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Quality and Stability of Amoxicillin-Potassium Clavulanate Drugs Marketed in Yemen: Influence of Tropical Storage Conditions

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ABSTRACT

The study objective is to assess the quality and stability of amoxicillin-potassium clavulanate products. HPLC Method was used for drug content and in vitro dissolution tests that were performed on 10 generic products registered and marketed in city of Sana'a at purchase time, after one month, three months and six months storage under simulated tropical conditions (40 ±2°C and 75 ±5% RH) according to USP30. The results of study has shown that 2 of 10 tested products had a poor quality at purchase time which one was lower than limit and the another was upper than limit. After six months storage 4 of 10 and 7 of 10 of the tested products were fail in amoxicillin and clavulanate content respectively, and 7 of 10, and 8 of 10 of the tested products were fail in amoxicillin and clavulanate release after 30 min respectively. Study shown that 8 of 10 (80%) of tested products was substandard and don't comply with USP30. Good manufacturing process and storage conditions may affect the quality of amoxicillin–potassium clavulanate products in Yemeni market. Stringent quality control measures and stability tests should be applied to maintain the quality of amoxicillin–potassium clavulanate products in the market.

Keywords*:* Amoxicillin Potassium clavulanate, Stability, quality, Yemen, tropical storage condition.

INTRODUCTION

In 1981, the clavulanic acid (C8H9NO5) and amoxicillin (C16H19N3O5S) combination was introduced in the U.K and became the drug of choice against several bacterial infections [1, 2]. The preparations of this combination contain 125mg of clavulanic acid. Therefore, amoxicillin–clavulanic acid sufficiently inhibits β-lactamase–producing organisms. In addition, it has higher activity to eradicate H. pylori [3, 4]. But, the resistance development of antibiotics may be increased due to inadequate doses as well as random use [5].

In order to reach into the typical quality of the drug and its conforming to the modern definition, the product should contain the sufficient amount of each active pharmaceutical ingredient (API) supposed to be labeled within the applicable limits of its specifications, dose to dose consistency, free from unessential substances, potency maintenance, availability of appearance and therapeutic until it is used, and it instantly release active pharmaceutical ingredient for full bioavailability upon administration [6]. For patient safety, Pharmaceutical drug products quality is deemed to be extremely important. Safety and effectiveness of Pharmaceutical product may be influenced by impurities of pharmaceuticals. In addition to potential degradation, the impurities may lead to alteration of pharmacological and chemical properties of drug products that have significant effect on product safety and quality. The drug stability is a major element that considered to be the secured manner for ensuring supply of therapeutic values to the patients [7-9]. To the maintenance the activity and product integrity it should be stored in appropriate conditions to protect the product composition that's sensitive and/or decomposed by environmental factors. "Stability is defined as the capacity of a drug substance or drug product to remain and maintain within its standard specifications of purity, strength, identity and quality throughout the retest or expiration dating periods" [10].

As a matter of fact, it is well understood that the minor target of pharmaceutical product stability is to find out the quality assurance that the product will absolutely remain at a desirable level of fitness throughout its validity during its existence in pharmacy or its treatment regimen [11].

Based on above mentioned target, one of the primary reason for stability testing is to create a confidence to the patient who is suffering from disease that we are taking care of his health to get recovered. In the other hand is to avoid the decomposition of unstable product to yield toxic material or losing its activity which lead to death due to the failure of treatment e.g. nitroglycerine tablets. Second reason is related to the drug manufacturer to protect his brand name of which evidenced and proved that it remain with effectiveness. In other way, the stability studies are used in manufactures during the development of drug formulation to select the suitable excipients, to distinguish the best storage conditions, to determine the claimed drug product shelf life for the confirmation of registration file the claimed shelf life, and finally to verify that the manufacturing method or drug formulation have no changes which can adversely influence the product stability [12, 13].

