Evaluation and comparison of in-vitro dissolution profiles for different brands of amoxicillin capsules

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Abstract

Background: Amoxicillin is an oral semi-synthetic, β-lactam antibiotic used to treat bacterial infections caused by susceptible micro organisms. It is usually prepared in capsule, tablet and powder for oral suspension form. Solid dosage forms for oral administration pose bioavailability problems related to the absorption process The World Health Organization (WHO) has promoted the use of generic brands in order to make the cost of medicines affordable. Generic substitution could be considered when a generic copy of a reference drug contains identical amounts of the same active ingredient in the same dose formulation and route of administration. However, the presences of generic products those are not interchangeable with that of the innovator and/or with each others have been reported.

Objective: To evaluate and compare the in-vitro dissolution profiles of different generic brands of amoxicillin capsules with the innovator that are available in Ethiopian market.

Methods: Dissolution profiles for nine brands of amoxicillin capsules contained amoxicillin 500 mg which are available in Ethiopian market were determined using a method from the United States Pharmacopoeia (USP, 2009). The obtained dissolution profile data of the eight brands were evaluated and compared with the innovator brand (Amoxil™) using two different statistical methods: the fit factors (*f1* & *f2*) and the dissolution efficiency (D.E.) model. Most generic brands of amoxicillin capsules (62.5% of the tested brands) are not interchangeable with the innovator brand.

Results: The calculated f1 factor for Brand A and Brand G are 10.1 and 1.1 respectively. However, for the rest six brands the f1 factors are greater than 15. The f2 factor for Brand G is 74.1 and for Brand A is 48.5 which is near to 50. Similarly, the f2 factors for the six brands are less than 50 which support the result of the f1 factors for the dissimilarity of these brands with the innovator brand. The mean dissolution efficiencies as well as the 95% confidence intervals are within ±10% only for two brands, Brand F and Brand G.

Conclusion: Most generic brands of amoxicillin capsules (62.5%) are not interchangeable with the innovator brand (Amoxil™).

Keywords: Dissolution profile, amoxicillin, evaluation and comparison

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Introduction

Amoxicillin is an oral semi-synthetic, β-lactam antibiotic used to treat bacterial infections caused by susceptible micro organisms[1]. It is usually the drug of choice within the class because it is better absorbed, following oral administration, than other β-lactam antibiotics. Amoxicillin is susceptible to degradation by β-lactamase producing bacteria, and so may be given with clavulanic acid to decrease its susceptibility. Amoxicillin acts by inhibiting the synthesis of bacterial cell walls. It inhibits cross-linkage between the linear peptidoglycan polymer chains that make up a major component of the cell wall of gram-positive bacteria[2] .

Solid dosage forms for oral administration are widely prescribed in clinical practice because they are practical, stable, economical, and usually safe. On the other hand, they pose bioavailability problems related to the absorption process. Drug absorption from a solid dosage form after oral administration depends on the release of the drug substance from the drug product, the solubilization of the drug under

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physiological conditions, and the permeability across the gastrointestinal tract. For that reason, the importance of dissolution tests and dissolution profile for the establishment of pharmaceutical equivalence must be highlighted[3, 4].

The World Health Organization (WHO) has advocated the use of generic brands in order to make the cost of medicines affordable especially for the developing countries[5]. However, this approach has not provided sufficient evidence for the substitution of one brand for another. In Ethiopia, the cost of a branded medicine may be as high as ten folds of the generic medicine. To become confident in substitution of branded with generics for affordability and at the same time to achieve therapeutic efficacy, bioequivalence studies become fundamental[6].

Generic substitution could be considered when a generic copy of a reference drug contains identical amounts of the same active ingredient in the same dose formulation and route of administration as well as meet standards for strength, purity, quality and identity[7]. However different reported studies over the last years revealed that marketed products with the same amount of active ingredient exhibit marked differences in their therapeutic responses. The presences of generic products that are not interchangeable with that of the innovator and/or with each others have been reported [4, 6, 8-10].

The study was set up to evaluate and compare the in-vitro dissolution profiles of different generic brands of amoxicillin capsules with the innovator that are available in Ethiopian market.

