Comparative *In vitro* Bioequivalence Evaluation of Different Brands of Amoxicillin Capsules Marketed in Tigray, Ethiopia

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**ABSTRACT**

The availability of multisource generic brands of amoxicillin in the market today places health professionals and patients in a difficult situation about the choice of a suitable product among numerous generic brands. The purpose of this study was to estimate the bioequivalence of amoxicillin capsules marketed in Ethiopia using *in vitro* tests in order to determine their interchangeability. The *in vitro* dissolution study was carried out on the six brands of amoxicillin capsules according to USP guidelines. To compare the dissolution profiles, a difference factor ($f_1$), similarity factor ($f_2$), dissolution efficiency (DE) and statistical methods were employed. Results have shown significant differences in the dissolution profiles of the brands based on the statistical analysis ($p<0.0001$). Pair-wise comparisons using Dunnett’s test indicated that the innovator brand has a significantly faster dissolution than the generic brands, except brand D. According to $f_1$, $f_2$ and DE calculations, only brand D was found to have similar dissolution profile with the innovator. Based on the *in vitro* studies, only brand D may be considered bioequivalent and interchangeable, while the other brands may not be considered bioequivalent and interchangeable with the innovator brand. This research highlights among other things the need for constant monitoring and surveillance on the marketed drugs by regulatory bodies to ascertain bioequivalence and quality medicines, especially for drugs like amoxicillin for which there exists evidence of non-bioequivalence from different firms, resulting in efficacy issues.

**KEYWORDS:** amoxicillin; bioequivalence; brand; dissolution; *in-vitro* bioavailability.

**Introduction**

Post-market monitoring of approved medicines has been employed to assess the quality, therapeutic effectiveness and safety of medicines (Hailu et al., 2011). The data and information obtained could be used for product improvement, development of standards and regulations (Chandrasekaran et al., 2011). Encouraging generic drugs from multiple sources into the healthcare system (Al-Ameri et al., 2012) is frequently promoted as a means of lowering healthcare costs (Hailu et al., 2011). This has, however, been accompanied by widespread of substandard drug products (Al-Ameri et al., 2012; Hailu et al., 2011). In the literature, large variation in the bioavailability of amoxicillin formulations has been reported. The most relevant was reported by Kyriacos et al. (2008) who evaluated amoxicillin capsules in some Arab countries, and found that 56% of amoxicillin capsules did not meet the USP requirements. Likewise, Bronnikova et al. (2008) and Caiaffa et al. (2002) indicated that variation in the dissolution profiles among amoxicillin brands were found in the Estonian and Brazilian markets. On the other hand, Del Tacca et al. (2009) and Manitpisitkul et al. (1998) found that some generic amoxicillin capsules were not bioequivalent to the innovator product on the basis of the *in vivo* studies in the Italian and Thai markets, respectively.

Drug absorption from a solid dosage form depends on the release of the drug substance and the solubilization of the drug (Ashraful-Islam et al., 2012a). Nowadays, *in vitro* dissolution study is considered as a fundamental requirement in the pharmaceutical industry and by regulatory authorities in order to guide formulation developments, predict *in vivo* performance and serve as a surrogate for bioequivalence (Ferraz and Carpentieri, 2007; Menegola et al., 2007). On a parallel basis, it allows stability studies (Ferraz and Carpentieri, 2007) and ensures continuing products quality after certain changes. Dissolution test is used as an *in vitro* bioequivalence test and to establish the similarity of dosage forms (Menegola et al., 2007). For that reason, the importance of dissolution profile for the establishment of bioequivalence must be highlighted (Hailu et al., 2011). In the literature, different methods are described for comparing dissolution profiles, such as statistical, model-dependent and model-independent methods. All the methods appear to be applicable and useful in comparing dissolution profiles (Eryol et al., 2004).

In this study, bioequivalence of selected multisource amoxicillin capsules was assessed using statistical,
difference factor ($f_1$), similarity factor ($f_2$) and dissolution efficiency (DE) methods in order to determine the appropriateness of their interchangeability and hence their bioequivalence.

**Materials and Methods**

**Materials**

High Performance Liquid Chromatography (Shimadzu, Japan), a Pharma Test dissolution tester (PTWS610, Germany), a LOGAN DST-3 disintegration tester (USA), a UV-1700 Pharma Spec (Shimadzu, Japan) and an electronic balance (Sartorius, Germany) provided by Addis Pharmaceutical Factory were used for the study. Likewise, acetonitrile HPLC grade (Sigma-Aldrich, Germany), sodium hydroxide (Sigma-Aldrich, Germany) and monobasic potassium phosphate (Sigma-Aldrich, Germany) were also used. Standard of amoxicillin trihydrate, batch number: MS22795 with purity of 99.60% obtained from Addis Pharmaceutical Company and six brands of 500 mg amoxicillin capsules purchased at various levels of the drug distribution chains were included. Table 1 shows the detailed information on amoxicillin capsules included in the study.

