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An *In-Vitro* Evaluation Study of the Quality Control of Different Brands of Amoxicillin Capsules Marketed in the South and West of the Republic of Yemen



Ahmed Ghaleb Al-Mekhlafi

*Pharmacy Department, Faculty of Medicine and Health
Sciences, Tamar University,
Republic of Yemen.*

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ABSTRACT

The Yemeni drug market is open, and most of the companies are non-research bases, and hence the medicines are degraded and lose their potencies due to the stressful climate in Aden and Hodeida cities. The former stands by the side of the Arabian Sea while the latter stands by the side of the Red Sea. Therefore, they have a high temperature and high humidity in summer. The samples of the study included eleven brands of amoxicillin capsules (6 locally, 3 Arabic, 1 Indian, and reference) which were collected from Aden and Hodeida governorates. The purpose of the study was to determine the quality of the brands according to standard criteria. The method of analysis was conducted by the HPLC instrument at wavelength 230 nm. The calibration curve achieved linearity, accuracy, and reproducibility and also the correlation coefficient was 0.997. The prerequisites that were required for the study have been taken to meet the USP and BP criteria. The results of the study showed that five brands out of eleven achieved an agreement with the Pharmacopeias specifications according to the physicochemical assays, whereas six brands failed in dissolution test that considered the important parameter to predict drug absorption and bioavailability. The conclusion of this study reflected that 55% of the brands were substandard and the results ensured that there were problems associated with substandard, adulterated, and counterfeit in the Yemeni market. In light of the results of this study, the investigator recommends that the authorities of health must conduct too many studies in physicochemical assays.

INTRODUCTION

Amoxicillin is a broad-spectrum, pharmacologically active beta-lactam antibiotic effective against Gram-positive and Gram-negative bacteria. It is stable in the gastrointestinal tract and has higher absorption than naturally occurring penicillin when administered orally. It is a widely used antibiotic in human and veterinary medicine for the treatment and prevention of respiratory, gastrointestinal, urinary and skin bacterial infections due to its pharmacological and pharmacokinetic properties ^{1,15}.

According to Sousa², Amoxicillin is de-activated by bacterial β -lactamase or penicillinases. In human medicine amoxicillin is commonly used in combination with clavulanic acid, a penicillinase inhibitor; it is not normally used with clavulanic acid in veterinary use ¹. The amoxicillin was introduced in the early of 1972 in the United Kingdom. The structure of amoxicillin achieves acid stability and β -lactamase resistance and hence in 1987 it was produced in a combination with amoxicillin-clavulanic acid to become more effective against the resistance of β -lactamase and penicillinase³. Amoxicillin acts by inhibiting the synthesis of bacterial cell walls. It also inhibits cross-linkage between the linear peptidoglycan polymer chains that make up a major component of the cell wall of Gram-positive and Gram-negative bacteria. It is usually the drug of choice within the class because it is better absorbed following orally route and it is resistant to gastric acid ⁴. The molecular chemical structure of amoxicillin is represented in Figure One below. The physicochemical properties of amoxicillin are characterized by the following: powder white crystalline solid, melting point 194 °C, pH 4.4-4.9 (0.25 w/v solution), slightly soluble about 3.43 mg/ml water, and optical rotation is \pm 390-315 °C ¹.

There are more than 200 generic brands in different dosage forms and strengths, according to the Annual Report of the Supreme Board of Drugs and Medical Appliances and most of the companies that market it in the Republic of Yemen (ROY) is non-research bases ⁵. The tablets and capsules of amoxicillin contain excipients such as diluents, binders, disintegrants, glidants, lubricants, coloring matter and flavoring substances which should be used in quantities that do not affect stability, dissolution rate release and bioavailability⁶.

Formulation parameters have been identified to influence tablet and capsules characteristics. Variations in the manufacturing process could consistently alter the disintegration, dissolution and consequently bioavailability of the active ingredients in a product ⁷.

The bioavailability of amoxicillin capsules 500 mg that excreted in the urine ranged between 57-63%, in eight hours ⁸. The physicochemical characteristics and adherence to the Current Good Manufacturing Practice (CGMP) during manufacturing are paramount to the predictability of its bioavailability and bioequivalence ⁹.

1. Previous Studies

Two previous studies were conducted in the quality control of different brands of amoxicillin capsules and tablets. First, Corazza et al ¹⁰ conducted a physicochemical quality evaluation of 13 brands of amoxicillin capsules in Brazil. The results of the study showed that all the brands passed the dissolution profiles test according to Brazilian Pharmacopeia in 90 minutes ¹¹, but when we take the US Pharmacopeia ¹² that determined the maximum limit to 60 minutes, none of the 13 brands passed the test. Again 11 brands failed in uniformity contents, and 3 brands in average weight.

