Quality Evaluation of Amoxicillin/Clavulanate Potassium Tablets Marketed in Mekelle, Ethiopia

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ABSTRACT

Background: The combination of amoxicillin and clavulinic acid (agumentin) is a widely used oral antibiotic consisting of semisynthetic aminopenicillin amoxicillin and a beta-lactamase inhibitor - clavulinate potassium. This comniation has a number of generics marketed in different brands in the Ethiopian market. Studies indicate that microbial resistance is becoming common phenomenon for agumentin.Frequent observation of therapeutic failures of medicinal products, especially the generics necessitates regular review andpostmarketing quality control of medicines circulating in the Ethiopian drug market and developing countries at large.

Objective: The aim of this study was to evaluate the *in-vitro* quality of Amoxicillin-Clavulinate potassium tablet combinationsmarketed in Mekelle, Ethiopia.

Methods: Qulaity attributes such as identification, weight uniformity, drug content, hardness, disintegration and dissolution tests were performed based on the British and United States pharmacopoeias. Comparisons among the generic brands and with the innovator product was made statistically using one-way-ANOVA. Dissolution profiles were compared using the similarity factor (f_2).

Results: All products passed the United States Pharmacopeia requirements for weight uniformity, identification and disintegration tests. Some products such as brand E for amoxicillin,brandB and brand F for clavulanic acid failed to meet pharmacopoeial specifications for drug content. However, all products complied with the requirements for *in vitro* release studies except product E. In addition, the similarity factor f_2 of all brands, except product E, was greater than 50 showing the similarity of their dissolution profiles as recommended by Food and Drug Administration.

Conclusions: The findings of this study imply that consistent quality assessment of drug products circulated in the market is essential to ensure the quality and interchangeability of generic brands of augmnetin.

Keywords: Augmentin; Quality evaluation; Similarity factor

Introduction

A generic drug is identical or bioequivalent to an approved/innovator drug in terms of active ingredient, dosage form, safety, strength, route of administration, quality, performance characteristics and intended use. Generic products are less expensive than the innovator productduetoseveral factors described elsewhere [1, 2]. Hence, their availability guarantees optimal healthcare due to their affordability in developing countries. However, the chemical and biopharmaceutical equivalence issue of generics to the innovator drug is of great concern among the health professionals.

Counterfeit and substandard antimicrobials are becoming serious treats to our health care system. These medicines may cause increased mortality, morbidity, antimicrobial resistance, treatment failure and side effects. The WHO estimates that up to 10% of all global pharmaceutical supply is counterfeit and substandard, reaching 50% in developing and sub-Saharan countries [3-5]. Antibiotics are the most counterfeited medicines and account for 28% of global counterfeit/substandard medicines [6].

Studies have shown that most generic products such as antibiotics are not bioequivalent to the approved drugs. For instance, three out of five brands of co-trimoxazole tablets [7] and four out of five brands of amoxicillin capsules [8] were not bioequivalent with the innovator brands on the basis of *in vitro* comparative studies in Mekelle, Ethiopia. The availability of sub-standard drug products such as erythromycin stearate in a market has been reported [9]. In addition, a study done in the central region of Ghana reveals poor quality of amoxicillin/clavulanic acid tablet products [10]

Antibiotics are very sensitive drugs and used in the microbial infections management. If they are not properly used, the tendency that the microbes involved may develop resistance against them and make them ineffective[11]. Resistance towards the medicines can equally be developed in cases related to substandard antibiotics, where they are under therapeutic dose. Studies [12, 13]have shown that inappropriate antibiotic use in Ethiopia resulted resistance to commonly prescribed antibiotcs and subsequently the existence of multidrug resistance. For instance, Wondemagegn et al [14] reported resistance of Gram positive bacteria to amoxicillin (85.1%) and of Gram negative bacteria isolates for the same 58.8%. For antibiotic resistance assessment in the

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study area, a two year data were collected from Ayder Comprehensive Specialized Hospital, Mekelle, Ethiopia. The data showed higher resistance among pathogens to augmentinand other antibiotics. Microorganisms specifically *Escherichia coli* and *Klebsiellapneumoniae*have highly developed resistance to augmentin, which is the combination of amoxicillin and clavulanic acid. Consequently, the authors have initiated to conduct the present study to evaluate the quality of commonly available brands of amoxicillin/clavulanic acid tablets in Mekelle, Ethiopia based on the United States Pharmacopeia [15]and British Pharmacopoeia [16] guidelines.