<table>
<thead>
<tr>
<th>Country</th>
<th>Code</th>
<th>Strength (mg)</th>
<th>Batch No</th>
<th>Mfg. Date</th>
<th>Exp. Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>England</td>
<td>A*</td>
<td>500</td>
<td>465880</td>
<td>Feb-10</td>
<td>Feb-15</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>B</td>
<td>500</td>
<td>9586</td>
<td>Mar-11</td>
<td>Mar-16</td>
</tr>
<tr>
<td>Cyprus</td>
<td>C</td>
<td>500</td>
<td>45396</td>
<td>Oct-10</td>
<td>Oct-15</td>
</tr>
<tr>
<td>India</td>
<td>D</td>
<td>500</td>
<td>S3650011</td>
<td>Apr-10</td>
<td>Mar-13</td>
</tr>
<tr>
<td>India</td>
<td>E</td>
<td>500</td>
<td>AG</td>
<td>Apr-10</td>
<td>Mar-13</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>F</td>
<td>500</td>
<td>1120011</td>
<td>---</td>
<td>Dec-15</td>
</tr>
</tbody>
</table>

*= innovator product

**Methods**

The tests for uniformity of weight and disintegration were carried out as described in BP 2009. High performance liquid chromatography method was used for the assay according to USP specification (USP, 2007). Similarly, the dissolution tests were carried out using the paddle method according to USP 2007 guidelines. While the pharmacopoeia specifies sampling at a single time point of 60 min, for this particular study samples were taken at 10, 20, 30, 45, 60 and 75 min time intervals by replacing with the same volume of dissolution medium. Each sample was filtered, diluted and the absorbance reading was determined at 272 nm using UV spectrophotometer.

**Data Analysis**

Analytical data obtained from the experiments were analyzed using GraphPad Prism 5 software program for statistical comparisons (p<0.05 was taken as the significant level). Difference factor ($f_1$) is the % difference between two curves at each point and is a measurement of the relative error between the two curves (Eq.1). While 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 % dissolution between two curves (Eq.2) (Ashraful-Islam et al., 2012a; Mostafa et al., 2011; Oishi et al., 2011):

$$f_1 = \left(1 - \frac{\sum_{i=1}^{n} R_i - T_i}{\sum_{i=1}^{n} R_i + T_i}\right) \times 100 \quad \text{.....(1)}$$

$$f_2 = 50 \log \left(1 + \frac{1}{n} \sum_{i=1}^{n} (R_i - T_i)^2 \right)^{-0.5} \times 100 \quad \text{.....(2)}$$

Where, n is the number of time points, $R_i$ is the dissolution value of innovator product at time $t_i$ and $T_i$ is the dissolution value for the test product at time $t_i$.

For curves to be considered similar $f_1$ values should be close to 0 and $f_2$ values should be close to 100. Generally, $f_1$ values up to 15 and $f_2$ values greater than 50, which means an average difference of no more than 10% at the sample time points (Nayak and Pal, 2010; Hasan et al., 2007) ensures equivalence of the two curves and thus of the performance of the test and innovator products (Ashraful-Islam et al., 2012b).

DE was also employed to compare the drug release from various brands. DE is the area under the dissolution curve within a time range ($t_1$ - $t_2$) expressed as a percentage of the dissolution curve at maximum dissolution $y_{\text{max}}$, over the same time frame (Eq.3) (Nayak and Pal, 2010; Oishi et al., 2011).

$$\text{DE} = \int_{t_1}^{t_2} \frac{y \, dt}{y_{\text{max}} (t_2 - t_1)} \times 100 \quad \text{.....(3)}$$

Where, $y$ is the percentage dissolved at time $t$.

The integral of the numerator which is the area under the curve was calculated using the trapezoidal method (Eq.4) (Ashraful-Islam et al., 2012a).

$$\text{AUC} = \sum_{i=1}^{n} \frac{(t_i - t_{i-1}) (y_i + y_{i+1})}{2} \quad \text{.....(4)}$$

Where $t_i$ is the $i$th time point, $y_i$ is the % of dissolved product at time $t_i$.

**Results**

The results of weight variation, disintegration and assay are depicted in Table 2. The weight variation for all the capsules showed compliance within the BP specifications, as none of the products deviated by 5% from their average weight (BP, 2009). However, the obtained values were statistically significant among the brands (p<0.0001). In the same way, all brands of...
amoxicillin capsules passed the pharmacopoeia standard for disintegration test. The results for the actual content show that all brands of amoxicillin capsules passed as per the USP specification (USP, 2007) and statistical comparison showed no significant difference in the drug content and disintegration time among the brands (p>0.05).

