USMANU DANFODIYO UNIVERSITY, SOKOTO
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Pharmaceutical Equivalence and *In vivo* Bioequivalence of Some Generic Brands of Ceftriaxone in Sokoto

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BY

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DEDICATION

This work is dedicated to my beloved family, friends, and well-wishers.

CERTIFICATION

This dissertation by Ode Edward Innocent (11210708120) has met the requirements for the award of the degree of Master of Science, Pharmacology (M.Sc Pharmacology), of Usmanu Danfodiyo University, Sokoto and is approved for its contribution to knowledge.

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LIST OF ABBREVIATIONS

AST	-	Aspartate aminotransferase
AUC	-	Area Under the Concentration-time curve
ALT	-	Alanine Aminotransferase
ANOVA	-	Analysis of Variance
BUN	-	Blood Urea Nitrogen
CL	-	Clearance
C max	-	Maximum Plasma Concentration
FDA	-	United States Food and Drug Administration
FBI	-	Federal Bureau of Investigation
GI	-	Gastrointestinal Tract
HPLC	-	High-Performance Liquid Chromatography
MRT	-	Mean Residence Time
S Cr	-	Serum Creatinine
T max	-	Time to reach maximum concentration
$T_{½}$	-	Elimination half-life
V_d	-	Volume of Distribution

ABSTRACT

A microbiological assay was used to compare pharmaceutical equivalence of six generic brands of ceftriaxone (Derfxone®, Costriax®, Cefzone®, Avicef®, Kembarth® and Biocef®) randomly selected from Pharmaceutical outlets in Sokoto and compared to the innovator product Rocephin®. Findings from regression analysis revealed the slope of the zone of inhibition against concentration for Biocef (4.959) to be closest to that of Rocephin (4.151). Biocef® was therefore chosen for comparison of its *in vivo* bioequivalence with Rocephin®. For the *in vivo* bioequivalence, a randomized two period, two sequence cross-over studies was conducted in ten healthy Sokoto red goats at a dose of 20mg/kg body weight. Plasma concentration of the two brands of ceftriaxone was determined using high-performance liquid chromatography and the various pharmacokinetic parameters of Rocephin® versus Biocef® were calculated and analyzed. The result showed no statistically significant difference between Biocef® and Rocephin®. The AUC for Biocef® was 5.171µg.hr/ml, and Rocephin® was 5.345µg.hr/ml. The result of 90% confidence interval of the ratio of Biocef® to Rocephin® was 0.8669 to 1.047 (86.69-104.7%) which is within the acceptance range of 0.8-1.25 (80-125%) stipulated by FDA for establishing bioequivalence. It can therefore be said with some degree of certainty that Biocef® is bioequivalent to Rocephin®.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The antibiotic penicillin was discovered accidentally by Sir Alexander Fleming in 1928. It was used extensively during the World War II. As time went by, new antibiotics started becoming increasingly available (Dosani, 2005).

Globally, antimicrobials are the most widely used agents in the world (WHO, 2004). This report also found out that up to 50% of antibiotic prescriptions are unnecessary. From 1992 to 1996, the World Health Organization (WHO) conducted a study in which it was discovered that 30% of antibiotics prescribed for upper respiratory tract infection (URTI) in middle and high-income countries were wrongly prescribed. Ronning *et al*., (2003) presented an article in which they reviewed antibacterial usage in 16 European countries and discovered that use of antibiotics was higher than necessary and in some cases irrational. This observation was supported by a report presented by the 14th European Congress of Clinical Microbiology and Infectious diseases in Prague in May 2004. The report suggested that antibiotics were used in 36% of children with symptoms of acute respiratory infections and diarrhoea-like syndrome (Kristiansson, 2004). Globally, about 50% of antibiotic prescriptions are unnecessary (WHO, 2004). According to study done and presented to WHO in 2000 suggested that 60% of antibiotic prescription in Nigeria was unnecessary (Gash, 2008). In Nepal, WHO suggested that 50% of antibiotic prescriptions in 1996 were unnecessary. In May 2005, the World Health Assembly warned that the irrational use of various antimicrobials is responsible for the promotion of resistance, especially in low-income countries. The World Health Organization (WHO) country data for 2002–2003 gave an estimate that prevalence of resistance to penicillins by

Neisseria gonorrhea was between 12% to 55% on a global scale (Gash, 2008). In 1992, the Ministry of Health of Malaysia in conjunction with National University of Malaysia did a joint study at six government hospitals. The breakdown showed that the Essential Medicine list recommended only 20 antibiotics but antibiotics preparations marketed in Malaysia were more than 200 (Gash, 2008). This factor can also lead to antibiotic overuse and the possibility of resistance. The working group on Health and Development (WEMOS) in Amsterdam pointed out that many preparations, including antibiotics, have been banned or recalled in some European countries, yet, in 1991, 75 of such products were found in some developing countries (Gash, 2008).

Infectious diseases are disorders caused by organisms such as bacteria, viruses, fungi, parasites and other disease-causing microorganisms. Infectious diseases have been known for thousands of years, although correct information on their etiology has only been available 100 years ago. In the teachings of Hippocrates, the cause of infections happening recurrently in a specific locality or during a specified period (epidemics) was sought in "change" in the air according to the theory of *miasmas* (Halliday, 2001). But Louis Pasteur in his time disproved the theory of spontaneous generation and established that without contamination, microorganisms could not develop. Towards the end of 19th century, microorganisms had been identified as the causal agents in many familiar diseases by applying the Henle-Koch postulates formulated by Roberts Koch in 1890 (Lerner & Lerner, 2003).

Bacteria are microorganisms that are found almost everywhere: in the air, water, food, and soil. Their nature can either be (1) rod-shaped, e.g., *Bacillus* and *Klebsiella spp*; or (2) in the cluster, e.g., *Staphylococcus aureus.* They can be gram positive or gram negative

depending on the thickness of their cell walls (Betsy & Keogh, 2005). Bacteria that are pathogenic can cause infections. Examples of bacterial infections include diarrhoea caused by *Escherichia coli*; skin infection due to *Staphylococcus aureus;* gonorrhea *by Neisseria gonorrhoeae*; Syphilis by *Treponema pallidum*; typhoid fever by *Salmonella typhimurium;* otitis media by *Streptococcus spp.* e.t.c. (Talaro & Chess., 2015).

People at extremes of age like elderly and neonates are at risk of developing infection due to *Escherichia coli* because of their weak immunity, the elderly particularly can quickly develop pneumonia caused by *Streptococcus pneumonia* (Thigpen *et al.,* 2011). Poor nutritional status can also predispose individuals to infection by lowering their immunity (Chandra, 1997). Nutrition can be improved using a balanced diet containing all the essential minerals and vitamins which function as cofactors and stimulate enzymes to carry out metabolic activities that will provide energy for the cells and fight infections. Another important risk factor for the development of bacterial infection is the genetic predisposition (Asner *et al.,* 2014). From the human genome projects, scientists have discovered that some people are more susceptible to certain infections or diseases than others because of their genetic makeup (Asner *et al.,* 2014). The World Health Organization report (1995) pointed out that 50% of deaths in developing countries in 1993 was due to communicable diseases. Out of 20 million deaths that occurred due to communicable diseases in that period, more than 16 million fatalities were due to infectious and parasitic diseases. Bacterial infections in Africa has taken a new dimension. For instance, invasive non-typhoidal salmonella disease caused by *salmonella enterca var typhimurium* has caused fatality between 20 to 25% in Sub-Saharan Africa (Feasy *et al.,* 2012). The disease presentation includes; fever, splenomegaly, diarrhea, etc. The risk

factors for the disease are; HIV infection, Malaria, and malnutrition in children. It has a prevalence of 175 to 388 cases per 100,000 in children and 2000 to 7500 cases per 100,000 HIV individuals (Feasy et al., 2012). In Nigeria, bacterial infection is also a problem. A study carried out by Jido and Garba in Aminu Kano Teaching Hospital (Jido & Garba, 2012) found that bacterial infections accounted for 9.1% of cases while *Staphylococcus aureus* was the implicating organism in 31.8% of cases recorded in surgical-site infection following caesarian section. The cephalosporins were the antibiotics that showed high sensitivity to the organism.

Bioequivalence and bioavailability are important concepts because of their application to innovator products and generic drugs. Over the years, regulatory agencies have come up with this idea as a criterion for approving generic drugs intended to be marketed. Generic drugs are important in the health care system because of their low cost and availability (Midha & Mckay, 2009). Before generic drugs are accepted and interchangeable with innovator products, they must demonstrate bioequivalence. The assessment of bioequivalence between an innovator and a generic is done through *in vivo* and *in vitro* studies (Meredith, 2003).

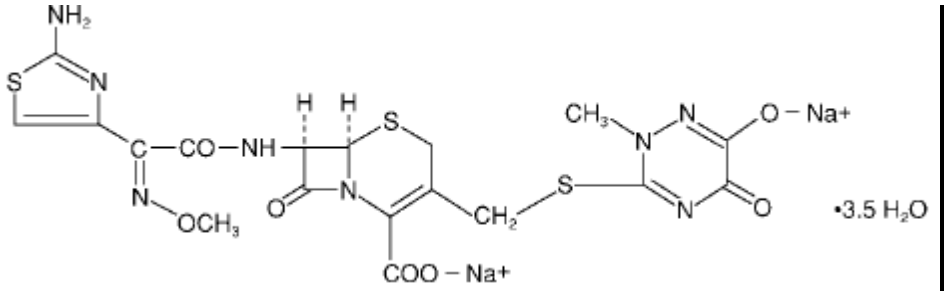
The quality of ceftriaxone in developing countries has become a source of concern. Several studies have been carried out on this. Some of the reviews have demonstrated that most generic versions of ceftriaxone were pharmaceutically and therapeutically equivalent to the innovator product (Okorie *et al.,* 2016) While some studies held negative views about the quality of the generic products. (Lambert & Conway, 2003), in their research publication, made some observations on the quality of 34 generic formulations of ceftriaxone when compared with the innovator product- Rocephin. According to them, all

the 34 ceftriaxone generics failed to match the requirements for Rocephin as specified by Roche. Also, eighteen of the generics did not meet the quality standards specified by European Pharmacopoeia, and four generics were not sterile; thirty did not meet the standard for clarity of 12% aqueous solution, while 33 contained thiotriazinone, a degradation product of ceftriaxone (Lambert & Conway, 2003). Based on the above-stated facts, comparative analysis of generic ceftriaxone is necessary to ensure compliance with the standard requirement.

Ceftriaxone, a semi-synthetic antibiotic, is a third generation cephalosporin widely used for modern-day clinical practices. This drug has been used in pre- and postoperative therapy against infections in gastrointestinal, pelvic, orthopaedic, cardiac and pulmonary diseases (Esposito *et al.,* 2004). It is a broad-spectrum antibiotic that is effective against both Gram-negative and Gram-positive bacteria. Regarding safety and efficacy, it is considered comparable to cefotaxime. It is marketed by Hoffmann –La Roche under the trade name Rocephin®. It can be used in combination but not directly with macrolide and aminoglycoside antibiotics for the treatment of community-acquired pneumonia (Roche, 2010). It is also a drug of choice for the treatment of bacterial meningitis. It is usually used in febrile infants of 4-8 weeks of age who are admitted to the hospital to rule out sepsis. It has also been used in the treatment of Lyme disease, typhoid fever and gonorrhoea (Roche, 2010).

Ceftriaxone is a white crystalline powder and is readily soluble in water but sparingly soluble in methanol or ethanol. The pH of a 1% aqueous solution is approximately 6.7 which makes it slightly acidic. The molecular formula of ceftriaxone sodium is C18 H16 N8 Na2 O7 S3 •3.5 H2O and has a molecular weight of 661.59 g/mol (Roche, 2010).

The structure of ceftriaxone is represented below



The mechanism of action of cephalosporins is similar to that of the penicillins. They inhibit transpeptidation reaction by binding covalently to the enzymes responsible for cross-linking the peptidoglycan strands which provide mechanical strength to the cell wall. The cephalosporins prevent cell wall synthesis and eventually lead to cell death. The exact mechanism of cell death is not entirely understood (Katzung *et al.*, 2007).

Ceftriaxone is considered as one of the most important medications needed in a primary health system and is also included on the World Health Organization's list of essential medicines (WHO, 2015).

The drug can be administered at a dose of 1 g in an adult. It has a maximum concentration (Cmax) of 123-151 µg/l, a half-life of 8 hours and volume of distribution of 10.7 l/kg in an adult.

Adverse reactions can be categorised as follows (Roche, 2010).

Hypersensitivity: maculopapular rash, urticaria, pruritus, anaphylaxis/angioedema, and eosinophilia.

Haematologic: hypoprothrombinaemia, neutropenia, leucopenia, and thrombocytopenia.

Gastrointestinal: diarrhoea and *Clostridium difficile* disease

Renal: interstitial nephritis (Roche, 2010).

It is contraindicated in hyperbilirubinaemic neonates. In this condition, there is an increased risk for bilirubin encephalopathy (Kernicterus) (Roche, 2010).

It can interact with calcium-containing diluents like Hartmann's solution. Other clinical situations that require precaution in the use of ceftriaxone include patients on cyclosporine due to an increased risk of cyclosporine toxicity (renal dysfunction, cholestasis, paraesthesias). Also, administration of ceftriaxone to patients on live typhoid vaccine may lead to a decreased immunological response to the vaccine (Roche, 2010).

The following parameters should be monitored when administering ceftriaxone.

Differential white blood cell count, culture and sensitivity result, Kidney function like blood urea nitrogen(BUN), serum creatinine should be done to ascertain the function of the liver, aspartate aminotransferase, (AST), alanine aminotransferase (ALT).For renally impaired patients and patients who have been receiving anticoagulant like warfarin, caution should be exercised. Nutritionally poor patients should also be monitored.. (Hoffman la Roche) Brand names/Manufacturer**:** Rocephin/Roche (Roche, 2010).The cost of innovator or branded antibacterials in developing countries is high. Most people in developing countries cannot afford branded drugs. Studies carried out in 36 developing countries on different antibiotics showed that about 50 – 70% savings could be achieved on the average with the use of generic drugs. About 73% savings was achieved for

ceftriaxone injection (Cameron *et al.*, 2009). The purpose of generic medicine or its promotion is often aimed at reducing cost and increasing consumer access to quality medicines. In most times originator brand medicines with patents are more expensive than their generic equivalents (Auta et al., 2014).

