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Assessment on the quality and influence of tropical storage conditions on amoxicillin formulations marketed in Ethiopia

W. MIKRE[1*] AND N. MEKONNEN[2]

[1]*Department of Pharmaceutical chemistry, School of pharmacy, Addis Ababa University*
[2]*Management Sciences for Health, Ethiopia*

The quality of drugs in some less developed countries is inadequate. The quality of drugs in countries having a tropical climate may be adversely affected if their formulations have not been optimized for stability under these conditions. Amoxicillin is broad-spectrum penicillin active against certain gram negative and gram-positive organisms. It is a widely used antibiotic and there are several formulations of it in Ethiopia. The aim of the present study was to investigate the physicochemical equivalence of eight brands of amoxicillin capsules and five brands of amoxicillin for oral suspension sourced from different retail Pharmacy outlets in Addis Ababa, Ethiopia market and to study the effect of tropical storage condition's on the their quality with respect to the USP and BP requirement's using the WHO guidelines.The quality, physicochemical equivalence and the influence of tropical storage conditions of on the previously mentioned commercial dosage forms were assessed. The assessment included the evaluation of uniformity of weight, identification, water content, dissolution tests and the pH values of the reconstituted oral suspensions in addition to the quantitative determination of the amoxicillin content for the studied formulations. Almost all of the amoxicillin formulations examined met pharmacopoeial requirements at zero time, three-month and six month after storage under tropical condition.

Keywords: Amoxicillin; Pharmacopoeial; Tropical storage condition, Dissolution; Dissolution profile comparison

INTRODUCTION

Generic drugs are a copy of brand drug made by the expiry of the patent covering innovator products [1]. Many developing countries must import a large proportion of their essential drugs requirements, as they do not have sufficient manufacturing facilities. Since most of these countries also lack well-established quality control laboratories to routinely verify the quality of the imported drugs, they mainly rely on the expertise and integrity of the manufacturer for the quality and stability of the drug formulations. Problems can arise especially in developing countries having a tropical climate. The high temperature combined with a high humidity, to which the drug formulations are exposed during transport, distribution and storage, adversely affects the biopharmaceutical properties of the drug [2-4].

The influence of climate conditions in tropical countries on the quality of essential drugs has been of concern to the WHO and regulatory bodies. The WHO has conducted studies on the stability of essential drugs during distribution and storage in tropical climates [5-6] and has recommended testing the stability of drugs manufactured for the global market under class IV conditions (40°C, 75% relative humidity). Drug regulatory authorities and Manufactures should pay more attention to WHO recommendation on the stability testing of drugs under tropical climatic condition. However, manufacturers are not obliged to adhere to this WHO recommendation.

*Author to whom correspondence may be addressed

Previous studies done in Ethiopia on locally manufactured and imported drugs indicate there are substandard drugs circulating in the country [7-8]. In this study, the equivalence of different brands (including the innovator product) of amoxicillin capsules and amoxicillin for oral suspensions sourced from retail pharmacies in Addis Ababa, Ethiopia will be determined using certain in vitro methods. The influence of tropical storage conditions on the potency and in vitro dissolution characteristics of the studied antimicrobial drug formulations available on the Ethiopian markets were also investigated.

The purpose of this study was to assess the overall quality (physicochemical aspects) of different formulations of essential antimicrobial drug (amoxicillin capsules and amoxicillin for oral suspensions) marketed in Ethiopia and to study the influence of tropical storage conditions on the quality and in vitro dissolution characteristics

EXPERIMENTAL

Apparatus

Dissolution apparatus (ERWEKA, DT700H, Germany), CE 4000 UV-VIS double beam spectrophotometer (Cecil instruments limited, England), DL18 Karl Fischer Titrator (Mettero Toledo, Switzerland), Stability chamber (Binder Gmbh Bergstr: 14 D-7852 Tuttlingeh, England), thermostatically controlled water bath GFL1092 (West Germany), Shaker (West Germany), analytical balance AB204, mettler Toledo (Mettero Toledo, Switzerland) and pH meter mettler Delta 320 model (Mettero Toledo, Switzerland) were used throughout this work.

