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FACULDADE DE CIÊNCIASFARMACÊUTICAS
Programa de Pós-Graduação em Fármaco e Medicamentos
Área de Produção e Controle Farmacêuticos

DISSOLUTION PROFILE OF DIFFERENT BRANDS OFLOW SOLUBILITY DRUGS AVAILABLE IN THE NIGERIAN AND BRAZILIAN PHARMACEUTICAL MARKETS

Henry Ifeanyi Ezeagu

Dissertation for Obtaining Master's Degree

Advisor: Prof. Dr. Humberto Gomes Ferraz

São Paulo

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Dissolution profile of different brands of low solubility drugs available in the Nigerian and Brazilian pharmaceutical markets

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1º. Examiner

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ABSTRACT

EZEAGU, H.I. Dissolution profile of different brands of low solubility drugs available in the Nigerian and Brazilian pharmaceutical markets. 2021. 69p. Dissertation (Masters) – Faculty of Pharmaceutical Sciences, University of Sao Paulo, Sao Paulo, 2021.

The purpose of this work was to elaborate a diagnosis of the dissolution test in Africa in comparison with Brazil, evaluating the dissolution profile of low solubility drugs such as albendazole, ibuprofen, furosemide, glibenclamide, hydrochlorothiazide and carvedilol to ascertain their quality. The dissolution profiles were evaluated by utilizing the United States Pharmacopeia (USP). The glibenclamide medicine was evaluated according to the Food and Drug Administration (FDA), while a dissolution method was developed for the carvedilol medicine. A filter selection test for all the drugs showed that cannula is suitable for all, except for carvedilol, which is centrifuged. The various brands of Nigerian and Brazilian medicines tested showed some statistical differences. The suitable conditions that allowed the dissolution of carvedilol to be determined were the USP type II apparatus at 75 rpm containing 900 mL of acetate buffer, pH 4.5. The results of the dissolution test showed that out of the 17 different brands of Brazilian medicines and 17 different products from Nigeria, 94.12% and 58.82% passed respectively.

Keywords: Dissolution Test. Dissolution Profile. Low Solubility Drugs. Tablets.

RESUMO

EZEAGU, H.I. Perfil de dissolução das diferentes marcas de medicamentos de baixa solubilidade disponíveis no mercado farmacêutico Nigeriano e Brasileiro. 2021. 69p. Dissertação (Mestrado) – Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, 2021.

O objetivo deste trabalho foi elaborar um diagnóstico do teste de dissolução na África em comparação ao Brasil, avaliando o perfil de dissolução de medicamentos de baixa solubilidade como albendazol, ibuprofeno, furosemida, glibenclamida, hidroclorotiazida e carvedilol para verificar sua qualidade.Os perfis de dissolução foram avaliados utilizando a Farmacopeia dos Estados Unidos (USP). O medicamento glibenclamida foi avaliado de acordo com a Food and Drug Administration (FDA), enquanto um método de dissolução foi desenvolvido para o medicamento carvedilol.Um teste de seleção de filtro para todos os medicamentos mostrou que a cânula é adequada para todos, exceto para o carvedilol, que é centrifugado. As diversas marcas de medicamentos Nigerianos e Brasileiros testadas apresentaram algumas diferenças estatísticas. As condições adequadas que permitiram a determinação da dissolução do carvedilol foram o aparelho USP tipo II a 75 rpm contendo 900 mL de tampão acetato, pH 4,5. Os resultados do teste de dissolução mostraram que das 17 diferentes marcas de medicamentos brasileiros e 17 diferentes produtos da Nigéria, 94,12% e 58,82% foram aprovados, respectivamente.

Palavras-chave: Teste de Dissolução. Perfil de dissolução. Farmacos de baixa solubilidade. Comprimidos.

SUMMARY

ANNEXES

I - Curriculum Vitae

II – Actualized Student Form

III – Catalog Card

CHAPTER 1

Literature review of the dissolution tests done on the available brands of albendazole, ibuprofen, furosemide, glibenclamide, hydrochlorothiazide and carvedilol tablets in Nigeria and Brazil

ABSTRACT

The present study is a literature review of the works done on the dissolution test of various brands of albendazole, ibuprofen, furosemide, glibenclamide, hydrochlorothiazide and carvedilol tablets marketed in Nigeria and Brazil. Databases such as Scopus, web of science, pubMed, scienceDirect, Google Search were used for the literature review. The review shows from the literature that all the samples of low solubility drugs listed in this present study which are available in the Brazilian pharmaceutical market passed the dissolution test as recommended by the United States Pharmacopeia (USP). The literature evaluation shows that most of the products marketed in Nigeria do not pass the dissolution test as it was observed that in Nigeria, among the 9 different brands of albendazole tablets marketed, 44.4% did not pass the dissolution test, 19 brands of ibuprofen tested, 57.9% failed the test, 100 % of the 3 different brands of glibenclamide tested failed.

Keywords: Dissolution Test. Literature review. Low Solubility Drugs. Tablets.

1. INTRODUCTION

The low quality of medicinal products in Nigeria and Africa in general has become a global source of concern as about 10% of estimated drugs in these countries are fake and of low quality which leads to the death of more than 120,000 people a year according to the World Health Organization (WHO) report in 2016. Most infant mortality rate in Africa today is caused by taking low quality medicines, some of which do not contain the right amount of active pharmaceutical ingredients (API) or do not contain active ingredients at all.

The introduction of generic drug products from numerous manufacturers into the health care system of many developing countries such as Nigeria, has been accompanied by a variety of problems of which the most critical is the widespread distribution of fake and substandard drug products (RAHEELA et al., 2011)

Nigeria which is Africa's most populous country has suffered heavily from the problem of medicine counterfeiting and trafficking, which has happened mostly during the period between 1985 and 2000 which was regarded as the period of faking and quackery, to an extent that counterfeit drugs, unlicensed drug vendors, quack doctors and illegal chemist shops became very common in the country (ERHUN et al., 2011).

In 2008, the WHO conducted a study to check the quality of antimalarial medicines in circulation in 14 African countries and the result showed that 64 percent of the antimalarial medicines in circulation in the Nigerian pharmaceutical market as of that time were either fake or substandard (OGUNDIPE, 2011).

In 2009, Nigeria seized a large consignment of fake anti-malaria drugs with the label of "made in India" but found out that the medicines were in fact produced in China and was imported into African countries (WHO, 2015).

According to the WHO, a counterfeit medicine is one which is manufactured and sold with the intent to deceptively represent its origin, authenticity or efficacy (BUOWARI, 2013). The WHO and Nigerian health officials estimated that 70% of drugs in circulation in the country are either fake or adulterated (WHO, 2015). The National Agency for Food and Drug Administration and Control, Nigeria (NAFDAC), which works under the Ministry of Health for the regulation and control of the quality standards of drugs, identified a number of different types of fake drugs in Nigeria,

including those with insufficient or an absence of active ingredients and some expired medicaments still in the Nigerian pharmaceutical market (AKUYILI, 2006).

The worldwide trade on fake drugs is a multi-billion dollars' industry which is flourishing in Africa at an alarming rate. The World Health Organization (WHO), estimated world sales of fake medicines to be above USD 75 billion in 2010 alone, which shows a rise of 90% from 5 years before and could be more than 10% of all medicines sold worldwide (WHO, 2010).

There are some possible strategies to control and prevent drug counterfeiting in Nigeria and some other African countries, some of these measures include; stopping the importation of counterfeit drugs to Nigeria at source of production. In a bid to stop the importation of fake drugs from the countries of production to Nigeria, NAFDAC have put in place some administrative guidelines which include: a factory must be Good Manufacturing Practice (GMP) certified before it can export drugs to Nigeria. The agencies official must inspect factories anywhere in the world before they register or renew registration for their medicines (AKUNYILI, 2015).

Furthermore, initiation of the West African Drug Regulatory Authorities Network (WADRAN). NAFDAC has initiated and is currently heading this network which is a forum where heads of drug regulatory authorities in West Africa can share strategies and experiences and carry each other along in the fight against drug counterfeiters. This was necessitated by the fact that when drug counterfeiters were chased out of Nigeria, they relocated to other West African countries and became a problem for them. Therefore, it became necessary to work in concert in order to ensure that these criminals do not find a safe haven anywhere in the sub-region (AKUNYILI, 2015).

However, there is no work in the literature comparing the dissolution profiles of Brazilian and Nigerian medicines. Comparison of results of the dissolution profiles of the drugs in the two countries will enable to elaborate a diagnosis of the quality of low solubility drugs in Nigeria.

The objective of this study was to do a literature review of the dissolution works on the low solubility drugs available in Brazil and Nigeria.

2. DISSOLUTION TEST AS A TOOL FOR QUALITY CONTROL OF PHARMACEUTICAL SOLID FORMS

Dissolution testing has in recent times emerged as a highly valuable *in vitro* tool to characterize the performance of pharmaceutical solid forms (VINOD, 2013).

Comparison of dissolution profile under appropriate conditions and criteria is used as biowaiver for a lower strength of an oral dosage form. Dissolution profile comparison has been extensively used to assess drug product similarity after scale-up and post approval changes (FDA, 2015).

Systemic absorption of drugs is a prerequisite for eliciting their therapeutic activity, whenever given non-instantaneously. All the oral dosage forms have to be evaluated for*in vivo* bioavailability, thus, generic manufacturers must provide detailed bioequivalence evidence showing head-to-head comparative performance of their product against reference. To conduct such a bioequivalence study is a very demanding task that involves series of technical, economical and ethical issues. Also, development and optimization of a formulation is a time consuming and costly process (FDA, 2015).

Thus, it would be very convenient if inexpensive *in vitro* experiments could substitute *in vivo* bioavailability tests. For *in vitro* dissolution to act as surrogate for bioavailability studies, an accurately validated correlation needs to be established between *in vitro* and *in vivo* performance of drug. Thus, by establishing IVIVC, *in vitro* dissolution can act as surrogate for bioequivalence studies (FDA, 2015).

The quality control of solid pharmaceutical dosage forms such as tablets involves several parameters such as hardness test, friability, content uniformity test, disintegration and dissolution test. Dissolution test is considered to be one of the most important of all the parameters for ascertaining drug quality. Poorly water soluble drugs often require high doses in order to release the required amount of drug in the plasma, required to elicit a therapeutic response after oral administration. The major problem encountered with formulation development of new chemical entities as well as generic development is low aqueous solubility (ZHU et al., 2009).

The Table1 is an illustration of the various factors affecting dissolution of solid pharmaceutical dosage forms. The table also shows the physicochemical and

physiological properties that affects dissolution of drugs in the gastrointestinal tract (FDA, 2015).

Table 1. Factors affecting dissolution of drugs

FACTORS AFFECTING DISSOLUTION OF DRUGS

FACTOR	PHYSICOCHEMICAL PROPERTIES	PHYSIOLOGICAL PROPERTIES
Surface area of drug	Particle size, wettability	Surfactants in gastric juice and bile
Diffusivity of drugs	Molecular size	Viscosity of luminal contents
Boundary layer thickness	Concentration of the drug	Motility patterns and flow rate
Solubility	Hydrophilicity, crystal structure, solubilization, polymorphism	pH, buffer capacity, bile and food composition
Amount of drug already dissolved	Hydrophilic/lipophilic nature of the drug	Permeability
Volume of solvent available	Depends upon type of body fluid	Secretion, co-administered fluids

Adapted: (FDA, 2015)

3. BIOPHARMACEUTICS CLASSIFICATION SYSTEM (BCS)

In the BCS, drugs are classified into one of four classes based solely on its solubility and intestinal permeability. The rationale for correlating *in vitro* drug product dissolution and *in vivo* bioavailability is because drug dissolution and gastrointestinal permeability are the parameters that control the rate and extent of drug absorption in the gastrointestinal tract (AMIDON et al., 1995).

The BCS's objective was to create a regulatory tool to replace some bioequivalence assays with *in vitro* dissolution tests, making it possible to reduce costs and time in the process of developing a drug, in addition to the unnecessary exposure of individuals in *in vivo* studies. Data observed by Cook (COOK, 2008) indicate that the use of BCS in the development stages can reflect considerable savings in resources for the pharmaceutical industries (LIPKA; AMIDON, 1999; LENNERNÄS; ABRAHAMSSON, 2005 ;).

Currently, BCS has been used to exempt clinical tests in formulations for oral use, especially for immediate release formulations, while for modified release

formulations; its use is still being studied (KU, 2008; LENNERNÄS; ABRAHAMSSON, 2005; LIPKA; AMIDON, 1999).

According to the BCS, drugs are divided into four classes, namely; Class I: drugs of high solubility and high permeability, ClassII: drugs of low solubility and high permeability, Class III: drugs of high solubility and low permeability, Class IV: drugs of low solubility and low permeability (AMIDON et al., 1995).

For various poorly soluble drugs (class II) such as albendazole, ibuprofen, glibenclamide, carvedilol and class IV drugs such as furosemide and hydrochlorothiazide, bioavailability is limited by the dissolution rate (VEMULA et al., 2010). The Table 2 shows the biopharmaceutics classification system of drugs with some examples in each group.

Table 2. Biopharmaceutics Classification System of drugs (WHO, 2006)

Drug class	Solubility	Permeability	Examples
I	High	High	Metoprolol Paracetamol Propranolol
II	Low	High	Glibenclamide Albendazole Carvedilol Ibuprofen
III	High	Low	Cimetidine Atenolol Ranitidine
IV	Low	Low	Furosemide Hydrochlorothiazide Ritonavir

The drugs in class II (glibenclamide, albendazole, carvedilol and ibuprofen) and class IV (furosemide and hydrochlorothiazide) where selected for this work because of their low solubility in water and therefore, cannot be considered for biowaver. Another reason for selecting them is their availability in the Nigerian and Brazilian pharmaceutical markets. They are also widely used drugs. A drug is said to be of low solubility in water when a maximum or highest dose strength of it is not soluble in 250 mL or less of aqueous media over the pH range of 1 to 7.5 (KETAN et al., 2012).

The volume estimate of 250 mL is derived from typical bioequivalence study protocols that prescribe administration of a drug product to fasting human volunteers with a glass of water (FDA, 2017).

