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Comparative Bioequivalence of Metformin Brands Available in Sokoto

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DEDICATION

This work is dedicated to Almighty Allah for His continued favour and blessings on me.

CERTIFICATION

This dissertation by AWESU ABDULHAKEEM ADIO (11210708142) has met the requirements for the award of the degree of Master of Science, Pharmacology (M. Sc. Pharmacology), of Usman Danfodiyo University, Sokoto and is approved for its contribution to knowledge.

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LIST OF ABBREVIATIONS

AUC	Area under Concentration-time Curve
AUMC	Area under the Mean Curve
BE	Bioequivalence
BP	British Pharmacopoeia
Cmax	Maximum Plasma Concentration
DM	Diabetic Mellitus
GC	Gas Chromatography
HPLC	High- Performance Liquid Chromatography
KEL	Elimination Constant
LC	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
PAR	Peak Area Ratio
PK	Pharmacokinetic
SEM	Standard Error of Mean
UV	Ultra Violet

ABSTRACT

The aim of this study was to establish if bioequivalence exists among Metformin brands marketed in Sokoto. Eight brands were randomly selected from survey conducted at drug outlets in Sokoto. In-vitro studies on Crushing strength, disintegration, dissolution, friability and weight uniformity was conducted. The brand with pharmaceutical equivalence to the innovator drug was selected for invivo study using High-performance Liquid Chromatography. A randomised, single dose, two-period, crossover study was conducted in 10 healthy Sokoto red goats. A single dose of 500mg of each brand was administered and plasma samples up to 24hours were obtained. Pharmacokinetic parameters, Cmax, Kel, Tmax, T½, AUC0-t and AUC0-∞, were analysed with PK Solution Software. All the brands had values within range specified by British Pharmacopoeia for uniformity of weight, disintegration, assay and dissolution test. The ratios of average Cmax, AUC0-t and AUC0-∞ of Metformin samples (test against reference) for the logarithm transformed data were 1.01,1 and 1 respectively. The corresponding 90% CI were 0.9512 – 1.0781, 0.978 – 1.022 and 0.9667 – 1.0251 which were within the acceptable range of 0.80 – 1.25. This study demonstrated bioequivalence of the generic Glumin with the innovator brand Glucophage. Hence, the two brands may be therapeutically equivalent and exchangeable in clinical practice.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Diabetes Mellitus (DM) was first documented by the Egyptians and it is characterised by weight loss and polyuria. However, the term Diabetes Mellitus was coined by the Greek physician Aertaeus. (Ahmad, 2013).This chronic metabolic disorder is a fast growing global problem with huge social, health, and economic consequences. In 2010, the global estimate of diabetes mellitus was 285 million approximately 6.4% of adult population. Between 2010 and 2030, the prevalence of diabetes among adults has been projected to increase by 69% in developing countries and 20% increase in developed countries (Shaw *et al*., 2010). According to the Atlas of International Diabetes Foundation the prevalence of Type 2 DM in European countries showed Portugal had highest prevalence of 12.4 while Norway was least with 4.7 (Whiting et al., 2011). The prevalence by year 2030 is estimated at 11.3 and 4.3 respectively, (Ginter and Simko, 2013a).

Diabetes is an important cause of prolonged ill health and premature mortality and claims more lives per year than HIV-AIDS with nearly one death every 10 seconds (Shamim, 2013)

Generic drugs are copies of innovator drug products with expired patents. They are advocated for use in practice because they are usually cheaper than the innovator products, thereby improving access to live- saving drugs especially in developing countries.

Generic drugs are required to have the same active ingredients, strength, dosage form, and route of administration as the brand-name product. However it is not mandatory that generic drugs should contain the same inactive ingredients as the brand name products.

In a study comparing the generic cardiovascular drug to their brand-name counterparts by evaluating the outcome of 38 published clinical trials, the findings showed no significant difference between the generic and branded drugs (Kesselheim et al., 2008)

Factors affecting drug response

Evidence has shown that different products with the same amount of active pharmaceutical ingredients have distinct differences in their therapeutic effects (Esimone et al., 2008; Fujii et al., 2008). This observation has been attributed to the differences in rate and extent of absorption, possibly due to the type of excipients, manufacturing variables such as the influence of mixing method and granulation procedure as well as coating parameters (Pillay and Fassihi, 1998; Maggio et al., 2008) Another factor affecting drug response is counterfeiting, which can apply to both branded and generic products and could include products with correct or wrong ingredients or with fake packaging (World Health Organization, 1999) Substandard drugs are genuine drug products which upon laboratory testing do not meet the quality specifications claimed by their manufacturers (Taylor et al., 2001)

Metformin

Metformin HCl (1,1-dimethylbiguanide HCl), first developed in 1957, is one of the most commonly used oral anti-hyperglycaemic agents for the treatment of Type II diabetes mellitus. The United Kingdom Prospective Diabetes Study has recommended Metformin

as monotherapy for the initial pharmacologic treatment of overweight Type II DM. (Boussageon et al., 2012)

The US Food and Drug Administration (FDA) has defined bioequivalence (BE) as the absence of a significant difference in the rate and extent of absorption of active ingredients when administered at the same dose under similar conditions in an appropriately designed study.

Ensuring uniformity in efficacy, standards of quality, and safety of pharmaceutical products is the fundamental responsibility of the pharmaceutical company and drug regulatory agencies. Reasonable assurance has to be provided that various products with the same active ingredients, marketed by different licensees are clinically equivalent and interchangeable (Adegbolagun et al., 2007).

The availability of many brands of metformin in the drug market today places clinicians and pharmacists in a dilemma of choice of a suitable brand or the possibility of alternative use. Furthermore, there are growing concerns that various formulations may have different bioavailability and that development of resistance will accelerate if suboptimal doses are used (Zakeri-Milani et al., 2012).

Bioequivalence studies should be conducted for the comparison of two medicinal products containing the same active substances. The studies should provide an objective means of critically assessing the possibility of an alternative use of the products.

Despite the considerable use of metformin in Sokoto, there are no reports on the bioavailability and bioequivalence of the various brands of the drug marketed in the state. Hence the present research is put forward.

1.2 Statement of the Research Problem

- Diabetes is a chronic debilitating disease with numerous complications. It affects all races and its management is a huge financial burden.
- In Nigeria, many patients are of low socio-economic status, coupled with low health insurance coverage; thus there is a need to find out cheaper bioequivalent drugs to innovator brand.
- Counterfeit and substandard medicines have been a serious problem facing health care delivery systems in developing countries. It is estimated that about 30% of drugs on sale in many countries in Africa and parts of Asia are counterfeit (WHO, 2003). Systemically assaying the various brands of Metformin in the drug market will expose the substandard ones.
- Use of poor-quality drugs has resulted in treatment failure in some instances (Petralanda, 1995).

1.3 Research Justification

- Diabetes remains one of the devastating diseases in sub- Saharan Africa with an enormous financial burden, hence the need to find out cheaper bioequivalent drugs to innovator brand.
- Growing concerns that various formulations of metformin may not be bioequivalent increases the likelihood of treatment failure if suboptimal doses are used.

- The increasing use of metformin in clinical practice creates the need to monitor and ascertain the quality of the various brands in the markets for quality control assessment and for generic substitution.
- Despite the considerable use of metformin in Sokoto, no report has been published on the bioequivalence of the generic brands hence the need for this study.

1.4 Aim and Objectives

Aim

The aim of this study is to determine the bioequivalence of eight brands of metformin marketed in Sokoto using *In-vitro* and *In-vivo* studies using red goats.

Objectives

- To conduct a market survey of available generic formulations in pharmaceutical outlets of Sokoto metropolis
- To determine in vitro pharmaceutical equivalence of some generic brands of Metformin marketed in Sokoto.
- To compare bioequivalence between innovator brand and the brand with the closest pharmaceutical equivalence profile from *in-vitro* studies.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

There are several publications on bioequivalence (BE) of metformin. These studies were conducted either in humans or animals. A larger percentage of these were published outside Nigeria. Bioequivalence studies consist of both *in-vitro* and *in-vivo.* Considering the first two step in absorption, in-*vitro* dissolution may be applicable to the prediction of *in vivo* bioequivalence (Polli, 2008).

The quality of drug assessment carried out in Nigeria indicated that 48% were out of range of the specification of British Pharmacopoeia (BP) and about 40% of these drugs were manufactured in India (Okunlola et al., 2009).Based on these facts, most of the health care professionals and other health workers were of the view that only innovator pharmaceutical products that are expensive are effective (Odeniyi and Adegoke, 2003). But this view may not always be the case because some generic versions of innovator products have been proven to be bioequivalent. Research carried out on the analysis of pharmaceutical qualities of paracetamol and ibuprofen tablets in Nigerian market showed that some of the paracetamol and Ibuprofen tablets conformed to the standard requirement while some do not (Okunlola et al., 2009).The eleven (11) brands of paracetamol tested were chemically and physically equivalent to the innovator products. In order to ensure compliance, regulatory agencies need to regularly monitor imported generics and those drugs manufactured locally in Nigeria.