Reagents and solvents

All reagents used during the determinations were of analytical reagent grade. Chloroform was obtained from Merck (Darmstadt, Germany). Ninhydrin, pyridine, Karl Fischer pyridine free reagent, Boric acid, methanol and HPLC grade methanol were obtained from BDH Laboratory supplies (Poole, England). Acetic anhydride, dioxone, Imidazole, mercuric chloride was obtained from Riedel –De haën ag seelze-(Hannover, Germany). Toluene was

obtained from May and Baker ltd (Dagenham, England). BPCRS reference standard amoxicillin trihydrate form British Pharmacopoeia Commission laboratory (London, UK). TLC silica gel 60 F254 pre-coated aluminum sheets (20 × 20) was bought from Merck (Darmstadt, Germany). Karl Fischer water standard, 1ml containing 5mg of H2O from BDH (Poole, England). Whatman filter paper 541was obtained from Whatman international ltd England. USP reference standard amoxicillin trihydrate was purchased from the USP (Rockville, USA).

Samples

Formulations sampled for the study were those having a remaining shelf life of at least 24 months (as claimed by the manufacturer) at the time of sampling except for Hiconcil capsules and Hiconcil powder for reconstitution with shelf life of 17 and 19 months respectively. Record of the name of the brand, strength, cost, batch number, manufacturer's name and the expiry dates were kept (Table 1). The samples were bought from the two most frequently visited community pharmacy in Addis Ababa. The Ethiopian Red Cross society pharmacy obtains its drug supply through international tender using WHO certification and almost all products are not tested for quality by DACA quality control laboratory.

Methods

Identity, water content of the formulation, weight uniformity, pH values of reconstituted solution and dissolution tests were performed as described in the United States Pharmacopoeia (9). Assay for content of active ingredients was done as described in British pharmacopoeia (10). Determinations were done in triplicate for the assay and water content. The dissolution profiles were determined six times as in the pharmacopoeia. Drug content, water content, weight uniformity, pH values of reconstituted solution and the dissolution profiles of each drug were determined immediately after purchase,

after 3 and 6 months storage under simulated tropical conditions (75% relative humidity, 40 °C) using the methods described in the USP and BP monographs for amoxicillin capsules and amoxicillin for oral suspension [9-10]. The capsules and the powder for reconstitution were stored at simulated tropical conditions in their original package

Procedures

Identification test

The identity for each of the studied formulations was confirmed by the thin layer chromatographic method (TLC) using a mobile phase consisting of a mixture of methanol, chloroform, water and pyridine (90:80:30:10) as mentioned by the official method [9].

Weight uniformity test

The test was carried out as described in the USP [9] for each batch of the amoxicillin capsules. The weights of 10 capsules were determined individually taking care to preserve the identity of each capsule. The weights of the intact capsules and their empty shell were recorded. The net weight of each capsule is obtained by subtracting the weight of the shell from the respective gross weight and the deviation of these individual weights from the mean capsule content weight was computed.

Dissolution tests

Dissolution tests for capsules were carried out by using the ERWEKA type DT6L dissolution apparatus (Germany) fitted with a basket rotated at 100 rpm. The dissolution medium consisted of 900 ml of distilled water in a thermostatically controlled water bath at 37 ± 0.5 ° C. Samples (10 ml) were withdrawn after 10, 20, 30, 40, 50 and 60 minutes and an equivalent amount of the dissolution medium were immediately introduced as replacement. The samples were filtered and suitably diluted with distilled water and assayed for the drug content by measuring the absorbance at 272 nm using CE 4000 UV-VIS double beam spectrophotometer. Distilled water was used as a blank and the necessary correction for dilution was made when calculating for drug content and the determinations were done six times as in pharmacopoeia [9].

Assay of amoxicillin

All the samples were assayed for the drug content immediately after purchase. The BP mercuric–imidazole derivatisation method that is reported as stability indicating [9] method was used for the assay of both amoxicillin capsules and amoxicillin for oral suspension (BP 2000).

