Evaluation of *In-vitro* Bioequivalence of Commonly Prescribed Generics of Poorly Water Soluble Drug-Atorvastatin Calcium in Pakistan

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**ABSTRACT**

Atorvastatin is a HMG-CoA reductase inhibitor which reduces the blood cholesterol levels and is commonly used for the treatment of hypertension. It is a poorly water soluble drug and has 14% oral bioavailability therefore mild formulation factors may affect its oral absorption and bioavailability. This study was aimed to conduct bioequivalence study for six commonly prescribed brands of atorvastatin. In this study *in vitro* bioavailability of six generic brands of atorvastatin were compared with the standard brand leader using their dissolution profiles and evaluation of pharmacokinetic data model independent approach (f1 and f2). Only one generic brand exhibits similar release profile and its f1 and f2 values were within range and the rest of the five brands did not show similar release patterns in comparison to the brand leader. The results of other physical test were found to be within range. It is concluded that all generic brands of atorvastatin are not bioequivalent in terms of their *in vitro* release behavior. Thus *in vitro* testing should be declared as mandatory requirement for registration of any pharmaceutical generic in Pakistan.

**KEYWORDS:** Atorvastatin, bio equivalence, generics, *in vitro* dissolution, model independent approach.

**INTRODUCTION**

Hypercholesterolemia is a risk factor for development of atherosclerotic disease. Atorvastatin is a synthetic hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, which reduces levels of low-density lipoprotein (LDL) cholesterol, very low density lipoprotein (VLDL) cholesterol and triglycerides. It also increases the levels of high density lipoprotein (HDL) cholesterol in patients with a wide variety of dyslipidemias.¹

Atorvastatin is administered in acid form as calcium salt and is lipid soluble and permeable. Atorvastatin is completely absorbed in the stomach after oral administration; however, it is subject to extensive first-pass effect which occurs in the gut wall and liver contributing to an overall oral bioavailability of 14%. The drug is 98% plasma protein bound and has a volume of distribution 381L. It is extensively metabolized in both the liver and gut by oxidation, lactonisation and glucuronidation. Cytochrome P450 (CYP) 3A4 is responsible for the biotransformation of atorvastatin and it forms two active metabolites and the lactone forms of atorvastatin. Atorvastatin and its metabolites further undergo glucuronidation mediated by uridinediphospho glucuronyl transferases 1A1 and 1A3. The metabolites produced are majorly (>99%) eliminated by biliary secretion and direct secretion from blood to the intestine and very little (<1%) via renal excretion.

The elimination half-life (t½) of atorvastatin has been reported about 7 hours² and mean elimination half-life of about 14 hours, but as a result of active metabolites the half-life of atorvastatin becomes 20 to 30 hours.³

Multiple daily doses of 2.5 to 80mg yields a steady-state maximum plasma concentration (Cmax) 1.95 to 252 µg/L within 2 to 4 hours after administration and area under the plasma concentration-time curve (AUC) values from 25.2 to 1293 µg/L.h. The Cmax and AUC values may increase in patients with hepatic impairment. As the renal excretion of atorvastatin is <1% therefore renal impairment has no effect on the Cmax and AUC values. During clinical trials little drug accumulation was seen in the elderly, but this accumulation did not produce clinically significant changes in its pharmacodynamics.³

In usual daily dosages of 10 to 80 mg, atorvastatin works by lowering the plasma levels of low-density lipoprotein (LDL) cholesterol by inhibition of HMG-CoA reductase. The mean pharmacodynamics dose-response relationship has been shown to be log-linear for atorvastatin, but plasma concentrations of atorvastatin and its metabolites do not correlate with the pharmacodynamics effect (LDL-cholesterol reduction) at a given dose.²

Atorvastatin oral bioavailability is limited by factors such as the membrane permeability of the gastrointestinal tract, the solubility of the drug, and the dissolution rate of the drug. Specially, the solubility and
the dissolution rate of a sparingly water soluble drug is a critical factor for its oral bioavailability. As the membrane permeability and the solubility are constant factors so the most important variable deciding the oral bioavailability is the dissolution rate of the formulated drug.\(^5\)