Valizadeh *et al*. (2014) conducted a study in Iran evaluating the pharmacokinetics (PK) and bioequivalence (BE) of two metformin tablets. The design was a randomized, single dose, two-period, cross over study in healthy male fasting volunteers. A 2-week washout period separated the two periods. Blood sampling was performed before and after drug administration in various time points up to 12 h for analysis of PK parameters. Concentration of metformin in plasma was analysed using a developed high performance liquid chromatography method. Both formulations passed the assay, content uniformity, and dissolution tests acceptance value. PK parameters, representing the rate and the extent of metformin absorption were calculated and analyzed for two formulations. The 90 % CI obtained by analysis of variance for the ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 92.14–110.95, 92.72–107.37 and 89.42–110.23 % respectively, meeting the criteria for BE (80–125 %).

A study by Wu et al.(2006) in China studied the bioequivalence of metformin hydrochloride tablets in healthy volunteers. A single oral dose (1000 mg) and multiple oral doses (1000 mg/d for 7 days) of reference or test metformin hydrochloride preparations were given to 20 healthy male volunteers according to an open randomized crossover study. The plasma concentrations of metformin hydrochloride were determined by RP-HPLC method. The pharmacokinetic parameters and bioavailability of test preparation were compared with reference preparation. *In-Vitro* bioequivalence study of 8 brands of metformin by Zakeri-Milani et al.(2012) evaluated the bioequivalence between different formulations of metformin 500 mg and 1000 mg tablets which were marketed in Iran, and innovator brand. Dissolution profiles were taken and compared through two model independent methods, difference factor (f1) and similarity factor (f2). All the Eight

tested brands released more than 80% of drug content within 30 minutes and contained 95-96.3% of labeled amount except two drugs (brands b and c). The acceptance value in all cases was below 15. Therefore, it is evident that test products except two drugs were bioequivalent to the reference product, and could be used as a generic substitute for the innovator product. Results emphasize the need for post marketing investigation for new formulations.

A study on *in-vitro* equivalence of generic metformin hydrochloride tablets and Propanolol hydrochloride tablets under biowaver conditions in Lagos, Nigeria by Oyetunde et al.(2012) found that none of the generic samples tested met biowaiver conditions; therefore, in vivo bioequivalence studies are required to ascertain therapeutic equivalence. To take advantage of the cost savings of using in vitro dissolution as a surrogate for bioequivalence studies, manufacturers of generic products need to consider factors that affect solubility and permeability of their products when formulating them.

2.2 Definition of Diabetes Mellitus

Diabetes mellitus (DM) is a syndrome of chronic hyperglycaemia due to Insulin resistance or relative deficiency or both. Several distinct types of DM are caused by interplay of genetics and environmental factors.

Based on the aetiology of the DM, factors contributing to hyperglycemia include reduced insulin secretion, increased glucose production and decreased glucose utilisation. The metabolic dysregulation associated with DM causes subsequent pathophysiologic changes

in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system.

2.3 Epidemiology of Diabetes Mellitus

In 2010, the global prevalence of diabetes mellitus among adults (aged 20–79 years) was 6.4%, affecting about 285 million adults, in 2010, and estimated to increase to 7.7% and 439 million adults by 2030,(Shaw et al., 2010). According to the Atlas of International Diabetes Foundation (IDF) 2011, the prevalence of Type 2 DM in European countries showed Portugal had the highest prevalence of 12.4 while Norway was least with 4.7. The prevalence by the year 2030 is estimated at 11.3 and 4.3 respectively,(Ginter and Simko, 2013b) . The estimated prevalence of DM in Africa is 1% in rural communities and within the range of 5 – 7% in urban sub- Saharan Africa (Kengne et al., 2005). International Diabetes Federation (IDF) gave the prevalence of DM in Nigeria adult population (20- 79 years) to be 1.9% (IDF, 2015) Ejike et al.(2015) reported the prevalence of DM in southeastern Nigeria to be 3% (3.6% for females and 2.3% for males). In Nigeria, the number of adult death due to Diabetes is quoted as 40,815 (IDF, 2015).

2.4 Classification of Diabetes Mellitus

Assigning a type of diabetes to an individual often depends on the circumstances present at the time of diagnosis, and many diabetic individuals do not easily fit into a single class. DM has been classified into Type 1, Type 2, gestational and other specific types. Type 1 diabetes usually autoimmune destruction of the β cells of the pancreas leading to absolute insulin deficiency. Type 2 diabetes (ranging from predominantly insulin resistance with

relative insulin deficiency to predominantly insulin secretory defect with insulin resistance).

Pharmacological treatment of DM consists of both insulin and oral glucose-lowering drugs and at times complementary and alternative medicine. Effective usage of insulin in the management of glycaemia remains a challenge in developing countries like Nigeria, and about a fifth of persons with T2DM are on insulin therapy solely or in combination with oral glucose agents (Ogbera and Kuku, 2012).

2.5 Pharmacological Treatment of Diabetes Mellitus

There are different classes of oral hypoglycaemic agents (OHA) notably Biguanides, DPP-4 Inhibitors, α-glucosidase inhibitor, sulphonylurea, thiazolidenediones, meglitides and incretin mimetics.

Sulphonylureas have been the mainstay in the early 1950's. They act by stimulating insulin release from the insulin secreting ß-cells located in the pancreas (Aguilar-Bryan *et al.*,1995) and may slightly improve insulin resistance in peripheral target tissues (muscle, fat) (Bressler and Johnson, 1997). All sulphonylureas have been associated with weight gain, the choice of which is primarily based on cost and availability, because their efficacy against microvascular and cardiovascular complications is similar (Zoungas et al., 2009).

Thiazolinediones improve glycaemia reducing insulin resistance and preserving pancreatic beta-cell function mainly by enhancing peripheral uptake and utilisation of glucose in muscle and fat, finally decreasing liver glucose production (Petersen et al., 2000). Rosiglitazone treatment has been found to increase expression of genes involved in

promoting lipid storage in human adipocytes, as well as decrease expression of genes associated with inflammation, such IL-6 (Kolak et al., 2007). These drugs activate one or more peroxisome proliferator-activated receptors (PPARs), which regulate gene expression in response to ligand binding (Vidal-Puig et al., 1997).

Biguanides are old drugs that act by decreasing hepatic glucose output and, to a lesser extent, enhancing insulin sensitivity in hepatic and peripheral tissues. They include Phenformin and Metformin. The latter is the one currently in use today.

2.5.1 METFORMIN

Metformin, marketed under the trade name **Glucophage** among others, was discovered in 1922 (Fischer and Ganellin, 2006). and was introduced as a drug in France in 1957 and the United states in 1995. It is the first-line medication for the treatment of type 2 diabetes (Maruthur et al., 2016). This is particularly true in people who are overweight (Wang et al., 2008). Found to be useful in the treatment of polycystic ovary syndrome. Limited evidence suggests metformin may prevent the cardiovascular disease and cancer complications of diabetes (Malek et al., 2013). It is not associated with weight gain. Metformin decreases glucose production by the liver and increase the insulin sensitivity of body tissues.

Metformin is an oral hypoglycaemic agent that belongs to the class known as biguanides which improve peripheral glucose tolerance, decreases hepatic glucose output and improves muscle sensitivity to insulin and glucose uptake in NIDDM. The primary effect is to reduce hepatic glucose production through activation of the enzyme AMP- activated protein kinase (AMPK) (Katzung 11th edition). In patients receiving metformin, a

significant reduction in hepatic glucose output has been observed (Adikwu et al., 2004; Andújar-Plata et al., 2012; Kim et al., 2009; Ali et al., 2007; Hu et al., 2006).

2.5.1.1 Chemistry

Metformin HCl (1,1- dimethyl lbiguanide HCl) is synthesised from the reaction of dimethylamine and 2- cyanoguanidine over heat. It precipitates with a 96% yield after cooling.

2.5.1.2 Structure

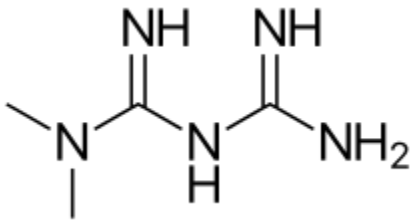

Figure 2.1: Chemical Structure of Metformin

2.5.1.3 Pharmacokinetics

Absorption: Bioavailability of 50- 60%, peak plasma time for regular release is 2-3hr while for extended release is 4-8hr. Metformin is minimally protein bound with volume of distribution (Vd) of 650 L for regular release tablet. It is not metabolised in the liver. It is excreted in the urine (90%, by tubular secretion), with an elimination half-life of 4-9 hours and a renal clearance of 450- 540 mL/min (regular release).