1.2 Statement of the Problem

Ceftriaxone, a broad spectrum antibiotic, is used extensively by clinicians for managing many bacterial infections. Because of the vast number generics of ceftriaxone, clinicians sometimes are faced with the huddles of choosing from different brands. Some of the generic brands of ceftriaxone sometimes don't give the expected results when they are used (Lambert & Conway, 2003) . This leaves most clinicians with no option than to prescribe the innovator product Rocephin. The fact that majority of Nigerian population are poor and the health insurance coverage is very poor in the country meant that many patients residing in Sokoto may not be able to afford the high cost of the original brand of ceftriaxone (Cameron *et al.*, 2009). In developing countries like Nigeria, it is recommended that Antibiotic prescription should be based on generics. There is gap of knowledge about bioequivalence of the generic brands of ceftriaxone in Nigeria.

1.3 Justification

The quality of ceftriaxone marketed in developing countries has become a source of concern. Some do not meet the requirements stipulated by regulatory agencies and official books. It is worrisome because such pharmaceutical products will not produce the needed therapeutic effects when used (Lambert & Conway, 2003). Several studies have also been done in Africa, Asia, and South America in an attempt to compare various brands of ceftriaxone to the innovator product, Rocephin® (Arnet et al., 2014). Research carried out

by some researchers in Eastern Asia indicated that some brands of ceftriaxone did not meet *in vitro* bioequivalence requirements (Arnet *et al.,* 2014; Thirumurugan *et al.,* 2015). Many clinicians in Usmanu Danfodio University Teaching Hospital Sokoto use Ceftriaxone for both pre and post-operative cases; they also used it for empirical treatment especially for people on admission before laboratory investigations are done (personal communication). It will be imperative for both pharmaceutical equivalence and *in vivo* bioequivalence to be carried out to ascertain the quality of ceftriaxone use.

1.4 Aim and Objectives

The study aimed to evaluate and validate the pharmaceutical equivalence and *in vivo* bioequivalence of some generic brands of ceftriaxone marketed in Sokoto.

The objectives of the study are:

- To conduct a market survey of generic brands of ceftriaxone available in Sokoto
- To determine pharmaceutical equivalence of some generic brands of ceftriaxone by using microbiological assay.
- To determine the generic with closest pharmaceutical equivalence to the innovator product and compare it with the innovator product using *in vivo* bioequivalence in Sokoto red goats

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Ceftriaxone

There are a lot of published studies on bioequivalence of ceftriaxone. Most of these studies were carried out in different countries using either animals or humans. (Brogden et al., 1984; Brogden & Ward, 1988) did an appraisal of its antibacterial activity, pharmacokinetics properties, therapeutic uses and adverse effects in human. Because of the vast number of generic brands of ceftriaxone marketed in the world, there is the tendency that some may not be bioequivalent or measure up to the essential quality specified by regulatory agencies (Odeniyi *et al.,* 2003). It is evident that the prevalence of resistance will be high in developing countries if all the generics available are used without assessing their quality. 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% were manufactured in India (Okunlola *et al.,* 2009). Based on these facts, most of the healthcare professionals and other health workers were of the view that only innovator pharmaceutical products that are expensive are effective (Odeniyi et al., 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 a 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 product. To

ensure compliance, there is the need for regulatory agencies to continually monitor imported generics and those drugs manufactured locally in Nigeria.

A generic drug is defined as a pharmaceutical product that is intended to be interchangeable with an innovator product, is manufactured without a license from the innovator company and is marketed after the expiry date of the patent or other exclusive right (WHO, 2015). A generic medicine contains the same active pharmaceutical ingredients like the innovator product. It is used at the same dose, strength and route in treatment of the same disease. Because of the huge global healthcare expenditure, many developing countries have been encouraged to utilise generic medicines in order to cut cost (WHO, 2014). The National Health Insurance Scheme (NHIS) which is a program under the National Health Policy of Nigeria recommends the prescription of NHIS drugs to be strictly on generics, unless otherwise necessitated. The only way generic can be compared with the innovative product and the result be satisfactory is through bioequivalence studies. Some antibiotics sold in Calabar did not have the required active ingredients needed for effective therapy (Nkang *et al.*, 2010). Various methods have been used to demonstrate bioequivalence in antibiotics, either *in vitro* or *in vivo* (Rodriguez et al., 2010). Bioequivalence of two formulations is assessed in terms of the Area under the curve (AUC), Time to reach maximum concentration (T_{max}), and the Peak Plasma Concentration (C_{max}) If there is no statistically significant difference between the test and the reference, then the formulations are said to be bioequivalent (FDA, 2010). Bioequivalence studies for formulations can be carried out using human volunteers or animals. For example, in a two treatment crossover study, bioequivalence of two brands of cefuroxime 500mg tablets (cefuzine® and Zinnat®) was carried out in healthy human

volunteers following a single dose, two-treatment,two-period crossover design, and the ratio of the test to the reference was in agreement with acceptable range of 80-125% (AL-Said *et al.,* 2000). Also, a comparative bioavailability of two intramuscular preparations of ceftriaxone in healthy Thai volunteers carried out by (Kanthawatana et al., 2011). The study was a randomised, double-blind two periods cross-over design involving 20 healthy volunteers. They received a 1 g intramuscular injection of a generic ceftriaxone (Cet-3) and the innovator preparation (Rocephin). Plasma ceftriaxone samples were collected and analysed using High- Performance Liquid Chromatography (HPLC) and the relevant pharmacokinetic parameters of Cet-3® and Rocephin® were compared. The result showed that Cet-3 could be used in place of the more expensive innovator product. Animal studies carried out by researchers using ceftriaxone showed that some generic formulations ceftriaxone have pharmacokinetics comparable to that of the innovator product. Tiwari et al. (2009) determined the pharmacokinetics of ceftriaxone in goats. In the study, they used a dose rate of 20 mg/kg. Following intramuscular administration, plasma ceftriaxone was determined using High Performance Liquid Chromatography (Hakim *et al.,* 1988). The peak plasma concentration of ceftriaxone was 21.51± 0.6 mg/ml, Time to reach maximum concentration was 0.5 hrs, ceftriaxone concentration was determined up to 12hours, apparent volume of distribution was 0.53±0.05 l/kg, Area under the curve (AUC) was 66.78±4.9 mg.hr/ml, Elimination half-life was 2.03±0.09 hr, and the clearance was 3.04±0.34ml/min/kg, respectively. The intramuscular bioavailability was 59.0±4.0%. They concluded that at a dose of 20 mg/kg, ceftriaxone could be used to treat bacterial infections in sheep. In another research work, Tiwari and some researchers determined pharmacokinetics and bioavailability of ceftriaxone in Patanwadi sheep

(Tiwari *et al.,* 2010). They concluded that a dose of 10mg/kg body weight can be used to treat various bacterial infections in Sheep.

2.1.1 Pharmacokinetics of ceftriaxone

Pharmacokinetics deals with the changes in the concentration of a drug and/or its metabolite(s) in the human or animals body following administration of the drug product, i.e., the changes of drug concentration in different body fluids and tissues in the dynamic systems of liberation, absorption, distribution, body storage, binding, metabolism and excretion (Bauer, 2008).

2.1.2 Linear and Non-Linear Pharmacokinetics of ceftriaxone

Majority of drugs follow linear pharmacokinetics, concentration of the drug changes proportionally with dosing.Non-linear kinetics or saturation kinetics refers to a change of one or more of the pharmacokinetics parameters during absorption, distribution, metabolism and excretion by saturation or overloading of the process due to increased dose sizes (Bauer, 2008). Most drugs like ceftriaxone, erythromycin, paracetamol follow linear pharmacokinetics or first order kinetics. Non-linear pharmacokinetics is usually due to saturation occurring in one of the pharmacokinetic mechanisms such as protein binding, hepatic metabolism or active renal transport of the drug (Goodman *et al.,* 2011).

Elimination is saturable when plasma concentration of the drug increases and the elimination rate reaches its maximum capacity. In non-linear elimination kinetics, the drug clearance decreases with increasing drug concentration (Goodman *et al.,* 2011).

For saturable protein binding, as the concentration of drug increases, the fraction of drugs that are unbound will eventually increase because all the available binding sites are saturated. Concentrations in plasma are in the range of 10s to 100s of ug/ml). For a drug

that is metabolised by the liver with a low intrinsic clearance- extraction ratio, saturation of plasma binding will cause both volumes of distribution and clearance to increase; $t_{½}$ may remain constant. For such drug that is cleared with the high intrinsic clearance-extraction ratio, the steady-state concentration may remain linearly proportional to the rate of drug administration (Goodman *et al.*, 2011).

2.2 Pharmacokinetics Parameters

The four most important pharmacokinetic parameters are clearance, the volume of distribution, elimination half-life and bioavailability.

Bioavailability is the fraction of drug administered that reaches the systemic circulation. It is also the rate and extent of absorption of a drug from a dosage form. Some drugs, eg., neomycin sulphate and sulphaguanidine are either not absorbed at all or poorly absorbed following oral administration. Some factors can influence the bioavailability of a drug. They include Solubility of drugs, dosage form and route of administration, first pass effect, formulation factors as well as physiological factors. Bioavailability parameters include C_{max}, T_{max}, and AUC. When the AUC, C_{max}, and T_{max} are the same within the statistical limit for two dosage forms, they are considered bioequivalent (Goodman *et al.*, 2011).

Clearance is the measure of the body's ability to eliminate a drug. Clearance is viewed as the most critical parameter to describe the pharmacokinetic of a drug. It is the most important concept to be considered when a rational regimen for long-term drug administration is to be designed. Clearance of a drug by several organs is additive. Drug elimination in the body is the total of the elimination of some important organs in the body. These organs are liver, gastrointestinal tract, and kidney. The rate of elimination of

each of these organs will result in their respective clearances. The total of this clearance will represent the systemic clearance (Katzung *et al.*, 2007).

CL renal +CL hepatic+ CL other = CL

Clearance (CL) = Rate of elimination/concentration

Renal Clearance

Renal clearance results in the appearance of a drug in the urine. It depends on the physicochemical properties and the binding ability of the drug plasma proteins, and the physiology of the kidney. The filtration rate of a drug is dependent on the quantity of fluid that the glomerulus filters and also the concentration of the unbound drugs to the plasma. Also, the secretion rate of drugs by the kidney is dependent on active secretion of the drug as a result of clearance by the endogenous transport system. These transporters can get saturated with drugs at a certain concentration, and that can affect delivery rate to the secretory site (Goodman *et al.*, 2011).

Hepatic Clearance

For a drug that is efficiently removed from the blood by the hepatic process (metabolism and excretion of the drug into the bile), the concentration of the drug leaving the liver will be low. The extraction ratio will approach unity, and clearance of drug from the blood will become limited by hepatic blood flow (e.g., drugs with systemic clearance greater than 6ml/min/kg). They are restricted in their rate of elimination by the rate at which they can be transported in the blood to the hepatic sites of elimination and not by intrahepatic process. Such drugs include imipramine, lidocaine, morphine, chlorpromazine, and

propranolol. Some factors can influence clearance. The rate of flow of blood to the organ and the dosage (Goodman *et al.,* 2011).

(1) Albumin concentration: some drugs bind strongly to plasma albumin. Examples of these drugs are phenytoin, theophylline, and the salicylates. In disease condition, albumin level tends to be low, and that can affect drug concentration.

(2) Alpha 1 acid glycoprotein concentration: α_1 acid glycoprotein is an important binding protein with binding sites for drugs. Even though drug elimination may remain unchanged sometimes, the concentration of some drugs like quinidine, propranolol, and lignocaine are increased in some disorders like acute inflammation that causes a change in plasma concentration.

(3) Capacity-limited protein binding: the binding of drugs to plasma protein is capacity-limited. The therapeutic concentration of salicylates and prednisolone shows concentration-dependent protein binding. Because unbound drug concentration is determined by dosing rate and clearance which is not altered, in the case of these low extraction ratio drugs, by protein binding- increase in dosing rate will cause corresponding changes in the pharmacodynamically important unbound concentration. Total drug concentration will increase less rapidly than the dosing rate would suggest as protein binding approaches saturation at higher concentrations (Goodman *et al.,* 2011).

The volume of distribution (V_d) relates the amount of a drug in the body to the concentration of the drug in the blood or plasma (Katzung *et al.,* 2007). It is the theoretical size (volume) of space necessary to contain the amount of a drug in the body given its concentration in plasma. It is useful for estimating a loading dose. It does not necessarily

refer to an identifiable physiological volume, but merely to the fluid volume that would be required to contain the entire drug in the body as the same concentration as in plasma. The volume of distribution is a direct measure of the extent of distribution. It rarely corresponds to the real volume. For example, the plasma volume of an average 70 kg man is 3 litres, blood volume is 5 litres, extracellular fluid volume outside plasma is 12 litres, and volume of the total body water is approximately 42 liters. However, many drugs exhibit volume of distribution far in excess of these values. For example, if 500 µg of digoxin is in the body of a 70 kg subject, as the concentration of approximately 0.7 ng/ml will be observed (Katzung *et al.*, 2007).

$$\text{i.e} \qquad \frac{500{,}000\text{ng}}{0.7\text{ ng/ml}} = 71 \text{ litres}$$

This value is almost two times greater than total body water of a 70 kg man. Digoxin is relatively hydrophobic. It distributes preferentially to muscle and adipose tissue and its specific receptors, leaving a small amount of drug in the plasma. The whole blood drug concentration is used for assessing the distribution of drug into and its elimination from tissues because it is the drug in the whole blood, not that restricted to plasma, which is delivered in tissues. Some drugs are strongly bound to plasma proteins but are not bound to some tissue component in such a case their volume of distribution will be equal to that of plasma. In contrast, certain drugs have a high volume of distribution even though most of the drug in the circulation is bound to albumin because such drugs are sequestered elsewhere. Changes in either tissue or plasma binding can change the apparent volume of distribution determined from plasma concentration measurement. For older adults, their volume of distribution is small because they have lean muscle mass. For a drug like

digoxin which binds to muscle proteins, the volume of distribution may be low and not real. As in the case with tissue, the apparent volume of distribution of theophylline is proportional to body weight. For obese patients, the volume of distribution might be overestimated because of their body weight because digoxin does not bind to fat. In the obese patients. Abnormal accumulation of fluid in conditions such as oedema, ascites and pleural effusion can markedly increase the volume of distribution of drugs such as gentamycin that is hydrophilic and has a small volume of distribution. Lipophilic drugs, such as the sedative drug thiopental accumulate in fat. These agents are released slowly from the fat stores. Thus an obese person might be sedated for a greater period than a lean person when the same dose of thiopental is administered (Katzung *et al.*, 2007).