The BCS when combined with the dissolution of the drug product takes into consideration three major factors that govern the rate and extent of absorption from immediate release solid oral dosage forms which include dissolution, solubility and intestinal permeability (AMIDON et al., 1995).

It is important to increase the dissolution of class II and IV drugs to give maximum therapeutic effect. This can be achieved if the aqueous solubility of the API is enhanced. The aqueous solubility of an API is an important factor in evaluating the oral bioavailability of orally administered poorly water-soluble drugs (BALVINDER et al., 2014).

According to the US FDA Guidance, based on the BCS, Class I drugs are eligible for biowaiver, because for a biowaiver, the dissolution of the dosage form should be compared with the dissolution of the reference drug (FDA, 2015).

Class I drugs have high permeability and high solubility in an aqueous medium.Thosecompoundsarewellabsorbedandtheirabsorptionrateisusually higher than excretion. Examples of class I drug is shown in the Table 2 (FDA, 2017).

Class II drugs have high permeability and low solubility. The bioavailability of those products is limited by their solvation rate. A correlation between the *in vivo* bioavailability and the *in vitro* solvation can be found. Examples of class II drugs include those shown in the Table 2 (FDA, 2017).

Class III drugs have low permeability and high solubility. The absorption is limited by the permeation rate but the drug is solvated very fast. If the formulation did not change the permeability or gastro-intestinal duration time, then class I

criteria can be applied. Examples of class III drugs include the above listed drugs in Table 2 (FDA, 2017).

Class IV drugs have low permeability and low solubility. Those compounds have a poor bioavailability. Usually they are not well absorbed over the intestinal mucosa and a high variability is expected. Examples of class IV drugs include the drugs listed in Table 2 (FDA, 2017).

For dissolution class boundaries, an immediate release product is considered rapidly dissolving when no less than 85% of the labeled amount of the drug substance dissolves within 15minutes using USP Dissolution Apparatus 1 at 100 RPM or Apparatus 2 at 50 RPM in a volume of 900 ml or less in the following media: 0.1 N HCl or simulated gastric fluid or pH 4.5 buffer and pH 6.8 buffer or simulated intestinal fluid (FDA, 2017).

4. WORKS DONE ON DISSOLUTION OF AFRICAN MEDICINES

There are few studies on dissolution of products containing the above mentioned drugs, in Nigeria, only one article presented the dissolution of furosemide, hydrochlorothiazide, albendazole and ibuprofen, there is no published work on the dissolution of glibenclamide and carvedilol tablets available in Nigeria.

JULIDE et al (2008) did a dissolution characteristic of 3 different brands of commercial furosemide tablets from different manufacturers in Ghana, it was observed that at pH 4.6 there is a large variation in the dissolution rates of these brands. Furthermore, the effect of methods of tablet processing on furosemide release was also studied, a poor dissolution profile was observed with the tablet prepared by direct compression. The best result was obtained with the wet-granulation.

CHINAKA and his group in 2017 carried out a dissolution test on five different brands of furosemide tablets marketed in Port Harcourt Nigeria by using United States Pharmacopeial (USP) method as a reference standard. They observed from the work that 100% of the samples tested passed the dissolution test as stipulated by USP because all the brands released more than 80% of their API within 30 min of the assay and 100% in 60 min.

ODENIRAN and his collaborators in 2012 did a dissolution test of three different brands of hydrochlorothiazide tablets marketed in the Nigerian pharmaceutical market. It was observed that the three different brands passed the dissolution test as they released more than 80% of the API in 30 min and 100% in 60 min.

ELHAMILI et al., (2014), evaluated the quality of 3 different brands of glibenclamide tablets available in the Libyan Market according to the British Pharmacopeia (BP, 2009). From the study, the obtained dissolution profile indicated that there are significant differences between the tested products. All tested samples did not release a significant percentage, since (80%) of the drug should be dissolved within 30 min according to BP.

AONDOVER et al. (2014), did a study on the quality control properties of 9 brands of veterinary albendazole boluses common in Nigeria, it was observed that 44.4% of the total number of albendazole tablets tested did not pass the dissolution test.

EICHIE et al. (2009), did an *in vitro* evaluation of the pharmaceutical quality of some ibuprofen tablets dispensed in Nigeria, it was discovered that out of the 19 different brands of ibuprofen tablets assayed, 4 of them passed dissolution test as recommended by the BP 2003 which states that 70% of the tablet drug should dissolve within 40 min, out of the remaining 15 tablets, 4 did not undergo dissolution test as they did not disintegrate after 30 min, while the remaining 11 tablets failed the dissolution test.

TAYLOR et al. (2001) analyzed 581antimalarial tablets of 27 different brands marketed in Nigeria. The medicines were analyzed for drug content by validated chromatographic methods and the results obtained was compared with pharmacopeial requirements. Out of the total samples analyzed, 279 (48%) did not comply with set pharmacopeial limits, and this proportion was uniform for the various types of drugs tested.

ERAGA and his collaborators did a dissolution test of 15 different brands of metformin hydrochloride tablets marketed in South-East Nigeria, the result showed that out of the 15 different brands of metformin hydrochloride tablets analyzed, 12 of the brands passed the dissolution test while 3 failed the dissolution test (ERAGA,

2020). It shows that the situation of the quality of drugs marketed in the Nigerian Pharmaceutical market is not optimal when compared to the reference standard. Table 3 indicates that the situation observed for metformin is the same for most of the drugs

The Table 3 below shows the various works, which have been done on the dissolution, test of various brands of low solubility drugs available in African pharmaceutical market.

Table 3. Dissolution works done on the African medicines found in the scientific literature

Drug	Number of brands	Country	Author	Year	Observation
Albendazole	9	Nigeria	Aondover et al.	2014	44.4% failed
Ibuprofen	19	Nigeria	Eichie et al.	2009	57.9 % failed
Hydrochlorothiazide	3	Nigeria	Odeniran et al.	2012	100% passed
Furosemide	3	Ghana	Julide et al.	2008	100% failed
Furosemide	5	Nigeria	Chinaka et al.	2017	100 % passed
Glibenclamide	3	Libya	Elhamili et al.	2014	100% failed
Metformin HCl	15	Nigeria	Eraga et al.	2020	80% passed

5. WORKS DONE ON DISSOLUTION OF BRAZILIAN MEDICINES

MAHLE et al. (2008) did an in vitro evaluation of tablets of four different brands containing hydrochlorothiazide marketed in Brazil. The market reference (R), another nominally similar brand and two generic forms (G1 & G2) were analyzed. The results showed all of the analyzed forms (S, G1 & G2) to be similar to the reference (R). All the formulations fulfilled the specifications laid down in the Pharmacopeial monograph, that 60% of the drug should be released within 60 min of analysis.

MADUREIRA and collaborators (2016) did a dissolution test of six different brands of ibuprofen tablets marketed in Brazil. Out of the six brands, one is a reference sample, one a generic sample and four are similar. The dissolution test was carried out by using the method stipulated by the Brazilian pharmacopeia 4th

edition. It was observed that all the samples passed the dissolution test as they all complied with the specifications of the Brazilian pharmacopeia.

GIANOTTO and group (2007) did a dissolution study on three commercially available products of glibenclamide using USP apparatus II. The results showed that all three samples passed the dissolution test as they released 100% at 60 min.

FERREIRA et al., (2016), did a comparative in vitro analysis of dissolution profiles of three different brands of furosemide tablets marketed in Bahia. The analysis was done by utilizing the Brazilian pharmacopeia 5th edition and the USP 34th. The results showed that all the brands tested passed the dissolution test as they released more than 80% of the API in 30 min of the test as stipulated by both the Brazilian Pharmacopeia and the United States Pharmacopeia. The Table 4 below shows some dissolution works done on the low solubility drugs available in the Brazilian pharmaceutical market.

Table 4. Dissolution works done on the Brazilian medicines

Drug	Number of brands	Country	Author	Year	Observation
Ibuprofen	6	Brazil	Madureira et al.	2016	100 % passed
Hydrochlorothiazide	4	Brazil	Mahle et al.	2008	100% passed
Furosemide	3	Brazil	Ferreira et al.	2016	100 % passed
Glibenclamide	3	Brazil	Gianotto et al.	2007	100% passed

6. CONCLUSION

From the results of the dissolution tests of some brands of medicines sold in Nigerian market, it can be shown that a major percentage of the medicines failed quality control test, using dissolution as the quality control tool. From World Health Organization (WHO) report, a majority of African children and even adults die from low quality of medicines, this situation can be improved if the diagnosis of the quality situation of medicines in African market is known and checked by regulatory agents. It can also be concluded that from the dissolution works done on the low solubility drugs available in the Brazilian pharmaceutical markets, all the brands passed the dissolution test. The high percentage of Brazilian medicines which were observed to pass the test may be due to the introduction of generic medicine into the Brazilian market, however, most products marketed in Nigeria were imported from other countries like India, Tukey, China and Pakistan, most of these products were not generic, and most at times subvert the drug regulatory agents in Nigeria, as a result, it became highly possible to introduce fake or counterfeit medicines into the Nigerian market.

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CHAPTER 2

DEVELOPMENT OF DISSOLUTION TEST METHOD FOR CARVEDILOL

ABSTRACT

The present work describes the development of a dissolution test method for carvedilol tablets. The dissolution test was performed using a dissolution apparatus USP Dissolution Apparatus II (paddle), Agilent Technologies 708-DS (Agilent USA). Based on the results of the solubility test, 0.1 N HCl and acetate buffer pH 7.2 were selected and the release was evaluated for 1 hour. The filter selection test indicated that there was drug retention with both the cannula and PVDF filters, with no total recoveries possible, centrifugation yielded 100% recovery and thus was the indicated method. Analysis of variance showed no significant difference in dissolution in 0.1 N HCl and acetate buffer. From surface response graph and Pareto chart, dissolution in acetate buffer, pH 4.5 at 75 rpm was seen to yield a better result. Conditions that allowed the dissolution to be determined were the USP type II apparatus at 75 rpm containing 900 mL of acetate buffer, pH 4.5.

Keywords: Buffers. Carvedilol. Filters. Solubility.

1. INTRODUCTION

Carvedilol (CVD) is a non-selective beta-blocker indicated in the treatment of mild to moderate congestive heart failure (CHF) and high blood pressure (HBP). It blocks beta-1 and beta-2 adrenergic receptors as well as the alpha-1 adrenergic receptors (TSUME et al., 2012). Carvedilol belongs to class II in the biopharmaceutics classification system (BCS). It has high permeability; however, precipitation of the drug may occur when it reaches the intestinal tracts due to its poor aqueous solubility (KOSTEWICZ et al., 2004). It has a logP value of 3.8 with a basic pKa of 7.8 (TSUME et al., 2014) and an acidic pKa of 15.0 (ChemAxon Software).

CVD is highly soluble in dimethylsulfoxide, methylene chloride and methanol; it is sparingly soluble in 95% ethanol and isopropanol, slightly soluble in ethyl ether and practically insoluble in water, gastric fluid and intestinal fluid (Hari et al., 2009).

The solubility of carvedilol is pH dependent (KUKEC et al., 2012). In its ionized form, it dissolves in the acidic pH of the stomach. Carvedilol exhibits poor bioavailaibility due to its very low aqueous solubility (LOFTSSON et al., 2008).

Generally, the rate of dissolution of BCS class II drugs can be affected by three major characteristics of the GI fluids, which are physiological factors like pH, ionic strength, and buffer capacity (GALIA et al., 2008). Figure 1 below is the chemical structure of carvedilol molecule showing the carboxylic acid end, the amino acid group and the ionizable hydroxyl group.

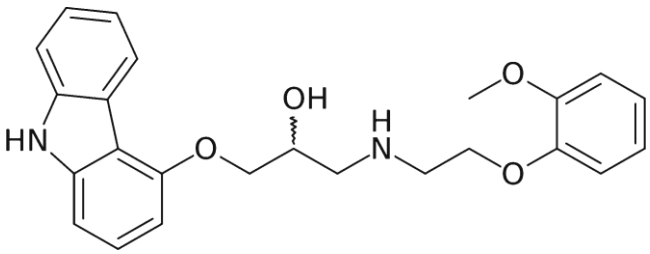


Figure 1- Chemical structure of carvedilol (source: EJPS, 2014)

The bioavailability of poorly water-soluble drugs such as carvedilol is a challenge that faces the development of such dosage forms. Because of the

poor water solubility of such drugs, bad dissolution profile is usually accompanying those drugs (GU et al., 2007).

Recent advances in biotechnology, combinatorial chemistry, and parallel synthesis are increasing the number of lipophilic molecules, which are difficult to deliver due to bioavailability issues (VARMA et al., 2004). CVD is currently approved for the treatment of mild to severe congestive heart failure (CHF), post-myocardial infarction, high blood pressure and ischemic heart diseases (PACKER et al., 2006).

There is no established dissolution test method for carvedilol in the United States Pharmacopeia (USP) or the Food and Drug Administration (FDA) list of medicines, as a result, the objective of this present work is to develop a discriminatory dissolution test method for carvedilol following standard practices.

2. MATERIALS AND METHODS

2.1. MATERIAL

2.1.1. DRUG

Carvedilol active ingredient was donated by Sigma-Aldrich (Missouri, United States). The API was within the expiry date limit and in good packaging and storage conditions. Analytical balance was utilized in the weighing of the sample. Osmotic water was also used throughout the analysis. The Brazilian samples utilized in this work include Divelol by Laboratórios Baldacci LTDA and Ictus by Biolab. The Nigerian samples include Viedilol by Ciron drugs &Pharmaceuticals PVT. Ltd India and Carvedilol by Teva UK Ltd. The Nigerian brand of medicines were bought from pharmacy outlets in Nigeria by Professor Sabinus Ofoefule and brought to Brazil through DHL.

2.2. METHODS

2.2.1. Analytical curve

The analytical curve of carvedilol was performed by considering the dosage of the drug, which is 12.5 mg, and volume of the dissolution medium, which is 900 mL. 1.39 mg of the carvedilol reference sample was weighed out in

an analytical balance and transferred to a 100 mL volumetric flask, the sample was dissolved in 20 mL methanol and the volume was made up to the meniscus with osmotic water, the same method was triplicated.