TABLE 2
A summary of the quality control tests undertaken on amoxicillin capsules.

<table>
<thead>
<tr>
<th>Product</th>
<th>Average weight ±SD</th>
<th>Mean % deviation</th>
<th>Disintegration time (min) ±SD</th>
<th>Assay % (w/w) ±RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>594.47±5.55</td>
<td>0.8</td>
<td>8.33±0.40</td>
<td>99.81±0.04</td>
</tr>
<tr>
<td>B</td>
<td>579.88±3.81**</td>
<td>0.52</td>
<td>7.09±0.32</td>
<td>97.02±0.01</td>
</tr>
<tr>
<td>C</td>
<td>587.68±10.72</td>
<td>1.61</td>
<td>10.13±1.26</td>
<td>97.39±0.02</td>
</tr>
<tr>
<td>D</td>
<td>604.90±10.47**</td>
<td>1.43</td>
<td>9.06±0.38</td>
<td>100.28±0.04</td>
</tr>
<tr>
<td>E</td>
<td>622.20±6.58**</td>
<td>0.85</td>
<td>7.58±0.35</td>
<td>97.55±0.07</td>
</tr>
<tr>
<td>F</td>
<td>600.18±13.48</td>
<td>1.82</td>
<td>10.25±0.82</td>
<td>101.61±0.03</td>
</tr>
</tbody>
</table>

SD=standard deviation; RSD=Relative Standard Deviation; (n=20); **(n=6); **=p<0.01

The dissolution behaviour of the different brands of amoxicillin capsules are presented in Table 3, while the release profiles are shown in Figure 1. It was evident that brand D, showed faster dissolution among the brands. And all the products tested showed greater than 80% of the labelled amount of amoxicillin dissolved in 60 min as stipulated in the official pharmacopoeia (USP, 2007). The % dissolved of amoxicillin was tested statistically to ascertain differences among brands using one-way analysis of variance (ANOVA) (Table 4) while Dunnett’s test was employed to ascertain where the difference arose with regard to the innovator product (Table 5). One way ANOVA was undertaken for time points 10, 30 and the Pharmacopoeially specified time, 60 min. The results of ANOVA as shown in Table 4 indicated that the % dissolved of all the generic brands and innovator brand were significantly different at the three time points (p<0.0001), nevertheless, brand D and the innovator showed similar dissolution rate at the 60 min time point.

TABLE 3
Dissolution behaviour of amoxicillin capsules using paddle method (n=12).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Sum of squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F-value</th>
<th>Signif. (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Between groups</td>
<td>4834</td>
<td>5</td>
<td>966.7</td>
<td>41.22</td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>703.5</td>
<td>30</td>
<td>23.45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>5537</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Between groups</td>
<td>3953</td>
<td>5</td>
<td>790.6</td>
<td>81.35</td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>291.6</td>
<td>30</td>
<td>9.719</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>4244</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Between groups</td>
<td>1677</td>
<td>5</td>
<td>335.5</td>
<td>38.59</td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>260.8</td>
<td>30</td>
<td>8.695</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1938</td>
<td>35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Mean dissolution profile of the six brands (A-F) of amoxicillin capsules.
by various pharmacopoeias, use of dissolution profile

DE = Dissolution Efficiency; Difference of % DE (innovator product - brand product)

Results of dissolution efficiency, \( f_1 \) and \( f_2 \) of the six brands of amoxicillin capsules

The multiplicity of brands often put health professionals into a difficult situation of choice, and the possibility of interchangeability among brands. To prove two or more drugs bioequivalent, a similarity in the rate and extent to which the drug in the dosage form becomes available for absorption need to be demonstrated (Esimone et al., 2008). The quantitative analysis of the values obtained in dissolution tests is easier when mathematical formulae that express the dissolution results as a function of some of the dosage forms characteristics are used (Hasan et al., 2007). When fit factors, \( f_1 \) and \( f_2 \), were employed in data treatment, it became apparent that the selection and determination of the dissolution end points play a critical role in the calculation of the values (Eryol et al., 2004). Since \( f_2 \) is sensitive to the measurements obtained, limiting to no more than one sampling time point after 85% dissolution is a useful recommendation (Esimone et al., 2008). For this reason, the values of fit factors \( f \) and \( E \) were calculated separately for the dissolution time points of 10, 20 and 30 min.

In cases where greater than 85% of drug is dissolved within 15 min, dissolution profiles are usually accepted as similar without further mathematical evaluation (Hailu et al., 2011). The rate of dissolution of product B, C, E and F, however, did not meet the criterion of 85% dissolution and were subjected to further mathematical evaluation to demonstrate bioequivalence (Esimone et al., 2008). Results show that all the generic brands of amoxicillin, except brand D, were not similar with the innovator brand as \( E \) values were less than 50 and \( f \) values were greater than 15 (Table 6) and so may not be used interchangeably. In other studies, \( E \) values less than 50 were reported on three brands of co-trimoxazole tablets, respectively. On the other hand, Ashraful-Islam and colleagues showed that all the ciprofloxacin brands and acetaminophen immediate released tablets were not significantly different from the innovator brand, respectively (Ashraful-Islam et al., 2012ab).