Elimination half-life ($t_{½}$) is the length of time required to eliminate 50% of the remaining amount of drug in the body. It is useful in determining dose interval and time to reach steady state. The half-life of a drug is important when one is considering questions involving time, such as how long will it take a patient to reach steady state on a constant dosage regimen or how long will it take for all the drug to be eliminated from the body. It may also be used to estimate the appropriate dosage interval during maintenance therapy or to estimate when the steady state will be reached. It takes one half-life to reach 50% of the steady state, two half-lives to reach 75%, three half-lives to reach 87.5% and four half-lives to reach 93.75% of the steady state. The difference between clearance and half-life is important in defining the underlying mechanism for the effect of disease state on drug disposition. Clearance of some drugs does not change with age. For example, the long half-life of diazepam in older patients is because of changes in the volume of distribution with age (Katzung *et al.*, 2007).

Mathematically $t_{½} = 0.698\ V_d/CL$

Where V_d= volume of distribution

Cl= clearance

Other secondary parameters include:

A C_{max}: This is the maximum drug concentration achieved in the systemic circulation following drug administration.

B T_{max}: It is the time required to achieve maximum drug concentration in the system circulation

C AUC_{o-t}: Area Under the plasma concentration-time curve from 0 hr to the last quantifiable concentration to be calculated using trapezoidal rule.

D AUC_{0-9}: Area Under the plasma concentration-time curve from 0 hours to the least quantifiable concentration to be calculated using trapezoidal rule.

2.3 Reasons why Bioequivalence Studies are Done

To compare a definitive dosage form (industrial batch) with the dosage form used in clinical trials developed and evaluated (relative bioavailability)

To evaluate two dosage forms administered by the same way, but with formulations or manufacturing process different, in the same company.

When a new product is introduced by one manufacturer and a similar product is already licensed to another (e.g., innovator).

When a drug is used to treat life-threatening diseases and assurance of therapeutic response is required, e.g., digoxin, warfarin.

When a drug has a narrow therapeutic windows/safety margin (e.g., digoxin, theophylline)

2.4 Chromatography

Chromatography is a method of separating the component of a mixture by taking advantage of their different rates of movement between the mobile phase and stationary phase. It is a method used by scientists for separating organic and inorganic compounds so that they can be analysed and studied. Chromatography is an excellent physical method for observing mixtures and solvents (Chris, 2009).

The word chromatography means "colour writing" which is a way that a chemist can test liquid mixtures. While studying the colouring materials in plant life, a Russian botanist discovered chromatography in 1903. Researchers use chromatography to determine unknown substance, either solid or liquid. Law enforcement agencies also use this method in their forensic analysis to detect the presence of hard drugs like amphetamine and cocaine in the urine, alcohol in blood, heavy metals like mercury and lead in water, etc. The principle of chromatography is based on the movement of the substances across the mobile phase and the retention of others in the stationary phase. The stationary phase as the name implies the phase that is static and does not move. The mobile phase is the phase that moves through the stationary phase by attracting the substances to be tested. Different components of the materials to be tested have varying degrees of affinity to the stationary phase and the mobile phase. In paper chromatography, the paper is the stationary phase while the solvent is the mobile phase. This type of chromatography use capillary action to move the solvent through the stationary phase (Chris, 2009)

2.4.1 Retention Factor (RF*)*

This is a quantitative measure of how far a particular compound moves in a particular solvent. The RF value is a good measure of whether an unknown compound and a known compound are the same, or not the same. The retention factor, RF, is defined as RF = distance the solute (D1) moves divided by the distance traveled by the solvent front (D2).

Rf = D1 / D2

Where D1 = distance that the colour traveled, measured from the center of the band of colour to the point where the food colour was applied

D2 = total distance that solvent travelled (Ettre, 1993).

2.4.2 Different Types of Chromatography

There are four main kinds of chromatography. Liquid chromatography, gas chromatography, thin-layer chromatography and paper chromatography (Ettre, 1993).

2.4.2.1 Liquid Chromatography

It can be used to test water samples and look for toxic waste in lakes and rivers. It is also used to evaluate metal ions and organic compounds in solutions. Liquid Chromatography uses liquids which may incorporate hydrophilic, insoluble molecules (Ettre, 1993).

2.4.2.2 Gas Chromatography

It is used in airports to detect the explosive device. It is also used for forensic analysis in many diverse ways. It is also employed to assess blood obtained from a crime scene.

Helium moves a mixture of gases through the stationary phase which is a column of absorbent material (Chris, 2009; Ettre, 1993).

2.4.2.3 Thin-layer Chromatography

As the name implies, it uses an absorbent material placed on a plastic or glass plates. It is a quick and simple method of checking levels of impurities of organic materials. The level of insecticides, pesticides, and other harmful organic substances can be determined in food using this means. It could also be used to provide forensic evidence especially when dye components are present in fibre (Chris, 2009; Ettre, 1993).

2.4.2.4 Paper Chromatography

It is one of the most common kinds of chromatography. It uses a strip of paper as the stationary phase. Capillary action is used to push the solvents up through the paper and separate the solutes. This technique can be used in separating amino acids in the mixture of proteins and anions, performing RNA fingerprinting, as well as separating and testing for the presence of histamines, antibiotics. The paper is the stationary phase. The solvent which is the mobile phase moves along the paper which is the stationary phase through capillary action (Chris, 2009; Ettre, 1993).

2.4.2.5 High-Performance Liquid Chromatography (HPLC)

It is also called high-pressure chromatography.The stationary phase is usually packed with tiny particles in the range of 3 to 5μm in diameter. The mobile phase which is the fluid moves as a result of high pressure coming from the pump (Chris, 2009; Ettre, 1993).

In principle, LC and HPLC work similarly except that the pace, effectiveness, sensitivity, and simplicity of operation of HPLC is more superior. These components are separated from each other with the help of the column as a result of chemical and physical

interactions of the particles in the column and the molecules of the substance. The components that are separated are detected at the exit point of this column by a device. What comes out of this detector is called a "**liquid chromatogram** (Chris, 2009; Ettre, 1993).

HPLC has the various components of their functions**:**

The pump in HPLC is a device that forces the mobile phase which is the liquid at a determined flow rate through the liquid chromatograph. The unit in which the flow rate is expressed is millilitres per minutes (ml/min).The flow rate is 2 ml/min. The pressure of the pump can be between 6000 to 9000 PSI which is equivalent to 400 to 600 bar. During analysis of a substance, there may be a continuous delivery of the mobile phase which is called "Isocratic" or increasing delivery of the mobile phase, which is called "gradient" (Chris, 2009; Ettre, 1993).

The injector in HPLC is the device that introduces the liquid sample into the mobile phase. Usually, 5 to 20 microlitres volumes of sample are injected. The injector should be able to withstand the high pressures of the liquid system. An autosampler can analyse many samples. It is the automatic version of the injector which is used when the manual injection is not practicable (Chris, 2009; Ettre, 1993).

The column is like the heart of the chromatogram; it separates sample components of interest using different physical and chemical parameters. The small particles inside the column at normal flow rate can cause the high back pressure. The pump must overcome the pressure and resistance to move the mobile phase along the column.

The detector is a device that detects the individual molecules that come out (elute) from the column.A detector measures the amount of those molecules so that the sample component can be quantitatively analysed. The detector provides an output to a recorder or computer that produces the liquid chromatogram (i.e., the graph of the detector response) (Chris, 2009; Ettre, 1993).

The computer is also called the data system; the computer controls all the operations of the HPLC instrument. The time of elution is determined by the detector which has a signal. The time of elution is the retention time. The signal also detects the amount of sample (quantitative analysis) (Chris, 2009; Ettre, 1993).

HPLC can be used for the separation of biological substances and chemicals that are not volatile; non-volatile substances include pharmaceuticals like paracetamol, ibuprofen, aspirin, etc. Potassium phosphate, and sodium chloride salts; proteinous materials like blood and white egg portion; organic materials like polymers, asphalt or motor oil that are the hydrocarbon in nature, etc.; plant extracts and herbal products, thermolabile compounds like trinitrotoluene, etc. The most important parameter for identifying a compound is dependent on its retention time. The retention time is the time taken for a compound to elute after it is injected into the column (Chris, 2009; Ettre, 1993).

The HPLC uses the following principles in analysing substances:

1. Quantitative Analysis: It is the measurement of the quantity of a compound in a sample (concentration); meaning, how much is there? There are two main ways to interpret a chromatogram.

a. Determination of the peak height of a chromatogram is measured from the baseline;

b. Determination of the peak area; For a quantitative analysis of the compound to be made, the compounds will be injected, and the result comes out in the form of a peak, and the area can be measured from the peak. The peak area observed from the chromatogram is directly related to the concentration or the amount of substance injected (Brown et al., 1997; Snyder & Kirkland, 2009).

2. Preparation of Pure Compound(s): A pure compound can be prepared by collecting the chromatographic peak and concentrating the compound (analyte) at the exit point of the detector. This is done by evaporating the solvent.A pure substance can be prepared for future use, for example in organic synthesis, clinical studies, toxicological evaluation, etc. (Brown *et al.,* 1997; Snyder & Kirkland, 2009).

3. Trace analysis: A compound whose concentration is less than 1% by weight is called a trace compound. Trace compound is important in pharmaceutical and biological analysis. This is because of the harmful effects these compounds can have on the environment or human tissue. It is often difficult to separate or detect substances in a chromatogram. It requires detectors that are very sensitive to high resolution. Four major techniques are employed to separate most substances. They are reverse phase column chromatography, normal or adsorption chromatography, ion exchange chromatography and size exclusion chromatography (Brown *et al.,* 1997; Snyder & Kirkland, 2009) In reversed-phase column chromatography (RPC), the packing consists of nonpolar substances (e.g., C3, C18, C9, phenyl, etc.). The mobile phase comprises of water-

miscible organic solvent; examples include acetonitrile, methanol, ethanol, etc. More than 90% of analysts use reverse phase column chromatography. The method can be used for non-polar, polar, ionizable and ionic molecules making RPC very versatile. For samples containing different ranges of compounds, gradient elution is often used. In this method, a principally water-based mobile phase is started with and then organic solvents are added as a function of time. Solvent strength increases when an organic solvent is used, and the compound that is eluted are strongly retained in reverse phase chromatography packing (Brown *et al.,* 1997; Snyder & Kirkland, 2009).

For normal or adsorption chromatography, the parking in the column could be amino-bonded, silica gel, cyanopropyl-bonded, etc. The mobile phase is non- polar. Examples of non-polar substance include ethyl acetate, isooctane, methylene chloride, etc. Normal phase separations are rarely performed, in most cases, it is less than 10% achieved. This method is important for compounds that are water sensitive, chiral compound, geometric isomers. (Brown *et al.,* 1997; Snyder & Kirkland, 2009).

In ion exchange chromatography, the parking in the column are ionic groups like tetraalkylammonium and sulfonic). About 20% of liquid chromatographers use this method. This method is useful for separating both organic substances that are cationic and anionic in aqueous solution. Proteins, ionic dyes and amino acid compounds that are salt in brine water can be separated by ion exchange. Examples include basic proteins on strong cation exchanger like (-SO3), RNA polymerase, proteolytic enzyme (Chymotrypsinogen) Lysozyme, etc. (Brown *et al.,* 1997; Snyder & Kirkland, 2009).

In size exclusion chromatography (SEC), there is no contact between materials in the column and the sample substance. What happens is that molecules diffuse depending on their size into a pore of a porous medium. Molecules that are bigger than the pore opening cannot diffuse into the particles, but molecules smaller than the pore opening can enter the opening readily and hence easily separated. Larger molecules will elute first, then smaller molecules later. Size exclusion chromatography method is used by 10 to 15% of people using chromatography. It is mainly used for characterization of proteins and polymers. There are two methods: non-aqueous SEC [sometimes termed Gel Permeation Chromatography (GPC)] and aqueous SEC [sometimes referred to as Gel Filtration Chromatography (GFC) (Brown *et al.*, 1997; Snyder & Kirkland, 2009).

In HPLC temperature control is critical to achieving the desired goal. Retention in HPLC is dependent on temperature. It should be reproducible. If the temperature is not consistent, then it may be difficult to get a precise peak for a compound. The peak height or area may vary (Brown *et al.*, 1997; Snyder & Kirkland, 2009).

Solubility is also crucial in HPLC**;** for substances that have low solubility, there may be difficulty in their precipitation if they are injected into the mobile phase. Biological compounds like proteins, enzymes, hormones may be denatured by temperature. So temperature control is important. Temperature control can be achieved in three ways using the oven, heater block or water bath.

There are many detection methods used to detect the compounds eluting from an HPLC column. The most common types are spectroscopic detection, refractive index detection, fluorescence detection and ultraviolet (UV) absorption. In ultraviolet absorption, a sensor

measures light beam as it passes through a flow cell. The amount of light energy passing through the sensor changes if the compounds that elute from the column absorb this light energy. A recorder or data system captures the amplified electrical signal. A UV spectrum is also obtained which can aid in the detection of substances or series of compounds (Brown *et al.,* 1997; Snyder & Kirkland, 2009).

In mass spectroscopy (MS)**,** the compound eluting from the HPLC column is detected by the sensor that ionises it. This is done by fragmenting the molecules into smaller units that are unique to the compound or by measuring its mass. The detector in mass spectroscopy can identify compounds directly in most cases because mass spectroscopy works similarly to fingerprint and is unique only hydrogen atom of that particular compound.

In refractive index (RI), the compound or solvent ability to deflect light is a measure used for detectors. In a refractive index, the ability of molecules to deflect light in the mobile phase in a flow cell used as a reference. The refractive index detector is used universally, but its sensitivity is low (Brown *et al.,* 1997; Snyder & Kirkland, 2009).

