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EVALUATION AND COMPARISON OF DISSOLUTION PROFILES FOR DIFFERENT BRANDS OF AMOXICILLIN CAPSULES AVAILABLE IN RWANDA

Thesis submitted in partial fulfillment of the requirements for the Degree of Master of Pharmaceutical Sciences, Drug Quality Assurance & Quality Control

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Kigali, August 2019

DECLARATION

I do hereby declare that this thesis titled **"EVALUATION AND COMPARISON OF DISSOLUTION PROFILES FOR DIFFERENT BRANDS OF AMOXICILLIN CAPSULES AVAILABLE IN RWANDA"** submitted in partial fulfillment of the requirements for Masters of pharmaceutical sciences: Quality Assurance and Quality Control, at University of Rwanda, is my own work and effort to the best of my knowledge; it has not been submitted anywhere for any award. Where other sources of information have been used, they have been acknowledged.

Signature:

Gaston KAZENEZA

Date: …….

APPROVAL

This is to certify that Gaston KAZENEZA has carried out a research work titled **"EVALUATION AND COMPARISON OF DISSOLUTION PROFILES FOR DIFFERENT BRANDS OF AMOXICILLIN CAPSULES AVAILABLE IN RWANDA"**.

Signature: ……

Prof. KADIMA NTOKAMUNDA Justin Leonard

Research supervisor

Date……

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To all of you GOD BLESSES

Abbreviations and acronyms

API: Active Pharmaceutical Ingredient
BCS: Biopharmaceutical Classification System
EMEA: European Medicines Agency
ER: Extended-Release
GIT: Gastro-Intestinal Tract
RSB: Rwanda Standards Board
SUPAC: Scale Up and Post Approval Changes
US FDA: United States Food and Drug Authority
USP: United States Pharmacopeia
UV: Ultra Violet
WHO: World Health Organization

ABSTRACT

Background: Amoxicillin is an oral semi-synthetic, β-lactam antibiotic used to treat bacterial infections caused by susceptible microorganisms. It is usually prepared in capsule and powder for oral suspension form. Solid dosage forms for oral administration pose bioavailability problems related to the absorption process.

The World Health Organization (WHO) has promoted the use of generic brands in order to make the cost of medicines affordable. Generic substitution could be considered when a generic copy of a reference drug contains identical amounts of the same active ingredient in the same dose formulation and route of administration. However, the presence of generic products that are not interchangeable each other have been reported.

Objective: To evaluate the in-vitro dissolution profiles of different generic brands of amoxicillin capsules 500 mg available on Rwandan market in comparison to Clamoxyl™.

Methods: We studied six products (coded as A, B, C, D, E, F) according to the monography described in the United States Pharmacopoeia (USP 41 NF 36,2018). The comparison was processed using statistical fit factor/similarity factor procedure.

Results: All six products released rapidly more than 85% of the labelled amount within 15min in acid media. Only 62.5% of the tested brands were declared interchangeable with the reference innovator brand and 37.5% were not interchangeable to (Clamoxyl^{TM}). However, all the products would pass the dissolution test standard in accordance with the USP requirement for amoxicillin capsule ($Q \geq 85\%$ at 60 min).

Conclusion: The generic brands of amoxicillin capsules tested satisfied USP specifications but we cannot completely rule out the presence of bad formulations in the country.

Keywords: Amoxicillin capsule, generic products, in vitro dissolution profiles, Rwanda

Chapter IINTRODUCTION

I.1 Background

Amoxicillin is an antimicrobial drug belonging to the penicillin group, and its antimicrobial activity covers many Gram-positive and Gram-negative microorganisms. It's a semi-synthetic compound with large stability in acid medium (W.A, 2001)and has been largely used for treatment of respiratory, urinary tract, ear and many others infections (W.A, 2001; Nascimento-Carvalho, 2004; Tavares et Al, 2008). It's one of the most sold antibiotics in Rwanda. Although amoxicillin has higher oral bioavailability than other penicillin antibiotics (i.e. ampicillin and benzylpenicillin), its absorption rate does not exceed 90%, which classifies this drug at the limit between drugs poorly and fairly permeable (Lima et al ,2008; Berquó et al,2004; Legen et al , 2006). The occurrence of variations in the amount of drug available for absorption may result in sub-therapeutic concentrations, which may cause an ineffective treatment (Horter et al, 1997). The bioavailability of drugs is related with the dissolution process (Manadas et al, 2002; Marcolongo, R. 2002) The speed and extend to which a drug dissolves from its solid oral dosage forms in the Gastrointestinal Tract (GIT) exerts direct influence on its concentration in plasma. In vitro dissolution tests are intended to mimic the physiological conditions of the GIT, and consist of sensitive and reliable predictive methods of in vivo availability of drugs (Marcolongo, R. 2002; Dressman et al, 1998; Brazil - National Agency for Sanitary Surveillance, 2002).

Thereby, monitoring the dissolution profile of an oral solid dosage form allows the prediction of bioavailability. However, the complexity of the GIT as well as their physical and chemical properties makes it impossible the perfect simulation in vitro (Singh S, 2012). In addition, there are other aspects such as age, diet, health conditions and genetic factors that may exert some influence on the dissolution process (Abuzarur-aloul et al,1997), but it is still possible to identify and simulate some of the variables to which the solid oral dosage forms is exposed. Due to these factors, only drugs covered as Class I (high solubility and high permeability) and some Class III (high solubility and low permeability) by the Biopharmaceutical Classification System (BCS) issued by the Food, Drug and Administration (FDA) may have their bioequivalence assessed only by the dissolution test (Bonamici, 2009). Amoxicillin is framed by BCS as a Class III drug, and currently does not have bio waivers, but studies are directed to frame it (Tsume Y, 2010).

I.2Problem statement

In Africa Poor-quality medicines present a serious public health problem, and may have a significant impact on the national clinical and economic burden. Attention has largely focused on the increasing availability of deliberately falsified drugs, but substandard medicines are also reaching patients because of poor manufacturing and quality-control practices in the production of genuine drugs (either branded or generic). Substandard antibiotic medicines are widespread and represent a threat to health because they can inadvertently lead to healthcare failures, such as antibiotic resistance and the spread of disease within a community, as well as death or additional illness in individuals. (Antholl and David W Holt, 2013)

The government of Rwanda has established Rwanda FDA that is mandated to assure the quality of all pharmaceutical products that enter the country, this is good step in fighting against falsified medicines in Rwanda but even if the country has set several measures to combating falsified medicines, Rwanda is not vaccinated against being entered with fake medicines the reason why It’s a responsibility for us Scientists to regularly check the quality of medicines we use.

The World Health Organization (WHO) has advocated the use of generic brands in order to make the cost of medicines affordable especially for the developing countries (WHO, 2004). However, this approach has not provided sufficient evidence for the substitution of one brand for another. In Rwanda, the cost of a branded medicine may be as high as ten folds of the generic

medicine. To become confident in substitution of branded with generics for affordability and at the same time to achieve therapeutic efficacy, bioequivalence studies become fundamental (Ngwuluka NC et al, 2009). Generic substitution could be considered when a generic copy of a reference drug contains identical amounts of the same active ingredient in the same dose formulation and route of administration as well as meet standards for strength, purity, quality and identity (Meredith P, 2003). However different reported studies over the last years revealed that marketed products with the same amount of active ingredient exhibit marked differences in their therapeutic responses. The presences of generic products that are not interchangeable with that of the innovator and/or with each other have been reported (Ferraz HG, 2007; Ngwuluka et al, 2009; El-Sayed et al, 2007; Hamdan II et al, 2010). The study was set up to evaluate and compare the in-vitro dissolution profiles of different generic brands of amoxicillin capsules with the innovator's Clamoxyl$^{(R)}$ that are available in Rwandan market.