2.5.1.4 Toxicity

Acute oral toxicity (LD_{50}): 350 mg/kg [Rabbit]. Metformin is generally well tolerated. The most common side effects attributed to metformin are gastrointestinal, including a metallic taste in the mouth, mild anorexia, nausea, abdominal discomfort and diarrhoea (Campbell and Howlett, 1995; Currie et al., 2009). High blood lactic acid level is a concern if the drug is prescribed inappropriately and in overly large doses (Lipska et al., 2011). When used according to current prescribing recommendations, however, the risk of metformin-induced lactic acidosis is close to zero (Tahrani et al., 2007).

2.6 *InVitro* and *InVivo* Studies

2.6.1 Bioavailability

The fraction of a dose of drug that is absorbed from its site of administration and reaches the systemic circulation in an unchanged form is termed bioavailability. It is proportional to the area under the concentration-time curve (AUC). Absorption describes the movement of unchanged drug from its site of administration to systemic circulation. Intestinal absorption is a function of (1) intestinal permeability, (2) aqueous solubility and (3) drug stability (Charman et al., 1997).

2.6.2 Relative Bioavailability

Relative Bioavailability is one of the measures used to asses bioequivalence between two products it is dependent on the route of administration (Pottenger et al., 2000). Relative bioavailability measures the bioavailability of a formulation (A) of a certain drug when compared to another formulation (B) of the same drug. Intravenously administered drugs have 100% bioavailability. *F*rel = 100. AUC.D

2.6.3 Biopharmaceutical (BCS) Classification of Drugs

The Biopharmaceutical Classification System is a system to differentiate the drugs on the basis of their solubility and permeability. It is a guide for predicting the intestinal drug absorption provided by the U.S Food and Drug Administration. Amidon et al. (1995) first proposed a biopharmaceutical classification system (BCS) that classified compounds based on their aqueous solubility and intestinal permeability. According to Biopharmaceutical classification system, drugs are classified as follows:

Class 1: High permeability, high solubility. They are well absorbed and absorption rate is higher than excretion, the crucial step is gastric emptying. Metropolol is an example.

Class 2: High permeability, low solubility, the rate limiting step is dissolution. A correlation between *invivo* bioavailability and *invitro* solvation can be found, examples are metformin, glibenclamide,.

Class 3: High solubility, low permeability. It is limited by intestinal permeability. If the formulation does not change the permeability or gastro-intestinal duration time, then class 1 criteria can be applied. Cimetidine is a member of this class.

Class 4: Low solubility, low permeability. These compounds have a poor bioavailability, they are not well absorbed over the intestinal mucosa and a high variability is expected. Hydrochlorothiazide is a drug in this class.

2.6.4 Factors Influencing Bioavailability

Varied factors affect bioavailability which may be drug related or the physiological process in the gastro intestinal system. These include physical properties of a drug (hydrophobicity, solubility), drug formulation (immediate or modified release).Drug-food

interaction, gastric emptying rate, enzyme induction/ inhibition by other drugs/food. In addition, individual variation in metabolic differences (age, gender diet and phenotypic changes) and disease states (hepatic or renal insufficiency).

2.7 *In vitro* Measurements

2.7.1 Disintegration

This test determines whether dosage forms like capsules, suppositories and tablets disintegrate within a prescribed time when placed in a liquid medium under certain experimental conditions. Disintegration is defined as that state in which no residue of the unit under test remains on the screen of the apparatus. The apparatus consist of a basket-rack assembly, a 1-litre beaker, a thermostatic arrangement for heating the fluid and a mechanical device for raising and lowering the basket in the immersion fluid at a constant frequency rate. The tablets pass the test if all six tablets have disintegrated within 30 minutes. (British Pharmacopoeia 2012.).

2.7.2 Dissolution

The rate at which a drug is released is called the dissolution rate. It is a buffer based medium with varied dissolution time and temperature for immediate or modified release tablets. The principle function of the dissolution test includes: a) prediction of *invivo* availability i.e. bioavailability. 2) Routine assessment of production quality to ensure uniformity between production batches. 3) Optimization of therapeutic effectiveness during product development and stability assessment.

2.7.3 Friability

This is a test to determine physical strength of uncoated tablets upon exposure to mechanical shock and attrition. The result is inspected for broken tablets, and the percentage of tablet mass loss through chipping. A tablet fails this test if it fragments or has greater than 1% friability.

2.8 *In vivo* Methods

In vivo method largely involves the use of chromatography to assay plasma and urine.

2.8.1 Chromatography

This is the separation of a complex mixture into individual components by exploiting the partition effect which distributes the molecules into the different phases. The distribution of a molecule between two phases (a stationary and a mobile phase) is given by a distribution coefficient, kd. The mobile phase or buffer is usually a solvent, while a stationary phase or matrix is a column packed with basic, acidic or neutral material mostly silica or alumina. The principle of Chromatography is applied in different fields, it is used in pharmaceutical industries to compare generics with innovator and to characterize and identify phytochemicals. It is also used as a forensic tool in the analysis of samples from crime scene.

There are different forms of chromatography. Partition chromatography in which analytes distribute themselves into two phases, liquid stationary and mobile phase. It is simple technique with broad specificity and low cost. It is further divided into bonded-phase liquid chromatography and liquid-liquid chromatography.

Adsorption chromatography in which matrix molecules has ability to hold the analyte on their surface through a mutual interaction due to forces such as hydrogen bonding and vander waal. The examples are affinity chromatography, ion-exchange and hydrophobic interaction chromatography.

Based on the pressure level, liquid chromatography can be classified into these categories. Low pressure liquid chromatography: pressure limit less than 5 Bar.

Medium Pressure Liquid Chromatography: Intermediate pressure limits (6-50 bar).

High Pressure Liquid Chromatography: pressure limit more than 50-350 bar.

2.9 Pharmacokinetic Studies

The design and conduct of pharmacokinetic studies includes: study design, study population, study conditions, investigated parameters in bioequivalence study and bioanalytical methodology.

Study Design

The design of choice in comparing two formulations is the crossover design ideally separated by a washout period equal to or more than five half-life's of the moieties to be measured. Alternative study designs include the parallel design for very long half-life substances or the replicate design for substances with highly variable disposition.

Single dose studies generally suffice, however the following situations may warrant a steady-state study design: (a) Dose or time dependent pharmacokinetics (b) some modified release products (in addition to single dose investigation).

Characteristics to be investigated during bioavailability/ bioequivalence studies

Evaluation of bioavailability and bioequivalence is usually based upon measured concentrations of the active drug substances in the biological matrix. In certain instances, however the measurement of an active or inactive metabolite may be necessary. These include: (a) where the concentration of the drugs may be too low to accurately measure in the biological matrix (b) unstable drugs (c) limitations in analytical method (d) pro drugs (e) drugs with short half-life.

The plasma-time concentration curve is mostly used to assess the rate and extent of absorption of the study drug. These include pharmacokinetic parameters such as Cmax, Tmax, AUC0-t, AUC0-∞. For studies in the steady state AUC0-t, Cmax, Tmax and degree of fluctuation should be calculated.

2.10 Bioequivalence

Bioequivalence of a drug product is achieved if its extent and rate of absorption are not statistically significantly different from those of reference product when administered at the same molar dose under similar conditions in an appropriately designed study (Gupta et al., 2006).

Pharmaceutical equivalent: These are drug product that has identical amounts of the identical active drug ingredient, that is the same salt or ester of the same therapeutic moiety in identical dosage forms, but not necessarily containing the same inactive ingredients Pharmaceutical equivalence does not necessarily confer therapeutic equivalence (Zuluaga et al., 2009).

Therapeutic equivalent: These are drug products that contain the same active substance or therapeutic moiety and clinically demonstrate the same efficacy and safety.

Essential similar product: When drug products are qualitatively and quantitatively similar as per active substances and the pharmaceutical form when necessary.

Generic: Generic drugs are copies of innovator drug products with expired patents. It is identical or bioequivalent to an innovator in strength, dosage form, quality, safety, route of administration, performance and intended use. A generic company can market their products when patency or period of exclusivity of innovator expires (Gupta et al., 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

The drug samples for the study were all conventional, immediate release, solid oral dosage forms. Eight metformin formulations with 500 mg strength were purchased from registered pharmacies in Sokoto labelled A – H. Analytical grade of reagents were used: Sodium hydroxide (BDH Chemicals, UK) and Potassium dihydrogen phosphate (BDH Chemicals, UK). Metformin Hydrochloride standard and Methanol (Sigma Aldrich, USA).

3.2 Methods

This study involved *In-vitro* and *in-vivo* studies. It was done in three phases.

In-vitro studies assess the physicochemical properties of the drugs.

In- vivo studies were conducted in laboratory animals (Red goats) and the drug concentration of the blood samples was analysed using High-Performance Liquid Chromatography (HPLC).

Phase 1

A market survey was conducted to identify the various brands of Metformin available in Sokoto with price ranges. Eight of the brands were randomly selected.

Phase 2

An *in-vitro* bioequivalence test comprising dissolution study, assay and other physicochemical methods were conducted on the above brands. The brand with the closest profile to the innovator product was selected for head to head comparison in the *in- vivo* test.