From the stock solution of 13.9 ug/mL, lower concentrations of 2 ug/mL, 4 ug/mL, 6 ug/mL, 8 ug/mL, 10 ug/mL were made in six different volumetric flasks of 10 mL each with the following volumes 1.439 mL, 2.878 mL, 4.317 mL, 5.755 mL and 7.194 mL pipetted from the stock solution respectively, and the volume made up to the meniscus with osmotic water. The volumetric flasks were then taken to the UV spectrophotometer and the absorbance was recorded at a wavelength of 241 nm.

2.2.2 Filter selection test

2.2.2.1. PVDF and Cannula filters

The filter selection test was performed by making a stock solution of the carvedilol reference sample. From carvedilol dosage of 12.5 mg, a stock solution of 13.9 ug/mL was made by dissolving about 1.39 mg of the sample in 20 mL methanol in a 100 mL volumetric flask and making up the volume to meniscus level with osmotic water. From the stock solution above, lower concentrations of 4 ug/mL, 8 ug/mL, 12 ug/mL were made by doing appropriate dilutions of the stock solution. The absorbance was read in the UV spectrophotometer and recorded at 241 nm.

Then, syringe filters of diameter 0.45 um and 25 mm pore size was used to filter each of the 50 mL solutions and the absorbance of the filtrates was also recorded. The percentage recovery was then calculated in Microsoft excel as follows; (absorbance of the filtrate/ absorbance of the stock solutions) x 100. The same procedure was carried out for the cannula filter of pore size 1 um.

2.2.2.2. Centrifugation method

Centrifugation was done because the carvedilol sample did not yield 100% release with both the cannula filter and the PVDF filter, this shows that there is a retention of the drug when those filters are used, therefore, the need for centrifugation. The centrifugation was done by making a stock solution of 13.9 ug/mL of the sample by dissolving about 1.39 mg of carvedilol in a 100 mL volumetric flask with methanol and making up to volume with osmotic water.

The absorbance of the stock solutions was recorded and the solutions were centrifuged for about 15 min at a speed of 4000 rpm. Then 3 mL of the centrifuged solution was carefully pipetted into test tube and the absorbance recorded. The percentage recovery was then calculated by dividing the absorbance of the centrifuged solution by the absorbance of the stock solution and multiplying the result by 100%.

2.2.3. Solubility test

The shake flask method or the equilibrium method was used to carry out a solubility test determination of the carvedilol sample. Different dissolution media such as water (H_2O), phosphate buffer pH 6.8, phosphate buffer pH 7.2, 0.1N HCl and 0.05M acetate buffer pH 4.5 was used to perform the test in order to determine in which of the medium, the drug has the highest solubility.

About 20 mg of the carvedilol sample was weighed in the analytical balance and transferred to a 100 mL volume flask, 20 mL of the dissolution medium was carefully measured and transferred to the 100mL flask to make a saturated solution. The procedure was triplicated and the flasks well covered and taken to the shake flask equipment (Tecnal, Sao Paulo, Brazil). The equipment was set at 37°Cand 150 rpm and allowed to shake the flasks for 72 hours.

At the end of the 72 hours run of the equipment, the flasks containing the saturated solution were brought out and about 5 mL of the saturated solution was pipetted out into the test tube and put into the centrifuge, the solution was allowed to centrifuge for 15minutes at 4000rpm. Then about 2 mL of the centrifuged solution was pipetted into another test tube and proper dilutions was done and carried to the UV Spectrophotometer to read the absorbance values at a wavelength of 241 nm. The absorbance value obtained was then used to calculate the solubility of the drug in each of the dissolution medium.

The solubility was calculated from the absorbance value by making use of the equation of linearity of the analytical curve of carvedilol. The Equation 1 was used in calculating the solubility of the carvedilol sample;

$S = ((((y+0.0028)/0.1122) *DF)/1000) *250$ …….. Equation 1

Where S = solubility (mg/mL), y= absorbance value, DF = dilution factor, 250= solubility of drug in 250 mL of water according to the BCS.

2.2.4. Dissolution test method

2.2.4.1. Design of the experiments

A complete fractional experimental design (Table1) of the dissolution experiment was performed by considering two factors and two levels; the dissolution medium (acetate buffer and HCl) and the rotation speed (50 rpm and 75 rpm). Therefore, a complete factorial design of 2^2 was obtained which gave rise to 4 experiments.

Table 1. Complete fractional design of the dissolution experiment

Design: 2**(2-0) design = 4 experiments		
Run	**Velocity (rpm)**	**Medium**
4	50	Acet. Buffer, pH 4.5
1	75	0.1 N HCl
3	75	Acet. Buffer, pH 4.5
2	50	0.1 N HCl

2.2.4.2. Dissolution test

The dissolution tests were performed by using an USP Dissolution Apparatus 2- Paddle, Agilent Technologies 708-DS dissolution device (Agilent USA). The assays were done by following the results obtained from the method development. Two dissolution media (acetate buffer, pH 4.5) were the drug has the highest solubility and (0.1 N HCl) to ensure a discriminatory method were both employed for the dissolution of the medicines. Both the rotation speed of the equipment and the dissolution medium were selected based on the factorial design above, the temperature of the equipment was set at 37 °C to simulate in vivo drug release.

Three tablets from the batch of carvedilol medicine donated by Brazilian pharmaceutical companies (Baldacci and Biolab) was used in carrying out the dissolution test under conditions observed from the method development and the fractional experimental design in order to obtain a discriminatory dissolution test

method. After about 5, 10, 15, 20, 30, 45, and 60 min of the assay, about 5mL of aliquots was withdrawn and the vessel volume was compensated with same volume of the fresh dissolution medium to maintain sink condition. The aliquots were subjected to centrifugation at a speed of 4000 rpm for 15 min, then carried to the UV spectrophotometer (EVOLUTION 201) to read off the absorbance. The dissolution profile was expressed as graph of percentage drug dissolved versus time.

A dissolution test of a simulated sample of carvedilol was done with an increased binder content to challenge if the selected dissolution method is discriminatory. The increased binder content was to retain the drug in the formulation. The ingredients used in the formulation of a CVD tablet of weight 150 mg include; carvedilol – 12.5 mg (drug), MCC 102 – 53.5 mg (binder), calcium phosphate – 80 mg (binder), magnesium stearate – 4 mg (diluent).

3. RESULTS AND DISCUSSIONS

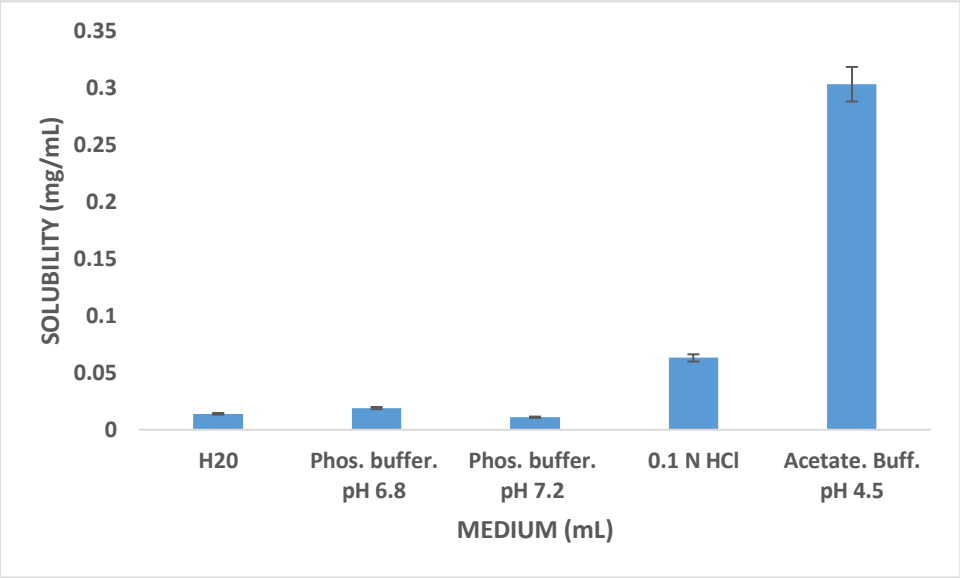
As can be shown in Table 2, the solubility of carvedilol sample in the different media of H_2O, acetate buffer pH 4.5, phosphate buffer pH 6.8, phosphate buffer pH 7.2, and 0.1 N HCl varies significantly from each other. The results show that carvedilol has lowest solubility in phosphate buffer, pH 7.2 of about 2.4 mg/mL in 250 mL, followed by its solubility in H_2O of about 2.9 mg/mL. It shows a considerable high solubility in 0.1 N HCl with a solubility value of about 13.6 mg/mL in 250 mL, but a highest solubility of the drug was observed in acetate buffer, pH 4.5, with a solubility value of about 75.9 mg/mL. Therefore, the two media (0.1 N HCl and acetate buffer, pH 4.5) where considered for dissolution test.

Table 2: Solubility test result of carvedilol in different media (UV: 241 nm)

Medium	Absorbance (nm)				Dil. Factor	Solubility		
	A	B	C	Mean		(ug/ mL)	(mg/mL)	(mg/ mL) * 250
H2O	0.339	0.332	0.305	0.325	4	13.7	0.01	2.5
Phos. Buffer, pH 6.8	0.350	0.362	0.366	0.359	5	18.9	0.02	5.0
Phos. Buffer, pH 7.2	0.238	0.275	0.270	0.261	4	11.1	0.01	2.5
0.1 N HCl	0.600	0.638	0.587	0.608	10	63.4	0.10	25.0
Acet. Buffer, pH 4.5	0.360	0.324	0.331	0.338	100	303.7	0.30	75.0

The Figure 2 shows the graph of the solubility of carvedilol in different dissolution medium. From the graph, it showed that carvedilol has the highest solubility in acetate buffer, pH 4.5 followed by its dissolution in 0.1 N HCl.

Figure 2: Solubility of carvedilol in different media



The Table 3 showed that carvedilol has some interactions with the cannula filter as it produces a % recovery of 41.4% at a concentration of 4 ug/mL and a discard volume of 6 mL, and a % recovery of 64.9% at a concentration of 8 ug/mL, even at a higher concentration of 12 ug/mL a % recovery of about 80.9% was observed.

Table 3: Filter selection test of carvedilol with cannula

Conditions	Disc. Vol. (mL)	Absorbance (nm) A	Absorbance (nm) B	Recu. (%) A	Recu. (%) B	MEAN	SD
4 ug/mL ABS. =0.270 nm	0	0.034	0.035	12.59	12.96	12.78	0.26
	2	0.044	0.071	16.30	26.30	21.30	7.07
	4	0.084	0.103	31.11	38.15	34.63	4.98
	6	0.106	0.132	39.26	48.89	44.07	6.81
8 ug/mL ABS. = 0.5905 nm	0	0.255	0.219	43.18	37.09	40.14	4.31
	2	0.382	0.370	64.69	62.66	63.67	1.44
	4	0.402	0.441	68.08	74.68	71.38	4.67
	6	0.443	0.430	75.02	72.82	73.92	1.56
12 ug/mL ABS. = 0.9165 nm	0	0.399	0.405	43.54	44.19	43.86	0.46
	2	0.578	0.551	63.07	60.12	61.59	2.08
	4	0.599	0.654	65.36	71.36	68.36	4.24

6	0.661	0.660	72.12	72.01	72.07	0.08

The Table 4 showed the analysis of variance of CVD with cannula filter, the analysis shows a p-value of about 0.003, which is less than the 0.05 level of significant, meaning there is a significant interaction between the CVD sample and cannula filter, which means that cannula is not suitable for CVD filtration.

Table 4. Analysis of variance of cannula filter for carvedilol

Source	Df	ss	ms	F-value	p-value
Conditions	2	4717	2358.5	12.03	0.003
Error	9	1765	196		
Total	11	6482			

Figure 3 showed a confidence interval graph of percentage recovery against varying concentrations of carvedilol sample, the graph shows an increasing percentage recovery with an increase in concentration of CVD, it shows a more % recovery of 80.9% at a concentration of 12 ug/mL.

Figure 3. Percentage recovery against different concentrations of CVD sample in cannula filter

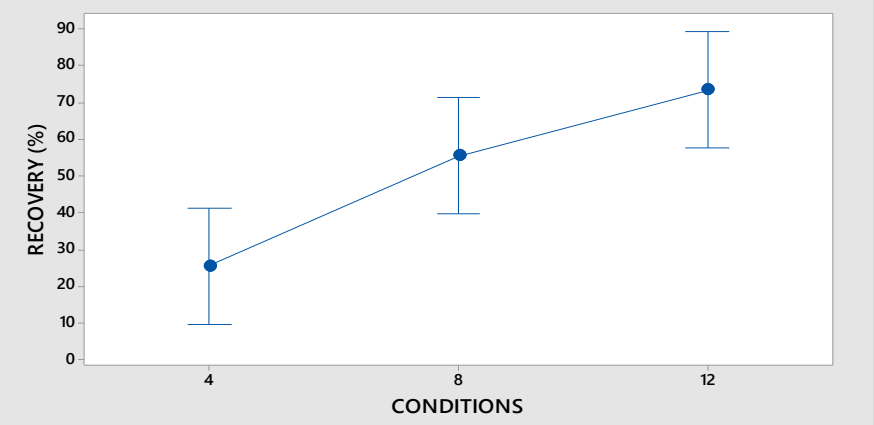


Table 5 shows that carvedilol when filtered through a PVDF at a concentration of 4 ug/mL yields a 44.1% recuperation, at a concentration of 8 ug/mL, a yield of 73.9% recuperation was observed and at a higher concentration of 12 ug/mL, it produces a recuperation of about 72.1% which shows that carvedilol has some interaction with PVDF.