In developing countries such as Yemen, drugs are often procured without the quality standards of sufficient references. In some settings, the quality restricted procedures are not implemented by nongovernmental organizations (NGOs) which work in such countries during drug purchase [14]. Furthermore, the technical assessment of the drug quality monitoring is imitated. It was reported by WHO that useful drug regulatory systems are only used by little countries. Even if the efforts of such drug quality monitoring are being made in developing countries, only simple pharmacopeial test methods for quality confirmation exist and the stability test for drug products will not be performed [15,16]. For drug purchase, the registration in resource-limited countries is usually approved under a prerequisite of a stability test; however, the approval of a drug in such countries is registered based on a simple review of documentation. Therefore, the stability for products can never be ensured without its assessment [14]· The study objective was to assess the quality and stability of different brands of amoxicillin-potassium clavulanate oral solid dosage forms in the Yemeni markets.

EXPERIMENTAL SECTION

Sample: A selection of antimicrobial drugs as the most commonly used in Yemen was listed below. Totally, ten products of such drugs were collected from Sana'a market. The shelf life of each tasted products was at least twenty two months. They were purchased and stored in their original package till they were tested. Detailed information about the tested products are shown in Table (1). Before the first test, the investigator was blinded to know the origin of drugs during the test because reference and all tested products were coded after erasing the products information.

Table(1): Detailed information about the tested products

Products name	Manufacturer-origin	Package Content Tablets	Batch No	Price per package (USD)
AUGMENTIN	GalaxoSmithKline. Unitedkingdom	Box 14tablets	661533	16$
AUGMEN	Modern PHARMA CO - Yemen	Box 15 tablets	14071	9$
AUGMACLAV	Global PHARMA CO -Yemen	Box 15 tablets	13559	11$
AMOKLAVIN	DEVA. Turkey	Box 14tablets	A0-30415	7$
JULMENTIN	Julphar. UAE	Box 15 tablets	B-186	12$
KLAMOKS	Bilim Pharmaceutical. Turkey	Box 10 tablets	4256028A	12$
KLAVOX	SPIMACO. Saudi Arabia	Box 14 tablets	74128	11$
MEGAMOX	JPI. Saudi Arabia	Box 14 tablets	135583	11$
GLOCLAV	Globalpharma. UAE	Box 14 tablets	8180	14$
CLAVIMOX	PHARMACARE INT-Yemen	Box 14 tablets	1306225	10$

Study design:
In vitro dissolution and analyzing drug content studied the drug quality. For all formulations, they were evaluated directly at the purchase time and after one, three and six months of storage which was performed based on "the International Conference on Harmonization (ICH) harmonized tripartite guidelines on stability testing of new drug substances and products" [17,18] by mean of accelerated testing conditions (40±2°C and 75±5% RH.). By using the methods specified in the monograph of US Pharmacopeia and National Formulary (USP30–NF24), all the drugs were tested[19]. The difference in drug content and dissolution characteristics between test formulations at purchase and after one, three and six months of storage was compared. The USP30 acceptance limits for drug content were 90–110% of the label claim, while the limits of released amount of drugs for "*in vitro*" dissolution after 30 minutes were not less than 85% for amoxicillin and not less than 80 for clavulanate. Three assays were often repeated, and the standard deviation was taken.

Drug standard and reagent: Amoxicillin trihydrate and potassium clavulanate RS, acetonetril and methanol were obtained from (Global Pharmaceutical Industry, Yemen). During the drug assay, reagents of HPLC grade were used, the other reagents were of analytical grade.

Dissolution testing and stability test
The stability of "in vitro" dissolution tests were performed using the USP paddle method (Method 2), (Dissolution apparatus 2010 Pharmatest, German) at a rotational speed of 75 rpm. The dissolution vessels were filled with 900ml of distilled water, maintained at 37 ±0.5°C. 20 ml of samples were withdrawn after 30 min. The samples were diluted with the mobile phase before being analyzed by HPLC.

Drug content (Standard preparation):
Weigh accurately powder equivalent to 25 mg of Clavulanic acid and equivalent to 100 mg of amoxicillin was transferred into 200 ml volumetric flask then mobile phase added for dissolving, and the mixture was sonicated for 5 min, then the volume was dilute and adjusted with the mobile phase and homogenized. (0.125 mg/ml of Clavulanic acid and 0.5 mg/ml of Amoxicillin) . 20 µl volumes of standards were injected into HPLC.