Chapter II: LITERATURE REVIEW

II.2.1. Defining the Problem

The spread of substandard medicines is attributable to a number of factors. These include weak supply chains, failed distribution networks, an abundance of small-scale suppliers, lack of integration of regulatory actors, poor information technology systems, and limited financial resources.

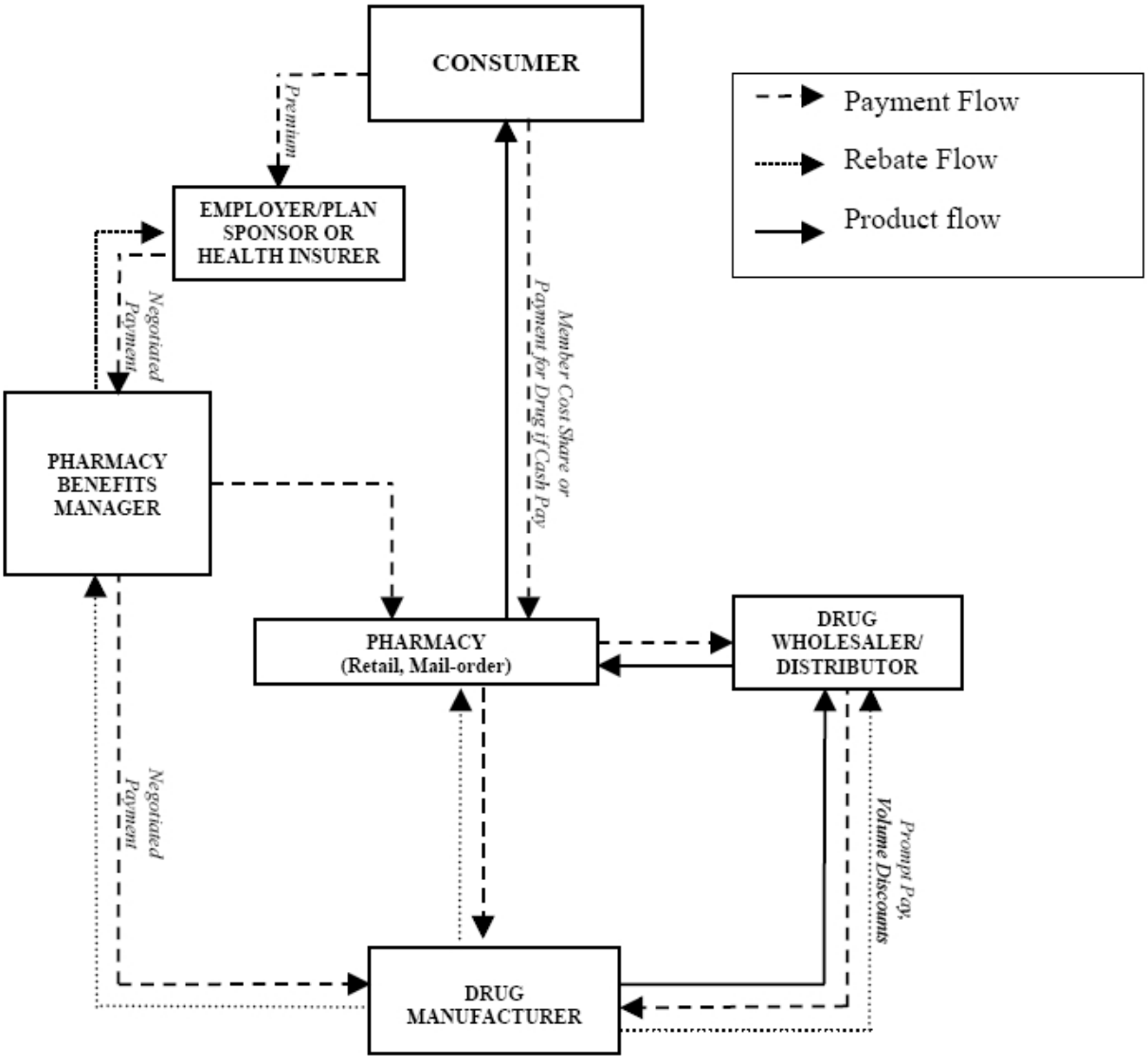


Figure1: supply chain of Pharmaceuticals process

II.2.2. Difference between Substandard and Falsified Medicines

Substandard medicines are made by licensed manufacturers operating within the framework of national pharmaceutical regulatory standards. Also referred to as “out of specification” products, these include medicines sold past their expiration date, medicines that have been compromised in shipping or storage, and medicines that are missing active ingredients or contain the wrong ratio of active ingredients (World Health Organization). Substandard medicines may arise due to human error, negligence, or resource restrictions (World Health Organization , 2003). They may result from both inadvertent and deliberate actions by a legitimate manufacturer.

Caudron et al. (2008) identified ten categories of substandard medicines:

- overconcentration of active ingredient
- under-concentration of active ingredient
- irregular filling of vials
- contamination
- mislabeling (not counterfeit)
- problems with active ingredient
- problems with excipients (inactive ingredients used as carriers for active ingredients in medicines)
- poor stability
- packing problems
- unsatisfactory dissolution profiles.

These categories exemplify the diverse number of ways medicines may be rendered substandard.

By contrast, falsified medicines, defined by the World Health Organization (WHO) as “spurious/falsely-labeled/falsified/counterfeit” medicines, are “deliberately and fraudulently mislabeled with respect to identity and/or source” (World Health Organization, 2010). Although substandard and falsified medicines are similar in that both have serious public health implications, falsified are not produced by licensed manufacturers. Although falsified medicines tend to be substandard (in that they do not contain correct amounts of active ingredient), this is

not inherent in the definition of falsified medicines. The difference in manufacturers means the problems with substandard and falsified medicines are distinct.

Substandard medicines, for example, can be controlled through effective regulation and enforcement, because manufacturers are known and licensed. falsified medicines, however, can be produced in homes, small industries, and backyards, and are harder to regulate (International Medical Products Anti-Counterfeiting Taskforce 2008).

In this study, we explored one dimension of poor-quality medicines that is "substandard medicines" as we studied dissolution profiles.

