KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF HEALTH SCIENCES

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

BIOEQUIVALENCE STUDY OF LOCALLY MANUFACTURED CIPROFLOXACIN HYDROCHLORIDE TABLETS (CIFLOX) USING URINARY EXCRETION DATA

BY

DZIGBORDI YAO AGBITOR (B.Pharm)

JULY, 2013

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY (M.PHIL.) IN PHARMACEUTICAL CHEMISTRY

JULY , 2013

DECLARATION

The experimental work described in this thesis was carried out at the Quality Control Laboratory of Ernest Chemists Ltd., Tema. This work has not been submitted for any other degree.

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ABSTRACT

Bioequivalence studies were conducted in two brands of Ciprofloxacin 500mg tablets (Ciflox and Ciprinol) in ten (10) healthy male adult volunteers using urinary excretory data. Ciflox was the test product and Ciprinol served as the reference. There was no significant statistical difference between the means and variances of the Cumulative Amount of Ciprofloxacin Excreted, the Maximum Excretory Rates and the Area under the Excretory Rate-Time Curve of Ciprofloxacin in both products. The Maximum Excretory Rate of Ciprofloxacin was reached in 1.85 hours when the reference product was administered and 2.45hours on administration of the test product. However, the 90% Confidence interval calculated for the ratio of the pharmacokinetic bioequivalence parameters of Ciflox to Ciprinol; 63.77% - 82.02% for the Cumulative Amount of Ciprofloxacin Excreted in seven hours, 57.39% - 92.67% for the Maximum Excretory Rate and 63.15% - 100.18% for the Area under the Excretory Rate -Time Curve were all outside the acceptable bioequivalence range of 80% - 125%. A decision of Ciflox not being bioequivalent to Ciprinol was therefore arrived at.

The method for the quantification of the Ciprofloxacin excreted in the urine involved the use of HPLC with UV detection set to 278nm. A mobile phase of 10% Acetonitrile and 90% of a 0.245%w/w Orthophosphoric acid was employed in an isocratic mode. A Waters Xbridge C18, 4.6x150mm, 5µ column set at a temperature of 40^0C was used. The method was found to be Accurate (with Accuracy of 102.7519%- 105.2587%), Precise (with RSDs of less than 2% between experimental values) and linear (with an R^2 value of 0.9988) within the range of 0.05µg/ml - 20µg/ml of Ciprofloxacin prepared in the urine sample.

DEDICATION

I dedicate this work to my family.

ACKNOWLEDGEMENT

I thank the Almighty God for successfully seeing me through my course of study.

My profound gratitude goes to Prof. Adosraku (my supervisor) for his patience, guidance and insight he brought to bear on this work.

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To all my colleagues at Ernest Chemists Ltd. who were the subjects used in the study and to my General Manager, Mr. Mark Owiredu, I say a big thank you for the diverse assistance given.

To my course mates; Agatha, Bright, Sammy and Mahmood, I say thank you all.

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DEFINITION OF TERMS

Pharmaceutical Equivalents

It refers to drug products, which contain the same active ingredient in the same strength (concentration) and dosage form, and is intended for the same route of administration. In general, it has the same labeling and meets compendia and other standards of strength, quality, purity, and identity.

Pharmaceutical equivalent does not necessarily imply therapeutic equivalence as differences in the excipients and/or the manufacturing process can lead to differences in product performance.

Pharmaceutical Alternatives

Drug products are considered a pharmaceutical alternative if they contain the same therapeutic moiety, but are different salts, esters, or complexes of that moiety, or are different dosage forms or strengths.

Therapeutic Equivalence

Two pharmaceutical products are therapeutically equivalent if they are pharmaceutically equivalent and after administration in the same molar dose their effects, with respect to both efficacy and safety, will be essentially the same as can be derived from appropriate studies (bioequivalence, pharmacodynamics, clinical or in-vitro studies). Therapeutically equivalent drug products are interchangeable.

Innovator Drug Product

Generally, the innovator pharmaceutical product is that which was authorized for marketing (normally as a patented drug) on the basis of documentation of efficacy, safety and quality (according to contemporary requirements).

Reference Product

A reference product is a pharmaceutical product with which the new product is intended to be interchangeable in clinical practice. The reference product would normally be the innovator product for which efficacy, safety and quality have been established. When the innovator product is not available the product which is the market leader may be used as a reference product, provided that it has been authorized for marketing and its efficacy, safety and quality have been established and documented.

Generic Product

A pharmaceutical product, usually intended to be interchangeable with the innovator product, which is usually manufactured without a license from the innovator company and marketed after expiry of patent or other exclusivity rights.

Interchangeable Pharmaceutical Product

An interchangeable pharmaceutical product is one, which is therapeutically equivalent to a reference product.

Multi-source Pharmaceutical Products

Multi-source pharmaceutical products are pharmaceutically equivalent products that may or may not be therapeutically equivalent. Multi-source pharmaceutical products that are therapeutically equivalent are interchangeable.

Steady State

Is the state when the plasma concentration of drug at any time point during any dosing interval should be identical to the concentration at the same time during any other dosing interval. The steady state drug concentration fluctuates between a maximum and minimum steady state concentration within each of the dosing intervals.

Bio batch

A batch of product manufactured for use in bioequivalence study

OTC- Over the counter

C18

Octyldecylsilane stationary phase material

ANOVA

Analysis of Variance

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Ciprofloxacin is a second-generation fluoroquinolone antibiotic (Nelson et al.,2007). Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal, and abdominal infections, including Gram-(-) (*Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae, Legionella pneumophila, Moraxella catarrhalis, Proteus mirabilis,* and *Pseudomonas aeruginosa*), and Gram-(+) (methicillin-sensitive but not methicillin-resistant *Staphylococcus aureus, Streptococcus pneumoniae, Staphylococcus epidermidis, Enterococcus faecalis,* and *Streptococcus pyogenes*) bacterial pathogens. Ciprofloxacin and other fluoroquinolones are valued for this broad spectrum of activity, excellent tissue penetration, and for their availability in both oral and intravenous formulations (Brunton et al., 2005).

Ciprofloxacin is used alone or in combination with other antibacterial drugs in the empiric treatment of infections for which the bacterial pathogen has not been identified including urinary tract infections and abdominal infections among others. Alone, Ciprofloxacin is used to treat a number of infections, including infections of bones and joints, endocarditis, gastroenteritis, malignant otitis external, respiratory tract infections, cellulitis, anthrax and chancroid as well as:

- Acute uncomplicated cystitis in females
- Chronic bacterial prostatitis (recommended as a first-line antibiotic choice)
- Acute sinusitis (not recommended as a first-line antibiotic choice)
- Skin and skin structure infections

- Infectious diarrhea
- Typhoid fever (enteric fever) caused by *Salmonella typhi*
- Uncomplicated cervical and urethral gonorrhea (due to *N. gonorrhoeae*)

It is also used in combination with other specific drugs:

- Complicated intra-abdominal infections (in combination with metronidazole);
- Empirical therapy for febrile neutropenic patients (in combination with piperacillin)

Oral and intravenous fluoroquinolones are approved by the FDA for use in children for only two indications due to the risk of permanent injury to the musculoskeletal system;

Complicated urinary tract infections and pyelonephritis due to *Escherichia coli* (Albrecht, 2004) and Inhalational anthrax (postexposure) (Murphy, 2000).

Ciprofloxacin is available for medical use as tablets, syrups, intravenous solutions, and eye and ear drops.

Ciprofloxacin is contraindicatded in the following (FDA, 2011);

- Co-administration of ciprofloxacin with other drugs primarily metabolized by CYP1A2 results in increased plasma concentrations of these drugs and could lead to clinically significant adverse events of the co-administered drug.
- Concomitant administration with tizanidine is contraindicated.
- Ciprofloxacin is contraindicated in persons with a history of hypersensitivity to ciprofloxacin, any member of the quinolone class of antimicrobial agents, or any of the product components.
- Local IV site reactions are more frequent if the infusion time is 30 minutes or less.

These may appear as local skin reactions that resolve rapidly upon completion of the infusion. Subsequent intravenous administration is not contraindicated unless the reactions recur or worsen.

Ciprofloxacin is also considered to be contraindicated within the pediatric population (except for the indications outlined above), pregnancy, nursing mothers and in patients with epilepsy or other seizure disorders.

The safety of fluoroquinolones is similar to that of other antibiotics. In most cases, adverse reactions are mild to moderate; but serious adverse effects occur on occasions. Most of the adverse events reported were described as only mild or moderate in severity, abated soon after the drug was discontinued, and required no treatment. The most frequently reported drug-related events are nausea, diarrhea, abnormal liver function tests , vomiting and rash .

The administration of Ciprofloxacin has been associated with a number of rare but serious side effects, including peripheral neuropathy(Zehnder et al.,1995), acute liver failure or serious liver injury (hepatitis), QT interval prolongation/torsades de pointes (US FDA, 2008), toxic epidermal necrolysis (TEN) (Jongen-Lavrencic et al.,2003) and Stevens–Johnson syndrome, severe central nervous system disorders (CNS) as well as photosensitivity/phototoxicity reactions.

Psychotic reactions and confusional states, acute pancreatitis (Mann and Thillainayagam,2000), interstitial nephritis and hemolytic anemia may also occur during ciprofloxacin therapy. Additional serious adverse reactions include temporary loss of vision, double vision and drug induced psychosis, impaired color vision, exanthema, abdominal pain, malaise, drug fever, dysaesthesia and eosinophilia.

Children and the elderly are at a much greater risk of experiencing such adverse reactions.

Ciprofloxacin interacts with other drugs and herbal and natural supplements, a characteristic it shares with other widely used antibacterial drugs, such as amoxicillin, trimethoprim, azithromycin, cephalexin and doxycycline (Cooper et al.,2005)

Concurrent administration of ciprofloxacin with magnesium or aluminium antacids, sucralfate or products containing calcium, iron or zinc (including multivitamins or other dietary supplements) may substantially decrease the absorption of ciprofloxacin, resulting in serum and urine levels considerably lower than desired(US FDA,2009).

Serum levels of certain drugs metabolised by the cytochrome P450 system is enhanced by concomitant use of some quinolones. Co-administration may dangerously increase coumadin (warfarin) activity; INR should be monitored closely. Levels of tizanidine and methylxanthines (for example, theophylline and caffeine) may be increased due to ciprofloxacin's interaction with the cytochrome P-450 enzyme CYP1A2.

Overdose of ciprofloxacin may result in reversible renal toxicity. Treatment of overdose includes emptying of the stomach by induced vomiting or gastric lavage. Careful monitoring and supportive treatment is given during treatment of overdose.

Bioavailability of Ciprofloxacin is approximately 70-80%, with no significant first pass effect. Biotransformation is hepatic with elimination half life of 4hours (US FDA, 2008). Elimination is principally renal with about 40% of the drug excreted unchanged in urine.

Ciprofloxacin functions by inhibiting DNA gyrase, a type II topoisomerase and topoisomerase IV (Drlica and Zhao,1997), enzymes necessary to separate bacterial DNA thereby inhibiting cell division.

Ciprofloxacin is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid. Its empirical formula is $C_{17}H_{18}FN_3O_3$ and its molecular weight is 331.4 g/mol. Ciprofloxacin hydrochloride (USP) is the monohydrochloride monohydrate salt of ciprofloxacin. It is a faintly yellowish to light yellow crystalline substance with a molecular weight of 385.8 g/mol. Its empirical formula is $C_{17}H_{18}FN_3O_3HCl{\bullet}H_2O$

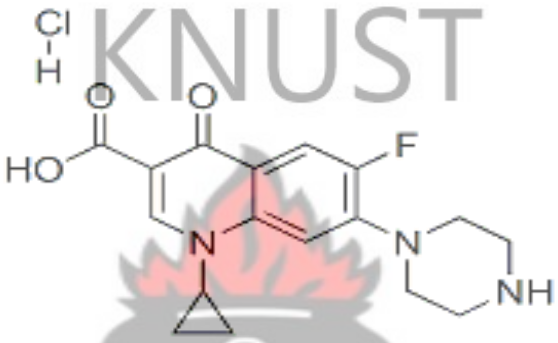

Figure 1: Structure of Ciprofloxacin Hydrochloride

When a new drug entity or product is developed, it is binding on the manufacturer of the new entity to demonstrate the drug product is capable of eliciting the intended therapeutic response. This is usually done through clinical trial studies. These studies are usually laborious and very expensive to carry out but are mandatory in order to ensure safety, efficacy and quality of the product. These products containing the new drug entity known as the innovator products are patented. After the expiration of the patent, the drug product may be multi-sourced as many manufacturing houses may come up with their own brands of the product (normally referred to as the generic product). The generic products contain the same drug entity as the innovator product. However, the excipients used in the formulation of the generic product are mostly different from those used in the manufacture of the innovator product and this may impact on the quality, safety and efficacy of the generic product. This is because the excipients cannot always be considered as inactive or inert (Borgherini,2003).

Some patients could have individual reactions or sensitivities to a change in excipients (Birkett, 2003). Besides, other factors such as differences in the drug particle size, manufacturing process, manufacturing site, equipment, batch size may impact on the therapeutic outcome of the generic product.

In order to assure the pharmaceutical quality, safety, and efficacy, generics should therefore be reliably compared with the corresponding innovator drugs (brand-name drugs). In the United States for example the US Food and Drug Administration (FDA) publishes a list of drug products and equivalents known as the Approved Drug Products with Therapeutic Equivalence Evaluations, commonly called the "Orange Book". The FDA's designation of therapeutic equivalence indicates that the generic formulation is (among other things) bioequivalent to the innovator formulation and signifies the FDA's expectation that the formulations are likely to have equivalent clinical effect and no difference in their potential for adverse effect.

The comparison assures that the generic and innovator products are interchangeable. The assessment of "interchangeability" between the innovator and generic products is carried out by a study of "in vivo equivalence" or "bioequivalence" (Midhal and McKay, 2009).

Bioequivalence study of generic products has now become very important in obtaining marketing authorization in many jurisdictions.

1.2 AIM OF THE STUDY

To determine whether Ciflox 500 (Ciprofloxacin HCl equivalent to Ciprofloxacin 500mg) which is a locally manufactured brand of Ciprofloxacin is bioequivalent to the reference product, Ciprinol in a cross-over non replicate bioequivalence study using urinary excretion data of Ciprofloxacin in ten human subjects.

1.3 OBJECTIVES OF THE STUDY

(i) To design a method capable of quantifying Ciprofloxacin in urine using HPLC

(ii) To validate the HPLC method of quantification of Ciprofloxacin in urine

(iii) To apply the validated method to quantify the concentration of Ciprofloxacin excreted in the urine of subjects after oral administration of the test product(Ciflox) and the reference (Ciprinol)

(iv) To determine the urinary bioequivalence parameters for both Ciflox and Ciprinol and to perform statistical analysis on these parameters in order to determine whether a bioequivalence relationship exists.

1.4 JUSTIFICATION OF THE STUDY

The rising cost of medications (branded or innovator products) has been contributing to the total overall cost of health care and this has received a lot of attention globally. A major strategy for lowering the cost of medication, and thereby reducing its contribution to total health care costs, has been the introduction of generic equivalents of brand-name drugs or innovator drugs(Midhal and McKay,2009). This strategy has reduced prescription cost by 11% without sacrificing quality (Haas et al.,2005).

In recent times, generic drugs have captured more than 65% of the global market and account for 66% of prescriptions filled in the United States but for less than 13% of the cost (Shrank et al.,2009).

The increasing use of the generics therefore demands that their pharmaceutical quality, safety and efficacy be compared to the corresponding innovator drug or market leader. The assessment of bioequivalence addresses these concerns which cannot be determined just by performing the usual physiochemical tests.

For this reason, many Drug Regulatory Authorities are beginning to demand proof of bioequivalence as an evidence of assuring safety, quality, efficacy and ultimately as an assessment of "interchangeability" between the innovator and generic product prior to marketing authorization.

In this study, the establishment of bioequivalence between Ciflox and Ciprinol would boost prescribers' and users' confidence in the use of Ciflox as a therapeutic equivalent or substitute to Ciprinol and put to rest any concerns about the quality, safety and efficacy of Ciflox.

1.5 LIMITATIONS OF THE STUDY

(i) Inability to confine subjects in order to monitor fluid intake, food intake, abstinence from alcohol, control of posture etc may impact on the outcome of the study, i.e. a standardized and controlled environment cannot be ensured.

(ii) Inability to assess the health status of subjects enrolled on the study through relevant tests e.g. hematological, hepatic, liver and renal function tests which may also impact the outcome of the study.

(iii) Absence of adequate storage facility for samples and a dedicated HPLC chromatograph necessitated subject being handled in groups on different days. This may introduce some bias into the study.

1.6 LITERATURE REVIEW

1.6.1 Definition of Bioequivalence

Bioequivalence has been variously described by various authors. Bioequivalence has been described as the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when

administered at the same molar dose under similar conditions in an appropriately designed study (US FDA, 2003).

Birkett (2003) also defined bioequivalence by stating that, "two pharmaceutical products are bioequivalent if they are pharmaceutically equivalent and their bioavailabilities (rate and extent of availability) after administration in the same molar dose are similar to such a degree that their effects, with respect to both efficacy and safety, can be expected to be essentially the same.

Two medicinal products containing the same active substance are considered bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives and their bioavailabilities (rate and extent) after administration in the same molar dose lie within acceptable predefined limits. These limits are set to ensure comparable in vivo performance, i.e. similarity in terms of safety and efficacy (European Medicines Agency, 2010).

Bioequivalence of a drug product is achieved if its extent and rate of absorption are not statistically significantly different from those of the reference product when administered at the same molar dose (India Central Drugs Standard Control Organization, 2005).

Bioequivalence is different from Bioavailability in that bioavailability simply refers to the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action((Chen et al., 2001). There is absolutely no comparison between any products.

1.6.2 Test and Reference Products

In bioequivalence study two drugs are compared; the test product (generic product) which is the product for which bioequivalence is to be established and the reference or innovator product which is usually the patented drug which was authorized for global marketing on the basis of efficacy, safety and quality. When the innovator product is not available, the product which is the market leader is used as a reference product, provided that it has been authorized for marketing and its efficacy, safety and quality have been established and documented (US FDA, 2003).

The test product used in the study must be representative of the product to be marketed. For solid oral dosage forms for example, the test product is usually from a pilot batch which is at least 1/10 of production scale or 100,000 units, whichever is greater. In case of a production batch smaller than 100,000 units, a full production batch will be required (European Medicine Agency, 2010).

Key quality attributes of the drug products (both test and reference), such as dissolution and the assay should be established. As a rule of thumb, the assayed content of the batch used as test product should not differ by more than 5% from that of the batch used as reference product determined with the test procedure proposed for routine quality testing of the test product (Saudi Arabia FDA, 2005). Many other guidelines stipulate this requirement.

1.6.3 Methods of Assessment of Bioequivalence

In a number of guidelines established to shed light on bioequivalence studies, these studies are generally classified as:

1. Pharmacokinetic endpoint studies – in this study the active drug substance or one or more metabolites are measured in an accessible biologic fluid such as plasma, blood or urine.

2. Pharmacodynamic endpoint studies- Pharmacodynamic evaluation involves the measurement of the effect on a pathophysiological process, such as a function of time, after administration of two different products to serve as a basis for BE assessment. Where Pharmacodynamic endpoint are used, Regulatory authorities request justification for its use. Two main conditions necessitate the use of pharmacodynamics studies to establish bioequivalence;

   i) if the drug and/or metabolite(s) in plasma or urine cannot be analyzed quantitatively with sufficient accuracy and sensitivity and ii) if drug concentration measurement cannot be used as surrogate endpoints for the demonstration of efficacy and safety of the particular pharmaceutical product

3. Clinical endpoint studies- these studies is used in the absence of pharmacokinetic and pharmacodynamics approaches. Adequate and well-controlled clinical trials may be used to establish BA/BE and several international regulatory authorities provide general information about the conduct of clinical studies to establish BE.

4. In vitro endpoint studies- this is used for highly permeable, highly soluble drug substances formulated into rapidly dissolving drug products. For such products, an only in vitro dissolution study is used to establish Bioequivalence (Davit et al., 2008).

The classification of drugs as either having high or low solubility and permeability and drug products as exhibiting rapid dissolution has been carried out by the Biopharmaceutics Classification System (BCS) (Amidon et al.,1995).

The general descending order of preference of the various studies includes pharmacokinetic, pharmacodynamic, clinical, and in vitro studies (Chen et al., 2001).

1.6.4 Subjects Used in Bioequivalence Study and Ethical Considerations

In conducting bioequivalence studies human subjects are used. The general norm in selection of subjects is to choose healthy male adult volunteers (usually 18 - 55years of age). A number of guidelines on conducting bioequivalence studies such as those written by the Food and Drugs Board of Ghana and the Food and Drug Authority of Saudi Arabia and India require that the volunteers should be non-smoking.

In some cases females were included in the bioequivalence study. However where they were included, the guidelines specify that the effects of gender differences and menstrual cycle (if applicable) must be statistically examined.

The health status of subjects enrolled onto the study is normally assessed prior to the commencement of the bioequivalence study. The assessment is done through physical examination, medical history (administered within 30 days prior to the initiation of the study), routine blood chemistry, haematology, kidney function tests, and urinalysis in order to ensure subjects have normal hepatic, haematological and renal functions (Health Canada, 1992). It was also ensured that subjects were free of any history of serious gastrointestinal, renal, hepatic, cardiovascular or haematological disorders and had no history of adverse reactions to the drug (or its class) under study.

During the conduct of bioequivalence studies, the health conditions of subjects are usually closely monitored especially for the side effects of the drug being studied (Saudi Arabia FDA, 2005).

Although healthy subjects are usually employed in bioequivalence studies, in studying the bioequivalence of specific classes of drugs such as cytotoxic drugs, drugs solely recommended for a very specific population or gender, etc., a targeted patient population are enrolled in the study instead of healthy volunteers (US FDA 2003).

Generally, for the purpose of performing bioequivalence studies, most international guidelines require a minimum of 12 to 24 subjects. However, in some cases (e.g., for highly variable drugs) more than 24 subjects may be required for acceptable bioequivalence study. The number of subjects employed is usually determined using appropriate methods taking into account the error variance associated with the primary parameters to be studied (as estimated for a pilot experiment, from previous studies or from published data), the significance level desired ($\alpha = 0.05$), and the deviation from the reference product compatible with bioequivalence (± 20%) and compatible with safety and efficacy ((Saudi Arabia FDA, 2005).

The number enrolled on a study is always in excess of the required number so as to allow for drop outs. Sometimes Bioequivalence may not be demonstrated because of an insufficient number of subjects. In such instances an add-on subject study may be performed using not less than half the number of subjects in the initial study (Shaik et al.,2011).

Bioequivalence studies using human subjects just as any other research involving humans are performed in accordance with the ethical principles contained in the Declaration of Helsinki (Saudi Arabia FDA, 2005).

As part of the declaration, an Ethical Review Committee reviews and confirms that the protocol for the conduct of the study complies with ethical standards for research on human subjects. A voluntary informed consent of the volunteers to participate in the study is obtained mostly in the form of Informed Consent Forms (ICF) which are signed by the subjects. Information provided in the form includes details of the study, the role of the participants, the risk associated with participation as well as information regarding the right of the subject to withdraw at any time from participation without any jeopardy.

The subjects are not permitted to take any prescription or over-the-counter drug products within two weeks of the start of the study. Ingestion of alcohol or caffeine or related xanthine containing food or beverages is not allowed within 48 hours of the study or during the study (Health Canada, 1992).

In order to closely monitor the subjects and ensure compliance to the rules governing the study, subjects are usually confined during the period of the study.

1.6.5 Study Designs

Cross over and Parallel designs are two main designs which have been widely employed in conducting bioequivalence study (Gordon, 2011). The above designs are based on the sequence in which the drugs are administered to the subjects.

Cross over study: In the cross over design the test product and the innovator product (comparator) are administered to each subject separated by a wash out period. The wash out period should be long enough to eliminate the possibility of carry -over of the earlier administered product. Generally, a wash out period of at least five (5) times the half life of the drug is employed (European Medicine Agency, 2010).

A minimum wash out period of at least 7 days has however been recommended (Gordon, 2011) for drugs with very short elimination half lives . The wash out period must take into account the slow metabolizers. Cross over design is the most preferred design as it allows within subject comparison to be made. In addition, it minimises genetic variations due to ethnic background as well as variations in food habits and metabolic variations. It is usually conducted as a randomised, two-period, two-sequence single dose cross over study.

Parallel Design: In the Parallel design, each subject is administered either the test product or the comparator. It permits between subject comparison and is only recommended for extremely long half- life drugs (Gordon, 2011).