The second study was carried out by Kerly et al ¹³ who studied the design of amoxicillin tablets of 8 brands. Their results showed that all the brands passed physicochemical assays taking the time of the official dissolution during 90 minutes, but when we take the US Pharmacopeia ¹² as previously mentioned, none of the 8 brands passed the test.

Both the above studies reflect that the quality of medicines in developing countries was worse and consequently, drug evaluated is necessary and intended to ensure the efficacy, safety, and quality of medicines; a crucial role for any pharmacist for public health ¹⁰. However, the climatic conditions, in the (ROY) are widely different and can affect the medicines during the storage especially in the west and south of Yemen which are very hot and high relative humidity in the summer such as Aden and Hodeida. These are our target areas in conducting this study. They are also considered as tropical areas ¹⁴. The drug quality control in the ROY analyzes 800 samples per year for registration purposes, not more. All the aforementioned facts mentioned above, justify researching the drug quality control.

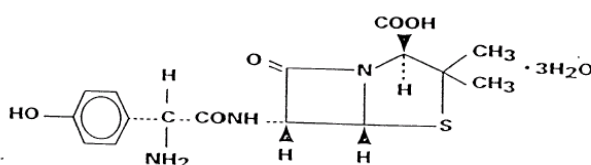


Figure 1 ¹⁵

3. MATERIALS AND METHODS

Collection of Samples

The samples of this study were collected randomly from the retail Pharmacies in Aden and Hodeida which included eleven different brands as follows: six brands are local products; three Arabic; one Indian and reference Amoxil capsules 500 mg which are coded as represented in Table (1) below.

Table No. (1): The Samples of Amoxil Brands

Sample code	Batch no	Sample code	Batch no	Sample code	Batch no
Amox1-cap	11131	Amox5-cap	11062	Amox9-cap	2583
Amox2-cap	11367	Amox6-cap	11B062	Amox10-cap	7540
Amox3-cap	5071	Amox7-cap	50410	Amox11	1535
Amox4-cap	A185	Amox8-cap	S365010		

Experiment;

Apparatus

Waters HPLC apparatus Model 1500, Detector Model 2489 Waters, Japan

Analytical balance, Model: HM 2000, Japan

pH – meter apparatus, Sartorius AG, Germany

Disintegration test apparatus, Erweka, Germany

Dissolution test apparatus, Hand Son Research Corporation, USA, Apparatus 1, USP 24.

Chromatographic conditioning

Column: C18, Flow rate: 2 ml/min, detector UV-visible, detection wavelength 230 nm, pressure 1082 Mpa, injection volume 20 µl, room temperature. The mobile phase consists of monobasic potassium phosphate 6.5 gm./1000 ml purified water, adjusted the pH buffer with KOH 4.5% to 5. Mobile phase buffer: acetonitrile (96:4).

Materials and Reagents: All reagents used were of analytical grade purchased from the local market; BDH, Germany. Concentrated HCL 35%, and its specific gravity 1.18.

Standard phosphate buffer solutions pH 4: composition of citric acid/NaOH and NaCl.

Amoxicillin was kindly supplied by a local manufacturer: Modern Pharma.

Preparation of Standard Calibration Curve

Prepare a stock solution by dissolving a suitable quantity of reference amoxicillin tri-hydrate that is equivalent to 100 mg of anhydrous amoxicillin by using an analytical balance and transfer the weighed quantity to 100 ml volumetric flask, complete the volume with purified water to produce concentration 1mg/ml.

Series of standard solutions with different concentration of standard amoxicillin trihydrate e.g. 20, 40, 60, 80, and 100 $\mu\text{g/ml}$ were prepared by pipetting 2 ml, 4 ml, 6ml, 8 ml, and 10 ml in five 100 ml volumetric flasks and complete the volume to 100 ml of each by purified water.

The above 5-point concentrations analysis by HPLC that repeated daily for seven days, the results achieved accuracy, reproducibility, and linearity. The relative standard deviation percent at lower and upper concentrations were 6.24 – 0.83 respectively. The correlation coefficient was 0.994 as represented in Figure 2.

