 were calculated from the dissolution data. The 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 difference factor f_1 and similarity factor f_2

$$f_1 = \left\{ \frac{\left[\sum_{t=1}^n |R_t - T_t| \right]}{\left[\sum_{t=1}^n R_t \right]} \right\} \times 100$$

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

where n is the number of time points, R_t is the dissolution value of reference product at time t , and T_t is the dissolution value for the test product at time t . Dissolution efficiency (%DE) is the area under the dissolution curve within a time range (t_1 – t_2). Table 1 shows the f_1 , f_2 , and %DE values of different brands with respect to the reference product.

Dissolution profiles in pH 6.8 buffer were similar without statistical treatment (percent cumulative release was greater than 85% within 15 min for test and reference products). For pH 4.5, the similarity factor f_2 value was greater than 50, and f_1 was less than 15; therefore, the dissolution profile of the test product is similar to that of the reference product at pH 4.5. Dissolution profiles in pH 1.2 buffer are considered not similar because the calculated factors do not meet the acceptance criteria ($50 \leq f_2 \leq 100$; $0 \leq f_1 \leq 15$). Test products may have contained alkalizing agents that possibly increased atorvastatin solubility, which caused the dissolution profile dissimilarity. The

addition of alkalizing agents in a formulation significantly increases the solubility of atorvastatin, especially at low pH (14).

Dissolution efficiency (%DE) was also employed to compare drug release from various brands. %DE is the area under the dissolution curve within a time range (t_1 – t_2) and is defined as

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

where y is the percentage of dissolved product. Table 1 shows the dissolution efficiency of different brands. The reference and test products are considered equivalent if the difference between their dissolution efficiencies is within appropriate limits ($\pm 10\%$ is often used) (15). Dissolution efficiencies of the test products were almost similar to the reference products in pH 4.5 and 6.8 media. However, test products were not similar to the reference product in pH 1.2 buffer due to the difference between their %DE values.

CONCLUSION

The dissolution profiles of the test products were similar to that of the reference product in pH 4.5 and 6.8 buffers, but not similar in pH 1.2 buffer using the paddle method at 75 rpm, although test brand B showed higher drug release at pH 1.2 within the testing time period. However, in vivo testing may be required for final comments regarding the similarity between marketed brands and the reference products.

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