The bioavailability of atorvastatin is one of the key parameters for successful treatment of many therapeutic conditions. Since there are differences in the solubility among individual atorvastatin forms, it also produces an indirect impact on their bioavailability. It is very important to ensure uniformity of the drug substance employed in a pharmaceutical formulation of the atorvastatin calcium for the effective pharmacodynamics response.\(^5\)

Pharmacoeconomic studies have shown lipid-lowering with atorvastatin to be cost effective in patients with CHD, men with at least one risk factor for CHD and women with multiple risk factors for CHD. In available studies atorvastatin was more cost effective than most other HMG-CoA reductase inhibitors in achieving target LDL-cholesterol levels.\(^1\)

This study was aimed to compare the \textit{in vitro} equivalence of commonly prescribed brands of atorvastatin in Pakistan and to help healthcare providers select the most economical brand of atorvastatin having better \textit{in vitro} performance.

**MATERIAL & METHODS**

**Chemical**

Standard Atorvastatin calcium powder was gifted by Pearl Pharmaceutical Islamabad, Pakistan. Methanol used for extraction and distilled water used for dilution was of analytical reagent grade.

**Sampling**

To study the \textit{in vitro} drug release five national and two multinational brands of Atorvastatin were collected randomly from pharmacies of Rawalpindi, Islamabad. The samples were checked for their manufacturing license number, batch number, manufacturing and expiry dates. These samples were randomly coded as A, B, C, D, E, F, and the reference brand and stored properly. All the collected samples have labeled active ingredient Atorvastatin 10 mg and were packaged in blister packing.

Following tests were carried out for each sample.

**Tests of physicochemical parameters:**

The average weight for each brand as well as the percentage deviation from the mean value were determined by weighing 20 tablets of each brand with an analytical balance (B.P 110S, Sartorius, Germany). The crushing strength (Hardness) was determined using an Automatic digital Tablet Hardness Tester (HT-901, Curio digital, Pakistan) for 5 tablets of each brand and the mean hardness was determined.

Friability test was applied on twenty tablets of each brand which were weighed and subjected to abrasion by employing a friabilitator (FB-0498, Curio, China) at 25 rev/min for 4 minutes. The tablets were then weighed, the weight was compared with the initial weight, and the percentage friability was calculated. For disintegration test six tablets from each brand were employed in distilled water using a Tablet Disintegration Tester (Model DS-0702, Curio, China) at 37 °C. The disintegration time was taken as the time when no particle remained on the basket of the system. The test was repeated three times for each brand to take mean disintegration time.

**In vitro dissolution Study**

The dissolution test was conducted using USP type II apparatus (DL-0601, Curio, China) at 37±0.5°C and 75 rpm with six sections assembly according to the USP 30 procedure (USP 30 and NF 25, 2007). Dissolution media used was USP Phosphate buffer pH 6.8.

The medium was maintained at 37 ± 0.5 °C. 5 mL of dissolution sample was withdrawn at 5, 15 and 45 min and replaced with an equal volume to maintain sink conditions. Samples were filtered and assayed by a validated UV method. The concentration of each sample was determined from a calibration curve obtained from pure samples of atorvastatin.

**RESULTS AND DISCUSSION**

The weight variation of all the brands was low (S.D < 3.5%) except brand B while the hardness of all brands was found to be within limits (<10 Kg/cm²). Disintegration time of tablets in all brands was found to be within range (<10 mins) except brand F. Drug contents of tablets in all batches were within specified limits (90-110%). All tablets were found to be of good quality in terms of physical appearance. The brand leader (G) was found to have lowest mean weight (153.7mg) with standard deviation 1.64, and ideal hardness (10 Kg/cm²) with lowest disintegration time (1.1 min). The results are given in the following table.