Materials and Methods

Materials

The innovator product (Augmentin, UK) and five brands of amoxicillin/clavulanate 625 mg tablets were collected from public and private pharmacies in Mekelle, Ethiopia. The survey was done within the shelf-lives of the products. Methanol HPLC grade (LobaChemie, India), phosphoric acid analytical grade (AR) (Sigma-Aldrich, Germany), Sodium hydroxide AR (Sigma-Aldrich, Germany), Monobasic potassium phosphate AR (Unichem, India) and double distilled water were used in this study.

Methods

Uniformity of weight: Twenty tablets from each product were individually weighed on an analytical balance (Adam, United Kingdom). Weight variation for tablets over 250 mg should not be more than 5% as recommended by the USP. The percent deviations and standard deviations of the means were reported.

Tablet thickness, length and width: The thickness, length and width of tablets were determined individually for ten tablets. The means, standard deviations and overall relative standard deviations were reported.

Hardness test: From each product, ten tablets were individually placed between the plates of integrated hardness tester (Pharma Test, Germany). The means and standard deviations were reported.

Identification: The identification of amoxicillin and clavulanate potassium tablets was tested by HPLC according to the USP procedures .

Disintegration test: Disintegration test was determined in 900 mL of distilled water as stipulated in the USP using the disintegration apparatus (Pharma Test, Germany). The

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temperature of the medium was maintained at 37 ± 0.5 °C. One tablet was placed in each of the six tubes.The tablets were considered disintegrated when all of the particles pass through the mesh screen. If any residue remains, it must be fragments of insoluble coating. The time in minutes required to disintegrate for each tablet was recorded and average disintegration time for each product was calculated.

Dissolution test: The drug release of amoxicillin and clavulanate potassium tablets were determined according to the USP using apparatus type II (Pharma Test, Germany) with the rotational speed of the paddle at 75 rpm. The dissolution medium was 900 ml of distilled water maintained at the temperature of 37±0.5°C. One intact tablet was added into each of the six dissolution vessels and sample solutions were withdrawn at the end of 5, 10, 15, 30, 45 and 60 minutes. The samples were filtered before injection into the HPLC system. According to the specification stipulated in the USP monograph, amoxicillincalvulanate tables should release more than 85% of the labeled amount of amoxicillin and clavulanic acid. To get more insight on the drug release, dissolution profile was constructed from 5 to 60 minutes interval.

The dissolution profiles for brand products were compared to the innovator drug using f_2 similarity factor according to Eq. 1 [17] as recommended by FDA.

$$f_2 = 50 * \log\{[1 + (1/n)\sum_{t=1}^{n} |R_t - T_t|^2]^{-0.5} * 100\}$$

where *n* is the number of time points used to assess the amount of amoxicillin and clavulanate potassium tablets dissolved, R_t and T_t are the mean percentage of the innovator and brand products dissolved at a specific time t, respectively. The similarity factor greater than 50 indicates the equivalence of dissolution profiles and thus, the similarity of the performance of the test and reference products according to FDA requirements.

Assay of amoxicillin-clavulanate tablets: The assay of amoxicillin and clavulanate potassium tablets was done using HPLC method by following the procedures described in the United States Pharmacopeia [15]. Liquid chromatography analyses were done on an apparatus from Agilent Technologies (Waldbronn, Germany) equipped with an infinity binary pump (G1312B), infinity high performance degasser (G4225A), infinity high performance autosampler (G1367E), infinity thermostat column compartment (G1316C) and photodiode array detector (G4212B). Data processing and acquisition was supported by Chemstationsoftwareversion B.04 from Agilent Technologies. A pH meter (Adwa,

Romania) was employed to measure the pH of the mobile phase composed of sodium phosphate buffer and methanol (95:5, v/v). Chromatographic separations were achieved on a C-18 (250 mm × 4.6 mm, 5 μm) column (Shodex, Japan).

The suitability of the HPLC system was evaluated using the relative standard deviation (RSD) of the peak areas of amoxicillin and ckavulanic acid. RSDs below 2% are acceptable for the dissolution and content assay of the tablets [16].