Water content

The determinations of the amount of water in the amoxicillin capsules and amoxicillin for oral suspension investigated in this work was done according to USP procedure using the Karl Fischer titration (Method I) [9].

pH values of amoxicillin for oral suspension after reconstitution

The reconstituted solutions were freshly prepared as described in the label and the pH values were measured using a pH meter (Mettler Delta 320, Switzerland) standardized with standard separate buffer solution of pH 4.0 and 7.0 (9).

RESULT AND DISCUSSION

Identification test of amoxicillin capsules and amoxicillin for oral suspensions

The Rf values of the sample and the reference standards were the same irrespective of the brand of the samples tested. This indicates the entire samples examined pass the identification test for the active drug.

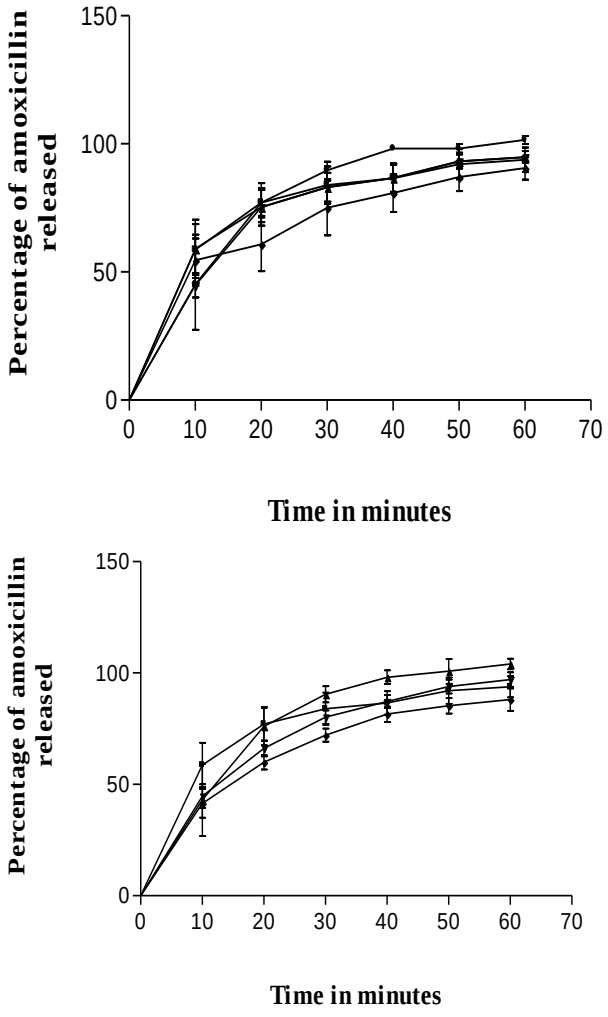
Weight uniformity test of amoxicillin capsules

The importance of this test is to ensure the capsules in each batch are within the appropriate size range. Failure to comply with weight uniformity test may be considered less serious provided the products fulfill the requirement for the content of the active ingredient. If the overall

weight of the capsules varies too much it is evidence that there will a great variation in the content of the active component. The result indicates that all the brands showed acceptable uniformity of weight as none of them had relative standard deviation in greater than 6 and range of weight within the prescribed pharmacopoeial range (85%-115%). This shows that all the capsules in each brands are within the appropriate size range. Although all products comply with the USP limits, the mass range indicated by the lowest and the highest mass was usually wider for the copies than for the reference product (Amoxil).

Table 1: Information on amoxicillin 500 mg capsule and amoxicillin for oral suspension 250 mg/5ml formulations evaluated for quality

Brand Code No.	Batch number	Date of manufacture	Expiry Date
CI	403249-1	03/2004	03/2007
CII	85555 B	01/2002	01/2007
CIII	414	02/2004	01/2007
CIV	3K78808	07/2003	07/2006
CV	X0049	03/2004	03/2007
CVI	408	07/2004	07/2007
CVII	22132	02/2004	02/2007
CVIII	E-4008	06/2004	05/2007
SI	92385 B	04/2002	04/2007
SII	3G67899	05/2003	05/2006
SIII	XS048	03/2004	03/2007
SIV	40097	02/2004	01/2007
SV	0401001	01/2004	01/2007