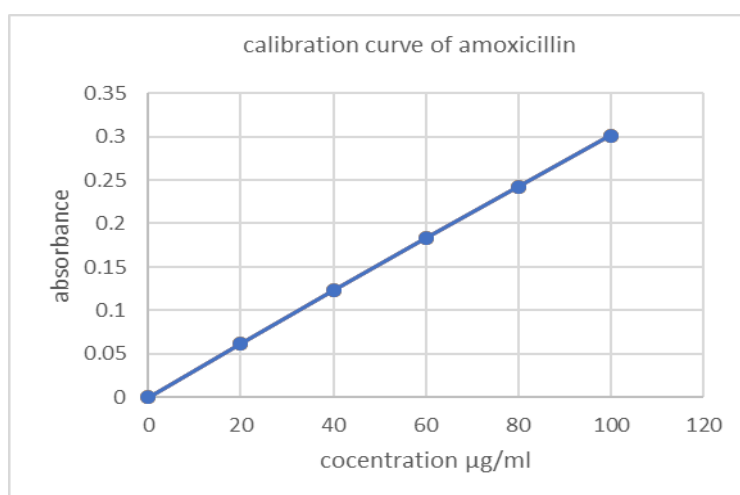


Figure No. (2): Calibration Curve of Amoxicillin

Uniformity of Weight

The British Pharmacopeia ¹⁶ method was adapted by using twenty randomly selected capsules from each batch and the average weight and not more $\pm 5\%$ deviate from the average.

Disintegration Time Test

The British Pharmacopeia ¹⁶ method for determination of disintegration time for uncoated tablets was adopted using a disintegrating apparatus (Erweka) and the medium was purified water at 37 ± 1 °C. Six tablets were placed in each six open-ended transparent tubes for determination of the test, a device to raising and lowering the basket is 30 cycles per minute.

Dissolution Test

USP apparatus 1 method was used to determine the dissolution test. The medium was purified water at 37 ± 0.5 C° and heated by auto-heater, the rotation per minute was 50.

Uniformity of Contents

The British Pharmacopeia ¹⁶ determined the active ingredient between 90-120%.

4. RESULTS

This study included eleven brands of amoxicillin 500 mg capsules that were available in the Yemeni market. The brands of the study and their original had been mentioned in Table (1) above. The instrument that was used in the physical tests such as disintegration, dissolution tests were calibrated. The high-performance liquid chromatography (HPLC) was used to determine the qualitative and quantitative analysis at 230 nm. The method of analysis was valid (See Methodology, above). The capsules products were examined according to the British and the United States Pharmacopoeias ^{16,12} (BP, 2008, USP 24). The physical essential examinations for capsules such as weight variations, disintegrations, and dissolutions tests were carried out for capsules and also chemical assays according to USP and BP. The results show that five brands that are coded Amox. 4, 7, 8, 9 and 10 agreed with the standard in physicochemical tests as illustrated in Table (2) below. The other six brands that are coded as Amox. 1, 2, 3, 5, 6, and 11 were satisfied in the quantitative analysis but all of them failed in the dissolution test as represented in Table (2) below and Figure (3). The results of the study ensured that 54.5% of the sample were substandard medicines in the ROY.

5. DISCUSSION

Weight Variation

Table (2) below shows that all the eleven brands gave good results to this test (average $\pm 5\%$) according to the British Pharmacopeia ¹⁶ (1998) specifies that not more than two individual weight should deviate from the average weight by more than 5% and none should deviate by 10%. These results reflect that capsules machines were calibrated and normal flow ability had been achieved and gave homogeneous fluidity and fallen the body of the capsules in a pattern that achieved specifications.

Disintegration Time Tests

The results of all the eleven brands in disintegration time ranged between 7 and 15 minutes (Table 2). The limit of this test was less or equal to fifteen minutes. The eleven-brands passed the disintegration test, and they reflect that the disintegrants were compromised with the components of the formula, and also occurred that the instruments that were used in this test were maintained and calibrated in a specific period time.