Phase 3

In-vivo bioequivalence was conducted between innovator (Glucophage) and generic (Glumin) with the closest variation using HPLC on red goats.

3.2.1 *In-Vitro* Tests

Uniformity of weight: Sample tablets (20) from each of the eight brands were randomly selected, weighed together and average weight determined. Each tablet was subsequently weighed individually on analytical weighing balance and percentage (%) deviation determined.

Hardness Test: Sample tablets (10) of each brand were randomly selected, then placed between the spindle of the Erweka hardness tester machine and pressure was applied by turning the knurled knot just sufficient to hold the tablet in position. The pressure was uniformly increased until the tablet breaks and the pressure required to break the tablet was then recorded.

Friability Test: Ten tablets from each brand were randomly selected and weighed on the analytical balance. These tablets were put in an automated fibrillator, spun at 100 rev/ min to roll and fall within the rotating apparatus. After spinning, the tablets were reweighed

after all loose particles were excluded by blowing off gently with a hand fan. The friability of the tablets was calculated as

% Friability =[(initial Wt- final Wt)/initial Wt]*100

Disintegration Test: The disintegration time of six randomly selected tablets from each brand was determined at 37^0C in distilled water using a multi-unit disintegration tester apparatus. The disintegration time was taken to be the time no granule of any tablet was left on the mesh.

Dissolution studies: A volume 900 ml of each of the following media was used: 0.01 N hydrochloric acid (pH 1.2), phosphate buffer (pH 6.8). The study was done using a 7-compartment Veego® dissolution test apparatus (basket type) maintained at 37 $\pm 0.5^0$C with a fixed speed of 100rpm. A tablet was put in each of the compartments and the machine operated. Five (5ml) sample was taken manually with syringes fitted with stainless tubing to ensure reproducibility of sampling location. Samples were withdrawn at specified time intervals (5, 10, 15, 30, 45 and 60 min) and replaced with a fresh 5 ml of the dissolution medium to maintain the sink conditions. Withdrawn samples were filtered using 0.45-µm Millipore filters, the filtrate was diluted and its absorbance at 233nm was measured using a UV-visible spectrophotometer. The concentration of Metformin hydrochloride in the samples was calculated according to metformin monograph in British Pharmacopoeia.

Assay of Tablets: This was done according to BP 2012 specification. Twenty (20) tablets of each metformin brand were weighed and made into powder form. A quantity of the powder containing 0.1 g of metformin hydrochloride was vortexed with 70 ml of water for

15 min. Thereafter, the volume was diluted to 100 ml with water and filtered. The first 20 ml was discarded. Ten (10) ml of the filtrate was diluted to 100 ml and 10 ml of the resulting solution was also diluted to 100 ml with water. The absorbance of the resulting solution was measured at 232 nm.

3.3 *In-vivo* Study

3.3.1 Chromatographic Conditions for HPLC Analysis

Quantification of metformin in plasma samples is essential for Bioequivalence tests.

Wavelength: 232nm

Injection volume: 20 µl

Flow rate: 1ml/min

Temperature: 25^{o}C (Ambient)

Mobile phase: Acetonitrile: KH_2PO_4 (40:60)

Chromatographic analysis was performed at ambient temperature using Agilent® Technologies 1260 infinity equipped with a 1260 quaternary pump coupled with an auto-sampler (1260 ALS) sample injector with a variable loop and an Agilent VWD (G1314f) prominence UV detector.

Isocratic method was applied, and the mobile phase consisting of a mixture of phosphate buffer and Acetonitrile (60: 40) was filtered through a 0.45 µm Millipore filter. The instrument was coupled to a degasser, and the column compartment was thermo- stated. Separation was carried out on a stainless Steel Eclipse plus C18 column 4.6×150mm, 3.5 µm particle size (Agilent).

Detector output was quantified and analysed using the Chemstation chromatography software.

3.3.1.1 Calibration Curve

The calibration curve was constructed in a replicate of five by plotting peak area of analytical versus metformin concentrations in 6 concentration levels (500-4000 ng/mL) which were prepared in 600 μL blank plasma. The method was validated for selectivity, accuracy and precision.

3.3.1.2 Validation of Method of Analysis

3.3.1.3 Selectivity and Linearity

The metformin standard spiked in plasma and the plasma sample obtained were injected into the HPLC to compare the retention time of metformin from both sources. Linearity was evaluated from gradient concentrations of metformin extracted from plasma. Linearity was determined using the coefficient of determination (r^2) which must not be less than 0.9.

3.3.1.4 Precision

Three different concentrations (500, 1000 and 2000 ng/ml) of metformin standard spiked in plasma (N=6) were analysed thrice a day for three days to assess both intra and inter day precisions. The coefficient of variation or otherwise called relative standard deviation was calculated.

3.3.1.5 Recovery Rates

The recovery rates were determined using the three concentrations prepared in metformin and compared with the metformin extracted from plasma. The ratio was multiplied by 100 to obtain the percentage recovery.

3.3.2 Experimental Animals and Study Design

A single-dose, randomised, two-period and crossover study design was used to evaluate the bioequivalence of two oral formulations of Metformin.

The experiment was conducted on ten healthy Sokoto Red Male goats with an average age of 12- 18 months and weighing 16 – 20 kg. The animals were examined clinically by a Veterinary Physician to ascertain their health status. The animals were dewormed with the administration of albendazole suspension at 12.5 mg/kg body weight. They also had injection oxytetracycline 20mg/kg body weight and Injection multivitamin 10kg/ml according to the protocol of the College of Veterinary Medicine, Usmanu Danfodiyo University Sokoto. The animals were housed in 2 pens containing 5 animals each. They were monitored closely for two weeks before commencement of the experiment. All the animals were fed with Bean offal, Cowpea hay, Wheat bran and allowed access to water ad libitum. Before commencement of the experiment, the animals were assigned numbers from 1 to 10; thereafter they were divided into two groups of five animals each using computer randomization. Group A had animals labelled as 1, 4, 5, 8 and 9 while Group B had animals 2, 3, 6, 7 and 10. Each group was fasted for 8 hours prior to drug administration, 2 hours after which standard ration was provided. The first group of animals (group A) received oral formulation of the innovator brand of 500 mg Metformin Hydrochloride (Glucophage) while group (B) was treated orally with 500 mg of a generic brand of Metformin Hydrochloride (Glumin). A washout period of 2 weeks was observed between treatments, Group (A) received Glumin while group (B) received Glucophage.

3.3.3 Collection of Samples

Blood samples (3 ml each) was collected through intravenous catheter fixed in contralateral jugular vein into Heparised test tubes at 0 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 12 hr and 24 hr. Plasma was separated by centrifugation at 3000 revolutions per min for 10 min at room temperature and stored in a refrigerator at -20^0C before assay.

3.3.4 Sample Preparation

To prepare samples Into 600 µL of plasma, 600 µL of trichloroacetic acid 10 % (w/v) was added and vortexed for 3 min. Short-term stability studies showed that the drug is stable in acidic media at least for 12 hr at room temperature. After centrifuging at 5,000 rpm for 5 min and precipitation of proteins, supernatant was transferred to another tube containing 36 µL NaOH (4N) and ultimately obtained clear supernatant was injected into the HPLC column.

3.4 Statistical Method and Data Analysis

The pharmacokinetic parameters including the area under the concentration-time curve from time 0 to α ($AUC_{0-\alpha}$), elimination half-life ($T_{1/2}$), elimination rate constant (K_{el}) the maximum serum concentration (C_{max}), time to reach the maximum serum concentrations (T_{max}), volume distribution (V_d), plasma clearance (Cl_p) and absorption rate constant derived with software or manually with log-transformed data are used. (Frey.2013). The difference in pharmacokinetics between the generic and innovator was analysed using – paired T-Test of each of these parameters at 90% confidence level (CI) (90-110), the *p* value of <0.05 was considered statistically significant.

3.5 Software

There are numerous advanced software applications which facilitate pharmacodynamics and pharmacokinetic analysis. Examples are CAPCIL, DATA KINETICS,GraphPad Prism Monolix, Nomen, PK CALC, PK Line, PK Solution Sumfit, Topfit, Winolin, etc.

The most used categories include: PK/PD Modeling based solution, Clinical pharmacology strategy, model based Meta analysis and regulatory and medical writing.

PK Solution

This is an Excel-based program for analysis of pharmacokinetic data. The key features includes: Graphical and programmatic interfaces, functionality for fitting data and estimating parameters using nonlinear mixed effects, diagnostic plots of individual and population fits. It computes plasma concentration/ time data of drugs or chemical substances given by oral and parenteral routes. It also perform statistical analysis on the data such as calculating the mean, area under the curve (AUC), maximum plasma concentration (C_{max}), time to get to maximum concentration (T_{max}), and elimination constant (K_{el}).