Fluorescence detectors are highly selective and sensitive. They can quantify impurities compounds in complex matrices even at low concentration level. Fluorescence detector can only detect a substance that shows fluorescence when analysed (trace level analysis) (Brown *et al.,* 1997; Snyder & Kirkland, 2009)

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Plate 1: Picture of a typical HPLC

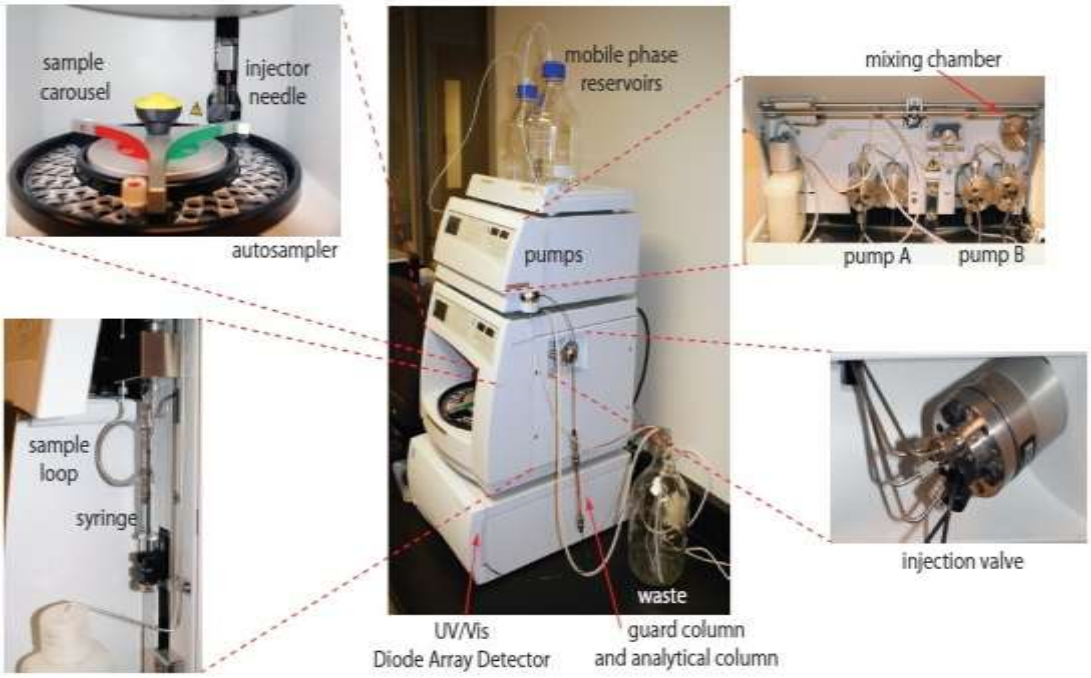

Plate 2: Picture of HPLC and its Components

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Market Survey of Different Brands of Ceftriaxone

A survey of various brands of ceftriaxone was carried out at 18 Pharmacy outlets in Sokoto Metropolis using convenience sampling method. The following brands were identified (Derfxone, Biocef, Avicef, Paucocef, Jawacef, Oxezone, Safetax, Cefzone, Rocephin, Kembarth and Costriax).

3.2 Materials

3.2.1 Procurement of Standard Drug

An analytical grade of Ceftriaxone sodium, Pharmacopoeia Secondary Standard was purchased from SIGMA-ALDRICH Germany. Ceftriaxone reference Rocephin® was obtained from the Department of Pharmacy, Usmanu Danfodiyo University Teaching Hospital, Sokoto.

3.3 Methods

3.3.1 Selection of Microorganisms for the Microbiological Assay

The prominent clinical isolates of bacteria available as at January 2014: *Pseudomonas aureginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were obtained from the Department of Microbiology, Usmanu Danfodiyo University Teaching Hospital, Sokoto. The isolates were identified by Gram staining technique. The four isolates were subjected to agar well diffusion method (Perez et al., 1990; Zaidan et al., 2005) to determine which one was most sensitive to ceftriaxone.

3.4 Preparation of Stock Solution and Serial Dilution

A stock solution was prepared in a test tube by adding distilled water to 6.4mg of pure ceftriaxone (Analytical grade) in a 10 ml test tube to give a concentration of 0.6 mg/ml (640 µg/ml). Nine test tubes were filled with 5 ml distilled water to do a serial dilution. A

5 ml was pipette from the stock solution and transferred into the next test tube to get a concentration of 320 µg/ml. A 5ml was then transferred from the second to the third test tube to get a concentration of 160 µg/ml. The same procedure was repeated for the remaining test tubes to get serial concentrations of 80 µg/ml, 40 µg/ml, 20 µg/ml, ten µg/ml, five µg/ml, 2.5 µg/ml, and 1.25 µg/ml.

3.5 Preparation of Agar Medium, Inoculation and Measurement of Zones of Inhibition

About 250 ml of nutrient agar was prepared according to the manufacturer's direction. A 20 ml of the nutrient agar was poured into each of the ten petri dishes. The content was allowed to set, and a 6 mm cork borer was used to cut out three holes from the already set agar in the petri dish. A file was used to remove the cut-out sections in the petri dish. The same procedure was repeated for the remaining dishes. About 0.5 ml of bacteria suspension (*Escherichia coli*) corresponding to 10^6 of colony forming unit (CFU) was adjusted to 0.5Mc Farland Standard. It was used in inoculating the nutrient agar by using a sterile swab stick to streak the petri dish (Perez *et al.,* 1990; Zaidan *et al.,* 2005). A microsyringe was used in filling the three holes on each dish with the concentration of pure ceftriaxone in the range of 1.25 µg/ml, to 640 µg/ml. The same procedure was done for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella Pneumoniae*. The petri dishes were allowed to dry for 30 minutes and then incubated at 37^0C for 24 hours.

After 24 hours, the petri dishes were brought out, and the zones of inhibition of each dish were measured using a Vernier calliper. The values were recorded against respective concentrations (in mm) and the average for each concentration was calculated (Perez *et al.,* 1990; Zaidan *et al.,* 2005).

3.6 Microbiological Assay for the Six (6) Selected Generic Brands of Ceftriaxone Using Agar Well Diffusion Method

Computer randomization was done using Decision analyst, and six generic brands were selected out of the ten brands identified, the 11th brand was Rocephin® so it was not included because it is the reference that will be used in bioequivalence studies. The Six (6) generic brands selected were: Avicef®, Derfxone®, Kembarth®, Costriax®, Cefzone®, and Biocef®. These six (6) brands were compared with Rocephin®, and the standard (Analytical grade). A Stock solution of 0.64mg/ml (640µg/ml) was prepared by adding distilled water to 6.4mg of Standard ceftriaxone in a 10ml test tube. The same procedure was done for Rocephin® and the remaining generic brands. After that 5 ml of the stock solution was pipetted and transferred into the second test tube that was filled with 5 ml of distilled water to give a concentration of 320 µg/ml. The same serial dilution was done until a final concentration of 1.25 µg/ml was achieved for all the generic brands. Sterile swab stick was used to collect 0.5 ml of the test organism (*Escherichia coli*) and was used in inoculating the agar plate. A micropipette was used in filling the holes with the known concentration of ceftriaxone ranging from 1.25 µg/ml to 640 µg/ml for the Standard (Analytical grade), Rocephin, and the remaining six (6) generic brands. The petri dishes were incubated at 37^{0}C for 24 hours. After 24 hours, the dishes were brought out, and the various zones of inhibition corresponding to various concentrations of the samples were measured using Vanier Calliper (Perez *et al.*, 1990; Zaidan *et al.*, 2005).

3.7 In vivo Study

Based on the result of the regression analysis, Biocef® (found to have the highest co-efficient of correlation between concentration and zone of inhibition) and Rocephin® were compared in a two-treatment two-period, two-sequence, single-dose crossover study.

Two phases of treatment were done with a wash-out period of three (3) weeks which was more than five half-lives of the drug.

3.7.1 Experimental Animals and Drug Administration

Ten male Sokoto red goats weighing between 16 kg to 20 kg were purchased from Kara market in Sokoto South Local Government Area of Sokoto, Sokoto State, Nigeria. The animals were examined clinically by a Veterinary Physician to ascertain their health status. The animals were administered albendazole suspension 12.5 mg/kg body weight, oxytetracycline injection 20 mg/kg body weight and multivitamin injection 10kg/ml according to the protocol of College of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto. The animals were weighed and housed in two separate pens containing five animals each. They were monitored closely for two weeks for any sign of ill health and were allowed access to a local feed of "Kowa" and "Dusa" and water *ad libitum*.

Before commencement of the experiment, the animals were assigned numbers, from 1 to 10. Computer randomization was done using Decision Stat Analyst to divide the animals into two groups. Group one has animals with numbers 1, 2, 3, 6 and ten while group two has 4,5,7,8 and 9. Group one received an intravenous injection of Biocef® at a dose of 20 mg/kg body weight, while group two received an intravenous injection of Rocephin®).

3.7.2 Collection of Samples

Blood samples of 3 ml each were collected through intravenous catheter fixed in contralateral jugular vein into heparinized test tubes before drug administration then at 30

minutes, I hour, 2 hours, 4 hours, 8 hours, 12 hours and 24 hours post-dose. Plasma was separated by centrifugation at 3000 revolutions per

minutes for 10 minutes at room temperature. The plasma was stored in a refrigerator at -20^0C before the commencement of the assay.

3.7.3 Extraction of Ceftriaxone From Plasma

The plasma was allowed to defreeze, and 1 ml was transferred into 10 ml plain bottle. Three ml of acetonitrile analytical grade was added to 1 ml of plasma, and the mixture was vortexed for 1 minute. The mixture was then centrifuged for 10 minutes at 5000 revolutions per minute; this was done to remove the proteins from the drugs (Hakim et al., 1988). After centrifugation about 3 ml of the clear supernatant fluid was withdrawn and filtered into 2 ml plain bottles using 0.45 µm syringe filter. The same procedure was carried out for both Rocephin® in and Biocef®.

3.7.4 HPLC Analysis

Ceftriaxone standards in the following range (0.19, 0.26, 0.52, 1.68, 4.93, 14.94, 49.79, 76.59, 90.11, 100.12 ug/mL) were prepared using two-fold dilution according to the method described by (Hakim et al., 1988). A calibration curve was prepared for drug concentrations in the following range 0.19 to 100.12 µg/mL and was used to quantify the drug concentration in samples. The supernatant solution was loaded in the autosampler tray, and 50 µl was then injected into the column, and peak heights were recorded. The computer display from the screen which is the peak height from the chromatogram is directly proportional to the concentration.The equation ($Y = 140501X$) generated from the calibration curve was used to calculate the corresponding concentration from the peak height.

CHAPTER FOUR

4.0 RESULTS

4.1 Market Survey of Different Brands of Ceftriaxone

Table 4.1 presents the names, cost and availability of the various brands of ceftriaxone in Sokoto market.

Table 4.1: The Cost and Availability of the Brands of Ceftriaxone in Sokoto Pharmacies as at 2014.

BRAND	COST IN NAIRA	AVAILABILITY(PACKS)
DERFXONE	130	2
BIOCEF	500	5
AVICEF	500	7
PAUCOCEF	150	3
JAWACEF	300	5
OXEZONE	1600	1
SAFETAX	200	6
CEFZONE	300	3
ROCEPHIN	2000	8
COSTRIAX	150	4
KEMBARTH	130	4

4.2 The Selection of Six Brands of Ceftriaxone

Out of the eleven brands of ceftriaxone found at the Pharmaceutical outlets of Sokoto metropolis six were randomly selected (Table 4.2). The brand name, manufacturer, marketer, batch number, NAFDAC number, manufacture date and expiry date of the products were highlighted.

Table 4.2: Descriptions of the Ceftriaxone Sodium (1000mg) Brands Analysed

Product	Manufacturer	Marketing company	Batch number	NAFDAC number	Manufacture date	Expiry date
ROCEPHIN®	Hoffman La Roche, Switzerland	Swiss Pharma Nigeria Limited	B0082B03	F0497	May-13	May-15
BIOCEF®	Shenzhen Pharmaceutical China	Biofem Pharmaceutical Ltd Lagos Nigeria	20130802	A4-154	Mar-12	Mar-15
DERFXONE®	Rayoung Pharmaceutical Ltd China	Anderson Pharmaceutical Ltd Lagos, Nigeria	14040541	A4-5828	Apr-14	Mar-17
AVICEF®	Furen Pharmaceutical China	Tyonex Nigeria Ltd Lagos	20130715	A4-7076	Jul-13	Jul-16
CEFZONE®	Bharat Parenterals India	Evans Medicals Ltd Ogun State, Nigeria	B4071	A4-3306	May-14	Apr-16
COSTRIAX®	Shijianzhuang Pharma China	Cossyvet Pharmaceuticals Ltd, Anambra, Nigeria	659140416	A4-4226	Apr-14	Apr-17
KEMBATH®	Yanzhou Pharmaceuticals China	Kembath Pharmaceuticals Anambra, Nigeria	140356	A4-3707	Mar-14	Mar-17

4.3 Screening of Microorganisms by Microbiological Assay

Out of the four screened microbes, *Escherichia coli* and *Klebsiella pneumonia* were found to be sensitive to ceftriaxone at the measured concentration of 1.25 µg/ml to 640ug/ml, while *Staphylococcus aureus* and *Pseudomonas aureginosa* were resistant (Table 4.4). The sensitive organisms were inhibited at concentrations ranging between 20 µg/ml and 640 µg/ml (Table 4.3). The 2 microbes were discovered to have a co-efficient of determination (R^2) ≥0.99 (Fig 5 and 7).

Table 4.3: Selection of the Test Microorganism for Microbiological Assay of Ceftriaxone Using Regression Analysis

Zones of Inhibition(mm)								
E .coli					***Klebsiella pneumonia***			
Concentration(µg/ml)	**Z1**	**Z2**	**Z3**	**Mean±SEM**	**Z1**	**Z2**	**Z3**	**Mean±SEM**
1.25	0	0	0	0	0	0	0	0
2.5	0	0	0	0	0	0	0	0
5.0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
20	15	15	17	15.6±0.67	17	16	17	16.7±0.33
40	18	17	15	16.7±0.88	18	20	20	19.30±0.67
80	18	20	16	18±1.15	20	20	21	20.3±0.33
160	22	20	23	21.7±0.88	22	23	23	22.7±0.33
320	24	25	20	23±1.53	25	26	25	25.30±0.33
640	25	26	27	26.0±0.58	27	28	30	28.30±0.88

Key: SEM=Standard error of mean; Z = zone of inhibition

4.4 Screening of the Selected Brands of Ceftriaxone Using Microbiological Assay

There was a direct positive relationship between the concentration of the selected brands and zone of inhibition. Except for Rocephin and Biocef (with R^2 values of 0.9947 and 0.9914 respectively), all the tested brands inhibited the selected microorganism (*Escherichia coli*) with a co-efficient of determination (R^2) <0.99 (Fig 3-Fig 9). The selected microbe was found to be inhibited by all the selected brands of ceftriaxone at the range of concentration of 160-640 µg/ml (Fig 3-Fig 9, Table 4.3 and Plate 3). Only Rocephin, Biocef and the Standard powder inhibited the microbe at a concentration of 20 µg/ml.