Drug content (Sample preparation):
20 tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to 25 mg of Clavulanic acid and equivalent to 100 mg of amoxicillin was transferred into 200 ml volumetric flask then mobile phase added for dissolving and the mixture was sonicated for 5 min, then the volume was dilute and adjusted with the mobile phase and homogenized. 20 µl volumes of sample was injected into HPLC and three injections were made per sample.

Instrument and chromatography condition:
The drug concentration in sample and standard solutions were determined by using HPLC method (1525 HPLC Gradient, Waters breeze system, USA) with UV detector. The column was a symmetry C18 (250 x 6.4mm, 5µm packing material) analytical column (Waters, USA).

mobile phase consist of mixture of 50 volume methanol and 950 volume of sodium dihydrogen phosphate solution of PH 4.4. The flow rate was 2ml/min. The detection wavelength was 220nm.

Statistical Analysis:
Data were summarized as mean ±SD. Independent (student) t-test was used to conduct the significance of association between drugs at different time using SPSS program version 21. Difference were considered significant at *P* values of less than 0.05.

RESULTS

For drugs content:
The relative standard deviation (RSD) for replicate injections of the standard or sample preparation for "within day" and "between day" and the next analyses which adjusted 0.01-1.6% and it was according to the USP 30, (RSD should be less than 2%. At purchase time as result in Table (2) that has shown all products of amoxicillin-clavulanic acid formulations were within standards in account of assay content of amoxicillin that comply with USP30 requirements. All products had the range 91.3%-106%. After one month storage in stability conditions one formulation was near the lower content acceptance limit for amoxicillin that for AMO-CLV3 (90.8%). While for clavulanate content as result shows at purchase time two products were shown don't comply with USP30 requirements as one was less than lower limit (**85.20%)** and the other one showed more than the upper limit of USP30 requirements (**123.40%).** 3 of 10 tested products became substandard in content of clavulanate after one month storage in test condition in accelerated stability conditions (40 ±2°C and 75 ±5% RH) in terms of content test according to USP30 requirements. After storage for three months 1 of 10 amoxicillin (AMO-CLV2) became degraded to 83.3% in that was showed less than acceptance limit of USP30 specifications for drugs content, 2 of 10were near to the lower of limit 90.1% and 90.7%. As for clavulanate content after 3 months storage in stability conditions it has shown 6 of 10 (60%) of products became degraded that's 41.20%, 47.10%, 23.40%, 85.90%, 73.50% and 77.90% that means less than the limit. After 6-month storage at accelerated testing conditions 4 of 10 (40%) formulations became substandard and don't comply with USP30 requirements for amoxicillin content and gave the result of 67.10%, 69.80%, 81.20% and 81.70%. also the result has shown that 3 of 10 products became at lower of acceptance limit (91.30%), (91.10%) and (91%). For clavulanate content after 6-month storage at accelerated testing the drug content for 7 of 10 test products became substandard (Table2). Out of the failed products, the smallest content of clavulanic acid after 6-month storage was 9.6% and the largest was 85.6%. So 7 of 10 (70%) of investigated products don't comply with USP30 requirements for clavulanic acid content.

Table (2): Percentage of Amoxicillin and clavulanate content before and after stored at study conditions (40°C,75 RH). USP 30 requirement: not less than 90%.