Table 5. Filter selection test of carvedilol with PVDF

Conditions	Disc. Vol. (mL)	Absorbance (nm)	Recu. (%) A	Recu. (%) B	MEAN	SD

		A	B				
4 ug/mL ABS. =0.270 nm	0	0.034	0.035	12.59	12.96	12.78	0.26
	2	0.044	0.071	16.30	26.30	21.30	7.07
	4	0.084	0.103	31.11	38.15	34.63	4.98
	6	0.106	0.132	39.26	48.89	44.07	6.81
8 ug/mL ABS. = 0.5905 nm	0	0.255	0.219	43.18	37.09	40.14	4.31
	2	0.382	0.370	64.69	62.66	63.67	1.44
	4	0.402	0.441	68.08	74.68	71.38	4.67
	6	0.443	0.430	75.02	72.82	73.92	1.56
12 ug/mL ABS. = 0.9165 nm	0	0.399	0.405	43.54	44.19	43.86	0.46
	2	0.578	0.551	63.07	60.12	61.59	2.08
	4	0.599	0.654	65.36	71.36	68.36	4.24
	6	0.661	0.660	72.12	72.01	72.07	0.08

Table 6 shows the analysis of variance of CVD with PVDF filter, the ANOVA showed a p-value of about 0.011, which is less than the 0.05 level of significant, meaning, there is a significant interaction between the carvedilol sample and PVDF, which means that this filter is not suitable to filter carvedilol.

Table 6. Analysis of variance of PVDF filter for carvedilol

Source	Df	ss	ms	F-value	p- value
Conditions	2	3026	1513.1	7.74	0.011
Error	9	1759	195.5		
Total	11	4785			

Table 7 showed that CVD centrifugation yields a 100% recovery at all concentrations (5 ug/mL, 10 ug/mL, 15 ug/mL) which means, it is ideal for the CVD.

Table 7. CVD recovery after centrifugation method

Stock sol. (ug/mL)	Abs. of stock sol.(nm)	Abs. of sol. After centrifugation (nm)		Reco. A (%)	Reco. B (%)	mean % reco.
		A	B			
5 ug/mL	0.314	0.312	0.317	99.36	100.96	100.16
10 ug/mL	0.632	0.635	0.631	100.47	99.84	100.16
15 ug/mL	0.934	0.938	0.934	100.43	100.00	100.21

Reco. = recovery

Table 8 is a dissolution test of CVD (Divelol®) in acetate buffer, pH 4.5 at 75 rpm and 50 rpm. From the table, it was observed that at 20 minutes, the drug has already released 100% of its active ingredients when the rotation speed of the paddle is maintained at 75 rpm. With 75 rpm, additionally, more than 80% of the API is released within 5 min of the assay while an equivalent amount of drug is released from 10 min of the test with a paddle velocity of 50 rpm. It was also observed from the table that the drug did not release all API content even at 60 min of the test with 50 rpm, except at infinity with an increased rotation speed of about 250 rpm. The standard deviation gave a uniform value from 60 min to infinity when 75 rpm was used showing a total release of the active ingredients in 60 min, but when 50 rpm was used, there was a 98.5 % release of the API at 60 min, also, a large margin of difference in the standard deviation from 60 min to infinity with a value of 4.1 and 11.3 respectively; this shows a retention of the drug and incomplete release. Based on these results, 75 rpm produces a better release of the drugs in acetate buffer pH 4.5 than 50 rpm.

Table 8. Dissolution Test of CVD in acetate buffer pH 4.5

Time	Mean % dissolved	
	75 rpm	50 rpm
0	0	0
5	86.3± 5.5	79.7± 5.8
10	91.2± 3.8	89.9± 3.2
15	98.1± 3.9	93.8± 3.0
20	100.4± 3.3	95.5± 4.8
30	103.3± 1.5	95.7± 2.1
45	104.3± 1.3	95.7± 1.1
60	104.5± 1.1	98.5± 4.1
Infinity	104.5± 1.1	104.3±11.3

Figure 4 showed the release pattern of the drug. It was observed from the graph that at 20 minutes, there is already a 100% release with the 75 rpm. The drug shows a uniform release pattern from 20 min to 60 min with the 75 rpm. The graph of 50 rpm showed that the drug released less than 100 % from 5 min to 60 min of the assay. The profile shows a more rapid drug release with 75 rpm than 50 rpm. The profile also shows that the drugs are immediate release as more than 80% of the API was released within 10 min of the assay. The profile shows a high standard deviation in dissolution of the drugs from the start of the assay.

Figure 4. Dissolution profile of CVD in acetate buffer pH 4.5

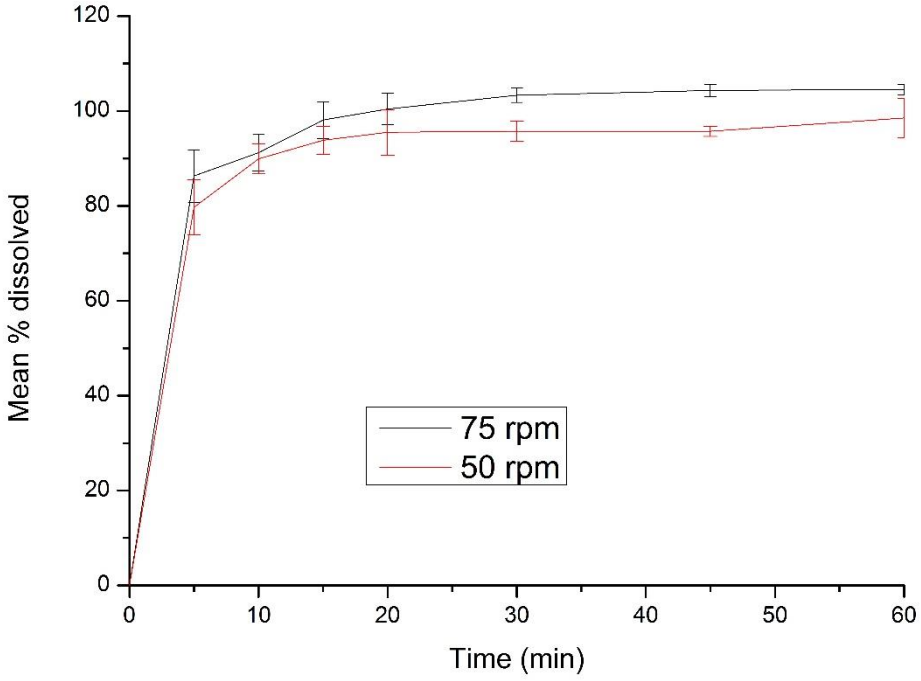


Table 9 showed the dissolution test of carvedilol in 0.1 N HCl at 75 rpm and 50 rpm. The Table shows that within 5 min of the assay, the drug has released more than 80% of its API with a rotation speed of either 75 rpm or 50 rpm. It was also observed that at a speed of 75 rpm, the drug releases about 100% of its API within 20 min, while an equivalent amount was released at a speed of 50 rpm within 45 min. The result also showed that within 60 min of the test, the drug has released almost all its API as more than 100% release was observed in both rotation speed of 75 rpm and 50 rpm.

The standard deviation (SD) did not vary significantly at infinite time of the assay, with the 75-rpm and 50-rpm speed having SD values of 1.9 and 1.9 respectively. This means that a change in the velocity of the paddles does not significantly affect the release pattern when 0.1 N HCl was used as the dissolution medium.

Table 9. Dissolution test of carvedilol in 0.1 N HCl

Time	Mean % dissolved	
	75 rpm	50 rpm
0	0	0

5	98.1 ± 0.6	87.6 ± 1.6
10	98.7 ± 0.6	92.1 ± 0.4
15	99.1 ± 1.3	95.3 ± 1.5
20	100.6 ± 1.7	95.7 ± 1.5
30	100.9 ± 1.4	99.1 ± 1.9
45	101.6 ± 0.6	100.6 ± 1.7
60	102.5 ± 0.6	102.5 ± 2.3
Infinity	103.8 ± 1.9	103.3 ± 1.9

Figure 5 showed the dissolution profile of carvedilol in 0.1 N HCl at 75rpm and 50 rpm, from the profiles, it showed that CVD has a more rapid liberation pattern when the paddles are set at a speed of 75 rpm. It also showed that from 30 min to 60 min of the assay, the drug releases almost the same amount of API in both the rotation speeds.

Figure 5. Dissolution profile of carvedilol in 0.1 N HCl

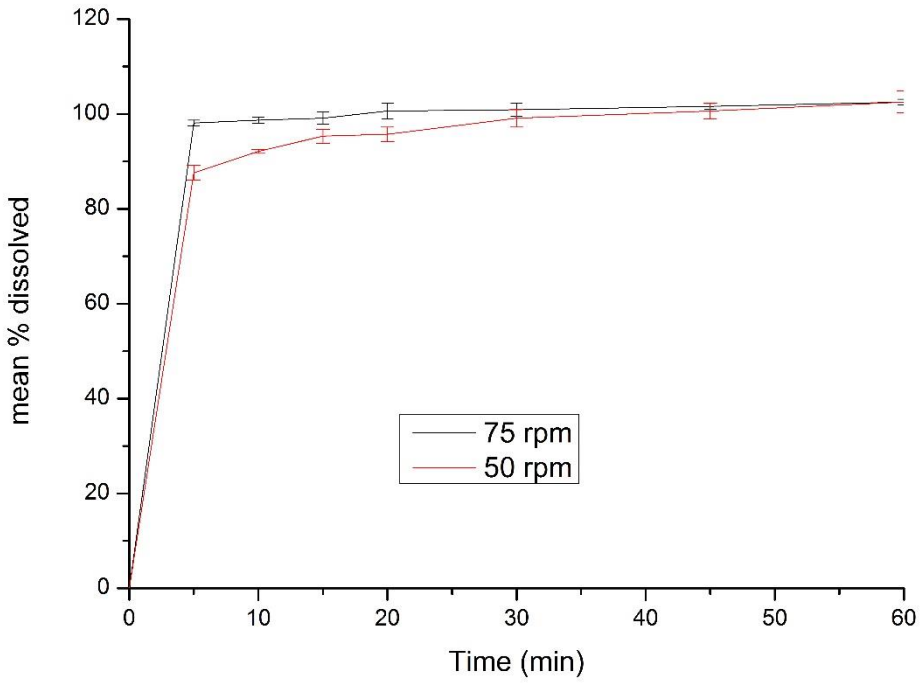


Table 10 represents the dissolution efficiency (ED) of Divelol in different dissolution media and rpm, the table shows a 96% ED of Divelol in both acetate buffer, pH 4.5 and 0.1 N HCl at 75 rpm, and 93% and 90% ED in 0.1 N HCl and acetate buffer respectively at 50 rpm.

Table 10. Dissolution efficiency (ED) of Divelol brand of CVD in different media and rpm

Brand	Medium	RPM	ED (%)
Divelol	acet. Buffer, pH 4.5	75	96
Divelol	acet. Buffer, pH 4.5	50	90
Divelol	0.1 N HCl	75	96
Divelol	0.1 N HCl	50	93

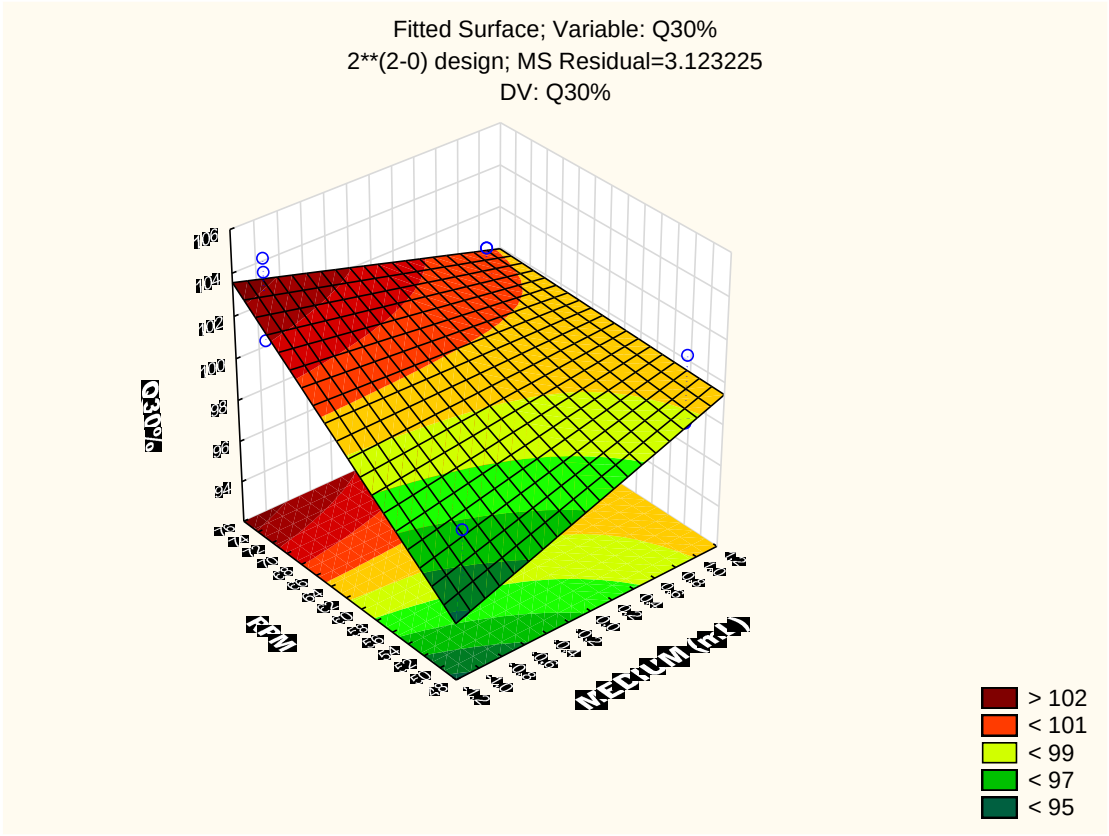
Table 11 shows the analysis of variance (ANOVA) of the dissolution test of the CVD in the two different media of acetate buffer pH 4.5 and 0.1 N HCl, at different rotation speeds of 75 rpm and 50 rpm. The ANOVA gave a p-value of about 0.001, which is less than the 0.05 significant level; this means that there is a significant difference in the results of the dissolution. The difference may be in the medium used or the rotation speed employed.

