

**COMPARATIVE BIOEQUIVALENCE STUDIES OF SOME SELECTED BRANDS OF
CIPROFLOXACIN TABLETS MARKETED IN ZARIA**

By

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CIPROFLOXACIN TABLETS MARKETED IN ZARIA**

By

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**DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY
FACULTY OF PHARMACEUTICAL SCIENCE
AHMADU BELLO UNIVERSITY, ZARIA**

NOVEMBER, 2014

DECLARATION

I declare that the work in this Thesis titled “Comparative Bioequivalence Studies of some selected Brands of Ciprofloxacin Tablets marketed in Zaria” has been carried out by me in the Department of Pharmaceutical and Medicinal Chemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

MOHAMMED Fatima Zongoma

Signature

Date

CERTIFICATION

This thesis titled COMPARATIVE BIOEQUIVALENCE STUDIES OF SOME SELECTED BRANDS OF CIPROFLOXACIN TABLETS MARKETED IN ZARIA by FATIMA ZONGOMA MOHAMMED meets the regulations governing the award of the degree of Master in pharmaceutical chemistry of the Ahmadu Bello University, and is approved for its contribution to Knowledge and literary presentation.

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DEDICATION

This project is dedicated to Almighty Allah, the creator of all things of life, wisdom and knowledge.

ACKNOWLEDGEMENT

In the name of Allah, the most Gracious, the most Merciful; all praises be to Allah, the lord of the world and prayers be upon His last prophet and messenger, Mohammed (S.A.W) Thanks be to Allah for his guidance, mercy, protection and blessings.

My appreciation goes to my Dad, May your gentle soul rest in perfect peace Ameen and Mum who are the building block of my success, May Allah (S.W.T) in his infinite mercy reward you both with Al-jannatul Firdausi, Ameen.

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My warmest regards goes to my siblings Aliyu, Aisha, Umar, Abdurrahman, Aminu, Hauwa.

Wishing you Allah's guidance throughout your lives

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ABSTRACT

Comparative bioequivalence studies of six selected brands of ciprofloxacin tablets was successfully conducted. In-vitro quality control studies were carried out on six randomly selected brands of ciprofloxacin to establish identity, weight uniformity, friability, crushing strength, disintegration, dissolution and assay according to BP 2002 and 2009.

Cheap, simple and available method of analysis of ciprofloxacin in saliva sample was developed by U.V Spectrophotometer with variable wavelengths. The developed method was validated based on ICH guidelines. Bioequivalence parameters (C_{max} , T_{max} and AUC) were evaluated on the six brands of ciprofloxacin to determine their interchangeability after a wash-out period of one week.

From the result of in-vitro studies all brands passed the test indicating pharmaceutical equivalence. All the brands were found to have the labeled active ingredient as they were within the accepted range of 95-105%. The percentage recovery was within the accepted range of 95 - 105%. Calibration curve was constructed and was linear within the range of 1-6 μ g/ml as the correlation coefficient was 0.9889. The regression equation was $y = 0.1092x + 0.2015$. Distilled water was used as solvent and 277nm was found to be wavelength of maximum absorption at pH4. The developed method was found to be accurate and precise.

The C_{max} (μ g/ml) of brands A (Reference), B, C, D, E and F were 2.83, 2.61, 2.51, 2.79, 2.67 and 2.78 respectively. Mean T_{max} (hrs) of Brands A, B, C, D, E and F was 2hrs. AUC (μ g/ml/hr) of Brands A, B, C, D, E and F were 14.06, 12.09, 12.095, 12.18, 11.43 and 12.38 respectively. Point estimate ratio for C_{max} and AUC of Brands A and B was 92.23% and 85.99%, A and C was 88.69% and 86.02%, A and D was 98.59% and 86.62%, A and E was

94.35% and 80.65%, A and F was 98.23% and 88.05% respectively. Acceptable range for bioequivalence parameters is 80-125%.

Based on the result from the in-vitro quality control studies, all the brands complied with the pharmacopoeial specifications. The UV Spectrophotometric method developed is simple, cheap, effective, rapid, precise and available. There was no significant difference in pharmacokinetic parameters used to assess bioequivalence. Therefore, it is concluded that the test drug product is bioequivalent to the reference brand and therefore interchangeable.

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ABBREVIATIONS

AUC	area under the curve
Tmax	time to reach maximum plasma concentration
Cmax	maximum plasma concentration
BP	British pharmacopoeia
GMP	good manufacturing practice
ICH	international conference on harmonization
IP	Indian pharmacopoeia
KgF	kilogram force
NAFDAC	Nigeria agency for food and drug administration
PR	percentage recovery
RSD	relative standard deviation
SD	standard deviation
SEM	standard error of the mean
U.V	ultraviolet
USP	United state pharmacopoeia
λ_{max}	maximum wavelength

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Preamble

There is growing trade in substandard and counterfeit drugs including antibiotics around the world. The prevalence of such substandard drugs in Nigerian markets has been worrisome to both regulatory agencies and all concerned. The availability of drugs, especially antibiotics in open markets has led to indiscriminate use and may contribute to high incidence of antibiotic resistant strains (Mukhtar *et al.*, 2010). The need to select one product from several generic drug products of the same active ingredients during the course of therapy is also a cause for concern to healthcare practitioners (Adegbolagun *et al.*, 2007).

Generic drug is a product sold under the chemical name of a branded drug, after the expiry of the patent for a branded drug. Both the branded and the generic versions must have the same potency, be available in the same dosage forms (i.e. tablets, liquids, injectables) and be demonstrated to be safe and effective. The generic drugs are less expensive as compared to branded drugs as generic manufacturers do not have the investment costs of the developer of a new drug. New drugs are generally developed under patent protection. Generic drugs are usually far cheaper than branded drugs, sometime up to 90%. Efficacy and safety of pharmaceutical product depends on its standards preset to assure the desired purpose. The efficiency of pharmaceutical products depends on its formulation properties and manufacturing methods, hence it is likely that the quality of pharmaceutical products may vary (Yogananda *et al.*, 2009).

The first stage in ascertaining the therapeutic equivalence of any drug product involves ascertaining the chemical and biopharmaceutical equivalency of such drug product. Drug products that are chemically and biopharmaceutically equivalent must be identical in strength, quality, purity as well as content uniformity, disintegration and dissolution rate (Adegbolagun *et al.*, 2007). The need to ensure that the generic and branded drug products are pharmaceutically and therapeutically equivalent cannot be over emphasized. The safety and efficacy of drug products can be guaranteed when their quality is reliable and reproducible from batch to batch. To ensure the requisite quality, drug manufacturers are required to test their products during and after manufacturing and at various intervals during the shelf life of the product (Getu and Awot, 2010).

"Bioequivalence" is a comparison of the bioavailability of two or more drug products. Thus, two products or formulations containing the same active ingredient are bioequivalent if their rates and extent of absorption are the same. When a new formulation of an existing drug is developed, its bioavailability is generally evaluated relative to the standard formulation of the originator (Rasma, 1996). For a generic drug to be considered bioequivalent to a pioneer product there must be no statistical differences (as specified in the accepted criteria) between their plasma concentration-time profiles. Because two products rarely exhibit absolutely identical profiles, some degree of difference must be considered acceptable (Rasma, 1996).

Since the concentration of a drug in blood is used as an assessment of its clinical performance, inherent in the demonstration that two preparations containing equivalent amounts of the same drug produce similar concentrations of the drug entity in blood is the assumption that they will elicit equivalent drug responses. Thus, two products that are

deemed to be bioequivalent are also assumed to be therapeutically equivalent, and therefore interchangeable (Rasma, 1996). In determining bioequivalence, for example, between two products such as a commercially-available brand product and a potential to-be-marketed generic product, pharmacokinetic studies are conducted whereby each of the preparations are administered in a cross-over study to volunteer subjects, generally healthy individuals but occasionally in patients. Serum/plasma samples are obtained at regular intervals and assayed for parent drug (or occasionally metabolite) concentration. Occasionally, blood concentration levels are neither feasible nor possible to compare the two products (e.g. inhaled corticosteroids), and then pharmacodynamic end points rather than pharmacokinetic end points are used for comparison. For a pharmacokinetic comparison, the plasma concentration data are used to assess key pharmacokinetic parameters such as area under the curve (AUC), peak concentration (C_{max}), time to peak concentration (T_{max}), and absorption lag time (t_{lag}). Testing should be conducted at several different doses, especially when the drug displays non-linear pharmacokinetics (Birkett, 2003).

In general, the FDA considers two products to be "therapeutic equivalents" if they each meet the following criteria;

1. They are pharmaceutically equivalent
2. They are bioequivalent (demonstrated either by a bioavailability measurement or an in-vitro standard)
3. They are in compliance with compendial standards for strength, quality, purity and identity
4. They are adequately labeled

5. They have been manufactured in compliance with Good Manufacturing Practices as established by the FDA (Rasma, 1996).

Ciprofloxacin hydrochloride is 1-cyclo-propyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazine-1-yl) quinoline-3-carboxylic acid hydrochloride. Ciprofloxacin is a quinolone carboxylic acid derivative with an extensive antibacterial spectrum of activity. Ciprofloxacin given as an oral tablet is rapidly and well absorbed from the gastrointestinal tract and this can be satisfactorily described as a zero-order process. It also exhibits a rapid onset of action, and lacks cross-reactivity with penicillin, cephalosporins and the amino glycosides. In the 1990's, there were just a few brands of ciprofloxacin in the Nigerian market but recently many brands have flooded the market. The absolute bioavailability is approximately 70% with no substantial loss by first pass metabolism. Maximum (peak) serum concentration is attained 1 to 2 hours after oral dosing. Serum concentrations increase proportionately with doses up to 1000mg (Osonwa *et al.*, 2011). Ciprofloxacin shows bactericidal action by inhibition of DNA super coiling in the bacteria (Yogananda *et al.*, 2009).

The drug has been shown to be active against most strains of the following microorganisms both *in vitro* and in clinical infections:

Aerobic Gram-positive Microorganisms; *Enterococcus faecalis*, *Streptococcus pneumonia*, *Streptococcus pyrogenes*, *Staphylococcus saprophyticus*, *Staphylococcus aureus*.

Aerobic Gram-negative Microorganisms; *Enterobacter cloacae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumonia*, *Neisseria gonorrhoeae*, *Shigella sonnei*.

Ciprofloxacin is indicated in infections of the urinary, gastro intestinal, respiratory tracts, tissue infections, gonorrhoea and septicaemia caused by sensitive organisms (Osonwa *et al.*, 2011).

1.2 Statement of Research Problem

The increase in the number of generic drug products from multiple sources has placed prescribers to select one out of the several products. However, there is high level of drug faking and substandard drugs are widespread in Nigerian markets. This has a negative impact on the patient as well as the healthcare delivery system with rapid emergence of bacterial drug resistance.

1.3 Justification

With the high levels of drug faking and widespread of substandard drugs in Nigerian markets, there is a need to assess the various brands of Ciprofloxacin tablets available to ascertain whether they are bioequivalent, meet the official requirement for quality and thus can be used interchangeably.

1.4 Aims and Objectives of the Study

- To evaluate and compare selected brands of Ciprofloxacin marketed in Zaria
- To carry out pharmaceutical equivalence studies on Ciprofloxacin tablets
- To develop and validate UV spectrophotometric method for analysis of Ciprofloxacin in tablets
- To evaluate pharmacokinetic parameters (C_{\max} , T_{\max} and AUC)

1.5 Hypothesis

There is no statistical difference between branded ciprofloxacin tablet and other generic brands of Ciprofloxacin tablets

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Bioequivalence

Bioequivalence is a term in pharmacokinetics used to assess the expected in-vivo biological equivalence of two proprietary preparations of a drug. If two products are said to be bioequivalent it means that they would be expected to be, for all intents and purposes, the same. 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. Pharmaceutical equivalence implies the same amount of the same active substance(s), in the same dosage form, for the same route of administration and meeting the same or comparable standards (Birkett, 2003).

The United States Food and Drug Administration (FDA) has defined bioequivalence 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." (SenthilKumar *et al.*, 2012)

2.1.1 Regulatory definitions

2.1.1.1 Australia

In Australia, the Therapeutics Goods Administration (TGA) considers preparations to be bioequivalent if the 90% confidence intervals (90% CI) of the rate ratios, between the

two preparations, of C_{\max} and AUC lie in the range 0.80-1.25. T_{\max} should also be similar between the products. There are tighter requirements for drugs with a narrow therapeutic index and/or saturable metabolism, thus no generic products exist on the Australian market for digoxin or phenytoin for instance (Bikett, 2003).

2.1.1.2 Europe

According to regulations applicable in the European Economic Area two medicinal products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives and if their bioavailabilities after administration in the same molar dose are similar to such a degree that their effects, with respect to both efficacy and safety, will be essentially the same. This is considered demonstrated if the 90% confidence intervals (90% CI) of the ratios for AUC_{0-t} and C_{\max} between the two preparations lie in the range 80.00 – 125.00% (EMA, 2010).

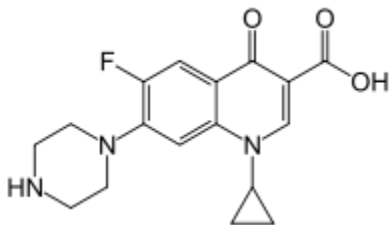
2.1.1.3 United States

The FDA considers two products bioequivalent if the 90% CI of the relative mean C_{\max} , $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of the test (e.g. generic formulation) to reference (e.g. innovator brand formulation) should be within 80.00% to 125.00% in the fasting state. Although there are a few exceptions, generally a bioequivalent comparison of test to reference formulations also requires administration after an appropriate meal at a specified time before taking the drug, a so-called "fed" or "food-effect" study. A food-effect study requires the same statistical evaluation as the fasting study (CDER, 2003).

2.2 Ciprofloxacin

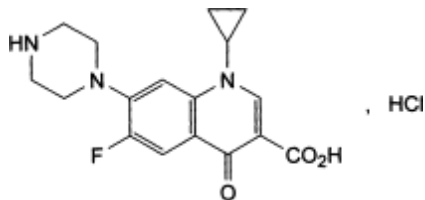
2.2.1 Description

Ciprofloxacin is a synthetic antibiotic of the fluoroquinolone drug class. It is a second-generation fluoroquinolone antibacterial (Pranshu *et al.*, 2012). Ciprofloxacin is a 1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid. Its empirical formula is $C_{17}H_{18}FN_3O_3$ and its molecular weight is 331.4 g/mol (BP, 2009).