Results and Discussion

Evaluation of amoxicillin and clavulanate potassium tablets was performed on physicochemical parameters such asuniformity of weight, thickness, length, width, hardness, identification, disintegration and dissolution tests. The presence of both active ingredients in the samples were confirmed through the correspondence of the major peaks retention times in the chromatograms of both assay and standard preparations as stated in the USP.

Table 1 shows uniformity of weight, thickness, length, width and hardness of amoxicillin/clavulanate potassium tablets. Results from the measurement of thickness, length and width of products showed that the tablets were within the pharmacopoeial specifications. It is important to control thickness of a tablet as it affects packaging in blister or plastic container. The relative standard deviation of the examined tablets from different products for thickness was below 5%, which is acceptable range while the overall RSDs of tablets for length and width were 3.71% and 2.39%, respectively. The weight uniformity results for all products revealed compliance within the USP specifications, as none of them deviated 5% from the mean weight. This parameter is importand since variation in amount of the APIs may lead to toxicity, ineffectiveness or may eventually cause resistance to the antibiotics. The mechanical strengths of tablets were high and acceptable as the crushing strength of all tablets were higher than 50 N [16]. Relatively high crushing strength helps the tablets to withstand the mechanical shocks of manufacturing, packaging and shipping. Results from ANOVA revealed significant differences among the various drug products withrespect to the thickness, length, width and hardness ($P < 0.05$). Product E was the only drug product with significantly lower resistance to crushing strength of tablets.

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Thedisintegration time, assay and dissolution study of amoxicillin and clavulanate potassium tablets results are shown in Table 2. The disintegration of all brands were within the range of 7.68 and 10.12 minutes. All the brand products complied with the official requirements of less than 15 minutes disintegration time for uncoated tablets though the crushing forces of the tablets were relatively high. Al-Tabakha et al. [18] reported similar findings where the dissolution of tablets and resistance to crushing strength were not well correlated. Formulation factors such as disintegrants and diluents might perform great roles in the tablet disintegration as well as dissolution profiles. Disintegration of tablets is the rate limiting step in drug dissolution and absorption [16]. These findings illustrate the ability of the product tablets to disintegrate and readily release their contents in the human gastrointestinal tract.

The assay acceptance criteria are between 90% and 120% of the labeled amounts of amoxicillin and clavulanic acid [15]. All products complied the compendial specification for content assay except brand product E (89.56%) for amoxicillin, and brands B (124.42%) and F (123.22%) for clavulanic acid. Nettey et al. [10] reported alarming results from the analyses of 14 brands of augmentin tablets for drug content indicating 100% failure for amoxicillin and 14% failure for clavulanic acid. The possible explanation for the failure of the amoxicillin content is the instability of drug products which could be affected by factors related to poor storage conditions, microbial contamination, packaging materials used for the drug product and transportation.

Table 1**:** Results of the physicochemical characteristics of amoxicillin/clavulanate potassium tablets

Product	**Weight uniformity Deviation(%)±SEM**	**Thickness (mm) ± SD**	**Length (mm) ± SD**	**Width (mm) ±SD**	**Hardness (N) ±SD**
Innovatorproduct A	0.64±0.12	6.86±0.03	20.11±0.03	9.28±0.01	253.34±24.93
Brandproduct B	0.77±0.15	6.27±0.05	21.64±0.03	10.15±0.02	246.47±10.41
Brandproduct C	1.78±0.21	7.18±0.05	21.63±0.03	10.13±0.03	239.94±16.26
Brandproduct D	0.76±0.11	7.30±0.06	20.65±0.01	9.99±0.04	218.07±3.95
Brandproduct E	0.81±0.11	6.83±0.03	20.18±0.02	9.72±0.04	139.48±1.02
Brandproduct F	0.84±0.14	6.98±0.1	19.66±0.03	9.63±0.02	233.33±16.06

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Table 2**:** Results of the disintegration, dissolution and assay of amoxicillin/clavulanate potassium tablets