II.2.3 Harmful Effects of Substandard Medicines

Medicines may be rendered substandard at any point along the medical supply chain, from the point of manufacture through the point of distribution. At manufacture, medicines may be produced with impure or improper proportions of active ingredients. But even if produced properly, medicines may be compromised during transportation, warehousing, distribution, or even as a result of improper storage by the consumer. Regardless of where along the supply chain substandard medicines are compromised, they pose serious public health risks. Use of substandard medicines increases mortality and morbidity and may result in harmful side effects or allergies or engender drug-resistant pathogens that limit the therapeutic effectiveness of legitimate medicines (Newton et al. 2011; Newton et al. 2010; Nsimba 2008; Hogerzeil et al. 1992). Substandard medicines also contribute to the spread of infectious diseases (Nsimba 2008) and, if contaminated with pathogens (fungi, bacteria, viruses, or parasites) or other toxic elements, can cause further illness or poisoning (Bate 2012b). At worst, substandard medicines result in death (Caudron et al. 2008; O'Brien et al. 1998; Aldhous 2005). For example, contaminated paracetamol cough syrup resulted in 89 deaths in Haiti in 1995 and 30 infant deaths in India in 1998. The WHO also estimates that "of the one million deaths that occur from malaria annually, as many as 200,000 would be avoidable if the medicines available were effective, of good quality and used correctly" (World Health Organization 2003). Substandard medicines also have social and economic effects, as they may reduce patients' confidence in their doctors, pharmacists, and even in modern medicines as a whole (Nsimba 2008). Patients

who consume substandard medicines also suffer economic losses, as they spend income on ineffective medication.

In the developing world, where medicines can constitute a substantial percentage of 4 individual incomes, such economic losses may be significant. Illness and death affect individual income and national economies, as they result in loss of productive worker time.

Furthermore, since the use of substandard medicines often leads to illness, additional costs for health-care workers are incurred. The need to guard against substandard medicines also results in costs for regulatory agencies and enforcement authorities (Newton et al. 2010). These additional health-care and regulatory costs include personnel costs for health-care workers and regulatory and enforcement agents, equipment costs for medical equipment and drug testing laboratories, and administrative costs.

Finally, the spread of substandard medicines has political ramifications (World Health Organization 2010b). Substandard medicines undermine governments' investments in health delivery systems. They erode citizens' trust in their governments' ability to maintain and enforce regulatory standards. Their spread also undermines governments' credibility with respect to providing quality health care.

II.2.4. Extent of the Problem

Substandard medicines are present throughout the global supply chain; in developing countries, the problem is acute. The WHO estimates that up to 25 percent of medicines consumed in developing countries are substandard (World Health Organization 2003).

According to the WHO, 30 percent of countries have either "no drug regulation, or a capacity that hardly functions" (Newton et al. 2011, 18). Even in places where national medicine distribution channels have been created to ensure drug quality and safety, those channels have proven incapable of eliminating the problem of substandard medicines (World Health Organization 1999). This problem is further confounded by the Internet, where "illegal sites that conceal their physical address" may sell falsified medicines (World Health Organization 2010b).

A case in Bangladesh in the early 1990s exemplifies the difficulty in detecting the source of substandard medicines, monitoring the drug supply chain, and enforcing pharmaceutical legislation in a developing country.

At some point along the supply chain, foreign or local manufacturers, importers, or local distributors substituted diethylene glycol for the more expensive propylene glycol. This drug appeared in the Bangladesh hospital, Dhaka Shishu, and resulted in an outbreak of diethylene glycol poisoning, which continued for almost three years. When ingested, diethylene glycol causes fatal renal failure. This high percentage compares to isolated cases in the United States, where concern over substandard relates to high imports of medicines and active ingredients from developing countries. By its own account, the U.S. Food and Drug Administration (FDA) is not able to fully regulate pharmaceutical imports (U.S. Food and Drug Administration n.d.). Recent and ongoing studies by the Institute of Medicine analyze the FDA's and international actors' potential for addressing shortfalls in regulations and safeguards against substandard medicines internationally (Riviere and Buckley 2012; Institute of Medicine n.d.).

These medicines may have originated from the legitimate manufacturer, or they may have been falsified medicines. Substandard medicines often do not pass even the most basic quality control tests, but data related to their propagation are scarce (Shakoor et al. 1997, 839), Although many studies run quality control tests on samples of medicines, reporting on the detected proportion of substandard medicines, they do not all follow the same standards. Some studies, for example, use the terms counterfeit and substandard interchangeably. Few studies analyze the prevalence of substandard medicines alone, and many studies focus only on the quality of antimalarial, tuberculosis, and antibacterial medicines. Sample size varies greatly and studies sometimes reach contradictory results.

II.2.5 Similar studies

Nilufer Yuskel, et al (2000) had used different comparison methods to dissolution profiles of immediate release commercial film coated tablets of naproxen sodium in order to evaluate each method in terms of easy application and usefulness. The applied methods for the comparison of in vitro dissolution profiles are ANOVA based methods, model dependent methods and model independent methods including difference factor, f1 and similarity factor, f2.

Difference factor seems to be easier to apply and interpret; only one value is obtained to describe the closeness of the two dissolution profiles.

Paulo Costa, et al (2001) had studied drug release from solid dosage form, it was necessary to ensure that drug dissolution occurs in an appropriate manner. The drug dissolution from solid dosage forms has been described by kinetic models in which the dissolved amount of drug (Q) is a function of time(t.)
Some are model dependent and some are model independent that can be used to characterize release profiles or dissolution.

Prior, et al (2004) had performed comparison of therapeutic performance of two medicinal products containing the active substance is critical means of assessing the possibilities of alternative using between some essentially similar medicinal products.
A study was basically a comparative study designed to establish equivalence between test and reference products.

Esra Demlrttrk et al (2005) had employed comparison method in evaluating scale-up and post-approval changes such as manufacturing site changes, component and composition changes, equipment changes and process changes. Two-point specifications are suggested for characterizing the quality of drug product and for accepting product sameness under SUPAC – relating changes.
In the presence of certain minor changes, the single point dissolution test may be adequate to ensure unchanged product quality and performance. For more major changes a dissolution profile comparison performed under identical conditions for the product before and after the changes is recommended. Dissolution profiles may be considered similar by virtue of overall profile similarity and similarity at every dissolution sample time point.

Samaha, et al (2009) has shown that generic drugs offer a cost-effective alternative to brand name products. However, the main concern with modified release formulations is the substitution of one product for another.

Accordingly, the first objective of that study was to assess the interchangeability of the available diltiazem extended-release (ER) products on the basis of their in vitro dissolution characteristics using USP Apparatus 2 and 3. The second objective was to compare dissolution profiles in simulated fasted and fed states and determine whether there is a change in the mechanism of drug release. Dissolution profiles were characterized using Apparatus 2 or 3 under fasted conditions were similar.

Kassaye L. et al (2013) explained different methods which can be used to compare dissolution profile data. In this study the two most important and widely engaged methods have been used: the fit factors and dissolution efficiency (D.E.). The fit factors can be expressed by two approaches:

- difference factor, f1 and similarity factor, f2.
- The second comparison method employed in that study was dissolution efficiency (D.E.) model.

The calculation was made for each individual vessel, Thus, the mean D.E. > for each brand with its 95% confidence interval was obtained and compared by measuring the difference between the mean D.B. the test brands.

CHAP III. OBJECTIVES OF THE STUDY

III.1. General Objective

To evaluate and compare the in-vitro dissolution profiles of different generic brands of amoxicillin capsules with the innovator's that are available on Rwandan market.

III.2. Specific objectives

1. To determine Dissolution test conditions for amoxicillin capsule as per USP 41NF36, 2018
2. To provide information about the drug release characteristics of different brands of amoxicillin under test.
3. To ensure in vitro adequate and reproducible bioavailability of different brands of amoxicillin under test.