CHAPTER FOUR

4.0 RESULTS

4.1 Market Survey of Metformin Brands

The survey of pharmaceutical outlets in Sokoto metropolis yielded a total number of 18 brands of metformin. Eight brands were finally selected (Table 4.1) based on certain factors (availability, cost and rapidity of release). The period of study was within the expiry date.

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Table 4.1: Characteristics of the Selected Metformin Brands (October- December 2014)

Formulation Code	Product	Manufacturer	Marketing Company	Batch Number	Manufactured Date	Expired Date	NAFDAC Number
A	Glucophage®	Merck Santes s.a.s France.	Biofem Pharmaceuticals Ltd	102890	01/2014	12/2018	04-6233
B	Glumin®	Rajat PharmaChem.India	Seagreen Pharmaceuticals Lagos.	RA4001	02/2014	01/2017	A4-3332
C	Diabetmin®	-	Phamatex Nigeria Ltd	BD12578	12/2013	12/2016	04-0810
D	Degluco®	Fredun Pharmaceuticals.India	Promedix Nigeria	FT168	08/2013	07/2016	A4-9357
E	Gluformin®	Nigerian German Chemicals Plc.	-	HO803	08/2013	08/2016	04-6426
F	BG Lophage®	Stallion Laboratories PVT LTD. India	BG Pharma & Health Care Limited	N-660	02/2013	01/2016	A4-3122
G	Glucoform®	Drugfield Pharmaceuticals LTD		581002	10/2012	09/2016	04-7120
H	Metforca®p	Watson Global Pharmaceuticals Nigeria	Laider Pharmaceuticals Nigeria	20131201	12/2013	11/2018	A4-1811

4.2 Weight Uniformity Values of Eight Brands of Metformin.

The weight deviation for the tablets of the different brands ranged from 0.77% for Glucophage to 3.01% for Gluformin (Table 4.2).

Reference value ±5% deviation from the Mean

Table 4.2: Weight Uniformity Testing for Eight Metformin Brands Marketed in Sokoto

Brand	Mean weight (in gram)	SD	RSD
Glucophage	0.5408	0.0042	0.7654
Glumin	0.5566	0.0116	2.0868
Diabetmin	0.5574	0.0063	1.1274
Degluco	0.5486	0.0099	1.7992
Gluformin	0.5369	0.0162	3.0088
BG Lophage	0.5662	0.0119	2.1081
Glucoform	0.6136	0.0117	1.9023
Metforcap	0.6284	0.008696	1.3838

KEY: SD= Standard deviation; RSD= Relative Standard Deviation

4.3 Determination of Disintegration Time (in Min) of Eight Brands of Metformin

The disintegration time for the tablets of metformin brands varied from 1.49 for Gluformin to 23.45 for Metforcap (Table 4.3). According to BP disintegration time should not exceed 30 Minutes for film coated tablets.

Table 4.3: Disintegration Time (min) of Eight Brands of Metformin

Brand	Disintegration time (in min)
Glucophage	8.43± 0.08
Glumin	9.82±0.35
Diabetmin	10.00±0.33
Degluco	8.46±0.32
Gluformin	1.49±0.50
BG Lophage	13.12±3.43
Glucoform	7.62±1.82
Metforcap	23.45±1.08

4.4 Determination of the Hardness for the Tablets of the Metformin Brands.

The values of the findings from hardness test vary from 10.88 KgF for Glumin to 21.78 KgF for Metforcap (Table 4.4).

Table 4.4: Result of Hardness Test of Eight Brands of Metformin

Brand	Hardness (Kgf) ±SEM
Glucophage	19.86 ± 0.64
Glumin	10.89 ± 0.37
Diabetmin	21.70 ± 0.00
Degluco	17.94 ± 0.71
Gluformin	17.12 ± 0.38
BG Lophage	14.71 ± 0.83
Glucoform	18.40 ± 0.10
Metforcap	21.78 ± 0.04

4.5 Friability Test

The values for the result of friability test vary from 0-1.6% (Table 4.5).

According to BP no batch should have a friability value greater than 1.0%w/w.

Table 4.5: Result of Friability Test of Eight Brands of Metformin

Brand	Initial weight	Final Weight in mg (Mean±SD)	% Friability
Glucophage	5.263	5.263±0.046	0.0
Glumin	5.55	5.55±0.008	0.0
Diabetmin	5.588	5.588±0.007	0.0
Degluco	5.584	5.584±0.008	0.0
Gluformin	5.316	5.23±0.021	1.6
BG Lophage	5.358	5.357±0.013	0.0
Glucoform	5.983	5.964±0.011	0.3
Metforcap	6.296	6.281±0.008	0.2

4.6 Determination of Drug Release

Table 4.6 presents result of the drug release profile versus time for the 8 brands of metformin at pH 6.8. All the brands, with the exception of Glucoform and Metforcap, released 85% of their drug contents within 15 minutes (Table 4.6: Fig 5).

Table 4.6: Percentage of Drug Released at PH 6.8 from Dissolution Studies of Metformin Brands

% Released(±SD) at pH 6.8 n= 2 tablets per brand								
Time/min	Glucophage	Glumin	Diabetmin	Degluco	Gluformin	BG Lophage	Glucoform	Metforcap
5	53.42±6.2	50.34±4.4	34.79±0.1	65.30±16.1	91.39±4.1	49.01±17.4	54.89±3.2	25.37±1.9
10	82.14±7.0	84.59±7.01	65.84±10.1	90.32±8.0	96.53±2.7	79.95±18.7	59.27±19.9	40.11±0.7
15	87.68±9.4	94.73±3.5	86.48±7.4	93.17±2.6	95.76±6.4	92.19±7.8	67.58±2.75	51.59±1.6
30	95.67±3.0	98.80±6.0	96.88±8.1	96.35±9.9	96.03±1.5	99.43±4.3	67.27±4.1	68.30±5.4
45	97.33±1.1	98.79±5.8	93.80±8.1	93.49±2.4	91.52±7.5	98.49±4.2	67.27±4.1	68.74±4.4
60	95.09±3.6	87.32±1.7	92.86±13.0	95.23±5.3	92.37±4.5	97.19±15.0	66.42±3.3	72.23±0.8

Similarly, at pH 1.2, all the 8 brands released 85% of their drug contents within 15 minutes except Metforcap (Table 4.7; Fig 6)

Table 4.7: Percentage of Drug Released at PH 1.2 From Dissolution Studies of Metformin Brands

	% released(±SD) at pH 1.2 with 2 tablets per brand							
TIME (M)	Glucophage	Glumin	Diabetmin	Degluco	Gluformin	BG Lophage	Glucoform	Metforcap
5	24.12±12.8	46.53±7.3	40.50±11.4	57.60±13.3	89.91±1.3	49.86±13.8	82.35±4.1	38.07±2.4
10	43.65±8.7	77.31±6.5	75.60±8.3	87.75±8.9	91.98±1.6	79.74±11.5	88.92±24.9	60.21±0.9
15	61.29±11.8	91.53±0.1	90.00±3.9	92.25±3.2	93.15±2.4	91.53±1.8	101.34±3.5	77.40±2.0
30	88.56±9.0	93.15±0.9	87.93±1.5	91.17±2.6	90.81±2.7	102.69±2.5	100.89±5.1	102.42±6.8
45	93.51±0.8	91.89±0.8	89.91±3.0	89.37±4.0	89.73±2.7	103.14±2.4	100.89±3.8	97.11±16.4
60	92.79±4.7	92.07±4.7	89.19±4.9	90.99±1.3	95.67±1.7	100.35±3.3	99.63±4.2	68.85±6.1

4.7 Assay of the Metformin Tablets for the Different Brands

Table 4.8 presents the results of the assay test for the tablets in each of the brands.

The Monograph Specification for Assay is 95- 105% of claimed value.

Table 4.8: Assay Test of Metformin Tablets (n= 20)

Brand	Assay (%)+SD
Glucophage	97.53±0.5
Glumin	99.51±0.7
Diabetmin	99.75±1.1
Degluco	97.41±1.2
Gluformin	96.05±1.8
BG Lophage	95.93±1.9
Glucoform	97.53±1.3
Metforcap	95.80±2.5

4.8 Determination of the Degree of Similarity of the Selected Generics Relative to the Innovator Product

Table 4.9 shows the F2 similarity values for the brands compared to the innovator. At pH 1.2, each of the brands had F2 similarity value less than 50, while only 3 of the brands (Gluformin, Glucoform and Metforcap) tested at pH 6.8 had F2 values lower than 50.