Ciprofloxacin hydrochloride (USP) is the monohydrochloride monohydrate salt of ciprofloxacin. It is a 1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid hydrochloride. It is a pale yellow, crystalline powder, slightly hygroscopic with a molecular weight of 367.8g/mol.

Its empirical formula is $C_{17}H_{18}FN_3O_3 \cdot HCl$ (BP, 2009)



Ciprofloxacin tablets are available as film-coated tablets in 250 mg, 500 mg and 750 mg (ciprofloxacin equivalent) strengths. Ciprofloxacin tablets are white to off-white. The inactive ingredients are colloidal silicon dioxide, croscarmellose sodium, magnesium

stearate, microcrystalline cellulose, and povidone. The film coating contains hypromellose, polyethylene glycol, and titanium dioxide (Bayer healthcare pharmaceuticals, 2009).

2.2.2 Clinical pharmacology

2.2.2.1 Pharmacokinetics

Ciprofloxacin is rapidly and well absorbed from the gastrointestinal tract. Oral bioavailability is about 70 to 80% and a peak serum concentration of about 2.4 micrograms/ml occurs 1 to 2 hours after a 500-mg oral dose. Absorption of ciprofloxacin tablets may be delayed by the presence of food, but is not substantially affected overall. Plasma protein binding ranges from 20 to 40%. Ciprofloxacin is widely distributed in the body and tissue penetration is generally good. It appears in the cerebro-spinal fluid (CSF), but concentrations are only about 10% of those in serum when the meninges are not inflamed. Ciprofloxacin crosses the placenta and is also distributed into breast milk. High concentrations are achieved in bile (Sean, 2009).

The elimination half-life is about 3 to 5 hours and there is evidence of modest accumulation. Half-life may be prolonged in renal impairment (a value of 8 hours has been reported in end-stage renal disease) and to some extent in the elderly. However, no dose adjustment is usually necessary in patients with renal impairment unless it is severe. Similarly, usual doses can be given to the elderly except in those with severe renal impairment. There is limited information on the effect of hepatic impairment. The half-life of ciprofloxacin has been reported to be slightly prolonged in patients with severe

cirrhosis of the liver. With one or two exceptions, most studies have shown that the pharmacokinetics of ciprofloxacin is not markedly affected by cystic fibrosis. Ciprofloxacin is eliminated principally by urinary excretion, but non-renal clearance may account for about one-third of elimination and includes hepatic metabolism, biliary excretion, and possibly transluminal secretion across the intestinal mucosa (Sean, 2009). Four metabolites have been identified in human. The metabolites have antimicrobial activity, but are less active than unchanged ciprofloxacin. The primary metabolites are Oxociprofloxacin and sulfociprofloxacin as major fecal metabolite, each accounting for roughly 3% to 8% of the total dose. Other minor metabolites are desethyleneciprofloxacin and formylciprofloxacin (Bayer Healthcare Pharmaceuticals, 2009). Urinary excretion is by active tubular secretion as well as glomerular filtration and is reduced by probenecid, it is virtually complete within 24 hours. About 40 to 50% of an oral dose is excreted unchanged in the urine and about 15% as metabolites. Up to 70% of a parenteral dose may be excreted unchanged within 24 hours and 10% as metabolites. Faecal excretion over 5 days has accounted for 20 to 35% of an oral dose and 15% of an intravenous dose. Only small amounts of ciprofloxacin are removed by haemodialysis or peritoneal dialysis (Sean, 2009).

2.2.2.2 Interactions

Fluoroquinolones, including ciprofloxacin, are known to inhibit the cytochrome P450 isoenzyme (CYP1A2) and may increase plasma concentrations of drugs, such as theophylline and tizanidine, which are metabolized by this isoenzyme. Use of

ciprofloxacin with tizanidine is contra-indicated, although theophylline may be used providing its dose is reduced and concentrations monitored.

Ciprofloxacin is reported to enhance the effect of oral anticoagulants such as warfarin and the oral antidiabetic glibenclamide. Severe hypoglycaemia, sometimes fatal, has occurred in patients also taking glibenclamide. Renal tubular secretion of methotrexate may be inhibited by ciprofloxacin, potentially increasing its toxicity.

The excretion of ciprofloxacin or related drugs is reduced and plasma concentrations may be increased by probenecid. Cations such as aluminium, calcium, magnesium, or iron reduce the absorption of oral ciprofloxacin or related drugs when given together. Changes in the pharmacokinetics of fluoroquinolones have been reported when given with histamine H₂ antagonists, possibly due to changes in gastric pH, but do not seem to be of much clinical significance.

Transient increase in serum creatinine has occurred when ciprofloxacin is given with ciclosporin. Monitoring of serum creatinine concentrations is recommended. Altered serum concentrations of phenytoin have been reported in patients also receiving ciprofloxacin.

Some fluoroquinolones have the potential to prolong the QT interval and should be avoided in patients also receiving class Ia antiarrhythmic drugs (such as quinidine and procainamide) or class III antiarrhythmics (such as amiodarone and sotalol). In addition, caution should be exercised when they are used with other drugs known to have this

effect (such as the antihistamines astemizole and terfenadine, cisapride, erythromycin, pentamidine, phenothiazines, or tricyclic anti-depressants) (Sean, 2009).

The Committee on the Safety of Medicines and the FDA warn that central nervous system (CNS) adverse effects, including seizure risk, may be increased when NSAIDs are combined with quinolones. The interaction between quinolones and NSAIDs is important, because it has the potential for considerable CNS toxicity. The mechanism for this interaction is believed to be due to a synergistic increased antagonism of GABA neurotransmission (Sean, 2009).

2.2.3 Antimicrobial action

Ciprofloxacin is bactericidal and acts by inhibiting DNA gyrase and topoisomerase IV, which are essential enzymes in the reproduction of bacterial DNA. It has a broader spectrum of activity and is more potent in vitro than the non-fluorinated quinolone nalidixic acid although resistance to many species or strains previously sensitive is emerging. Activity may be reduced in acid media and in the presence of urine but not of serum (Sean, 2009).

2.2.3.1 Spectrum of activity

Among Gram-negative aerobic bacteria, ciprofloxacin may be active in-vitro against Enterobacteriaceae including *Escherichia coli* and *Citrobacter*, *Enterobacter*, *Klebsiella*, *Proteus*, *Providencia*, *Salmonella*, *Serratia*, *Shigella*, and *Yersinia spp.* It may also exhibit activity against *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae*. *H. influenzae*, *Moraxella catarrhalis* (*Branhamella catarrhalis*), and *N. meningitidis* are all

sensitive. Other Gram-negative aerobic bacteria reported to be sensitive to ciprofloxacin includes *Gardnerella vaginalis*, *Helicobacter pylori*, *Legionella spp.*, *Pasteurella multocida*, and *Vibriospp.* Variable activity has been reported against *Acinetobacter spp.*, *Brucella melitensis*, and *Campylobacter spp.*

Among Gram-positive aerobic bacteria, ciprofloxacin is active against staphylococci, including penicillinase-producing and penicillinase-non producing strains and against some MRSA. Streptococci, in particular *Streptococcus pneumonia* and enterococci, are less susceptible. Other Gram-positive bacteria sensitive to ciprofloxacin in vitro are *Bacillus spp.* Variable activity has been noted for *Corynebacterium spp.*

Most anaerobic bacteria, including *Bacteroides fragilis* and *Clostridium difficile*, are resistant to ciprofloxacin, although some other *Clostridium spp.* may be susceptible. Ciprofloxacin has some activity against *mycobacteria*, *mycoplasmas*, *rickettsias*, *Chlamydia trachomatis*, and *Ureaplasma urealyticum* (Sean, 2009).

2.2.3.2 Acquired resistance

Resistant strains, particularly of MRSA, *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumoniae*, *C. jejuni*, *N. gonorrhoeae*, and *Streptococcus pneumonia* have emerged during treatment with ciprofloxacin although there are widely differing patterns of resistance geographically. Resistance to ciprofloxacin has usually been chromosomally mediated although plasma-mediated resistance has recently been noted (Sean, 2009).

2.2.4 Uses and administration

Ciprofloxacin has been used in the treatment of infections including anthrax, biliary tract infections, infected bites and stings, bone and joint infections, cat scratch disease, chancroid, exacerbations of cystic fibrosis, ear, nose, and throat infections (including otitis externa, otitis media, and sinusitis), HACEK endocarditis, gastro-enteritis (including travellers' diarrhoea and campylobacter enteritis, cholera, salmonella enteritis, shigellosis, and yersinia enteritis), gonorrhoea, granuloma inguinale, infections in immunocompromised patients (neutropenia), legionnaires' disease, pelvic inflammatory disease, peritonitis, plague, lower respiratory-tract infections (including pseudomonal infections in cystic fibrosis, but excluding infections due to *Streptococcus pneumoniae* such as pneumococcal pneumonia), rickettsial infections (including Q fever, spotted fevers, and typhus), septicaemia, skin infections (including soft-tissue infections), typhoid and paratyphoid fever, and urinary-tract infections including chronic bacterial prostatitis. Ciprofloxacin is used for meningococcal meningitis prophylaxis. It is also used for surgical infection prophylaxis and in the treatment of non tuberculous mycobacterial infections and tuberculosis. Ciprofloxacin is used topically in the treatment of eye and ear infections (Sean, 2009).

2.2.5 Contraindications

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 coadministered 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 under licensed use), pregnancy, nursing mothers, and in patients with epilepsy or other seizure disorders (Bayer Healthcare Pharmaceuticals, 2009)

2.2.6 Precautions

Ciprofloxacin should be used with caution in patients with epilepsy or a history of CNS disorders. Care is also necessary in those with renal impairment, G6PD deficiency, or myasthenia gravis. An adequate fluid intake should be maintained during treatment with ciprofloxacin and excessive alkalinity of the urine avoided because of the risk of crystalluria (Sean, 2009).

Since ciprofloxacin and related fluoroquinolones have, like nalidixic acid, been shown to cause degenerative changes in weight-bearing joints of young animals, it has been suggested that these drugs should not generally be used in patients aged less than 18 years, pregnant women, or breast-feeding mothers unless the benefits outweigh the risks. Tendon damage may occur rarely with fluoroquinolones and treatment should be stopped if patients experience tendon pain, inflammation, or rupture. Subsequent use of fluoroquinolones is contra-indicated in these patients. Exposure to strong sunlight or sunlamps should be avoided during treatment with ciprofloxacin. The ability to drive or operate machinery may be impaired, especially when alcohol is also taken. Some fluoroquinolones have the potential to prolong the QT interval and should be avoided or used with caution in patients with QT prolongation or relevant risk factors such as uncorrected electrolyte disturbances, bradycardia, or pre-existing cardiac disease. Certain drugs may also increase the risk. Ciprofloxacin and other fluoroquinolones should be avoided in MRSA infections because of the high level of resistance (Sean, 2009).

2.2.7 Adverse reactions

Ciprofloxacin is generally well tolerated. The range of adverse effects associated with ciprofloxacin and the other fluoroquinolones is broadly similar to that of earlier quinolones such as nalidixic acid. They most often involve the gastrointestinal tract, CNS, or skin. Gastrointestinal disturbances include nausea, vomiting, diarrhoea, abdominal pain, and dyspepsia and are the most frequent adverse effects. Pseudo membranous colitis, pancreatitis, and dysphagia have been reported rarely (Sean, 2009).

Headache, dizziness, confusion, insomnia, and restlessness are among the commonest effects on the CNS. Others include tremor, drowsiness, nightmares, visual and other sensory disturbances, hallucinations, psychotic reactions, depression, convulsions, and intracranial hypertension. Paraesthesia and peripheral neuropathy have also been reported (Sean, 2009).

In addition to rash and pruritus, hypersensitivity type reactions affecting the skin include rarely vasculitis, erythema multiforme, Stevens-Johnson syndrome, and toxic epidermal necrolysis. Photosensitivity has occurred, although it may be more frequent with some other fluoroquinolones such as lomefloxacin and sparfloxacin. Anaphylaxis has been associated with ciprofloxacin and some other quinolones. As with other quinolones, reversible arthralgia or myalgia has sometimes occurred and joint erosions have been documented in immature animals. Tendon damage has also been reported (Sean, 2009).

Other adverse effects reported with ciprofloxacin include crystalluria, transient increases in serum creatinine or blood urea nitrogen and, rarely, acute renal failure secondary to

interstitial nephritis. Elevated liver enzyme values, jaundice, and hepatitis have occurred, as have haematological disturbances including eosinophilia, leucopenia, thrombocytopenia and, very rarely, pancytopenia, haemolytic anaemia or agranulocytosis. Cardiovascular adverse effects include tachycardia, hypotension, oedema, syncope, hot flushes, and sweating. Some fluoroquinolones may rarely cause prolongation of the QT interval and ventricular arrhythmias, including torsade de pointes (Sean, 2009).

As with other antibacterials, super infection with organisms not very susceptible to ciprofloxacin is possible. Such organisms include *Candida*, *Clostridium difficile*, and *Streptococcus pneumoniae*. There is some evidence that fluoroquinolone use may be associated with an increased risk of colonisation by MRSA (Sean, 2009).

Pain and irritation may occur at the site of infusion accompanied rarely by phlebitis or thrombophlebitis (Sean, 2009).

Adverse effects reported after ocular use of ciprofloxacin include local burning or discomfort, keratopathy, corneal staining, corneal precipitates or infiltrates, and photophobia. Local discomfort, pain or pruritus has occurred after use of ear drops containing ciprofloxacin (Sean, 2009).

2.2.8 Overdosage

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, monitoring of renal function, and maintaining adequate hydration is recommended by the manufacturer. Administration of magnesium, aluminium, or calcium-containing antacids can reduce the absorption of ciprofloxacin. Hemodialysis or peritoneal dialysis removes only less than 10% of ciprofloxacin. Ciprofloxacin may be quantified in plasma or serum to monitor for drug accumulation in patients with hepatic dysfunction or to confirm a diagnosis of poisoning in acute overdose victims (Bayer Healthcare pharmaceuticals, 2009).