Besides the crossover and parallel designs, a replicate or non replicate designs have been adopted in the study of bioequivalence based on the type of product been assessed. Non replicate design which is highly recommended has been extensively used for bioequivalence studies of most orally administered immediate-release and modified –release dosage forms. Replicate study designs on the other hand have been used for the bioequivalence study of highly variable drug products (i.e. drugs having high intra-subject or within subject variation with coefficient of variation of ≥30)(Haidar et al.2008).

These drugs may be immediate release, modified release and other orally administered drug products. Though the lesser recommended design, the replicate study design offers some advantages compared to the non replicate design;

(a) Allows comparisons of within subject variance for the test and reference products.

(b) Indicates whether a test product exhibits higher or lower within-subject variability in the bioavailability measures when compared to the reference product.

(c) Provides more information about the intrinsic factors underlying formulation performance

(d) Suggests whether a subject-by-formulation interaction may be present.

(e) Reduces the number of subjects needed in the bioequivalent study.

The cross over or parallel designed studies are conducted either as a non replicate or replicate studies.

1.6.6 Doses used in bioequivalence studies and Standardization of the Experimental Conditions

In conducting bioequivalence studies, a single dose of the drug product consisting usually of the highest recommended dose stated on the product label or the highest approved strength is mostly used. The rationale for the preference of the single dose study is the fact that single doses are generally more sensitive in assessing the release of the drug substance from the drug product into the systemic circulation (US FDA, 2003). Single dose studies are widely employed for both immediate and modified-release drug products. Multiple doses may also be administered. However, where a multiple dose study design is used, appropriate dosage administration and sampling is carried out to document attainment of a steady state (India Central Drugs Standard Control Organisation, 2005)

The experimental conditions for carrying out bioequivalence studies are always standardised. The purpose of the standardisation is to minimise the variability of all factors involved except that of the products being tested. Standardisation of the diet, fluid intake and exercise is therefore highly recommended.

For this reason the dose administered in bioequivalence studies is administered mostly in the fasting state; the dose is administered with sufficient fluid after at least 10 hours of fasting which is continued for at least 4 hours post-dose (Health Canada, 1992). Alcohol and xanthine free fluid may be given the night prior to the study. However, when it is recommended that the study drug be given with food or where the dosage form is a modified release product, fed state studies have been carried out (European Medicines Agency, 2010). Studies in the fed state usually involve consumption of a high fat diet prior to the dosing. Fed state studies have also been conducted when fasting state studies make assessment of bioequivalent parameters such as C_{max} and T_{max} difficult and in cases where the drug may produce gastric irritation under fasting conditions e.g. NSAIDs (US FDA, 2003).

The appropriate choice of the meal's timing and its contents should be carefully considered.

On the morning of the study, up to 250 mL of water may be permitted up to two hours before drug administration. The dose should be taken with water of a standard volume (e.g., 150 mL) and at a standard temperature. Two hours after drug administration, up to about 250 mL of xanthine-free fluids are permitted. Four hours after drug administration, a standard meal may be taken. All meals should be standardized and repeated on each study day.

For most drugs, subjects should not be allowed to recline until at least two hours after drug ingestion. Physical activity and posture should be standardized as much as possible. The standardization is important in order to limit effects on gastrointestinal blood flow and motility (Health Canada, 1992). It is imperative that the same pattern of posture and activity should be maintained for each study day.

When the sample collected is urine samples, a standard volume of water (e.g. 150ml) is given to each subject after the excretion of urine at all the various time intervals.

1.6.7 Biological Matrix, Moieties to be Measured, Sampling Schedule and Handling of Samples

Blood, plasma, serum and urine are the biological matrices mostly sampled in bioequivalence studies. Plasma or serum is the matrix of choice in most studies. Whole blood may be used when appropriate. However, in instances where the parent drug is not metabolized and is largely excreted unchanged and can be suitably assayed in the urine, urinary drug levels may be used to assess bioequivalence, if plasma/ serum concentrations of the drug cannot be reliably measured. Generally, urinary drug levels may be employed for a drug which has >40% of it being excreted as the unchanged drug (New Zealand Regulatory Guidelines, 2001).

A number of drugs show poor correlation between the urinary levels of the drug and the plasma levels. For this reason, when using urinary data, any available data supporting that urinary excretion will reflect plasma exposure must be demonstrated (European Medicine Agency, 2010).

The moieties measured in the biological matrix are either the parent drug substance or it metabolites. For most part, bioequivalence is determined by measurement of the parent drug in body fluids. In some cases, however, monitoring a metabolite is considered more appropriate. A number of reasons for use of metabolite have been put forward.

Midha et al.,(2004) stated reasons such as:

i) The parent drug is an inactive prodrug

ii) Plasma concentrations of the parent drug are too low to monitor because of inadequate assay sensitivity

iii) The parent drug is metabolized rapidly to an active metabolite, and

iv) The parent drug and a metabolite both have therapeutic activities but the metabolite is present in higher concentrations when the parent drug is rapidly and extensively metabolized such that only metabolite(s) data are available.

In bioequivalence studies, samples of the biological fluid/ matrix are taken at predetermined intervals over a scheduled period of time. The length of time over which the samples are collected must be enough to allow for the determination of the various pharmacokinetic parameters. Various national guidelines spell out the length of time over which biological samples must be collected in order to meet their respective requirements. A period of three (3) to seven (7) halve lives of drugs is usually considered as the period over which sampling is to be carried out.

The samples are appropriately processed and stored carefully under conditions that preserve the integrity of the analyte(s) (usually in a freezer) until the day of analysis.

1.6.8 Bioequivalence Parameters

The parameters which are determined in pharmacokinetic bioequivalence studies depend on whether the data to be used is obtained from plasma/blood/serum or from urinary excretion data.

Though different authors and guidelines specify additional parameter to be assessed , assessment of the C_{max} (peak drug concentration), AUC [$AUC_{0\rightarrow t}$(area under the blood/serum/plasma concentration-time curve from time zero to time t, where t is the last time point with measurable concentration) and $AUC_{0\rightarrow\infty}$ (area under the plasma/serum/blood concentration-time curve from time zero to time infinity] are parameters which are mandatorily required by various drug regulatory authorities

when blood/plasma/serum is used. The AUCs are determined using the trapezoidal rule. In some cases T_{max} (time to maximum concentration) is also assessed.

When urine samples are used, U_{0-t} (the cumulative amount/concentration of drug excreted in the urine from time zero to the final sampling time t), R_{max} (the maximum urinary excretion rate) and Ae_{τ} (area under the excretion rate-time curve) are the parameters that are assessed. These parameters are those determined in a single dose study. If a multiple dose studies (steady-state studies) were performed, the following pharmacokinetic parameters are examined (India Central Drugs Standard Control Organization, 2005):

$AUC0 \rightarrow T_{ss}$ = Area under the curve (from time 0 to dosing interval) over a dosing interval at steady-state.

C_{maxss} = Maximum concentration at steady state.

C_{minss} = Minimum concentration at steady state.

C_{avgss} = Average concentration at steady state.

T_{maxss} = Time to maximum concentration at steady state.

% Swing = $100 (C_{maxss} - C_{minss}) / C_{minss}$

% Fluctuation = $100 (C_{maxss} - C_{minss}) / C_{avgss}$

1.6.9 Data Treatment, Statistical Analysis and Acceptance Criteria

The pharmacokinetic parameters are determined for each of the subjects used in the study for both the test and reference products. Many drugs regulatory authorities recommend that the parameters are logarithmically transformed.

The various logarithmically transformed pharmacokinetic parameters derived from the study are subjected to ANOVA in which the variance is partitioned into components due to subjects, periods, and treatments (Rani and Pargal, 2004). This involves complex statistical evaluations.

A number of programs have therefore been developed to ease the calculations involved in this statistical evaluation. Programs such as SAS® (Statistical Analysis System, SAS Institute, Cary, NC) or WinNonlin® (Pharsight Corporation, St. Louis, MO) etc which allows contributions from subjects, period, product/formulation, and interactions between these to be examined (Midhal and McKay, 2009) are available.

Although the variances as described above, means, standard deviations, test of significant difference between the pharmacokinetic indices among others are determined, the key estimate of bioequivalence is the estimation of the 90% Confidence Interval(CI) of the ratio of the pharmacokinetic parameter of the test and reference product(Midhal and McKay,2009). Most drug regulatory authorities corroborate this approach for the determination of bioequivalence.

Westlake (1972) gave the classical 90% CI of the difference between the bioequivalence parameter of the test and innovator products as $[U_T-U_R) \pm S\sqrt{2/nt(0.05)v}]$. Therefore for the logarithmically transformed parameters, the CI is given as;

$90\%\ CI = Exp\ [U_T-U_R) \pm S\sqrt{2/nt(0.05)v}]$

U_T=Mean of the bioequivalent parameter calculated for the test product

U_R= Mean of the bioequivalent parameter calculated for the reference product

n = Number of subjects per period

S= √MSE

MSE= Error Mean Sum of Square (from ANOVA analysis)

t(0.05)v= Critical value of t at α= 0.05

v = Number of degrees of freedom associated with the MSE

The lower and upper confidence intervals so obtained is then maintained as a ratio or transformed into percentages.

Bioequivalence is established when the 90% CI as described falls in the range 0.8 – 1.25 or 80 – 125% (various regulatory guidelines).

The 90% CI ranges as stated represent the BE limits for conventional solid oral dosage forms and the limits are widely recommended. For some special classes of drugs such as those with a narrow therapeutic index or highly variable drugs the approaches and limits are different.

1.6.10 Phases of Bioequivalence study

A two phase approach to the determination of bioequivalence in the appropriate body fluid i.e. plasma, serum, blood or urine is adopted in bioequivalence studies;

(a) **Pre-study phase**: this phase involves validation of the bio-analytical method used in quantification of the analyte in the body fluid. The body fluid is spiked with the reference standard of the analyte and the internal standard (IS) at known concentrations. The spiked body fluid is then used for the validation of the proposed bio-analytical method. Characteristics of the bio-analytical methods which are validated include:

(i) Specificity/selectivity

Selectivity is the ability of an analytical method to differentiate and quantify the analyte and the internal standard (IS) in the presence of other components in the sample. Potential interfering substances in a biological matrix include endogenous matrix components, metabolites, decomposition products, and in the actual study, concomitant medications, etc. Absence of interfering components is accepted where the response is less than 20% of the limit of quantitation for the analyte (European Medicines Agency, 2009)

(ii) Accuracy

Accuracy of an analytical method has been described as the closeness of the mean test results obtained by the method to the true value (concentration) of the analyte. Accuracy of bio-analytical methods have been established by analysing known amounts of the analyte in the spiked body fluid and comparing it to a control (usually the same analyte in the proposed diluent or mobile phase).Accuracy is established if the amount of analyte recovered in the body fluid is within 15% of the nominal value (European Medicines Agency, 2009)

(iii) Precision

It is the closeness of individual measures/responses of an analyte when the procedure is applied repeatedly to replicate samples. The coefficient of variation (CV) or relative standard deviation (RSD) between the set of responses obtained for the replicate analysis are computed. Precision of a bio-analytical method as described is referred to as repeatability. Since it is practically impossible to analyse all samples at one go, samples are usually stored (frozen). For this reason it is required that the effect of storage on the sample be investigated; the samples are analysed after an adequate storage period and the RSD computed between the responses obtained for the stored and fresh samples. This is widely referred to as stability test.

An RSD of ±15% demonstrates a bionalytical method is precise (European Medicines Agency, 2009)

(iv)Linearity/ Calibration curve

Linearity studies investigate the relationship between the response and the concentration of the analyte. A sufficient number of standard concentrations of the analyte are used to adequately define the relationship between concentration and response. The straight line obtained in the study serves as the calibration curve for the

determination of unknown concentrations of the analyte in the body fluid. A minimum of seven concentration level are used.

(v)Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD is the lowest concentration of the analyte which is detected by the method but cannot be accurately quantified. The LOQ is the lowest amount of analyte in a sample which can be quantified reliably, with an acceptable accuracy and precision. For the sake of convenience these parameters are determined from the calibration curve.

As a requirement Accuracy and Precision are examined using a minimum of three concentrations which are within the range of concentrations to be analysed (US FDA, 2001).

A pilot study in a very small number of subjects are also been carried out at this phase in order to optimise sample collection time intervals and provide other information e.g. for conventional immediate-release products, careful timing of initial samples were carried out to avoid a subsequent finding in a full-scale study that the first sample collection occurs after the plasma concentration peak. For modified-release products, a pilot study has helped to determine the sampling schedule to assess lag time and dose dumping.

(b) **Study phase**: the validated analytical method is applied to quantify the analyte in the appropriate sampled body fluid and the required pharmacokinetic determinations made.

1.6.11 Products Requiring Bioequivalence Study

In-vivo bioequivalence studies have been performed on different categories of drug products. For certain drugs and dosage forms, in vivo documentation of equivalence through either a bioequivalence study, a comparative clinical pharmacodynamics study or a comparative clinical trial is regarded important (India Central Drugs Standard Control Organisation, 2005). The products for which bioequivalent studies are required include but not limited to the following classes of drugs;

(a) Oral immediate release pharmaceutical products with systemic action when one or more of the following criteria apply;

(i) indicated for a serious condition requiring assured therapeutic response.

(ii) narrow therapeutic window and or narrow safety margin or step dose-response curve.

(iii) pharmacokinetics complicated by variable or incomplete absorption or absorption window, nonlinear pharmacokinetics, pre-systemic elimination and/ or high first pass metabolism greater than 70%.

(iv) unfavourable physic-chemical properties such as low solubility, instability, poor permeability, unfavourable ionization.

(v) documented evidence for bioavailability problems related to the drug or drugs similar in chemical structure or formulations.

(vi) where a high ratio of excipients to active ingredients exists.

(b) Non-oral and non-parenteral pharmaceutical products designed to act by systemic absorption such as transdermal patches and suppositories.

(c) Sustained or otherwise modified release pharmaceutical designed to act by systemic absorption

(d) Fixed OTC combination products with systemic action

(e) Non-solution pharmaceutical products which are for non systemic use such as oral, nasal, ocular, dermal, rectal and vaginal applications

1.6.12 Products Requiring Waiver of Bioequivalence Study

Although the requirement for demonstration of bioequivalence is never waived in many drug applications, for certain formulations and circumstances, equivalence between two pharmaceutical products are considered self-evident (India Central Drugs Standard Control Organisation, 2005). Examples include;

(a) When multi-source pharmaceutical products are to be administered parenterally (e.g, intravenous, intramuscular, subcutaneous, intrathecal administration) as aqueous solutions and contain the same active substance(s) in the same concentration and the same excipients in comparable concentrations.

(b) When multi-source pharmaceutical products are solutions for oral use, contain the active substance in the same concentration, and do not contain an excipient that is known or suspected to affect gastrointestinal transit or absorption of the active substance.

(c) When multi-source pharmaceutical products are gases.

(d) When multi-source pharmaceutical products are powders for reconstitution as a solution .

(e) When multi-source pharmaceutical products are optic or ophthalmic products prepared as aqueous solutions and contain the same active substance(s) in the same concentration and essentially the same excipients in comparable concentrations.

(f) When multi-source pharmaceutical products are topical products prepared as aqueous solutions and contain the same active substance(s) in the same concentration and essentially the same excipients in comparable concentrations.

(g) When multi-source pharmaceutical products are inhalation products or nasal sprays, tested to be administered with or without essentially the same device, prepared as aqueous solution and contain the same active substance(s) in the same concentration and essentially the same excipients in comparable concentrations.

CHAPTER TWO

EXPERIMENTAL

2.1 INSTRUMENTS AND MATERIALS

- Agilent Series 1200 HPLC Chromatograph
- Waters Xbridge C18 Column, 4.6 x 150mm, 5µ
- Hanna HI 2215 pH/ORP Meter
- Fistreem™ Calypso water distillation apparatus and Fistreem™ AquaRec UV Deioniser
- Copley DIS 6000 dissolution bath
- AB104-S Mettler Toledo weighing scale
- Hot plate
- Vacuum pump
- Samarth Ultrasonic bath
- 0.45µ syringe filter
- 0.45µ sintered glass filter
- Stuart Magnetic stirrer
- 10ml syringe
- Volumetric flasks (50ml,100ml,200m,500mll)
- Beaker (100ml)
- Conical flask (1L,2L)
- Measuring cylinder (10ml, 20ml,500ml)
- Graduated pipettes (1ml, 5ml)
- Glass funnel
- Thermometer

- Whatman filter paper(no. 1)
- Amber coloured glass bottle and plastic cap

2.2 REAGENTS AND SAMPLES

- Hydrochloric acid (35%w/w) (Merck)
- Triethylamine, TEA(HPLC grade- BDH)
- Orthophosphoric acid (HPLC grade-)
- Acetonitrile (HPLC grade- Merck)
- Methanol
- Distilled and deionised water

Table 1: Pure Sample Used

Sample	Source	Batch/Lot Number	Man. Date	Exp. Date	Purity
Paracetamol	Hebei Jiheng (Group) Pharmaceuticals Co. Ltd., China	1101098	14/01/2011	13/01/2015	99.5%
Ciprofloxacin HCl	Zhejiang Xinhua Pharmaceutical Co. Ltd., China	HB 00N120478	13/04/2012	12/04/2015	100.8

Table 2: Brands of Tablets Used (Ciprofloxacin Hydrochloride equivalent to ciprofloxacin 500mg)

Brand	Code	Batch Number	Man. Date	Exp. Date	Country of Origin
Ciprinol	A	N 88473	02 – 2012	02- 2017	Slovenia
Ciflox	B	1010M	10 – 2012	02 - 2016	Ghana

2.3 METHODS

2.3.1 Preparation of Reagents

- **Preparation of 0.01M HCl TS(Dissolution Medium)**

36.5g HCl in 1000ml ≡ 1M HCl

0.365g HCl in 1000ml ≡ 0.01M HCl

0.730g HCl in 2000ml ≡ 0.01M HCl

Purity of HCl= 35%

Amount of HCl required= 100/ 35 x 0.730 = 2.0857g

Weight per ml of HCl = 1.18g/ml

Volume of HCl required = 2.0857/1.18 = 1.77ml

1.80ml of concentrated Hydrochloric acid was measured using a 10ml measuring cylinder and carefully transferred into 1800ml of distilled water in a 2Litre conical flask. The solution was made up to 2 litres with the distilled water. The preparation was repeated three more times to get a total of eight (8) litres of 0.01MHCl.

The dissolution medium was warmed to a temperature of 41^0C. It was filtered under vacuum through a sintered glass filter of 0.45µ porosity with vigorous stirring. The stirring was continues for 5 minutes. This was done to degas the dissolution medium.

- **Preparation of 0.245%w/w Orthophosphoric acid adjusted to pH 3.0**

0.245g Orthophosphoric acid in 100g water ≡ 0.245%w/v

2.45g Orthophosphoric acid in 1000g water ≡ 0.245%w/w

Weight per ml of Orthophosphoric acid= 1.71g/ml

Volume required= 2.45/ 1.71= 1.43ml

Density of water = 1

Volume of water required= 1000ml

1.45ml of orthophophoric acid was measured using a 10ml measuring cylinder and carefully transferred into 800ml of distilled and deionised water (prepared by passing distilled water obtained through distillation using Fistreem™ Calypso water distillation unit through the Fistreem™ AquaRec UV deioniser) in a 1litre conical flask. Using the Hanna HI 2215 pH meter, the pH of the solution was adjusted to pH 3.0± 0.05 with Triethylamine(TEA).

2.3.2 Assay of Tablets (Test and Reference Product)

10 tablets of Ciflox were weighed and the average tablet weight was determined. The tablets were finely powdered. Two different weights of the powdered sample containing an equivalent of 100mg of Ciprofloxacin were weighed and quantitatively transferred with the aid of the mobile phase into a 200ml volumetric flask. The volume was made up to about 150ml with the mobile phase. The mixture was then sonicated for 15minutes and made up to 200ml with the mobile phase. The mixture was well mixed and filtered through a 0.45µ membrane syringe filter into samples vials. The procedure was repeated using Ciprinol.

An equivalent of 100mg of Ciprofloxacin pure sample was weighed and dissolved in 200ml of the mobile phase to serve as the standard solution.

The sample and standard solutions were then subjected to chromatographic analysis using the following chromatographic conditions;

Column: Waters Xbridge C18, 4.6 x 150mm, 5µ

Mobile Phase: 60% of 0.245%w/w Orthophosphoric acid pH 3.0: 40% Acetonitrile

Flow rate: 1.5ml

Injection volume: 5µl

Column temperature: 40^0C

Wavelength of detection: 278nm

The content of Ciprofloxacin in the tablets were then determined by the comparison of the average area under the chromatograms obtained for the sample and standard solutions.

2.3.3 Dissolution Test for Tablets

900ml of the degassed dissolution medium was measured into each of the six vessels of the dissolution tester. The medium was allowed to equilibrate to a temperature of 37 ± 0.5^0C. 1 tablet of Ciflox 500mg was placed into each of the six dissolution vessels. The tester was operated for 30minutes with the blades rotating at 50 revolutions per minute.

At the end of the 30minutes, about 10ml of the dissolution medium was withdrawn, filtered through Whatman filter paper and 0.5ml diluted to 50ml with the dissolution medium to give a concentration of 0.00056%w/v Ciprofloxacin.

The absorbance of the resulting solution was then read at a wavelength of 276nm. The percentage of Ciprofloxacin dissolved was then determined by comparison of the absorbance of this test solution to that of a standard solution of 0.00056%w/v Ciprofloxacin(prepared by dissolving the equivalent of 0.100g of Ciprofloxacin

Hydrochloride in 100ml of the dissolution medium and diluting 0.56ml to 100ml with the dissolution medium).

Sample solution: 0.5g Ciprofloxacin/900ml $\xrightarrow{0.5ml}$ 50ml ≡ 0.00056%w/v

Standard solution: 0.100g Ciprofloxacin/100ml $\xrightarrow{0.56ml}$ 100ml≡ 0.00056%w/v

The procedure was then repeated using Ciprinol 500mg.

2.3.4 Preparation of Validation Samples, Conduct of Bioanaytical Method Validation and Method Optimization

2.3.4.1 Preparation of Validation Samples

Urine was collected into a 100ml plastic capped amber coloured glass bottle. 5ml of the urine was diluted 100 fold to 500ml with the diluent (i.e. 0.245%w/v orthophosphoric acid adjusted to pH 3.0 with triethylamine) to give the blank urine sample in a 500ml conical flask.

A weight of Ciprofloxacin Hydrochloride pure sample equivalent to 0.05g (0.0582g) of Ciprofloxacin was weighed and dissolved in 50ml of the blank urine sample to give a stock solution of 0.001g/ml Ciprofloxacin. 0.05ml of the stock solution was then pipetted using a 1ml pipette (with 0.01ml graduations) into a 50ml volumetric flask.

1ml of a stock solution of 10mg/ml Paracetamol (prepared by dissolving 1g of Paracetamol in 100ml methanol) was pipette and added to the 50ml volumetric flask and the volume made up to 50ml with the blank urine sample. The Paracetamol serves as the Internal Standard (IS).

This dilution gave concentrations of 1µg/ml for the Ciprofloxacin and 200µg/ml for Paracetamol;

Ciprofloxacin: 0.05g/50ml (Blank urine) $\xrightarrow{0.05ml}$ 50m (Blank Urine) ≡ 1µg/ml

Paracetamol: 1g/100ml(methanol) $\xrightarrow{1ml}$ 50ml(Blank Urine) ≡ 200µg/ml

Other concentrations of the Ciprofloxacin ; 5µg/ml,10µg/ml, 15µg/ml and 20µg/ml were similarly prepared by pipetting 0.25ml, 0.50ml, 0.75ml and 1ml respectively of the 0.001g/ml Ciprofloxacin stock solution and making up to 50ml with the blank urine sample.

To each of these concentrations, 1ml of the Paracetamol Stock solution was added before being made up to volume.

Two more concentrations, 0.1µg/ml and 0.05µg/ml were prepared by respecting pipetting 5ml and 2.5ml of a 1ug/ml prepared as described (but without the Paracetamol Internal Standard) into 50ml volumetric flasks. 1ml of the Paracetamol stock solution was added to each of the solutions before being made up to volume with the blank urine.

Thus the blank urine sample was spiked with various amounts of Ciprofloxacin to yield seven concentrations of 0.05µg/ml, 0.1µg/ml, 1µg/ml, 5µg/ml, 10µg/ml, 15µg/ml and 20µg/ml (a range of 0.05 – 20µg/ml). All these samples contained 200µg/ml Paracetamol as Internal Standard.

A 50µg/ml of Ciprofloxacin in urine (with 200µg/ml Paracetamol) was also prepared.

Three concentrations of Ciprofloxacin were similarly prepared in the diluent to give control /reference solutions of 1µg/ml, 10µg/ml and 20µg/ml. Each contained a 200µg/ml Paracetamol as Internal Standard. All solution for validation were prepared in triplicates.