III.3. Hypothesis

Some generic brands of amoxicillin capsules available on Rwandan market are not interchangeable with the innovator brand (Clamoxyl TM) capsules.

III.4. Interest of the study

I selected to conduct this study because no study has been conducted about evaluation and comparison of dissolution profiles of different amoxicillin brands available on Rwandan market, in addition in vitro dissolution tests are used to predict in vivo bioavailability.

The results of this study could provide information about the similarity between the reference brand Clamoxyl^{R} and other available brands on Rwandan market, and should predict interchangeability.

CHAPTER IV: MATERIAL AND METHODS

IV. 1 MATERIAL

IV.1.1 Test products

Amoxicillin

This was an analytical study that consisted on conducting several dissolution tests for different amoxicillin brands available on Rwandan market in March/2019.

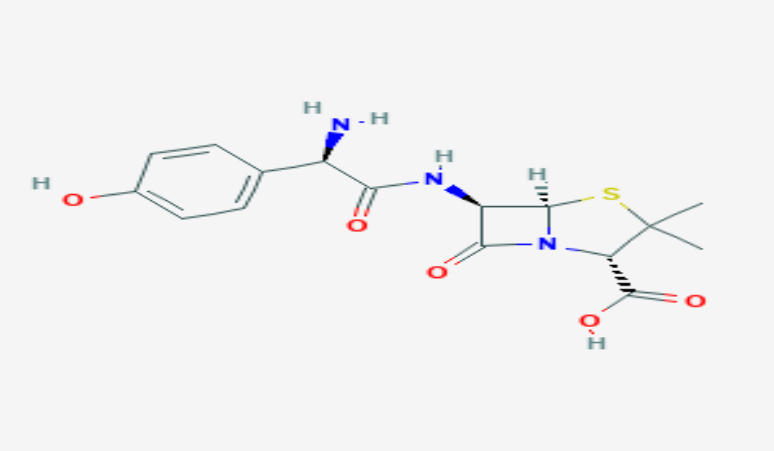


Figure 2: Chemical structure of amoxicillin

Amoxicillin USP reference standard (potency =864µg mg-1);

Six brands of amoxicillin capsules as shown in table 1.

The sampling method that was used is a random sampling and six samples of different amoxicillin brands were tested during this study.

IV.1.2. Equipment

Dissolution test apparatus (708-DS, Agilent technologies, USA),

UV-Visible spectrophotometer (Cary 60, Agilent technologies, USA),

Electronic balance (KERN, Max: 120gr, min: 1mg, USA),

PH meter (HI 2210, Hanna instruments), distilled water

Table 1 : Samples of amoxicillin capsules coded from A,B,C,D ,E and Innovator Clamoxyl

Samples code/brand	Country of origin / Manufacturer	Sample collection site	Mfg date	Exp date
Brand A	China/ Reyoung Pharmaceuticals Co Ltd	KIBOGORA HOSPITAL/ WESTERN PROVINCE	May-18	Apr-21
Brand B	China/CSPC ZHONGNUO PHARMACEUTICAL CO.LTD	PACIS PHARMACY/ SOUTHERN PROVINCE	Aug-18	Jul-21
Brand C	France / Laboratoire Innotech International	IRIS Pharmacy/ Kigali city	Feb-17	Jan-20
Brand D	Kenya/ Dawa Ltd	SANA Pharmacy/ Kigali city	Dec-17	Nov-20
Brand E	India/ Sparsh Bio-Tech PVT LTD	TWITEKUBUZIMA PHARMACY/ WESTERN PROVINCE	Oct-17	Sep-20
Innovator (Clamoxyl$^{(R)}$	France / Laboratoire Glaxo Smith Kline	IRAGUHA PHARMACY / Northern Province	Sep-18	08/20121

The label claim for all samples is amoxicillin 500 mg

IV. 2. METHODS

The study was conducted at National Medical and Pharmaceutical laboratory, in Rwanda Standards Board (RSB), in Kicukiro District / Kigali; in that building, three rooms were commonly used: Dissolution and Disintegration room, sample preparation room and weighing room and its execution necessitated conducting dissolution tests for sampled amoxicillin capsules.

IV.2.1 Standard preparation

Stock standard solution (1 mg /mL) was prepared by dissolving 100 mg equivalent of anhydrous amoxicillin USP reference standard in 100 mL of distilled water. Six different concentration levels of calibration solutions (0.01 to 1 mg/mL) were freshly prepared by diluting suitable volumes of the stock standard solution in appropriate volumetric flasks.

IV.2.2 Buffer preparation

Medium 1: Hydrochloric acid 0.1N, pH: 1.2

57.3 ml of Hydrochloric acid was mixed with 6942.7 ml of distilled water, we obtained a mixture of hydrochloric acid medium of 1.2 PH, 7 liters of this mixture were filled in dissolution vessels (900mls per each vessel)

Medium 2: Acetate buffer, pH: 4.5

20.9 gr of sodium acetate and 11.2mls of acetic acid were mixed up to 7litres with distilled water, we obtained a mixture of acetate buffer of 4.5 pH, 7 liters of this mixture were used to fill dissolution vessels (900mls per each vessel)

Medium 3: Phosphate buffer, pH: 6.8

6.272 gr of sodium hydroxide and 47.6gr of KH_2PO_4(Potassium Dihydrogen Phosphate) were mixed up to 7litres with distilled water, we obtained a mixture of phosphate buffer of 6.8 pH, 7 liters of this mixture were used to fill dissolution vessels (900mls per each vessel)

IV.2.3 Dissolution test and sample preparation

One capsule was placed in each of the six vessels of the dissolution apparatus, and each amoxicillin brand was tested in two replicates in hydrochloric acid medium, in acetate buffer and in phosphate buffer. for each test six vessels were filled with 900 ml of medium per each vessel. Dissolution media were previously heated and bath temperature was maintained at 37.3 ºC± 0.5. 10 mL of sample was withdrawn from each vessel after 5, 10, 15 ,20 , 30, 45 and 60 minutes.
The sampling was done automatically by the dissolution apparatus which was connected to UV-VIS Spectrophotometer. Absorbance of the solution was measured at 272 nm. Employed conditions for the dissolution test are shown in table 2.

Table 2: Dissolution test conditions for amoxicillin capsule as per USP 41nf 36(2018)

Media	
Media Volume (ml)	900
Spindle	
Apparatus Type	Baskets
Spindle (RPM) Spin duration (min) 1.0	100
Spin tolerance (%)	.5
Temperature	
Bath temperature (°C)	37.3
Temperature tolerance (± °C)	0.5
Stabilization Delay (sec)	12
Log intervals (min)	5.0
Evaporation Rate (ml/min)	0.00

Sampling Parameters	
Prime Volume (ml)	10.0
Pause Time (sec)	5
Purge Volume (ml)	20.0
Plunger Speed (steps/sec) 5900	5900
Aspiration Dwell (sec)	3
Detection	
Detection	Absorbance at 272 nm

Dissolution test is specified for its compliance in the individual monographs of pharmacopoeias, particularly for tablets and capsules.

A number of apparatus is available to conduct dissolution studies, because no single equipment is adequate for the study of all drugs and dosage forms.