2.3 Saliva as an Analytical Tool

In recent years saliva has attracted much attention, in particular among people interested in the determination of drug concentrations, who suggest that saliva might be substituted for plasma in the areas of pharmacokinetic studies and drug monitoring. The traditional biological samples for the qualitative and quantitative measurement of most drugs are blood, plasma and urine. Many substances and their metabolites are present in different concentrations in these media. Blood or plasma provides an estimate of the current circulating concentration of the analyte of interest. Urine permits measurement of the accumulated concentration of analytes since the last void of the bladder. Previous publications have made it clear that for many drugs the monitoring of saliva is a real alternative for determining plasma levels because saliva lacks "the drama of blood, the sincerity of sweat and the emotional appeal of tears". However, a clear interpretation of

the quantitative significance of saliva drug concentrations to explain the mechanisms of drug secretion into saliva has not been achieved. Therefore, it is important to use saliva concentrations in conjunction with concentrations recorded from paired plasma samples (Karin *et al.*, 1999).

2.3.1 The collection and analysis of saliva

Many of the advantages of measuring drugs in saliva relate to the noninvasive nature of the easy collection procedure. For the collection of samples on a patient basis, mixed whole saliva is the only practical alternative. Therefore, if the measurement of a drug level in saliva is to be of general clinical value it will need to be done on mixed (whole) saliva. Several methods have been described for the collection of mixed saliva. Without stimulation the normal human salivary glands do not secrete saliva. However, many different stimuli will cause salivation and even during sleep there is usually sufficient stimulation to elicit a very small flow of saliva (typically 0.05 ml/min). In many studies claiming to have utilized unstimulated whole saliva, the subjects have usually been asked to spit directly into a collection tube. This spitting itself is usually a sufficient stimulus to elicit a flow of about 0.5 ml/min. In healthy subjects, gingival crevicular fluid (from the tooth/gum margin) may constitute up to 0.5% of the volume of mixed saliva: this proportion may be markedly increase in patients with gingivitis. Plasma exudate from minor abrasions in the mouth may also contribute to saliva. The protein content of gingival fluid is similar to that of plasma and thus it provides a potential route for the entry of many drugs into saliva. Therefore, it is usually recommended that subjects

should not brush their teeth or practice any other methods of oral hygiene for several hours before collecting a saliva sample (Karin *et al.*, 1999).

Although most patients prefer donating saliva rather than blood, a substantial social barrier exists to "spitting". For this and other reasons, subjects often experience decreased salivary secretion (dry mouth) if asked to provide a sample. Many researchers have found it advantageous to further stimulate salivation and a number of stimuli has been used. Chewing paraffin wax, parafilm[®], rubber bands, pieces of Teflon or chewing gum (Dawes *et al.*, 1992) will usually elicit a flow of 1 to 3 ml/min. recommends that, when these types of stimuli are used, the subject should allow saliva to accumulate in the mouth until the desire to swallow occurs, at which time the fluid can be expelled smoothly into a vessel. Repeated expectorations should be avoided since this introduces bubbles, which may result in changes in pH leading to errors in interpretation of the saliva/plasma concentration ratio (S/P ratio). The use of acid lemon drops or a few drops of 0.5 mol/l citric acid are among the most potent of taste stimuli and will generally induce a maximal secretion of 5 to 10 ml/min (Karin *et al.*, 1999).

In general, the secretion rate increases with the size of the bolus and the pressure required to chew it. If chewing is unilateral, then the glands on the active side may secrete copiously while those on the inactive site secrete very little. For studies requiring high saliva flow rates for extended periods of time, secretion stimulating drugs, such as the parasympathomimetic drug pilocarpine, have sometimes been used orally, subcutaneously, or intravenously. However, in doses sufficient to produce very high flow rates, parasympathomimetic drugs have undesirable side effects such as flushing,

palpitations, colicky abdominal pains, and an urgent desire to micturate. In addition, they appear to cause an enlargement of the tight junctions of the secretory end pieces and thus result in the appearance in the saliva of compounds of higher molecular weight than would normally be expected (Karin *et al.*, 1999).

There are several advantages of stimulating salivary flow.

1. Large volumes of saliva can be obtained within a short time
2. The pH of stimulated saliva mostly lies within a narrow range around the value of 7.4, whereas the pH of unstimulated saliva shows a larger variability, which may be of importance for the salivary secretion of weak acidic and basic compounds. In particular, Parafilm[®] has been shown to absorb highly lipophilic molecules, leading to an apparent reduction in drug level. Gustatory stimulants (e.g., citric acid) can result in changes in salivary pH that can consequently lead to erroneous results when calculating S/P ratios.

2.3.2 Mechanisms of drug transfer from blood to saliva

The possible routes which may lead to a drug being present in mixed saliva are passive transcellular diffusion, ultra filtration, active transport and pinocytosis. Clearly, if a patient has just received a drug orally there may be a spurious elevation of the salivary drug level. Even hard gelatin capsules containing amphetamine have produced oral drug retention. Usually the problem can be overcome by washing residual drug from the mouth with water prior to sampling, but one has to be careful of dilution effects. Similarly, the handling of drugs by subjects has been suggested as a possible source of saliva contamination. The contribution of gingival crevicular fluid to mixed saliva and

the need to refrain from vigorous brushing of the teeth to prevent contamination of the saliva by blood (Karin *et al.*, 1999).

2.4 Spectroscopy

It is the branch of science that deals with the study of interaction between electromagnetic radiation and matter. It is a most powerful tool available for the study of atomic and molecular structure/s and is used in the analysis of wide range of samples. Optical spectroscopy includes the region on electromagnetic spectrum between 100 Å and 400 μm (Behera *et al.*, 2012).

2.4.1 Ultraviolet visible spectroscopy

UV-Visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measure the ratio, or function of ratio, of the intensity of two beams of light in the U.V-Visible region are called Ultraviolet-Visible spectrophotometers (Behera *et al.*, 2012).

In qualitative analysis, organic compounds can be identified by use of spectrophotometer, if any recorded data is available, and quantitative spectrophotometric analysis is used to ascertain the quantity of molecular species absorbing the radiation. Spectrophotometric technique is simple, rapid, moderately specific and applicable to small quantities of compounds (Behera *et al.*, 2012).

Quantitative analysis basically relates concentration of analyte and the intensity of light absorbed. In addition, features of absorption spectra such as the molar absorptivity, spectral position, and shape and breadth of the absorption band are related to molecular structure and environment and therefore can be used for qualitative analysis. The fundamental law that governs the quantitative spectrophotometric analysis is the Beer -Lambert law (Behera *et al.*, 2012).

2.4.2 Beer's law

It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially with the number of absorbing molecules. In other words, absorbance is proportional to the concentration (Behera *et al.*, 2012).

2.4.3 Lambert's law

It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially as it passes through a medium of homogeneous thickness (*Behera et al.*, 2012).

Table 2.1 Regions of electromagnetic spectrum

Region	Wavelength
Far (or vacuum)ultraviolet	10-200 nm
Near ultraviolet	200-400 nm
Visible	400-750 nm
Near infrared	0.75- 2.2 μm
Mid infrared	2.5-50 μm
Far infrared	50-1000 μm

2.4.4 Beer-Lambert's law

When beam of light is passed through a transparent cell containing a solution of an absorbing substance, reduction of the intensity of light may occur. Mathematically, Beer- Lambert law is expressed as $A = a b c$

Where, A=absorbance or optical density

a=absorptivity or extinction coefficient

b=path length of radiation through sample (cm)

c=concentration of solute in solution.

Both b and a are constant so a is directly proportional to the concentration c

Quantification of medicinal substance using spectrophotometer may be carried out by preparing solution in transparent solvent and measuring its absorbance at suitable wavelength. The wavelength normally selected is wavelength of maximum absorption (λ_{max}), where small error in setting the wavelength scale has little effect on measured absorbance. Ideally, concentration should be adjusted to give an absorbance of approximately 0.9, around which the accuracy and precision of the measurements are optimal (Behera *et al*, 2012).

2.5 Quality Control of Tablets (Evaluation Tests)

The quantitative evaluation and assessment of a tablet's chemical, physical and bioavailability properties are important in the design of tablets and to monitor product quality. These properties are important since chemical breakdown or interactions between tablet components may alter the physical tablet properties, and greatly affect the bioavailability of the tablet system. Operations in tableting property are meaningless unless appropriate test methods and specifications are derived which are capable of guaranteeing quality of batch to batch basis. It is also called physicochemical property.

There are various standards that have been set in the various pharmacopoeias regarding the quality of pharmaceutical tablets. These include the diameter, size, shape, thickness, weight, hardness, disintegration and dissolution characters. The diameters and shape depends on the die and punches selected for the compression of tablets. The remaining specifications assure that tablets do not vary from one production lot to another. These tests are carried out based on the official books, British Pharmacopoeia (BP, 2002, 2009). The following standard or quality control tests should be carried out.

2.5.1 General appearance

It is the visual identity and overall elegance which is essential for consumer acceptance. This includes size, shape, and thickness: this is important to facilitate packaging and to decide which tablet compressing machine to use. Organoleptic properties like color and odor of the tablets. Tablet color is important because it affects rapid identification of the medication, and odor is important to identify any stability problems. An example is aspirin which gives the odor of acetic acid if any stability problem occurred.

2.5.2 Non-official tests

Non-Official Tests includes hardness, friability, Tensile strength, Brittle fracture index (BFI)

2.5.3 Hardness (Crushing strength)

Hardness is the load required to crush the tablet when placed on its edge. The small and portable hardness tester was manufactured and introduced by Monsanto in the Mid 1930s. It is now designated as either the Monsanto or Stokes hardness tester. The instrument measures the force required to break the tablet when the force generated by a coil spring is applied diametrically to the tablet. The Strong-Cobb Pfizer and Schleuniger apparatus which were later introduced measures the diametrically applied force required to break the tablet.

Hardness is now more appropriately called crushing strength. Determinations are made during tablet production and are used to determine the need for pressure adjustment on tablet machine. If the tablet is too hard, it may not disintegrate in the required period of time to meet the dissolution specifications; if it is too soft, it may not be able to withstand the handling during subsequent processing such as coating or packaging and shipping operations.

Why do we measure hardness?

Hardness determinations are made throughout the tablet runs to determine the need for pressure adjustments on the tableting machine.

Hardness can affect the disintegration. So if the tablet is too hard, it may not disintegrate in the required period of time. And if the tablet is too soft, it will not withstand the handling during subsequent processing such as coating or packaging.

In general, if the tablet hardness is too high, we first check its disintegration before rejecting the patch. And if the disintegration is within limit, we accept the batch. ie

if hardness is high + disintegration is within time = accept the batch.

2.5.3.1 Factors affecting the hardness

1. Compression of the tablet and compressive force.
2. Amount of binder. (More binder more hardness)
3. Method of granulation in preparing the tablet (Wet granulation method gives highest hardness)

4. Characteristics of the drug.

2.5.3.2 Limits

The force required to break the tablet is measured in kilograms and a crushing strength of 4Kg is usually considered to be the minimum for satisfactory tablets. Oral tablets normally have a hardness of 4 to 10kg however, hypodermic and chewable tablets are usually much softer (3 kg) and some sustained release tablets are much harder (10-20 kg). Tablet hardness has been associated with other tablet properties such as density and porosity. Hardness generally increases with normal storage of tablets and depends on the shape, chemical properties, binding agent and pressure applied during compression.

2.5.4 Friability

Friction and shock are the forces that most often cause tablets to chip, cap or break. The friability test is closely related to tablet hardness and is designed to evaluate the ability of the tablet to withstand abrasion in packaging, handling and shipping. It is usually measured by the use of the Roche friabilator. A number of tablets are weighed and placed in the apparatus where they are exposed to rolling and repeated shocks as they fall 6 inches in each turn within the apparatus. After four minutes of this treatment or 100 revolutions, the tablets are weighed and the weight compared with the initial weight. The loss due to abrasion is a measure of the tablet friability. The value is expressed as a percentage. A maximum weight loss of not more than 1% of the weight of the tablets being tested during the friability test is considered generally acceptable and any broken or smashed tablets are not picked up. Normally, when capping occurs, friability values are not calculated. A thick tablet may have less tendency to cap whereas thin tablets of large

diameter often show extensive capping, thus indicating that tablets with greater thickness have reduced internal stress.

2.5.5 Official tests

Official Tests includes Weight variation, disintegration, dissolution, drug content.

2.5.6 Disintegration

It is the time required for the tablet to break into particles, the disintegration test is a measure only of the time required under a given set of conditions for a group of tablets to disintegrate into particles. In the present disintegration test the particles are those that will pass through a 10-mesh screen. Complete disintegration occurs when no residue of the tablet still present on the screen except the insoluble ingredients as the shell or the coat of the tablet.

2.5.6.1 Liquids used in disintegration

Water, simulated gastric fluid (pH = 1.2 HCl), or Simulated intestinal fluid (pH = 7.5, KH_2PO_4 (phosphate buffer) + pancreatin enzyme + NaOH)

2.5.6.2 Limits

Table 2.2 Uncoated tablets

Medium	Temperature	Time limit
According to U.S.P	37°C	not exceed 30mins

Simulated gastric fluid

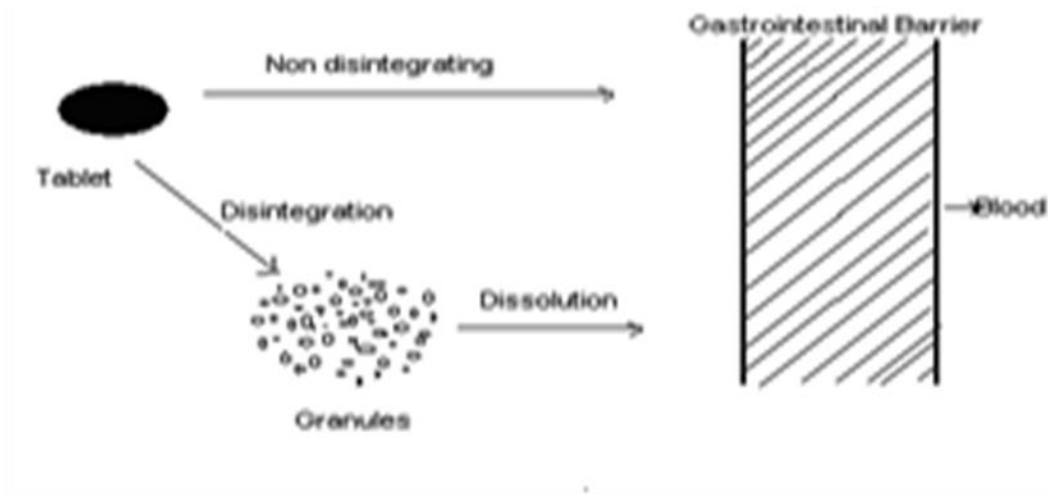
According to B.P	Water	37°C	not exceed 15mins
According to I.P	Water	37°C	not exceed 15 (unless Otherwise directed)

2.5.7 Dissolution

Dissolution is the process by which a solid solute enters a solution. In the pharmaceutical industry, it may be defined as the amount of drug substance that goes into solution per unit time under standardized conditions of liquid/solid interface, temperature and solvent composition.