The validation samples (including the blank urine) were then subjected to chromatographic analysis using the following chromatographic conditions:

Column- Waters Xbridge C18, 150x4.6mm, 5µ

Mobile Phase: 10% Acetonitrile: 90%(0.245%w/w) H_3PO_4 adjusted to pH 3.0 with triethylamine.

Column Temperature; 40^0C

Flow Rate: 1ml/min

Detection wavelength: 278nm

Injection Volume: 20µl

2.3.4.2 Conduct of Bioanaytical Method Validation

The areas under the chromatograms for the Ciprofloxacin and Paracetamol peaks for all the validation samples were obtained from the respective chromatograms. The Ciprofloxacin-Paracetamol peak area ratios were then determined at the various concentrations. Using the peak area ratios, the following parameters were examined:

- **Accuracy:**

Accuracy was determined at Ciprofloxacin concentrations of 1µg/ml, 10µg/ml and 20µg/ml which are all with the range of 0.05- 20µg/ml. The ratio of the peak areas of the Ciprofloxacin peak and the Paracetamol peak in the urine sample was compared to the ratios obtained for samples prepared in the diluent (orthophosphoric acid) which served as the reference solution

- **Precision**

Repeatability: The Relative Standard Deviation (RSD) between the peak area ratios (i..e. Cipro-paracetamol peak area ratio) obtained at1µg/ml, 10µg/ml and 20µg/ml Ciprofloxacin in urine was computed.at each of the concentration levels.

Stability: The 50ug/ml Ciprofloxacin in urine sample were stored in the freezer for 5 days. At the end of the 5th day, the sample was taken out, sonicated and subjected to chromatographic analyssis already described. The RSD was then computed between the set of values obtained on the first and fifth days of analysis.

- **Linearity/Calibration Curve**

The Ciprofloxacin-Paracetamol peak area ratio obtained for all seven concentrations of Ciprofloxacin prepared in the urine were plotted as a function of the concentration of Ciprofloxacin in the urine. A linear regression line was then drawn through the points. The equation of the straight line and the coefficient of determination were then determined.

- **Specificity**

The chromatogram obtained for the blank urine sample was inspected and checked for the occurrence of extraneous peaks at the retention times of Ciprofloxacin and Paracetamol.

- **Limit of Detection(LOD) and Limit of Quantification (LOQ)**

The LOD and LOQ of the method were determined from the residual standard deviation and slope of calibration curve.

2.3.4.3 Method Optimization

Prior to deciding on the approach to quantifying Ciprofloxacin in urine, the investigator took a single dose of Ciprinol on two different occasions and excreted urine over a period of time. The concentrated urine and various dilutions of it in the diluent were subjected to the proposed chromatographic analysis. The dilution which gave the least interference from other components in the urine matrix (1 in 100 dilution) was adopted.

The excretion profile of Ciprofloxacin was also monitored and it was discovered that the maximum excretion of Ciprofloxacin occurs between 1.50-3.0hrs. Urine samples were therefore collected at closer time interval s (30mins) between 1-3hours in order to ensure that the maximum excretion was not missed in the study.

2.3.5 Conduct of the Bioequivalence Study

2.3.5.1 Selection of Subjects

Ten (10) seemingly healthy male adult volunteers between the ages of 23 to 38years were enrolled on the study. All subjects confirmed taking Ciprofloxacin at one time or the other without any observed adverse drug reactions. The subjects were selected among staff of Ernest Chemists Ltd, Manufacturing Division in Tema. Subjects were coded as A, B, C, D, E, F, G, H, I and J. Due to lack of adequate storage facility for all samples when collected at the same time and unavailability of a dedicated HPLC chromatograph, subjects were divided into four groups; ABC, DEF, GHI and J and handled on different days. A written informed consent was also obtained from participants.

2.3.5.2 Coding of Products

The products were taken out of their blisters prior to administration to subjects and placed into pill envelopes. The reference and test products were then coded respectively as A and B. The coding was done so as to 'blind' the product so subjects were not aware of which particular product they took.

2.3.5.3 Dose Administration

Subjects were asked not take breakfast on the day of administration of the dose in order to mimic the study being conducted in the fasting state. A single dose of 500mg of either the reference product (Ciprinol) or the test product (Ciflox) was administered

to the subjects with about 150ml of water after the collection of the initial urine sample. The doses were administered between the hours of 8.00-9.00am on each day of the study. A meal of rice was served during lunch (1.00pm) to all subjects on the study days.

After a wash out period of at least seven (7) days, a second dose of either the test product(Ciflox) or reference product(Ciprinol) was given to the subjects, depending on what product was previously given (a subject who had taken Ciflox was given Ciprinol after the wash out period or vice versa).

2.3.5.4 Sample Collection and Disposal

An initial sample of urine was collected from subjects prior to the administration of the doses, i.e. at time t=0. After administration of the dose, further urine samples were collected over a seven (7) hour period at the following time points; 1.00hr, 1.50hrs, 2.00hrs, 2.50hrs, 3.00hrs, 4.00hrs, 5.00hrs, 6.00hrs and 7.00hrs. After the collection of urine at each time point, subjects were made to take about 200ml of water. Samples were collected into labeled 100ml amber coloured glass bottles and capped with plastic caps and placed in the refrigerator. The labels bore the code of the subject and time of urine collection.

At the end of analysis urine samples were poured into the WC and flushed. The bottles were crushed and discarded in the refuse bin.

2.3.5.5 Analysis and Quantification of Ciprofloxacin in Urine of Subjects

The quantification of Ciprofloxacin in the urine samples was done on the day of collection of urine samples. 0.5ml of the urine sample collected at each time point was pipetted into a 50ml volumetric flask (100 fold dilution of the urine as prepared

for the validation samples). 1ml of the stock Paracetamol internal standard solution (i.e. 10mg/ml Paracetamol) was added.

The solution was made up to volume with the orthophosphoric acid diluent, well mixed and transferred into labeled amber glass bottles and capped with plastic caps. The solutions were then filtered through a 0.45µ syringe filter and subjected to chromatographic analysis as described for the validation;

Column- Waters Xbridge C18, 150x4.6mm, 5µ

Mobile Phase: 10% Acetonitrile: 90%(0.245%w/w)H_3PO_4 adjusted to pH 3.0 with triethylamine.

Column Temperature; 40^0C

Flow Rate: 1ml/min

Detection wavelength: 278nm

Injection Volume: 20µl

From the chromatograms obtained for the Ciprofloxacin and Paracetamol internal standard, the Ciprofloxacin-Paracetamol peak area ratios were calculated at each time point. The concentrations of Ciprofloxacin excreted in the urine samples over time were then deduced from the equation of the calibration curve. The concentrations obtained were then multiplied by a dilution factor of 100 to get the concentration of Ciprofloxacin in the original undiluted (excreted) urine sample.

2.3.5.6 Data Treatment and Determination of Bioequivalence

The Ciprofloxacin concentration –time data was fed into an Excel Spreadsheet. The cumulative amount of Ciprofloxacin excreted and the excretory rates were calculated for each subject. The maximum excretory rate and time to the maximum excretory rates were then deduced.

A mean excretory rate time curve was also plotted. However the area under the respective excretory rate-time curve for each subject was calculated using the Trapezoidal rule.

The key pharmacokinetic parameters; Cumulative amount excreted (U_{0-7}), Maximum excretory rate (R_{max}) and the Area under the excretory rate time curve (Ae_T) for all subjects were then logarithmically transformed using the formula In(pharmacokinetic parameter).

The F and t- tests of significance were performed on the sets of data for the test and reference product for each pharmacokinetic parameter. ANOVA was also carried out to quantify some of the variations in the study. The error mean sum of squares (MSE) was obtained for each of the pharmacokinetic parameters and employed in the determination of the 90% confidence interval of the ratio of the test and reference products as given by the formula;

90% CI = $Exp^{[U_T-U_R)\pm S\sqrt{2/nt(0.05)v}]}$ where

U_T=Mean of the bioequivalent parameter calculated for the test product

U_R= Mean of the bioequivalent parameter calculated for the reference product

n = Number of subjects per period

S= √MSE, MSE= Error Mean Sum of Square (from ANOVA analysis)

t(0.05)v= Critical value of t at α= 0.05

v = Number of degrees of freedom associated with the MSE. The lower and upper confidence intervals so obtained were then transformed into percentages.

CHAPTER THREE

RESULTS AND CALCULATIONS

3.1 ASSAYS OF TABLETS

$$\text{Percentage (\%) Ciprofloxacin in Ciprinol} = \frac{\text{Average area of Ciprinol peak}}{\text{Average area of standard solution peak}} \times 100$$

$$= \frac{2497.0151}{2551.9554} \times 100$$

= 98.3085%

Same was done for Ciflox

Table 3: Area under Ciprinol and Ciflox Assay Chromatograms and Assay of Tablets.

Area under curve		
Ciprofloxacin std Solution	**Ciprinol**	**Ciflox**
2521.4273	2572.0210	2542.6738
2520.2908	2414.5415	2543.6965
2549.4023	2410.3682	2523.7759
2546.7090	2504.5371	2561.1704
2550.4553	2583.6079	2588.4602
Mean area =2537.6569	Mean Area =2497.0151	Mean Area=2551.9554
Assay: % Ciprofloxacin	98.3985	100.5635

3.2 DISSOLUTION OF TABLETS

% Content of Ciprofloxacin dissolved in vessel 1 (for Ciprinol) =

absorbance of solution of Ciprinol in vessel 1 x 100
absorbance of standard solution of Ciprofloxacin

= 0.7168 x 100
0.7269

= 98.6105%

Same calculation was done for the other five vessels for Ciprinol and for Ciflox

Table 4: Absorbances and Percentage Dissolution Values Obtained for Ciprinol

Sample designation	Absorbance 1	Absorbance 2	Mean Absorbance	% Dissolution
Standard Ciprofloxacin Solution	0.7279	0.7259	0.7269	-
Vessel 1	0.718	0.7156	0.7168	98.6105
Vessel 2	0.7194	0.7185	0.71895	98.9063
Vessel 3	0.7429	0.7431	0.743	102.2149
Vessel 4	0.7255	0.7254	0.72545	99.8005
Vessel 5	0.7448	0.7442	0.7445	102.4212
Vessel 6	0.7175	0.7409	0.7292	100.3164

Table 5: Absorbances and Percentage Dissolution Values Obtained for Ciflox

Sample designation	Absorbance 1	Absorbance 2	Mean Absorbance	% Dissolution
Standard Ciprofloxacin Solution	0.7279	0.7323	0.7301	-
Vessel 1	0.7312	0.7311	0.7312	100.1438
Vessel 2	0.6965	0.6966	0.6966	95.4047
Vessel 3	0.75	0.7507	0.7504	102.7736
Vessel 4	0.6916	0.6912	0.6914	94.6994
Vessel 5	0.7386	0.7384	0.7385	101.1505
Vessel 6	0.7008	0.7002	0.7005	95.9458

3.3 BIOANALYTICAL METHOD VALIDATION RESULTS

3.3.1 Accuracy of Method

Accuracy (% Recovery) at 1µg/ml =

$$\frac{\text{Mean Ciprofloxacin-Paracetamol peak area ratio at 1µg/ml in Urine sample}}{\text{Mean Ciprofloxacin-Paracetamol peak area ratio at 1µg/ml in standard solution (diluent)}} \times 100$$

$$= \frac{0.0501}{0.0487} \times 100 = 102.8747\%$$

Same was done to calculate the accuracy of the method at 10µg/ml and 20µg/ml

Table 6: Accuracy

Concentration of Ciprofloxacin	Mean Ciprofloxacin-Paracetamol peak are ratio in urine sample	Mean Ciprofloxacin-Paracetamol peak are ratio in mobile phase	Accuracy(%Recovery)
1µg/ml	0.0501	0.0487	102.8747
10ug/ml	0.4690	0.4456	105.2513
20µg/ml	0.9233	0.8986	102.7487

3.3.2 Precision

Precision was established by calculation of the coefficient of variation(RSD) between the set of peak area ratios obtained at each of the three concentrations stated below using the appropriate formula in Excel spreadsheet;

Table 7: Repeatability

Concentration of Ciprofloxacin in urine sample	Peak area ratio	Mean	Standard Deviation	Relative Standard Deviation (RSD)
1µg/ml	0.0511	0.0501	0.0009	1.7182
	0.0497			
	0.0495			
10µg/ml	0.4716	0.4690	0.0023	0.4947
	0.4684			
	0.4671			
20µg/ml	0.9145	0.9233	0.0155	1.6758
	0.9412			
	0.9142			

Table 8: Stability of Ciprofloxacin in Urine Sample

Day of analysis	Peak area ratio	Mean	Standard Deviation	Relative Standard Deviation (RSD)
First day	3.4888	3.4696	0.0486	1.4021
	3.5393			
	3.5061			
Fifth day (after 5 days of storage)	3.4294			
	3.4291			
	3.4246			

3.3.3 Linearity

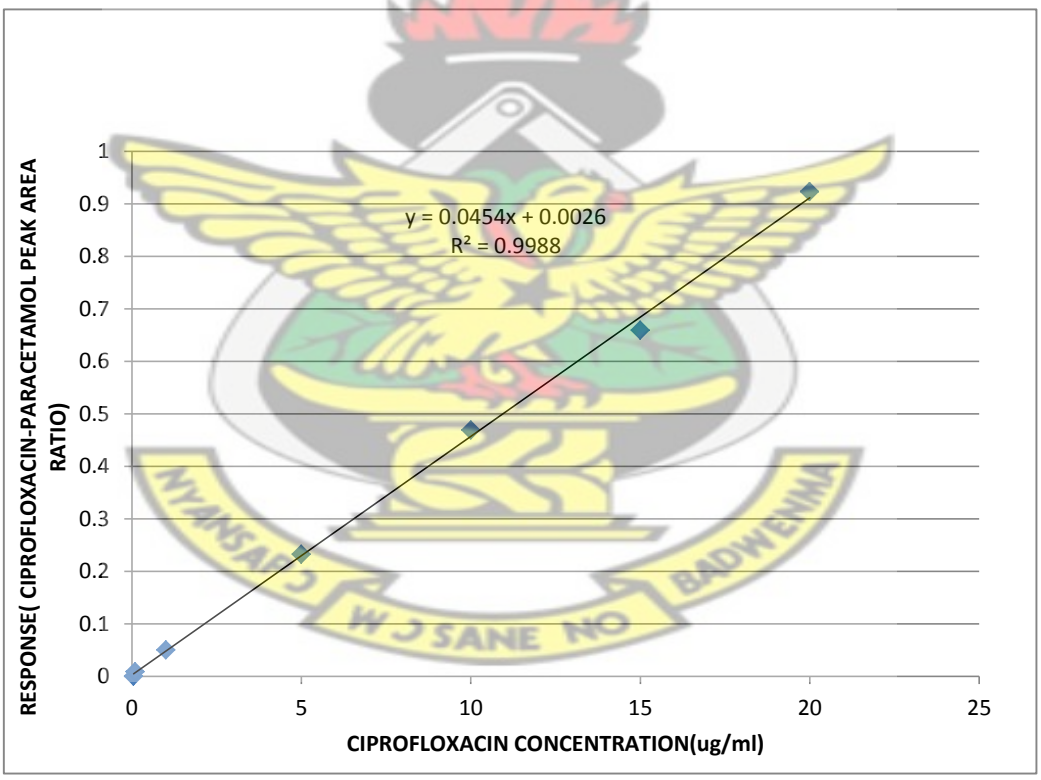

Figure 2: Ciprofloxacin - Paracetamol Peak area ratio against concentration of Ciprofloxacin in Urine Calibration Curve

Table 9: Calibration Curve Parameters

Parameter	Value
Range	0.05µg/ml - 20µg/ml
Slope	0.0454
Intercept	0.0026
R^2	0.9988

3.3.4 Limit of Detection (LOD) and Limit of Quantification (LOQ)

$$LOD = \frac{3.3\sigma}{S}$$

Where,

σ = the standard deviation of the regression line

S = the slope of the calibration curve

From calibration curve, σ = 0.01369

Slope= 0.0454

$$LOD = \frac{3.3 \times 0.01369}{0.0454}$$

= 0.9951µg/ml

$$LOQ = \frac{10\ \sigma}{S}$$

Where,

σ = the standard deviation of the regression line

S = the slope of the calibration curve

From calibration curve, σ = 0.01369

Slope= 0.0454

LOQ= <u>10 x 0.01369</u>

0.0454

= 3.0154µg/ml

3.3.5 Specificity

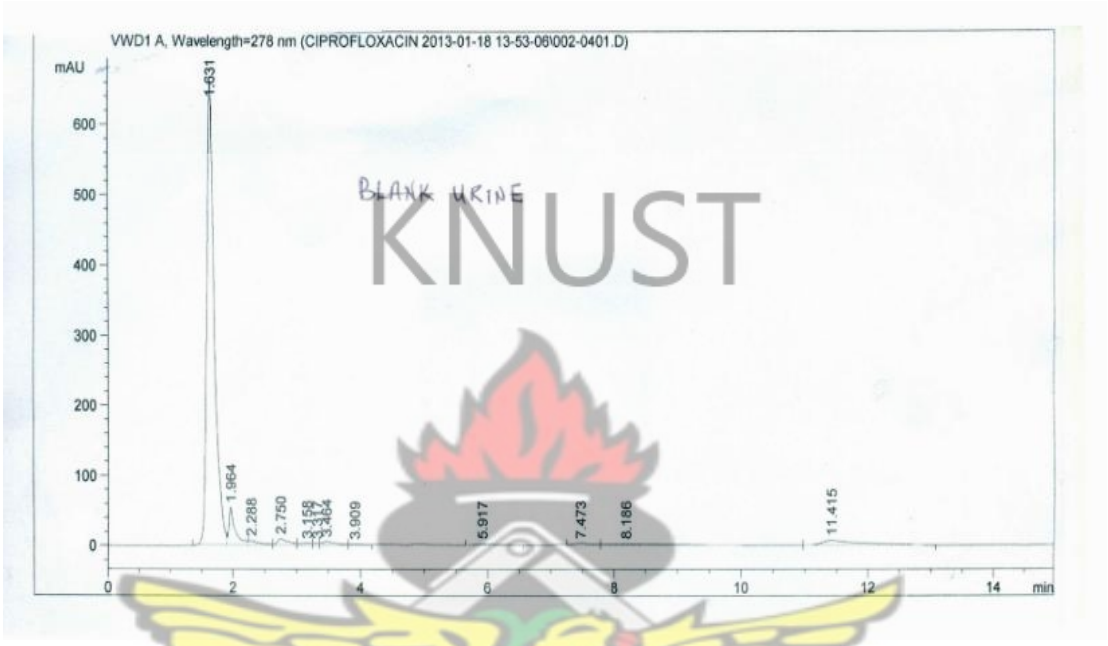

Figure 3: Chromatogram for Blank Urine Sample

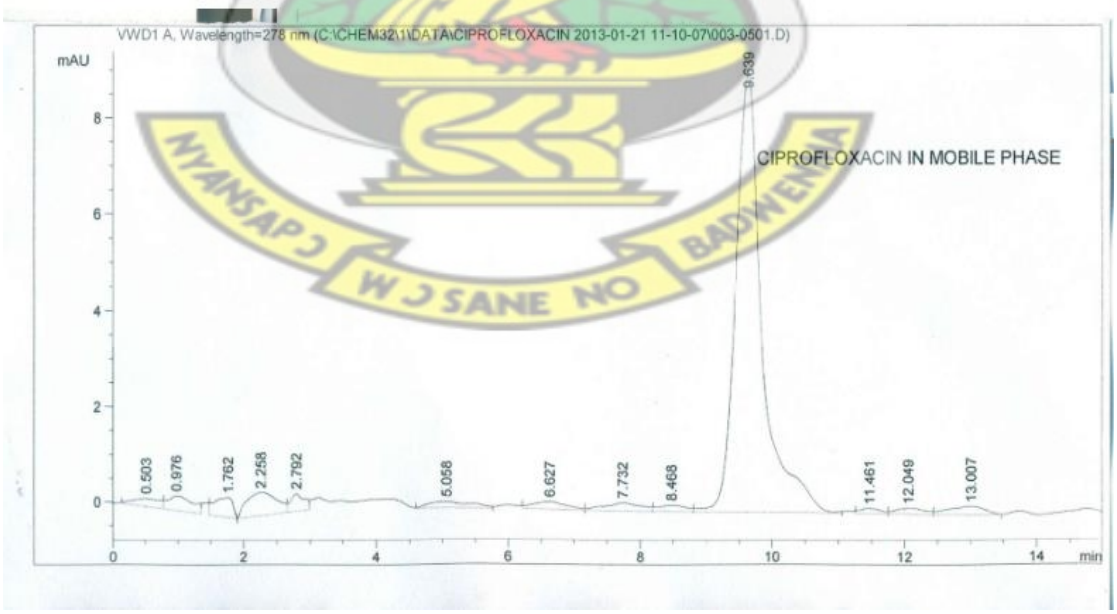

Figure 4: Chromatogram of Ciprofloxacin in Mobile Phase

Figure 5: Chromatogram for Paracetamol in Mobile Phase

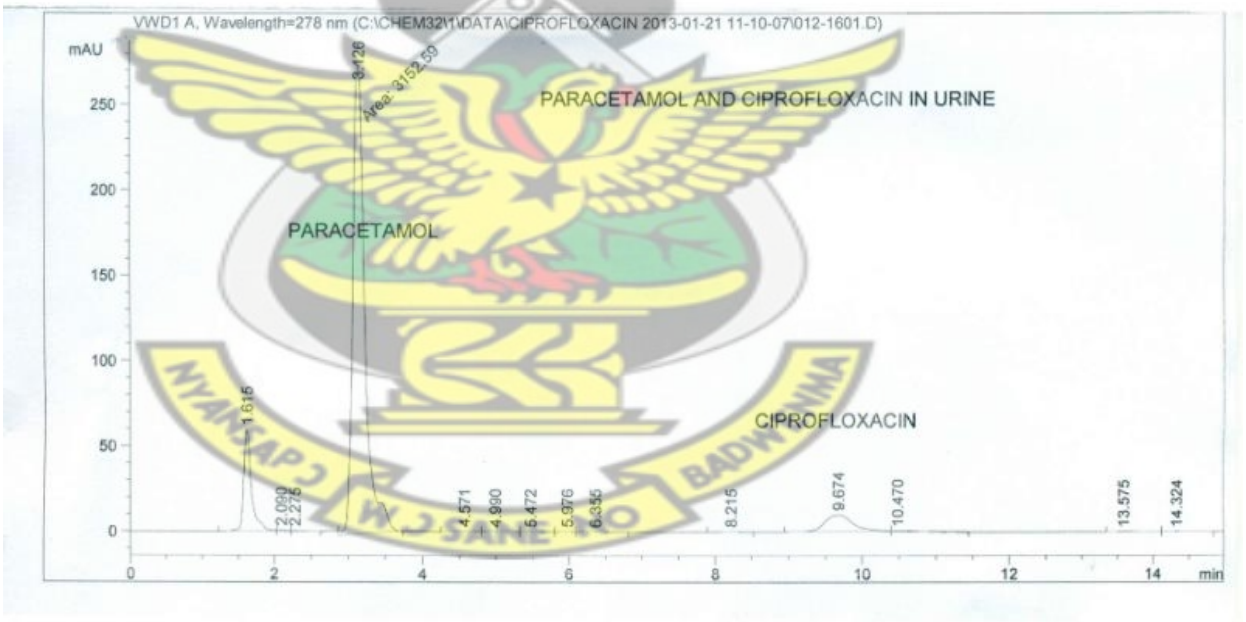

Figure 6: Chromatogram for Paracetamol and Ciprofloxacin in Urine

Table 10: Parameters of Chromatograms of Analytes

Component	Retention time	Symmetry
Paracetamol	3.1±0.3s	0.72
Ciprofloxacin	9.6±0.3s	0.83

No interfering peaks occurred at the retention times of both Ciprofloxacin and Paracetamol

3.4 RESULTS OF DETERMINATION OF BIOEQUIVALENCE PHARMACOKINETIC PARAMETERS

3.4.1 Statistical Evaluation of Cumulative Amount of Ciprofloxacin Excreted

Table 11: Cumulative Amount of Ciprofloxacin Excreted

SUBJECT	Cumulative amount excreted (U_{0-7})/µg/ml		Log transformed data In(U_{0-7})	
	CIPRINOL	CIFLOX	CIPRINOL	CIFLOX
A	8290.9922	6685.8848	9.0246	8.8093
B	3933.7426	1052.3746	8.2788	6.9601
C	4580.2256	2139.0214	8.4310	7.6695
D	856.1749	610.1309	6.7537	6.4148
E	2648.7174	2066.1205	7.8833	7.6348
F	894.9002	1946.7579	6.7979	7.5753
G	3433.9955	2910.9651	8.1429	7.9777
H	7872.6534	7972.1303	8.9728	8.9853
I	2936.7664	1696.4868	7.9865	7.4377
J	1287.3836	834.6408	7.1617	6.7282
MEAN	3673.5553	2791.4513	-	-