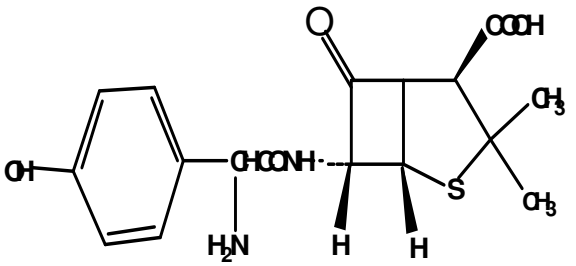

Figure 1: Chemical structure of amoxicillin

Methods

Materials and equipments

Amoxicillin USP reference standard (potency = 864μg mg^{-1}), Nine brands of amoxicillin capsules as shown in table 1, dissolution test apparatus (PTW II, Pharma Test, Germany), UV-Visible spectrophotometer (Shimadzu, Japan) distilled water and Microsoft excel to treat data statistically were used.

Standard preparation

Stock standard solution (1 mg mL^{-1}) was prepared by dissolving 100 mg equivalent of anhydrous amoxicillin USP reference standard in 100 mL of distilled water. Six different concentration levels of calibration solutions (0.01 to 1 mg mL^{-1}) were freshly prepared by diluting suitable volumes of the stock standard solution in appropriate volumetric flasks.

Table 1: Samples of amoxicillin capsules

Samples code	Country of origin	Mfg date	Exp date
Amoxil™	United Kingdom	06/2009	06/2014
A	India	09/2009	08/2012
B	India	10/2009	09/2012
C	Cyprus	07/2009	07/2014
D	Ethiopia	03/2010	03/2014
E	India	08/2009	07/2012
F	Ethiopia	11/2009	11/2014
G	India	03/2009	02/2013
H	India	12/2009	11/2011

The label claim for all samples is amoxicillin 500 mg

Dissolution test and sample preparation

One capsule was placed in each of the six vessels of the dissolution apparatus contained 900 ml of water which were previously heated and maintained at 37 ± 0.5 °C. 5 mL of sample was withdrawn from each vessel after 5, 10, 20, 30, 40, 60 and 70 minutes. After each withdrawal, an equal volume of water that had been maintained at the same temperature was replaced in order to maintain the total volume of the medium constant. 2 mL of the sample solution was quantitatively taken in to 10 mL volumetric flask after being filtered through whatman filter paper and diluted to volume with distilled water. Absorbance of the solution was measured at 272 nm. Employed conditions for the dissolution test have been shown in table 2.

Table 2: Dissolution test conditions for amoxicillin capsule as per USP (2009)

Medium	Water, 900 mL
Apparatus type	II
Stirring rate	75 rpm
Detection	Absorbance at 272 nm

Statistical methods

Fit factors

Fit factors or similarity indices are defined as follows: Where *Rt* is the percentage of dissolved product for a reference batch at time point *t*, *Tt* is the percentage of dissolved product for the test batch, *n* is the number of time points. For each brand, the calculations were made on the mean values for the six vessels. The factor, *f*1, is the average % difference over all time points in the amount of test brand dissolved as compared to the reference brand. The *f*1 value is 0 when the test and the reference profiles are identical and increases proportionally with the dissimilarity between the two profiles. The *f*2 value is between 0 and 100. The value is 100 when the test and the reference profiles are identical and approaches zero as the dissimilarity increases [6, 11, 12].

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

and

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

Dissolution efficiency

This concept is defined as follows:

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

Where, *y* is the percentage of dissolved product. D.E. is the area under the dissolution curve between time points *t*1 and *t*2 expressed as a percentage of the curve at maximum dissolution, *y*100, over the same time period. For a capsule product, *t*1 can be set to the period corresponding to disintegration of the capsule shell. The integral of the numerator, i.e. the area under the curve is calculated by a model independent method, the trapezoidal one. The area under the curve is the sum of all the trapeziums defined by:

$$AUC = \sum_{i=1}^{i=n} \frac{(t_1 - t_{i-1})\,(y_{i-1} + y_i)}{2}$$

Where t_i is the i^{th} time point, y_i is the percentage of dissolved product at time t_i [6, 12].