Product code	at purchase		One month		three months		Six months	
	Amoxicillin average ±SD	Clavulanate average ±SD	Amoxicillin average ± SD	Clavulanate average ± SD	Amoxicillin average ± SD	Clavulanate average ± SD	Amoxicillin average ±SD	Clavulanate average ±SD
AMO-CLV1	106.0±0.56	102.9±1.09	106.0±1.17	101.3±1.38	102.1±0.66[b]	90.4±1.16[a]	99.3±0.20[a]	85.6±0.40[a]
AMO-CLV2	95.1±0.88	104.3±0.17	91.4±1.43[c]	83.2±1.29[b]	83.3±0.50[a]	41.2±0.08[a]	81.7±0.80[a]	33.4±0.12[a]
AMO-CLV3	91.3±1.45	85.2±0.40	90.8±1.50	73.9±1.10[a]	90.1±0.53	47.1±0.30[a]	81.2±0.70[a]	40.2±0.60[a]
AMO-CLV4	103.0±0.21	99.2±0.14	100.8±1.30[c]	52.8±1.15[a]	90.6±1.54[b]	23.4±0.18[a]	69.8±0.18[a]	9.6±0.17[a]
AMO-CLV5	102.3±0.26	115.1±1.10	100.6±1.22	91.2±0.86[a]	96.5±1.35[b]	85.9±1.21[a]	93.0±0.27[a]	58.9±1.30[a]
AMO-CLV6	99.4±0.28	116.6±0.53	97.8±0.83[c]	116.5±1.01	98.0±1.27	113.9±1.41[c]	91.1±0.50[a]	111.8±0.28[a]
AMO-CLV7	100.2±0.32	111.0±0.65	100.0±1.57	109.1±1.23	95.9±1.30[b]	73.5±1.20[a]	91.0±0.61[a]	46.8±0.56[a]
AMO-CLV8	104.3±0.50	113.7±0.54	101.0±0.73[b]	105.7±0.84[a]	100.7±1.36[b]	77.9±0.88[a]	98.5±0.68[a]	62.4±0.42[a]
AMO-CLV9	97.0±0.18	112.0±0.20	96.4±1.11	105.0±1.44[a]	91.2±0.40[a]	106.1±1.19[b]	67.1±0.12[a]	93.7±0.90[a]
AMO-CLV10	105.7±0.80	123.4±0.90	100.4±0.65[a]	113.3±1.12[a]	92.2±1.00[a]	95.4±0.53[a]	91.3±0.61[a]	90.0±0.64[a]

Note: [a]p value <0.001, [b]p value <0.01,[c]p value <0.05 compared to drug at purchase. Data were expressed as means ±standard deviation.

Table (3):Percentage of Amoxicillin and clavulanate release before and after stored at study conditions (40°C,75 RH). USP 30 requirement: more than 85 is released for Amoxicillin and more than 80 for clavulanate within 30 min

Product code	at purchase		one month		three months		six months	
	Amoxicillin average ± SD	Clavulanate average ± SD	Amoxicillin average ± SD	Clavulanate average ± SD	Amoxicillin average ± SD	Clavulanate average ± SD	Amoxicillin average ± SD	Clavulanate average ± SD
AMO-CLV1	100.4± 0.15	100.9±0.19	99.5±0.22[b]	99.0±0.30[a]	99.3±0.11[a]	95.3±0.60 [a]	**72.8**±0.18[a]	**72.7**±0.21[a]
AMO-CLV2	116.6±0.28	111.3±0.13	105.0±0.83[a]	96.2±0.81[a]	98.5±0.62[a]	82.69±0.40[a]	**61.89**±0.26[a]	**40.8**±0.16[a]
AMO-CLV3	101.8±0.07	103.0±0.06	100.1±0.85	**66.0**±0.91[a]	98.6±0.65[a]	**65.8**±0.62[a]	**57**.0±0.05[a]	**30.9**±0.04a
AMO-CLV4	96.4±0.06	85.8±0.04	95.6±0.34[c]	**32.7**±0.31[a]	90.7±0.31[a]	**17.25±0.13**[a]	**60.18**±0.08[a]	**10.4**±0.02[a]
AMO-CLV5	112.4±0.15	105.3±0.22	101.5±0.15[a]	**79.0**±0.04 [a]	95.1±0.17[a]	**57.3**±0.02 [a]	**68.2**±0.12 [a]	**34.3**±0.18[a]
AMO-CLV6	101.8±0.10	110.0±0.07	91.7±0.11[a]	109.1±0.05[a]	92.39±0.14[a]	90.77±0.07[a]	**83.94**±0.11[a]	89.23±0.09[a]
AMO-CLV7	101.0±0.12	107.0±0.62	99.3±0.15[a]	102.7±0.24[a]	96.1±0.19[a]	99.7±0.24[a]	94.3±0.13[a]	**47.7**±0.65[a]
AMO-CLV8	112.7±0.11	109.2±0.15	105.5±0.03[a]	97.38±0.15[a]	104.1±0.06[a]	87.87±0.12[a]	99.96±0.15[a]	**52.08**±0.13[a]
AMO-CLV9	99.3±0.08	98.5±0.16	99.1±0.27	97.5±0.19 [b]	97.2±0.27[a]	92.4±0.14[a]	**61.7**±0.10[a]	**78.2**±0.17[a]
AMO-CLV10	98.7±0.30	106.7±0.20	96.6±0.06[a]	102.95±0.05[a]	95.28±0.08[a]	99.3±0.06[a]	90.0±0.28[a]	99.0±0.24 [a]

