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In Vitro Dissolution Testing to Assess Pharmaceutical Equivalence of Selected Amoxicillin Products Available in Sri Lanka: A Post-Marketing Study

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ABSTRACT

Guidance documents on biowaivers and published literature, including the biowaiver monograph on amoxicillin, recommend conducting biopharmaceutics classification system (BCS)-based in vitro dissolution studies as a surrogate for in vivo bioequivalence (BE) studies for solid oral amoxicillin products. Until now, comparative in vitro dissolution studies have not been used in regulatory submissions in Sri Lanka. This post-marketing study was conducted to compare dissolution profiles of three registered amoxicillin products. In vitro dissolution testing was conducted according to the WHO guideline for biowaiver studies. We compared three generic solid oral amoxicillin products of 500 mg (generic A, B, and C) available in Sri Lanka with the innovator product. Dissolution samples were quantified using a validated high-performance liquid chromatography (HPLC) method with ultraviolet (UV) absorption at 229 nm. The results showed that all products complied with pharmacopoeial specifications, but variation of dissolution data greater than 20% was noted at the 10-min time point, and 10% variation was noted at later time points with product A at pH 4.5 and product C at pH 4.5 and 6.8. Therefore, both conventional similarity factor (f_2) and model-independent multivariate confidence region procedures (bootstrap approach) were used to compare the dissolution profiles. Only generic A met dissolution study criteria at all three pH conditions. Generic B failed to meet dissolution study criteria at pH 4.5, and generic C, with highly variable dissolution data, met the dissolution criteria at pH 4 using bootstrap f_2 and failed to meet dissolution criteria at pH 6.8. This study highlights the need for careful consideration of an appropriate mathematical model or models for comparing dissolution profiles, especially when the coefficient of variation (CV) warrants application of two models for different pH conditions. The international guidelines on BCS-based dissolution studies do not provide adequate guidance on these issues.

KEYWORDS: Biowaivers, amoxicillin, dissolution, generic drugs

INTRODUCTION

Amoxicillin is an aminopenicillin that is widely prescribed in outpatient setting in Sri Lanka. There are 13 generic solid oral amoxicillin products registered with the National Medicines Regulatory Authority (NMRA) (*1*). These include both locally manufactured and imported solid products including the innovator product. Regulatory requirements for registration of generic products include submission of evidence to show therapeutic equivalence of the generic product with a suitable comparator, often the innovator product by a bioequivalence (BE) study. In Sri Lanka, BE of generic products was not assessed during the regulatory approval process until 2012. With new regulations introduced in 2014 by the NMRA, all generic antibiotic products need to show BE at the time of registration. There are six local amoxicillin manufacturers in Sri Lanka and importers who need to show BE for their amoxicillin products. An alternative to in vivo BE is conducting a BCS-based in vitro biowaiver study; however, the concept of biowaiver based on comparative dissolution studies is not yet formally recognized in the Sri Lankan drug registration process.

Amoxicillin (500-mg capsules or tablets) is categorized as a biopharmaceutics classification system (BCS) class 1 drug (*2*). A recently published biowaiver monograph on amoxicillin trihydrate recommends submission of either comparative in vitro dissolution data or in vivo BE data as evidence to establish therapeutic equivalence of generic solid oral amoxicillin products of 250 and 500 mg (*2*).

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The BCS-based biowaiver methods for dissolution require use of pH 1.2, 4.5, and 6.8 media as the dissolution medium. According to the published biowaiver guidelines of the United States Food and Drugs Administration (FDA) and the World Health Organization (WHO), the specification for the BCS-based biowaiver requires at least 85% release in 30 min for rapid dissolution or 15 min for very rapid dissolution (*3, 4*). Three BCS-based in vitro dissolution studies have been reported in the literature for amoxicillin products. Löbenberg et al reported dissolution profile comparison of nine amoxicillin products (500-mg tablets) marketed in Argentina, Chile, and Peru using amoxicillin Sandoz as the comparator (*5*). The dissolution studies were conducted according to the recommended BCS-based dissolution study procedures, and dissolution profiles were compared using the similarity factor (f_2) metric (*5*). They found that only three generic products showed in vitro equivalence to the comparator pharmaceutical product. Similar BCS-based dissolution studies for 14 different amoxicillin products (500-mg capsules) conducted by Reddy et al. reported none of the products complied with the WHO biowaiver criteria (*6*). The third study by Stuart et al. from Trinidad and Tobago evaluated seven products of amoxicillin under similar study conditions to those used by Löbenberg et al. and reported that none of the products met the WHO biowaiver criteria (*7*). Thus, according to the guidance documents on biowaivers and published literature, including the biowaiver monograph on amoxicillin, conducting BCS-based in vitro dissolution studies as a surrogate for in vivo BE is possible. Until now, comparative in vitro dissolution studies have not been used much in regulatory submissions in Sri Lanka. In this environment, this study was conducted as a post-marketing study to show the possibility of conducting BCS-based comparative in vitro dissolution studies with dissolution profile comparison.