Therefore, the objectives of the test are defined first and depending on the nature of active ingredients and the types of dosage forms, an efficient dissolution method can be developed. (Banakar U. V, 2010; J. Dressman et al 2005, Lachman L et al, 1987; Subrahmanyam CVS,2013;USP29 NF24, 2005 ;Yadav A. V et al ,2011)

To date, in vitro dissolution tests seems to be the most sensitive and reliable predictors of in vivo performance

IV.2.4. Measurement variables

Dissolution

According to Augsburger L. A.et al (2008), Kuchekar B. S(2013), Shah V. P et al (1998)and Sweetman S. C (2011). A drug is expected to be released from solid dosage forms (granules, tablets, capsules, etc.) and immediately go into molecular solution. This process is called dissolution. It is a critical step for performance of a drug as well as dosage form, because it is a prerequisite for the drug absorption. Absorption of drug is possible only when itis present in

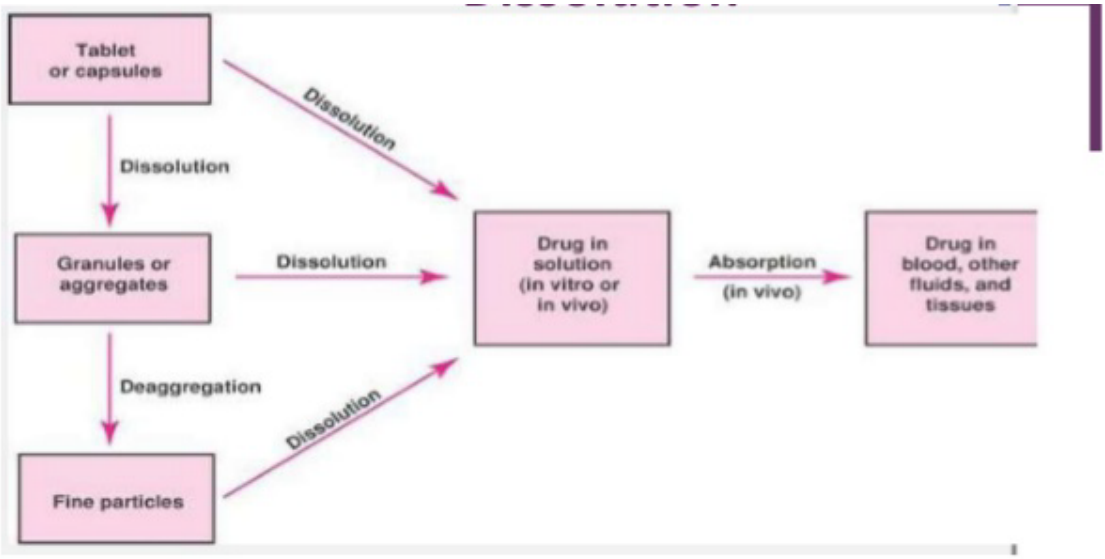
solution form, wherein the molecules are independent and assume molecular dispersion. Each molecule is absorbed independently through biological membranes.

Factors affecting dissolution

A. "Factors relating dissolution apparatus and Dissolution test parameters
   - ✓ Instrumental factors: -temp, agitation, speed, dissolution medium & pH

B. Factors related to drug):
   - ✓ Physicochemical factors: -particle size, shape, surface area, form (amorphous, crystalline) or state of drug (salt), polymorphism

C. Factors related to dosage form: -
   - ✓ excipient related factors: -diluent, disintegrant, binder, lubricant, surfactant, coating
   - ✓ processing related factor: - method of granulation, compression force"(Banakar U. V, 2010; Brahmankar D. M et al , 2013; Singh S, 2012; Tipnis H. A. et al , 2010).



Absorption depends some what on

1- The rate of disintegration of the dosage forms
2- Deaggregation of the granules
3- More importance is the dissolution rate of the solid drug.
Frequently, dissolution is the limiting or rate-controlling step in the absorption of **drugs with low solubility**

Figure3:Dissolution process of medicinal product

Similarity Factor (f2)

The similarity factor is a logarithmic reciprocal square root transformation of one plus the average means squared differences in percent dissolved between the test (Tt) and reference (Rt) products over all time points (n). It stresses on the comparison of closeness of two comparative formulations.

The US FDA and EMEA suggests that two dissolution profiles are declared similar if f2is between 50 to 100. It can be computed using the formula.

$$f_2 = 50\log\left\{\left(1+\frac{1}{n}\sum\left(R_t - T_t\right)^2\right)^{-0.5} x100\right\}$$

Equation: Similarity factor F2

where,

n = number of dissolution sample times.

Rt and Tt = individual or mean percent dissolved at each time point t, for the reference and test dissolution profiles, respectively.

The similarity factor should be between 0 and 100. It is 100 when two comparative groups of reference and test are identical and approaches 0 as the dissimilarity increases. This factor is endorsed by the FDA as acceptable and preferred method for dissolution profile comparison. The main advantage of f2 equation is that it is easy to compute and provide a single no. to describe the comparison of dissolution profile data.

To evaluate of similarity between dissolution is based on following parameters:

- Minimum of three dissolution time points are measured.
- Number of drug product tested for dissolution is 12 for both test and reference.
- Not more than one mean value of > 85% dissolved for each product.
- Standard deviation of mean of any product should not be more than 10% from the second to last dissolution time points.

Fit factors or similarity indices are defined as follows:

$$f_1 = \left\{\left[\sum_{t=1}^{n}\left|R_t - T_t\right|\right] \Big/ \left[\sum_{t=1}^{n} R_t\right]\right\} \times 100$$

Formula for F1 Calculation (CDER/FDA, 1997, 2000;

Swami R1 et al, 2011)[39,40,41]

$$f_2 = 50 \cdot \log\left\{\left[1+\frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right\}$$ Formula for F2 Calculation (CDER/FDA, 1997, 2000; Swami R1 et al, 2011)[39,40,41]

Where Rt is the percentage of dissolved product for a reference batch at time point t, Tt is the percentage of dissolved product for the test batch, n is the number of time points. For each brand, the calculations were made on the mean values for the six vessels. The factor, f1,is the average % difference over all time points in the amount of test brand dissolved as compared to the reference brand. The f1 value is 0 when the test and the reference profiles are identical and increases proportionally with the dissimilarity between the two profiles. The f2 value is between 0 and 100.The valueis100 when the test and the reference profiles are identical and approaches zero as the dissimilarity increases (Ngwuluka NC et al, 2009; Polli JE et al, 1997; Anderson NH et al, 1999).

time points t1 and t2 expressed as a percentage of the curve at maximum dissolution, y100, over the same time period. For a capsule product, t1 can beset to the period corresponding to disintegration of the capsule shell.

IV.2.4. Data analysis Statistical calculations

Excel Windows was used

CHAPTER V: RESULTS AND DISCUSSION

V.1 DISSOLUTION RESULTS

V.1.1. Dissolution in acid media

In acid media 0.1N HCl(pH=1.2), 4 brands(A,B,C,D) and reference product show ≥ 85% dissolution within 15 minutes; the brand E released less than 70% (Figure 4).