Table 12: t- Test for Comparison of Means for Cumulative Amount of Ciprofloxacin Excreted

	CIPRINOL	CIFLOX
Mean	7.9433	7.6193
Variance	0.6625	0.6809
Observations	10	10
Pearson Correlation	0.7840	
Hypothesized Mean Difference	0	
Df	9	
t Stat	1.9019	
P(T<=t) one-tail	0.0448	
t Critical one-tail	1.8331	
P(T<=t) two-tail	0.0896	
t Critical two-tail	2.2622	

Table 13: F- Test for the Comparison of Variances for the Cumulative Amount of Ciprofloxacin Excreted

	CIFLOX	CIPRINOL
Mean	7.6193	7.9433
Variance	0.6809	0.6625
Observations	10	10
Df	9	9
F	1.0277	
P(F<=f) one-tail	0.4841	
F Critical one-tail	3.1789	
F Critical two tail	4.0260	

Table 14: ANOVA of the Cumulative Amount of Ciprofloxacin Excreted

SUMMARY	Count	Sum	Average	Variance
SUBJECT A	2	17.8349	8.9169	0.0232
SUBJECT B	2	15.2389	7.6194	0.8696
SUBJECT C	2	16.1005	8.0503	0.2900
SUBJECT D	2	13.1685	6.5843	0.0574
SUBJECT E	2	15.5181	7.7590	0.0309
SUBJECT F	2	14.3732	7.1866	0.3021
SUBJECT G	2	16.1206	8.0603	0.0137
SUBJECT H	2	17.9581	8.9790	7.89E-05
SUBJECT I	2	15.4242	7.7121	0.1506
SUBJECT J	2	13.8899	6.9449	0.0939
CIPRINOL	10	79.4332	7.9433	0.6625
CIFLOX	10	76.1927	7.6193	0.6809

ANOVA						
Source of Variation	SS	Df	MS	F	P-value	F crit
Rows (Intra Subject)	10.7840	9	1.1982	8.2549	0.0021	3.1789
Columns (Inter Subject)	0.5250	1	0.5250	3.6171	0.0896	5.1174
Error	1.3064	9	0.1452	--	-	-
Total	12.6155	19	-	-	-	-

Determination of 90% CI for the Ratio of Cumulative Amount of Ciprofloxacin Excreted

90% CI for the ratio of cumulative amount of Ciprofloxacin excreted =

$\text{Exp}^{[U_T-U_R)\pm S\sqrt{2/nt(0.05)v}]}$

U_T=Mean cumulative amount of Ciflox excreted (i.e. Test Product) = 7.6193

U_R=Mean cumulative amount of Ciprinol excreted (Reference Product) = 7.9433

n = Number of subjects per period

S= √MSE = 0.3810

MSE= Error Mean Sum of Square (from ANOVA analysis above) = 0.1452

t(0.05)v= Critical value of t at α= 0.05= 1.833

v = Number of degrees of freedom associated with the MSE= 9

$U_T - U_R$ = -0.3240

n = 10

nt = 18.33

2/nt = 0.1091

√2/nt = 0.3303

S * √2/nt= 0.1258

Lower Limit = $Exp^{(-0.3240- 0.1258)}$

= $Exp^{(-0.4499)}$

= 0.6377 = 63.77%

Upper Limit = $Exp^{(-0.3240+ 0.1258)}$

= Exp(-0.1982)

= 0.8202 = 82.02%

90% CI of the ratio of the Cummulative Amount of Ciflox to Ciprinol=

63.77 – 82.02%

3.4.2 Statistical Evaluation of Maximum Excretory Rates

Table 15: Maximum Excretory Rate of Ciprofloxacin

SUBJECT	MAXIMUM EXCRETORY RATE(ug/ml/hr)- R_{max}		Log Transformed Data (In R_{max})	
	CIPRINOL	CIFLOX	CIPRINOL	CIFLOX
A	1914.8729	2006.6029	7.5588	7.6056
B	1571.3355	364.4767	7.3610	5.8995
C	1358.1872	593.7174	7.2152	6.3876
D	167.5442	461.8004	5.1222	6.1362
E	2873.6635	982.9305	7.9648	6.8918
F	159.0816	592.3503	5.0703	6.3852
G	1446.9294	298.8443	7.2785	5.7009
H	2014.5577	3691.9851	7.6095	8.2154
I	554.6717	229.0501	6.3195	5.4349
J	568.3136	414.8386	6.3438	6.0290

Table 16: t - Test for Comparison of Means for Maximum Excretory Rate of Ciprofloxacin

	CIPRINOL	CIFLOX
Mean	6.7844	6.4686
Variance	1.0608	0.7580
Observations	10	10
Pearson Correlation	0.4273	
Hypothesized Mean Difference	0	
Df	9	
t Stat	0.9733	
P(T<=t) one-tail	0.1779	
t Critical one-tail	1.8331	
P(T<=t) two-tail	0.3558	
t Critical two-tail	2.2622	

Table 17: F- Test for the Comparison of Variances for the Maximum Excretory Rate for Ciprofloxacin

	CIPRINOL	CIFLOX
Mean	6.7844	6.4686
Variance	1.0608	0.7580
Observations	10	10
Df	9	9
F	1.3995	
P(F<=f) one-tail	0.3123	
F Critical one-tail	3.1789	
F Critical two tail	4.0620	

Table 18: ANOVA of Maximum Excretory Rates

SUMMARY	Count	Sum	Average	Variance
SUBJECT A	2	15.1643	7.5822	0.0011
SUBJECT B	2	13.2605	6.6303	1.0680
SUBJECT C	2	13.6028	6.8014	0.3425
SUBJECT D	2	11.2584	5.6292	0.5142
SUBJECT E	2	14.8566	7.4283	0.5757
SUBJECT F	2	11.4556	5.7278	0.8645
SUBJECT G	2	12.9795	6.4897	1.2443
SUBJECT H	2	15.8249	7.9125	0.1835
SUBJECT I	2	11.7544	5.8772	0.3913
SUBJECT J	2	12.3728	6.1864	0.0496
CIPRINOL	10	67.8436	6.7844	1.0608
CIFLOX	10	64.6862	6.4686	0.7580

ANOVA

Source of Variation	SS	Df	MS	F	P-value	F crit
Rows(Intra subject)	11.6331	9.0000	1.2926	2.4562	0.0984	3.1789
Columns(Inter Subject)	0.4985	1.0000	0.4985	0.9473	0.3558	5.1174
Error	4.7361	9.0000	0.5262			
Total	16.8677	19.0000				

Determination of 90% CI for the Ratio of the Maximum Excretory Rate of Ciprofloxacin Excreted

90% CI for the ratio of Max . Excretory rate = $Exp^{[U_T-U_R)\pm S\sqrt{2/nt(0.05)v}]}$

U_T=Mean maximum excretory rate of Ciflox (i.e. Test Product) = 6.4686

U_R=Mean Maximum excretory rate of Ciprinol (Reference Product) = 6.7844

n = Number of subjects per period

S= √MSE = 0.7254

MSE= Error Mean Sum of Square (from ANOVA analysis above) = 0.5262

t(0.05)v= Critical value of t at α= 0.05= 1.833

v = Number of degrees of freedom associated with the MSE= 9

$U_T - U_R$ = -0.3157

n = 10

nt = 18.33

2/nt = 0.1091

√2/nt = 0.3303

S * √2/nt= 0.2396

Lower Limit = Exp(-0.3157- 0.2396)

= Exp(-0.5553)

= 0.5739= 57.39%

Upper Limit = Exp(-0.3157+ 0.2396)

= Exp(-0.0761)

= 0.9267 = 92.67%

90% CI of the ratio of the maximum excretory rates of Ciflox to Ciprinol=

57.39 – 92.67%

3.4.3 Statistical Evaluation of Area Under Excretory Rate –Time Curve

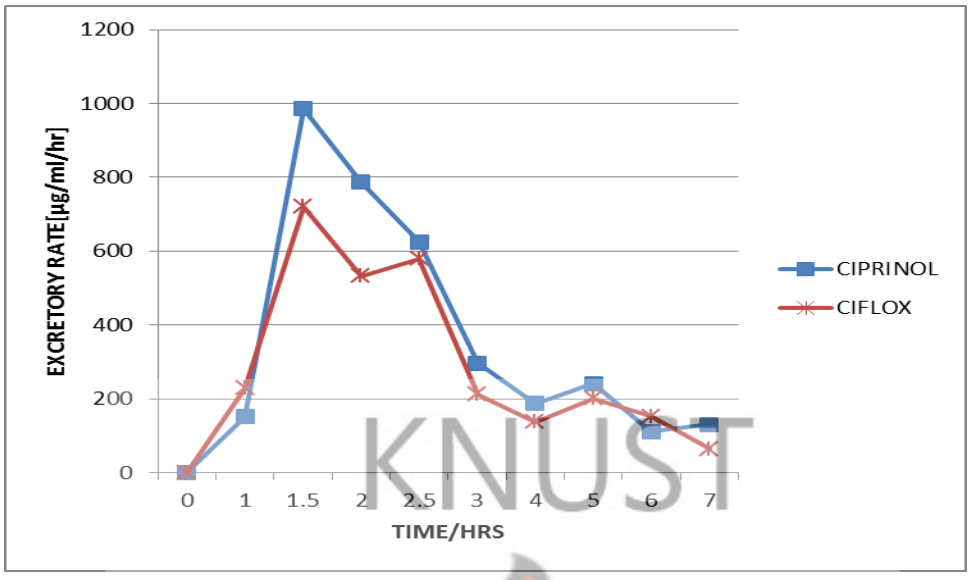

Figure 7: Graph of Mean Ciprofloxacin Excretory Rate - Time Curve

Table 19: Area Under Excretory Rate - Time Curve

SUBJECT	Area under curve $Ae_{(0-7)}$		Log transformed data (In ($Ae_{(0-7)}$))	
	CIPRINOL	CIFLOX	CIPRINOL	CIFLOX
A	4059.9508	4623.6967	8.3104	8.4405
B	2818.2186	933.5006	7.9453	6.8402
C	2675.7802	955.4725	7.8934	6.8634
D	282.2609	545.2524	5.6438	6.3024
E	3219.9962	959.2772	8.0786	6.8674
F	301.4980	1839.4204	5.7098	7.5186
G	1905.1938	644.8728	7.5537	6.4702
H	3748.1525	5595.4679	8.2305	8.6313
I	1174.2935	592.3994	7.0697	6.3853
J	1202.8134	1011.6325	7.0937	6.9206
MEAN	2138.8157	1770.0992	-	-

Table 20: t- Test for the Comparison of the Means of the Area under the Excretory Rate - Time

	CIPRINOL	CIFLOX
Mean	7.3529	7.1240
Variance	0.9628	0.6738
Observations	10	10
Pearson Correlation	0.4104	
Hypothesized Mean Difference	0	
Df	9	
t Stat	0.7329	
P(T<=t) one-tail	0.2411	
t Critical one-tail	1.8331	
P(T<=t) two-tail	0.4823	
t Critical two-tail	2.2622	

Table 21: F- Test for the Comparison of the Variance of the Area under the Excretory Rate - Time Curve

	CIPRINOL	CIFLOX
Mean	7.3529	7.1240
Variance	0.9628	0.6738
Observations	10	10
Df	9	9
F	1.4288	
P(F<=f) one-tail	0.3018	
F Critical one-tail	3.1789	
F Critical two tail	4.0260	

Table 22: ANOVA of the Area under the Excretory Rate - Time Curve

SUMMARY	Count	Sum	Average	Variance
SUBJECT A	2	16.7509	8.3754	0.0085
SUBJECT B	2	14.7855	7.3927	0.6106
SUBJECT C	2	14.7569	7.3784	0.5304
SUBJECT D	2	11.9462	5.9731	0.2168
SUBJECT E	2	14.9460	7.4730	0.7335
SUBJECT F	2	13.2284	6.6142	1.6358
SUBJECT G	2	14.0239	7.0120	0.5870
SUBJECT H	2	16.8618	8.4309	0.0803
SUBJECT I	2	13.4550	6.7275	0.2342
SUBJECT J	2	14.0143	7.0071	0.0150
CIPRINOL	10	73.5290	7.3529	0.9628
CIFLOX	10	71.2398	7.1240	0.6738

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows (Intra Subject)	10.3394	9	1.1488	2.3552	0.1090	3.1789
Columns (Inter Subject)	0.2620	1	0.2620	0.5372	0.4823	5.1174
Error	4.3901	9	0.4878			
Total	**14.9915**	**19**				

Determination of 90% CI for the Ratio of the Area Under theExcretory Rate-Time Curve of Ciprinol and Ciflox

90% CI for the ratio of the area under the Excretory rate-time curve =

$Exp^{[U_T-U_R)\pm S\sqrt{2/nt(0.05)v}]}$

U_T=Mean area under curve of Ciflox (i.e. Test Product) = 7.1240

U_R=Mean area under curve of Ciprinol (Reference Product) = 7.3529

n = Number of subjects per period=10

S= √MSE = 0.6984

MSE= Error Mean Sum of Square (from ANOVA analysis) = 0.4878

t(0.05)v= Critical value of t at α= 0.05= 1.833

v = Number of degrees of freedom associated with the MSE= 9

$U_T - U_R$ = -0.2289

nt = 18.33

2/nt = 0.1091

√2/nt = 0.3303

S * √2/nt= 0.2307

Lower Limit = Exp(-0.2289- 0.2307)

= Exp(-0.4596)

= 0.6315= 63.15%

Upper Limit = Exp(-0.2289+ 0.2307)

= Exp(0.0018)

= 1.0018 = 100.18%

90% CI of the ratio of the area under the excretory rate-time curve of Ciflox to Ciprino l= 63.15 – 100.18%

3.4.4 Statistical Evaluation of Time to Maximum Excretory Rate

Table 23: Time to Maximum Excretory Rate

SUBJECT	TIME/Hrs (Tmax)	
	CIPRINOL	CIFLOX
A	2	1.5
B	1.5	5
C	2	2.5
D	2.5	2
E	1.5	1.5
F	1.5	3
G	1.5	1
H	1.5	1.5
I	1.5	1.5
J	3	5

Table 24: t- Test for the Mean of the Time to Maximum Excretory Rate

	CIFLOX	CIPRINOL
Mean	2.45	1.85
Variance	2.1361	0.2806
Observations	10	10
Pearson Correlation	0.4198	
Hypothesized Mean Difference	0	
Df	9	
t Stat	1.4275	
P(T<=t) one-tail	0.0936	
t Critical one-tail	1.8331	
P(T<=t) two-tail	0.1872	
t Critical two-tail	2.2622	

Table 25: F- Test for the Comparison of the Variances of the Time to Maximum Excretory Rate

	CIFLOX	CIPRINOL
Mean	2.45	1.85
Variance	2.1361	0.2806
Observations	10	10
Df	9	9
F	7.6139	
P(F<=f) one-tail	0.0029	
F Critical one-tail	3.1789	
F Critical two tail	4.0260	

CHAPTER FOUR

DISCUSSION, CONCLUSION AND RECOMMENDATION

4.1 DISCUSSION

4.1.1 Reference Samples

Reference samples whether primary or secondary/pure samples are of critical essence in quantitative analysis or determinations. The quantity of analyte determined in any analysis in which these reference samples are used depends to a large extent on the quality of these reference samples. It is therefore important for the reference samples to be of the desired quality(purity). The quantification of Ciprofloxacin in the urine samples was done employing Ciprofloxacin Hydrochloride pure sample supplied by Zhejiang Xinhua Pharmaceutical Co. Ltd, China with a purity of 100.8% and Paracetamol pure sample supplied by Hebei Jiheng (Group) Pharmaceutical Co. Ltd., China with a purity of 99.5%. The pure samples used complied with USP specifications.The acceptance criteria for the purity are; 98-102% (on anhydrous basis) for Ciprofloxacin and 99 – 101% for Paracetamol.

4.1.2 Assay

The assay of the reference and test products describes the percentage content of Ciprofloxacin present in the tablets. Ciprinol (reference product) had a percentage content of 98.40% and Ciflox (test product) had an assay of 100.56%. Both tablets comply with the USP assay specification of 90-110%. The difference in the assay values for the two batches of products used was 2.16% and this difference is less than the limit of not more than 5% difference in assay of the reference and test products allowed for batches of products used in bioequivalence studies (bio batches). Hence the two batches of the products can conveniently be employed in the study.

4.1.3 Dissolution

Dissolution testing of solid oral dosage forms is an in-vitro test carried out to demonstrate the extent of absorption of drug products on administration. Together with the assay, they form two major physiochemical tests carried out to assess the quality of drug products. As a requirement for bioequivalence study, the products used must pass the test for dissolution as prescribed by Compendia standards or non compendia validated method. The dissolution value obtained for Ciprinol(reference product) was 98.61% - 102.42% while Ciflox (test product) had a dissolution value of 94.70%- 102.77%. The dissolution values obtained for both products conform to the USP specification for dissolution of Ciprofloxacin which states that not less than 80% of the labeled amount of Ciprofloxacin is dissolved in 30minutes.

4.1.4 Bio -analytical Method Validation Results

4.1.4.1 Accuracy

The accuracy of the method at the three different concentrations of 1µg/ml, 10µg/ml and 20µg/ml of Ciprofloxacin was 102.85%, 105.26% and 102.75% respectively. The values obtained for the accuracy within the range of concentrations used for the study fell within the acceptance criteria of 85-115% (i.e. 15% of the nominal value) of accuracy for bio-analytical methods.

4.1.4.2 Precision

4.1.4.2.1 Repeatability

The relative standard deviation (RSD) obtained for the set of results at the test concentrations of 1µg/ml, 10µg/ml and 20µg/ml of Ciprofloxacin were 1.71%, 0.49% and 1.68%. All the RSDs met the RSD requirement of ±15% between sets of results obtained at the same concentration.

4.1.4.2.2 Stability Ciprofloxacin in Urine

The RSD between the sets of results obtained on the first day of analysis and on the fifth day (after storage in the refrigerator for 5 days) was 1.40% which conforms to the acceptance criteria of ±15%. Hence storage as described (in a refrigerator) for five days did not affect the stability of Ciprofloxacin in the urine samples collected.

4.1.4.3 Linearity, LOD and LOQ

Linearity was observed with concentrations of Ciprofloxacin in the range of 0.05µg/ml- 20µg/ml. An R^2 (Coefficient of determination) of 0.9988 was obtained for the calibration curve. The high value of the R^2 indicates a strong linear (proportional) relationship between the concentrations of Ciprofloxacin in the range and the ratio of the peak area of Ciprofloxacin to Paracetamol. The Limit of detection (LOD) and Limit of Quantification (LOQ) calculated from the standard deviation of the calibration curve were 0.9951µg/ml and 3.0154µg/ml respectively.

4.1.4.4 Specificity

Specificity is achieved when there are no interfering peaks at the retention times of analytes or in bio-analytical method validation, when the area of any interfering peak is less than 20% of the total area obtained at the retention time. From the chromatogram obtained for the blank urine sample, no peak was observed at the retention times of 3.1mins and 9.6mins for Paracetamol and Ciprofloxacin respectively. However, some noise was recorded in the region of 3.1minutes but this did not affect the quantification of the Paracetamol internal standard.

4.1.5 Statistical Analysis of Urinary Excretory Values of Ciprofloxacin

4.1.5.1 Cumulative Amount of Ciprofloxacin Excreted

The mean cumulative concentrations of Ciprofloxacin excreted by subjects over the seven hour period for Ciprinol and Ciflox were 3673.5552µg/ml and 2791.4513µg/ml respectively.

The mean of the log transformed data of the cumulative amounts of Ciprofloxacin excreted was 7.9433 for Ciprinol and 7.6193 for Ciflox.

A t-test tests the hypothesis of whether there is a significant statistical difference between the means of two sets of values. This is done by calculating the t-value for the sets of values and comparing the result to the t- values indicated in the t-statistical table (referred to as the t critical value). A decision of no significant statistical difference between the means is made when the calculated 't' value is less than the 't' critical value obtained from the t- table. The calculated 't' value for the means of the cumulative amount of ciprofloxacin excreted in urine for the two products was 1.9019 which is less than the t-critical value of 2.2622 for a two tail test.

Statistically, it can be said that there is no significant difference between the means of the logarithmically transformed cumulative amounts of Ciprofloxacin excreted in the urine on oral administration of Ciprinol and Ciflox.

An F test also compares the variances of different sets of data and tests the hypothesis of whether a significant statistical difference exists between the variances. The variances obtained for the sets of log transformed data for Ciprinol and Ciflox were 0.6625 and 0.6809 respectively. The F test yielded an F value of 1.0277 which is less than the F critical value of 4.0260. Statistically, there is no difference between the variances of the two sets of data.

The 90% Confidence Interval calculated for the ratio of the cumulative amount of Ciprofloxacin excreted in Ciflox to Ciprinol was 63.77 - 82.02%. The observed Confidence Interval was outside the bioequivalence acceptance criteria range of 80% - 125%.

4.1.5.2 Maximum Excretory Rate of Ciprofloxacin

The mean maximum excretory rate of Ciprofloxacin was 1216.5928µg/ml/hr for Ciprinol and 963.6596µg/ml/hr for Ciflox. The means of the logarithmically transformed data were 6.7567 for Ciprinol and 6.4686 for Ciflox. The calculated t value of 0.9733(between the two logarithmically transformed excretory values) was less than the t-critical value of 2.2622. Hence there is no statistical difference between the means obtained for the maximum excretory rates after the transformation. The calculated F value of 1.3995 was also less than the F critical value of 4.0260 and therefore no significant statistical difference was observed in the variances for the two sets of data. The 90% Confidence Interval calculated for the ratio of the maximum excretory rates of Ciprofloxacin excreted in Ciflox to Ciprinol was 57.39% - 92.67%. The observed Confidence Interval is outside the bioequivalence acceptance criteria range of 80% - 125%.

4.1.5.3 Area under the Excretory Rate- Time Curve

The mean area under the Ciprofloxacin excretory rate-time curve for Ciprinol was 2138.8157 and that for Ciflox was 1770.0992. The means for the logarithmically transformed areas under the curve were 7.3529 and 7.1240 for Ciprinol and Ciflox respectively. Statistically, no significant difference between the means of the logarithmically transformed areas under the curve exist since the calculated t value of 0.7329 was less that t-critical value of 2.2622. The F calculated value for the variances of the two sets of data stood at 1.4288 which is also less than the critical

value of 4.0260. This demonstrates the absence of significant statistical difference between the two variances of 0.9628(for Ciprinol) and 0.6738(for Ciflox).

The established 90% confidence interval for the ratio of the area under the curve for Ciflox to Ciprinol of 63.15% - 100.18% is outside the bioequivalence acceptance criteria range of 80% - 125%.

4.1.5.4 Time to Maximum Excretory Rate

An average time to maximum excretory rate of 1.85 hours and 2.45hours was observed for Ciprinol and Ciflox respectively. Whilst the calculated t value of this means indicates the absence of significant statistical difference (calculated t-value of 1.4275 is less than t-critical of 2.2622), the F test demonstrates a significant difference exists between the two variances of 0.3222 (for Ciprinol) and 2.1361(for Ciflox). The F value of 7.6139 is much greater than the F critical value of 4.0260

The 90%CI established for all three main pharmacokinetic bioequivalence parameters; 63.77% - 82.02% for the cumulative amount of ciprofloxacin excreted, 58.80% - 95.60% for the maximum excretory rate and 63.15% - 100.18% for the area under the mean excretory rate time curve are all outside the bioequivalence acceptance range of 80% - 125%.

A number of reasons may be adduced to the deviation of the parameters from the acceptance criteria;

(i) In this study it was not possible to confine subjects. In a controlled environment in bioequivalence study, subjects are normally confined or quarantined. In quarantine, food and fluid intake prior to and during the study, physical exercise etc are standardized and regulated to ensure all subjects are exposed to the same study

conditions. Unfortunately, in this study it was impossible to confine subjects and to strictly regulate the study conditions.

(ii) The number of subjects used in the study may not be large enough to demonstrate bioequivalence.

(iii) Though subjects were well educated on their role in the study, it is possible subjects urinated in between urine collection time points. This may have affected the concentrations of Ciprofloxacin recovered in the urine samples.

(iv) Any pre-existing conditions of health may have impacted on the excretion of Ciprofloxacin since medical examination could not be conducted to screen subjects prior to the study.