Product	**Disintegration time (min)**	**Amoxicillin**		**Clavulanate potassium**	
		Drug released (%w/v) 30 minutes	**Assay (%)**	**Drug released (%w/v) 30 minutes**	**Assay (%)**
Innovator product A	10.12	95.55	96.64	109.85	108.91
Brand product B	7.85	90.25	107.69	120.14	124.42
Brand product C	9.81	86.16	91.18	111.98	111.14
Brand product D	7.93	88.27	97.44	109.81	116.50
Brand product E	7.68	49.09	89.56	108.92	111.23
Brand product F	8.57	89.11	98.15	121.01	123.22

The dissolution values for all amoxicillin and clavulanate potassium tablets showed more than 85% of the active ingredient dissolved within 30 minutes except brand product E with 49.09% of amoxicillin release (Table 2). Fig. 1 and Fig. 2 indicate dissolution profiles of amoxicillin and clavulanate potassium, respectively. The United States of Pharmacopia has recommended not less than 85% and 80% of the labeled amounts of amoxicillin and clavulanate dissolved in 30 minutes, respectively. Dissolution of a drug product is a prerequisite for absorption and hence determines bioavailability of the drug. It is usually performed as a tool for bioavailability comparison of the same drug produced by various manufacturers. It is a primary quality control test for the evaluation of *in vitro* drug release studies [19]. Therefore, it is required for all solid oral dosage forms in which absorption of the drug is necessary for the product to exert the desired therapeutic effect. Dissolution test as a quality control measure ensures continued quality of a drug during the shelf life [20]. In this work, dissolution failure of product E to release amoxicillin within a specified time possibly related to the nature of excipients employed in manufacturing process and low active pharmaceutical ingredient in the product. A study conducted in Ghana reveals similar findings where one out of 14 brands of amoxicillin and clavulanic acid failed to comply compendial requirements for both assay content and dissolution test [10]. Olanrewaju, Paul &Olus [21] also reported amoxicillin and clavulanic acid tablets failed parameters such as hardness, disintegration and dissolution tests. On the other hand, brand products B and F reached the plateau within 30 minutes by releasing just above

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120% of labeled clavulanic acid (Fig. 2), which supports why these products failed content uniformity requirements for this ingredient.

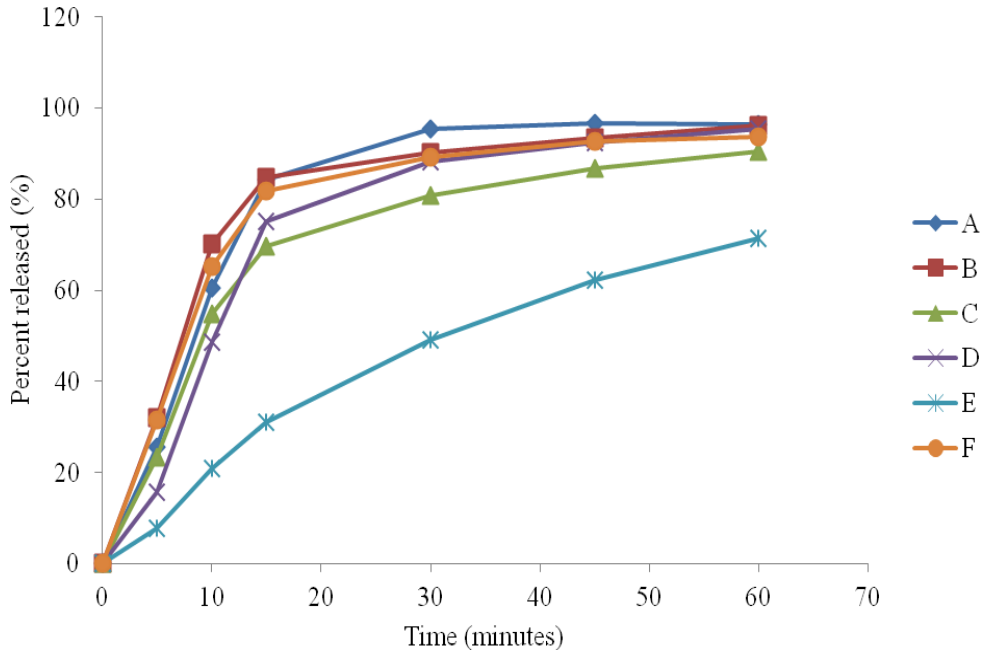

Figure 1 Dissolution profile of amoxicillin from six brands of amoxicillin/clavulanate tablets