Table 11. ANOVA of the dissolution of CVD in acetate buffer, pH 4.5 and 0.1 N HCl at 75 rpm and 50 rpm

Source	Df	ss	ms	F-value	p-value
Conditions	6	580.5	96.75	5.76	0.001
Error	21	352.8	16.80		
Total	27	933.3			

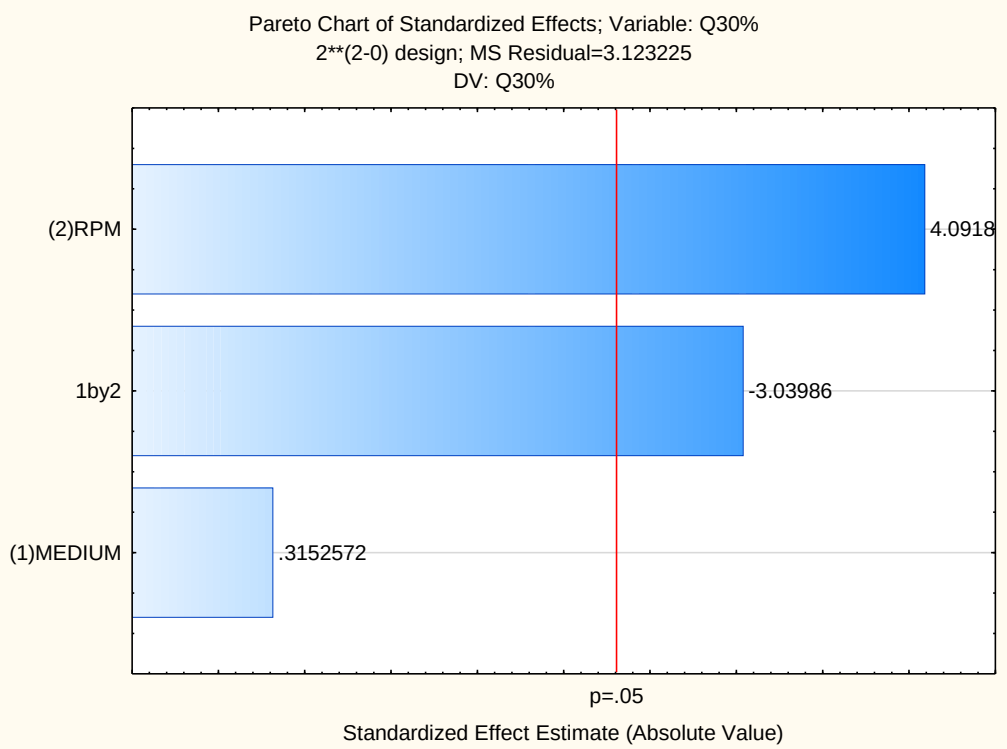
From the surface response graph (SRg) in Figure 6, it was observed that a high % drug release is obtained at increasing RPM and pH of the medium, considering the colour shades of the graph. This means that 75 rpm will produce a more drug release than 50 rpm.

Figure 6. Surface response graph (SRg) of the rpm against medium



The Pareto chart in Figure 9 shows a comparison of the rotation speed with medium, from the chart, it was observed that a comparison of the 50 rpm and 75 rpm has a significant difference. A comparison of the RPM and Medium also shows a significant difference, while a comparison of the two media (0.1N HCl and Acetate buffer, pH 4.5) showed no significant difference. This means that either of the two medium (acetate buffer, pH 4.5 and 0.1 N HCl), is suitable for the assay at a velocity of 75 rpm.

Figure 9. Pareto chart of standardized effects of the rpm and medium



4. METHOD CHALLENGE

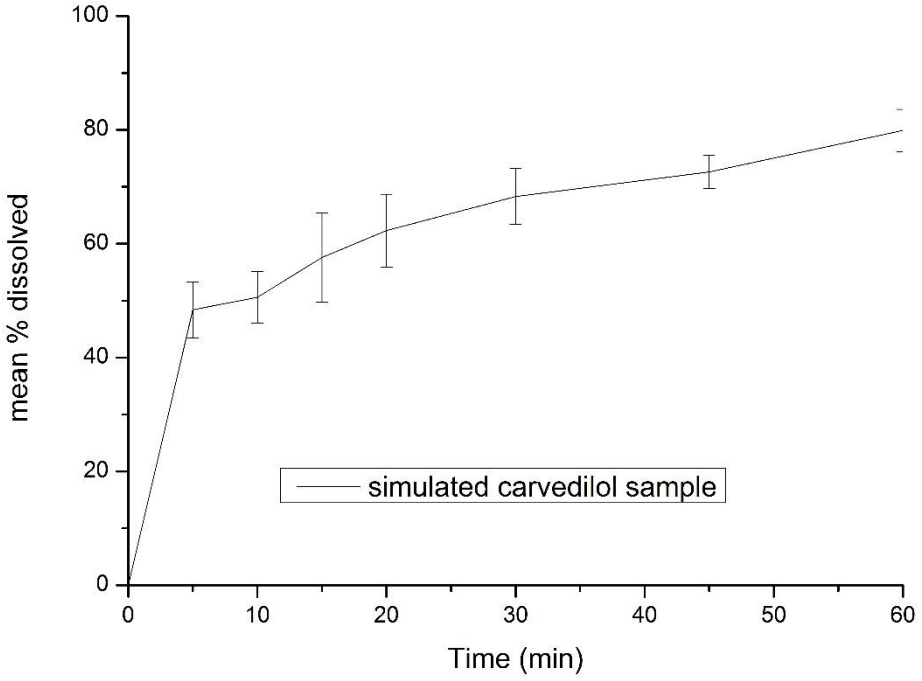
Table 12 is the result of the dissolution test of the simulated formulation of CVD in the selected medium and rpm. The table shows that the drug releases less than 100 % of the API in 60 min of the assay, even at infinity, 80.9 % was liberated showing a possible retention of the active ingredients in the formulation, this proves that the selected method of dissolution was discriminatory, because with the market brand of medicine, a 100 % of the API was released.

Table 12. Dissolution test of simulated carvedilol in the selected dissolution method (acetate buffer, pH 4.5, 75 rpm)

Time (min)	Mean % dissolved
0	0
5	48.4± 4.9
10	50.6± 4.5
15	57.6± 7.8
20	62.3± 6.4
30	68.3± 4.9
45	72.6± 2.9
60	79.9± 3.7
Infinity	80.9± 4.3

Figure 10 shows the dissolution profile of the CVD in the selected medium of acetate buffer, pH 4.5 and 75 rpm. The profile shows a non-uniform release of the active ingredients, it also showed that a less than 100% of the API is released in 60 min. When the profile is compared with that of the market brand of CVD, it can be ascertained that the method selected is discriminatory as a uniform release of the drug was observed with the market brands.

Figure 10. Dissolution profile of the Simulated CVD in the selected dissolution method



5. CONCLUSION

Development of the dissolution method for carvedilol showed that the drug has very high solubility of 303.7 ug/mL in acetate buffer, pH 4.5, it also has a considerable solubility of about 63.4 ug/mL in 0.1 N HCl. Centrifugation is the best method for carvedilol. A dissolution study shows a 100 % release of the drug in either of the media. Pareto chart of standardized effects show that a high velocity of the paddles (75rpm) yields a better result than 50 rpm. Surface response graph showed a significant difference in dissolution between the 75

rpm and 50 rpm. Analysis of variance of the methods showed a statistical significant difference in the rotation speeds but from the pharmaceutical point of view, there is no difference as both methods yielded 100% release of the drug. The selected dissolution method was challenged with a simulated carvedilol sample and it was observed that the method is discriminatory. Therefore, can be concluded that the best method for the dissolution test of carvedilol is centrifugation in 900 mL acetate buffer of pH 4.5 at 75 rpm for 60 minutes.

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CHAPTER 3

DISSOLUTION PROFILE OF DIFFERENT BRANDS OF LOW SOLUBILITY DRUGS AVAILABLE IN THE NIGERIAN AND BRAZILIAN PHARMACEUTICAL MARKETS

ABSTRACT

The present work describes the dissolution profiles of different brands of furosemide, glibenclamide, albendazole, ibuprofen, carvedilol and hydrochlorothiazide tablets available in the Nigerian and Brazilian pharmaceutical markets. The dissolution test was performed by following the recommendation of the United States Pharmacopeia (USP), FDA and developed method (carvedilol). A filter selection test was done for the drugs; the results obtained indicated that cannula filter was ideal for all the drugs with the exception of carvedilol which was centrifuged. The ANOVA of the filter selection showed no significant retention of drug with cannula filter ($p > 0.05$), with the exception of carvedilol ($p< 0.05$). The data obtained from the dissolution test was subjected to statistical analysis in a Microsoft excel and Minitab 17 (USA). Dissolution efficiency (%DE) was calculated for the formulations to evaluate their in vitro biopharmaceutical features. Tukey grouping, ANOVA and confidence interval (CI) were obtained for the comparison of the results. The ANOVA of the results indicated that the brands of (albendazole, ibuprofen, furosemide, glibenclamide, & carvedilol) were statistically different ($p< 0.05$). Hydrochlorothiazide brands were pharmaceutical equivalents ($p > 0.05$). A comparison of the results showed that 94.1% and 58.8% of the Brazilian and Nigerian brands passed respectively.

KEYWORDS: Dissolution profile, Dissolution test, Low solubility drugs, Tablets

1. INTRODUCTION

Drug dissolution testing is an analytical technique used to assess release profiles of drugs from medicines, generally, from oral products such as tablets and capsules. It plays an important role as a routine quality control test, for characterizing the quality of a product (FDA, 2015). Dissolution profile comparison has been extensively used to assess drug product similarity after scale-up and post approval changes (FDA, 2007).

Dissolution testing is considered to be one of the important parameters for ascertaining drug quality (ZHU et al., 2009). This process involves the breakdown of tablets into granular or fine particulate form followed by de-aggregation, leading to its availability for systemic circulation (RAHUL et al., 2017). It also involves the interaction of solid drug with the medium resulting in the movement of drug molecules into the bulk solution (QIU et al., 2016).

Systemic absorption of drugs is a prerequisite for eliciting their therapeutic activity, whenever given non-instantaneously. Drugs of low solubility have to be evaluated for *in vivo* bioavailability, thus, generic manufacturers must provide detailed bioequivalence evidence showing head-to-head comparative performance of their product against reference (FDA, 2017).

Considering that dissolution is an *in vitro* method that characterizes how an API is extracted out of a solid dosage form, it is related with the initial stages after oral administration, that comprises the disintegration and dissolution process, thus it is a very useful tool to evaluate the quality of immediate release tablet formulations, mainly derived from class II and IV (low solubility drugs) of BCS (FDA, 1997; FERRAZ; CARPENTIERI; WATANABE, 2007; PITA; PRATES; FERRAZ, 2004).

The Table 3 in chapter 1 shows the various works, which have been done on the dissolution test of various brands of low solubility drugs available in the African pharmaceutical market. It can be observed from the table that most of the low solubility drugs marketed in the African market do not pass the dissolution test. It is equally shown that most of the dissolution tests have been for a long time (5 years, 7 years, 8 years, 10 years and 13 years), therefore, this present work will focus on the current evaluation of the available products in the African market.

However, Table 4 in chapter 1 shows the various works done on the dissolution test of Brazilian brands of low solubility drugs, it was observed from the results that 100 % of the drugs marketed in Brazil passed the dissolution test. It further illustrates that Brazilian brands of medicines comply with Pharmacopeial standard. As a result, the objective of this present work was to perform dissolution test of the different brands of low solubility drugs (albendazole, ibuprofen, glibenclamide, furosemide, hydrochlorothiazide and carvedilol) available in the Nigerian and Brazilian Pharmaceutical markets, by using the established methods (USP, FDA) to elaborate a diagnosis of the dissolution test in Nigeria in comparison with Brazil, evaluating the dissolution profile of the low solubility drugs to ascertain their quality.

2. Materials and methods

2.1. Material

2.1.1. Drug

The Brazilian brands of medicines were acquired from pharmaceutical shops in the city of Sao Paulo. Prof. Sabinus Ofoefule of the University of Nigeria bought the Nigerian brands from different Pharmacy stores in Nigeria. The various brands of Brazilian and Nigerian medicines utilized in this present work are shown in the table below.

Table 2. Nigerian and Brazilian products utilized in the study

DRUG	BRAZIL			NIGERIA		
ALBENDAZOLE	ALBBR1	ALBBR2	ALBBR3	ALBNG1	ALBNG2	ALBNG3
IBUPROFEN	IBUBR1	IBUBR2	IBUBR3	IBUNG1	IBUNG2	IBUNG3
FUROSEMIDE	FURBR1	FURBR2	FURBR3	FURNG1	FURNG2	FURNG3
GLIBENCLAMIDE	GLIBR1	GLIBR2	GLIBR3	GLING1	GLING2	GLING3
HYDROCHLOROTHIAZIDE	HYDBR1	HYDBR2	HYDBR3	HYDNG1	HYDNG2	HYDNG3
CARVEDILOL	CARBR1	CARBR2	....	CARNG1	CARNG2	....

2.2. Methods

2.2.1. Dissolution test method

The dissolution test for albendazole, ibuprofen, furosemide and hydrochlorothiazide was done by following the criteria recommended by the United States Pharmacopeia (USP, 2016). Glibenclamide was assayed by using the FDA method, while for carvedilol; a dissolution test method was developed for its assay. Apparatus 2 (paddle) was utilized for all the dissolution tests. A quantification of each drug was realized through a calibration curve which was previously constructed for each of the drugs, a straight-line graph generated from the calibration curve was used to calculate the percentage of drug released, the percentage of drug released was plotted against time to generate a dissolution profile. The filter used was previously subjected to a filter selection test and cannula was ideal for all the drugs, with the exception of carvedilol which was centrifuged.

Table 3 shows the dissolution test method for each of the drug and their tolerance range.