Dissolution Tests:

The dissolution of an oral pharmaceutical product is important because it indicates the percentage of the drug that is available for absorption after a specific time. Table (3) below shows the results of dissolution behaviors for the eleven various brands, five of them had passed this test, whereas brands no 1, 2, 3, 5, 6 and 11 failed to meet the standard when compared with the reference no 4 as represented in Figure (3) below. The failure in this test could be attributed to different factors such as increasing the particle size diameter of raw materials of amoxicillin, bad granulation process, and may increase the binder in excess amount and lubricant such as magnesium stearate. In addition to the formulation dosage form there are hydrophobic compounds, besides the physicochemical properties e.g. amorphous polymorphisms, and salvation and also the environmental conditions such as humidity during production and storage conditions. This high ratio defective reflects a low bioavailability which may lead to therapeutics ineffectiveness and spread of resistance, and finally may lead to death. Moreover, the brands that were deviated from the limit of dissolution profiles passed also in quantitative analysis (Table 2). The previous reasons can lead to a lack of homogeneity in excipients and so lead to co-precipitation or complexation and hence

decrease the dissolution rate process of amoxicillin capsules at the time allowance. Furthermore, the failure in the dissolution test ensured that the experienced in developing countries are not enough and reformulation is mandatory in drug manufacturing where most of the companies in the Yemeni market are non-research bases and hence the absence of the CGMP in most of these layouts.

Our findings were comparable with two studies: the first one was carried out by Kerly et al.¹³ who investigated the amoxicillin tablets of 8 brands¹². Their results showed that all brands passed the physicochemical assays taking the time of the official dissolution for 90 minutes, but when we take the US Pharmacopeia¹² that determined the maximum limit as 60 minutes none of the 8 brands passed the test.

The second study was carried out in Brazil by Corraza et al.¹⁰. on 13 brands of amoxicillin 500 mg capsules, all of the brands passed the dissolution profiles test according to Brazilian Pharmacopeia in 90 minutes¹¹, but when we take the US Pharmacopeia¹² as mentioned above, none of the 13 brands passed the test.

Uniformity of Contents:

Table (2) and figure (4) show that the five brands that are coded Amox. 4, 7, 8, 9 and 10 passed the physicochemical tests, whereas the other six brands failed in the official dissolution test as aforementioned, and passed the quantitative analysis. In my opinion the grinding of granules in capsules made them fine powdered before the samples of analysis were weighed, and hence this process facilitated the solubility of the active ingredients, and hence the brands passed these chemical assays, but the prediction of absorption in vivo was exactly very bad and most of them led to malabsorption. In the previous study that was conducted by Corraza et al.¹⁰ on 13 brands of amoxicillin 500 mg capsules, only two brands out of 13 passed the quantitative analysis, whereas the other 11 brands failed in uniformity contents. Also, our finding agrees with the study that was conducted by WHO¹⁶ and Stephen¹⁷ who estimated that 10 percent of the global pharmaceutical market consists of counterfeit, substandard, and adulterated drugs, but this estimate increases to 25% for developing countries and may exceed to 50% in certain countries. The final estimated of our study proved that 55% of the samples were substandard.

The Conclusion of these Studies

The failure in dissolution tests in six brands can cause a low bioavailability which may lead to therapeutic ineffectiveness and spread of resistance and may lead to death. Defect in the formulation compromising between the amoxicillin and additive materials are the main reasons for failing the test and hence this led to poor quality of medicines in the Yemeni market. The CGMP is still far away in the ROY. The researcher recommends the authority of health to conduct too many studies in an in-vitro quality control, besides the application of bioavailability and bioequivalence studies and also drug stability to achieve drug safety, efficacy and quality according to the Yemeni law of medicines no 333 for 2004¹⁸.

Table No. (2): The Results of Various Brands of Amoxicillin Capsules 500 mg

Essential tests					
Sample Code	WV-mg Av ± 5%	Disin Time min ≤ 15	Dissoln after 60 m ≥ 85%	Assays Limit: 90 -120%	Results
Amox1	600.43	9	84.30	97	N. CPY
Amox2	601.75	9	83.70	102.5	N. CPY
Amox3	577.55	15	83.10	92.7	N. CPY
Amox4	616.14	12	93.4	100.7	CPY
Amox5	629.70	7	84.10	101.85	N. CPY
Amox6	580.00	7	80.80	98.00	N. CPY
Amox7	595.82	10	92.80	99.1	CPY
Amox8	601.20	8	90.00	96	CPY
Amoxi9	606.50	12	90.40	102.6	CPY
Amox10	609.80	7	91.90	102.6	CPY
Amox11	586.90	10	84.10	98.2	N. CPY

Disin: disintegration, dissoln: dissolution, WV: weight variation, N: not, CPY: Comply,

Table No. (3): Dissolution Rate of Various Brands of Amoxicillin 500 mg- Capsules