Table 4.4: Microbiological Assay of Six Generic Brands of Ceftriaxone against at a concentration of 1.25µg/ml to 640µg/ml Using Agar Well Diffusion Method with Escherichia coli as the representative organism

CONC (µg/ml)	ROCEPHIN®	CEFXONE®	DEFXONE®	KEMBATH®	BIOCEF®	COSTRIAX®	AVICEF®	STANDARD CEFTRIAXONE®
				MEAN ZONES OF INHIBITION (mm)				
1.25	0	0	0	0	0	0	0	0
2.5	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
20	12.6±0.67	0	0	0	11.0±0.67	0	0	12.7±0.33
40	15.6±0.33	0	0	0	14.3±0.33	0	0	15.3±0.53
80	17.7±1.53	15.6±0.3	0	0	17.7±0.33	14.7±0.33	0	19.3±0.12
160	21.7±0.88	17.3±0.23	11.0±0.33	15.3±0.33	21.3±0.53	15.3±1.53	16.0±0.6	22.0±0.33
320	26.0±0.58	19.0±0.50	15.7±0.88	19.3±1.58	26.0±0.56	22.7±0.67	20.7±0.3	24.3±1.52
640	28.0±0.58	21.7±0.88	21.7±0.88	17.6±0.88	28.0±0.58	25.3±0.58	22±.0.53	30.7±1.53

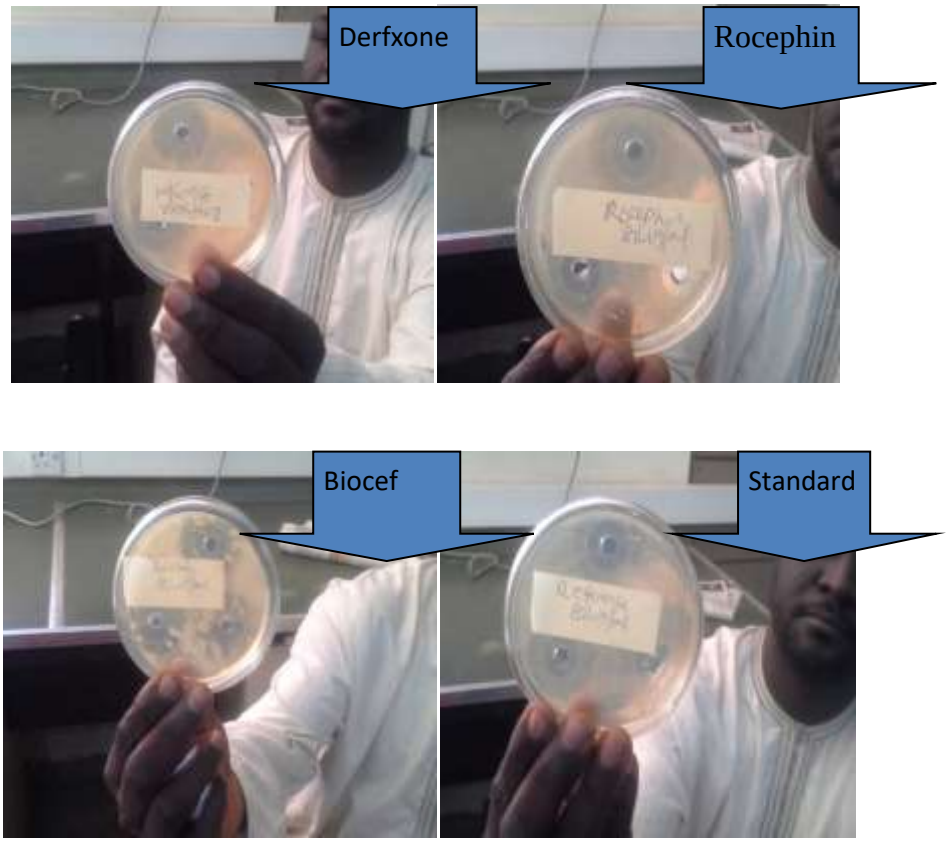

Plate 3: Pictures showing zones of inhibition of some brands of ceftriaxone

The values for the slopes of the zone of inhibition-concentration lines for Biocef® (4.95) and Rocephin® (4.12) were found to be similar. The ratio of the 2 slopes was 1.195 (Figure 4.1).

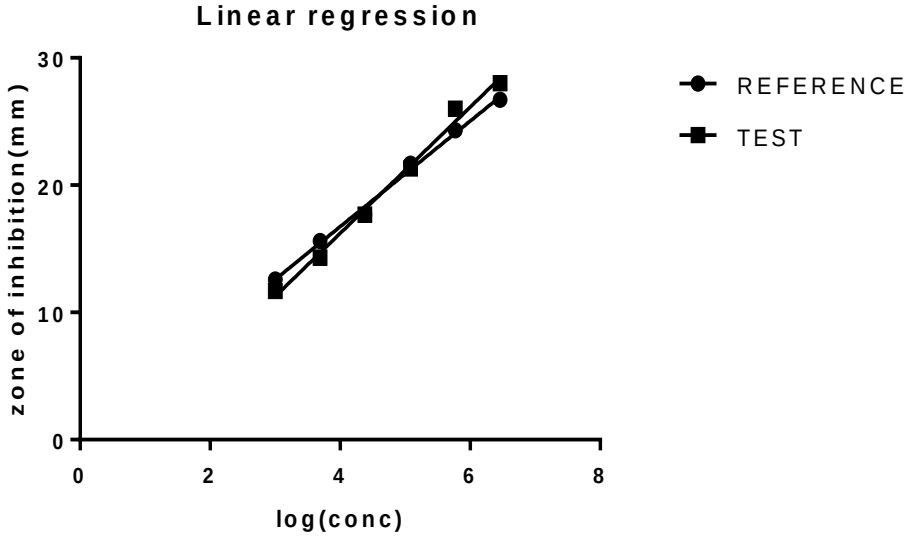

Figure 4.1: Linear regression of the zone of inhibition-concentration lines for Rocephin and Biocef using graph pad prism 7

Key: Reference=Rocephin® Test=Biocef®. Conc is in µg/ml.

4.5 In vivo Bioequivalence Study

4.5.1 Determination of Plasma Concentration of the Test and Reference Brands of Ceftriaxone

At a dose of 20 mg/kg dose of each of the 2 brands selected for *in vivo* study (Rocephin and Biocef), there was a reduction in the plasma concentration for the drugs from 30 minutes to 12 hours. The difference in the concentrations of the two drugs at various time-points was not statistically significant (Table 4.5). The standard and semilog plots of the two drugs at various time-points was also not statistically significant (Fig 2, Fig 10a and 11a; Fig 10b and 11b).

Table 4.5 a : Plasma Concentrations of Ceftriaxone for Individual and The Overall Mean of Sokoto Red Goats for Rocephin® and Biocef® Following Intravenous Administration at 20mg/Kg Body Weight. N=10

Time	0.5	1	2	4	8	12
R1	60.10	30.00	11.72	10.80	2.60	1.10
R2	58.3	40.4	18.11	7.23	3.70	0.81
R3	82.25	22.70	9,03	3.63	3.00	0.57
R4	29.50	25.50	10.00	5.50	3,30	0.50
R5	49.70	25.60	8.00	4.50	3.00	0.30
R6	24.070	16.20	11.20	4.67	2.24	1.26
R7	29,00	20.60	6.30	5.00	3.50	0.29
R8	27.60	14.60	7.00	2.00	1.71	0.82
R9	39.10	28.40	11.00	10.10	4.12	0.47
R10	50.00	47.50	20.40	13.93	1.82	1.26
Mean±SEM	**45.03±5.85**	**27.15±3.20**	**11.28±1.50**	**6.73±1.10**	**2.90±0.25**	**0.74±0.11**

Not statistically significant at $P \leq 0.05$ using unpaired Student t-test

Key: R=Rocephin®, SEM = Standard Error of mean

Table 4.5 b: Plasma Concentrations of Ceftriaxone for Individual and The Overall Mean of Sokoto Red Goats for Biocef® Following Intravenous Administration at 20mg/Kg Body Weight. N=10

Time	0.5	1	2	4	8	12
T1	28.3	12.86	8.10	1.47	1.02	0.12
T2	10.9	9.57	5.96	1.96	0.74	0.68
T3	29.45	12.73	7.55	5.05	5.01	2.14
T4	41.61	39.88	33.70	14.43	4.62	2.50
T5	57.00	41.65	22.20	6.43	3.16	2.00
T6	20.30	19.28	8.99	5.78	3.88	2.80
T7	52.37	39.96	17.25	6.00	4.43	2.24
T8	38.48	35.89	20.28	10.00	5.46	2.70
T9	60.33	40.90	29.55	17.06	5.55	0.47
T10	65.88	40.10	17.85	6.53	4.92	2.15
Mean±SEM	**40.46±5.78**	**29.28±4.35**	**17.14±3.0**	**7.47±1.60**	**3.88±0.55**	**1.78±0.31**

Not statistically significant at $P \leq 0.05$ using unpaired Student t-test

Key: T=Biocef®, SEM = Standard Error of mean

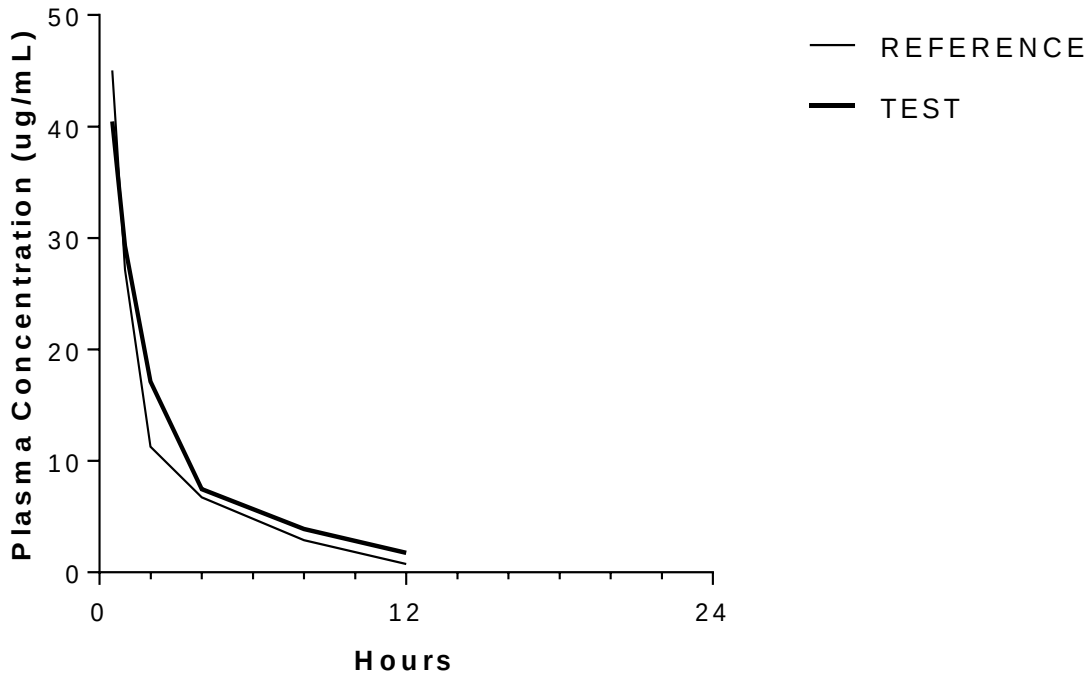

Figure 4.2: Concentration-time curve of Rocephin® and Biocef® following intravenous administration in Sokoto red goats at 20mg/kg body weight generated from graph pad prism 7. N=10

Reference=Rocephin® Test=Biocef®

4.5.2 Plasma concentration of ceftriaxone without outliers

The lack of significant difference in the plasma concentrations between the brands persisted even after removing three goats that appear as outliers. Their plasma concentrations were inconsistent when compared with others. (Table 4.6; Fig 12a and 12b, Fig 13a and 13b). The drug was not detected after 24 hours.

Table 4.6: Plasma Concentration of Ceftriaxone Following Intravenous Administration of Rocephin® and Biocef® at 20mg/Kg Body Weight With 3 Outliers Removed (N=7).

	0.5	1	2	4	8	12
R1	60.10	30.00	10.80	10.80	2.60	1.10
R3	82.50	22.70	3.63	3.63	3.00	0.57
R4	29.00	25.5	5.50	5.50	3.30	0.50
R7	29.00	20.60	5.00	5.00	3.50	0.29
R8	27.60	14.60	2.00	2.00	1.71	0.82
R9	39.10	28.40	10.10	10.10	4.12	0.47
R10	39.10	47.5	13.9	13.9	1.82	1.26
Mean±SEM	**45.40±7.70**	**27.04±3.90**	**10.78±1.77**	**7.28±1.64**	**2.86±0.50**	**0.72±0.14**

Time	0.5	1	2	3	4	5
T1	28.30	12.86	1.47	1.47	1.02	0.12
T3	29.45	12.73	5.05	5.05	5.01	2.14
T4	41.61	39.88	14.43	14.43	4.62	2.50
T7	52.37	39.96	6.00	6.00	4.43	2.24
T8	38.48	35.89	10.00	10.00	5.46	2.70
T9	60.33	40.60	17.06	17.06	5.55	0.47
T10	65.88	40.10	6.53	6.53	4.92	2.15
Mean±SEM	**45.40±7.70**	**27.04±3.90**	**10.78±1.77**	**7.28±1.64**	**2.86±0.50**	**0.72±0.14**

Not statistically significant at $p \leq 0.05$ using Student t test unpaired
KEY R=ROCEPHIN®, T=BIOCEF®

4.5.3 Determination of Pharmacokinetic Parameters

In the determination of pharmacokinetic parametres, there was a wide individual variation in the value of the area under the curve between the animals under study (1-10) from 30 minutes to 12 hours. There was no statistically significant difference between the AUCs of the two groups of animals administered with either Rocephin® or Biocef®, with a ratio of the 2 AUCs having a 90% confidence interval of 0.8669 to 1.074 (Table 4.7). The volume of distribution, half-life, area under the curve and mean residence time for Rocephin® were lower than Biocef® respectively. Only the clearance for Rocephin® was higher than Biocef® (Table 4.8). The lack of significant difference between the 2 drugs persisted even after removing three goats that appeared as outliers (Table 4.9).