(a)
(b)

Figure 1: **Dissolution profile (n= 6) of amoxicillin capsules evaluated after zero month of storage under simulated tropical conditions (75%RH: 40^{0}C) against the innovator products(Amoxil). (a) [■ Amoxil, ▲ Hiconcil, ▼Epharm, ◆Amoxy, ● Rivamox] and (b) [■ Amoxil, ▲ Winpen, ▼Amoxicare, ◆Amoxicillin (Cyperus)]**

Table 2: Mean drug content ± SD (n=3) of amoxicillin capsules and amoxicillin for oral suspension at zero, three and six months storage under simulated tropical condition (75% RH, 40 °C)

Brand Code No.	Percentage label claim immediately after purchase *	Percentage label claim after three months*	Percentage label claim after six months*
C1	92.20±2.40	91.34±0.93	89.80±1.05
C2	101.67±1.16	98.75±1.19	96.47±1.98
C3	95.54±1.35	92.86±1.21	92.58±1.17
C4	104.06±1.90	100.8±2.64	97.25±2.52
C5	96.35±1.69	93.27±1.30	92.59±1.8
C6	97.38±1.90	96.08±1.15	93.33±1.27
C7	95.74±1.30	93.42±2.42	92.16±3.19
C8	99.56±1.58	97.89±2.32	96.03±1.87
S6	114.15±2.32	112.46±0.76	109.07±0.84
S5	113.2±1.50	109.50±1.97	105.16±1.23
S2	115.65±2.40	113.52±1.51	111.84±1.48
S3	109.78±1.36	105.46±2.90	100.55±1.70
S4	117.97±138	114.93±1.9	113.16±2.87

Table 4: Values of calculated fit factors to compare dissolution profiles of the amoxicillin capsules against the innovator product and the effect of storage condition on the in vitro dissolution

	Fit factor values for amoxicillin capsules against the innovator							
Fit factor	**C2**	**C4**	**C1**	**C3**	**C5**	**C8**	**C6**	**C7**
f1		92.85	48.05	58.15	55.48	49.32	51.97	44.79
f2		0.98	12.33	6.91	7.69	11.16	9.41	14.38
	Fit factors to compare the change in dissolution profile after three months storage							
f1	69.19	67.38	55.30	78.21	42.02	86.48	86.66	79.25
f2	5.45	4.87	10.63	2.92	8.90	1.48	1.95	2.48
	Fit factors to compare the change in dissolution profile after six months storage							
f1	57.93	59.22	62.75	63.62	42.87	78.57	77.84	77.33
f2	7.75	8.79	7.00	7.00	17.35	3.45	1.71	2.78

Table 3: Results of mean ± SD water content of the amoxicillin capsules at zero, three and six months stored under simulated tropical condition (75% relative humidity, 40 °C)

Brand name	Water content at zero month*	Water content after three month*	Water content after six month *
C7	9.41±0.36	12.39±0.64	13.00±0.04
C2	11.45±0.11	12.12±0.20	12.72±0.28
C3	11.24±0.08	12.21±0.10	12.82±0.07
C4	11.15±0.11	12.06±0.08	12.63± 0.28
C5	11.04 ±0.06	11.51±0.08	12.54± 0.06
C6	10.53±0.97	12.91±0.22	12.84± 0.15
C1	1.04 ±0.11	10.74±0.16	12.67± 0.05
C8	11.38±0.10	12.06±0.09	12.98± 0.06
S6	3.99 ± 0.11	4.13 ± 0.06	4.30 ± 0.03
S5	1.95 ± 0.09	2.19 ± 0.07	2.35 ± 0.15
S2	1.36 ± 0.03	1.40 ± 0.03	1.63 ± 0.06
S3	1.50 ± 0.01	1.49 ± 0.04	1.69 ± 0.02
S4	2.19 ± 0.08	2.34 ± 0.21	2.23 ± 0.05

Assay of amoxicillin capsules

The British Pharmacopoeia states that the amoxicillin capsules must contain not less than 92.5 % and not more than 110% of the label claim of amoxicillin in a capsule of average weight [10]. The amoxicillin content in the sample immediately after purchase (before storage under simulated tropical condition) ranged from 92.7 to 101.67 as shown in table 2 immediately after purchase. This result is within the acceptable range of 92.5-110% prescribed by BP [10], indicating all the products tested are confirmed as being compendial quality in terms of content of active ingredient. During the 6 months of storage under simulated tropical conditions all formulation except amoxicillin manufactured by Remeda (% claim after 3 and 6 month storage) conformed to the BP 2000 tolerance limits.