Sample code	% of drug release after 15 minutes	% of drug release after 30 minutes	% of drug release after 45 minutes	% of drug release after 60 minutes
Amox1	56.4	70.4	77.4	84.3
Amox2	59.7	70.2	77.7	83.7
Amox3	58.5	71.9	80.1	83.1
Amox4	70.8	86.2	90.2	93.4
Amox5	63.3	74.2	80.0	84.1
Amox6	58.4	69.1	76.1	80.8
Amox7	79.6	89.9	93.0	92.8
Amox8	69.4	81.8	87.8	90.0
Amox9	70.6	80.4	87.0	90.4
Amox10	72.7	83.1	89.0	91.9
Amox11	43.1	67.4	78.1	84.1

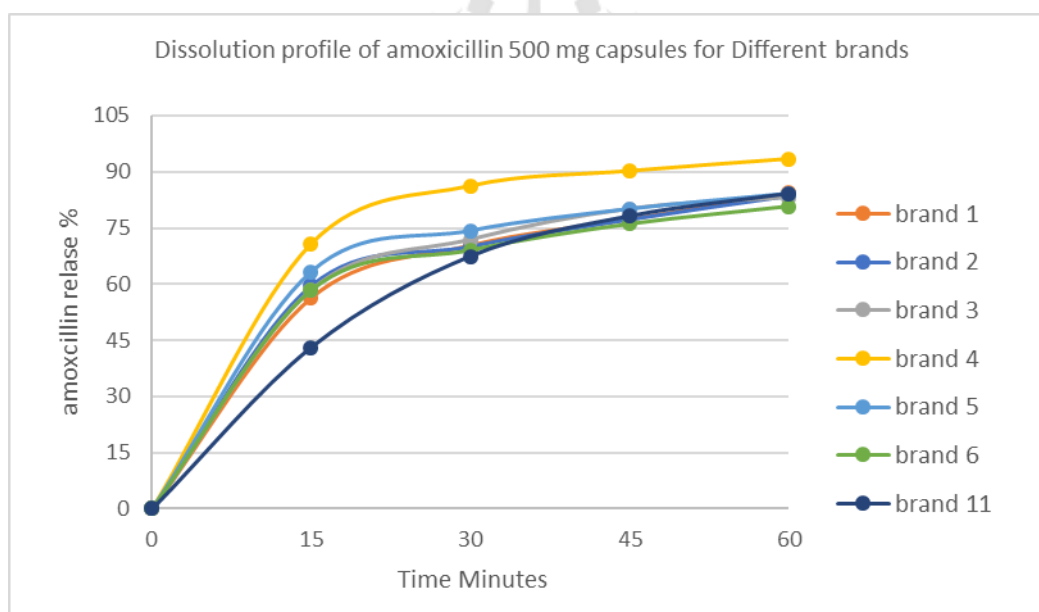


Figure No. 3

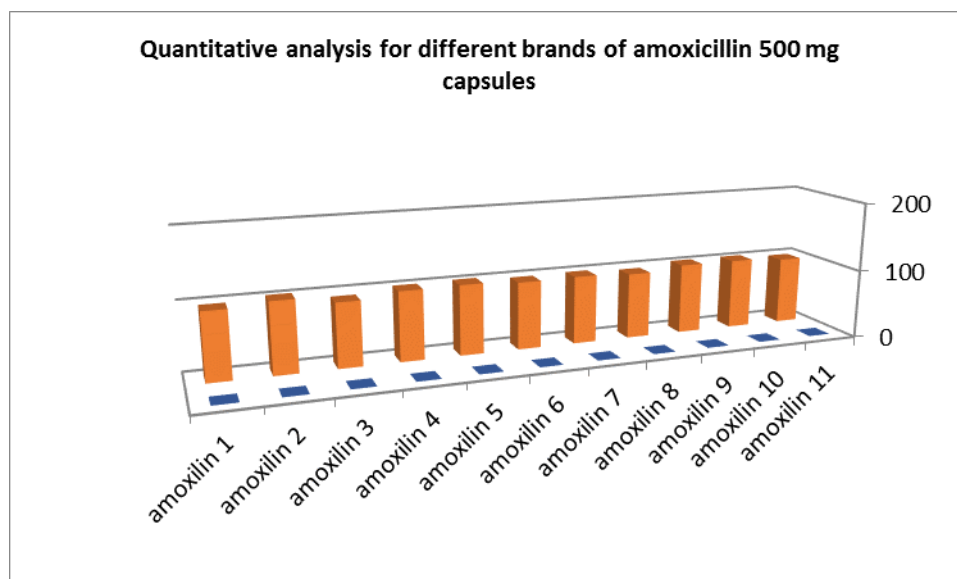


Figure No. 4

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