Table 4.9: F2 Statistical Values for the Generics Relative to Innovator Product

Brands	F2 Values						
PH	Glumin	Diabetmin	Degluco	Gluformin	BG Lophage	Glucoform	Metforcap
1.2	34	36	29	23	32	23	40
6.8	65	50	58	38	74	31	25

4.9 Determination of the Calibration Curve

Figure 4.1 shows a graph plot of peak area of metformin standard against metformin concentration (ng/ml) at 500, 1000, 2000 and 4000 ng/ml

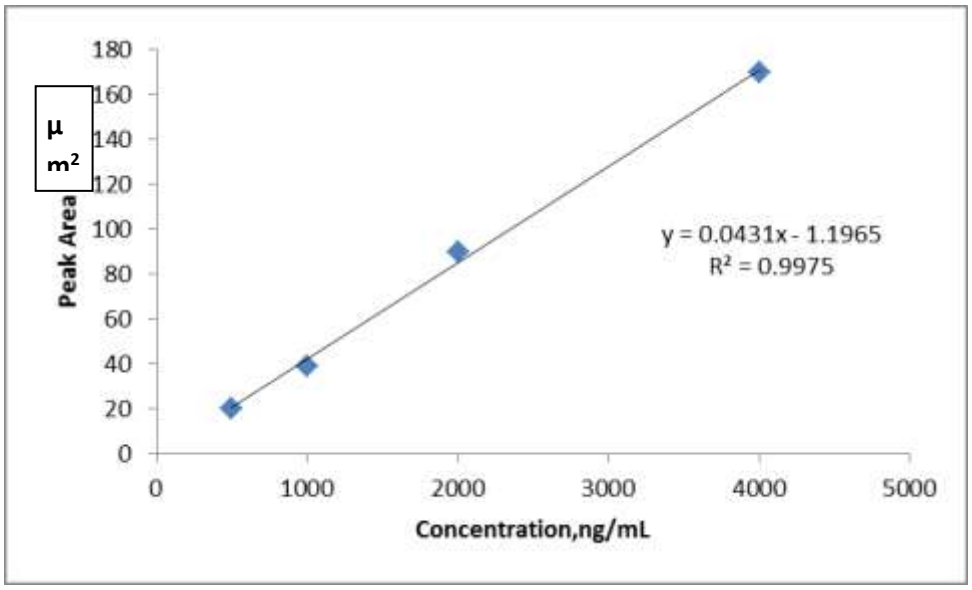

Figure 4.1: Graph of Calibration of Metformin

4.10: Precision of Analytical method for Metformin

The table shows the concentration (ng/ml) and coefficient of variation (%) for both Intra and Inter day assay at three concentration levels (250, 500 and 1000).

Table 4.10: Precision of Analytical method for Metformin

	Concentration ng/mL	CV %	n
Intra-day assay			
	250	5.3	6
	500	3.2	6
	1000	2.1	6
Inter-day assay			
	250	8.1	6
	500	6.2	6
	1000	5.4	6

CV = Coefficient of Variation, n = number of samples

The Correlation coefficient for linearity of the gradient concentration is 0.998 with a slope of 0.043 and the intercept is at -1.196.

The average recovery for metformin was 88.5 ± 0.6%

4.11 Determination of Plasma Concentrations for Glucophage

The concentration- time curves (standard and semi-log plots) of Glucophage, following oral administration of 500 mg tablet to 10 Goat is shown is Fig 3a and 3b.

ReferenceAverageGlucophage

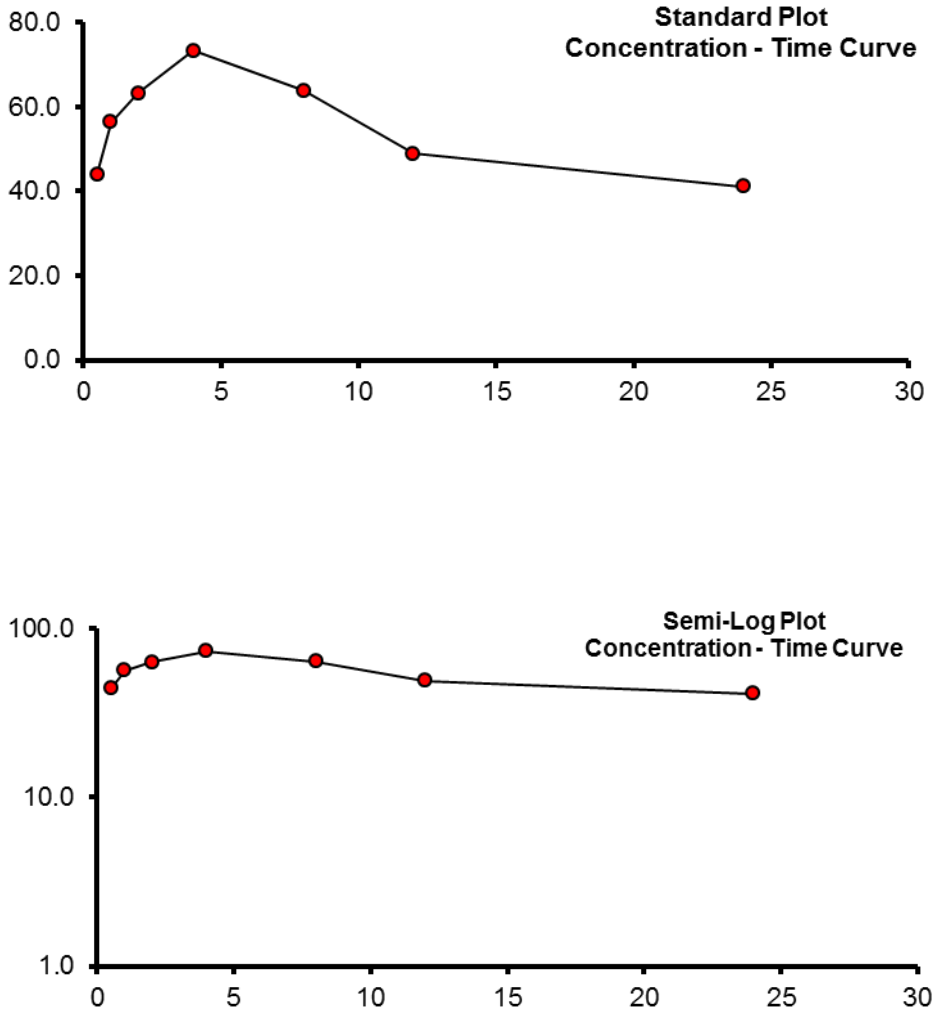

Figure 4.2a and 2b : Concentration time curve of Metformin (Reference) following oral administration of 500mg Tablet Sokoto Red Goats, Generated from pK Software N=10

4.12 Determination of Plasma Concentrations for Glumin

The concentration- time curves (standard and semi-log plots) of Glumin, following oral administration of 500 mg tablet to 10 Goat is shown . The corresponding curves using average values for the 10 animals are shown in Fig 4a and 4b.

Test Average Glumin

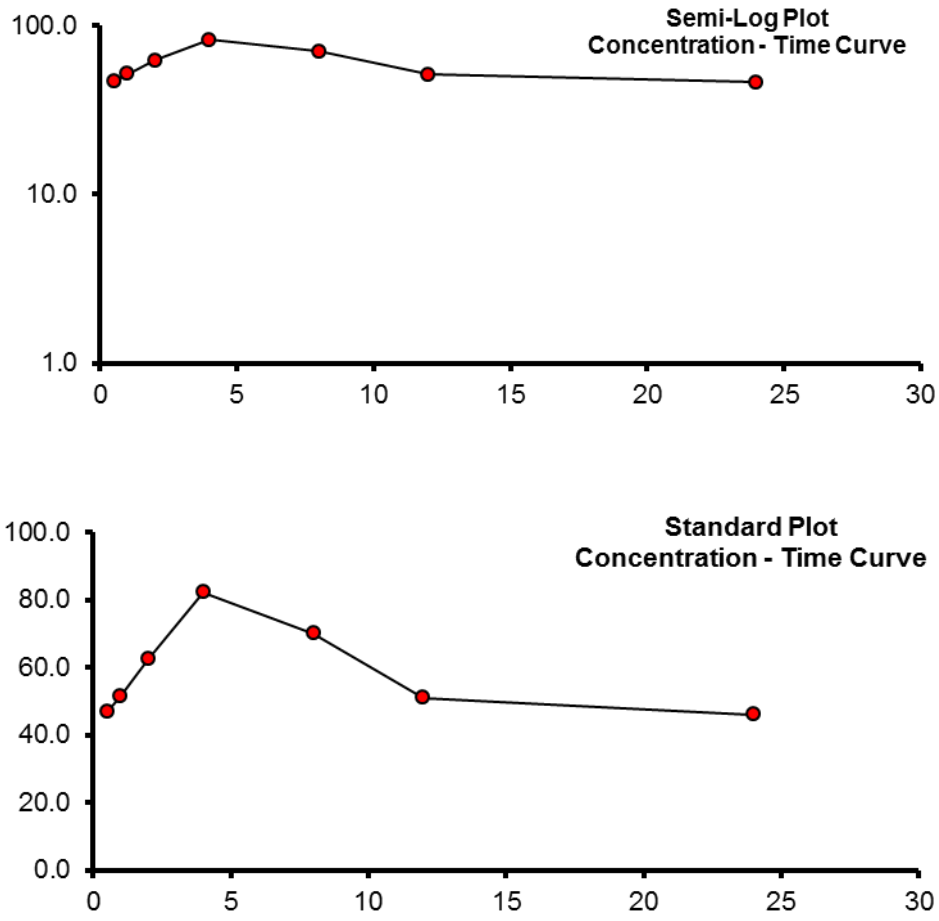

Figure 4.3a and 3b: Concentration Time Curve of Glumin(Test) following Oral Administration of 500mg Tablet Sokoto Red Goats, Generated from pK Software N=10

4.13 Pharmacokinetic Parameters of Glumin and Glucophage

Table 4.11 summarises the values of selected PK parameters (Cmax, Tmax, t½, Kel, AUC0-∞, and AUC0-t) of 10 Sokoto red goats after oral administration of a single 500 mg dose of either the test (Glumin) or the reference (Glucophage) brand.