Table 1: Price in (PKR Rs) and Values of different tests of all brands

<table>
<thead>
<tr>
<th>Code</th>
<th>Price / Tab Mean Weight ± S.D (Kg/cm²)</th>
<th>Hardness D. Time Assay (%) ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15.5 189.28 ± 3.02</td>
<td>10 7.7 ± 0.42 107.9 ± 5.1</td>
</tr>
<tr>
<td>B</td>
<td>9 244.60 ± 11.39</td>
<td>7.6 9.5 ± 6.36 102.6 ± 0.5</td>
</tr>
<tr>
<td>C</td>
<td>14.5 153.00 ± 3.44</td>
<td>9 3.1 ± 1.56 100.8 ± 0.5</td>
</tr>
<tr>
<td>D</td>
<td>15 198.14 ± 2.31</td>
<td>11 5.1 ± 1.27 106.6 ± 10.3</td>
</tr>
<tr>
<td>E</td>
<td>9.9 195.40 ± 4.33</td>
<td>7.4 1.65 ± 0.92 109.2 ± 0.4</td>
</tr>
<tr>
<td>F</td>
<td>10 171.20 ± 2.86</td>
<td>5.5 45 ± 7.07 99.9 ± 3.1</td>
</tr>
<tr>
<td>Reference 66.2 153.70 ± 1.64</td>
<td>10 1.1 ± 0.14 98.2 ± 1.6</td>
<td></td>
</tr>
</tbody>
</table>

The result of dissolution studies was evaluated using model independent approach. The values for f1 (Difference factor) and f2 (Similarity factor) were calculated. The formulations were considered similar if f1 and f2 were below 15 and 50, respectively. The similarity factor for all formulations was found to be within limits, indicating similarity in dissolution behavior.
larity factor) were calculated from the dissolution profile data shown in Figure-1.

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

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

Where \( R_t \) is dissolution value of reference brand at time \( t \), and \( T_t \) is the dissolution value of test brand at time \( t \) and \( n \) is the number of time points. For a test brand to be bioequivalent the difference factor \( (f_1) \) must be from zero to fifteen (0-15) and the similarity factor \( (f_2) \) must be from fifty to hundred (50-100). A product with 0 difference factor \( (f_1) \) and 100 similarity factor \( (f_2) \) is considered 100% bioequivalent. The \( f_1 \) and \( f_2 \) values of all the test brands are given in the following Table-2.

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>( f_1 )</th>
<th>( f_2 )</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15.09</td>
<td>34.40</td>
<td>Not Equivalent</td>
</tr>
<tr>
<td>B</td>
<td>39.98</td>
<td>15.18</td>
<td>Not Equivalent</td>
</tr>
<tr>
<td>C</td>
<td>4.85</td>
<td>59.39</td>
<td>Equivalent</td>
</tr>
<tr>
<td>D</td>
<td>15.23</td>
<td>34.82</td>
<td>Not Equivalent</td>
</tr>
<tr>
<td>E</td>
<td>10.04</td>
<td>44.22</td>
<td>Not Equivalent</td>
</tr>
<tr>
<td>F</td>
<td>56.73</td>
<td>9.77</td>
<td>Not Equivalent</td>
</tr>
</tbody>
</table>

When compared with the reference Brand C was found to be equivalent to the reference product as has \( f_1 \) 4.85 and \( f_2 \) 59.39 while rest of the brands were found to have different dissolution profiles. There is a difference of Rs. 51.7 per tablet in the price of reference brand and Brand C having similar dissolution profile and found to be bioequivalent through model independent approach.

CONCLUSION

The dissolution profile of only one brand of atorvastatin was found to be similar to the reference brand; while other commonly prescribed brands have different release behavior and dissolution profile. As the difference in dissolution profile may affect bioavailability (rate and extent of drug absorption) therefore these brands may not produce same drug levels within the body and therefore optimum therapeutic effects may not be achieved. Cost effectiveness is an important step in enhancing patient compliance of such drugs, but therapeutic effects of the drug should not be compromised in such cases. Therefore drug regulatory authority of Pakistan should make it compulsory to submit in vitro dissolution profile \( (f_1 \) and \( f_2 \)) and stability testing data for registration of a generic brand after the expiry of patent period.

REFERENCES


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