Table 3. Dissolution test method for the medicines

Drug	Medium	RPM	UV (nm)	Tolerance (NLT)
albendazole	0.1 N HCl, 900 mL	100	311	80 % in 30 min
ibuprofen	phosphate buffer, pH 7.2, 900 mL	50	228	80 % in 60 min
furosemide	phosphate buffer, pH 5.8, 900 mL	50	274	80 % in 60 min
glibenclamide (FDA method)	phosphate buffer, pH 7.5, 900 mL	50	208	80 % in 60 min
hydrochlorothiazide	0.1 N HCl, 900 mL	100	272	60 % in 60 min
carvedilol (methoddevelopment)	acetate buffer, pH 4.5, 900 mL	75	241	80 % in 60 min

NLT = not less than

2.2.1.1. Statistical analysis

The results obtained from the dissolution test was analyzed statistically by using Minitab 17 (Minitab, USA) application. This was done to determine the ANOVA, Tukey grouping and confidence interval of the samples. The percentage of drug dissolved as a function of time was used in the statistical analysis. The results of the analysis will enable to know if the medicines have significant difference between them or they are similar.

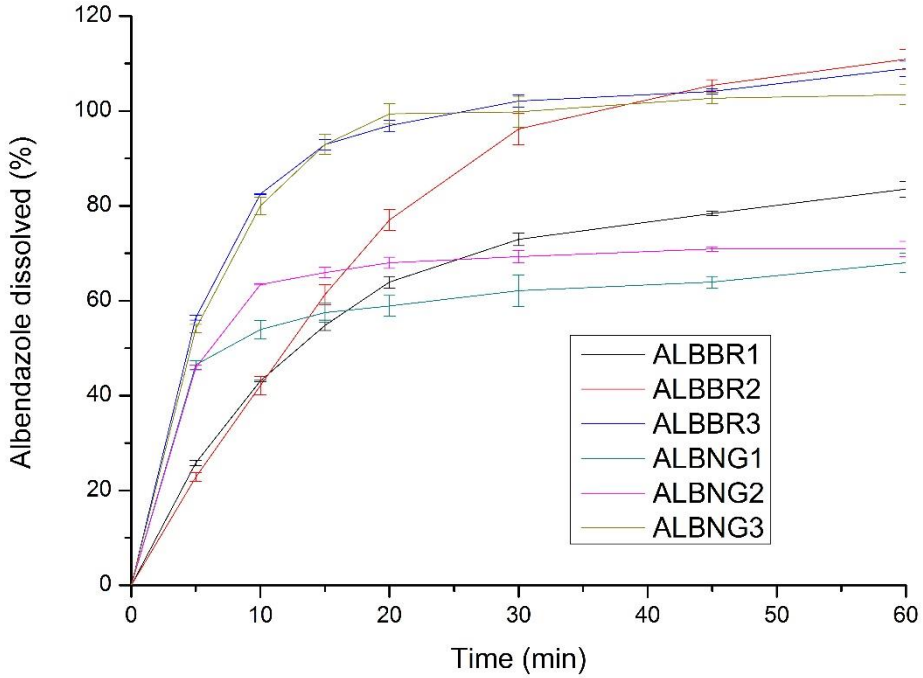
3. Results and Discussions

3.1. Albendazole

Figure 6 shows the dissolution profile of the brands of albendazole. From the graph also, it shows that ALBBR3 brand has more rapid release pattern followed by the ALBNG3 brand and then ALBBR2 respectively. The graph also shows that both the ALBBR3 brand and ALBNG3 has the same characteristics release pattern. At 45 min of the assay, ALBBR3, ALBNG3 and ALBBR2 have an equivalent amount of drug released. The results of the dissolution test showed that ALBBR1, ALBNG1 and ALBNG2 brands do not comply with the United States Pharmacopeia recommendation which states that at 30 min of the assay, not less than 80% of the drug will be released; but what was observed was 72.9%, 62.1% and 69.3% release for the ALBBR1, ALBNG1 and ALBNG2 brands respectively. This shows that these medicines do not pass the dissolution test.

It was also observed that, at 30 min of the assay, 96.2 %, 102.1 % and 99.8 % of the active ingredients was released for the ALBBR2, ALBBR3 and ALBNG3 brands of medicines respectively. This is in compliance with the USP recommendations, meaning that these products passed the dissolution test.

Figure 6. Dissolution profile of the brands of albendazole (400 mg)



The ANOVA of the albendazole dissolved gave a p-value of about 0.006, which is less than the 0.05 significant level; this means that the brands of medicines have significant difference between them.

Table 5 is a Tukey test of the brands of albendazole, from the table, it shows that the medicines are placed into groups with ALBBR3 belonging to group A, ALBNG3 belonging to group AB, ALBBR2 and ALBNG2 belonging to group ABC, ALBBR1 belonging to group BC and ALBNG1 to group C. ALBBR3 in group A has some similarities with ALBNG3, ALBBR2 and ALBNG2. ALBNG3 has some similarities with ALBBR2, ALBNG2, and ALBBR1. ALBBR2 has some similarities with ALBNG2, ALBBR1 and ALBNG1. ALBBR3 has significant difference with ALBBR1 and ALBNG1; also, ALBNG3 has significant difference with ALBNG1. In the Tukey grouping, the mean % of drug released against time (min) was considered.

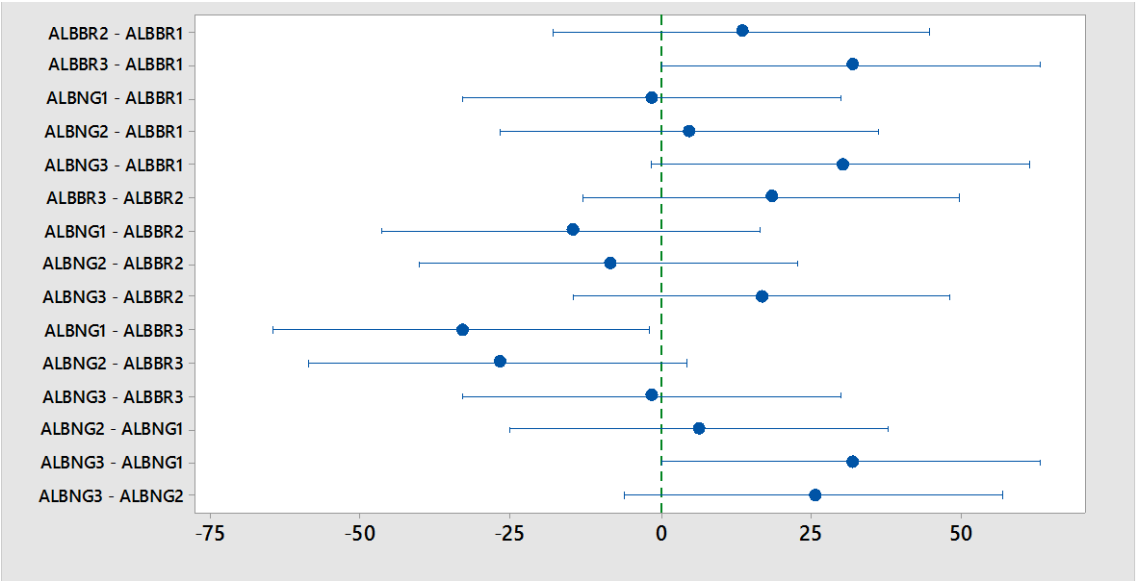
Table 5. Tukey test of the brands of albendazole

Brands	N	Mean	Grouping
ALBBR3	7	91.9	A
ALBNG3	7	90.3	AB

ALBBR2	7	73.7	ABC
ALBNG2	7	64.9	ABC
ALBBR1	7	60.4	BC
ALBNG1	7	58.7	C

Figure 7 shows a 95% Confidence interval of the brands of albendazole. Intervals that contain the zero line such as ALBBR2-ALBNG1, ALBNG2-ALBNG1, ALBBR1-ALBNG1, ALBNG3-ALBBR2, ALBBR3-ALBBR2 and ALBBR3-ALBNG3 has no significant difference between them, while the interval such as ALBBR3-ALBNG1, ALBNG3-ALBNG1 and ALBBR3-ALBBR1 which do not contain the zero line has statistical significant difference.

Figure 7. Confidence Interval of the Tukey grouping of the brands of albendazole



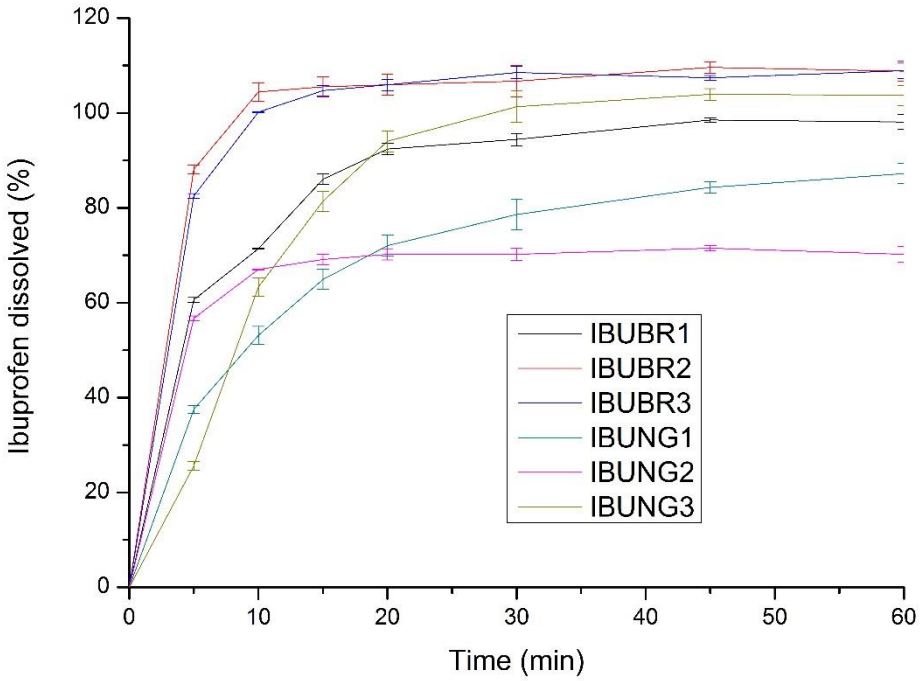
3.2. Ibuprofen

Figure 8 shows the dissolution profile of the brands of Nigerian and Brazilian ibuprofen. The profile shows that IBUBR2 brand of ibuprofen has a more rapid liberation pattern followed by IBUBR3. The graph shows also that within 20 min of the assay, all the medicines have a steady liberation pattern until the 60 min time of the dissolution test. It is also observed from the profile that from 45 to 60 min of the assay, both IBUBR2 and IBUBR3 has almost the same amount of drug release, the same is obtainable at 15 and 20 min of the assay. IBUNG3 and IBUBR1 show the same amount of drug release at 20 min of the assay.

IBUNG2 and IBUNG1 Show the same % drug release at 20 min of the dissolution test also.

Ibuprofen dissolution showed that at 60 min of the assay, all the medicines have released more than 80% of their active ingredients with the exception of IBUNG2, which released 72.2% of its active ingredient. This shows that all the brands passed the dissolution test as they comply with the recommendation of the USP method but the IBUNG2 brand do not pass as it does not comply with the USP method which states that within 60 min of the assay, not less than 80% of the active constituents will be released.

Figure 8. Dissolution profiles of the brands of ibuprofen medicines



The ANOVA of the ibuprofen dissolved gave a P-value of 0.000, which is less than the 0.05 level of significance; this means that the different brands of medicines have significant difference between them.

Table 10 is a Tukey grouping of the brands of ibuprofen. The Tukey grouped the medicines into groups A, AB and B. IBUBR2 and IBUBR3 are grouped into A showing that the two does not have significant difference, IBUBR1 and IBUNG3 are grouped into AB showing that the both medicines does not have

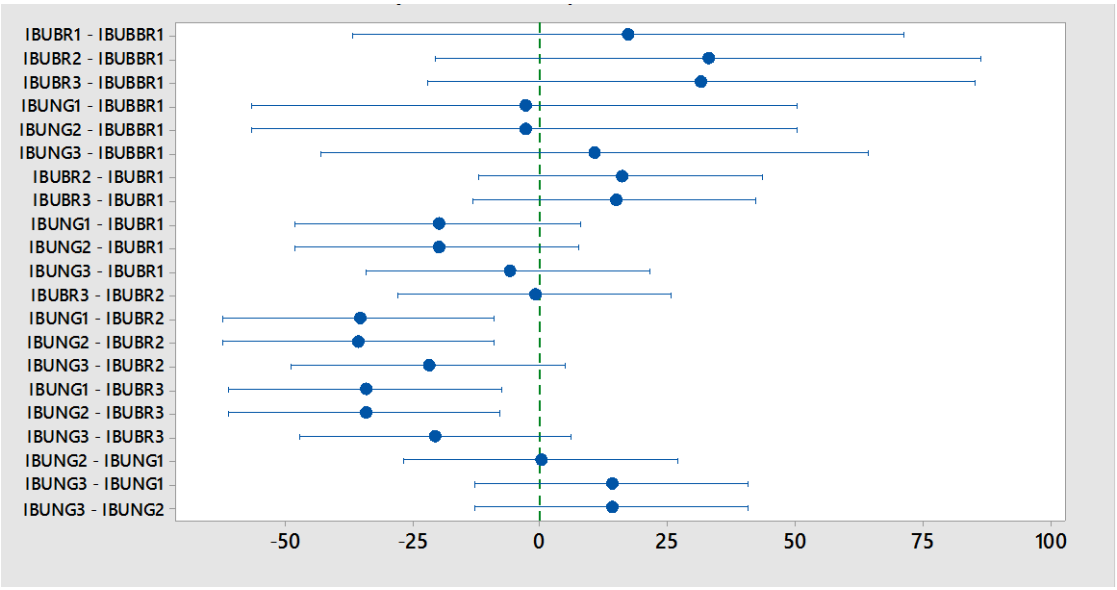
significant difference. IBUNG1 and IBUNG2 are grouped into B showing they do not have significant difference. IBUBR1 and IBUNG3 have some similarities with IBUBR2, IBUBR3, IBUNG1 andIBUNG2 while IBUBR2 and IBUBR3 have significant difference with IBUNG1 and IBUNG2.

Table 6. Tukey test of the brands of ibuprofen medicine

Brands	N	Mean	Grouping
IBUBR2	7	104.2	A
IBUBR3	7	102.6	A
IBUBR1	7	85.9	AB
IBUNG3	7	81.9	AB
IBUNG1	7	68.2	B
IBUNG2	7	67.9	B

Figure 9 is the 95% confidence interval of the different brands of ibuprofen medicine. The interval does a comparison of the different brands based on their differences and similarity to each other. Intervals that contain the zero line do not have significant difference between them. While intervals such as IBUBR3-IBUNG1, IBUBR2-IBUNG1, IBUBR3-IBUNG2 and IBUBR2-IBUNG2, which do not contain the zero line, have significant difference between them.