Table 4.11: Comparison of the Mean PK Parameters (Cmax, Tmax, t½, Kel, AUC0-∞, and AUC0-t) of 10 Sokoto red Goats after Oral Administration of Single Dose Test(T)and Reference (R) Metformin 500mg doses. Using Graph pad Prism Software.

Parameter	Glucophage			Glumin		
	Mean±SD	CV (%)	90% CI	Mean±SD	CV (%)	90%CI
T_{max} (h)	4.7±2.50	53.12%	3.25-6.15	4.2 ± 2.20	52.40%	2.92-5.48
C_{max} (ng/ml)	85.15±25.18	29.57%	70.55-99.74	94.66±42.41	44.81%	70.07-119.20
K_{el}	0.03±0.02	86.21%	0.02-0.04	0.023±0.008	35.59%	0.02-0.03
$T_{1/2}$(h)	30.59±10.07	32.91%	24.76-36.43	34.13±11.22	32.86%	27.63-40.63
AUC0-t (ng/ml/h)	1262.67±103.78	8.22%	1203.00-1323.00	1344.04±363.25	27.03%	1133.00-1555.00
AUC0-∞ (ng/ml/h)	3752.81±1421.32	37.87%	2929.00-4577.00	3536.64±1088.60	30.78%	2906.00-4168.00

KEY: CV Coefficient of variation, SD Standard deviation

Data	Mean	Variance
Glucophage	855.99	2.258E6
Glumin	835.62	2.027E6

T= 0.024 p= 0.981 at the 0.05 level no significant difference between Glucophage and Glumin

Table 4.12 presents Summary of Confidence Intervals (95% and 90%) for the ratio of the values for Glumin to Glucophage of log-transformed Pharmacokinetic Parameters measuring extent of absorption.

Table 4.12: Confidence Intervals (95% and 90%) for the ratio of the values for Glumin to Glucophage of log-transformed Pharmacokinetic Parameters measuring extent of absorption (n=10)

	Glucophage (Mean±SD)	**Glumin (Mean±SD)**	**Ratio of Test/ Reference**	**95% Confidence Interval**	**90% Confidence Interval**
				0.9383 -	0.9512 - 1.0781
Cmax	4.41±0.28	4.47±0.42	1.0134	1.0921	
				0.9667 -	0.9667 - 1.0251
AUC0-∞	8.17±0.37	8.13±0.30	0.9955	1.0315	
AUC0-t	7.14±0.08	7.17± 0.27	1.0049	0.974 - 1.026	0.978 -1.022

CHAPTER FIVE

DISCUSSION AND CONCLUSION

Eight randomly selected brands of Metformin Hydrochloride were purchased from different pharmacy stores within Sokoto town. The brands were subjected to a number of pharmacopoeial tests (friability, uniformity of weight, disintegration, hardness and dissolution as well determination of chemical content) to ascertain their bioequivalence.

All the brands passed the weight uniformity test, since the variation (relative standard deviation) of the tablets in each brand was lower than 5% of the mean value. This is in compliance with the British pharmacopoeial standards (BP 2012).the finding is similar to that reported in a study in Iran by Zakeri-Milani et al.(2012).

The result of the friability test showed that seven out of the eight brands passed the test with values ranging from 0.04% to 0.32%. The only exception is Gluformin with a friability of 1.62% which is beyond the acceptable limit of 1%. According to the recommendation of British pharmacopoeia no brand should have a value greater than 1.0%w/w, hence brand E failed friability test. This finding was comparable to a study done by Olusola et al.(2012).

From the results of the disintegration test, all the brands passed the test, with all the tablets disintegrating in less than 30 min, which is the upper limit for film –coated tablets as specified by British pharmacopoeia (BP, 2012). This is similar to a study from Iran by (Zakeri-Milani et al., 2012). Disintegration is an important step in drug release from immediate release dosage forms. The rate of disintegration is influenced by the rate of

influx of water into the drug which is dependent on the porosity of the tablets. It can be used as a measure to predict product behaviour in vivo (Papadopoulou et al., 2008).

With the exception of Metforcap, all the brands released 85% of their drug contents within 15 minutes; thus meeting the criteria for Biopharmaceutical classification system (BCS) class 3 drugs. Dissolution test is currently used as an in vitro bioequivalence (BE) test, for establishing the similarity of pharmaceutical dosage forms and determining dissolution profile (Amidon et al., 1995; Jang, et al, 2010; Esimone et al., 2008)

The values of similarity factor "F2" comparing the dissolution of seven generic brands with innovator brand demonstrated equivalence of 4 brands (Glumin® , Diabetmin® , Degluco® , BG Lophage®) at pH 6.8, but none exhibited equivalence at pH 1.2. The use of fit factors is recommended for dissolution profile comparison in FDA's guides for industries. According to these guides, f1 values up to 15 (0-15) and f2 values of at least 50 (50-100) indicates similarity or equivalence of the two curves (Yuksel et al., 2000). The study done by Zakeri-Milani et al. (2012) had better similarity as all the brands were equivalent. The above result is, however, comparable to the report by (Olusola et al.(2012). All assay results achieved from analysis of active ingredients in the brands and innovator products, were within the range of 95 to 105% of labeled amount, which represent the limits specified by British Pharmacopoeia. (BP, 2012). Therefore regarding the in vitro tests of pharmaceutical bioequivalence, Glumin had the most impressive performance based on friability, disintegration, dissolution and drug content compared to the innovator product, hence was selected for the in vivo study.

High performance liquid chromatography (HPLC) was used in this study to quantify blood concentration of metformin. Several methods for determination and estimation of metformin in biological samples have been documented in the literature such as calorimetry, gas chromatography and UV spectrophotometry (Corti et al., 2008; Jang et al., 2010; Lee et al., 2011; Mistri et al., 2007; (Kim et al., 2009)Shin et al., 2011) Different HPLC methods have been used for this purpose. Techniques were Ion-pair, reverse- phase and cation-exchange HPLC (Cheng and Chou, 2001; Huttunen et al., 2009; Önal, 2009; Hu et al.,2006; Al-Rimawi, 2009).

The calibration graph, drawn up by plotting peak area of metformin standard versus metformin concentration, had good linearity within the range of 500- 4000 ng/ml (mean r^2= 0.9975). The r^2 value obtained in this study is similar to the work done by Gabr et al. (2010) also comparable to the research in Iran by Valizadeh et al.(2014) with a value of 0.9987.

The core pharmacokinetic parameters (Cmax, Tmax, AUC0-∞ and AUC0-t) showed no statistically significant difference between the test and reference formulations. The ratios of average Cmax, AUC0-t and AUC0-∞ of metformin samples (test against reference) for the logarithm transformed data were 1.0134, 1.0049 and 0.9955 respectively. The corresponding 90% CI were 0.9512 – 1.0781, 0.978 – 1.022 and 0.9667 – 1.0251 which were within the acceptable range of 0.80 – 1.25. These results had similar values to the study done in Iran by Valizadeh et al.(2014) and several other studies carried out on other dosage forms in different countries within Asian continent (Ali et al., 2007; Corti et al., 2008; Hu et al., 2006).

Conclusion

The current study demonstrated that at pH 6.8, 3 out of the 7 tested generics of metformin were not equivalent in vitro to the reference product Glucophage. In vivo experiment revealed that Glumin was bioequivalent to Glucophage with respect to the rate and extent of absorption. Glumin which was a low cost generic was proven to be bioequivalent to Glucophage; hence assumed to be interchangeable in clinical practice.

Recommendations

A detailed study in Humans should be conducted to corroborate the findings in Animal studies. More number of generic brands needed to be compared with the innovator brand in the in-vivo study to give room for more bioequivalent alternatives.

A multi dose study is further recommended to compare the effect of dosing interval.