Table 4.7. Log Transformed Data for Area Under The Curve For Rocephin and Biocef, Standard Deviation And Confidence Interval Calculated Using Graph Pad Quick Calcs Software

	AUC ROCEPHIN	AUC BIOCEF	LN(AUC) ROCEPHIN	LN(AUC) BIOCEF
	155.3	90	5.342	4.5
	162.5	73.6	5.883	4.299
	340	97.1	6.361	4.576
	209	225.1	4.253	4.765
	359	402.4	6.059	5.159
	94	117.3	5.045	5.417
	579	159	5.091	6
	70.3	160.3	5.829	5.069
	428	939.7	4.543	5.077
	170	174.1	5.136	6.846
MEAN	**256.71**	**243.65**	**5.354**	**5.171**
SD	**155.17**	**248**	**0.675**	**0.768**
SE	**51.73**	**82**	**0.214**	**0.243**

90% CI =0.8669 -1.074
95% CI =0.8470- 1.098

KEY SD= Standard deviation, SE=standard error,

AUC=Area under the curve , LN= Natural logarithm

Table 4.8: Summary of Pharmacokinetics Parameters for Rocephin® and Biocef® Using PK Solution Software N=10

	Rocephin®	Biocef®
Elimination Half-life (hr)	1.98	3.53
V_d (ml/kg)	475.2	763.2
Cl (ml/hr/kg)	166.3	149.68
AUC(0-ϕ) (ughr/ml)	120.3	133.6
AUMC (ughr*hr/ml)	291.4	495.8
MRT (hr)	2.4	3.7

Not statistically significant at $P \leq 0.05$ using Student T-test

Key CL=Clearance, AUMC=Area under the first moment curve,

AUC= Area under the curve, MRT=Mean residence time, $V_{d=}$ Volume of distribution

Table 4.9: Summary of Pharmacokinetics Parameters for Rocephin® and Biocef® Using PK Solution Software N=7

	Rocephin®	Biocef®
Elimination Half-life (hr)	2.0	3.0
V_d (ml/kg)	485.5	584.2
Cl (ml/hr/kg)	167.4	138.3
AUC(0-ϕ) (ughr/ml)	119.3	148.3
AUMC (ughr*hr/ml)	291.2	505
MRT (hr)	2.4	3.4

Not statistically significant at $P \leq 0.05$ using Student T-test

Key CL=Clearance, AUMC=Area under the first moment curve,

AUC= Area under the curve, MRT=Mean residence time, $V_{d=}$ Volume of distribution

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

Over the years, microbiological assay has been used to compare the potency of antibiotics (Dafale *et al.,* 2016). In Sokoto, *Escherichia coli* is one of the most susceptible microorganism to ceftriaxone (Abdulgafar *et al.*, 2011b). Although it has been argued that microbiological assay is prone to biological error and less reliable than other physiochemical methods like High- performance liquid chromatography (Lotfipour et al., 2010). Some researchers have used and validated microbiological assay like agar diffusion method and found it to be reliable, giving accurate results comparable to that obtained using high-performance liquid chromatography (Zuluaga *et al.,* 2009). Bioequivalence implies that the active pharmaceutical ingredients in the tested generics are within an approved range of 80– 125% (FDA, 2010) concerning the innovator products. Five of the generics analysed were not pharmaceutically equivalent to the innovator product evidenced by their failure to inhibit the growth of the organism at the lowest concentration of 20 µg/ml (3 started showing inhibitions at 160 µg/ml while 2 started at 80 µg/ml). Also, the results of regression analysis revealed a wide variation in the slopes of the five generics, outside the acceptable range of 0.8 to 1.25. This suggested that the generics were not pharmaceutically equivalent to the original product. The only generic brand that inhibited the growth of the microorganism at the lowest concentration tested (20 µg/ml) was Biocef. Also, the finding of regression analysis showed that the slope of Biocef was similar and comparable to that of the innovator brand Rocephin, thus establishing the fact that the 2 products are pharmaceutically equivalent. The ratio of the slope of the generic Biocef to that of Rocephin was 1.195 (about 120%), which was within the acceptable

range of 0.8 to 1.25 (FDA, 2010) with Biocef having the highest R^2 (0.9914). From the microbiological assay result, only Biocef was pharmaceutically equivalent to Rocephin.

Following intravenous injection of 20 mg/kg ceftriaxone, the plasma concentration of the drug was detectable up to 12 hours post administration. The elimination half-life of Rocephin® was 1.98 hours, which is in agreement with the finding of previous studies in goats (Ismail, 2005; Johal & Srivastava, 1988) but lower than 5.19 hours reported by (EL-Sayed *et al.,* 2015) in a similar study in goats. The volume of distribution for Rocephin was 475.2 ml/L which is similar to 485.3 ml/L found (Abdulgafar et al., 2011a), but slightly lower than 580±0.04 ml/L reported by Ismail (Ismail, 2005). The clearance for Rocephin® was 166.3 ml/hr/kg which is lower than the value reported in previous studies in goats (Tiwari *et al.,* 2009). These differences could be due to inter-animal variation or interspecies variation as a result of the difference in metabolic activities. The area under the curve of Rocephin® was 120.3 µg/hr/ml, a value similar to that reported in Red Sokoto goats (144.1±1.71) by Abdulgafar *et al.* (2011a), but higher than the value (77.51± 7.49) reported in a different strain of goats (Tiwari *et al.,* 2009). The AUMC and MRT for the reference brand were 291.4 and 2.4 hours respectively. These values were higher than 72.71±4.22 and 1.01±0.11 hours reported by Tiwari *et al.* (Tiwari et al., 2009). These differences could be due to inter-animal variation or interspecies variation as a result of the difference in metabolic activities. Although the values for the above-mentioned pharmacokinetic parameters for the test generic brand Biocef® tend to be higher than that of the reference brand(though not significant), the value of the average log-transformed ratio of the AUC for the 2 brands was within the acceptable range of 80 - 125%. Therefore, the 2 products are considered bioequivalent. The increase in the values

of the parameters for Biocef® compared to that of Rocephin may not be unrelated to the excipients contained in the product. Another possible factor contributory to the finding is the presence of 3 animals with abnormal pharmacokinetic parameters, which can be considered as outliers. Reanalysis of the data with the three outliers excluded still resulted in comparable pharmacokinetics parameters between the reference and the test brands.

5.1 CONCLUSION

In conclusion, the result of the microbiological assay and the regression analysis revealed that out of the six selected generic products of ceftriaxone only Biocef is pharmaceutically equivalent to the innovator product Rocephin. In the *in vivo* bioequivalence, 90% confidence interval of the ratio of the 2 AUCs for Biocef® and Rocephin® was within the acceptable range of 80-125%, i.e., 86.69-104.7. It can be said with some degree of certainty that Biocef® is bioequivalent to Rocephin®.

5.2 RECOMMENDATIONS

National Agency for Food and Drug Administration and Control (NAFDAC) should be more proactive and demand for bioequivalent studies of generics regularly to ascertain their switchability. Postgraduate students should be made to acquaint themselves with the use of sophisticated equipment in the college to increase their technical know-how. Also, a more detailed study is required like using the animal model of infection to determine therapeutic equivalence.

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APPENDIX
Appendix 1: DEFINITION OF TERMS

Bioequivalence

Two pharmaceutical products are bioequivalent if their bioavailability (rate and extent of availability) following administration in the same molar dose are similar to such a degree that their effects, concerning both efficacy and safety, can be expected to be the same for all intents and purposes (Birkett, 2003).

Therapeutic Equivalence

Drug products are considered to be therapeutically equivalent only if they have the same clinical effect and safety profile when administered to patients under the same condition (FDA, 2015).

Pharmaceutical Equivalence

Products are pharmaceutical equivalents if they contain the same molar amount of the same active pharmaceutical ingredients, in the same dosage form, that meet the same or comparable standards and are intended to be administered by the same route (FDA, 2015).

Pharmaceutical equivalence does not automatically imply bioequivalence or therapeutic equivalence as differences in the excipients or manufacturing procedure can lead to differences in the product performance.

Pharmaceutical Alternative

Medicinal products are pharmaceutical alternatives, if they contain the same active moiety but differ in chemical form (e.g., salt ester) or in the dosage form or strength. Examples are Tetracycline Hydrochloride 250 mg capsules versus Tetracycline phosphate complex 250 mg capsules (FDA, 2015).

Appendix 2

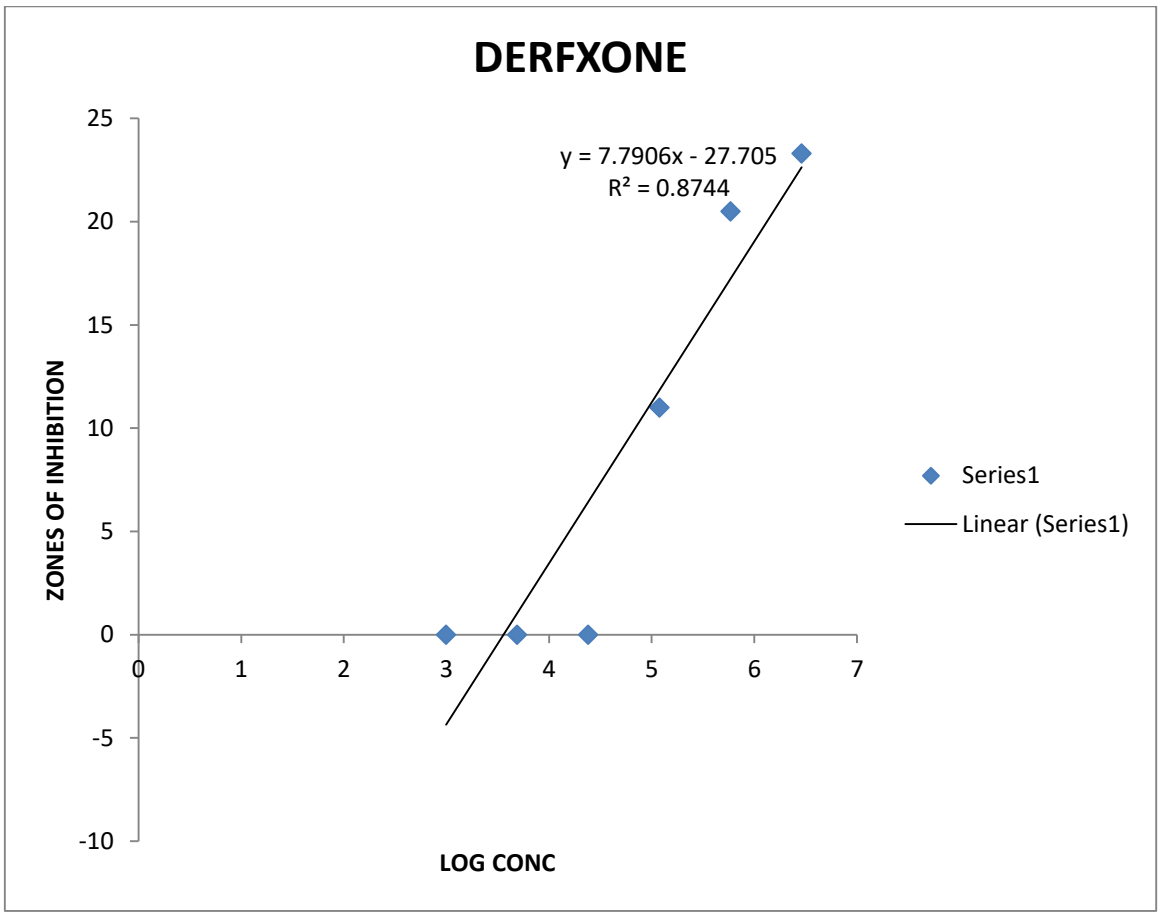

Figure 3: Graph of Zones of Inhibition Against Log Concentration for Derfxone.

Key: M=slope (potency), C=intercept, which is concentration when x=0.

Appendix 3

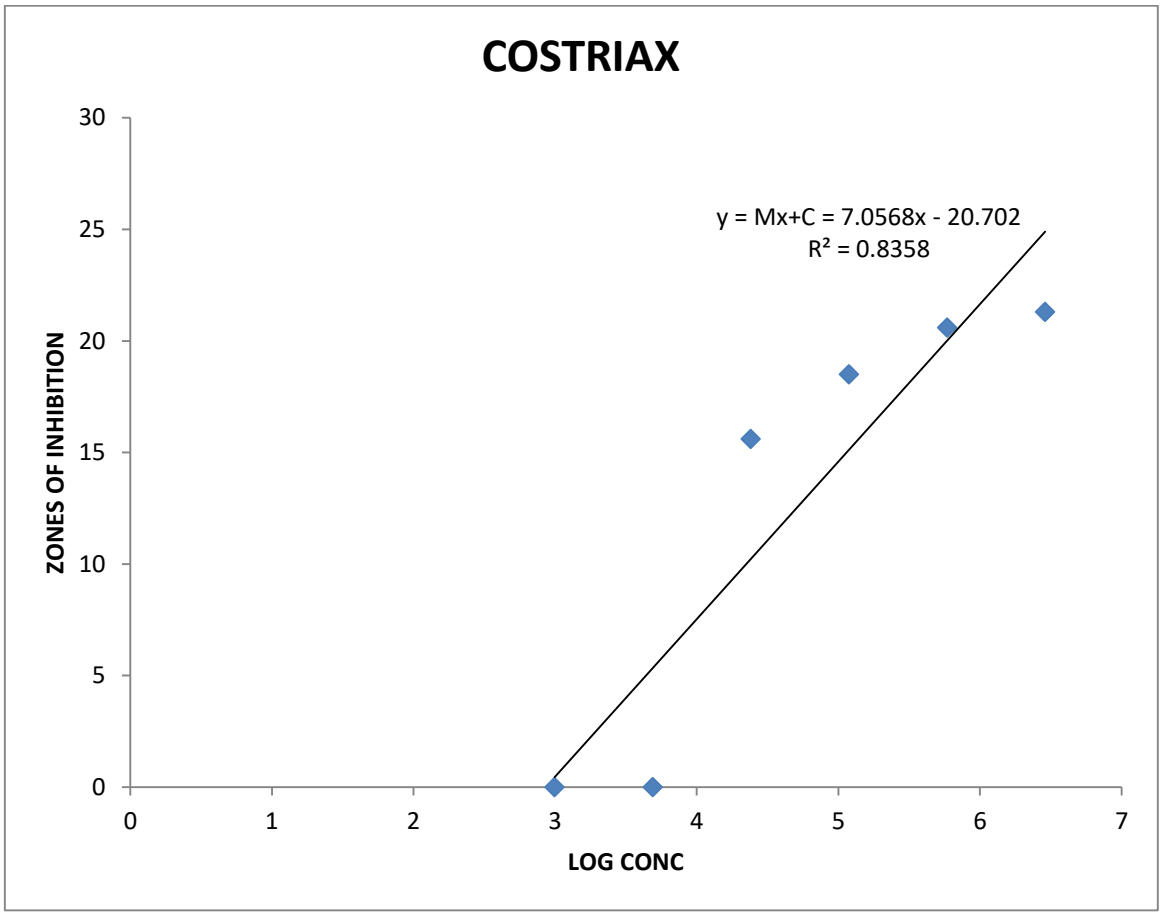

Figure 4: Graph of Zones of Inhibition vs. Log Concentration for Costriax

Key: M=slope (potency), C=intercept, which is concentration when x=0.