Dissolution is considered one of the most important quality control tests performed on pharmaceutical dosage forms and is now developing into a tool for predicting bioavailability, and in some cases, replacing clinical studies to determine bioequivalence. Dissolution behavior of drugs has a significant effect on their pharmacological activity. In fact, a direct relationship between in-vitro dissolution rate of many drugs and their bioavailability has been demonstrated and is generally referred to as in-vitro in-vivo correlation.

Fig 1 Schematic diagram of the dissolution process



2.5.8 Weight variation or Uniformity of weight

Twenty (20) tablets selected are weighed at random and the average weight calculated. Not more than two of the individual weights should deviate from the average weight by more than the percentage shown in the table below and none deviates by more than twice that percentage

Table 2.3 I.P Standard

Average weight of tablets	Percentage deviation
80 mg or less	10
More than 80 mg but less than 250 mg	7.5
250 mg or more	5

Table 2.4 USP Standard

Average weight	Percent difference
----------------	--------------------

130mg or less	10
More than 130mg through 324mg	7.5
More than 324mg	5

2.5.9 Content uniformity

The content uniformity test is used to ensure that every tablet contains the amount of drug substance intended with little variation among tablets within a batch. Due to increased awareness of physiological availability, the content uniformity test has been included in the monographs of all coated and uncoated tablets and all capsules intended for oral administration where the range of size of the dosage form available include 50mg or smaller sizes. Tablet monographs with a content uniformity requirement do not have weight variation requirements

2.6 Analytical Procedures

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc. (ICH, 1995)

2.6.1 Types of analytical procedures to be validated

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

1. Identification tests.
2. Quantitative tests for impurities' content.
3. Limit tests for the control of impurities.
4. Quantitative tests of the active moiety in samples of drug substance, drug product or other selected component(s) in the drug product.

There are many other analytical procedures, such as dissolution testing for drug products or particle size determination for drug substance (ICH, 1995). A brief description of the types of tests considered in this document is provided below.

2.6.1.1 Identification tests

Identification test are intended to ensure the identity of an analyte in a sample. This is normally achieved by comparison of a property of the sample (e.g., spectrum, chromatographic behavior, chemical reactivity etc.) to that of a reference standard.

2.6.1.2 Testing for impurities

Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test.

2.6.1.3 Assay procedures

Assay procedures are intended to measure the analyte present in a given sample. In the context of this document, the assay represents a quantitative measurement of the major component(s) in the drug substance. For the drug product, similar validation characteristics also apply when assaying for the active or other selected component(s). The same validation characteristics may also apply to assays associated with other analytical procedures (e.g., dissolution).

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated (ICH, 1995).

2.6.2 Validation of analytical method and characteristics

Typical validation characteristics which should be considered are listed below

- Accuracy
- Precision
- Repeatability
- Intermediate Precision
- Specificity
- Detection Limit
- Quantitation Limit
- Linearity
- Range
-

2.6.2.1 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

2.6.2.2 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

2.6.2.3 Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements (ICH, 1995).

- Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision

- Intermediate precision

Intermediate precision expresses within laboratories variations: different days different analysts, different equipment, etc.

- Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies usually applied to standardization of methodology).

2.6.2.4 Detection limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value (ICH, 1995)

2.6.2.5 Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products (ICH, 1995).

2.6.2.6 Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample (ICH, 1995).

2.6.2.7 Range

The range of an analytical procedure is the interval between the upper and lower Concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity (ICH, 1995).

2.6.2.8 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. (ICH, 1995)

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 Materials

3.1.1 Drugs

- Standard Ciprofloxacin Hydrochloride powder, donated by Juhel Nigeria Ltd.
- 6 brands of ciprofloxacin tablets

Brand A (Ciprotab -500®)

Manufacturer; GeltecPvt LTD, Marketed by Fidson healthcare LTD

NAFDAC No; 04-0723

Batch no; UG1308

Expiry Date; 01/16

Brand B (Ciprovam 500®)

Manufacturer; Tuyilpharmaceutical industry LTD

NAFDAC No; 04-7022

Batch no; TCV03

Expiry Date; 12/16

Brand C (Ciprofit-500®)

Manufacturer; Jiangsu RuinanQianjin Pharmaceutical Co LTD

NAFDAC No; 04-7405

Batch no; 121222

Expiry Date; 12/15

Brand D (Cipocil®)

Manufacturer; Greenfield pharmaceutical (Jiang su) Co., LTD. Distributed by

Justeen pharmaceutical LTD

NAFDAC No; 04-5495

Batch no; 120602

Expiry Date; 06/15

Brand E (Ciproflo^x 500®)

Manufacturer; Indonesian pharmaceutical industry

NAFDAC No; 04-0807

Batch no; 120029

Expiry Date; 12/19

Brand F (Cipromax fort 500®)

Manufacturer; Greenfield pharmaceutical (Jiang su) Co., LTD. Marketed by Climax

Pharmaceutical Chemist LTD

NAFDAC No; 04-4950

Batch no; 120109

Expiry Date; 01/15

3.1.2 Glass wares accessories

2×250ml Extraction tubes (Pyrex England)

2×100ml measuring cylinders (Pyrex England)

2×100ml and 2×250ml conical flasks (Pyrex England)

2×25ml and 2×50ml Beakers (Pyrex England)

6×10ml Test tubes (Pyrex England)

6×15ml centrifuge tubes (Pyrex England)

2×25ml, 2×50ml and 2×100ml volumetric flasks (Pyrex England)

20×Filter paper

3.1.3 Equipments

Electronic weighing balance (Metlers Pr 63 Switzerland)

Tablet friabilator (Erweka TA3, Germany)

Disintegration apparatus (Erweka G.M.B.H Germany)

Tablet hardness tester (Monsanto, Philip Harries Ltd, England)

Centrifugation machine (Gallen Kamp, Philip Harries Ltd, England)

Gallen Kamp Hot Air Oven (Philip Harries Ltd, England)

Porcelain pestle and mortar

Thermometer (Mc Donald Scientific International, England)

Dissolution machine (DA-6D, VeegoScientificdevicesMumbai, India)

UV Spectrophotometer (Model; Helioszeta UV-Vis, ser no; UV2-164917)

Stop watch (Smith England clock system)

3.1.4 Reagents

Glacial acetic acid (BDH Chemical, England)

Distilled water (BDH Chemical, England)

Ethanol (BDH Chemical, England)

Methanol (BDH Chemical, England)

Glacial acetic (BDH Chemical, England)

N-hexane (BDH Chemical, England)

Chloroform (BDH Chemical, England)

Conc. HCl (BDH Chemical, England)

NaOH pallet (BDH Chemical, England)

3.2 Subjects

Six healthy adult male volunteers (age- 22-43±5years, weight 55-65±5kg) were used for the study. All volunteers were instructed to abstain from analgesic, anti-inflammatory drugs or any other medication one week before commencement of the study. No subject had a history of hepatic, renal, gastro-intestinal, hematological disease or drug allergy. Prior to any screening procedures, consent was obtained from each subject participating in this study after adequate explanation of the aims, methods, objective, and potential hazards of the study.

3.3 Methodology

3.3.1 Sample collection

Six brands of ciprofloxacin tablets were purchased from registered pharmacies at Sabon Gari market, Zaria and were then coded as A, B, C, D, E and F. Brand A was considered as innovator.

3.3.2 In-vitro quality control (BP 2002, 2009)

3.3.2.1 Visual inspection

Physical identity of the samples and other NAFDAC requirements for labeling pharmaceutical products were determined.

3.3.2.2 Identification of Ciprofloxacin Hydrochloride powder

To confirm the powder, chloride test was carried out

0.1g of ciprofloxacin powder was dissolved in 10ml distilled water. To 2ml of the solution, 5ml 2M nitric acid was added then 1ml 5% AgNO₃ solution. The solution was left to stand for 5mins. Appearance of white precipitates indicates a positive chloride test (BP, 2002).



3.3.2.3 Weight variation

Twenty (20) tablets from each generic and innovator brand products were weighed individually using a weighing balance. The average weights of the tablet as well as their percentage deviation were calculated (BP, 2009).

3.3.2.4 Friability test

Friability test was conducted by weighing altogether Twenty (20) tablets from each generic and innovator brand products and friabilated using a Roche friabilator at 25 rev/min for 4 minutes. The tablets were dusted and reweighed again collectively (BP, 2009).

Percent friability was determined by using the following formula:

$$\% \text{ Friability} = (W_1 - W_2 / W_1) 100 \text{ (1)}$$

Where, W₁= Initial weight and, W₂= Final weight

3.3.2.5 Crushing strength

Hardness was determined using five (5) tablets from each generic and innovator brand products and a Mosanto hardness tester. The pressure at which each tablet is crushed was recorded. The average strength as well as their percentage deviation was calculated (BP, 2009).

3.3.2.6 Disintegration test

Six (6) tablets were randomly selected from each brand. One tablet was placed in one basket of unit of Erweka disintegration machine Germany, in 0.1N HCl solution maintained at $37 \pm 10^0\text{C}$ using Erweka disintegration machine Germany. The disintegration time was taken to be the time; when no particle remained on the basket. The average time as well as their percentage deviation was calculated (BP, 2009).

3.3.2.7 Dissolution behavior

In-vitro dissolution studies were carried out using Erweka dissolution apparatus Germany (basket type). The dissolution medium was 900 ml of 0.1N HCl which was maintained at $37 \pm 0.5 \text{ }^\circ\text{C}$. The paddles were rotated at a rotational speed of 50rpm. Ten (10 ml) of dissolution sample was withdrawn after 30minutes. The absorbance of the filtered sample measured for determination of released ciprofloxacin concentrations using a UV spectrophotometer against a blank at 276nm wavelength (BP, 2009).

3.3.2.8 Assay

Powdered amount from each generic and innovator brand products equivalent to 0.300g was dissolved in 80ml of anhydrous acetic acid then titrated with 0.1M perchloric acid using crystal violet solution as indicator until a bluish-green endpoint was observed (1ml of 0.1M perchloric acid is equivalent to 0.03314g ciprofloxacin) (BP, 2002).

3.3.3 Development and validation of the analytical methods

3.3.3.1 Development of the analytical method

In developing the method, the following tests were carried out

3.3.3.2 Preparation of stock solution

0.01g of standard ciprofloxacin powder was dissolved in 20ml distilled water contained in 100ml volumetric flask and was made up to 100ml with the same solvent to get a standard working solution of 100 μ g/ml stock solution .

3.3.3.3 Determination of wavelength of maximum absorption

1ml was withdrawn from the stock solution into a 10ml volumetric flask and made up to 10ml with distilled water to make 10µg/ml solution. This was then scanned through the workable wavelength (200-400nm) so as to obtain the wavelength of maximum absorption.

3.3.3.4 Determination of pH of maximum absorption

To 5 test-tubes each containing 3ml of a 10µg/ml ciprofloxacin solution, 3ml of phosphate buffer (pH 4, 5, 7, 9, 10) was respectively added and mixed vigorously. The solutions were then scanned through the workable wavelength (200-400nm) so as to obtain the pH of maximum absorption

3.3.4 Validation of the developed analytical method

The developed method was validated for its precision, accuracy and percentage recovery according to ICH guideline.

3.3.4.1 Precision

This is done by actual determination of 6 replicates of a fixed concentration of the drug (10µg/ml) within the Beer's range and finding out the absorbance by the proposed method. It consists of Within-day and between day precision. Expressed as $RSD \leq 2\%$

Within-day precision: 3ml of phosphate buffer (pH 4) was added to 3ml of 10 μ g/ml solution. Six absorbances were measured hourly at 277nm. Mean and percentage deviation was then calculated.

Between-day precision: This consists of measuring the absorbance of the 10 μ g/ml solution used for within-day three times for two different days in order to get six (6) absorbances.

3.3.4.2 Accuracy

The accuracy of the proposed method was tested by recovery studies at 80%, 100%, and 120% (8, 10 and 12 μ g/ml) by adding a known amount of pure drug to the pre-analyzed formulation of the 10 μ g/ml solution.

3.3.5 Extraction method

Sharma *et al.*, 2010 method was adopted and modified as follows; 2ml saliva was mixed with 2ml of the extracting solvent (distilled water) and buffered with 1ml of pH 4. This was then mixed for 2minutes and then centrifuged at 3000rpm for 10minutes. 2ml of the supernatant was taken and absorbance recorded at 277nm.

3.3.6 Percentage recovery

2ml blank saliva was collected prior to drug administration and then vigorously mixed with 2ml of extracting solvent (distilled water) then 1ml pH 4 buffer solution. This was

then centrifuged at 3000rpm for 10mins and the supernatant was collected forming the blank solution.

A 500mg ciprofloxacin tablet (Innovator brand) was given to a healthy male volunteer after an overnight fasting. 2ml of saliva was collected after 2hrs. The collected sample was stored at -4°C prior to analysis (Demiana *et al.*, 2006). 2ml of extracting solvent was added to the saliva sample followed by 1ml buffer (pH 4). The sample was mixed vigorously and centrifuged at 3000rpm for 10mins (Đorđević *et al.*, 2009). The supernatant layer was decanted and absorbance measured at 277nm against the previously prepared blank.

0.25ml (2.5µg/ml) was measured using a 2ml syringe from the standard 10µg/ml solution was transferred into a test-tube. The volume was made up to 10ml with 0.1N HCl. The absorbance was measured at 277nm against 0.1N HCl as blank.

$$\% \text{ Recovery} = \frac{\text{Drug concentration in saliva}}{\text{Drug concentration in 0.1N HCl}} \times 100 \quad \text{----- (3)}$$

3.3.7 Calibration curve

15ml saliva was collected from a volunteer with the aid of chewing gum and centrifuged. The supernatant was collected thereby discarding the mucus and particulate matters. 2ml of the blank saliva was measured into 6 sample bottles. 0.1,0.2,0.3,0.4,0.5 and 0.6ml of the 10µg/ml stock solution were withdrawn into the six labeled test tubes using a 2ml syringe and then made up to 10ml with distilled water making 1,2,3,4,5,6 µg/ml ciprofloxacin solution respectively. 2ml of the extracting solvent (distilled water) was added then lastly 1ml of phosphate buffer (pH 4). The sample was mixed vigorously and

centrifuged at 3000rpm for 10mins. The supernatant layer was decanted and the absorbance measured at 277nm.