Results

Disintegration could be directly related to dissolution and subsequently to bioavailability of a drug. A drug filled in a capsule shell is released rapidly as the capsule shell disintegrates; a fundamental step for immediate release dosage forms because the rate of disintegration affects the dissolution and afterward the therapeutic efficacy of the medicine. For comparison of dissolution profiles with dissolution efficiency model, the lag phase should be determined[12]. And hence, disintegration test was carried out as per USP, 2009 prior to the actual dissolution test and 90% of the studied brands have been averagely disintegrated within ten minutes. Summarized disintegration test results have been presented in table 3.

In the presented study, sample quantification was based on the previously constructed calibration curve. The calibration curve has correlation coefficient (r) and linear equation of 0.9994 and Y = 2.9001 X + 0.0336 respectively. It is linear in the ranges of 0.01 – 1.00 mg mL^{-1}.

Table 3: Summarized disintegration test results for the tested brands of amoxicillin.

Tested brands	Disintegration times (min : second)		
	Minimum	Maximum	Average
Amoxil™	5:34	6:16	5:50
A	3:40	5:48	4:18
B	5:07	7:45	6:22
C	4:43	6:39	5:33
D	4:32	6:24	5:19
E	3:37	4:49	4:14
F	3:41	8:13	5:40
G	7:06	13:00	11:14
H	3:11	4:47	3:51

The dissolution test according to USP 2009 requires that each unit not less than Q + 5% of the active ingredient should dissolve with in 60 minutes for the first six units (stage 1). But, if the requirement at stage 1 is not met, another six units will be tested and the mean percent dissolved for the twelve units is not less than Q% and no unit is less than Q – 15% (stage 2). In this study all the tested brands have satisfied these requirements and thus were in agreement with the USP 2009 specifications. However, six of the nine tested products have satisfied these requirements after passing stage 2 test. Dissolution test results have been presented in table 4.

Table 4: Dissolution results of the tested brands of amoxicillin at USP 2009, Test 1 sampling time (60 minutes)

% dissolved		Amoxil™	A	B	C	D	E	F	G	H
Stage 1	Min	94.4	85.7	80.3	83.1	77.8	69.9	78.9	93.8	75.5
	Max	98.6	96.7	86.5	87.6	86.5	82.9	87.9	99.5	87.9
	Mean (n=6)	96.5	91.1	81.3	84.0	80.4	76.7	81.5	95.1	81.0
Stage 2	Min			76.1	77.8	78.3	75.5	74.7		78.6
	Max			89.1	89.1	87.9	84.3	89.1		86.2
	Mean(n=12)			82.1	84.5	83.8	80.0	82.5		81.2

The dissolution profiles of the innovator product as well as the tested generic products have been presented in figure 2. According to the result, the innovator product and Brand G have shown superior dissolution performances over the rest of the tested generic products.

The percentage dissolved was tested statistically in order to compare the dissolution profiles of the tested eight brands of amoxicillin capsules with the innovator. In this study the two most important and widely engaged methods have been used: the fit factors and dissolution efficiency (D.E.).

The dissolution data with the result obtained from the calculation of the fit factors has been shown in table 5. Similarly, table 6 shows the calculated dissolution efficiencies with their respected 95% confidence intervals.