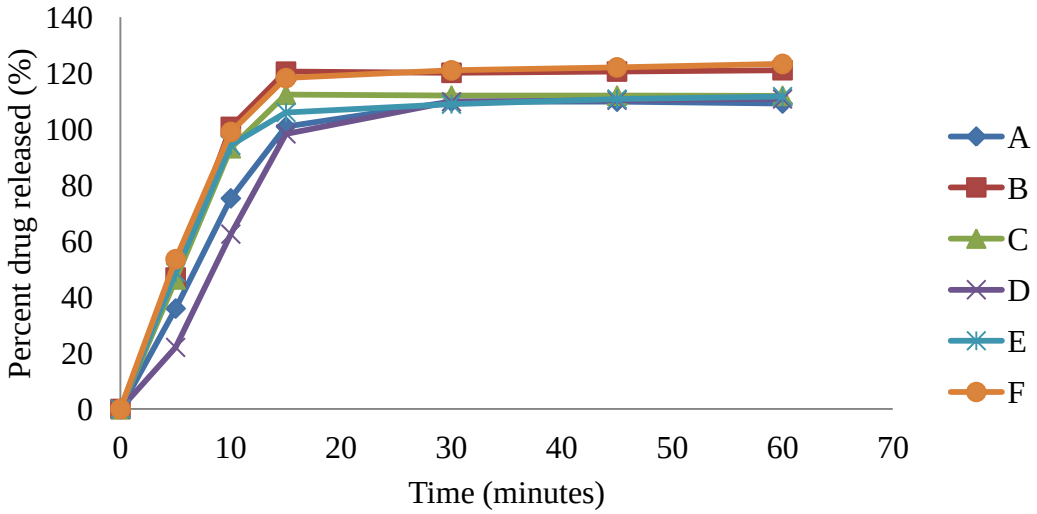

Figure 2 Dissolution profile of clavulanate from six brands of amoxicillin/clavulanate tablets

The f_2 statistical similarity factor comparison of the dissolution profiles of the five brand products with the innovator drug is shown in Fig. 3. The similarity factor of all brand products, except product E, ranged from 52 to 66 which indicates that they could be used interchangeably with the innovator drug according to FDA requirements. The similarity factor of product E (f_2 value of 26, which is less than 50) confirmed that this product is not *in vitro* bioequivalent to the innovator drug. The similarity factor result from other study supported the *in vitro* non-bioequivalence of a specific brandproduct, which had already failed the requirements for hardness, disintegration and dissolution tests [21].

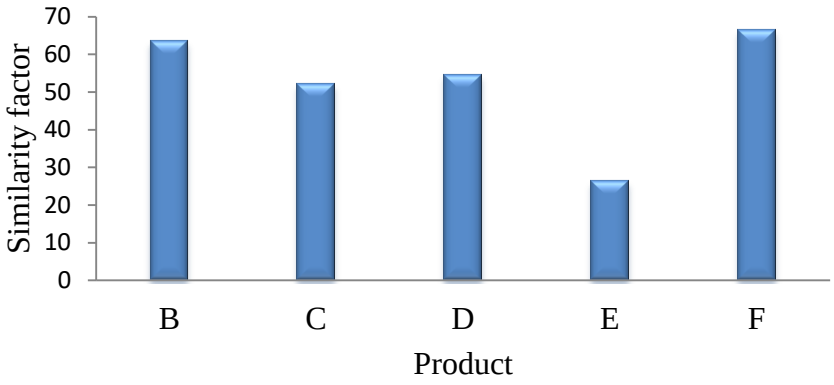

Figure 3 Similarity factor (f_2) values of different brands relative to the innovator drug

Conclusion

The findings of this study showed that all the analyzed samples for weight uniformity, identification and disintegr-ation tests met the compendial specificat-ions. However, brand products E for amoxicillin, B and F for clavulanic acid failed to comply pharmacopoeial requirements for drug content. Furthermore, the brand product E did not comply with compendial and FDA requirements for *in vitro* release studies. These results indicate the availability of drug products with poor quality, which could lead to therapeutic failure. Hence, regular post-marketing surveillance for quality assessment of drug products available in the market is essential to ensure the quality and interchangeability of generic brands.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

All authors conceived and designed the experiments, performed the experiments,analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, reviewed drafts of the paper.

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