The absorbances were plotted against concentrations with the aid of Microsoft excel 2007.

3.3.8 Study design

After an overnight fast, the volunteers were made to rinse their mouths, and 5ml saliva was collected prior to drug administration with the aid of chewing gum (Dawes *et al*, 1992). Each of the six adult male healthy volunteers were then administered a 500mg innovator with adequate water. Food and drinks were not allowed until 2 hours after drug administration. (This is the time maximum plasma concentration is said to occur). About 5ml of saliva was collected at 0, 0.5, 1, 2, 3, 4, 5,6,7,8 hrs after drug administration in a sample bottle. Saliva samples were stored at - 4°C (it has been shown that at this temperature no reaction is said to occur) and analyzed the next day. The procedure was repeated for the other brands after a wash out period of a week (Akinleye *et al.*, 2007).

3.3.9 Data analysis

The absorbances were converted to concentration terms using the calibration curve.

AUC was determined by trapezoidal method. Results were expressed as mean \pm SEM and statistical analysis was carried out using student's T-test 95% level of significance.

CHAPTER FOUR

4.0 RESULT

4.1 In-Vitro Quality Control

4.1.1 Physical identity of samples

NAFDAC registration number, manufacturing, expiry dates, batch number and country of origin of all the brands are as follows;

Table 4.1 Physical identity of samples

Brand Code	Brand	NAFDAC No	Mfd	Expd	Batch No	Country of Origin
A	Ciprotab 500	04-5495	6/12	06/15	120602	China
B	Ciprovam 500	04-7022	18/13	12/16	TCV03	Nigeria
C	Ciprofit-500	04-7405	12/12	12/15	121222	China
D	Cipocil	04-0723	2/13	01/16	UG1308	India
E	Ciprofloxx 500	04-0807	12/13	12/13	120029	Indonesia
F	Cipromaxfort 500	04-4950	01/15	01/15	120109	China

4.1.2 Identification of Ciprofloxacin Hydrochloride powder (Chloride test)

The appearance of white particles on addition of AgNO₃ to 2ml ciprofloxacin hydrochloride solution indicates a positive chloride test (BP, 2002).

4.1.3 Mean weight variation

The BP 2009 specification for uncoated tablets is that for tablets weighing more than 324mg, not more than two of the individual weights should deviate from the average weight by more than 5%. All brands passed the test as none of the tablets deviated from their average weight by 5%

Table 4.2 Mean weight variation

Brands	Mean Weight	±5% deviation	Remarks
--------	-------------	---------------	---------

	(mg) ± SD (n=20)	from mean weight	
A	783.0 ± 1.8	None	Passed
B	700.5 ± 2.6	None	Passed
C	910.5 ± 2.7	None	Passed
D	723.0 ± 3.0	None	Passed
E	817.0 ± 1.0	None	Passed
F	730.0 ± 1.0	None	Passed

4.1.4 Percentage friability (%)

BP 2009 specification for friability is that a maximum weight loss of not more than 1% of the weight of the tablets is considered generally acceptable. All brands passed the test with friability less than 1% as shown in the table.

Table 4.3 Percentage friability (%)

Brands	% Friability (n=6)	Remarks
--------	--------------------	---------

A	0.000	Passed
B	0.631	Passed
C	0.121	Passed
D	0.138	Passed
E	0.409	Passed
F	0.000	Passed

4.1.5 Mean crushing strength

The crushing strength of 4kg is usually considered to be the minimum force for satisfactory tablets according to BP 2009. All brands passed the test with a crushing strength greater than 4kgf as shown in the table.

Table 4.4 Mean crushing strength

Brands	Mean crushing strength (Kgf) \pm SD	Remarks
A	12.42 \pm 0.14	Passed
B	11.75 \pm 0.19	Passed

C	8.25±0.56	Passed
D	10.42±0.3	Passed
E	12.58±0.25	Passed
F	10.50±0.17	Passed

4.1.6 Mean disintegration (mins)

The time limit for disintegration of uncoated tablets in water at 37°C should not exceed 15mins according to BP 2009. All brands passed the test with a disintegration time less than 15mins as shown in the table.

Table 4.5 Mean disintegration (mins)

Brands	Mean disintegration (mins)	Remarks
	± SD	

A	3.32±0.56	Passed
B	4.24±0.25	Passed
C	2.74±0.11	Passed
D	9.50±0.23	Passed
E	8.27±0.33	Passed
F	2.17±0.12	Passed

4.1.7 Dissolution

The percentage drug release in 0.1N HCl at 30mins according to BP 2009 should be more than 80%. All brands passed the test as more than 80% of the drug was released at 30mins as shown in the table

Table 4.6 Dissolution rate in 0.1N HCl at 30mins (% drug release)

Time (mins)	Brand A	Brand B	Brand C	Brand D	Brand E	Brand F	Remarks
30	89.72	81.18	83.84	84.64	87.22	86.54	Passed

4.1.8 Assay of innovator and test brands of Ciprofloxacin

The acceptable limit for the percentage content of Ciprofloxacin tablet according to BP 2002 is 95-105%. All brands passed the test as their % contents were within the accepted limit as shown in the table.

Table 4.7 Assay of Innovator and Test brands of Ciprofloxacin

Brands	% content	Remarks
--------	-----------	---------

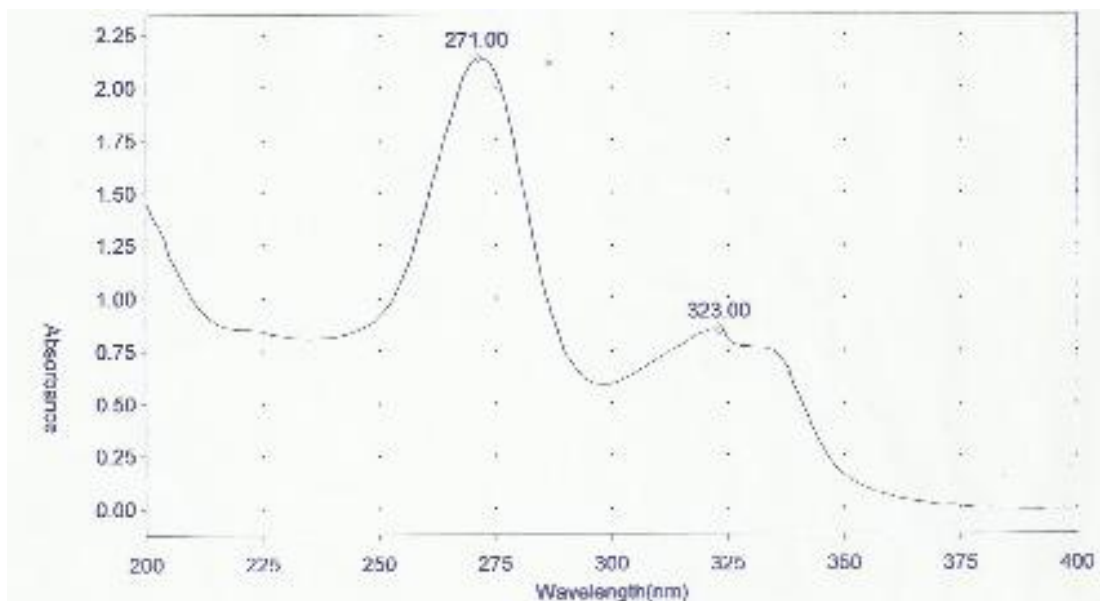
A	103.8	Passed
B	100.5	Passed
C	97.20	Passed
D	98.00	Passed
E	101.6	Passed
F	102.7	Passed

4.2 Development and Validation of the Analytical Methods

4.2.1 Development of the analytical methods

277nm was obtained as the analytical wavelength after scanning of 10µg/ml solution of Ciprofloxacin Hydrochloride through a workable wavelength of 200-400nm.

Figure 4.1 Spectrum of 10 μ g/ml solution of Ciprofloxacin Hydrochloride standard powder at 200 – 400nm



Wavelength - 271nm

Absorbance - 2.140

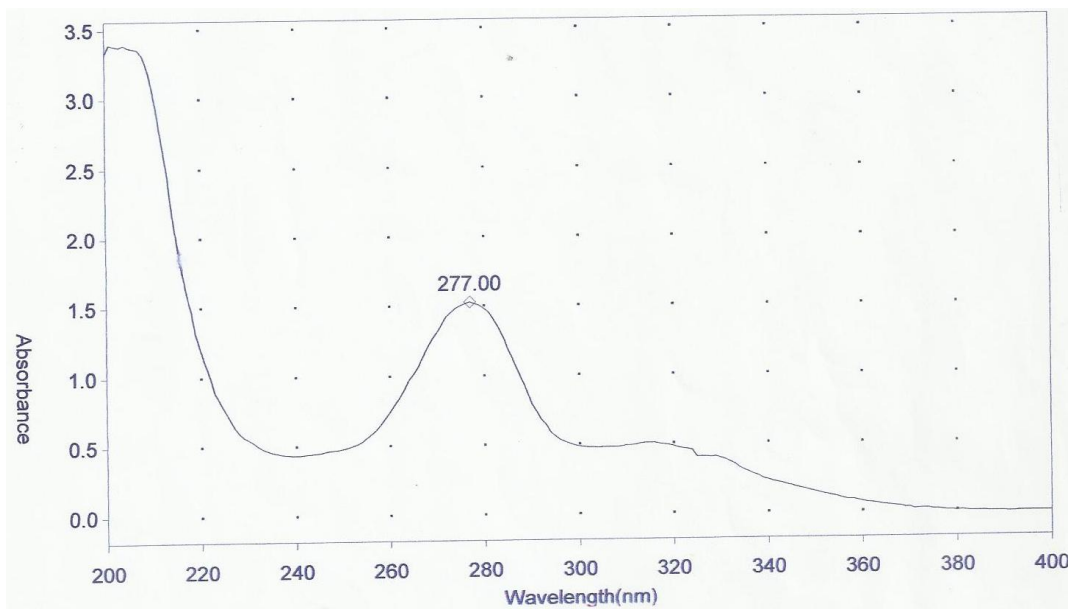
4.2.2 Effect of pH on wavelength of maximum absorption

pH 4 was seen to have the highest wavelength as compared to pH 5, 7, 9 and 10. The lower the pH the higher the wavelength of maximum absorption.

Table 4.8 Effect of pH on wavelength of maximum absorption

pH	λ_{max} (nm)	Absorbance
4	277	1.527
5	276	1.124
7	271	0.918
9	271	1.007
10	271	1.118

Figure 4.2 Spectrum of 10 μ g/ml solution of Ciprofloxacin Hydrochloride standard powder in distilled water at pH 4



Absorbance –1.527

Wavelength –277nm

4.2.3 Precision of the analytical method

The acceptable limit for RSD is less than 2% according to ICH 1995 guidelines; hence the developed method is precise.

Table 4.9 Within-day and Between-day precision of 10µg/ml Ciprofloxacin Hydrochloride solution

Concentration (µg/ml)	Within-day	Between-day
10	1.520	1.400
	1.512	1.423
	1.501	1.451
	1.479	1.463
	1.472	1.403
	1.485	1.412
Mean	1.49	1.43
RSD (%)	1.2	1.8

4.2.4 Accuracy and percentage recovery of standard Ciprofloxacin Hydrochloride powder spiked in blank saliva

Accuracy according to ICH 1995 guidelines is expressed as percentage recovery. The percentage recovery was found to be within the accepted limit of 98-102%

Table 4.10 Accuracy and percentage recovery of standard Ciprofloxacin Hydrochloride powder spiked in blank saliva

S/no	Amount added ($\mu\text{g/ml}$) (n=3)	Amount found ($\mu\text{g/ml}$) (n=3)	Percentage recovery
1	8	7.86	98.3
2	10	9.89	98.9
3	12	11.91	99.3

4.2.5 Validation parameters of the developed method

The results obtained for the maximum wavelength, range, regression equation, correlation coefficient, intercept, accuracy and precision are shown in the table below.

Table 4.11 Validation parameters of the developed method

S/no	Parameters	Results obtained
1	λ_{max}	277nm
2	Range	1-6 $\mu\text{g/ml}$
3	Regression equation	$y = 0.1092x + 0.2015$
4	Correlation coefficient	0.9889
5	Intercept	0.2015
6	Accuracy	98.3-99.3%
7	Precision:	
	Within-day (%RSD)	1.2
	Between-day (%RSD)	1.8

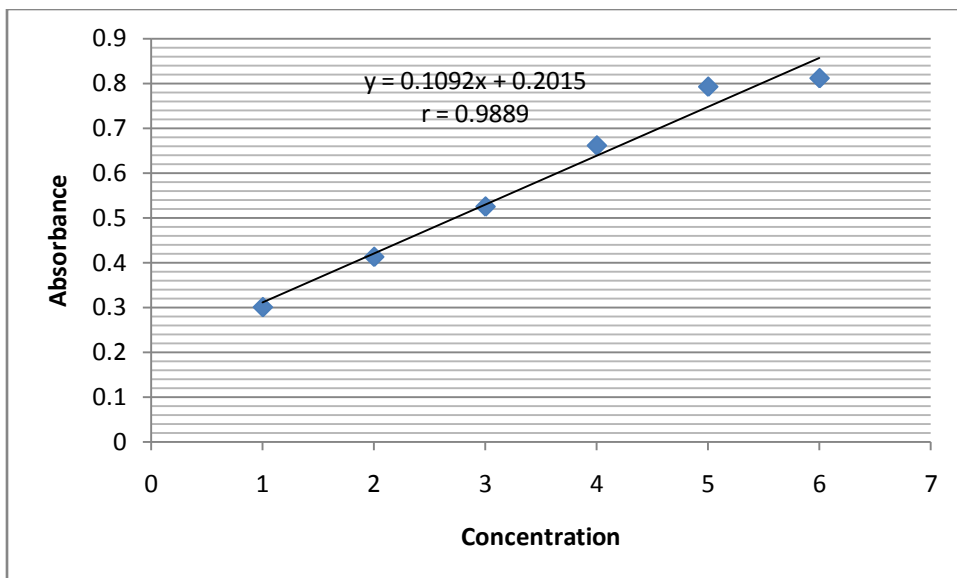
4.2.6 Calibration curve

Concentration of Ciprofloxacin Hydrochloride in $\mu\text{g/ml}$ and their corresponding absorbances are as follows;

Table 4.12 Calibration curve

Concentration ($\mu\text{g/ml}$)	Absorbance
1	0.301
2	0.401
3	0.525
4	0.661
5	0.792
6	0.811

Figure 4.3 Calibration curve of Ciprofloxacin



4.3 In-Vivo Result

4.3.1 Pharmacokinetic parameters of various brands of Ciprofloxacin tablets

Pharmacokinetic parameters used to assess bioequivalence (C_{max} , T_{max} and AUC) of all the selected brands are shown below.