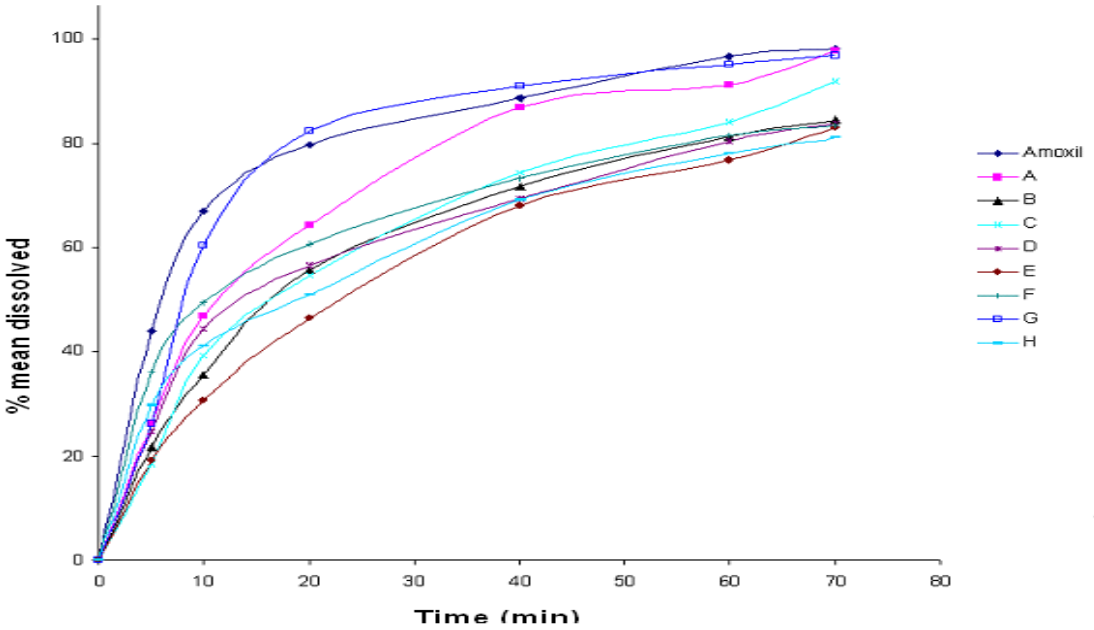

Figure 2: Dissolution profiles of nine brands of amoxicillin capsule

Table 5: Dissolution data for the calculation of *f1* and *f2*

Time (min)	% mean dissolved ± SD[a]								
	Amoxil™	A	B	C	D	E	F	G	H
5	44.0± 7.2	26.3± 7.4	21.6±7.2	18.4± 6.8	24.6± 6.9	19.0±4.5	36.2± 3.4	26.2± 11.5	29.7± 3.7
10	67.0± 9.7	46.7±3.2	35.6± 7.9	39.2± 6.4	44.5± 9.7	30.6±4.6	49.3± 5.4	60.4± 11.3	41.1± 5.5
20	79.7± 5.6	64.3± 7.0	55.6± 6.8	54.5± 3.9	56.5± 5.3	46.4± 5.1	60.6± 2.9	82.3± 7.1	51± 13.5
40	88.7± 3.3	86.8± 9.5	71.7± 3.9	74.2± 2.4	69.4± 4.6	68.1±7.6	73.2± 3.1	90.9± 4.9	69.1± 6.3
60	96.5± 2.0	91.1±4.3	81.3± 2.6	84± 1.6	80.4± 4.1	76.7± 5.2	81.5± 3.2	95.1± 2.1	77.9± 5.3
70	98.2± 1.6	97.6±4.5	84.5± 4.3	91.8± 2.5	83.8± 3.2	82.9±3.8	83.3± 2.3	96.8± 2.5	81.0± 4.4
		f1=10.1 f2= 48.5	f1=23.6 f2= 35.5	f1=20.1 f2= 37.9	f1=22.2 f2= 37.5	F1=29.1 f2= 30.9	f1=19.1 f2= 41.0	f1=1.1 f2= 74.1	F1=35.6 f2= 34.4

[a]SD: standard deviation f1: the difference factor f2: the similarity factor

Table 6: Dissolution efficiencies with 95% confidence intervals

Tested brands	Mean D.E(%) with CIS	D.E	CIS
Amoxil™	88.9 (86.9, 90.8)	0.0	0
A	80.1 (75.1, 85.1)	8.8	15.7
B	80.2 (76.4, 84.0)	8.7	4.4
C	77.1 (74.3, 79.9)	11.8	16.5
D	81.0 (77.8, 84.2)	7.9	13.0
E	75.8 (73.1, 78.6)	13.1	17.7
F	85.0 (83.6, 86.3)	3.9	7.2
G	90.2 (87.6, 92.7)	1.3	3.2
H	79.0 (75.9, 82.2)	9.9	14.9

D.E: Dissolution Efficiency
CIS: Confidence Intervals
" D.E = Innovator – test brand "CIS is calculated by considering the maximum possible mean
D.E. value of Innovator and minimum possible mean
D.E. value of other brands.