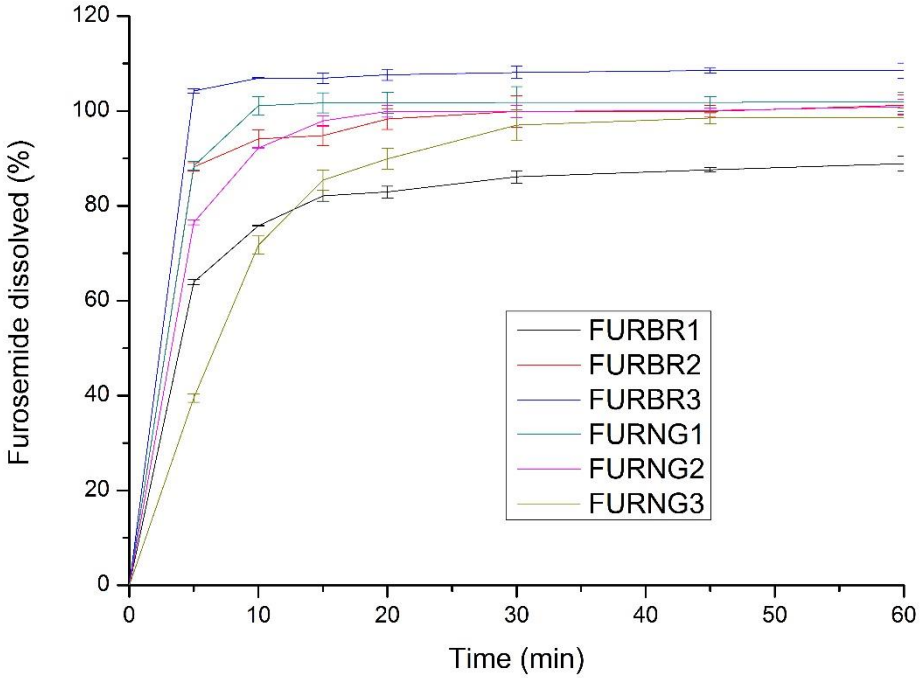
Figure 9: Confidence Interval of the Tukey of the brands of ibuprofen



3.3. Furosemide

The Figure 10 represents the dissolution profile of the brands of furosemide; it shows a steady release of the drug from 30 min of the assay to 60 minutes. The profile shows that FURBR3 has a more rapid release pattern followed by FURNG1, FURBR2, FURNG2 and FURNG3. The profile also shows that at 30 min of the assay, FURBR3 and FURNG1have released more than 100 % of their active ingredient. It was observed from the profile that from 30 min of the assay to 60 min, FURBR2, FURNG1 and FURNG2 released almost the same amount of active ingredients. The rapid release pattern observed with FURBR3 is not due to drug content because all the drugs liberate above 100% at infinity. FURBR1 does not release 100% of the API within 60 min of the assay but it passed the dissolution test because 88.9% of the active constituent was released in 60 min which is in compliance with the pharmacopeia recommendation of not less than 80% in 60 min. Furosemide dissolution showed that at 60 min of the assay, all the brands of the Brazilian and Nigerian furosemide has released more than 80 % of the active ingredients. With FURBR1, FURBR2, FURBR3, FURNG1, FURNG2 and FURNG3 releasing about 88.9 %, 101.2%, 108.5%, 101.2%, 100.9% and 98.6% respectively. These values passed the USP recommendation of not less than 80% in 60 min; this means that all the medicines passed the dissolution test in accordance with the USP method.

Figure 10. Dissolution profile of the brands of furosemide



The ANOVA of the furosemide dissolved gave a P-value of about 0.000, this value is less than the 0.05 significant level. This means that the various brands have significant difference between them.

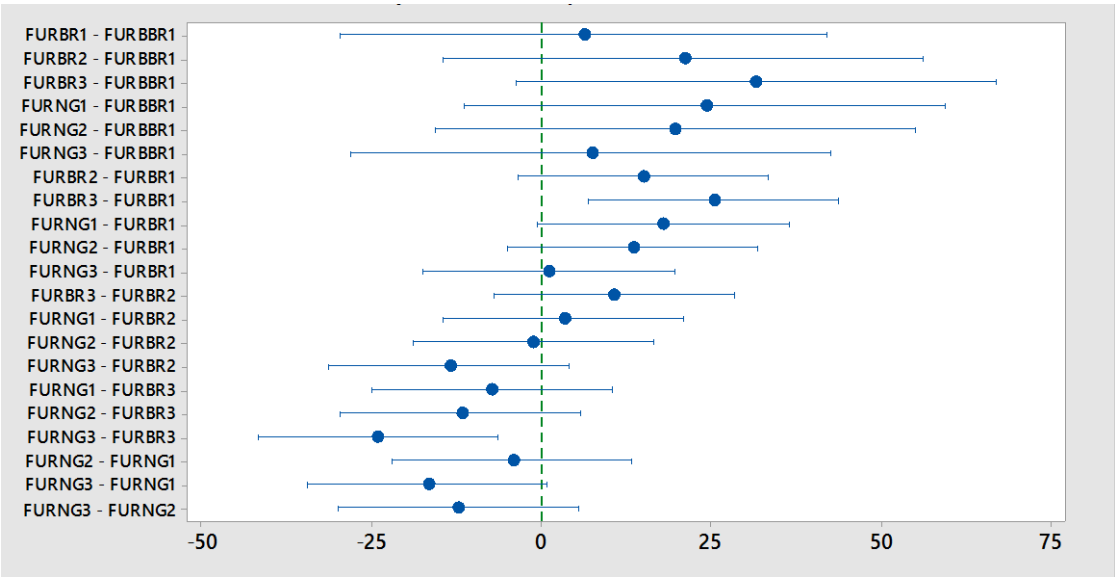
The Table 7 shows the Tukey test of the brands of medicine, from the table, it is observed that the medicines are placed in groups of A, AB, ABC, BC and C, the analysis indicate that FURBR2 and FURNG2 are similar. FURBR3 and FURNG3 have significant difference between them; FURBR3 and FURBR1 have significant difference between them also FURNG1 and FURBR1 have significant difference. FURBR3 and FURNG1 have some similarity; FURNG3 and FURBR1 have some similarity while all the brands have some similarity with FURBR2 and FURNG2.

Table 7. Tukey test of the brands of furosemide

Brands	N	Mean	Grouping
FURBR3	7	107.1	A
FURNG1	7	98.9	AB
FURBR2	7	96.6	ABC
FURNG2	7	95.3	ABC
FURNG3	7	82.9	BC
FURBR1	7	81.1	C

The Figure 11 is a 95% confidence interval of the brands of medicines. The interval is a comparison of each brand with another. It shows that FURBR3-FURNG2, FURNG1-FURNG2, FURBR2-FURNG2, FURBR2-FURBR1 and FURNG3-FURBR1 do not have significant difference between them as they contain the zero line of the interval. While FURBR3-FURBR1, FURNG1-FURBR1, FURNG3-FURBR3 and FURNG3-FURNG1 which do not contain the zero line have significant difference between them.

Figure 11. Confidence Interval of the Tukey of the brands of furosemide medicines



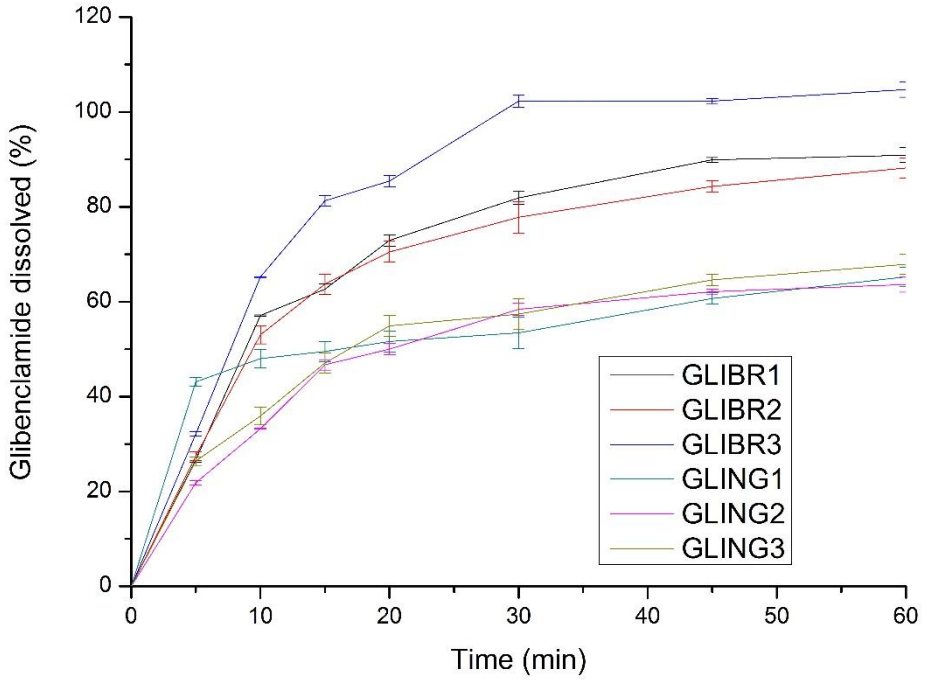
3.4. Glibenclamide

The Figure 12 is a dissolution profile of the brands of glibenclamide. GLING1 has the most rapid release pattern. The profile also shows that GLIBR1 and GLIBR2 have close liberation characteristics while GLING1, GLING2 and GLING3 also show close release characteristics. The figure also showed that the brands of GLING1, GLING2 and GLING3 do not liberate up to 80% of the API at 60 min of the assay.

Glibenclamide dissolution showed that at 60 min of the assay, GLIBR1, GLIBR2 and GLIBR3 has released 90.9 %, 88.2% and 104.7% of their active ingredients respectively, while GLING1, GLING2 and GLING3 released 65.2%, 63.6% and 67.9% of their active ingredients respectively. According to the FDA

recommendation of not less than 80% in 60 min, it therefore, means that GLIBR1, GLIBR2 and GLIBR3 passed the dissolution test as they released more than 80% of the active ingredients (API) in 60 min, while GLING1, GLING2 and GLING3 do not pass the test as they released less than 80% of the API in 60 min. This implies that all the Brazilian brands of glibenclamide passed the test while all the Nigerian brands failed the test.

Figure 12. Dissolution profile of the brands of glibenclamide medicine



The ANOVA of the glibenclamide dissolved gave a p-value of about 0.014, which is less than the 0.05 significant levels. This means that the brands have significant difference between them.

The Table 8 is a Tukey grouping of the brands of glibenclamide medicine. It shows that the medicines are placed into groups of A, AB and B. With GLIBR3 in group A, GLIBR1, GLIBR2 and GLING1 in group AB, while GLING3 and GLING2 are in the same group of B. GLING1 has statistical similarity to GLIBR1 and GLING3 but from pharmaceutical point of view, it is different from them because it did not pass the dissolution test and the amount of drug released in

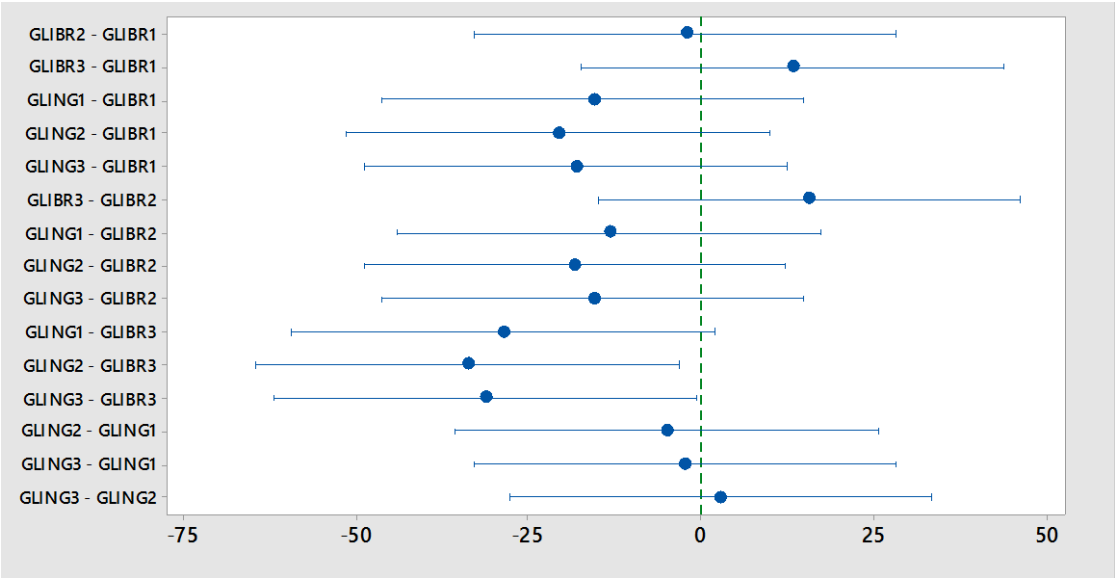
30 min of the assay is significantly different from that released by GLIBR1 and GLIBR2. The Tukey grouping was done by utilizing the mean % of drug released in 30 min of the assay against time (min).

Table 8. Tukey test of the brands of glibenclamide medicine

Brands	N	Mean	Grouping
GLIBR3	7	81.9	A
GLIBR1	7	68.9	AB
GLIBR2	7	66.4	AB
GLING1	7	53.1	AB
GLING3	7	50.6	B
GLING2	7	47.9	B

The Figure 13 is a 95% confidence interval of the Tukey of the brands of glibenclamide medicine. From the interval, it shows that GLING2-GLING1, GLING3-GLING1, GLING2-GLIBR1, GLING3-GLIBR1 and GLIBR2-GLIBR1 contain the zero line, this means that they have no significant difference between them, while the other comparisons, GLIBR3-GLING2 and GLIBR3-GLING3 that do not contain the zero line have significant difference between them.