Assay of amoxicillin for oral suspension

The British Pharmacopoeia states that the amoxicillin oral suspension when freshly constituted should not be more than 120,0% of the stated amount. When stored at the temperature and for the period stated on the label during which the oral suspension may be expected to be satisfactory for use, not less than 80 % of the stated amount. The amoxicillin content in the amoxicillin oral suspension sample immediately after purchase (before storage under simulated tropical condition) ranged from 109.78 to 117.97. This result is within the acceptable range of 80-120 % prescribed by BP [10], indicating all the products tested are complying the compendial quality in terms of content of active ingredient.

Water contents of amoxicillin capsules

Determination of water in pharmaceuticals is important in that many samples contain water as solvent, as absorbed water or as water of crystallization. When dealing with expensive materials and even with large quantities of less costly materials, water can be considered an

adulterant. Quantitative specifications for water present must be established. It is advantageous to keep water level to minimum because water itself can accelerate the degradation of drug substance. As with residual solvents, water level that are not within specifications for a drug substance can lead to modification of physical properties of a material leading to manufacturing difficulties in formulating drug products [12].

The water content of medicaments strongly influences their quality, shelf life, sticking and clumpiness, and stability as well as the release of the active substances. The determination of water therefore assumes great importance in pharmaceutical analysis. The United State Pharmacopoeia describes various methods for determining the water content of pharmaceuticals [9]. By far the most important method used to quantify water in pharmacopeial assays is the Karl Fischer titration.

The tolerance for water content of amoxicillin capsules in the pharmacopoeia is 14% [9]. The results given in Table 3 indicate the entire brand examined within the Pharmacopoeial limit before storage under simulated tropical condition. Among the products examined the lowest percentage of water content was 9.41 and while the highest was 11.45 as shown in table 3. The highest water content before storage under tropical storage was found to be the innovator product with a special outside aluminum cover. As indicated in Table 3 the water content of the entire samples after 6 months of storage under tropical condition was found to be with in the pharmacopoeial limits.

Water content of amoxicillin for oral suspension

Water content tolerance stated in the pharmacopoeia for amoxicillin for oral suspension is not more than 3.0%. All the products except Amoxil passed the test as indicated in Table 3. Water content of Amoxil was found to be 4.03 percent, which is out of the compendial specification. The percentage of water content of the other four products was within the range of 1.36- 2.19 percent of water thus satisfying the requirement for water content

Dissolution test of the amoxicillin capsules

The in vitro release or the dissolution of the product is dependent upon several factors: the drug itself, the active ingredients, and the formulation. Products with different formulations, different inactive ingredients and different manufacturing procedures may have different dissolution profile; and may have different bioavailability. The result of calibration curve for the determination of amoxicillin in dissolution testing using USP reference standard amoxicillin within the concentration range of 200 µg/ml to 20 µg/ml gave the regression equation [A = 2.339 × 10-3C-0.00753, r2 = 0.9999] and the concentration of amoxicillin in the dissolution media was determined using this equation

The dissolution test results indicate before storage all brands of amoxicillin in this study released at least 80% of their amoxicillin content within 60 minutes. All formulations conformed to the USP 26 dissolution tolerance limits (minimum 80% dissolved within 60 min) the percentage drug dissolved in 60 minutes ranging from 88.11 to 104.08% figure 1. After the 6 months storage under the tropical condition as shown in Tables 2, the percentage of amoxicillin released within 60minutes ranged from 85.58 to 101.54 indicating the products agreed well with the official requirement maintained above.