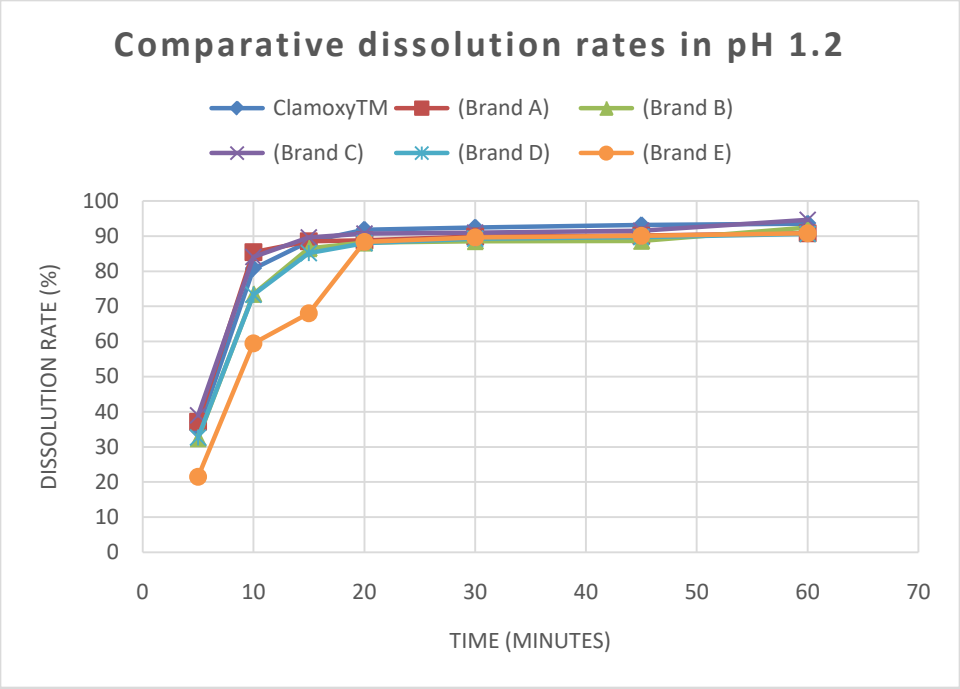


Figure 4 Comparative dissolution rates in pH 1.2

As, the profiles of brands A, B, C are regarded similar no calculation of F_2 is required. For brand E which requested F_2, f2 factor was 36, which is less than 50 which supports the dissimilarity of this brand with the innovator brand in acid media (Table 3).

Table 3: Dissolution Profiles in Medium 0.1N HCl (pH=1.2)

Time(min)	**Clamoxy™**	**(Brand A)**	**(Brand B)**	**(Brand C)**	**(Brand D)**	**(Brand E)**
5	34.9±1.1	37.2±5.3	32.4±2.1	39.0±4.8	32.7±8.1	21.5±1.8
10	80.7±5.1	85.4±2.6	73.5±0.8	84.0±3.5	73.3±0.2	59.5±2.1
15	**88.4±3.3**	**88.5±1.4**	**86.5±0.6**	**89.6±1.2**	**85.1±2.2**	**68.0±11.0**
20	91.8±0.7	88.8±1.6	88.1±0.3	90.7±0.5	88.0±1.8	88.3±0.1
30	92.4±0.7	89.9±2.8	88.5±0.5	91.0±0.3	89.2±1.9	89.6±0.8
45	93.1±0.7	90.2±0.3	88.6±0.6	91.5±0.7	89.7±2.3	90.1±0.4
60	93.5±1.3	90.8±0.8	92.4±0.6	94.6±0.1	90.6±2.5	90.8±0.6
F_2						F_2=36

V.1.2. Dissolution in acetate buffer

In acetate buffer (pH=4.5) none of the brand and reference product show ≥ 85% dissolution within 15 minutes (Figure 5)

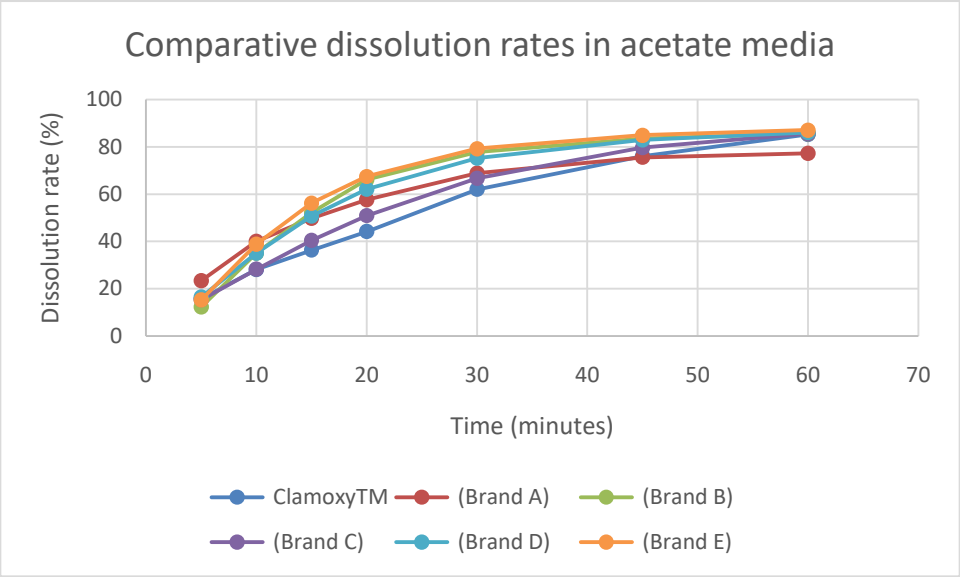


Figure 5 Comparative dissolution rates in acetate media

Calculations of F_2 are required. F_2 factors were 52, 62 and 71 for brands A, B, C respectively, greater than 50, which qualify them to have similar dissolution profiles to that of innovator. F_2 of Brand D and Brand E were 48 and 42 respectively which is less than 50 indicating dissimilarity with the innovator brand in acetate buffer (Table 4).

Table 4: Dissolution Profiles Dissolution Medium: Acetate buffer (pH=4.5)

Time(min)	**Clamoxy™**	**(Brand A)**	**(Brand B)**	**(Brand C)**	**(Brand D)**	**(Brand E)**
5	15.7±0.6	23.3. ±16.3	12.2±1.3	15.3±0.6	16.5±4.0	15.2±0.6
10	28.1±0.9	40.0±7.2	35.0±1.6	28.2±5.2	35.1±2.5	38.8±2.5
15	36.3±1.6	49.7±4.0	52.1±2.8	40.4±5.6	50.8±1.1	56.2±2.6
20	44.2±2.2	57.6±1.8	66.1±4.9	50.9±5.6	62.1±0.6	67.5±3.0
30	62.0±0.0	68.9±1.1	77.8±4.4	66.7±4.7	75.3±1.1	79.2±0.8

45	76.1±1.5	75.6±2.4	83.5±3.3	79.7±2.2	83.0±0.6	84.9±0.4
60	85.3±1.8	77.3	85.6±2.0	85.6±0.4	86.1±0.5	87.1±0.8
F_2		**F_2=52**	**F_2=60**	**F_2=71**	**F_2=48**	**F_2=42**
Conclusion		**Similar**	**Similar**	**Similar**	**Not Similar**	**Not Similar**

Average %Dissolved ±SD(n=12) Dissolution Media - pH 4.5 in acetate buffer

V.1.2. Dissolution in phosphate buffer

In phosphate buffer (pH=6.8) none of the brand and reference product show ≥ 85% dissolution within 15 minutes (Fig.6).