Appendix 4

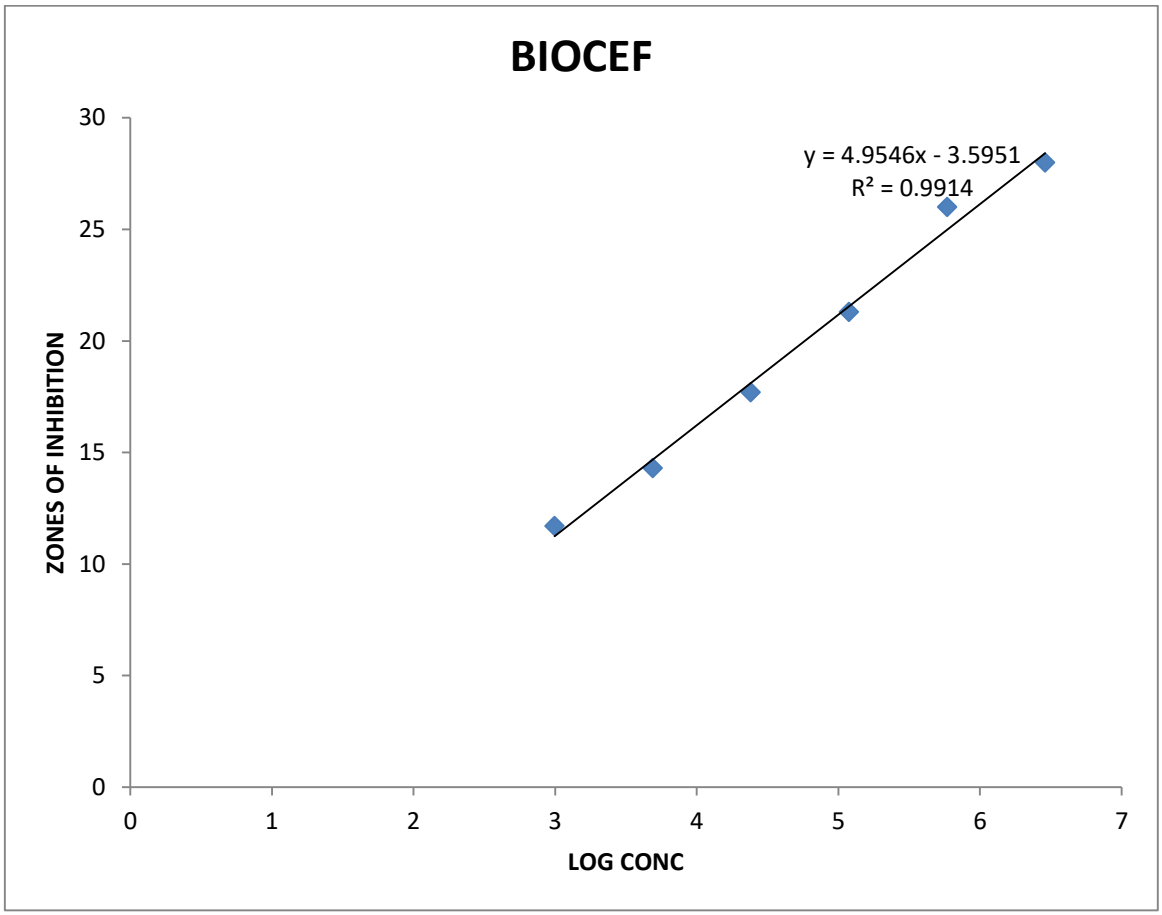

Fig 5: Graph of Zones of Inhibition vs. Log Concentration for Biocef

Key: M=slope (potency), C=intercept, which is concentration when x=0.

Appendix 5

Appendix 5

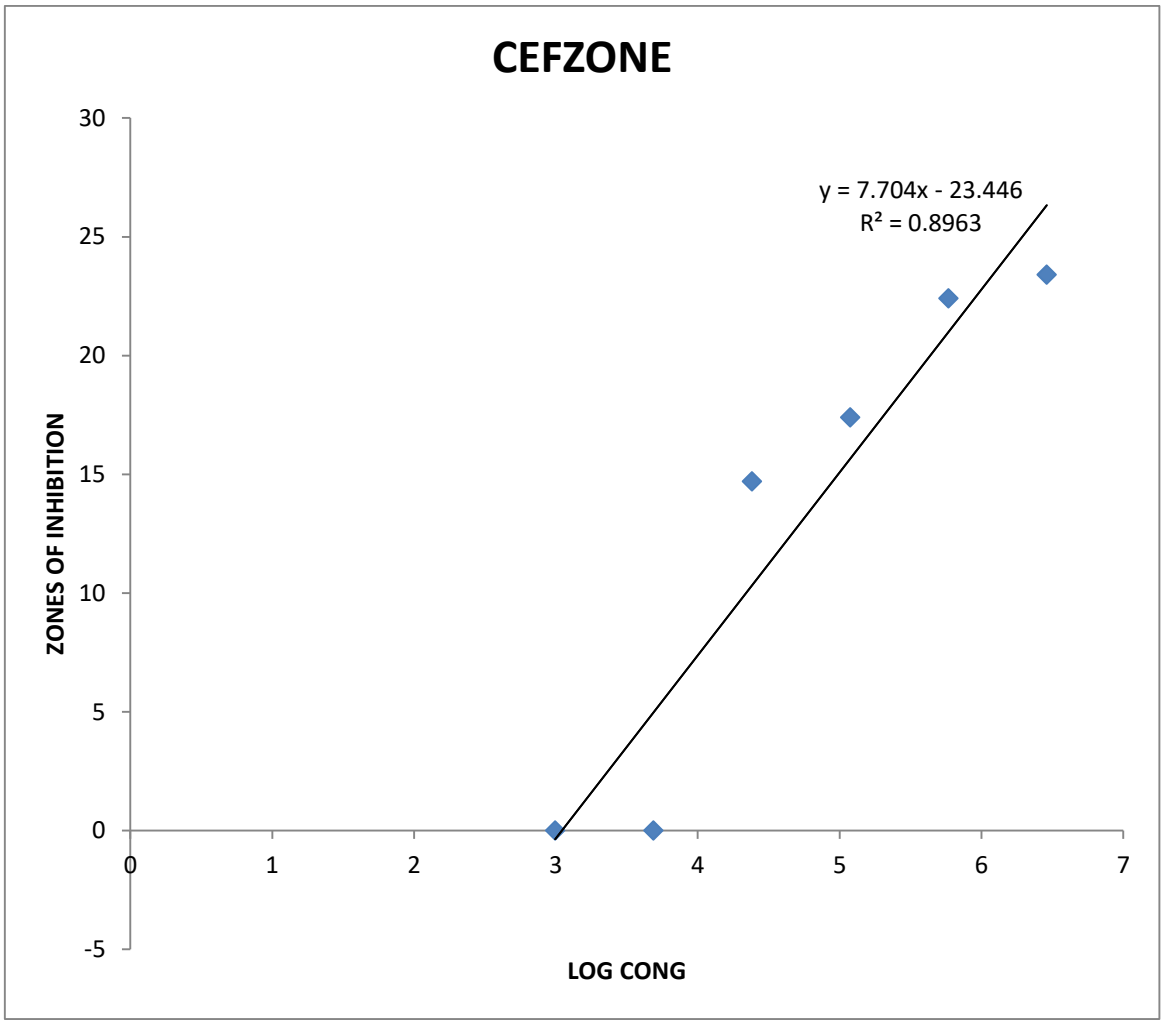

Figure 6: Graph of Zones of Inhibition vs. Log Concentration for Cefzone.

Key: M=slope (potency), C=intercept, which is concentration when x=0

Appendix 7

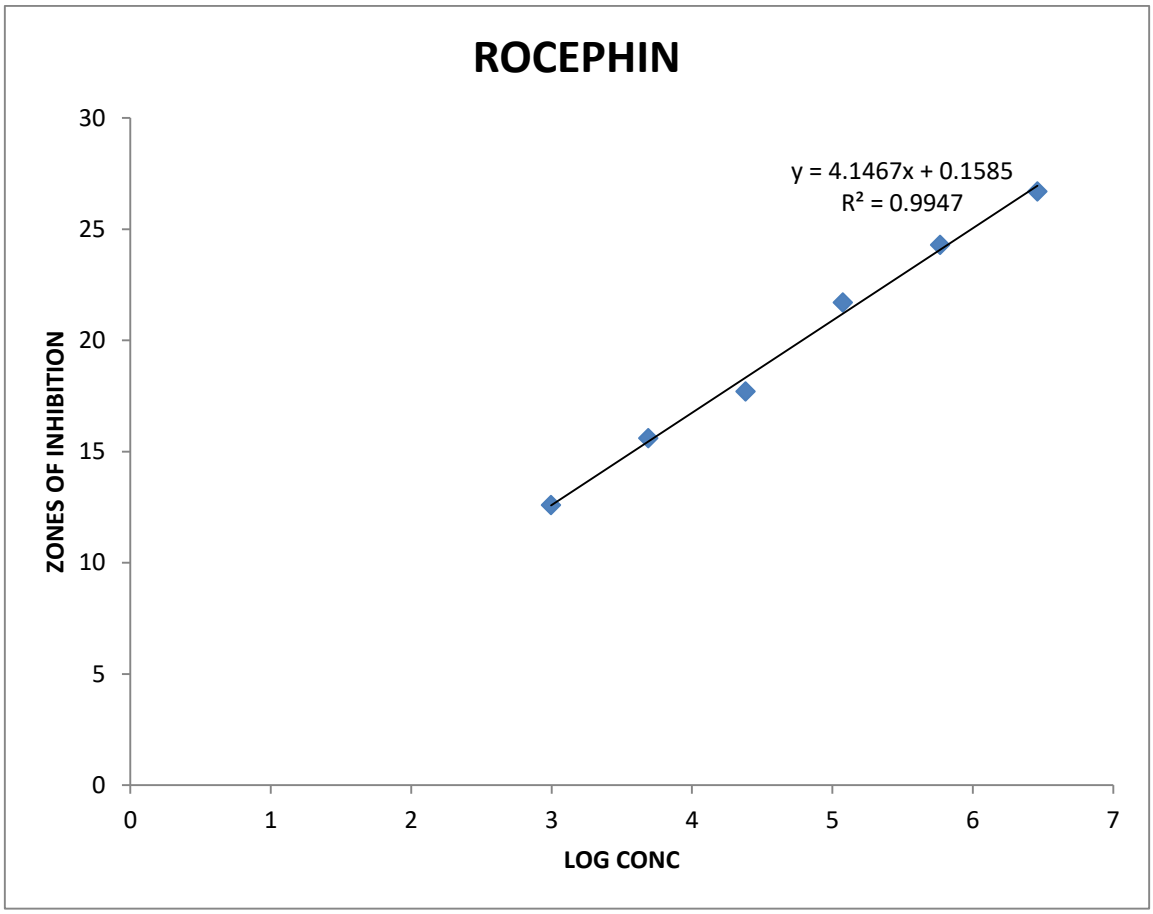

Figure 7: Graph of Zones of Inhibition vs. Log Concentration for Rocephin

Key: M=slope (potency), C=intercept, which is concentration when x=0

.

Appendix 8

Appendix 9

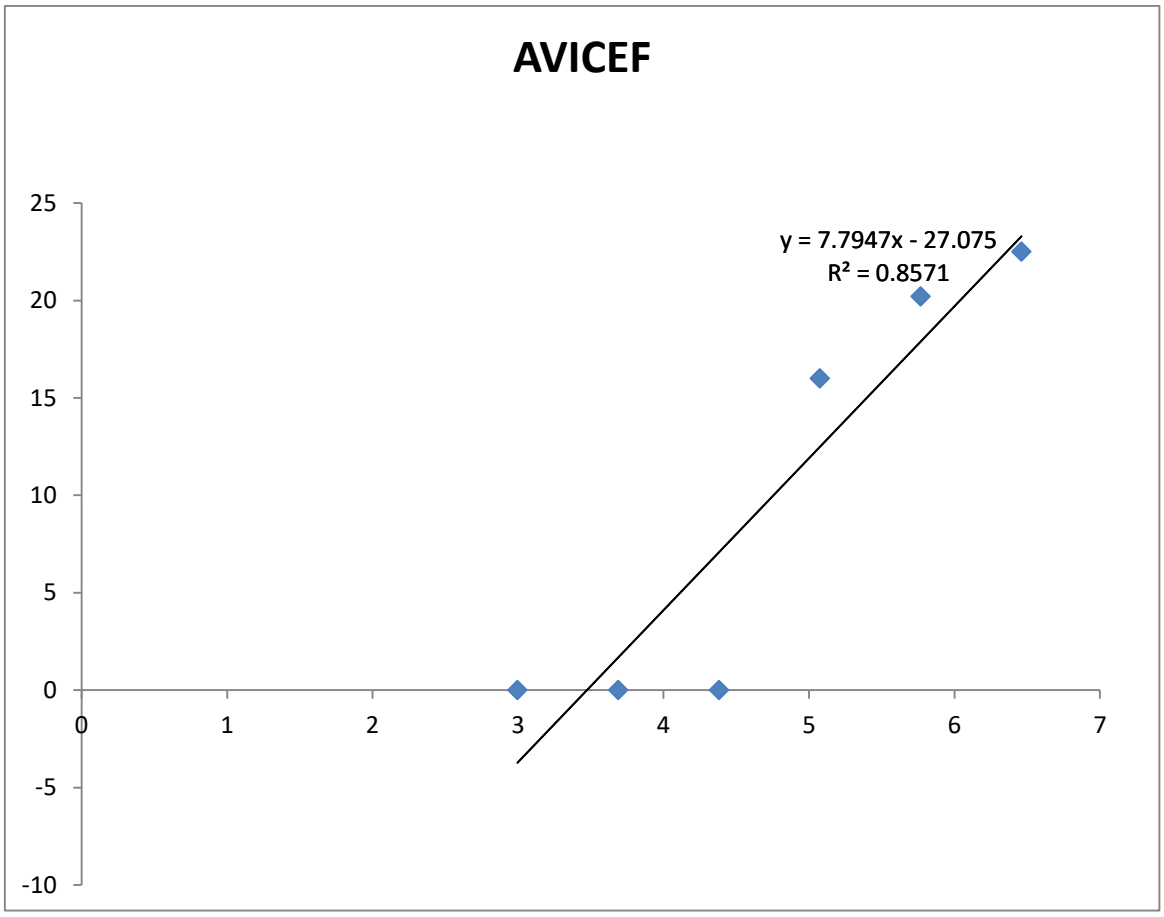

Figure 8: Graph of Zones of Inhibition vs. Log Concentration for Avicef.