To compare the similarity of the dissolution profile of the different brands with that of the innovator the difference (f1) and similarity (f2) factor were used. In contrast to the USP single point determination, the similarity factor takes into consideration all sampling points of the dissolution profiles. The fit factors may be used as a tool for product development and optimization. Generic producers try to mimic the dissolution profile of an innovator in their formulation in hope of getting wavier of the bioavailability/ bioequivalency study (13-14). Looking at the data presented in table 9, the data fulfill all the criteria set by the FDA guideline. Therefore, both f1 and f2 statistics can be used to evaluate the equivalency of the different products of amoxicillin investigated with the

innovator product. According to the US Food and Drug Administration's (FDA) guides for industry (13), generally, f1 values up to 15 (0–15) and f2 values greater than 50 ensure sameness of the two curves. The values of f2 and f1 factors for the different amoxicillin products versus the innovator product were calculated from the means of percent dissolved at each time point by using the equations 1 and 2 and are listed in below.

$$f_1 = \left\{ \frac{\sum_{t=1}^{n} |Rt - Tt|}{\sum_{t=1}^{n} Rt} \right\} \times 100 \quad (1)$$

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

Where f1 is difference factor, f2 is a similarity factor, Rt is the reference assay at time point t, Tt is the assay at time point t, n is the number of pull points. The f2 and f1 analysis showed the dissolution profiles are similar to innovator for Hiconcil, Rivamox, Amoxicare, Amoxy as the values of similarity factor f2 calculated were larger than 50 and f1 values were less than 9.41. With grater similarity to the innovator profile being observed in the case of Hiconcil. An f2 value is taken to indicate a potential difference in vivo dissolution [13-14]. A different from dissolution profiles of the innovator was found for Winpen, Amoxicillin (Epharm) and Amoxycillin. As seen in figure 1 amoxicillin (Cyprus) has the dissolution profile the furthest away from the profile of the innovator product. The f1 and f2 values for amoxicillin products were determined to observe if there is any effect tropical storage has on the dissolution profiles and the results are given in table 5.

Apparent pH values of amoxicillin for oral suspension

The tolerance in the pharmacopoeia is the pH value of the suspension after constitution as directed in the labeling be between 5.0 and 7.5. Except Amoxil all the products evaluated in this work passed the test for pH as shown in Table 4. The obtained pH values of the reconstituted Amoxil suspension was 4.53 far below the lower value of the officially required. The pH value of the other four products was within the range of 5.38 to 6.25 thus meeting the requirements for pH value of the reconstituted suspension. The pH of the solution affects the stability of amoxicillin. Amoxil failed in combination with the pH result twice the official requirement. The preparation was within the BP specification and its makes its claim on BP method.

CONCLUSION

Almost all of the amoxicillin formulations examined met pharmacopoeial requirements at zero time, three-month and six month after storage under tropical condition. One of the amoxicillin for oral suspension had failed the tests for pH values of the reconstituted solution and for the content of water in the three different months. With regard to the f1 and f2 values of the dissolution profiles of the different brands of the capsules against the innovator at zero month of storage, three of the products showed significant difference. Comparison of the dissolution profile of the zero month with the three and six month storage under tropical condition has shown a significant difference in the dissolution profiles only for one product.

This study provides baseline data for the presence of substandard products (possibly by decomposition) for the selected essential anti-microbial drug (amoxicillin capsules and amoxicillin for oral suspension). Drug quality is a source of great concern worldwide, particularly in developing countries like Ethiopia. One of the means to combat availability of poor drugs in the market is to increase post marketing surveillance for the quality of marketed drugs. This study provides an insight into the quality of marketed ant infective agents in the country. The accelerated stability testing under zone IV climatic condition for both imported and locally manufactured drugs would serve as a quality control tool to verify whether the products have been optimized

for maintenance of efficacy and safety during distribution and storage under tropical climatic conditions. The results of the study shows most of the products evaluated in this study satisfied official requirements with some failing to satisfy official requirements but there should be continuous follow up by the regulatory authority (DACA) to ensure that drugs on the market are consistently of good quality.

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