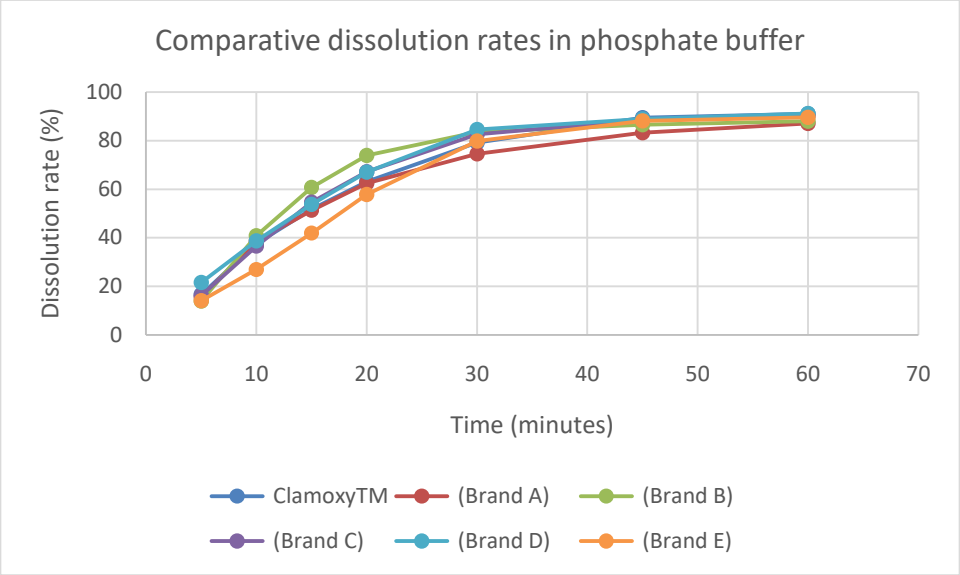


Figure 6 Comparative dissolution rates in phosphate buffer (pH 6.8)

All brands A, B, C D have F_2 which are greater than 50, which qualify them to have similar dissolution profiles to that of innovator in phosphate buffer (Table 5).

Table 5: Dissolution Profiles Dissolution Medium: Phosphate buffer (pH=6.8)

Time(min)	**Clamoxy™**	**(Brand A)**	**(Brand B)**	**(Brand C)**	**(Brand D)**	**(Brand E)**
5	16.6±4.0	15.5±0.1	13.9±4.5	16.2±1.6	21.5±3.2	14.0±1.7
10	37.5±11.2	38.6±0.3	40.8±10.6	36.5±1.2	38.7±3.4	26.9±2.3
15	51.8±14.0	51.3±0.3	60.7±10.0	54.6±1.8	53.7±6.1	41.9±3.3
20	63.1±14.4	62.4±0.3	73.9±4.9	67.2±0.7	67.0±6.2	57.8±4.4
30	79.1±9.1	74.6±4.9	83.5±3.4	82.6±0.4	84.6±3.3	79.8±4.3
45	89.4±2.1	83.3±0.6	86.5±2.1	88.5±0.5	89.0±0.8	88.1±0.8
60	91.0±0.1	87.1±0.7	88.0±0.6	90.3±0.4	91.2±2.2	89.6±04
F_2		**$F_{2=}$72**	**$F_{2=}$60**	**$F_{2=}$78**	**$F_{2=}$72**	**$F_{2=}$59**
Conclusion		**Similar**	**Similar**	**Similar**	**Similar**	**Similar**