Note: [a]p value <0.001, [b]p value <0.01,[c]p value <0.05 compared to drug at purchase. Data were expressed as means ±standard deviation.

For drugs dissolution, for amoxicillin release at purchase time and after one and three month storage at accelerated testing conditions, the dissolution test for amoxicillin release were within the limits of good quality in all products while after six months storage result has shown that 7 of 10 tested products shown reduction in amoxicillin release after 6 months (Table3) to reach a lower level than limit in comparison with their release at purchase time. Also for release of clavulanate all products shown good release at purchase time that comply with USP30 requirements in exception for one product at lower than limit of dissolution test (85.8%) as show in a Table (3). After one month storage in study conditions result shown that 3 of 10 products became substandard in dissolution of clavulanate as (66%), (32.7%) and (79%) in dissolution. as well as after three months storage in study conditions the same products showed continuation reducing in its clavulanate dissolution to became (**65.8**%), (**17.25**%) and (**57.3**%). The smallest % in reducing of clavulanate dissolution was AMO-CLV10 (7.4%), AMO-CLV1 (8.2%) and AMO-CLV7 (9.3%). For clavulanate release after six months storage in study conditions showed 8 of 10 (80%) tested products became substandard in dissolution of clavulanate and don't comply with USP30 requirements. As shown in result tables, comparison of drug content and drug release at purchase time with that's after one, three and six months storage shown significant change for 10 of 10 tested products.

Table (3) Represent number of drugs that pass and/or fail in the study condition after storage for six months

drugs	amoxicillin		clavulanate	
Product code / test	**Content**	**release**	**content**	**release**
AMO-CLV1	√	×	×	×
AMO-CLV2	×	×	×	×
AMO-CLV3	×	×	×	×
AMO-CLV4	×	×	×	×
AMO-CLV5	√	×	×	×
AMO-CLV6	√	√	√	√
AMO-CLV7	√	√	×	×
AMO-CLV8	√	√	×	×
AMO-CLV9	×	×	√	×
AMO-CLV10	√	√	√	√
Pass / Fail	**6/4**	**4/6**	**3/7**	**2/8**

As shown in Table 3, there are three Products that AMO-CLV 2, 3 and 4 shown don't comply with USP30 specifications in both content test and drug release for both amoxicillin and clavulanate. Tow Products that AMO-CLV6 and 10 shown they were comply with USP30 specifications in both content test and drug release for both amoxicillin and clavulanate. Products that AMO-CLV 1and 5 shown comply with USP30 specifications in amoxicillin content test only and don't comply in amoxicillin release and in clavulanate content test and release. Products that's AMO-CLV7and 8 shown comply with USP30 specifications in amoxicillin content test and release but don't comply in both content test and release of clavulanate. Only one product shown pass clavulanate content test but don't comply with USP30 specifications in release.

DISCUSSION

In order to achieve this study perfectly, Ten different brands of amoxicillin-clavulanate products that obtained from the local market in city of Sana'a have been subjected to accelerated testing conditions of (40 ±2°C and 75 ±5% RH.). The study has clearly shown that 2 of 10 tested products of AMO-CLV3 and AMO-CLV10 not accepted quality at purchase time as they are not in conformity with USP specifications.