MATERIALS AND METHODS

The study consisted of pharmaceutical quality testing of the selected products according to the *United States Pharmacopoeia* (USP) and *British Pharmacopoeia* (BP), including analytical method validation and BCS-based comparative in vitro dissolution testing to determine in vitro BE of selected solid oral amoxicillin products. Comparative in vitro dissolution studies were conducted in pH 1.2, 4.5, and 6.8 media according to the WHO guideline on conducting BCS-based dissolution studies.

Chemicals and Reagents

The innovator pharmaceutical product, Amoxil 500 mg capsules (GlaxoSmithKline Pharmaceuticals Ltd, Mumbai, India), was purchased from the local market and used as the comparator product. Two locally manufactured products of amoxicillin 500 mg capsules, named generic A and B, and one imported product named generic C, were used as test products. Quality testing was done according to pharmacopoeial requirements using standard reagents. Amoxicillin USP RS was used as the reference standard (Sigma Aldrich, St. Louis, USA), and analytical grade chemicals were used for the study and method validation.

Instruments and Equipment

Dissolution testing was conducted in a dissolution apparatus (PTWS610, Pharma Test Apparatebau AG, Germany) with an autosampler system. The amount of amoxicillin dissolved in the dissolution samples was quantified using a high-performance liquid chromatography (HPLC) system (Prominence, Shimadzu, Japan). A C_8 column (Restek, USA) was used for chromatographic separation of the analyte.

Quality Testing

Quality tests were conducted for all selected products prior to conducting biowaiver studies to confirm compliance to pharmacopoeial quality standards of the selected products. Quality tests and dissolution tests were carried out according to BP and USP, respectively (*8, 9*).

ANALYTICAL METHOD VALIDATION

Amoxicillin concentration in dissolution samples was assayed using a validated HPLC method with ultraviolet (UV) absorbance at 229 nm. The analytical method was validated for its suitability in pH 1.2, 4.5, and 6.8 media according to the USP 2011 (*9*). The linear range of the analytical method was selected based on the expected lowest (3%) and highest (120%) released concentrations of amoxicillin in 900 mL of dissolution medium. Accuracy, linearity, and precision of the method were determined as percentage recovery, correlation coefficient (*r*), and relative standard deviation (RSD), respectively. Percentage recovery falling within ±15% and ±20% of the true value at higher and lower concentrations, respectively, was considered as the expected accuracy of the analytical method (*9*). Correlation coefficient greater than 0.99 was used as the criterion of linearity, and RSD less than 15% was used as the criterion for the precision of the method.

In Vitro Dissolution Study

The in vitro dissolution study was conducted according to the biowaiver guideline published by the WHO (*10*). Dissolution media of pH 1.2, 4.5, and 6.8 were prepared according to the standard buffer solutions given in the USP

2011 and deaerated prior to dissolution testing. A validated media degassing method was used for the deaeration of the dissolution media. In vitro dissolution studies were performed for 12 dosage units of each test product (generic A, B, and C) and for the innovator product in 900 mL of standard buffer media in pH 1.2, 4.5, and 6.8, at 37 ° C ± 0.5 ° C separately. USP apparatus I (basket) at 100 rpm was used for the dissolution of capsules. Samples were withdrawn at 10, 15, 20, 30, 45, and 60 min using an auto sampler; total dissolution time was 60 min. Dissolved amoxicillin was quantified using the HPLC system with a C_8 column, pH 4.5 KH_2PO_4 buffer, and acetonitrile (95:5) at 229 nm. The percentage of drug dissolution was calculated at each time point for the 12 dosage units of both products using a calibration curve.