Table 4.13 Pharmacokinetic parameters of various brands of Ciprofloxacin tablets (n=6)

Parameters	Reference	Brand B	Brand C	Brand D	Brand E	Brand F
C_{max} ($\mu\text{g/ml}$)	2.83	2.61	2.51	2.79	2.67	2.78
T_{max} (hr)	2.00	2.00	2.00	2.00	2.00	2.00
AUC $0 \rightarrow 8$ ($\mu\text{g/ml/hr}$)	14.06	12.09	12.095	12.18	11.34	12.38

4.3.2 Bioequivalence ratio of reference and test brands

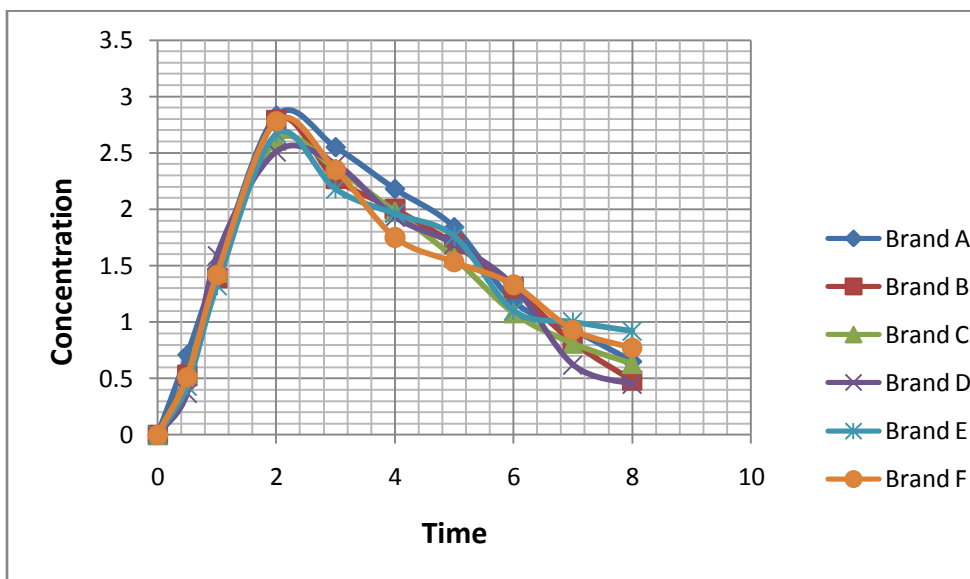
Cmax, AUC and bioequivalence ratio of reference and test brand products are shown below. Acceptable range for Bioequivalence according to Birkett, 2003 is 80-125%.

Table 4.14 Bioequivalence ratio of reference and test brands

Parameters	Cmax ($\mu\text{g/ml}$)	AUC ($\mu\text{g/ml/hr}$)
Brand A (Reference)	2.83	14.06
Brand B	2.61	12.09
Point of estimate	92.23%	85.99%
Acceptable range	80-125%	80-125%
Conclusion	Bioequivalent	Bioequivalent
Brand A (Reference)	2.83	14.06
Brand C	2.51	12.095
Point of estimate	88.69%	86.02%
Acceptable limit	80-125%	80-125%
Conclusion	Bioequivalent	Bioequivalent

Parameters	Cmax ($\mu\text{g/ml}$)	AUC ($\mu\text{g/ml/hr}$)
Brand A (Reference)	2.83	14.06
Brand D	2.79	12.18
Point of estimate	98.59%	86.62%
Acceptable limit	80-125%	80-125%
Conclusion	Bioequivalent	Bioequivalent
Brand A (Reference)	2.83	14.06
Brand E (Test)	2.67	11.34
Point of estimate	94.35%	80.65%
Acceptable limit	80-125%	80-125%
Conclusion	Bioequivalent	Bioequivalent
Brand A (Reference)	2.83	14.06
Brand F (Test)	2.78	12.38
Point of estimate	98.23%	88.05%
Acceptable limit	80-125%	80-125%
Conclusion	Bioequivalent	Bioequivalent

Figure 4.4 Super imposed mean salivary concentration–time graph of all brands



CHAPTER FIVE

5.0 DISCUSSION

From the result of in-vitro studies result of weight variation, friability, crushing strength, disintegration, dissolution and assay as specified by B.P (2002 and 2009) all brands passed the test indicating pharmaceutical equivalence giving the opportunity of initiating bioequivalence trial. Uniformity of weight, assay, disintegration and dissolution are compendial standards to assess the quality of tablets while hardness and friability are referred to as non-compendial standards although friability is now included in the United States Pharmacopeia (USP, 2000).

Uniformity of weight does serve as a pointer to good manufacturing practices (GMP) as well as amount of the active pharmaceutical ingredient (API), ciprofloxacin hydrochloride contained in the formulation. All the brands complied with the compendial specification for uniformity of weight which states that for tablets weighing more than 324 mg, weights of not more than 2 tablets should not differ from the average weight by more than $\pm 5\%$.

The hardness or crushing strength assesses the ability of tablets to withstand handling without fracturing or chipping. It can also influence friability and disintegration of the tablet. The harder a tablet, the less friable and the more time it takes to disintegrate. Brand C (8.25 kg/f) required the least pressure before fracture followed by brands D (10.42kg/f), F (10.50kg/f), B (11.75kg/f), A (12.42kg/f) then E (12.58kg/f). A force of about 4 kg is the minimum requirements for a satisfactory tablet hence the tablets of all brands were satisfactory for hardness.

Friability test is used to evaluate the tablets resistance to abrasion. The compendial specification for friability is less than 1% (BP, 2009). Friability for all the brands was below 1%.

All the brands complied with the compendial specifications for disintegration. Brand A (3.32 ± 0.56), Brand B (4.42 ± 0.25), Brand C (2.74 ± 0.11), Brand D (9.50 ± 0.23), Brand E (8.27 ± 0.33) and Brand F (2.17 ± 0.12). The BP specification is that film coated should disintegrate within 30mins (BP, 2009). The drug incorporated in a tablet is released rapidly as the tablet disintegrates. Therefore, disintegration is a vital quality parameter of tablet as this is directly related with drug dissolution and subsequent bioavailability of drug.

All the brands complied with the compendial specifications for dissolution. The USP and BP specifies that the amount of drug released (dissolution) should not be less than 80% of the labeled amount at 30 min. The tablet dissolution rates were in the order – A > E > F > C > D > B. All the tablets, both generics and the innovator brand contained ciprofloxacin within the percentage of the labeled claim (BP, 2009).

Ngwuluka *et al*, 2013 conducted similar study on six brands of ciprofloxacin and found out that all the brands passed the test.

The BP 2002 specifications for assay are that the ciprofloxacin content should not be less than 95 % and not more than 105 %. Therefore, the assay results [Brand A (103.8%) Brand B (100.5%) Brand C (97.2%) Brand D (98%) Brand E (101.6%) Brand F (102.7%) ascertained the presence and compendia quality of ciprofloxacin in all the products.

Ciprofloxacin powder was identified by chloride test appearance of white precipitate on addition of AgNO_3 to the 2ml ciprofloxacin hydrochloride solution indicated a positive chloride test. (BP 2002)

Cheap, specific and selective UV spectrophotometric method for analysis of ciprofloxacin in biological sample was developed. The method was validated according to international conference on Harmonization (ICH) guideline. Wavelength of maximum absorbance was obtained at 277nm when 10 $\mu\text{g/ml}$ ciprofloxacin solution was scanned through a workable wavelength of 200-400nm. Maximum absorbance was recorded at pH 4. Calibration curve was constructed by plotting absorbance against concentration (1-6 $\mu\text{g/ml}$). Beer-Lambert's law was obeyed in the concentrations of 1-6 $\mu\text{g/ml}$ as the correlation co-efficient was 0.9889.

In validating the developed method, relative standard deviation (RSD) values for within-day and between day precision were 1.2 and 1.8% respectively which is within the specified limit of $\text{RSD} \leq 2\%$ as specified by the ICH guidelines indicating that the developed method have good repeatability. Precision is the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Accuracy of the developed method was tested by determining percentage recovery at 80%, 100% and 120% (8, 10 and 12 $\mu\text{g/ml}$) by adding the corresponding amount of standard ciprofloxacin into the pre-analyzed 10 $\mu\text{g/ml}$ concentration and spiked with blanked saliva. The percentage recovery was found within the range of 98.3-99.3% which is within the accepted limit of 98-102% (Chan, 2008)

Six healthy adult male volunteers (age 22-43±5years, weight 55-65±5 kg) were used. No subject had a history of hepatic, renal, gastro-intestinal, hematological disease or drug allergy. Prior to any screening procedures consent was obtained from each subject participating in this study after adequate explanation of the aims, methods, objective, and potential hazards of the study.

Six brands of ciprofloxacin tablets were purchased from registered pharmacies at Sabonhari Market Zaria Kaduna State and were then coded as A, B, C, D, E and F. Brand A considered as innovator.

For a pharmacokinetic comparison, the pharmacokinetic parameters are area under the curve (AUC), peak concentration (C_{max}) and time to peak concentration (T_{max}). Individual plasma level-time curves were constructed and maximal concentration (C_{max}) and time to reach this maximum (T_{max}) were directly obtained from these curves. Area under the plasma concentration against time curve was calculated by the trapezoidal rule. Then, ratios of AUC and C_{max} for all the brands and reference was calculated, Bioequivalence was concluded since $p < 0.05$ at 95% confidence intervals.

The C_{max} and AUC values for reference and test brand B was (2.83 and 2.61) and (14.06 and 12.09) respectively with point of estimate ratio of 92.23% and 85.99%.

The C_{max} and AUC values for test reference test brand C was (2.83 and 2.51) and (14.06 and 12.095) respectively with point of estimate ratio of 88.69% and 86.02%

The C_{max} and AUC values for reference and test brand D was (2.83 and 2.79) and (14.06 and 12.18) respectively with point of estimate ratio of 98.59% and 86.62%

The C_{max} and AUC values for reference and test brand E was (2.83 and 2.67) and (14.06 and 11.34) respectively with point of estimate ratio of 94.35% and 80.65%

The C_{max} and AUC values for reference and test brand F was (2.83 and 2.78) and (14.06 and 12.38) respectively with point of estimate ratio of 98.23% and 88.05%

In a bioequivalence studies comparing the bioavailability of two oral formulations of 500mg ciprofloxacin widely used in Mexico, C_{max} and AUC values of the test and reference formulations was within the acceptable range of 80-125. It was concluded that the formulations tested are bioequivalent. (Daniel *et al*, 2003)

In a bioequivalence study of two different formulations of ciprofloxacin tablets in healthy volunteers the mean values for the test/reference were 95-107% for AUC and 90-107% for C_{max}, respectively. Both of these values were within the bioequivalence acceptance range of 80-125% (Hassan *et al*, 2007).

In a pharmacokinetic and bioequivalence evaluation of two brands of Ciprofloxacin 500 mg tablets in Iranian healthy volunteers the 90% confidence intervals of test/reference for AUC_{0-t}, AUC_{0-∞} and C_{max} were (95.6–109.9 %), (91.8–106.3 %) and (95.2–112.8 %), respectively and all were within the bioequivalence acceptance range of 80–125 % (Valizadeh *et al*, 2012).

In a bioequivalence and pharmacokinetic study of two oral formulations of Ciprofloxacin Tablets 250mg in healthy male volunteers all pharmacokinetic parameters are within the bioequivalence accepted range of 80%-125% (Mohammad *et al*, 2007).

In a randomized crossover study of the bioequivalence of two commercially available ciprofloxacin after administration of a single oral dose in healthy volunteers, there was no significant difference in pharmacokinetic parameters used to assess bioavailability between the two formulations both are similar in the rate and extent of absorption.

Therefore, it is concluded that the test drug product is bioequivalent to the reference brand (Demiana *et al*, 2006).

CHAPTER SIX

6.0 CONCLUSION

Based on the result from the in-vitro quality control studies, it can be concluded that all the brands complied with the pharmacopoeial specifications described in the BP and USP. Moreover, the ciprofloxacin tablets included in the study have mean drug content within the compendial tolerance limits. Statistical comparison for drug content indicates that with 95% confidence, that there is no significant difference among the brands ($P < 0.05$.)

The UV Spectrophotometric method developed is a simple, cheap, effective, rapid, precise and available method

There was no significant difference in pharmacokinetic parameters used to assess bioavailability. AUC, C_{max} and T_{max} between the test brands and innovator are similar in the rate and extent of absorption. Therefore, it is concluded that the test drug product is bioequivalent to the reference brand and therefore interchangeable.

RECOMMENDATIONS

Ciprofloxacin being one of the most commonly used antibiotics in Nigeria today to treat infections due to its spectrum of activity; several generic brand products are available. Bioequivalence studies of the different generic drugs in circulation is required to ascertain that the drugs being sold can actually be trusted to produce the desired effect similar to the standard drug.