Actually, this result is obviously in contrary with few studies which reported no formulation substandard in content at purchase time [20-22]. Our study is in exact conformity with study reported that the existence of substandard formulations at purchase time was because of manufacturer error[23]. Many factors that may affect the quality of manufactured drug products include storage conditions, humidity, light, packaging materials, transportation factors, components of drug formulation and the nature of the active ingredient which is the most important [23].

Study shown that after six months storage in study condition was 4 0f 10 (40%) and7 of 10 (70%) tested products was substandard in content of amoxicillin and clavulanic acid, also 70% and 80% was substandard in drug release of amoxicillin and clavulanic acid respectively, this study is in exact conformity with study at 2014 and obviously in contrary with others studies. The most logical explanation for the failure of the samples is the impurity of active pharmaceutical ingredient and storage conditions at transport also be attributed to poor storage at pharmacy and/or manufacturing conditions[24, 25].

The failure of clavulanic acid content in this study has shown more failure than amoxicillin and it is comparable with few studies[26, 27]. clavulanic acid was the main factor and faster in the degradation of products than amoxicillin may be due to that clavulanic acid is unstable and when exposed to high temperatures it became more volatile in addition to that clavulanic acid is hygroscopic; therefore, 30% RH or less is suitable for storage [28,29]. In other word, degradation of clavulanic acid may starts during manufacturing, in distribution or in storage at the pharmacy. The degradation of amoxicillin–clavulanic acid products might to be attributed to the packaging practices and materials [30].

In the present study dramatic changes in the dissolution behavior have been observed that's 8 of 10 (80%) of amoxicillin and/or clavulanic acid dissolution that became substandard. The remarkable reduction in the amount of drug released was clearly existing after three and six months of stability testing. This outcomes of study had strong association in failure between content and dissolution that all products which failed in assay content also failed in drug dissolution that refers to chemical instability of products, The products that passed assay content and failed in drug dissolution 3 of 10 (30%) of amoxicillin and/or clavulanic acid refers to formulation composition reasons, it is not possible to determine the cause of the failure in dissolution of the formulations dependent due to unavailability of exact formulation compositions[31].

A drug dissolution is the most requirement needed for the drug absorption into patient bodies as the dissolution rate is directly related to the bioavailability. If the dissolution rate is the rate-limiting step, then the dissolution rate determines the bioavailability. For this reason, dissolution testing is usually used as a measure to compare the bioavailability of the similar drugs manufactured by diverse companies. In addition, the dissolution test is the primary quality control test to determine whether a drug product is able to release its active pharmaceutical ingredient(s) in a timely style [32]. It is thus necessary for all solid oral dosage forms in which absorption of the drug is essential for the product to perform the required therapeutic effect. Dissolution is also used as a quality control measure for batch release to ensure continued quality during the shelf life and also to distinguish a poor quality batches [33].

The failure of (80%) of tested products to pass dissolution requirements after being subjected to a stability test infers that's the drug formulations are unsuitable for marketing in countries with tropical climatic conditions. This study also shown that both drug content and drug dissolution can be altered under accelerated testing conditions 2 of 10 (20%) tested products were the only that complied with USP30 specifications and 8 of 10 (80%) tested products don't comply with USP30 specifications and that drugs being within or at the lowest of the quality limits of drug release may have a reduced bioavailability as shown by inferior dissolution.

CONCLUSION

Stability tests are carried to ensure that the medicine is stable and effective throughout its shelf life. Study showed that both drug content and drug dissolution can be changed under accelerated testing conditions. Good manufacturing process and storage conditions affects the quality as well as stability of amoxicillin–potassium clavulanate solid preparations marketed in Yemen so the stringent quality control measures and stability tests of these products should necessarily be applied to maintain the standard quality of amoxicillin– potassium clavulanate products in markets.

Limitation of study:
In this study there are two limitation, the first was that the assay test of content and dissolution test were only performed, the second limitation is that the samples for study been collected from city of Sana'a only.

Recommendations:
Regular monitoring of the quality and stability in tropical conditions of the drugs in the market by the regulatory authority of Yemen (The Pharmacy Board) should be promoted for preventing the drug of inferior quality from accessing into Yemeni market.

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