Comparison of Dissolution Profiles

Dissolution data were taken as an average of the 12 dosage units of each product for a given time point. Cumulative dissolution profiles were generated with the mean dissolution data against time points. The dissolution profiles were compared using an f_2 test for the products with a CV of the dissolution data less than 20% for early time points up to 15 min and less than 10% for other time points (*4, 11*). For dissolution data that did not meet the CV requirement, a model-independent multivariate confidence region procedure (bootstrap approach) was used to compare the dissolution profiles (*12*). In the bootstrap approach, the confidence limit of f_2 using 5000 bootstraps was calculated using Microsoft Excel 2010 Add-In function (*12*). Dissolution profiles with an f_2 value greater than or equal to 50 are considered to be similar.

RESULTS AND DISCUSSION

Pharmacopoeial Quality Testing

Results of the quality testing of all products complied with the pharmacopoeial specification limits and are summarized in Table 1.

Analytical Method Validation

The analytical method was validated to quantify concentrations of amoxicillin in dissolution samples in the range of 0.10–1.00 mg/mL. Linearity of the method at all three pH levels was greater than 0.99, and the recovery percentage was greater than 99%. The RSD of the method at low, medium, and high concentrations in all three pH media were less than 1%. Results of the analytical method validation are given in Table 2.

Table 1. Pharmacopoeial Quality Testing of Selected Amoxicillin (500 mg)

Parameter	Specification	Results			
		Generic A	Generic B	Generic C	Innovator product
Identification by infrared spectroscopy	Pharmacopoeial reference spectrum	Complied	Complied	Complied	Complied
Identification by TLC	Similar R*f*	Complied	Complied	Complied	Complied
Dissolution test	Not < 80% of labeled amount of amoxicillin is dissolved in 90 min	116.8%	114.6%	109.00%	104.01%
Assay	Should contain 90.0%–120.0% of labeled amount of amoxicillin	100.3%	99%	102.96%	96.80%
Uniformity of weight	Deviation of individual net weight should not exceed ±7.5%	Complied	Complied	Complied	Complied

TLC, thin layer chromatography; Rf, retention factor.

Table 2. Analytical Method Validation Results

Parameter	Specification	pH 1.2	pH 4.5	pH 6.8
Linearity	$r > 0.99$	0.998	0.999	0.999
Accuracy	Recovery	99.99%	99.81%	99.78%
Precision	RSD < ± 15%	Low:0.72%, Medium:0.97% High: 0.75%	Low: 0.36%, Medium: 0.83% High: 0.32%	Low: 0.35%, Medium: 0.44% High: 0.03%
Range	NA	0.10–1.00 mg/mL	0.10–1.00 mg/mL	0.10–1.00 mg/mL
Specificity	No interference with placebo	Passed	Passed	Passed

Low, amoxicillin concentration of 0.05 mg/mL; med, 0.3 mg/mL; high, 0.6 mg/mL.
r, correlation coefficient; RSD, relative standard deviation; med, medium; NA, not applicable.

In Vitro Dissolution Testing

As shown in the cumulative dissolution profiles in Figure 1, all generic products showed very rapid dissolution in pH 1.2. Thus, f_2 calculation was not necessary to compare the dissolution profiles. According to the WHO biowaiver guideline, the generic products showed similar dissolution profiles as the innovator product in pH 1.2 medium (*4*). The percentage of drug released showed little reduction over the rest of the time points, indicating possible degradation of amoxicillin in the pH 1.2 medium. Similar observations have been reported by Löbenberg et al and Stuart et al, showing a declining dissolution curve after reaching a peak at about 10 to 15 min in pH 1.2 (*5, 7*). Amoxicillin is chemically unstable in acidic pH (*2*). However, Reddy et al reported increasing amoxicillin absorption with time in pH 1.2 medium with no degradation (*6*). Tsuji et al studied degradation of amoxicillin and reported the highest degradation rate at pH 1.09 (*13*). Tsuji et al also reported that the pH-apparent solubility profile of amoxicillin is U-shaped , with the highest solubility at pH less than 2 (*13*).