(v) Urine may not be the appropriate biological matrix to use for the determination of bioequivalence for Ciprofloxacin. There is evidence to show that a drug entity may demonstrate bioequivalence in one biological fluid but not the other.

4.2 CONCLUSION

Paracetamol and Ciprofloxacin pure samples used as reference samples comply with the United States Pharmacopoiea test requirements.

The assay of Ciprofloxacin in the reference and test products conforms to specifications. The difference in the content of Ciprofloxacin between the two products also met the stipulated criteria for the difference in assays of products employed in bioequivalence study.

Dissolution of the test and reference products complied with Pharmacopoeia requirements (USP).

The proposed HPLC method of analysis proved suitable for use in the quantification of Ciprofloxacin excreted in human urine.

No significant difference was statistically observed between the means of Ciprofloxacin excreted in the urine of subjects for both test and reference products as regards the cumulative amount, the maximum excretory rate, the area under the excretory rate –time curve and the time to maximum excretory rate of Ciprofloxacin.

Similarly, the variances for all the pharmacokinetic parameters for the test and reference products; cumulative amount, the maximum excretory rate, the area under the excretory rate –time curve of Ciprofloxacin excreted showed the absence of a significant statistical difference except for the time to the maximum excretory which showed significant difference in the variances for the test and reference products.

The test product (Ciflox) within the confines of this study is not bioequivalent to the reference product (Ciprinol) and thus may not be used as a therapeutic equivalent of the reference product.

4.3 RECOMMENDATION

(i) The study should be repeated in a well controlled or standardized environment

(ii) A number of subjects much higher than that used in this study should be employed in order to improve the power of the study.

(iii) Urine samples should be collected over durations of time e.g 0-2hrs, 2-4hrs etc instead of at specific time points as used in this study. With this urine collection schedule, the possibility of subjects urinating in-between specific collection time points which subsequently affects the concentration of drug excreted would be avoided. The various urine samples so collected over the duration are analysed and the mean concentrations used in the determination of bioequivalence.

(iv) Medical screening should be conducted for subjects prior to the study in order to assess the health status of subjects.

(v)A different biological matrix e.g. plasma could be used in place of urine in the determination of bioequivalence.

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APPENDIX

CERTIFICATES OF ANALYSIS (COA OF REFERENCE SAMPLES)

COA OF PARACETAMOL

IRREVOCABLE DOCUMENTARY CREDIT NO.
CL005DBDP11-0208

HEBEI JIHENG（GROUP）PHARMACEUTICAL CO.，LTD.

No. 368 Jianshe Street,Hengshui City,Hebei Province, 053000 P.R. China

CERTIFICATE OF ANALYSIS

Name of product	PARACETAMOL		
Lot No.	1101098	Report No.	01113
Quantity	6000kg	Test date	2011/01/19
Manufacture date	2011/01/14	Expiry date	2015/01/13
Quality standard	BP2007		

Test	Standards		Results
Characteristics	White,crystalline powder. Sparingly soluble in water, freely soluble in alcohol,very slightly soluble in methylene chloride.		*White,crystalline powder. Sparingly soluble in water, freely soluble in alcohol,very slightly soluble in methylene chloride.*
Identification	A:Melting point	168-172°C	*169.1-170.3°C*
	C:IR absorption	Complies	*Complies*
	B:UV absorption	Complies	*Complies*
	D,E:Chemical indentification	Complies	*Complies*
Related substance	Impurity J(chloroacetanilide)not more than 10 ppm		*3ppm*
	Impurity K(4-aminophenol)not more than 50 ppm		*7ppm*
	Impurity F(4-nitrophenol)not more than 0.05%		*Not detected*
	any other impurity not more than 0.05%		*0.02%*
	Total of other impurity not more than 0.1%		*0.02%*
Sulphated ash	Not more than 0.1%		*0.06%*
Heavy metals	Not more than 20ppm		*Less than 20ppm*
Loss on drying	Not more than 0.5%		0.1%
Assay	99.0-101.0% (dried substance)		99.5%
Organic volatile impurities	Residual content of acetic acid Not more than0.5%		0.1%
Conclusion :	Complies with BP2007		
Rechecker by	吴芳芳	Reported by	陈春荣
QC Manager	焦银蕾		

河北冀衡（集团）药业有限公司
HEBEI JIHENG (GROUP) PHARMACEUTICAL CO.,LTD.
王翠生

COA OF CIPROFLOXACIN

品名 Product Name	盐酸环丙沙星 CIPROFLOXACIN HCL	标准代号 Specification Code	QS-HA00U-05
批号 Batch/Lot No.	HB00N120478	生产日期 Mfg. Date	2012 年 04 月 13 日 Apr.13, 2012
包装规格 Pack size	25kg/桶 25kg/drum	失效日期 Expiry Date	2015 年 04 月 12 日 Apr.12, 2015
数量 Quantity	500kg	报告日期 Report Date	2012 年 04 月 17 日 Apr.17, 2012
检验依据 Standard	《美国药典》34 版/ *USP34*		

检验项目 TESTING ITEM	标准 SPECIFICATION	检验结果 RESULT
外观 Description	微黄色至淡黄色结晶 Faintly yellowish to light yellow crystals	微黄色结晶 Faintly yellowish crystals
【鉴别】 Identification	应符合规定 Meet the requirements	符合规定 Meets the requirements
【检查】Tests		
酸度 pH	3.0~4.5 Between 3.0 and 4.5	3.8
水分 Water	4.7%~6.7% Between 4.7% and 6.7%	5.3%
炽灼残渣 Residue on ignition	≤0.1% Not more than 0.1%	0.04%
重金属 Heavy metals	≤20ppm Not more than 20ppm	<20ppm
硫酸盐 Sulfate	≤0.04% Not more than 0.04%	<0.04%
氟喹啉酸 Fluoroquinolonic acid	≤0.2% Not more than 0.2%	<0.2%
【有关物质】Related compounds		
环丙沙星乙二胺类似物 Ciprofloxacin ethylenediamine analog	≤0.2% Not more than 0.2%	0.02%
其他单个杂质 Any other individual impurity	≤0.2% Not more than 0.2%	0.04%
总杂质 Total impurities	≤0.5% Not more than 0.5%	0.20%

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品名 Product Name	盐酸环丙沙星 CIPROFLOXACIN HCL	标准代号 Specification Code	QS-HA00U-05
批号 Batch/Lot No.	HB00N120478	生产日期 Mfg. Date	2012 年 04 月 13 日 Apr.13, 2012
包装规格 Pack size	25kg/桶 25kg/drum	失效日期 Expiry Date	2015 年 04 月 12 日 Apr.12, 2015
数量 Quantity	500kg	报告日期 Report Date	2012 年 04 月 17 日 Apr.17, 2012
检验依据 Standard	《美国药典》34 版/ *USP34*		

【含量】（以无水物计） Assay(on the anhydrous basis)	98.0%~102.0%($C_{17}H_{18}FN_3O_3$.HCl) Between 98.0% and 102.0%	100.8%
结论：符合《美国药典》34 版 Conclusion ： Meet the requirements of *USP34*		

Prepared by
QC Analyst

Reviewed and Approved by
QC Director

Approved and Certified by
QA Director

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Determination of Average Weight of Tablets and Calculation of Amount of Samples Used For Assay of Products

Ciprinol (Reference Product):

Weight of 20 film coated tablets = 16.0380g

Average weight of tablets = 0.8019g

0.5g Ciprofloxacin ≡0.8019g of tablet powder

0.100g Ciprofloxacin ≡ 0.1604g 0f tablet powder

Ciflox (Test Product):

Weight of 20 film coated tablets= 17.2860g

Average of tablets= 0.8643g

0.5g Ciprofloxacin ≡ 0.8643g of tablet powder

0.100g ≡ 0.1729g powder

Ciprofloxacin Reference Sample:

250g Ciprofloxacin ≡ 291.1g Ciprofloxacin Hydrochloride

0.05g Ciprofloxacin ≡ 0.0582g Ciprofloxacin Hydrochloride

Purity of Ciprofloxacin HCl Pure sample = 100.8%

Amount required= 100/100.8 x 0.0582g= 0.0577g

Paracetamol Reference Sample

Purity of Paracetamol pure sample= 99.5%

Amount required for preparation of stock Paracetamol internal standard solution=

100/99.5 x 1g= 1.0050g

Table 26 Peak Area and Peak Area Ratio Table-Bioanalytical Method Validation

Sample Designatiion	Peak Area-Paracetamol	Peak Area-Ciprofloxacin	Ciprofloxacin-Paracetamol Peak Area Ratio	Mean Peak Area Ratio
0.05ug/ml Cipro in Urine	3089.0056	0.0000	0.0000	0.0000
0.05µg/ml Cipro in Urine	3066.0640	0.0000	0.0000	
0.05µg/ml Cipro in Urine	3063.7400	0.0000	0.0000	
0.1µg/ml Cipro in Urine	3030.1355	25.0485	0.0081	0.0085
0.1µg/ml Cipro in Urine	3020.8721	27.0441	0.0090	
0.1µg/ml Cipro in Urine	3045.2817	22.5127	0.0074	
1µg/ml Cipro in Urine	3033.9016	154.9416	0.0511	0.0501
1µg/ml Cipro in Urine	3026.6582	150.4488	0.0497	
1µg/ml Cipro in Urine	3061.2544	151.4647	0.0495	
5µg/ml Cipro in Urine	3008.0339	697.4862	0.2319	0.2324
5µg/ml Cipro in Urine	3073.1521	693.0018	0.2255	
5µg/ml Cipro in Urine	3066.7642	735.1029	0.2397	
10µg/ml Cipro in Urine	3054.7322	1440.6096	0.4716	0.4690
10µg/ml Cipro in Urine	3057.6965	1432.2388	0.4684	
10µg/ml Cipro in Urine	3043.1943	1421.4369	0.4671	

Table 26 (Continued): Peak Area and Peak Area Ratio Table-Bioanalytical Method Validation

Sample Designatiion	Peak Area-Paracetamol	Peak Area-Ciprofloxacin	Ciprofloxacin-Paracetamol Peak Area Ratio	Mean Peak Area Ratio
15µg/ml Cipro in Urine	3036.3364	1995.4174	0.6572	0.6594
15µg/ml Cipro in Urine	3054.0107	2029.6643	0.6646	
15µg/ml Cipro in Urine	3094.2371	2031.1190	0.6564	
20µg/ml Cipro in Urine	3037.9946	2778.3748	0.9145	0.9233
20µg/ml Cipro in Urine	3021.3765	2843.5571	0.9411	
20µg/ml Cipro in Urine	3040.8762	2779.8372	0.9142	
1µg/ml Cipro in Mobile Phase	3011.9360	142.9620	0.0475	0.0487
1µg/ml Cipro in Mobile Phase	3014.3919	146.8466	0.0487	
1µg/ml Cipro in Mobile Phase	3045.5200	152.0119	0.0499	
10µg/ml Cipro in Mobile Phase	3016.1992	1292.0144	0.4284	0.4456
10µg/ml Cipro in Mobile Phase	3000.4890	1379.0853	0.4596	
10µg/ml Cipro in Mobile Phase	3081.8706	1383.1815	0.4488	
20µg/ml Cipro in Mobile Phase	3082.3335	2720.6228	0.8827	0.8986
20µg/ml Cipro in Mobile Phase	3064.5947	2820.8286	0.9205	
20µg/ml Cipro in Mobile Phase	3074.5645	2744.2214	0.8926	

Table 26 (Continued): Peak Area and Peak Area Ratio Table- Bioanalytical Method Validation

Sample Designatiion	Peak Area-Paracetamol	Peak Area-Ciprofloxacin	Ciprofloxacin-Paracetamol Peak Area Ratio	Mean Peak Area Ratio
Stability Sample				
50µg/ml Cipro in Urine(day 1)	3141.5638	10960.3000	3.4888	3.5114
50µg/ml Cipro in Urine(day 1)	3058.0208	10823.3000	3.5393	
50µg/ml Cipro in Urine(day 1)	3135.0948	10991.8000	3.5061	
50µg/ml Cipro in Urine(day 5)	3182.0611	10912.6000	3.4294	3.4277
50µg/ml Cipro in Urine(day 5)	3181.0144	10908.1000	3.4291	
50µg/ml Cipro in Urine(day 5)	3176.5732	10878.6000	3.4246	

Table 27 Concentration of Ciprofloxacin Excreted in the Urine of Subjects over Seven (7) Hours-µg/ml

SUBJECT	PRODUCT	0(Blank)	1hr	1.5hrs	2.0hrs	2.5hrs	3hrs	4hrs	5hrs	6hrs	7hrs
A	Paracetamol (IS)- Area	3066.1521	3109.7835	2976.0161	3078.1314	3009.0002	3103.3081	3226.5801	3285.7087	3061.7559	3190.8711
	Ciprinol- Area	0.0000	0.0000	577.3957	1935.1980	2883.2712	2926.9229	2222.1909	709.1508	271.6184	215.5995
	Area Ratio	0.0000	0.0000	0.1940	0.6287	0.9582	0.9432	0.6887	0.2158	0.0887	0.0676
	Test Concentration(µg/ml)	0.0000	0.0000	4.2162	13.7906	21.0488	20.7172	15.1126	4.6967	1.8968	1.4310
	Initial Concentation(µg/ml)	0.0000	0.0000	421.6218	1379.0583	2104.8804	2071.7229	1511.2643	469.6671	189.6769	143.1005
	Paracetamol(IS)-Area	4045.3005	4041.2390	4096.1851	3960.9849	4048.5913	3936.9907	3930.1802	4241.6216	4206.0625	4063.3987
	Ciflox- Area	0.0000	1175.7444	3057.5378	3838.0564	1361.7057	611.6550	1012.1447	524.0893	312.8251	427.8266
	Area Ratio	0.0000	0.2909	0.7464	0.9690	0.3363	0.1554	0.2575	0.1236	0.0744	0.1053
	Test Concentration(µg/ml)	0.0000	6.3510	16.3840	21.2856	7.3511	3.3648	5.6152	2.6643	1.5809	2.2618
	Initial Concentation(µg/ml)	0.0000	635.1027	1638.4041	2128.5577	735.1115	336.4781	561.5228	266.4289	158.0943	226.1847
B	Paracetamol (IS)- Area	3299.3264	3054.5925	3067.5969	3140.2382	3056.3274	3060.8713	2884.9177	3260.7168	3128.7363	3157.5015
	Ciflox- Area	0.0000	0.0000	68.7640	183.9716	146.5869	114.8257	126.3714	682.3915	139.0566	109.1710
	Area Ratio	0.0000	0.0000	0.0224	0.0586	0.0480	0.0375	0.0438	0.2093	0.0444	0.0346
	Test Concentration(µg/ml)	0.0000	0.0000	0.4365	1.2332	0.9992	0.7690	0.9076	4.5523	0.9217	0.7043
	Initial Concentation(µg/ml)	0.0000	0.0000	43.6481	123.3155	99.9158	76.9032	90.7580	455.2347	92.1695	70.4298
	Paracetamol(IS)- Area	3949.1792	3888.7131	3943.7776	3965.8337	3881.0327	3944.4768	4272.0039	3841.6780	4163.2393	3912.0715
	Ciprinol- Area	0.0000	1240.0432	2664.3208	1614.4316	333.9952	259.5191	181.1229	216.2522	221.7903	406.7068
	Area Ratio	0.0000	0.3189	0.6756	0.4071	0.0861	0.0658	0.0424	0.0563	0.0533	0.1040
	Test Concentration(µg/ml)	0.0000	6.9666	14.8233	8.9094	1.8383	1.3919	0.8766	1.1826	1.1162	2.2326
	Initial Concentation(µg/ml)	0.0000	696.6579	1482.3256	890.9362	183.8289	139.1917	87.6600	118.2623	111.6156	223.2643

Table 27 (Continued) - Concentration of Ciprofloxacin Excreted in the Urine of Subjects over Seven (7) Hours-µg/ml

SUBJECT	PRODUCT	0(Blank)	1hr	1.5hrs	2.0hrs	2.5hrs	3hrs	4hrs	5hrs	6hrs	7hrs
C	Paracetamol (IS)- Area	3119.9658	3111.7070	3020.7144	2990.3040	3103.5249	3103.5701	3171.9414	3261.7756	3128.7363	3264.7871
	Ciprinol- Area	0.0000	33.9318	183.2143	1103.3059	1814.5974	1538.9576	989.3555	263.1535	139.0566	472.4688
	Area Ratio	0.0000	0.0109	0.0607	0.3690	0.5847	0.4959	0.3119	0.0807	0.0444	0.1447
	Test Concentration(µg/ml)	0.0000	0.1829	1.2787	8.0696	12.8213	10.8649	6.8130	1.7198	0.9217	3.1303
	Initial Concentation(µg/ml)	0.0000	18.2920	127.8692	806.9628	1282.1347	1086.4909	681.2963	171.9779	92.1695	313.0321
	Paracetamol(IS)- Area	3820.2275	4005.8777	3979.2764	5037.3828	4120.4155	4029.3816	4010.9248	4020.6641	4148.6177	4083.0307
	Ciflox- Area	0.0000	69.6603	199.1284	333.4902	828.1085	875.9686	485.0046	265.8592	533.2245	518.2789
	Area Ratio	0.0000	0.0174	0.0500	0.0662	0.2010	0.2174	0.1209	0.0661	0.1285	0.1269
	Test Concentration(µg/ml)	0.0000	0.3258	1.0450	1.4009	4.3695	4.7312	2.6062	1.3992	2.7738	2.7387
	Initial Concentation(µg/ml)	0.0000	32.5760	104.4964	140.0949	436.9536	473.1174	260.6187	139.9190	277.3803	273.8653
D	Paracetamol (IS)- Area	3011.8010	2953.7864	3244.4111	3084.5349	3070.9678	3027.8445	3004.0044	3038.0962	3129.5781	3382.4795
	Ciflox- Area	0.0000	0.0000	13.7694	336.4387	324.1881	72.0626	43.9308	49.3522	40.2222	38.5482
	Area Ratio	0.0000	0.0000	0.0042	0.1091	0.1056	0.0238	0.0146	0.0162	0.0129	0.0114
	Test Concentration(µg/ml)	0.0000	0.0000	0.0362	2.3452	2.2680	0.4670	0.2648	0.3005	0.2258	0.1938
	Initial Concentation(µg/ml)	0.0000	0.0000	3.6213	234.5214	226.7961	46.6960	26.4848	30.0539	22.5821	19.3754
	Paracetamol(IS)- Area	2795.0996	2903.1077	2790.5706	2802.7727	2819.5327	2782.9805	2904.7319	2916.1035	2869.0273	2803.6516
	Ciprinol- Area	0.0000	25.6572	55.2664	106.8064	214.6791	205.2968	159.2593	135.7141	170.6474	97.0024
	Area Ratio	0.0000	0.0088	0.0198	0.0381	0.0761	0.0738	0.0548	0.0465	0.0595	0.0346
	Test Concentration(µg/ml)	0.0000	0.1374	0.3790	0.7821	1.6198	1.5676	1.1504	0.9678	1.2528	0.7048
	Initial Concentation(µg/ml)	0.0000	13.7398	37.8958	78.2102	161.9822	156.7592	115.0386	96.7831	125.2845	70.4815

Table 27 (Continued) - Concentration of Ciprofloxacin Excreted in the Urine of Subjects over Seven (7) Hours- µg/ml

SUBJECT	PRODUCT	0(Blank)	1hr	1.5hrs	2.0hrs	2.5hrs	3hrs	4hrs	5hrs	6hrs	7hrs
	Paracetamol (IS)- Area	3080.6301	3167.1375	3049.3289	3083.6816	3094.5325	3068.5920	3289.2010	3049.9028	2928.2983	3107.6707
	Ciprinol- Area	0.0000	0.0000	1997.0714	565.0242	216.4825	98.6805	148.2382	389.2210	295.8090	29.0504
	Area Ratio	0.0000	0.0000	0.6549	0.1832	0.0700	0.0322	0.0451	0.1276	0.1010	0.0093
	Test Concentration(µg/ml)	0.0000	0.0000	14.3683	3.9786	1.4836	0.6511	0.9354	2.7537	2.1678	0.1486
	Initial Concentation(µg/ml)	0.0000	0.0000	1436.8318	397.8643	148.3622	65.1062	93.5422	275.3690	216.7783	14.8634
E											
	Paracetamol(IS)- Area	2677.4680	2861.0627	2985.6301	2938.9334	2801.8630	2759.2634	3017.4185	2814.2102	2796.0195	2759.5815
	Ciflox- Area	0.0000	0.0000	673.9320	535.1325	389.2461	347.9493	217.2809	260.3206	215.3688	122.6505
	Area Ratio	0.0000	0.0000	0.2257	0.1821	0.1389	0.1261	0.0720	0.0925	0.0770	0.0444
	Test Concentration(µg/ml)	0.0000	0.0000	4.9147	3.9534	3.0027	2.7203	1.5288	1.9802	1.6394	0.9217
	Initial Concentation(µg/ml)	0.0000	0.0000	491.4652	395.3390	300.2732	272.0314	152.8829	198.0224	163.9360	92.1703
	Paracetamol (IS)- Area	3052.7412	3027.9050	3214.6624	2945.0374	3047.0884	3062.3032	3246.1714	3231.2979	3285.1287	3221.9004
	Ciflox- Area	0.0000	451.5711	456.6431	159.7462	138.4063	550.8655	133.9998	607.5660	326.9249	24.7093
	Area Ratio	0.0000	0.1491	0.1421	0.0542	0.0454	0.1799	0.0413	0.1880	0.0995	0.0077
	Test Concentration(µg/ml)	0.0000	3.2277	3.0716	1.1375	0.9432	3.9050	0.8520	4.0843	2.1347	0.1117
	Initial Concentation(µg/ml)	0.0000	322.7676	307.1588	113.7500	94.3226	390.4978	85.1968	408.4260	213.4727	11.1656
F											
	Paracetamol(IS)- Area	2766.0972	2772.6821	2780.7764	2845.3777	2884.7268	2754.5935	2688.9363	2706.1409	2669.9705	2661.8823
	Ciprinol- Area	0.0000	0.0000	107.6481	164.0522	238.4894	200.4554	197.5191	101.3265	90.8472	80.7831
	Area Ratio	0.0000	0.0000	0.0387	0.0577	0.0827	0.0728	0.0735	0.0374	0.0340	0.0303
	Test Concentration(µg/ml)	0.0000	0.0000	0.7954	1.2127	1.7637	1.5456	1.5607	0.7675	0.6922	0.6112
	Initial Concentation(µg/ml)	0.0000	0.0000	79.5408	121.2680	176.3725	154.5624	156.0710	76.7471	69.2193	61.1192

Table 27 (Continued) - Concentration of Ciprofloxacin Excreted in the Urine of Subjects Over Seven (7) Hours-µg/ml

SUBJECT	PRODUCT	0(Blank)	1hr	1.5hrs	2.0hrs	2.5hrs	3hrs	4hrs	5hrs	6hrs	7hrs
G	Paracetamol (IS)- Area	45512.7222	4438.4248	4390.1519	4270.4336	4360.6797	4534.0615	4538.3989	4319.1567	4639.8237	4935.7188
	Ciprinol- Area	0.0000	219.8109	1659.3787	1852.3155	708.8864	794.2970	863.5549	402.7170	285.1552	189.8049
	Area Ratio	0.0000	0.0495	0.3780	0.4338	0.1626	0.1752	0.1903	0.0932	0.0615	0.0385
	Test Concentration(µg/ml)	0.0000	1.0336	8.2682	9.4968	3.5234	3.8014	4.1339	1.9965	1.2964	0.7898
	Initial Concentation(µg/ml)	0.0000	103.3580	826.8227	949.6773	352.3420	380.1419	413.3864	199.6470	129.6436	78.9766
	Paracetamol(IS)- Area	3120.6406	3091.2795	3100.9961	3192.1282	3018.1001	3087.1890	3152.2544	3207.3845	3136.8464	3062.1367
	Ciflox- Area	0.0000	427.4476	447.8326	529.8259	614.7111	647.4251	494.5898	448.1010	300.2818	277.3778
	Area Ratio	0.0000	0.1383	0.1444	0.1660	0.2037	0.2097	0.1569	0.1397	0.0957	0.0906
	Test Concentration(µg/ml)	0.0000	2.9884	3.1237	3.5987	4.4290	4.5620	3.3987	3.0200	2.0513	1.9380
	Initial Concentation(µg/ml)	0.0000	298.8443	312.3694	359.8654	442.8962	456.1971	339.8686	302.0026	205.1262	193.7954
H	Paracetamol (IS)- Area	4331.5415	4202.2192	4344.1724	5281.9273	4134.7983	4473.0381	4267.1362	4481.6895	4535.1001	4328.9854
	Ciflox- Area	0.0000	1800.0465	5501.6196	3344.5859	933.5637	1028.9713	599.8558	1337.6779	1197.1152	674.8961
	Area Ratio	0.0000	0.4284	1.2664	0.6332	0.2258	0.2300	0.1406	0.2985	0.2640	0.1559
	Test Concentration(µg/ml)	0.0000	9.3779	27.8378	13.8902	4.9159	5.0097	3.0391	6.5171	5.7570	3.3767
	Initial Concentation(µg/ml)	0.0000	937.7889	2783.7814	1389.0158	491.5906	500.9660	303.9113	651.7098	575.6975	337.6689
	Paracetamol(IS)- Area	3132.3838	3061.7451	3009.1365	2975.9697	3088.3713	3162.1057	3125.7805	3142.0149	3111.7070	3146.5056
	Ciprinol- Area	0.0000	872.2379	2233.3425	2301.4036	2183.6985	1166.8809	732.2216	969.0908	475.3619	80.6976
	Area Ratio- Area	0.0000	0.2849	0.7422	0.7733	0.7071	0.3690	0.2343	0.3084	0.1528	0.0256
	Test Concentration(µg/ml)	0.0000	6.2177	16.2905	16.9764	15.5170	8.0709	5.1025	6.7363	3.3076	0.5076
	Initial Concentation(µg/ml)	0.0000	621.7678	1629.0467	1697.6409	1551.6987	807.0929	510.2476	673.6338	330.7613	50.7637