The statistical results indicate that amoxicillin capsules were not equivalent with respect to their in vitro release profile. Moreover, the values of \( f_2 \) and \( f_1 \) were out of limits (\( f_2 < 50 \) and \( f_1 > 15 \)) (Table 6), except for brand D. Table 6 also shows the DE of different brands of amoxicillin capsules along with the differences with innovator brand.

**TABLE 6**

Results of dissolution efficiency, \( f_1 \) and \( f_2 \) of the six brands of amoxicillin capsules

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**TABLE 5**

Dunnett’s test for amoxicillin release at 0.05 levels with critical value 2.66.*

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(Hailu et al., 2011), one brand of artesunate (Esimone et al., 2008), two brands of citalopram (Menegola et al., 2007), three brands of metformin hydrochloride (Hamdan and Jaber, 2010), six brands of atorvastatin (Oishi et al., 2011), all brands of acetaminophen extended release (Ashraful-Islam et al., 2012b) and enteric coated pantoprazole sodium sesquihydrate (Mostafa et al., 2011) products. On the other hand, the dissolution profile of brand D was similar and most probably bioequivalent with innovator brand as indicated by Nayak and Pal (2010) on six brands of ciprofloxacin, Oishi et al. (2011) on two brands of atorvastatin and Ashraf-ul-Islam et al. (2012b) on all brands of acetaminophen immediate release.

DE has been used as a quantitative approach to assess drug release profile. The innovator and the test products can be said to be equivalent if the difference between their dissolution efficiencies is within appropriate limits (±10%, which is often used) (Ashraful-Islam et al., 2012b; Eryol et al., 2004; Hasan et al., 2007). So, we can say that only brand D is equivalent with the innovator brand as difference of DE (test product – reference product) is less than 10 (Ashraful-Islam et al., 2012b), similar to the reports by Ashraf-ul-Islam et al. (2012a) and (2012b) on ciprofloxacin and acetaminophen immediate release tablets, respectively. However, the other generic brands are not similar with innovator brand as difference of DE is greater than 10, similar to the reports by Ashraf-ul-Islam et al. (2012b) and Oishi et al. (2011) on acetaminophen extended release and six brands of atorvastatin tablets, respectively. Particularly, generic brand E and F were very much away from the limit 17.73 and 16.39, respectively.

Comparison of the therapeutic performance of two or more formulations containing the same active substance is a critical means of assessing the possibility of alternative use between the innovator and any essentially similar formulation (Esimone et al., 2008). The results so far show that, except brand D, the four generic brands of amoxicillin were not equivalent with the innovator brand based on the results of ANOVA, fit factors and DE. Hence, our findings agree with similar studies done in both in vitro (Bronnikova et al., 2008; Caiaffa et al., 2002; Kyriacos et al., 2008) and in vivo (Del Tacca et al., 2009; Manitpisitkul et al., 1998) tests elsewhere. There are many potential factors that can explain the differences between the innovator brands and their generic counterparts. Those include the particle size of a drug, storage, dosage form and the level and type of excipients (Al-Ameri et al., 2012). Another possible reason for the difference in dissolution rate is the difference in amorphous or crystalline form of the drug (Ferraz and Carpentieri, 2007; Hasan et al., 2007). Differences in the patterns of drug release should have been caused by the manufacturers’ choice of method of achieving reduction or delay in the rate of drug release (Okoye and Iwuagwu, 2010). These differences in dissolution rate among the different brands could impact the drugs’ effectiveness and side-effects profiles (Al-Ameri et al., 2012). This finding is significant in therapy where drugs are expected to have satisfactory bioavailability (Hailu et al., 2011).

Conclusions

Six brands of amoxicillin capsules have been subjected to analysis according to the monograph of BP and USP. The results suggest that all the tested brands satisfied the pharmacopeial requirements. On the other hand, in vitro comparative dissolution profiles revealed potentially significant differences between the generic brands and the innovator brand. Only brand D could be said to be equivalent to the innovator while the other four were not equivalent based on the statistical, fit factors and DE approaches. Although the study was performed on limited in vitro experiments, the results clearly raise a question about the interchangeability between innovator brands and their generic counterparts and among generics themselves. This study highlights among other things the need for constant surveillance on the marketed drugs by the regulatory bodies with the view to ascertain bioequivalence and quality medicines, especially for drugs like amoxicillin for which there exists evidence of non-bioequivalence from different firms, resulting in efficacy issues.

Acknowledgements

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References


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