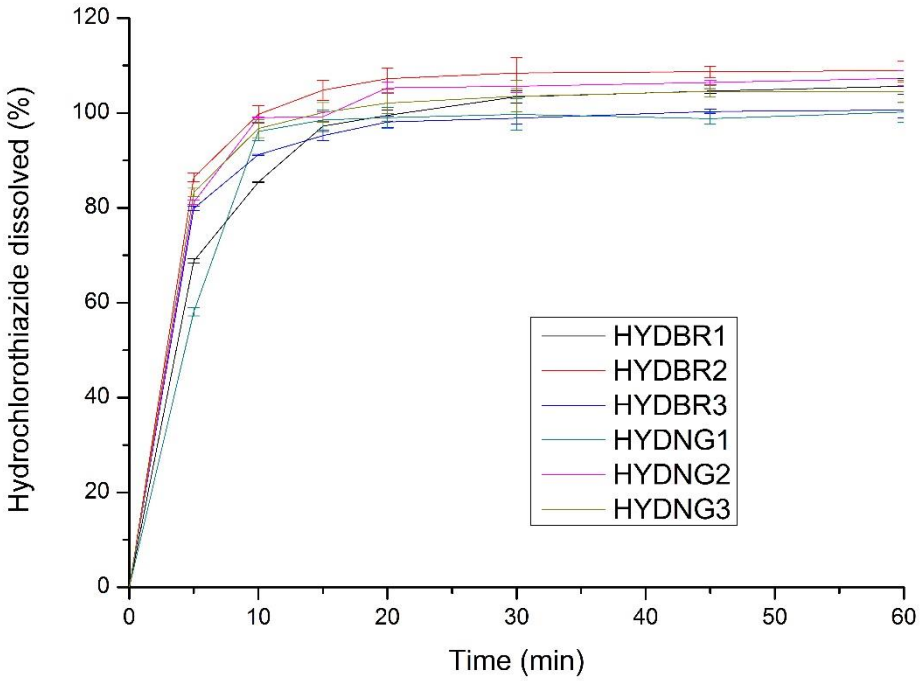
Figure 13. Confidence Interval of the Tukey of the brands of glibenclamide



3.5. Hydrochlorothiazide

The Figure 14 shows the dissolution profile of the hydrochlorothiazide medicines (HCTZ). All the brands show almost the same release characteristics. The profile shows that from 20 min of the assay to 60 min, there is a steady liberation. Hydrochlorothiazide dissolution showed that at 60 minutes of the assay, HYDBR1, HYDBR2, HYDBR3, HYDNG1, HYDNG2 and HYDNG3 has released 105.6%, 108.9%, 100.6%, 100.2%, 107.3% and 104.4% of the active ingredients respectively. These values comply with the USP recommendation of not less than 80% release of the API in 60 min. It therefore means that both the Nigerian and Brazilian brands of hydrochlorothiazide passed the dissolution test.

Figure 14. Dissolution profiles of the brands of HCTZ medicines



The ANOVA of the hydrochlorothiazide dissolved gave a p-value of 0.424, which is above the 0.05 significant levels; this means that the brands do not show significant difference between them.

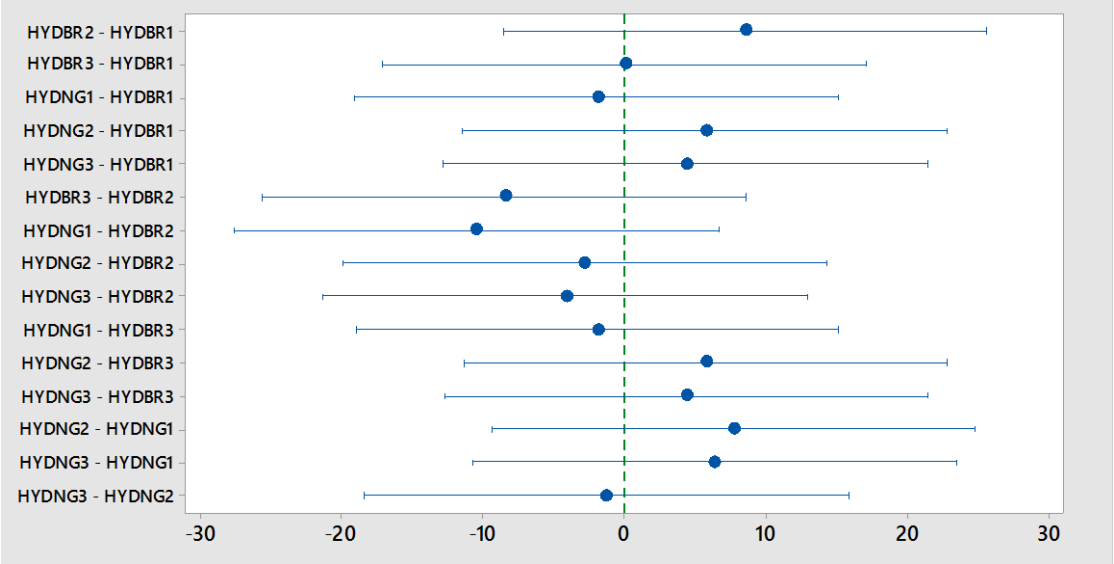
The table9 shows a Tukey test of the brands of medicine. All the medicines were placed in the same group of A, this means that the brands are similar.

Table 9. Tukey test of the brands of hydrochlorothiazide medicine

Brands	N	Mean	Grouping
HYDBR2	7	103.5	A
HYDNG2	7	100.6	A
HYDNG3	7	99.2	A
HYDBR1	7	94.9	A
HYDBR3	7	94.9	A
HYDNG1	7	92.9	A

The Figure 15 is a 95% confidence interval of the Tukey of the brands of hydrochlorothiazide medicine. The Figure shows a comparison of two brands and it was observed that all the brands contain the zero line; this means that there is no statistical significant difference between them.

Figure 15. Confidence interval of the Tukey of the brands of hydrochlorothiazide medicine



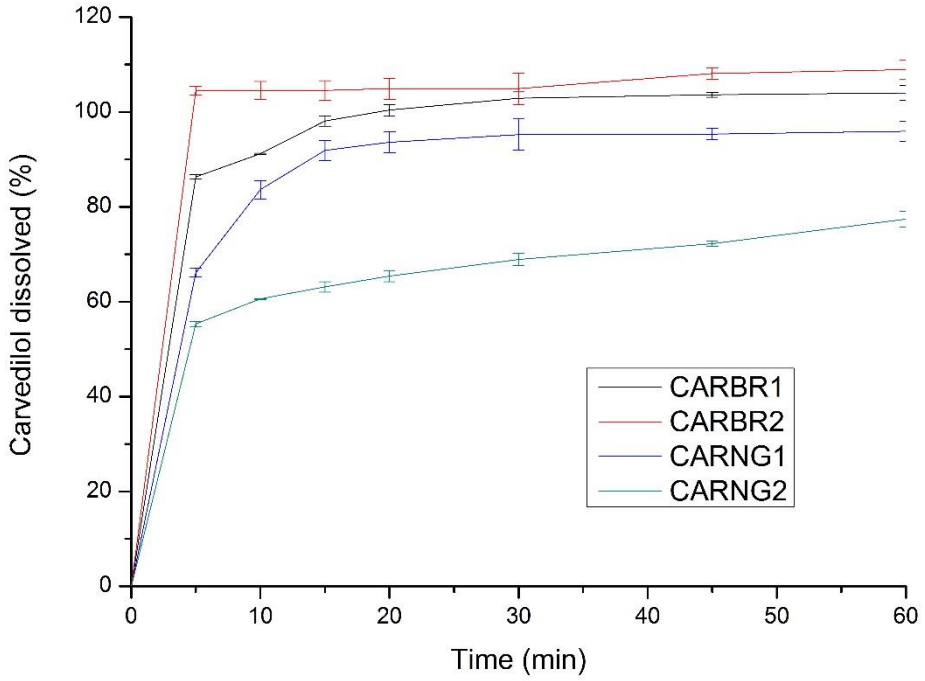
3.6. Carvedilol

The Figure 16 is the dissolution profiles of the various brands of carvedilol medicine. The profile shows that Ictus brand has the most rapid release pattern followed by CARBR1. It also showed that CARNG2 has the lowest release pattern. It is observed from the figure that the medicines have steady drug release from 15 min of the assay to 60 min. The profile also showed that all the medicines except CARNG2 have released more than 80% of their API within 15

min of the assay. CARBR2, CARBR1 and CARNG1 show the same liberation characteristics.

Carvedilol dissolution showed that CARBR2 carvedilol has already released more than 100% of its active ingredient within 5 min of the assay, CARBR1 released 100% of its active ingredient within 20 min of the assay, CARNG1 which is a Nigerian brand did not release 100% of its active content even at infinity time of the assay but released more than 80% within 10 min of the assay, another Nigerian brand (CARNG2) did not also release 100% of the API even at infinity time of the assay as only 83.817% was released, it also released less than 80% of its active content within 60 min of the assay. Therefore, based on the result of the dissolution, it then means that three (CARBR1, CARBR2, and CARNG1) out of the four brands passed the dissolution test while the CARNG2 brand did not pass. All the Brazilian brands passed while 50% of the Nigerian brands passed

Figure 16. Dissolution profiles of the brands of carvedilol medicine



The ANOVA of the carvedilol dissolved gave a p-value of 0.000, which is less than 0.05 level of significant. This means that there is a significant difference between the brands.

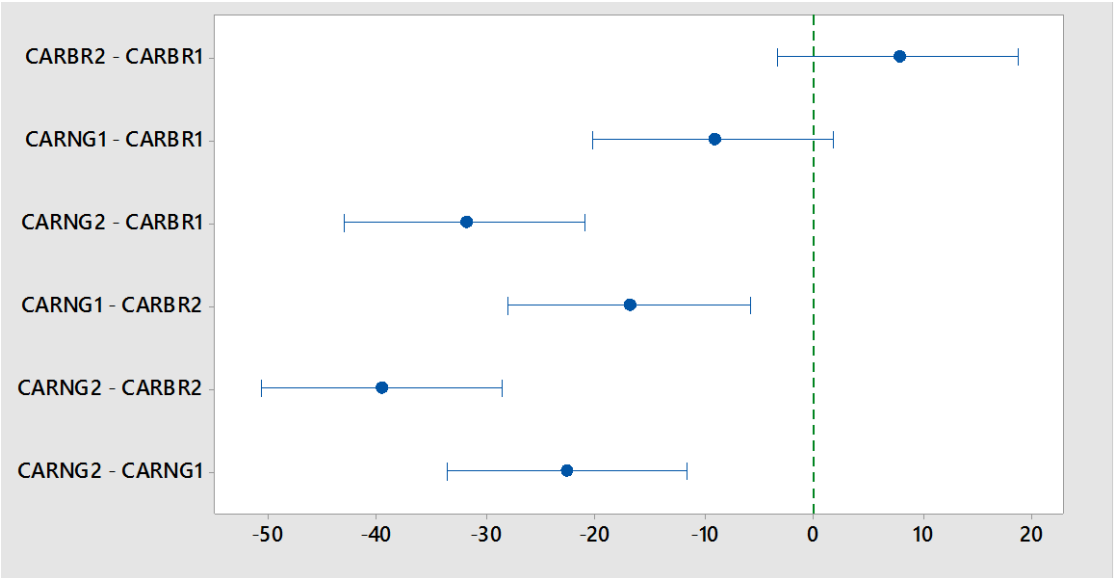
The Table 10 shows the Tukey grouping of the brands of carvedilol medicines. The medicines were found to be grouped into A, AB, B and C. Ictus was placed in group A due to the drug content of the product. Ictus and CARBR1 show some similarity between them also, CARNG1 and CARBR1 have some similarity. CARNG2 has significant difference with the other three products.

Table 10. Tukey test of the brands of carvedilol medicine

Brands	N	Mean	Grouping
CARBR2	7	105.5	A
CARBR1	7	98.1	AB
CARNG1	7	88.8	B
CARNG2	7	66.1	C

The Figure 17 is a 95% Confidence interval of the Tukey of the brands of carvedilol available in the Brazilian and Nigerian pharmaceutical market. From the Figure, it showed that CARBR2-CARBR1 and CARNG1-CARBR1 brands comparison contain the zero line, which means that they do not have statistical significant difference. While the other comparison; CARNG2-CARBR1, CARNG1-CARBR2, CARNG2-CARBR2and CARNG2-CARNG1, which do not contain the zero line, have significant difference.

Figure 17. Confidence interval of the brands of carvedilol medicine



4. General evaluation of the Brazilian and Nigerian brands

The table 23 showed the summary of the dissolution test of the brands of medicines in this present work. It was observed that out of the 17 brands of Brazilian medicines tested, 94.1% passed the dissolution test, also out of the 17 brands of Nigerian medicines tested, 58.8% passed. It can also be summarized that none of the glibenclamide products from Nigeria passed. The furosemide and hydrochlorothiazide samples from both countries passed the test. It is obvious that a higher percentage of Brazilian products passed the test than Nigerian products which may be attributed to the availability of generic medicine in Brazil while most Nigerian medicines are imported from foreign countries which may make it easy for adulteration or falsification of the products.

Table 11: Summary of the dissolution tests showing the percentage of products that passed

Drug	Brazilian Brands	Nigerian Brands
Albendazole	66.7%	33.3%
Ibuprofen	100%	66.7%
Furosemide	100%	100%
Glibenclamide	100%	0 %
Hydrochlorothiazide	100%	100%
Carvedilol	100%	50%

5. CONCLUSION

From the results of the dissolution works done on the Brazilian and Nigerian brands of medicines, it can be concluded that a higher percentage of Brazilian medicines (about 94.1%) passed the dissolution test as compared to a lower percentage of the Nigerian medicines (about 58.8%) that passed. The presence of generic medicines in the Brazilian pharmaceutical market may be one of the contributory factors to the differences in dissolution observed between the Nigerian and Brazilian brands. The result of the dissolution tests obtained for all the drugs in this present chapter validates the results shown in the literature review of chapter one, as a higher percentage of Nigerian medicines were observed not to have passed the dissolution test.

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ANNEXES