Table 27: (Continued) - Concentration of Ciprofloxacin Excreted in the Urine of Subjects over Seven (7) Hours-µg/ml

SUBJECT	PRODUCT	0(Blank)	1hr	1.5hrs	2.0hrs	2.5hrs	3hrs	4hrs	5hrs	6hrs	7hrs
	Paracetamol (IS)- Area	4146.2891	4331.9106	4468.9502	4115.0425	4451.1201	4360.6011	4397.9399	4629.5332	4390.0977	4351.1094
	Ciprinol- Area	0.0000	120.9519	687.4658	420.3813	463.9272	530.5693	1270.2067	1216.4739	938.3443	354.8677
	Area Ratio	0.0000	0.0279	0.1538	0.1022	0.1042	0.1217	0.2888	0.2628	0.2137	0.0816
	Test Concentration(µg/ml)	0.0000	0.5577	3.3311	2.1929	2.2385	2.6228	6.3044	5.7305	4.6507	1.7392
	Initial Concentation(µg/ml)	0.0000	55.7734	333.1093	219.2890	223.8482	262.2763	630.4374	573.0481	465.0685	173.9162
I											
	Paracetamol(IS)- Area	3067.9749	3128.4666	3100.2251	3034.5381	3136.4045	2993.1450	3084.1238	3097.4951	3313.7720	3087.2212
	Ciflox- Area	0.0000	98.3541	258.6605	132.3167	153.4226	117.2383	279.1085	368.9727	569.0478	511.5659
	Area Ratio	0.0000	0.0314	0.0834	0.0436	0.0489	0.0392	0.0905	0.1191	0.1717	0.1657
	Test Concentration(µg/ml)	0.0000	0.6352	1.7805	0.9032	1.0202	0.8055	1.9361	2.5665	3.7252	3.5926
	Initial Concentation(µg/ml)	0.0000	63.5208	178.0459	90.3162	102.0192	80.5483	193.6090	256.6513	372.5156	359.2606
	Paracetamol (IS)- Area	3150.4846	3144.2185	3127.3562	3064.7556	3077.5613	3075.9021	3009.2349	3043.2793	3229.6086	3290.7412
	Ciflox- Area	0.0000	0.0000	8.3120	14.1859	114.6718	43.3645	103.9931	678.3309	136.4353	136.1833
	Area Ratio	0.0000	0.0000	0.0027	0.0046	0.0373	0.0141	0.0346	0.2229	0.0422	0.0414
	Test Concentration(µg/ml)	0.0000	0.0000	0.0013	0.0447	0.7634	0.2533	0.7039	4.8523	0.8732	0.8543
J	Initial Concentation(µg/ml)	0.0000	0.0000	0.1274	4.4685	76.3449	25.3263	70.3920	485.2307	87.3241	85.4268
	Paracetamol(IS)- Area	3029.8269	3102.3298	3069.9907	3160.9531	3152.2083	3076.1252	3090.0850	3238.3464	3224.4819	3225.8831
	Ciprinol- Area	0.0000	0.0000	91.4033	493.8689	612.1615	200.5439	78.7809	251.0899	59.2142	124.0962
	Area Ratio	0.0000	0.0000	0.0298	0.1562	0.1942	0.0652	0.0255	0.0775	0.0184	0.0385
	Test Concentration(µg/ml)	0.0000	0.0000	0.5985	3.3842	4.2203	1.3787	0.5043	1.6506	0.3472	0.7901
	Initial Concentation(µg/ml)	0.0000	0.0000	59.8527	338.4152	422.0283	137.8715	50.4289	165.0583	34.7223	79.0064

Table 28: Ciprofloxacin Excretory Rates in Subjects

CIPRINOL EXCRETORY RATE/(µg/ml/hr)										
SUBJECT	0Hr	1.0Hr	1.5Hrs	2.0Hrs	2.5Hrs	3.0Hrs	4.0Hrs	5.0Hrs	6.0Hrs	7.0Hrs
A	0	0	843.2437	1914.8729	1451.6441	66.3150	560.4585	1041.5972	279.9903	46.5764
B	0	696.6579	1571.3355	1182.7787	1414.2146	89.2744	51.5317	30.6022	6.6466	111.6487
C	0	18.2920	219.1545	1358.1872	950.3438	391.2876	405.1947	509.3184	79.8084	220.8626
D	0	13.7398	48.3120	80.6288	167.5441	10.4461	41.7206	18.2555	28.5014	54.8030
E	0	0.0000	2873.6635	2077.9349	499.0042	166.5120	28.4359	181.8268	58.5906	201.9150
F	0	0.0000	159.0816	83.4545	110.2089	43.6202	1.5086	79.3239	7.5278	8.1001
G	0	103.3580	1446.9294	245.7092	1194.6706	55.5998	33.2445	213.7394	70.0033	50.6670
H	0	621.7678	2014.5577	137.1884	291.8843	1489.2116	296.8453	163.3862	342.8724	279.9976
I	0	55.7734	554.6717	227.6405	9.1183	76.8562	368.1611	57.3893	107.9796	291.1523
J	0	0.0000	119.7055	557.1249	167.2262	568.3136	87.4426	114.6293	130.3359	44.2841
MEAN	0	150.9589	985.0655	786.5520	625.5859	295.7437	187.4543	241.0068	111.2257	131.0007

Table28: (Continued) - Ciprofloxacin Excretory Rates In Subjects

CIFLOX EXCRETORY RATE/(µg/ml/hr)										
SUBJECT	0Hr	1.0Hr	1.5Hrs	2.0Hrs	2.5Hrs	3.0Hrs	4.0Hrs	5.0Hrs	6.0Hrs	7.0Hrs
A	0	635.1027	2006.6029	980.3071	2786.8923	797.2668	225.0447	295.0939	108.3346	68.0904
B	0	0.0000	87.2962	159.3348	46.7994	46.0251	13.8548	364.4767	363.0652	21.7398
C	0	32.5760	143.8407	71.1970	593.7174	72.3276	212.4987	120.6997	137.4613	3.5150
D	0	0.0000	7.2425	461.8004	15.4506	360.2003	20.2112	3.5691	7.4717	3.2068
E	0	0.0000	982.9305	192.2524	190.1317	56.4837	119.1484	45.1395	34.0865	71.7657
F	0	322.7676	31.2176	386.8175	38.8548	592.3503	305.3010	323.2292	194.9532	202.3071
G	0	298.8443	27.0503	94.9920	166.0615	26.6018	116.3285	37.8660	96.8764	11.3308
H	0	937.7889	3691.9851	2789.5313	1794.8503	18.7508	197.0547	347.7985	76.0123	238.0286
I	0	63.5208	229.0501	175.4593	23.4059	42.9418	113.0607	63.0423	115.8643	13.2550
J	0	0.0000	0.2548	8.6823	143.7528	102.0373	45.0657	414.8386	397.9066	1.8973
MEAN	0	229.0600	720.7471	532.0374	579.9917	211.4985	136.7568	201.5754	153.2032	63.5136

Table 29: Area under the Excretory Rate-Time Curve (By Trapezoidal Rule)

CIPRINOL										
	Subject A		Subject B		Subject C		Subject D		Subject E	
Time/hrs	Ex. Rate	Area Under Curve	Ex. Rate	Area Under Curve	Ex. Rate	Area Under Curve	Ex. Rate	Area Under Curve	Ex. Rate	Area Under Curve
0	0	0	0	348.3289	0	9.1460	0.0000	6.8699	0.0000	0.0000
1	0	210.8109	696.6579	566.9983	18.2920	59.3616	13.7398	15.5130	0.0000	718.4159
1.5	843.2437	689.5292	1571.3355	688.5286	219.1545	394.3354	48.3120	32.2352	2873.6635	1237.8996
2	1914.8729	841.6293	1182.7787	649.2483	1358.1872	577.1328	80.6288	62.0432	2077.9349	644.2348
2.5	1451.6441	379.4898	1414.2146	375.8723	950.3438	335.4079	167.5441	44.4976	499.0042	166.3790
3	66.3150	313.3868	89.2744	70.4031	391.2876	398.2411	10.4461	26.0834	166.5120	97.4739
4	560.4585	801.0279	51.5317	41.0670	405.1947	457.2565	41.7206	29.9880	28.4359	105.1314
5	1041.5972	660.7937	30.6022	18.6244	509.3184	294.5634	18.2555	23.3785	181.8268	120.2087
6	279.9903	163.2833	6.6466	59.1477	79.8084	150.3355	28.5014	41.6522	58.5906	130.2528
7	46.5764		111.6487		220.8626		54.8030		201.9150	
TOTAL		4059.9508		2818.2186		2675.7802		282.2609		3219.9962

Table 29: (Continued) - Area under the Excretory Rate-Time Curve (By Trapezoidal Rule)

CIPRINOL										
	Subject F		Subject G		Subject H		Subject I		Subject J	
Time/hrs	Ex. Rate	Area Under Curve	Ex. Rate	Area Under Curve	Ex. Rate	Area Under Curve	Ex. Rate	Area Under Curve	Ex. Rate	Area Under Curve
0	0	0	0	51.67900719	0	310.8839	0	27.8867	0	0
1	0	39.7704	103.3580	387.5718	621.7678	659.0814	55.7734	152.6113	0	29.9264
1.5	159.0816	60.6340	1446.9294	423.1596	2014.5577	537.9365	554.6717	195.5780	119.7054862	169.2076
2	83.4545	48.4158	245.7092	360.0949	137.1884	107.2682	227.6405	59.1897	557.1249159	181.0878
2.5	110.2089	38.4573	1194.6706	312.5676	291.8843	445.2740	9.1183	21.4936	167.2261757	183.8849
3	43.6202	22.5644	55.5998	44.4222	1489.2116	893.0285	76.8562	222.5086	568.3135579	327.8781
4	1.5086	40.4162	33.2445	123.4920	296.8453	230.1158	368.1611	212.7752	87.44259049	101.0360
5	79.3239	43.4258	213.7394	141.8714	163.3862	253.1293	57.3893	82.6844	114.6293426	122.4826
6	7.5278	7.8140	70.0033	60.3352	342.8724	311.4350	107.9796	199.5659	130.3359496	87.3100
7	8.1001		50.6670		279.9976		291.1523		44.2840596	
TOTAL		301.4980		1905.1938		3748.1525		1174.2935		1202.8134

Table 29: (Continued) - Area under the Excretory Rate-Time Curve (By Trapezoidal Rule)

CIFLOX										
	Subject A		Subject B		Subject C		Subject D		Subject E	
Time/hrs	Ex. Rate	Area Under Curve	Ex. Rate	Area Under Curve	Ex. Rate	Area Under Curve	Ex. Rate	Area Under Curve	Ex. Rate	Area Under Curve
0	0	317.5513	0.0000	0.0000	0.0000	16.2880	0.0000	0.0000	0.0000	0.0000
1	635.1026664	660.4264	0.0000	21.8240	32.5760	44.1042	0.0000	1.8106	0.0000	245.7326
1.5	2006.602926	746.7275	87.2962	61.6578	143.8407	53.7594	7.2425	117.2607	982.9305	293.7957
2	980.3070628	941.7999	159.3348	51.5336	71.1970	166.2286	461.8004	119.3127	192.2524	95.5960
2.5	2786.892349	896.0398	46.7994	23.2061	593.7174	166.5113	15.4506	93.9127	190.1317	61.6538
3	797.266785	511.1558	46.0251	29.9399	72.3276	142.4131	360.2003	190.2058	56.4837	87.8160
4	225.0447275	260.0693	13.8548	189.1657	212.4987	166.5992	20.2112	11.8901	119.1484	82.1440
5	295.0939449	201.7143	364.4767	363.7709	120.6997	129.0805	3.5691	5.5204	45.1395	39.6130
6	108.3345807	88.2125	363.0652	192.4025	137.4613	70.4881	7.4717	5.3392	34.0865	52.9261
7	68.0904445		21.7398		3.5150		3.2068		71.7657	
TOTAL		4623.6967		933.5006		955.4725		545.2524		959.2772

Table 29: (Continued) - Area under the Excretory Rate-Time Curve (By Trapezoidal Rule)

CIFLOX										
	Subject F		Subject G		Subject H		Subject I		Subject J	
Time/hrs	Ex. Rate	Area Under Curve	Ex. Rate	Area Under Curve	Ex. Rate	Area Under Curve	Ex. Rate	Area Under Curve	Ex. Rate	Area Under Curve
0	0	161.3838	0.0000	149.4221	0.0000	468.8945	0.0000	31.7604	0.0000	0.0000
1	322.7675859	88.4963	298.8443	81.4736	937.7889	1157.4435	63.5208	73.1427	0.0000	0.0637
1.5	31.21760304	104.5088	27.0503	30.5106	3691.9851	1620.3791	229.0501	101.1273	0.2548	2.2343
2	386.8175215	106.4181	94.9920	65.2634	2789.5313	1146.0954	175.4593	49.7163	8.6823	38.1088
2.5	38.85477674	157.8013	166.0615	48.1658	1794.8503	453.4003	23.4059	16.5869	143.7528	61.4475
3	592.3503285	448.8257	26.6018	71.4651	18.7508	107.9027	42.9418	78.0013	102.0373	73.5515
4	305.3010035	314.2651	116.3285	77.0972	197.0547	272.4266	113.0607	88.0515	45.0657	229.9522
5	323.2291742	259.0912	37.8660	67.3712	347.7985	211.9054	63.0423	89.4533	414.8386	406.3726
6	194.9532393	198.6302	96.8764	54.1036	76.0123	157.0205	115.8643	64.5596	397.9066	199.9019
7	202.3071321		11.3308		238.0286		13.2550		1.8973	
TOTAL		1839.4204		644.8728		5595.4679		592.3994		1011.6325

Sample Name: CIFLOX 1010M- 0.05%w/v(A)

```
==========================================================================
Acq. Operator    : DZIGBORDI AGBITOR              Seq. Line :  13
Acq. Instrument  : ERNEST CHEMISTS                 Location : Vial 4
Injection Date   : 2/11/2013 3:07:14 PM                Inj :   1
                                                 Inj Volume : 1 µl
Acq. Method      : C:\Chem32\1\DATA\CIPROFLOXACIN 2013-02-11 13-41-54\CIPRO BIOANALYTICAL METHOD.
                   M
Last changed     : 2/11/2013 3:06:30 PM by DZIGBORDI AGBITOR
                   (modified after loading)
Analysis Method  : C:\CHEM32\1\METHODS\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 7/29/2013 11:00:04 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Method Info      : ASSAY OF CIPROFLOXACIN IN URINE
                   COLUMN: WATERS XBRIDGE C18, 150X4.6mm, 5u
                   MOBILE PHASE: ACETONITRILE: ORTHOPHOSPHORIC ACID pH 3.0
                   DETECTION WAVELENGTH: 278nm
                   TEMPERATURE: 40 DEGREES CELCIUS
                   INJECTION VOLUME: 20ul
```


```
==========================================================================
                  Area Percent Report with Performance
==========================================================================

Multiplier              :      1.0000
Dilution                :      1.0000
Use Multiplier & Dilution Factor with ISTDs
Signal 1: VWD1 A, Wavelength=278 nm
```

RetTime [min]	k'	Area mAU *s	Height [mAU]	Symm.	Width [min]	Plates	Resol ution	Select ivity
0.357	-	3.05749	1.41961e-1	0.40	-	-	-	-
0.926	-	8.60183	6.32557e-1	0.61	0.1371	253	-	2.59
1.295	-	6.45163	7.14620e-1	0.28	0.1618	355	1.45	1.40
1.589	-	3.92993	5.98409e-1	0.75	0.1126	1103	1.26	1.23
1.967	-	2543.85645	429.38361	0.57	0.0777	3555	2.34	1.24
3.069	-	3.68736	3.37416e-1	0.47	0.1666	1879	5.30	1.56
3.688	-	1.91032	1.33192e-1	1.00	0.2451	1254	1.77	1.20
4.153	-	2.13334	1.23051e-1	2.07	0.3878	635	0.86	1.13
4.684	-	4.10298	1.38997e-1	0.56	0.5775	364	0.65	1.13
5.369	-	1.60450	1.17172e-1	1.27	0.2168	3398	1.01	1.15

```
==========================================================================
                        *** End of Report ***
```

Figure 8: Sample Chromatogram -Assay of Ciflox Tablet

Sample Name: CIPRINOL N88473- 0.05%w/v(A)

```
==========================================================================
Acq. Operator    : DZIGBORDI AGBITOR              Seq. Line :   1
Acq. Instrument  : ERNEST CHEMISTS                 Location : Vial 2
Injection Date   : 2/11/2013 1:42:40 PM                Inj :   1
                                                 Inj Volume : 1 µl
Acq. Method      : C:\Chem32\1\DATA\CIPROFLOXACIN 2013-02-11 13-41-54\CIPRO BIOANALYTICAL METHOD.
                   M
Last changed     : 2/11/2013 1:41:52 PM by DZIGBORDI AGBITOR
Analysis Method  : C:\CHEM32\1\METHODS\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 7/29/2013 11:00:04 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Method Info      : ASSAY OF CIPROFLOXACIN IN URINE
                   COLUMN: WATERS XBRIDGE C18, 150X4.6mm, 5u
                   MOBILE PHASE: ACETONITRILE: ORTHOPHOSPHORIC ACID pH 3.0
                   DETECTION WAVELENGTH: 278nm
                   TEMPERATURE: 40 DEGREES CELCIUS
                   INJECTION VOLUME: 20ul
```


```
==========================================================================
                Area Percent Report with Performance
==========================================================================

Multiplier                :      1.0000
Dilution                  :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=278 nm
```

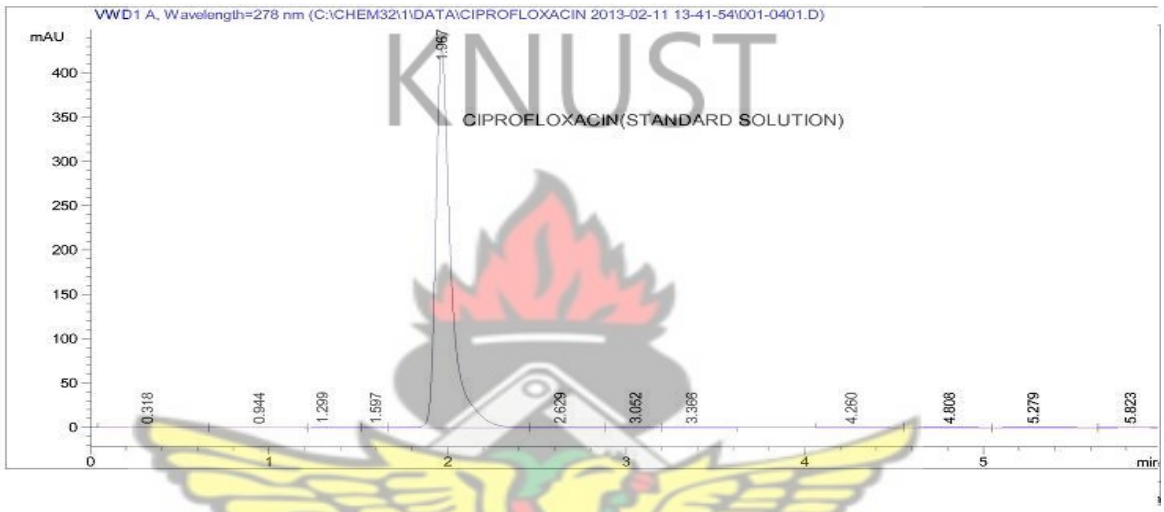
RetTime [min]	k'	Area mAU *s	Height [mAU]	Symm.	Width [min]	Plates	Resol	Selectivity
0.929	-	1.34980	1.38227e-1	1.01	0.1640	178	-	-
1.175	-	1.64748	1.80777e-1	2.96	-	-	-	1.26
1.286	-	2.90909	5.84394e-1	0.83	0.0926	1069	-	1.09
1.394	-	2.88823	4.83261e-1	0.76	0.1008	1060	0.66	1.08
1.569	-	1.11948	2.12734e-1	0.72	0.1015	1322	1.01	1.12
1.930	-	2451.54053	426.23660	0.59	0.0752	3645	2.40	1.23
4.426	-	3.55140	1.15689e-1	1.72	0.6091	293	4.29	2.29
5.027	-	2.02582	1.32058e-1	1.21	0.2536	2177	0.82	1.14
5.408	-	2.07431	1.41230e-1	1.29	0.2427	2751	0.90	1.08

```
==========================================================================
                        *** End of Report ***
```

Figure 9: Sample Chromatogram -Assay of Ciprinol Tablet

Sample Name: CIPRO STD.- 0.05%w/v

```
=====================================================================
Acq. Operator    : DZIGBORDI AGBITOR              Seq. Line :   4
Acq. Instrument  : ERNEST CHEMISTS                 Location : Vial 1
Injection Date   : 2/11/2013 2:04:18 PM               Inj :   1
                                                  Inj Volume : 1 µl
Acq. Method      : C:\Chem32\1\DATA\CIPROFLOXACIN 2013-02-11 13-41-54\CIPRO BIOANALYTICAL METHOD.
                   M
Last changed     : 2/11/2013 2:03:34 PM by DZIGBORDI AGBITOR
                   (modified after loading)
Analysis Method  : C:\CHEM32\1\METHODS\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 7/29/2013 11:00:04 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Method Info      : ASSAY OF CIPROFLOXACIN IN URINE
                   COLUMN: WATERS XBRIDGE C18, 150X4.6mm, 5u
                   MOBILE PHASE: ACETONITRILE: ORTHOPHOSPHORIC ACID pH 3.0
                   DETECTION WAVELENGTH: 278nm
                   TEMPERATURE: 40 DEGREES CELCIUS
                   INJECTION VOLUME: 20ul
```




```
=====================================================================
                Area Percent Report with Performance
=====================================================================

Multiplier              :      1.0000
Dilution                :      1.0000
Use Multiplier & Dilution Factor with ISTDs
Signal 1: VWD1 A, Wavelength=278 nm
```

RetTime [min]	k'	Area mAU *s	Height [mAU]	Symm.	Width [min]	Plates	Resol ution	Select ivity
0.318	-	3.52104	1.49657e-1	0.53	0.3769	4	-	-
0.944	-	5.76537	3.14598e-1	0.91	0.2973	56	1.09	2.97
1.299	-	5.84983	5.67521e-1	0.26	0.1820	282	0.87	1.38
1.597	-	3.05910	4.33467e-1	0.93	0.1309	825	1.12	1.23
1.967	-	2532.45825	428.42319	0.58	0.0793	3410	2.07	1.23
2.629	-	8.48958	4.31237e-1	0.86	0.3482	316	1.82	1.34
3.052	-	5.06442	3.80305e-1	0.97	0.2532	805	0.83	1.16
3.366	-	6.01840	3.34240e-1	0.85	0.3155	631	0.65	1.10
4.260	-	4.76179	2.09417e-1	0.73	0.4190	573	1.43	1.27
4.808	-	4.28977	1.96357e-1	1.09	0.4065	775	0.78	1.13
5.279	-	6.24320	2.77238e-1	0.70	0.3883	1024	0.70	1.10
5.823	-	1.15893	1.05699e-1	1.17	0.2289	3584	1.03	1.10

```
=====================================================================
                        *** End of Report ***
```

Figure 10: Sample Chromatogram -Standard Solution of Ciprofloxacin (for assay of products)