REFERENCE

- Adegbolagun, O.A, Olalade, O.A, Osumah, S.E. (2007). Comparative evaluation of the biopharmaceutical and chemical equivalence of some commercially available brands of ciprofloxacin hydrochloride tablets. *Tropical Journal of Pharmaceutical Research*, **6**(3): 737-745
- Adnan A. K, Badraddin M. A., Mohamed W. A., Ali S. A., Yousry M. El., Mohamed I.A. (2013). Comparative bioequivalence evaluation of two brands of ofloxacin 200mg tablets (Jedcoflacin vs Tarivid) in 24 health volunteers: a randomized, single dose, two period, and two sequence cross-over studies. *Digest journal of nanomaterials and biostructure*, **8**(1):239-246
- Akinleye M.O., Coker H.A.B., Chukwuani C.M., Adeoye A.W. (2007). Effect of Five alive® fruit juice on the dissolution and absorption profiles of Ciprofloxacin. *Nigerian quarterly journal of hospital medicine*, **17**(1):53-57
- Bayer Healthcare pharmaceuticals (2009). CIPRO (ciprofloxacin hydrochloride) Tablets and CIPRO (ciprofloxacin hydrochloride) Oral Suspension. Ciprofloxacin medication guide. Retrieved from <http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=888dc7f9-ad9c-4c00-8ddfd9bd27c0>. Retrieved 14/4/2013
- Behera S., Subhajt G., Fahad A., Saayak S., Sritoma B. (2012). UV-Visible Spectrophotometric Method Development and Validation of Assay of Paracetamol Tablet Formulation. *Journal Analytical and Bioanalytical Techniques*, **3**(6):2-6
- Birkett, D.J. (2003). "Generics - equal or not?" *Australian Prescriber*, **26**(4): 85-7
- British Pharmacopoeia (2009). *Medicinal and Pharmaceutical Substances*, (pp. 1381-85, 8351 – 8353). London: The Stationery Office on behalf of the Medicines and Healthcare products Regulatory Agency (MHRA)
- British Pharmacopoeia (2002). *Medicinal and Pharmaceutical Substances*, (pp. 1301). London: The Stationery Office on behalf of the Medicines and Healthcare products Regulatory Agency (MHRA)
- Center for Drug Evaluation and Research, United States Food and Drug Administration (2003). "Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products — General Considerations". (pp. 1-23). Retrieved from <http://www.fda.gov/cder/guidance/index.htm>. pdf. Retrieved 9/4/13

- Chan, C. C. (2008). Analytical method validation: principles and practices. In: S.C.Gad (Ed.), *Pharmaceutical Manufacturing Hand book: Regulations and Quality*, (pp. 727-742). USA: John Willey and Sons
- Daniel T., Alejandra T., Miriam D.C., María E. A., Herrera E., Francisco J. F. (2003). Comparative Bioavailability of Two Oral Formulations of Ciprofloxacin. *Proceedings of the Western Pharmacological Society*, **46**:134-135
- Dawes, C., Macpherson, L. M. D. (1992). Effects of 9 different chewing-gums and lozenges on salivary flow-rate and pH. *Caries Research*, **26**(3):176-182
- Demiana, I. N., Nisserin. F. A., LailaA., Abdulla. M. M. (2006). Randomized crossover studies of the bioequivalence of two commercially available ciprofloxacin after administration of a single oral dose in healthy volunteers, *HIV and AIDS Review*, **5**(1): 20-29.
- Dorđević S., Kilibarda V., Stojanović T. (2009). Determination of carbamazepine in serum and saliva samples by high performance liquid chromatography with ultraviolet detection. *Vojnosanitetski pregled*, **66**(5):347–352
- European Medicines Agency, Committee for Medicinal Products for Human Use (2010). "Guideline on the Investigation of Bioequivalence". (pp 1-27). Retrieved from <http://www.ema.europa.eu>. pdf. Retrieved 9/4/13
- Getu K., Awot G.E. (2010). Quality Assessment of the Commonly Prescribed Antimicrobial Drug, Ciprofloxacin Tablets, Marketed in Tigray, Ethiopia. *Momona Ethiopian Journal of Science*, **2**(1):93-107
- Hassan Y., Alfadly S.O., Azmin M.N., Peh K.K., Tan T.F., Noorizan A.A., Ismail O. (2007). Bioequivalence evaluation of two different formulations of ciprofloxacin tablets in healthy volunteers. *Singapore Medical Journal*, **48**(9):819-23
- ICH Topic Q2A (1995). Validation of analytical methods, Definition and Terminology. European Agency for the Evaluation of medicinal product human medicine Evaluation. Ref. CPMP / ICH / 381 / 95. Retrieved from <http://www.fda.gov/guidances/ucm073381.pdf>. Retrieved 9/4/13
- Karin M.H., Douwe D., Jan Z., Robert A.A. (1999). Saliva as an analytical tool in toxicology. *International Journal of Drug Testing*, **1**(1); 1-7
- Luthy S., Ambroise P. (2004). Bioequivalence study comparing a new Paracetamol solution for injection and Proparacetamol after single intravenous infusion in subjects. *International journal of clinical pharmacology*, **42**(1):50-57

- Mohammad AbulKalam A., Ashik U., MahbubLatif A.H.M. (2007). Pharmacokinetic Study of Two Oral Formulations of Ciprofloxacin Tablets in Healthy Male Volunteers. *Journal of applied research*, **7**(2):150-57
- Mukhtar M.D., Chedi B.Z., Aliyu M., Umar M.S., Abdullahi A.A. (2010). In-Vitro Assessment of Some Oral Ciprofloxacin Brands Traded in Kano, Nigeria. *International Journal of Pharmaceutical Sciences*, **2**(1):13-17
- Ngwuluka N.C., Lawal K., Olorunfemi P.O., Ochekepe, N.A. (2009). Post-market in vitro bioequivalence study of six brands of ciprofloxacin tablets/caplets in Jos, Nigeria. *Scientific Research and Essay*, **4**(4):298-305.
- OsonwaUduma E., AgbokeAyodeji A., Amadi R.C., Okorie O., Opurum C.C. (2011). Bioequivalence studies on some selected brands of ciprofloxacin hydrochloride tablets in the Nigerian market with ciprofloX® as innovator brand. *Journal of Applied Pharmaceutical Science*, **01**(06):80-84
- Pranshu Tangri, Ishwari Dutt (2012) Development and Validation of Dissolution Procedures for Ciprofloxacin. *Current Pharmaceutical Research*, **2**(3)553-556.
- Rasma Chereson (1996). Bioavailability, Bioequivalence and Drug Selection. In: Umesh Banakar (Ed). *Basic Pharmacokinetics*, (pp. 8-20). Easton Pennsylvania: Mack Publishing Company
- Sean C.S. (2009). Antibacterials. *Martindale, The complete Drug Reference 36*, (pp. 243-248). London: Pharmaceutical Press
- SenthilKumar K., Mozhiarasi M., Chirag K., Mahashree M. (2012). Bioequivalence studies in pharmacokinetics of drugs. *International journal of universal pharmacy and bio sciences*, **1**(2):103-109
- Sharma A., Arora S., Grewal P., Chand P., Dhillon V., Malik K., Goel A. (2010). comparative bioequivalence study of some brands of Ofloxacin by urine and salivary analysis in india. *International journal of drug development & research*, **2**(3):534-546
- United State Pharmacopoeia (2000). National Formulary. USP 26/NF 21 (pp.1882-1883). United States: Pharmacopoeia Convention Inc
- Valizadeh H., Hamishehkar H., Ghanbarzadeh S., Zabihian N., Zakeri-Milani P., (2012). Pharmacokinetics and Bioequivalence Evaluation of Two Brands of Ciprofloxacin 500 mg Tablets in Iranian Healthy Volunteers; *Arzneimittelforschung*, **62**(12):566-570

Yogananda R., Nagaraja T.S., Snehalatha, Jayadevaiah K.V., Vijay Kumar M.M.J.
(2009) Comparative in-vitro bioequivalence studies of designed, branded and generic tablets of ciprofloxacin-250. *International Journal of Pharmaceutical Science*, **1**(1):28-34

APPENDICES

APPENDIX I

Weight variation (mg) n=20

Brands A	B	C	D	E	F
780	710	900	740	820	730
780	720	970	720	820	730
780	700	960	730	810	730
800	660	890	720	820	730
780	720	920	710	820	720
770	710	910	750	810	730
790	700	930	710	820	730
790	700	960	730	820	730
780	690	830	710	820	730
780	700	960	710	810	740
780	700	910	730	820	730
780	700	730	730	820	730
780	710	970	710	820	730

APPENDIX II

Friability (%)

	Brands A F	B	C	D	E
W1	7.83 8.20	6.34	8.24	7.25	7.34
W2	7.83 8.20	6.30	8.23	7.24	7.31
%	0.000 0.000	0.631	0.121	0.138	0.409

APPENDIX III

Crushing strength (Kgf) n=6

Brands A	B	C	D	E	F
12.0	11.0	8.0	9.5	12.0	10.0
12.5	11.5	8.5	10.0	13.0	10.5
13.0	12.0	7.5	10.0	13.5	11.0
12.5	12.5	9.0	11.5	12.0	10.0
12.0	11.5	8.5	9.5	13.0	10.5
12.5	12.0	8.0	11.5	12.0	11.0

APPENDIX 1V

Disintegration (mins) n=6

Brands A	B	C	D	E	F
8.90	4.00	2.40	2.00	7.05	1.55
9.00	4.10	2.48	2.20	8.00	2.15
9.20	4.20	2.55	2.30	8.05	2.25
9.40	4.30	3.00	3.00	8.25	2.30
10.00	4.30	3.00	5.15	9.10	2.35
10.50	4.55	3.00	5.25	9.15	2.40

APPENDIX V

Dissolution at 30mins

Time	Brands					
	A	B	C	D	E	F
30	89.72	81.18	83.84	84.64	87.22	86.54

APPENDIX VI

Assay

Brands	Strength (g)	Weight (g)	Equivalent of 0.3g to dissolve (g)	Final Volume (ml)	%Content
A	0.5	0.75	0.45	9.40	103.8
B	0.5	0.66	0.40	9.10	100.5
C	0.5	0.81	0.49	8.80	97.2
D	0.5	0.69	0.41	8.90	98.0
E	0.5	0.61	0.37	9.20	101.6
F	0.5	0.71	0.43	9.30	102.7

APPENDIX VII

Preparation of 0.1N HCl

1.7ml of the conc. HCl solution was measured and made up to 200ml with distilled water to make 200ml of 0.1N HCl solution as follows:

$$C_1 = 12N, V_1 = ?, C_2 = 0.1N \text{ and } V_2 = 200\text{ml}$$

$$C_1 V_1 = C_2 V_2, 12 \times V_1 = 0.1 \times 200$$

$$V_1 = 1.7\text{ml}$$

APPENDIX VIII

Preparation of 0.1N NaOH

16g of NaOH was weighed and then dissolved into 200ml of distilled water contained in 500ml volumetric flask to make 200ml 2M NaOH solution as follows:

$$V = 200\text{ml (0.2ml)}, \text{Conc.} = 2M$$

$$\text{Mol} = 0.2 \times 2, \text{Mol} = 0.4\text{ml}$$

$$\text{Mass} = \text{Mol} \times \text{Mass } 0.4 \times 40$$

$$\text{Mass} = 16\text{g}$$

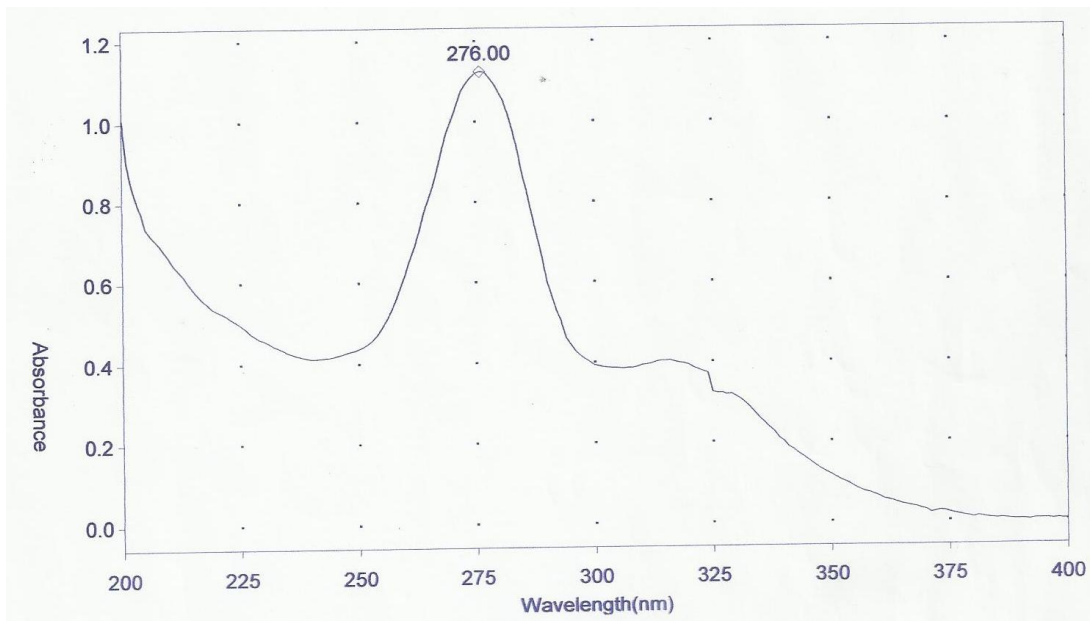
APPENDIX IX

Preparation of pH solution

pH solution of 4 and 5 were prepared by dissolving each of the pH 4 and pH 5 buffer tablets into 100ml distilled water. pH 9 and 10 were made by adjusting the previous pHs with either 0.1N HCl or 0.1N NaOH as the case may be.