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APPENDIXES

Appendix 1: Market Survey of Prices of Metformin Brands in Sokoto

Brand	Cost (Naira)	Availability
Diabetmin	12	9
Glucophage	33	8
Glumin	13	6
Gluconorm	20	4
Glucodix	15	1
Panfor	25	3
Degluco	6	3
Aldoxi	7	1
BG Lophage	14	3
Bondomet	6	1
Clabetic	15	1
Diabenon	10	1
Diamet	25	2
Glavamet	10	2
Glucoform	15	2
Gluformin	12	3
Glulife	15	1
Metforcap	10	2

Appendix 2: Pharmacokinetic Parameters of Test Product (Glumin)

The table presents the pharmacokinetic parameters of glumin (Tmax, Cmax, T½,AUC $_{0-t}$ AUC $_{0-\infty}$,

: Pharmacokinetic Parameters for Test Product

GLUMIN AVERAGE						
	Tmax	Cmax	Auc 0-t	Auc 0-∞	Kel	t½
T1	2	58.42	895.01	2745.28	0.019	37.12
T2	2	64.61	1165.91	3994.61	0.015	46.87
T3	4	68.1	1147.53	2193.65	0.032	21.68
T4	4	136.09	1804.54	3906.09	0.027	26.2
T5	8	122.27	1645.3	5838.4	0.014	45.61
T6	2	57.76	903.07	2958.56	0.017	40.58
T7	4	62.19	1162.54	4102.21	0.014	49.11
T8	4	69.76	1182.01	2296.56	0.031	22.26
T9	4	173.52	1745.87	3237.62	0.035	20.02
T10	8	133.84	1788.64	4093.43	0.022	31.87
MEAN	4.2	94.66	1344.04	3536.64	0.023	34.13
SD	2.20	42.41	363.25	1088.60	0.008	11.22

KEY: T= Test product ,SD= Standard Deviation

Appendix 3: Summary of Pharmacokinetic Parameters for Reference Product (Glucophage)

The table presents the pharmacokinetic parameters of glumin (Tmax, Cmax, T½,AUC 0-t ,AUC 0- ∞,)

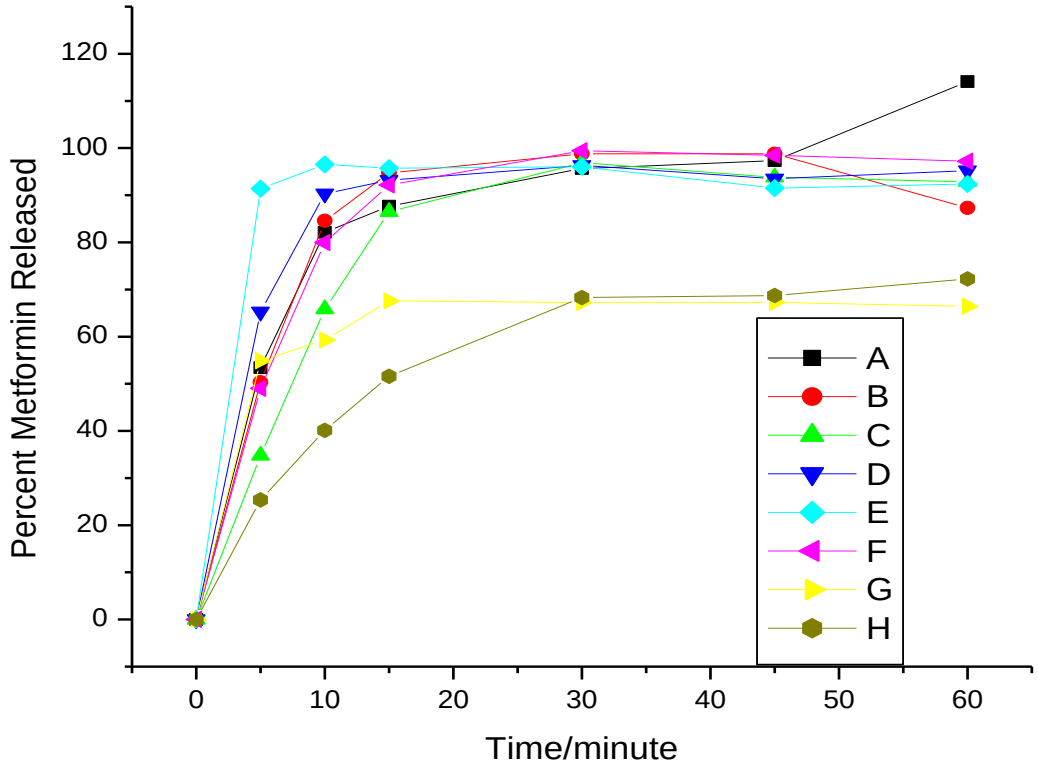
The mean and standard deviation are shown.

Summary of Pharmacokinetic Parameters for Reference Product

GLUCOPHAGE AVERAGE						
R1	Tmax	Cmax	Auc0-t	Auc 0-∞	Kel	t½
R1	2	60.14	1186.25	2579.71	0.03	27.97
R2	4	131.37	1463.36	2694.27	0.04	19.96
R3	8	63.18	1216.19	5248.59	0.01	32.07
R4	4	97.23	1265.7	2418.92	0.03	22.54
R5	4	73.06	1188.2	4879.87	0.01	37.66
R6	4	73.17	1247.99	2328.94	0.03	20.78
R7	1	98.13	1191.44	3467.05	0.01	37.08
R8	4	61.58	1185.13	5084.18	0.01	31.97
R9	8	119.49	1439.86	2733.46	0.03	23.35
R10	8	74.11	1242.53	6093.14	0.09	52.56
MEAN	4.7	85.15	1262.67	3752.81	0.03	30.59
SD	2.50	25.18	103.78	1421.32	0.03	10.07

KEY: R= Reference product , SD= Standard Deviation

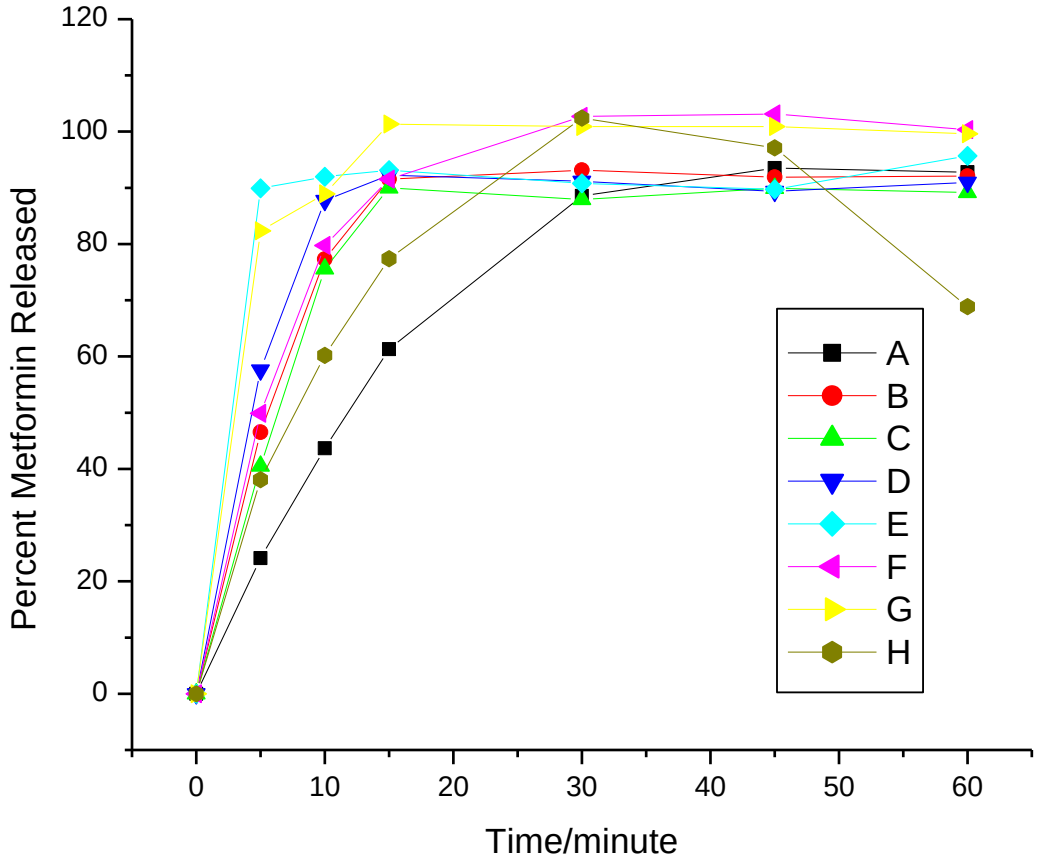
Appendix 4



Appendix 4: Dissolution Profile of Eight Brands of Metformin at PH 6.8

A= Glucophage, B= Glumin, C= Diabetmin, D= Degluco, E= Gluformin, F= BG Lophage, G= Glucoform, H= Metforcap

Appendix 5



Appendix 5: Dissolution Profile of Eight Brands of Metformin at pH 1.2

A= Glucophage, B= Glumin, C= Diabetmin, D= Degluco, E= Gluformin, F= BG Lophage, G= Glucoform, H= Metforcap