Discussion

In the literature, different methods which can be used to compare dissolution profiles data have been reported[3, 4, 11-14]. However, in this study the two most important and widely engaged methods have been used: the fit factors and dissolution efficiency (D.E.). The fit factors can be expressed by two approaches: *f*1 (the difference factor) and *f2* (the similarity factor). Two dissolution profiles to be considered similar and bioequivalent, f1 should be between 0 and 15 whereas f2 should be between 50 and 100[6].

In the calculations of fit factors and dissolution efficiency values, the mean percent dissolved at 5 minutes was excluded for the disintegration of the capsule shell had not been completed at this point of time. Therefore, as shown in table 5, the dissolution profiles of all brands except Brand G are not similar with the innovator brand (Amoxil™) using the f2 factor. But, using the f1 factor besides to Brand G, Brand A can probably be considered as bioequivalent with the innovator brand.

The second comparison method employed in this study was dissolution efficiency (D.E.) model. The calculations were made for each individual vessel. Thus, the mean D.E. for each brand with its 95% confidence intervals was obtained and compared by measuring the difference between the mean D.E. and confidence intervals of the innovator brand and the test brands. If the differences of the mean dissolution efficiencies as well as the 95% confidence intervals are within appropriate limits (±10%), one can conclude that the reference and test dissolution profiles are equivalent[12]. As shown in table 6, both conditions have been satisfied only for two brands, Brand F and Brand G. Therefore, the dissolution profiles of Brand G and Brand F are similar with each other and with the innovator as per this method.

The calculation of *f*1 and *f*2 is very simple but the calculation for D.E. is more complex. Fit factors comparison (*f*1 and *f*2) do not reveal the intra-batch variability because the calculations need to be made on the mean. Moreover, it is also said to be insensitive to the shapes of dissolution profiles and does not put into consideration unequal spacing between sampling time points. Even though *f*2 is quite closely correlated with *D.E.* it is more difficult to interpret *f*2 than D.E. data without reference to dissolution data or curves, since it relates to differences between curves, and because of its non-linear behaviour[12].

In this study, all the comparison methods have proven the similarity of the dissolution profile of only Brand G with the innovator. The f1 factor and the D.E. independent model have included Brand A and F respectively in addition to Brand G. In *f2* comparison method, these generic brands are also aspirant, have closest value to 50 in particular Brand A. Similarly, in *f1* comparison Brand F was the next brand with closest value to 15. In D.E. model, Brand A is slightly excluded due to the increment of the difference of the confidence intervals. Even though all the tested products have passed the USP (2009) specifications, most of the generic products are not interchangeable with the innovator product. The three brands: Brand A, Brand F and Brand G may probably interchangeable with each other and with the innovator brand (Amoxil™). However the rest five brands may not probably interchangeable with the innovator brand.

Limitations of the study

The content of the active ingredient of each tested product is not assessed against the label claim. Moreover; in-vitro dissolution test might be an indicator to investigate the interchangeability of products. The study has not been assisted by other methods like in-vivo bioequivalence study for better conclusion.

Conclusion

Most generic brands of amoxicillin capsules (62.5%) are not interchangeable with the innovator brand (Amoxil™).

Recommendations

Overall, it can be recommended that drug regulatory Authorities should be reinforced and capacitated in order to address proper post marketing surveillance for sensitive medicines like antibiotics. Any drug regulatory authorities should also stick to the requirement of bioequivalence studies during market authorization. Further studies shall be done on the tested products for better conclusion of the interchangeability of the generic products with the innovator. The samples need to be assessed in terms of dosage uniformity, water content and assay. Moreover, in-vivo bioequivalence study on the generic products is highly considerable.

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