Key: M=slope (potency), C=intercept, which is concentration when x=0

Appendix 10

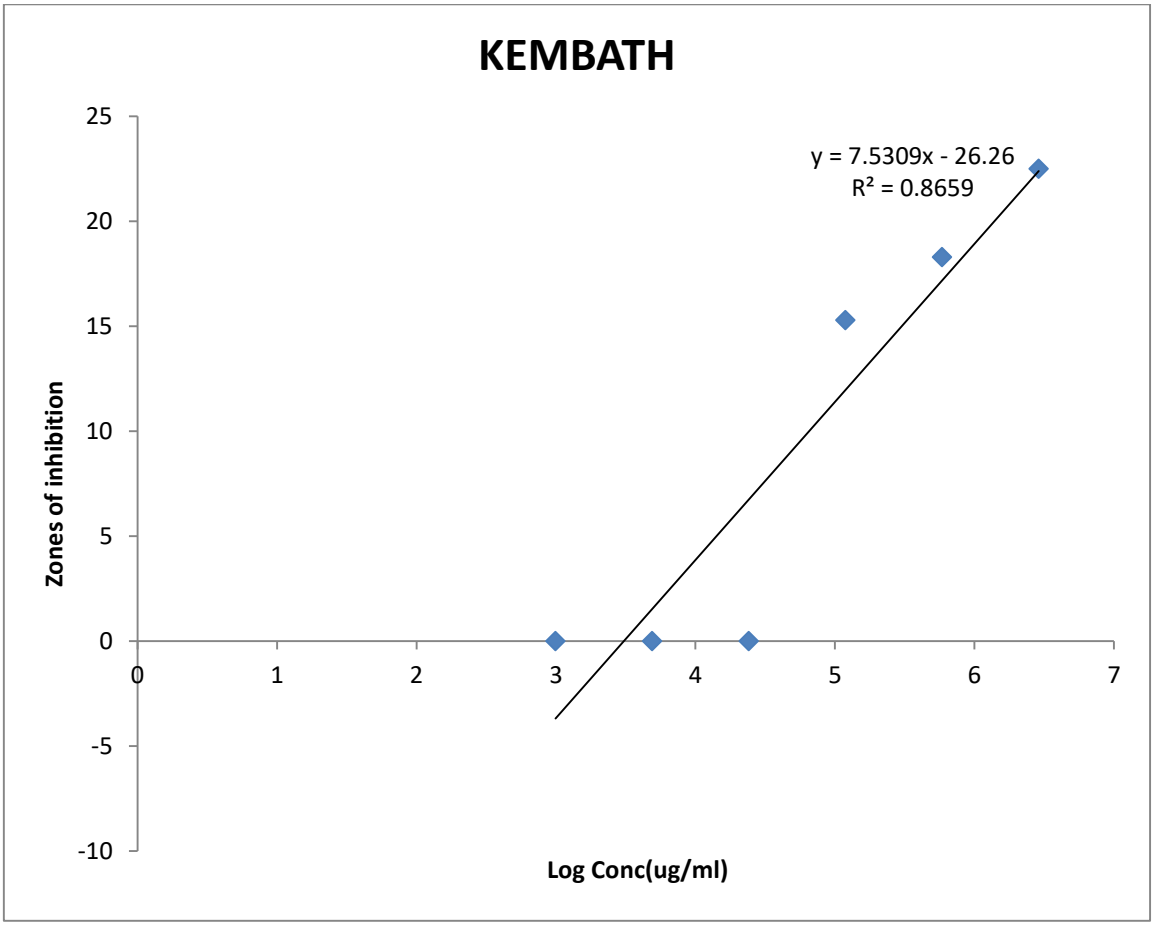

Figure 9: Graph of Zones of Inhibition vs. Log Concentration for Kembath

Key: M=slope (potency), C=intercept, which is concentration when x=0

Appendix 11

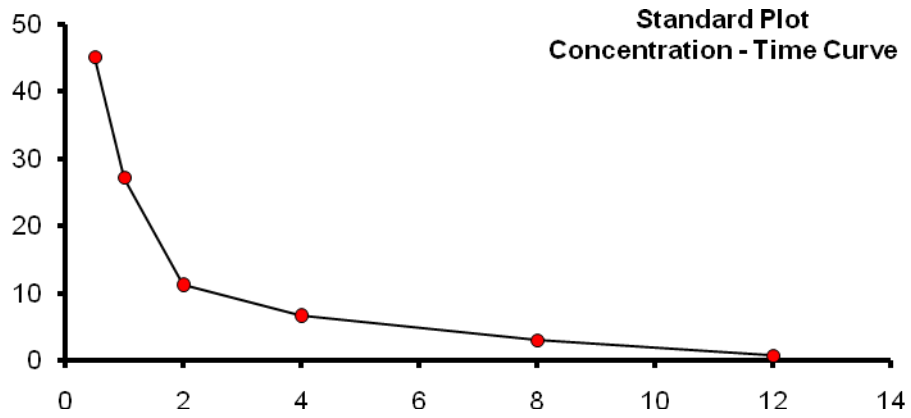

Figure 10a: Standard Concentration-time curve of Rocephin® following single intravenous administration in Sokoto red goats at 20 mg/kg body weight generated from PK Solution software. N=10

Appendix 12

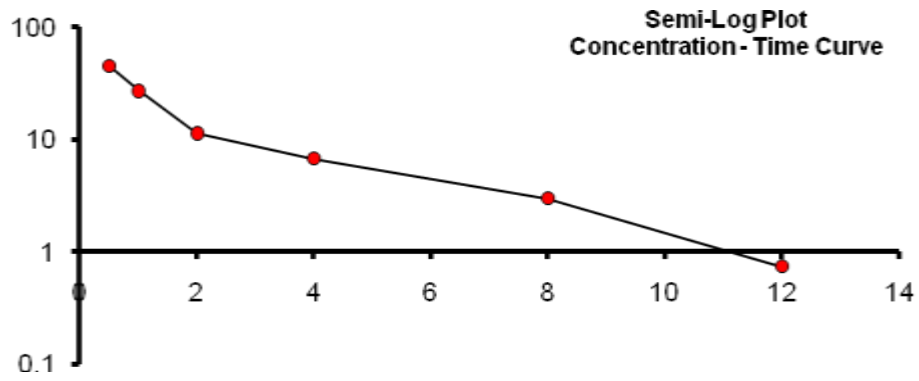

Figure 10b: Semi-log Concentration-time curve of Rocephin® following single intravenous administration in Sokoto red goats at 20mg/kg body weight generated from PK Solution software. N=10

Appendix 13

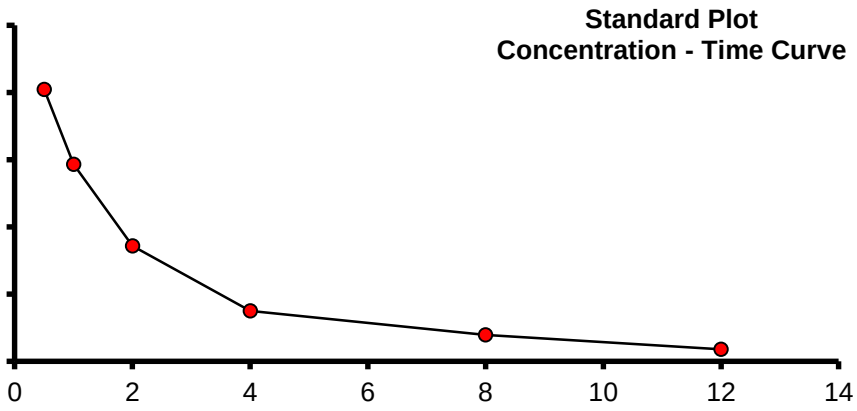

Figure 11a: Standard Concentration-time curve of Biocef® following intravenous administration in Sokoto red goats at 20mg/kg body weight generated from PK solution software. N=10

Appendix 14

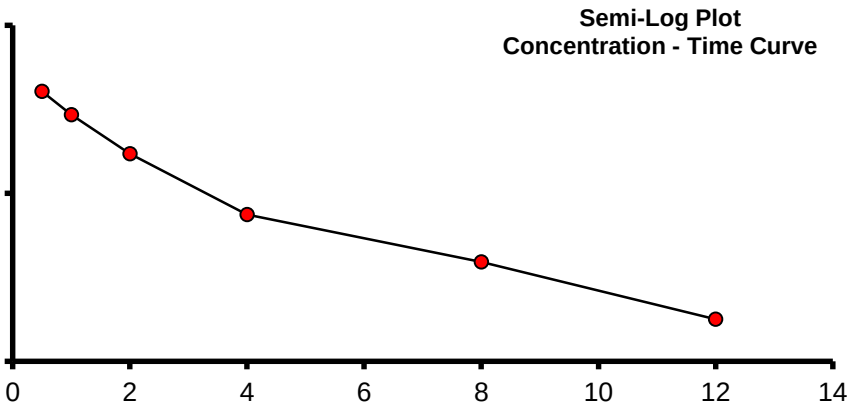

Figure 11b: Semi-log Concentration-time curve of Biocef® following intravenous administration in Sokoto red goats at 20mg/kg body weight generated from PK solution software. N=10

Appendix 15

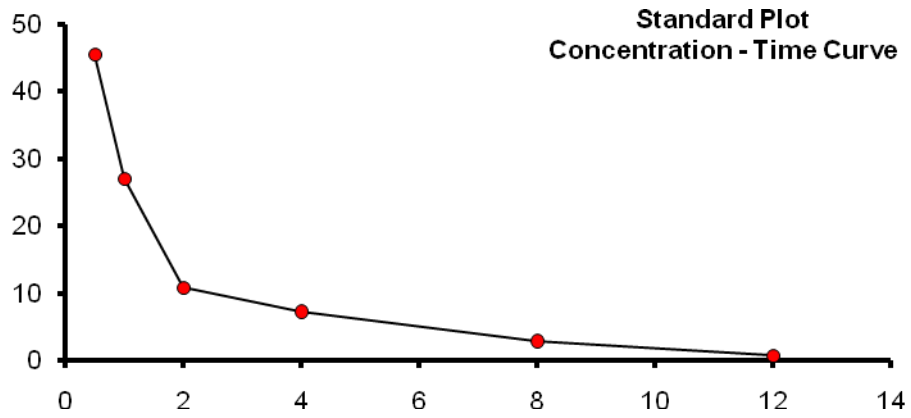

Figure 12a: Standard plot concentration-time curve of Rocephin® following intravenous administration in Sokoto red goats 20mg/kg body weight generated from pk solution software. n=7

Appendix 16

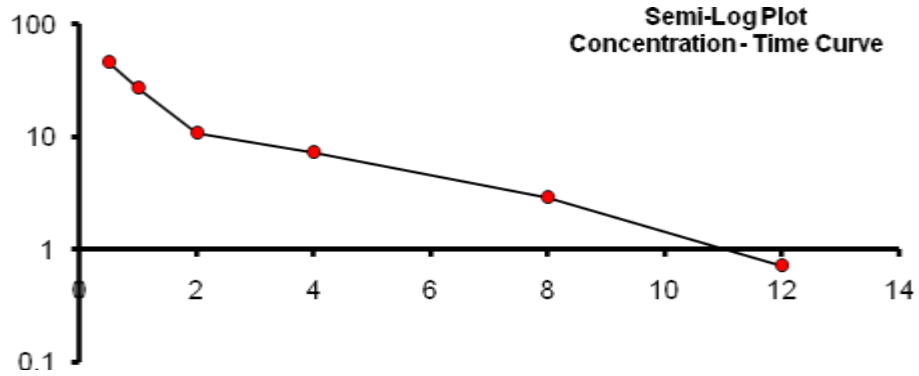

Figure 12b: Semi-Logplot concentration-time curve of Rocephin® following intravenous administration in Sokoto red goats 20mg/kg body weight generated from pk solution software. n=7

Appendix 17

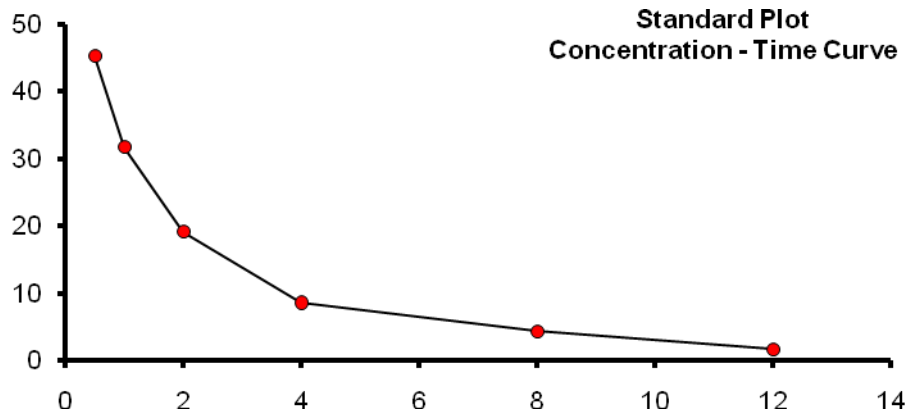

Figure 13a: StandardPlot Concentration-time curve of Biocef® following intravenous administration in Sokoto red goats at a dose of 20mg/kg body weight generated from PK solution software =7

Appendix 18

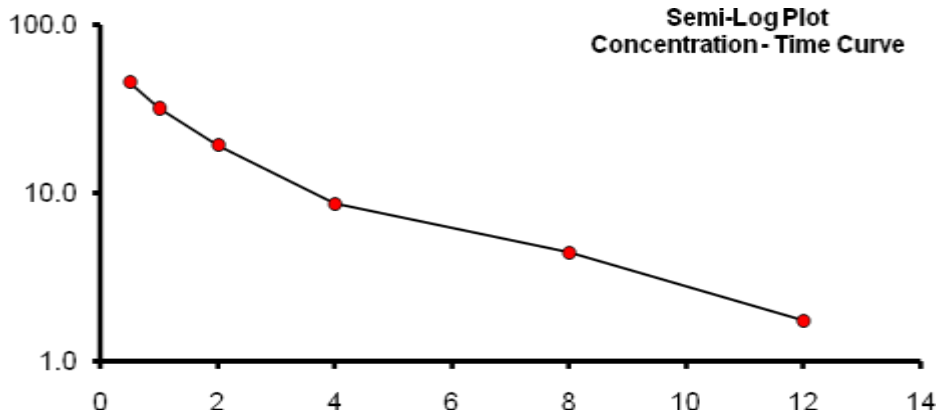

Figure 13bSemi-LogPlot Concentration-time curve of Biocef® following intravenous administration in Sokoto red goats at a dose of 20mg/kg body weight generated from PK solution software =7