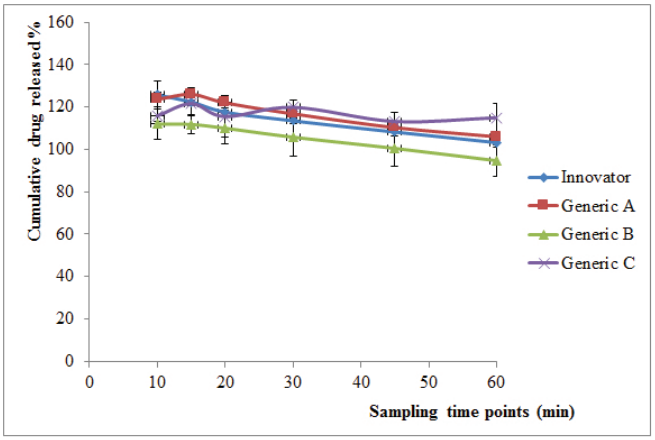

Figure 1. Cumulative dissolution profiles of selected amoxicillin (500 mg) products at pH 1.2.

Amoxicillin dissolution first increased with time in pH 4.5 for all products tested, and this result was comparable with the findings of Löbenberg et al, Reddy et al, and Stuart et al (*5–7*). However, only a few products in these studies released at least 85% of amoxicillin in 15 to 30 min to meet biowaiver criteria. Generic product A showed 28.7% CV at the initial time point, which is higher than recommended 20% CV (*3, 4*). The calculation of f_2 using the bootstrap method is recommended for highly variable dissolution data, so this method was applied. Figure 2 shows these cumulative dissolution profiles obtained for generic A, B, and C in pH 4.5. According to the results obtained for pH 4.5, the innovator product shows little variation in dissolution data, which may be due to formulation factors or inter-batch variation of the product. Amoxicillin has shown its lowest solubility at pH 4.5 (*2*).

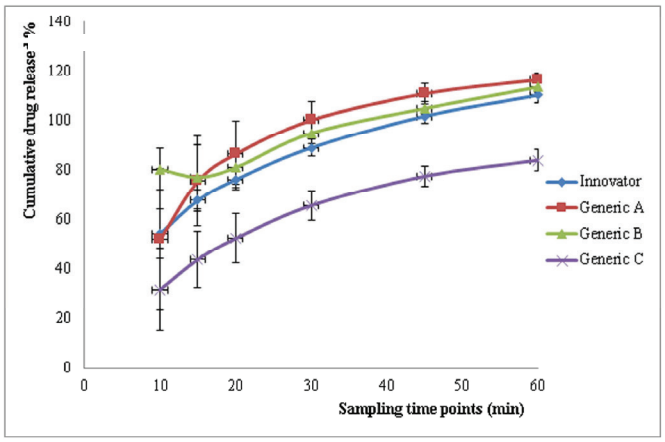

Figure 2. Cumulative dissolution profiles of selected amoxicillin (500 mg) products at pH 4.5.

At pH 6.8, generic C had 29.8% CV at the 10-min time point, exceeding the acceptable range for conventional f_2 test. The CVs of generic A and B were acceptable for dissolution profile comparison using conventional f_2 testing. Figure 3 shows the cumulative dissolution profiles of the generic products in pH 6.8 medium, which are comparable with the three published studies discussed above (*5–7*).

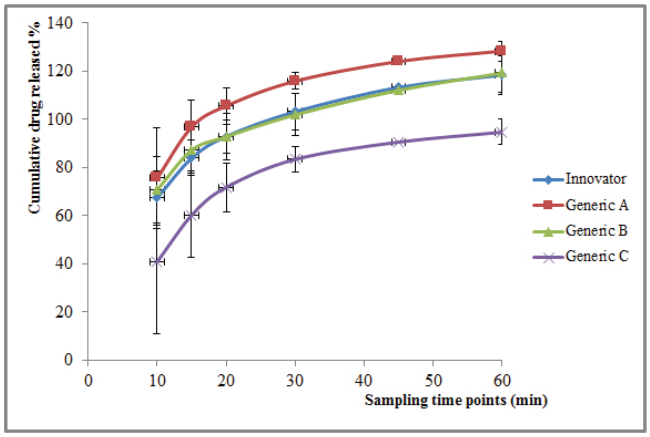

Figure 3. Cumulative dissolution profiles of selected amoxicillin (500 mg) products at pH 6.8.