Average %Dissolved ±SD(n=12) /Dissolution Media- pH 6.8 phosphate buffer

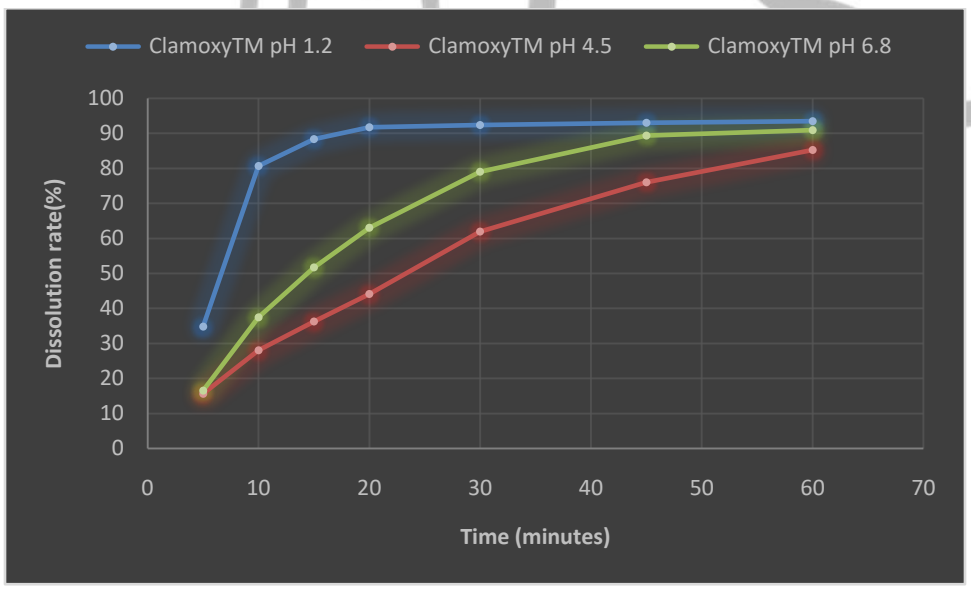


Figure 7 Clamoxyl dissolution rates in different media used

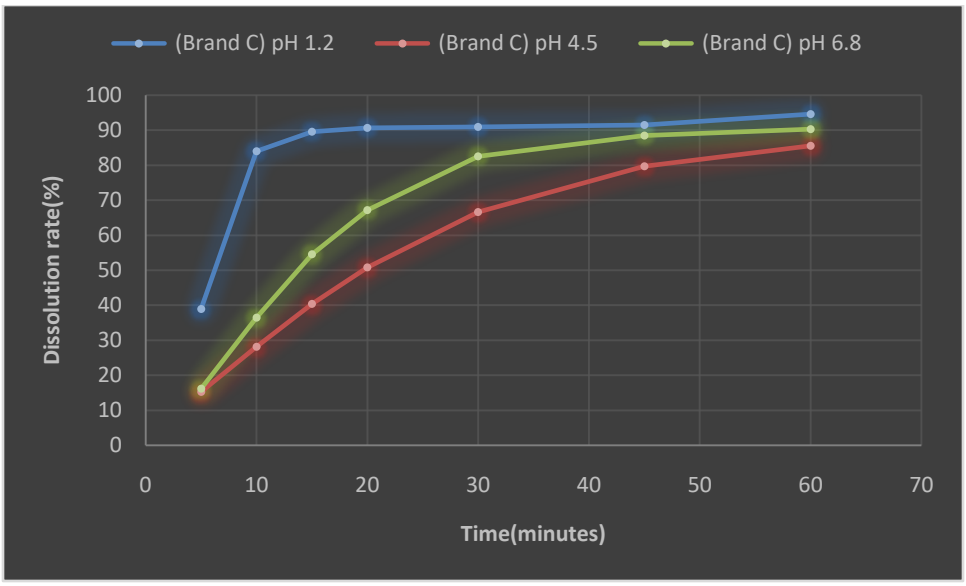


Figure 8 Brand C dissolution rates in different media used

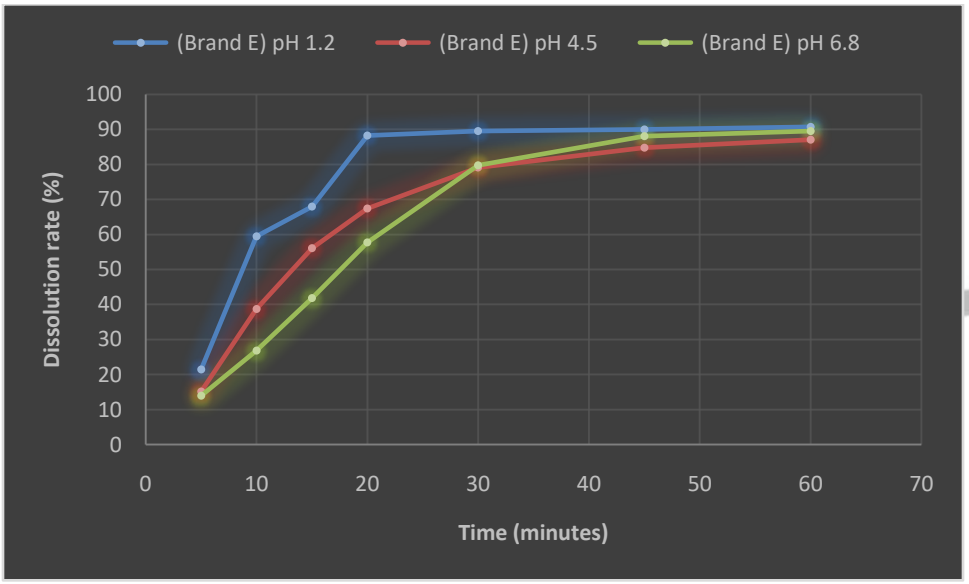


Figure 9 Brand E dissolution rates in different media used

Figures 7, 8 and 9 illustrate the dissolution profiles for individual brands in the three media used.

Reference made to dissolution profiles for individual brands presented on figure 7,8 and 9 We can conclude that Amoxicillin had higher dissolution rates in acid media pH:1.2 than in both acetate buffer pH:4.5 or Phosphate buffer pH:6.8 .

V.2 **DISCUSSION**

In the literature, different methods which can be used to compare dissolution profiles data have been reported, However, in this study the most important and widely engaged method has been used: the fit factors. The fit factors can be expressed by two approaches: f1 (the difference factor) and f2 (the similarity factor). Two dissolution profiles to be considered similar and bioequivalent, f1 should be between 0 and 15 whereas f2 should be between 50 and 100.

The dissolution test according to USP 41 NF36 ,2018 requires that each unit not less than Q + 5% of the active ingredient should dissolve within 60 minutes for the first six units (stage 1). But, if the requirement at stage 1 is not met, another six units will be tested and the mean percent dissolved for the twelve units is not less than Q% and no unit is less than Q – 15% (stage 2). In this study all the tested brands have satisfied these requirements at stage 1 and thus were in agreement with the USP 41 NF36 ,2018 specifications.

As such, the products would pass the dissolution test standard in accordance with the USP requirement for amoxicillin tablets (i.e., Q ≥ 85% at 30 min). In fact A B,C, are considered as very rapidly dissolving since at least 85% of labelled amoxicillin have dissolved in 15 min (In acid media 0.1N HCl(pH=1.2), 4 brands(A,B,C,D) and reference product show ≥ 85% dissolution within 15 minutes; the brand E released less than 70%). In these cases, similarity factor and difference factor calculations become unnecessary. The coefficient of variations for drug release at the time points 5 and 10 min were high but did not exceed 20% with any product.

FDA Guidance for biowaiver for immediate-release solid oral dosage forms states that “a product is said to be rapidly dissolving when not less than 85% of the labelled API dissolves within 30 minutes”5. The API

Two dissolution profiles are considered similar when the F_2 value is ≥ 50. To allow the use of mean data, the coefficient of variation should not be more than 20 percent at the earlier time points (e.g., 15 minutes), and should not be more than 10 percent at other time points. Only one measurement should be considered after 85 percent dissolution of both products. In addition, when both test and reference products dissolve 85 percent or more of the label amount of the

drug in 15 minutes using all three-dissolution media recommended above, the profile comparison with an f2 test is unnecessary (U.S. Food and Drug Administration /CDER, December 2017 Biopharmaceutics, page 18/point C)

Amoxicillin in test formulation showed almost complete dissolution in the pH 1.2 ,0.1N HCl, (within 15min) for only 4 brands(A,B,C and D).

By referring to results found, only Amoxicillin Brand A, B, C have similar dissolution profiles to that of Innovator (Clamoxyl^{R}) due to following reasons:

I. In acid media 0.1N HCl(pH=1.2), 3 brands(A,B,C,) show dissolution ≥ 85% within 15 minutes.
II. In acetate buffer (pH=4.5), F_2 factors for brands A, B, C were 52, 62 and 71 respectively, greater than 50, which qualify them to have similar dissolution profiles to that of innovator.
III. In phosphate buffer (pH=6.8), F_2 factors for brands A, B, C were 72, 60 and 78 respectively, greater than 50, which qualify them to have similar dissolution profiles to that of innovator.

Results found also show that brand D and E have Dissimilar dissolution profiles to that of Innovator (Clamoxyl^{R}) due to following reasons:

I. Brand E has F_2 which is less than 50 ($F_{2=}36$) in 0.1N HCl,and has ($F_{2=42}$) which is less than 50 in acetate buffer .
II. Brand D has F_2 which is less than 50 in acetate buffer ($F_{2=48}$) .

This supports the result of the Dissimilarity of both brand D and E with the innovator brands without considering similarity factor in other media because test products must show similarity in all 3 media

V.3 **Limitations of the study**

The content of the active ingredient of each tested product is not assessed against the label claim.

Moreover; in-vitro dissolution test might be an indicator to investigate the interchangeability of products.

The study has not been assisted by other methods like in-vivo bioequivalence study for better conclusions.

CHAPTER VI: CONCLUSION AND RECOMMENDATIONS

VI.1 **Conclusion**

Most generic brands of amoxicillin capsules (62.5%) are interchangeable with the innovator brand (Clamoxyl^{TM}).

VI.2 **Recommendations**

Overall, it is recommended that drug regulatory Authorities should be reinforced and capacitated in order to address proper post marketing surveillance for all medicines but specifically for sensitive medicines like antibiotics.

We recommend to Rwanda FDA to stick to the requirement of bioequivalence studies during market authorization.

We recommend to researchers to conduct Further studies on the tested products for better conclusion of the interchangeability of the generic products with the innovator. the samples need also to be assessed in terms of dosage uniformity, water content and assay. Besides, in-vivo bioequivalence study on the generic products is highly considerable

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