Table 30: Dissolution Data Sheet- Ciflox

Date : 30/1/2013 Time : 18:13:09

Wavelength Program

Date: 30/01/2013 Time: 18:03:57 AM Method: wp1
Slit: UV/VIS: 1.00 nm
Analyst: DZIGBORDI AGBITOR

Sample ID	Cyc	Factor	276.00	nm
CiproStd	1	1.0000	0.7279	Ciprofloxacin Standard solution
CiproStd	1	1.0000	0.7323	Ciprofloxacin Standard solution
vessel1	1	1.0000	0.7312	Ciflox sample solution- Batch No. 1010M
vessel1	1	1.0000	0.7311	Ciflox sample solution- Batch No. 1010M
vessel2	1	1.0000	0.6965	Ciflox sample solution- Batch No. 1010M
vessel2	1	1.0000	0.6966	Ciflox sample solution- Batch No. 1010M
vessel3	1	1.0000	0.7500	Ciflox sample solution- Batch No. 1010M
vessel3	1	1.0000	0.7507	Ciflox sample solution- Batch No. 1010M
vessel4	1	1.0000	0.6916	Ciflox sample solution- Batch No. 1010M
vessel4	1	1.0000	0.6912	Ciflox sample solution- Batch No. 1010M
vessel5	1	1.0000	0.7386	Ciflox sample solution- Batch No. 1010M
vessel5	1	1.0000	0.7384	Ciflox sample solution- Batch No. 1010M
vessel6	1	1.0000	0.7008	Ciflox sample solution- Batch No. 1010M
vessel6	1	1.0000	0.7002	Ciflox sample solution- Batch No. 1010M

Table 31: Dissolution Data Sheet- Ciprinol

Date : 30/1/2013 Time : 17:18:26

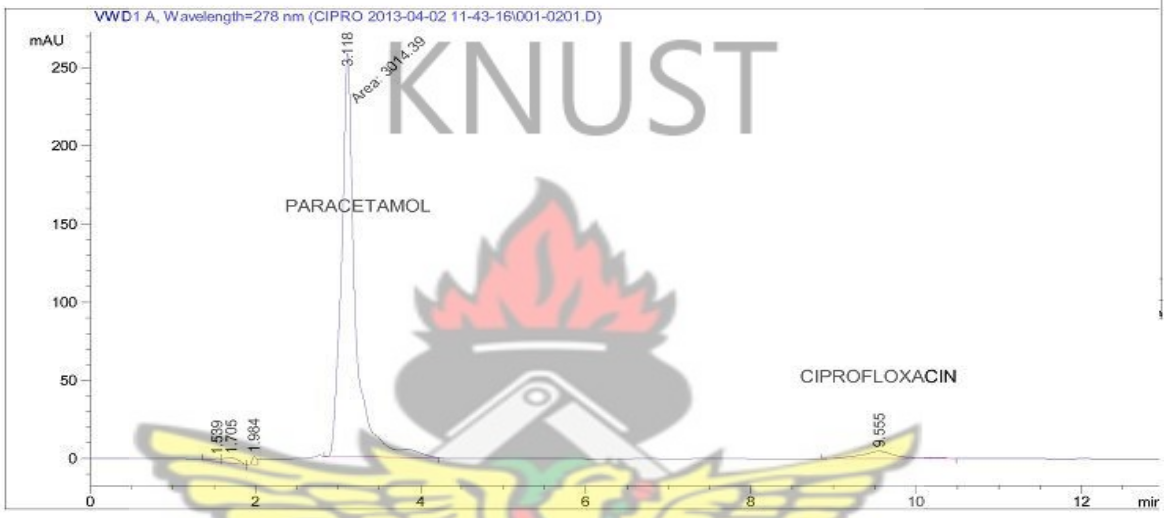
Wavelength Program

Date: 30/01/2013 Time: 17:08:50 AM Method: wpl
Slit: UV/VIS: 1.00 nm
Analyst: DZIGBORDI AGBITOR

Sample ID	Cyc	Factor	276.00	nm	
CiproStd	1	1.0000	0.7279		Ciprofloxacin Standard solution
CiproStd	1	1.0000	0.7259		Ciprofloxacin Standard solution
vessel1	1	1.0000	0.7180		Ciprinol sample solution- Batch No. N88473
vessel1	1	1.0000	0.7156		Ciprinol sample solution- Batch No. N88473
vessel2	1	1.0000	0.7194		Ciprinol sample solution- Batch No. N88473
vessel2	1	1.0000	0.7185		Ciprinol sample solution- Batch No. N88473
vessel3	1	1.0000	0.7429		Ciprinol sample solution- Batch No. N88473
vessel3	1	1.0000	0.7431		Ciprinol sample solution- Batch No. N88473
vessel4	1	1.0000	0.7255		Ciprinol sample solution- Batch No. N88473
vessel4	1	1.0000	0.7254		Ciprinol sample solution- Batch No. N88473
vessel5	1	1.0000	0.7448		Ciprinol sample solution- Batch No. N88473
vessel5	1	1.0000	0.7442		Ciprinol sample solution- Batch No. N88473
vessel6	1	1.0000	0.7175		Ciprinol sample solution- Batch No. N88473
vessel6	1	1.0000	0.7409		Ciprinol sample solution- Batch No. N88473

Sample Name: CIPRO IN MOBILE PHASE(1ug/ml)-(II)

```
==================================================================
Acq. Operator    : DZIGBORDI AGBITOR              Seq. Line :   2
Acq. Instrument  : ERNEST CHEMISTS                 Location : Vial 1
Injection Date   : 4/2/2013 11:58:47 AM               Inj :   1
                                                  Inj Volume : 20 µl
Acq. Method      : C:\Chem32\1\DATA\CIPRO 2013-04-02 11-43-16\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 4/2/2013 11:33:20 AM by DZIGBORDI AGBITOR
Analysis Method  : C:\CHEM32\1\METHODS\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 4/2/2013 1:40:28 PM by DZIGBORDI AGBITOR
                   (modified after loading)
Method Info      : ASSAY OF CIPROFLOXACIN IN URINE
                   COLUMN: WATERS XBRIDGE C18, 150X4.6mm, 5u
                   MOBILE PHASE: ACETONITRILE: ORTHOPHOSPHORIC ACID pH 3.0
                   DETECTION WAVELENGTH: 278nm
                   TEMPERATURE: 40 DEGREES CELCIUS
                   INJECTION VOLUME: 20ul
```




```
==================================================================
                Area Percent Report with Performance
==================================================================

Multiplier              :      1.0000
Dilution                :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=278 nm
```

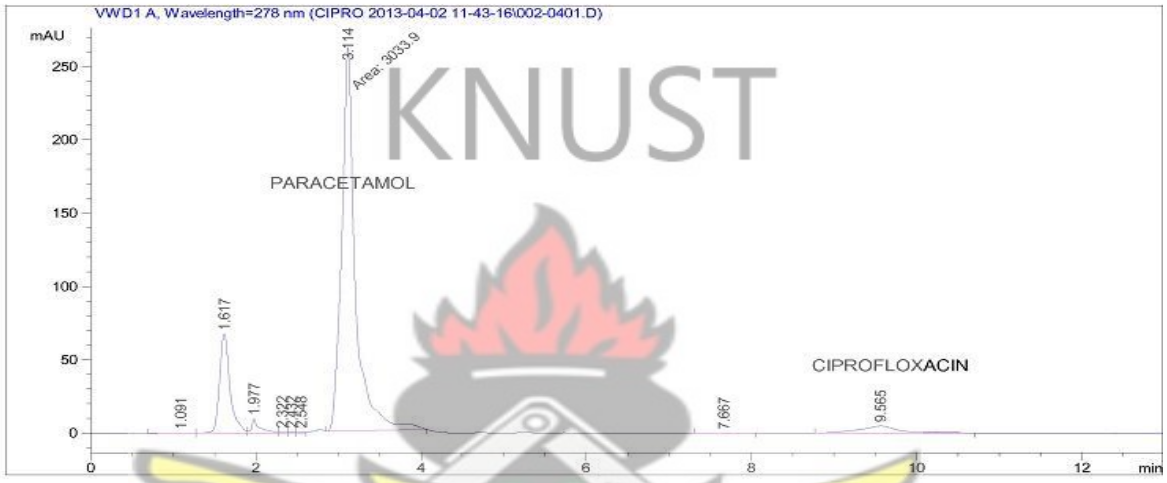
RetTime [min]	k'	Area mAU *s	Height [mAU]	Symm.	Width [min]	Plates	Resol ution	Select ivity
1.539	-	23.49025	2.10895	2.82	-	-	-	-
1.705	-	43.06792	3.58820	0.89	0.2125	357	-	1.11
1.984	-	20.27358	5.16980	0.65	0.0767	3708	1.13	1.16
3.118	-	3014.39185	258.53198	0.73	0.1359	2916	6.26	1.57
9.555	-	146.84656	4.78688	1.01	0.3883	3355	14.43	3.06

```
==================================================================
                        *** End of Report ***
```

Figure 11: Sample Validation Chromatogram - 1µg/ml Ciprofloxacin containing 200µg/ml Paracetamol (internal standard) in Mobile Phase

Sample Name: CIPRO-SPIKED URINE SAMPLE (1ug/ml)-(I)

```
==================================================================
Acq. Operator    : DZIGBORDI AGBITOR              Seq. Line :   4
Acq. Instrument  : ERNEST CHEMISTS                 Location : Vial 2
Injection Date   : 4/2/2013 12:27:34 PM                 Inj :   1
                                                 Inj Volume : 20 µl
Acq. Method      : C:\Chem32\1\DATA\CIPRO 2013-04-02 11-43-16\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 4/2/2013 11:33:20 AM by DZIGBORDI AGBITOR
Analysis Method  : C:\CHEM32\1\METHODS\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 4/2/2013 1:40:28 PM by DZIGBORDI AGBITOR
                   (modified after loading)
Method Info      : ASSAY OF CIPROFLOXACIN IN URINE
                   COLUMN: WATERS XBRIDGE C18, 150X4.6mm, 5u
                   MOBILE PHASE: ACETONITRILE: ORTHOPHOSPHORIC ACID pH 3.0
                   DETECTION WAVELENGTH: 278nm
                   TEMPERATURE: 40 DEGREES CELCIUS
                   INJECTION VOLUME: 20ul
```




```
==================================================================
                Area Percent Report with Performance
==================================================================

Multiplier              :      1.0000
Dilution                :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Sample Name: CIPRO-SPIKED URINE SAMPLE (1ug/ml)-(I)

    Signal 1: VWD1 A, Wavelength=278 nm
```

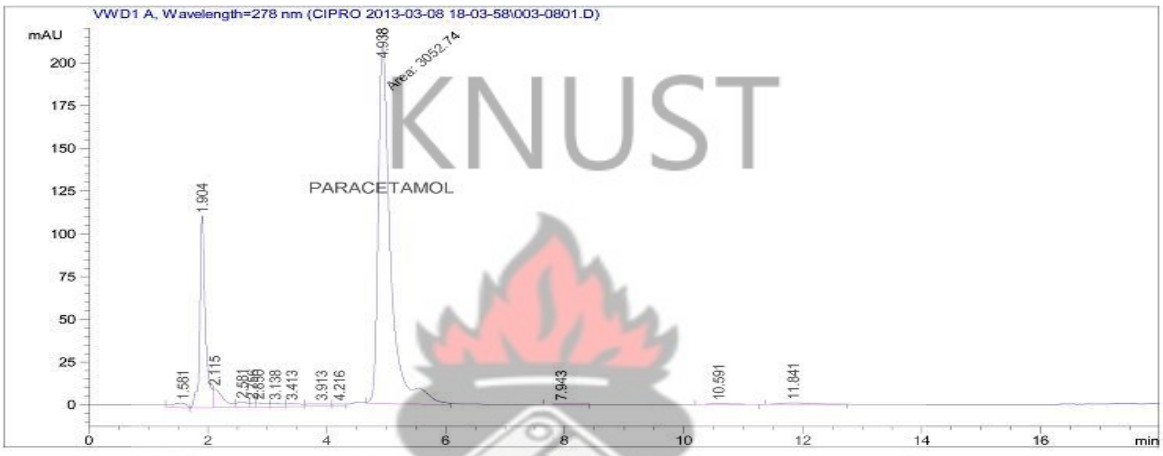
RetTime [min]	k'	Area mAU *s	Height [mAU]	Symm.	Width [min]	Plates	Resol ution	Select ivity
1.091	-	3.13820	1.69114e-1	1.48	0.3316	60	-	-
1.617	-	597.29071	67.66676	0.75	0.1213	984	1.36	1.48
1.977	-	67.01402	9.69489	0.34	0.0592	6176	2.34	1.22
2.322	-	4.83166	7.92696e-1	0.67	-	-	-	1.17
2.432	-	4.30248	7.46602e-1	0.87	-	-	-	1.05
2.548	-	5.04535	7.65835e-1	1.14	-	-	-	1.05
3.114	-	3033.90161	262.34802	0.75	0.1407	2712	-	1.22
7.667	-	4.55917	2.23069e-1	0.71	0.3397	2821	11.13	2.46
9.565	-	154.94159	4.78255	1.13	0.4271	2778	2.91	1.25

```
==================================================================
                        *** End of Report ***
```

Figure 12: Sample Validation Chromatogram - 1µg/ml Ciprofloxacin containing 200µg/ml Paracetamol (internal standard) in Urine

Sample Name: SUBJECT F-BLANK URINE SAMPLE

```
==================================================================
Acq. Operator    : DZIGBORDI AGBITOR              Seq. Line :   8
Acq. Instrument  : ERNEST CHEMISTS                 Location : Vial 3
Injection Date   : 3/8/2013 7:55:28 PM                 Inj :   1
                                                  Inj Volume : 20 µl
Acq. Method      : C:\Chem32\1\DATA\CIPRO 2013-03-08 18-03-58\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/8/2013 7:54:07 PM by DZIGBORDI AGBITOR
                   (modified after loading)
Analysis Method  : C:\CHEM32\1\METHODS\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/11/2013 8:51:07 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Method Info      : ASSAY OF CIPROFLOXACIN IN URINE
                   COLUMN: WATERS XBRIDGE C18, 150X4.6mm, 5u
                   MOBILE PHASE: ACETONITRILE: ORTHOPHOSPHORIC ACID pH 3.0
                   DETECTION WAVELENGTH: 278nm
                   TEMPERATURE: 40 DEGREES CELCIUS
                   INJECTION VOLUME: 20ul
```

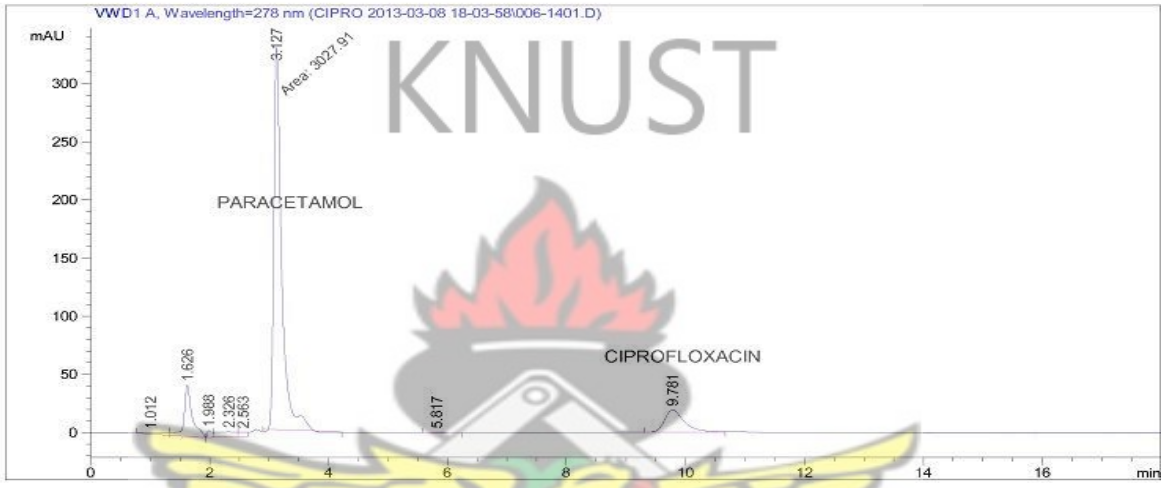



```
==================================================================
                      Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=278 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]      [min]  mAU   *s    [mAU   ]     %
----|-------|----|-------|----------|----------|--------|
   1   1.581 BV   0.2325   45.42768    2.64343   1.0755
   2   1.904 VV   0.0969  734.37158  111.94399  17.3860
   3   2.115 VV   0.1604  130.71712   10.89068   3.0947
   4   2.581 VV   0.1775   40.28219    3.23125   0.9537
   5   2.756 VV   0.0874   15.48973    2.47932   0.3667
   6   2.850 VV   0.1723   31.34449    2.45363   0.7421
   7   3.138 VV   0.1923   30.30823    2.12835   0.7175
   8   3.413 VV   0.2280   30.74051    1.80340   0.7278
   9   3.913 VV   0.3553   37.88957    1.40526   0.8970
  10   4.216 VV   0.1994   17.17764    1.26343   0.4067
  11   4.938 MM   0.2425 3052.74121  209.77200  72.2726
  12   7.943 BB   0.3564    7.56228 2.87471e-1   0.1790
  13  10.591 BB   0.3690   15.32029 5.59509e-1   0.3627
  14  11.841 BB   0.5020   34.55339 9.32995e-1   0.8180

Totals :                 4223.92590  351.79472

==================================================================
                      *** End of Report ***
```

Figure 13: Sample Chromatogram - Subject F (Blank Urine Sample)

Sample Name: SUBJECT F- CIFLOX(1HR AFTER DOSE)

```
==================================================================
Acq. Operator    : DZIGBORDI AGBITOR              Seq. Line :  14
Acq. Instrument  : ERNEST CHEMISTS                 Location : Vial 6
Injection Date   : 3/8/2013 9:51:55 PM                 Inj :   1
                                                  Inj Volume : 20 µl
Acq. Method      : C:\Chem32\1\DATA\CIPRO 2013-03-08 18-03-58\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/8/2013 9:50:34 PM by DZIGBORDI AGBITOR
                   (modified after loading)
Analysis Method  : C:\CHEM32\1\METHODS\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/11/2013 8:51:07 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Method Info      : ASSAY OF CIPROFLOXACIN IN URINE
                   COLUMN: WATERS XBRIDGE C18, 150X4.6mm, 5u
                   MOBILE PHASE: ACETONITRILE: ORTHOPHOSPHORIC ACID pH 3.0
                   DETECTION WAVELENGTH: 278nm
                   TEMPERATURE: 40 DEGREES CELCIUS
                   INJECTION VOLUME: 20ul
```




```
==================================================================
                        Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
Sample Name: SUBJECT F- CIFLOX(1HR AFTER DOSE)
```

Signal 1: VWD1 A, Wavelength=278 nm

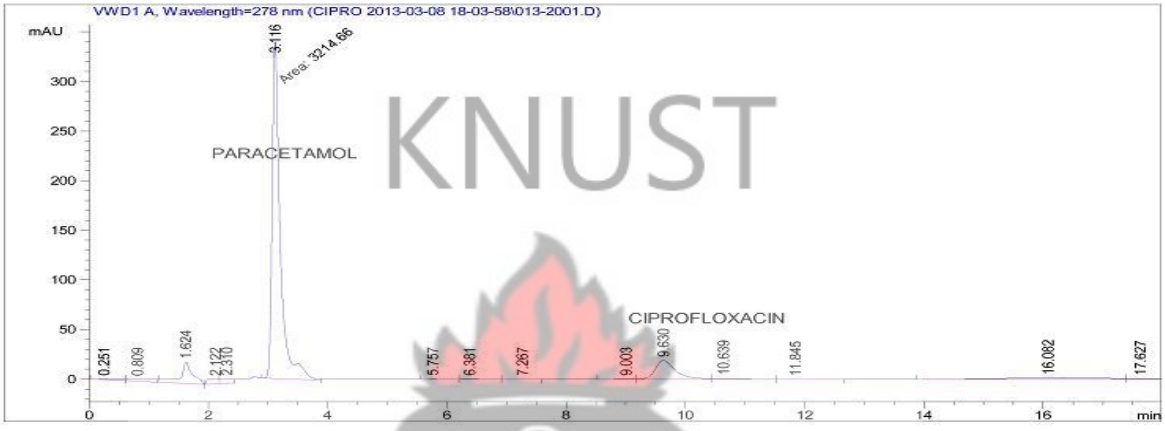
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	1.012	BV	0.4450	50.33595	1.54173	1.2161
2	1.626	VV	0.1349	422.49182	44.18375	10.2069
3	1.988	VV	0.0770	31.59095	5.89888	0.7632
4	2.326	VV	0.3062	108.59777	4.47837	2.6236
5	2.563	VV	0.1407	39.19137	3.99335	0.9468
6	3.127	MM	0.1533	3027.90503	329.26822	73.1506
7	5.817	BB	0.2496	7.59280	4.49253e-1	0.1834
8	9.781	VB	0.3483	451.57111	19.32615	10.9094
Totals :				4139.27680	409.13970	

```
==================================================================
                        *** End of Report ***
```

Figure 14: Sample Chromatogram -Subject F (1hr after oral dose)

Sample Name: SUBJECT F- CIFLOX(1.5HRS AFTER DOSE)

```
Acq. Operator    : DZIGBORDI AGBITOR              Seq. Line :  20
Acq. Instrument  : ERNEST CHEMISTS                 Location : Vial 13
Injection Date   : 3/8/2013 11:48:21 PM               Inj :   1
                                                  Inj Volume : 20 µl
Acq. Method      : C:\Chem32\1\DATA\CIPRO 2013-03-08 18-03-58\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/8/2013 11:46:56 PM by DZIGBORDI AGBITOR
                   (modified after loading)
Analysis Method  : C:\CHEM32\1\METHODS\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/11/2013 8:51:07 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Method Info      : ASSAY OF CIPROFLOXACIN IN URINE
                   COLUMN: WATERS XBRIDGE C18, 150X4.6mm, 5u
                   MOBILE PHASE: ACETONITRILE: ORTHOPHOSPHORIC ACID pH 3.0
                   DETECTION WAVELENGTH: 278nm
                   TEMPERATURE: 40 DEGREES CELCIUS
                   INJECTION VOLUME: 20ul
```



Area Percent Report

```
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: VWD1 A, Wavelength=278 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	0.251	BV	0.4877	30.19117	7.93203e-1	0.6736
2	0.809	VB	0.4260	74.09818	2.19431	1.6532
3	1.624	BV	0.1920	315.47263	21.81785	7.0386
4	2.122	VV	0.1800	65.17458	4.94482	1.4541
5	2.310	VV	0.2124	70.15521	4.53823	1.5653
6	3.116	MM	0.1575	3214.66235	340.13516	71.7237
7	5.757	BB	0.3383	4.64847	1.73752e-1	0.1037
8	6.381	BV	0.3180	7.41994	3.22365e-1	0.1655
9	7.267	VV	0.3002	4.56394	2.12408e-1	0.1018
10	9.003	BV	0.2870	9.81790	4.54464e-1	0.2191
11	9.630	VB	0.3540	456.64307	19.14140	10.1884
12	10.639	BV	0.4862	32.18393	9.84634e-1	0.7181
13	11.845	VB	0.4381	5.52140	1.88340e-1	0.1232
14	16.082	BV	1.3955	168.15474	1.49305	3.7518
15	17.627	VBA	0.4786	23.30384	7.27402e-1	0.5199
Totals :				4482.01135	398.12138	

*** End of Report ***

Figure 15: Sample Chromatogram - Subject F (1.5 hrs after oral dose)

Sample Name: SUBJECT F- CIFLOX(2HRS AFTER DOSE)

```
==================================================================
Acq. Operator    : DZIGBORDI AGBITOR              Seq. Line :  26
Acq. Instrument  : ERNEST CHEMISTS                 Location : Vial 16
Injection Date   : 3/9/2013 1:44:43 AM                 Inj :   1
                                                  Inj Volume : 20 µl
Acq. Method      : C:\Chem32\1\DATA\CIPRO 2013-03-08 18-03-58\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/9/2013 1:43:18 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Analysis Method  : C:\CHEM32\1\METHODS\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/11/2013 8:51:07 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Method Info      : ASSAY OF CIPROFLOXACIN IN URINE
                   COLUMN: WATERS XBRIDGE C18, 150X4.6mm, 5u
                   MOBILE PHASE: ACETONITRILE: ORTHOPHOSPHORIC ACID pH 3.0
                   DETECTION WAVELENGTH: 278nm
                   TEMPERATURE: 40 DEGREES CELCIUS
                   INJECTION VOLUME: 20ul
```