To compare the dissolution profiles, both conventional and bootstrap f_2 values were calculated. These results are summarized in Table 3. Shah et al recommend using bootstrapping to calculate a lower bound for f_2 for highly variable dissolution data (*12*). Both conventional and bootstrap f_2 calculations of generic A in pH 4.5 gave 53 and 53.92, respectively, indicating similar dissolution profiles. Generic A in pH 6.8 had an acceptable CV, but the bootstrap f_2 value (f_2 = 47.16) indicated a difference in

dissolution profiles and conventional f_2 testing indicated no difference (f_2 = 50). This calculation was done to compare the interpretation of the same results using both models.

Table 3. Similarity Factor (f_2), of generic Amoxicillin Formulations (500 mg) Compared to Innovator Product Calculated Conventionally and Using Bootstrap Method

Product	pH 1.2		pH 4.5		pH 6.8	
	f_2	Bootstrap f_2	f_2	Bootstrap f_2	f_2	Bootstrap f_2
Generic A	NA	NA	53[a]	53.92	50	47.16
Generic B	NA	NA	35	38.82	57	56.0
Generic C	NA	NA	46[b]	50.57	39[c]	42.15

[a]High CV reported in dissolution data (28.7%); [b]high CV reported in dissolution data (12.7%); [c]high CV reported in dissolution data (29.8%); NA, not applicable as all products showed very rapid dissolution; CV, coefficient of variation.

Results of both conventional and bootstrap f_2 calculations for generic B were similar at pH 4.5 (f_2 = 35 and 38.82, respectively) and 6.8 (f_2 = 57 and 56.0, respectively). According to the both methods in pH 4.5, the dissolution profile of the generic B was not similar to the innovator product, and in pH 6.8 the dissolution profile was similar to the innovator product. At pH 4.5, conventional f_2 calculation indicated a difference (f_2 = 46) in dissolution profiles of generic C, but a bootstrap f_2 of 50.57 indicated similar profiles. At pH 6.8, the conventional and bootstrap f_2 results indicated similar dissolution profiles for generic C (f_2 = 39 and 42.15, respectively).

According to these results, bootstrap f_2 calculation can be used for comparison of dissolution profiles of highly variable dissolution data. Löbenberg et al, Reddy et al, and Stuart et al did not report variability of dissolution data (*5–7*). In our study, bootstrap f_2 was a more conservative approach over conventional f_2 calculation for comparing the dissolution profiles within the maximum allowable CV. Generic A at pH 6.8 failed to meet biowaiver criteria using bootstrap f_2, but did meet the criteria using conventional f_2 testing. Generic B at pH 4.5 failed to meet biowaiver criteria. Generic C at pH 4.5 met biowaiver criteria using bootstrap f_2 but failed using conventional f_2 testing. Even though biowaiver guidelines provide comprehensive guidance on conducting in vitro dissolution studies with comparison of dissolution profiles, it does not provide guidance on selection of more than one model in a given study due to variability of dissolution data in different pH conditions.

CONCLUSIONS

Out of the three generic products tested, only generic product A met biowaiver criteria at pH 1.2, 4.5, and 6.8. Generic B failed dissolution study criteria in pH 4.5. Generic C had highly variable dissolution data in pH 4.5 and 6.8 that were not eligible for conventional f_2 testing for dissolution profile comparison. A bootstrap f_2 calculation was used in this instance, which also did not meet the biowaiver criteria at pH 6.8. This study, along with other similar published studies, shows the possibility of using BCS-based in vitro dissolution testing as a surrogate for in vivo BE studies. It further highlights considerations for appropriate mathematical models for dissolution profile comparison. International drug regulatory agencies need to provide guidance on applying biowaiver criteria when the CV warrants application of two comparison models at different pH conditions.

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CONFLICTS OF INTEREST

The authors disclosed no conflicts of interest related to this article.

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