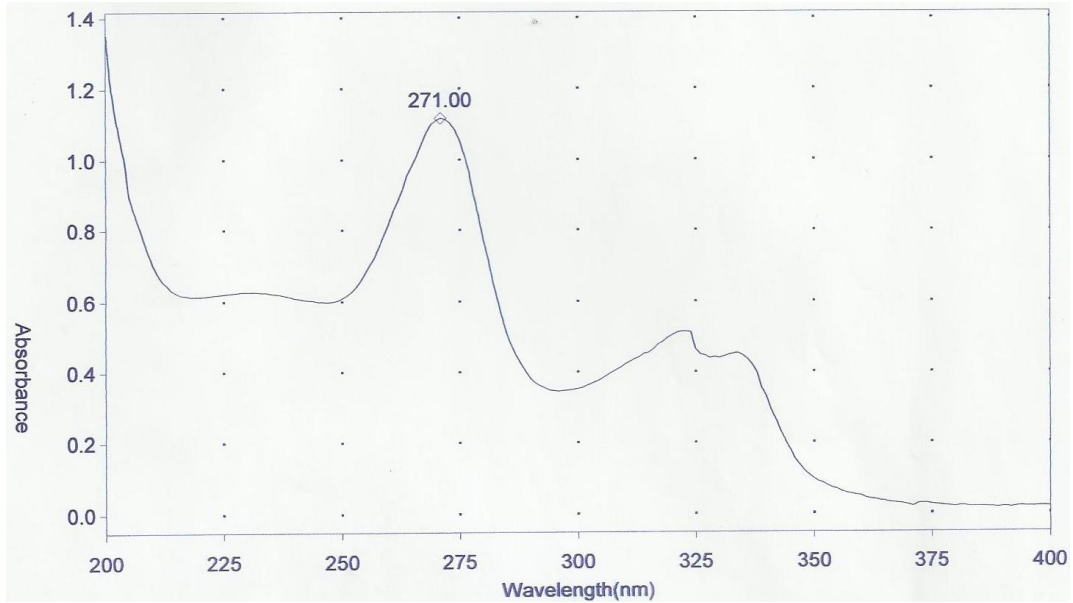
APPENDIX X

Spectrum of 10 μ g/ml solution of Ciprofloxacin Hydrochloride standard powder at pH 5



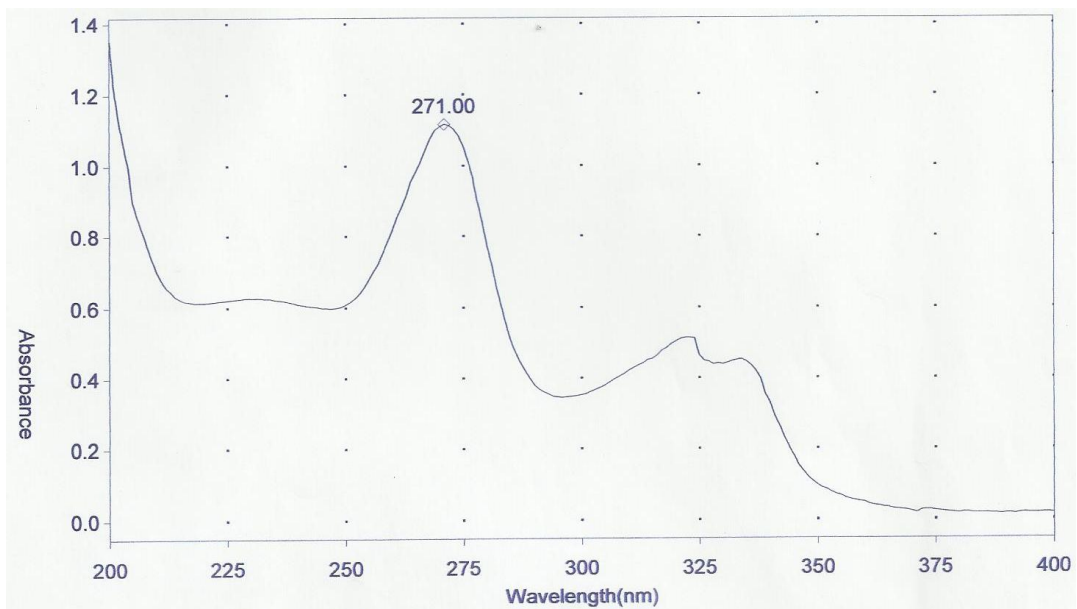
APPENDIX XI

Spectrum of 10 μ g/ml solution of Ciprofloxacin Hydrochloride standard powder at pH 9



APPENDIX XII

Spectrum of 10 μ g/ml solution of Ciprofloxacin Hydrochloride standard powder at pH 10



APPENDIX XIII

Precision

Within-day	Between-day
1.520	1.400
1.512	1.423
1.501	1.451
1.479	1.463
1.472	1.403
1.485	1.412

APPENDIX IV

Sample conversion of absorbances of innovator Brand into concentrations

Time(hr)	Absorbance	Concentration(mcg/ml)
0.5	0.279	0.71
1	0.362	1.47
2	0.511	2.83
3	0.480	2.55
4	0.440	2.18
5	0.402	1.84
6	0.330	1.18
7	0.300	0.93
8	0.270	0.65

APPENDIX XV

Sample conversion of absorbances of Brand B into concentrations

Time(hr)	Absorbance	Concentration(mcg/ml)
0.5	0.285	0.52
1	0.359	1.44
2	0.486	2.61
3	0.460	2.36
4	0.373	1.98
5	1.080	1.57
6	0.319	1.08
7	0.290	0.81
8	0.270	0.63

APPENDIX VI

Sample conversion of absorbances of Brand C into concentrations

Time(hr)	Absorbance	Concentration(mcg/ml)
0.5	0.242	0.37
1	0.376	1.59
2	0.475	2.51
3	0.464	2.40
4	0.410	1.93
5	0.387	1.69
6	0.346	1.32
7	0.269	0.62
8	0.251	0.45

APPENDIX VII

Sample conversion of absorbances of Brand D into concentrations

Time(hr)	Absorbance	Concentration(mcg/ml)
0.5	0.260	0.53
1	0.354	1.39
2	0.507	2.79
3	0.450	2.27
4	0.420	2.00
5	0.390	1.69
6	0.325	1.13
7	0.290	0.82
8	0.254	0.48

APPENDIX XVIII

Sample conversion of absorbances of Brand E into concentrations

Time(hr)	Absorbance	Concentration(mcg/ml)
0.5	0.248	0.43
1	0.346	1.32
2	0.493	2.67
3	0.439	2.18
4	0.415	1.96
5	0.394	1.76
6	0.320	1.10
7	0.311	1.00
8	0.302	0.92

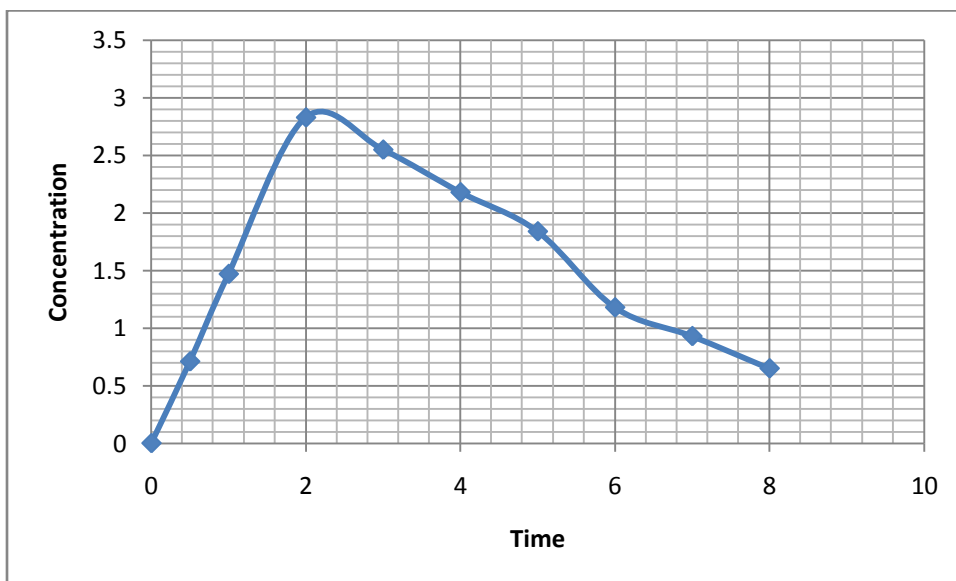
APPENDIX XIX

Sample conversion of absorbances of Brand F into concentrations

Time(hr)	Absorbance	Concentration(mcg/ml)
0.5	0.257	0.51
1	0.356	1.42
2	0.505	2.78
3	0.458	2.35
4	0.392	1.75
5	0.368	1.53
6	0.347	1.33
7	0.303	0.93
8	0.285	0.77

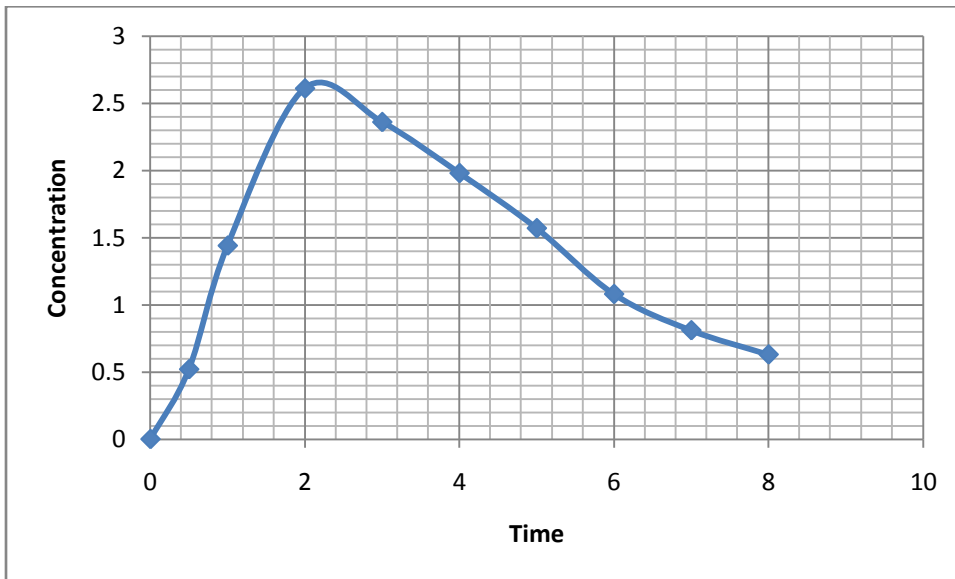
APPENDIX XX

Concentration-Time curve of Innovator Brand



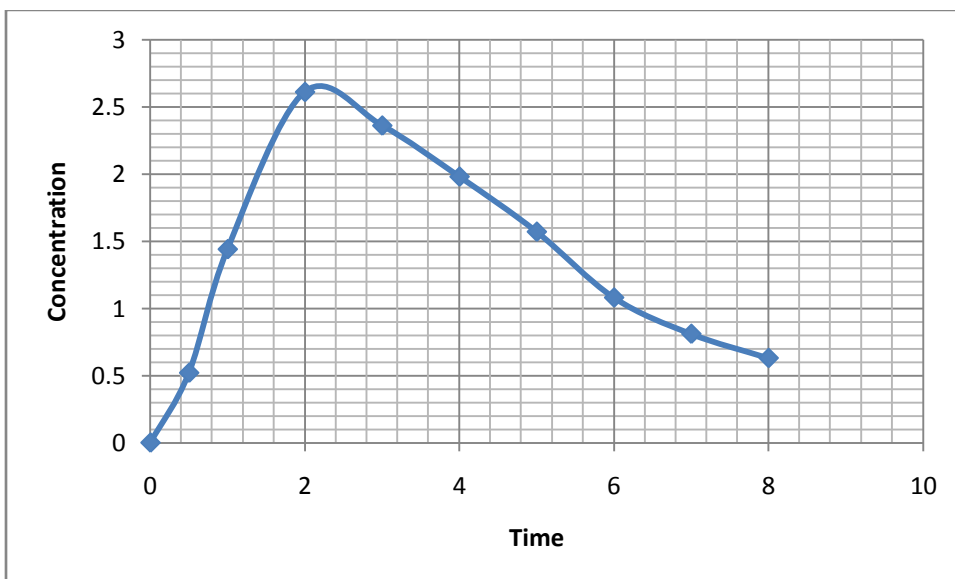
APPENDIX XXI

Concentration-Time curve of Brand B



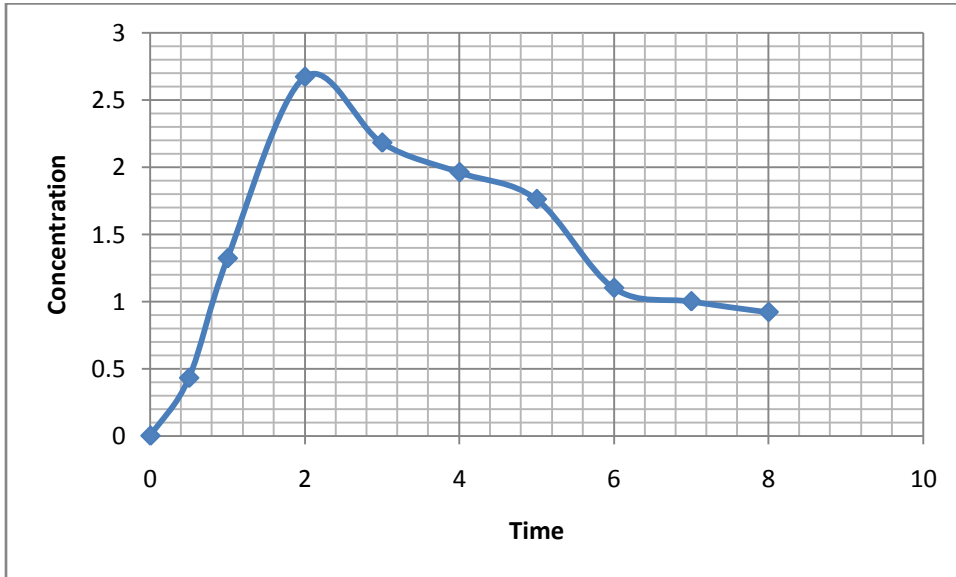
APPENDIX XXII

Concentration-Time curve of Brand C



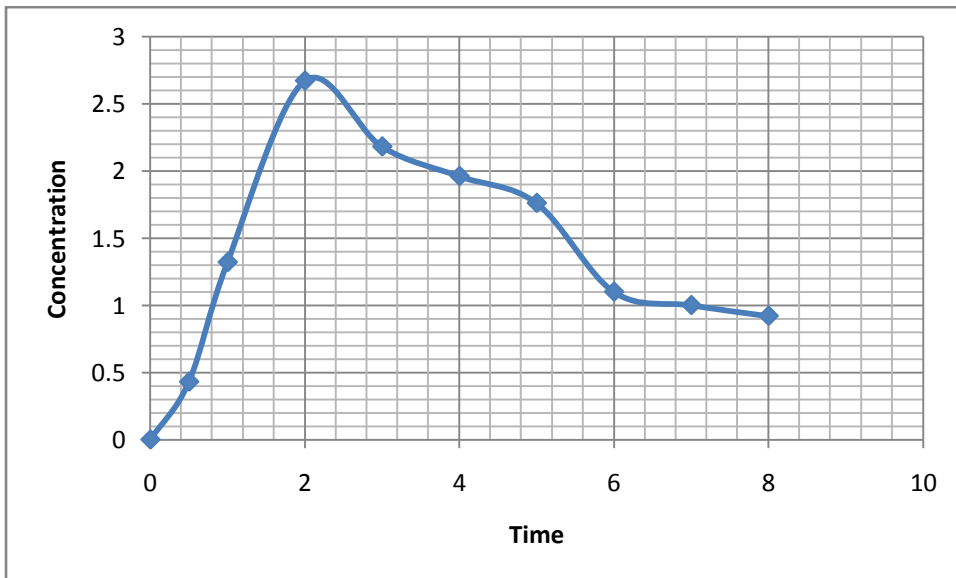
APPENDIX XXIII

Concentration-Time curve of Brand D



APPENDIX XXIV

Concentration-Time curve of Brand E



APPENDIX XXV

Concentration-Time curve of Brand F