```
==================================================================
                        Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
Sample Name: SUBJECT F- CIFLOX(2HRS AFTER DOSE)
```

Signal 1: VWD1 A, Wavelength=278 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	0.818	BB	0.4537	61.50893	1.71354	1.6171
2	1.625	BV	0.2307	211.49481	11.73428	5.5604
3	2.574	VV	0.4857	200.87494	5.06800	5.2812
4	2.771	VV	0.1828	90.25217	6.60092	2.3728
5	3.115	MM	0.1537	2945.03735	319.41211	77.4284
6	4.413	BB	0.5701	109.85796	2.38206	2.8883
7	9.613	BB	0.3414	159.74622	6.93762	4.1999
8	10.599	BB	0.4056	13.64672	4.43431e-1	0.3588
9	15.164	BV	0.5301	11.14302	2.57982e-1	0.2930
Totals :				3803.56211	354.54994	

```
==================================================================
                        *** End of Report ***
```

Figure 16: Sample Chromatogram - Subject F (2hrs after oral dose)

Sample Name: SUBJECT F- CIFLOX(2.5HRS AFTER DOSE)

```
==================================================================
Acq. Operator    : DZIGBORDI AGBITOR              Seq. Line :  32
Acq. Instrument  : ERNEST CHEMISTS                 Location : Vial 23
Injection Date   : 3/9/2013 3:40:57 AM                  Inj :   1
                                                 Inj Volume : 20 µl
Acq. Method      : C:\Chem32\1\DATA\CIPRO 2013-03-08 18-03-58\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/9/2013 3:39:34 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Analysis Method  : C:\CHEM32\1\METHODS\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/11/2013 8:51:07 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Method Info      : ASSAY OF CIPROFLOXACIN IN URINE
                   COLUMN: WATERS XBRIDGE C18, 150X4.6mm, 5u
                   MOBILE PHASE: ACETONITRILE: ORTHOPHOSPHORIC ACID pH 3.0
                   DETECTION WAVELENGTH: 278nm
                   TEMPERATURE: 40 DEGREES CELCIUS
                   INJECTION VOLUME: 20ul
```


```
==================================================================
                        Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=278 nm
```

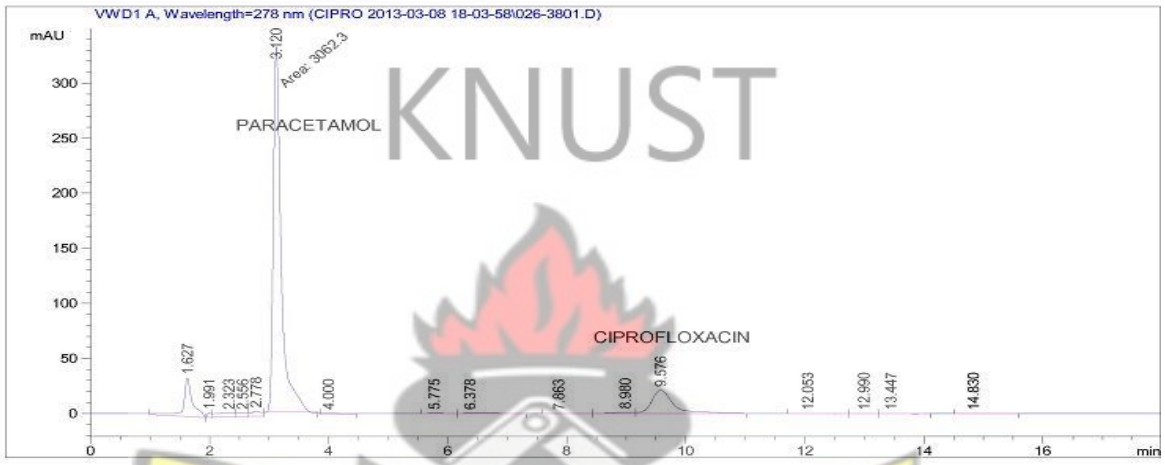
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	1.625	BV	0.1854	159.04720	11.24518	4.4215
2	2.560	VV	0.5247	175.38930	4.10465	4.8758
3	2.773	VV	0.1700	66.80183	5.31136	1.8571
4	3.116	MM	0.1518	3047.08838	334.50204	84.7095
5	4.995	BB	0.3246	3.45895	1.36558e-1	0.0962
6	5.801	BV	0.2428	2.31089	1.22813e-1	0.0642
7	7.704	BB	0.1635	9.81034e-1	9.55263e-2	0.0273
8	9.610	BB	0.3357	138.40630	6.28096	3.8477
9	14.875	BB	0.2422	2.19915	1.20421e-1	0.0611
10	17.699	BV	0.1987	1.42032	1.15557e-1	0.0395
Totals :				3597.10334	362.03507	

```
==================================================================
                        *** End of Report ***
```

Figure 17: Sample Chromatogram - Subject F (2.5 hrs after oral dose)

Sample Name: SUBJECT F- CIFLOX(3HRS AFTER DOSE)

```
==================================================================
Acq. Operator    : DZIGBORDI AGBITOR              Seq. Line :  38
Acq. Instrument  : ERNEST CHEMISTS                 Location : Vial 26
Injection Date   : 3/9/2013 5:37:24 AM                 Inj :   1
                                                  Inj Volume : 20 µl
Acq. Method      : C:\Chem32\1\DATA\CIPRO 2013-03-08 18-03-58\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/9/2013 5:35:59 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Analysis Method  : C:\CHEM32\1\METHODS\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/11/2013 8:51:07 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Method Info      : ASSAY OF CIPROFLOXACIN IN URINE
                   COLUMN: WATERS XBRIDGE C18, 150X4.6mm, 5u
                   MOBILE PHASE: ACETONITRILE: ORTHOPHOSPHORIC ACID pH 3.0
                   DETECTION WAVELENGTH: 278nm
                   TEMPERATURE: 40 DEGREES CELCIUS
                   INJECTION VOLUME: 20ul
```




```
==================================================================
                        Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
Signal 1: VWD1 A, Wavelength=278 nm
```

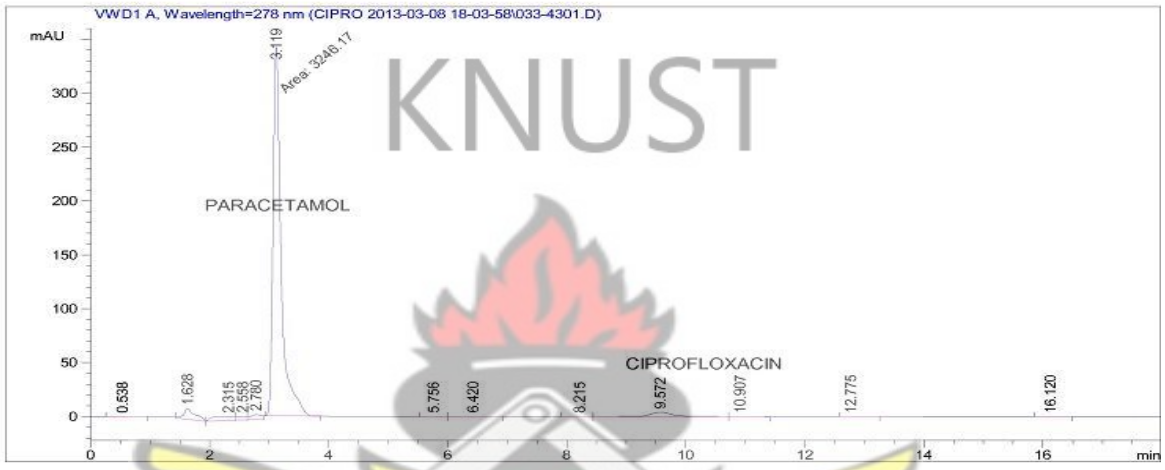
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	1.627	BV	0.1471	372.57248	35.11067	8.6757
2	1.991	VV	0.0708	20.59790	4.26985	0.4796
3	2.323	VV	0.2899	82.75639	3.62179	1.9271
4	2.556	VV	0.1641	38.39677	3.31784	0.8941
5	2.778	VV	0.1731	59.67989	4.64938	1.3897
6	3.120	MM	0.1537	3062.30322	332.12756	71.3083
7	4.000	VB	0.3521	34.12371	1.27870	0.7946
8	5.775	BV	0.2210	7.46502	5.07823e-1	0.1738
9	6.378	VB	0.4429	17.22288	5.10730e-1	0.4010
10	7.863	BB	0.3099	3.87527	1.67960e-1	0.0902
11	8.980	BV	0.3494	31.53722	1.30256	0.7344
12	9.576	VB	0.3899	550.86548	21.24569	12.8274
13	12.053	BB	0.2328	2.97415	2.12968e-1	0.0693
14	12.990	BV	0.2802	3.19449	1.82290e-1	0.0744
15	13.447	VB	0.2141	1.97617	1.56690e-1	0.0460
16	14.830	BB	0.3648	4.91480	1.98325e-1	0.1144
Totals :				4294.45586	408.86083	

```
==================================================================
```

Figure 18: Sample Chromatogram - Subject F (3hrs after oral dose)

Sample Name: SUBJECT F- CIFLOX(4HRS AFTER DOSE)

```
================================================================================
Acq. Operator    : DZIGBORDI AGBITOR                Seq. Line :  43
Acq. Instrument  : ERNEST CHEMISTS                   Location : Vial 33
Injection Date   : 3/9/2013 7:14:34 AM                   Inj :   1
                                                   Inj Volume : 20 µl
Acq. Method      : C:\Chem32\1\DATA\CIPRO 2013-03-08 18-03-58\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/9/2013 7:13:07 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Analysis Method  : C:\CHEM32\1\METHODS\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/11/2013 8:51:07 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Method Info      : ASSAY OF CIPROFLOXACIN IN URINE
                   COLUMN: WATERS XBRIDGE C18, 150X4.6mm, 5u
                   MOBILE PHASE: ACETONITRILE: ORTHOPHOSPHORIC ACID pH 3.0
                   DETECTION WAVELENGTH: 278nm
                   TEMPERATURE: 40 DEGREES CELCIUS
                   INJECTION VOLUME: 20ul
```

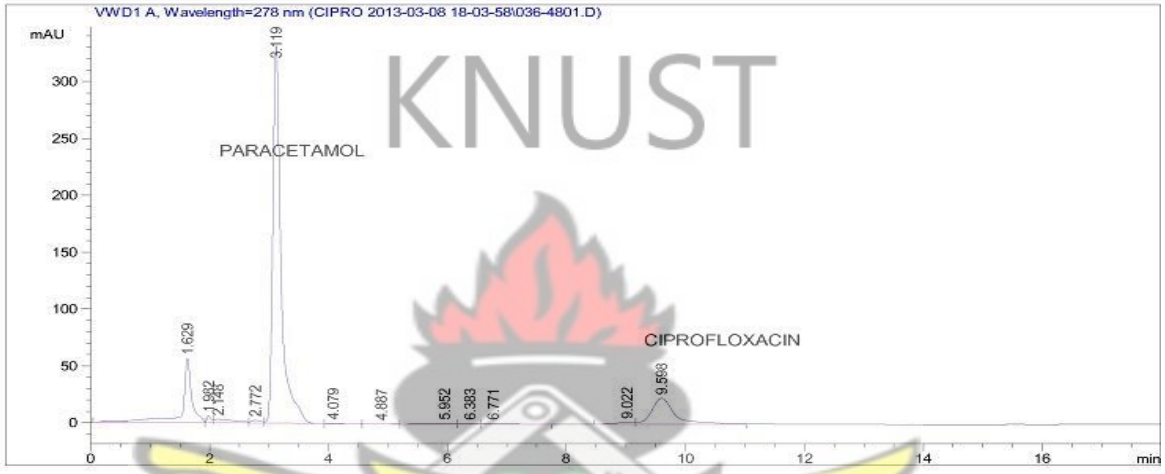



```
================================================================================
                          Area Percent Report
================================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=278 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]      [min]  mAU   *s    [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1   0.538 BB    0.2332    2.51105 1.41556e-1   0.0669
   2   1.628 BV    0.1795  140.53923   10.30707   3.7471
   3   2.315 VV    0.3441  103.62963    3.87419   2.7630
   4   2.558 VV    0.1654   40.55779    3.40022   1.0814
   5   2.780 VV    0.1853   61.69026    4.52270   1.6448
   6   3.119 MM    0.1579 3246.17139  342.63226  86.5493
   7   5.756 BV    0.2422    2.29876 1.48145e-1   0.0613
   8   6.420 VB    0.3208    5.23414 2.13601e-1   0.1396
   9   8.215 BV    0.2508    2.76803 1.58117e-1   0.0738
  10   9.572 VB    0.4598  133.99983    4.23036   3.5727
  11  10.907 BV    0.3475    7.52628 3.03595e-1   0.2007
  12  12.775 BB    0.3120    1.94610 7.94417e-2   0.0519
  13  16.120 BB    0.2042    1.78729 1.13452e-1   0.0477

Totals :                  3750.65978  370.12471

================================================================================
                          *** End of Report ***
```

Figure 19: Sample Chromatogram - Subject F (4hrs after oral dose)

Sample Name: SUBJECT F- CIFLOX(5HRS AFTER DOSE)

```
==================================================================
Acq. Operator    : DZIGBORDI AGBITOR              Seq. Line :  48
Acq. Instrument  : ERNEST CHEMISTS                 Location : Vial 36
Injection Date   : 3/9/2013 8:51:38 AM                Inj :   1
                                                  Inj Volume : 20 µl
Acq. Method      : C:\Chem32\1\DATA\CIPRO 2013-03-08 18-03-58\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/9/2013 8:50:10 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Analysis Method  : C:\CHEM32\1\METHODS\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/11/2013 8:51:07 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Method Info      : ASSAY OF CIPROFLOXACIN IN URINE
                   COLUMN: WATERS XBRIDGE C18, 150X4.6mm, 5u
                   MOBILE PHASE: ACETONITRILE: ORTHOPHOSPHORIC ACID pH 3.0
                   DETECTION WAVELENGTH: 278nm
                   TEMPERATURE: 40 DEGREES CELCIUS
                   INJECTION VOLUME: 20ul
```




```
==================================================================
                      Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=278 nm
```

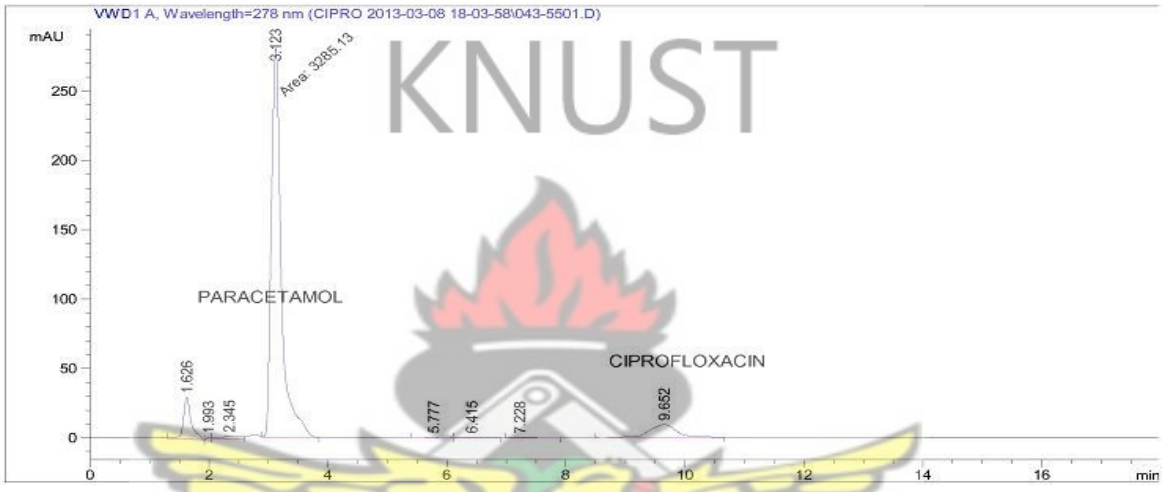
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	1.629	BV	0.1543	635.26514	56.57688	13.4969
2	1.982	VV	0.0691	31.56782	6.40883	0.6707
3	2.148	VV	0.3210	81.22242	3.24601	1.7257
4	2.772	VV	0.1545	27.66801	2.51690	0.5878
5	3.119	VV	0.1456	3231.29785	331.83493	68.6526
6	4.079	VV	0.2516	8.50467	4.77167e-1	0.1807
7	4.887	VV	0.2186	2.37305	1.46124e-1	0.0504
8	5.952	VV	0.2810	14.37440	7.33621e-1	0.3054
9	6.383	VV	0.2489	12.00608	7.23625e-1	0.2551
10	6.771	VB	0.3993	18.03076	5.42669e-1	0.3831
11	9.022	BV	0.3059	36.85898	1.73607	0.7831
12	9.598	VB	0.3981	607.56604	22.81130	12.9084
Totals :				4706.73522	427.75412	

```
==================================================================
                      *** End of Report ***
```

Figure 20: Sample Chromatogram - Subject F (5hrs after oral dose)

Sample Name: SUBJECT F- CIFLOX(6HRS AFTER DOSE)

```
==================================================================
Acq. Operator    : DZIGBORDI AGBITOR              Seq. Line :  55
Acq. Instrument  : ERNEST CHEMISTS                 Location : Vial 43
Injection Date   : 3/9/2013 11:07:36 AM                 Inj :  1
                                                 Inj Volume : 20 µl
Acq. Method      : C:\Chem32\1\DATA\CIPRO 2013-03-08 18-03-58\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/9/2013 11:06:08 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Analysis Method  : C:\CHEM32\1\METHODS\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/11/2013 8:51:07 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Method Info      : ASSAY OF CIPROFLOXACIN IN URINE
                   COLUMN: WATERS XBRIDGE C18, 150X4.6mm, 5u
                   MOBILE PHASE: ACETONITRILE: ORTHOPHOSPHORIC ACID pH 3.0
                   DETECTION WAVELENGTH: 278nm
                   TEMPERATURE: 40 DEGREES CELCIUS
                   INJECTION VOLUME: 20ul
```

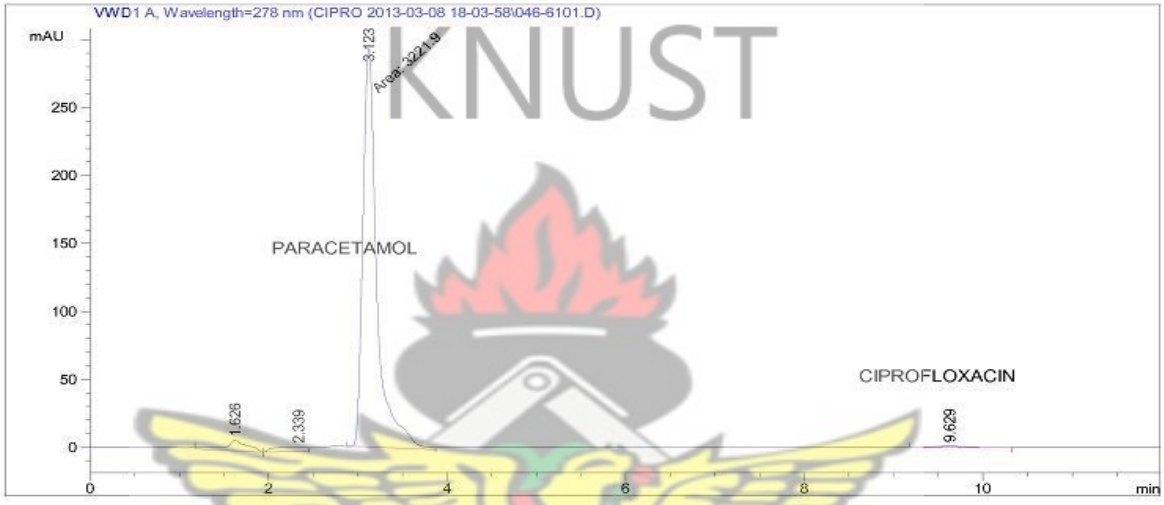



```
==================================================================
                        Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=278 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]      [min]  mAU   *s    [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1   1.626 BV    0.1327  277.34189   30.37704   6.9607
   2   1.993 VV    0.0678    9.08298    1.98941   0.2280
   3   2.345 VV    0.3528   62.77469    2.32554   1.5755
   4   3.123 MM    0.1949 3285.12866  280.89590  82.4504
   5   5.777 BV    0.2659    8.37995 4.34217e-1   0.2103
   6   6.415 VB    0.3545    8.72848 3.50617e-1   0.2191
   7   7.228 BB    0.3405    6.00880 2.13193e-1   0.1508
   8   9.652 BB    0.4978  326.92490    9.56438   8.2052

Totals :                   3984.37035  326.15031

==================================================================
                        *** End of Report ***
```

Figure 21: Sample Chromatogram - Subject F (6hrs after oral dose)

Sample Name: SUBJECT F- CIFLOX(7HRS AFTER DOSE)

```
==================================================================
Acq. Operator    : DZIGBORDI AGBITOR              Seq. Line :  61
Acq. Instrument  : ERNEST CHEMISTS                 Location : Vial 46
Injection Date   : 3/9/2013 1:04:12 PM                  Inj :   1
                                                 Inj Volume : 20 µl
Acq. Method      : C:\Chem32\1\DATA\CIPRO 2013-03-08 18-03-58\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/9/2013 1:06:43 PM by DZIGBORDI AGBITOR
                   (modified after loading)
Analysis Method  : C:\CHEM32\1\METHODS\CIPRO BIOANALYTICAL METHOD.M
Last changed     : 3/11/2013 8:51:07 AM by DZIGBORDI AGBITOR
                   (modified after loading)
Method Info      : ASSAY OF CIPROFLOXACIN IN URINE
                   COLUMN: WATERS XBRIDGE C18, 150X4.6mm, 5u
                   MOBILE PHASE: ACETONITRILE: ORTHOPHOSPHORIC ACID pH 3.0
                   DETECTION WAVELENGTH: 278nm
                   TEMPERATURE: 40 DEGREES CELCIUS
                   INJECTION VOLUME: 20ul
```




```
==================================================================
                        Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=278 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]      [min]  mAU   *s    [mAU   ]      %
----|-------|----|------|----------|----------|--------|
   1   1.626 BV    0.2354  140.77019    7.74283   4.0539
   2   2.339 VV    0.3520   85.04446    3.10044   2.4491
   3   3.123 MM    0.1828 3221.90039  293.72940  92.7853
   4   9.629 BB    0.4094   24.70934 8.95205e-1   0.7116

Totals :                  3472.42439  305.46788

==================================================================
                        *** End of Report ***
```

Figure 22: Sample Chromatogram - Subject F (7hrs after oral dose)

INFORMED CONSENT FORM- BIOEQUIVALENCE STUDY OF CIFLOX TABLETS

I am by this form asking for your voluntary participation in my project work. Please read the following information which provides an overview of my project. If you would like to participate, please indicate by signing the consent column provided.

TITLE OF PROJECT :Bioequivalent study of Ciflox 500 (CiprofloxacinHydrochloride equivalent to Ciprofloxacin 500mg)tabletusing urinary excretion data.

SUPERVISING AUTHORITY : Prof. R.K. Adosraku

Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi

NAME OF STUDENT UNDERTAKING PROJECT:Dzigbordi Yao Agbitor

PURPOSE OF PROJECT : To determine whetherCiflox tablet, a locally manufactured market brand of Ciprofloxacin in Ghana is bioequivalent to the referenceproductCiprinol, for which reason Cifloxcould be administered in place of the reference product and would be expected to work to the same extent as the reference product.

YOUR ROLE IF YOU DECIDE TO PARTICIPATE:

You would be required to take a single dose of either Ciflox or Ciprinol and express urine over a seven hour period (at start of the test,t=0, 1, 1.5,2,2.5,3, 4, 5,6 and 7hrs). After at least 7days, a dose of Ciflox or Ciprinol (i.e. different product from what was administered earlier) would be taken and the regime for the collection of the urine sample repeated.

After excretion of urine at each time point you are expected to drink about 250ml of water.

CONFIDENTIALITY:

Absolute confidentiality pertaining to your participation in the study is assured. A code would be assigned to you and your name would not appear anywhere in the conduct or reporting of the outcome of the study.

VOLUNTARY PARTCIPATION: Participation in the study is entirely voluntary. You may choose not to participate in the study or stop participating at anytime during the conduct of the study without any consequence whatsoever.Please note that the exercise is for academic purposes and no payments would be made to you for participating.

PLACE OF CONDUCT OF STUDY: Ernest Chemists Ltd.,
Manufacturing Division
Near DVLA Office
Tema

Consent:

By signing this form, I attest that I have ever taken Ciprofloxacin tablets with no observed side effects and have read and fully understand the information provided and I give my consent to participate in the study.

Patrick Frawoyife — Signature — 01-02-2013

Name | Signature | Date

Place of work: Ernest Chemists Limited