IN VITRO COMPARATIVE EVALUATION OF THE QUALITY AND PHARMACEUTICAL EQUIVALENCE OF SELECTED GENERIC CIPROFLOXACIN HYDROCHLORIDE TABLET BRANDS MARKETED IN KENYA

BY

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This thesis is my original work and has not been presented for examination in any other university.

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Dedication

To my friend, Lydia Abolo, for her support and encouragement.

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Abbreviations

°C	Degrees centigrade
Å	Angstrom
AIDS	Acquired immunodeficiency syndrome
amu	Atomic mass units
BP	British Pharmacopoeia
C_{18}	Octyldecylsilane stationary phase for chromatography
cm	Centimeter
CSF	Cerebral Spinal Fluid
DARU	Drug Analysis and Research Unit
DNA	Deoxyribonucleic acid
FDA	Food and Drug Administration
FTIR	Fourier-transform infra-red
GMP	Good manufacturing practices
h	Hour(s)
HIV	Human Immunodeficiency Virus
HPLC	High performance liquid chromatography
ICH	International Conference on Harmonisation
INN	International Nonproprietary Name
Kg	Kilogram

LC	Liquid chromatography
M	Molar
m	Metre
mg	Milligram
MIC	Minimum inhibitory concentration
min	Minute(s)
mL	Milliliter
mm	Millimeter
ms	Millisecond(s)
N	Newton
NF	National formulary
nm	Nanometer
Ph. Int.	International Pharmacopoeia
pK_a	Acid dissociation constant
PPB	Pharmacy and Poisons Board
QA	Quality assurance
QC	Quality control
RNA	Ribonucleic acid
rpm	Revolutions per minute
SD	Standard deviation
TLC	Thin layer chromatography

USP	United States Pharmacopoeia
UTI	Urinary tract infections
UV	Ultraviolet
WHO	World Health Organization
µm	Micrometer

Definition of terms

Bioavailability

"This term means the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action " (Shargel and Yu, 1985).

Bioequivalence

Bioequivalence is the absence of a significant difference in the rate at which, and the extent to which, the active ingredients in pharmaceutical equivalents become available at the site of drug action in the body when administered under similar experimental conditions in an appropriately designed study. A product may also be considered bioequivalent to an innovator product if (a) the difference in rate of drug absorption between the two products is intentional and (b) no significant difference is found in the extent of absorption of the two products when they are evaluated under similar experimental conditions (Shargel and Yu, 1985).

Pharmaceutical equivalence

To be considered pharmaceutically equivalent, two drug products must; (a) contain identical amounts of the same active ingredients in the same dosage form, (b) be formulated to meet the same compendial or other applicable standards of quality and purity, and (c) generally be labeled for the same indications. However, pharmaceutical equivalents may differ in the excipients (e.g., flavors, preservatives) that they contain, as well as in their shape, scoring, packaging, and in certain circumstances, their labeling (Shargel and Yu, 1985).

ABSTRACT

Introduction

Ciprofloxacin is a quinolone derivative antibiotic produced through structural modification by addition of the 7-piperazinyl group and a fluoride atom on the quinolone molecule. It has a broad spectrum of activity against both Gram-positive and Gram-negative bacteria. Because of this, it is used to treat a wide range of infections including urinary tract infections (UTIs), respiratory tract infection, skin and soft tissue infections and intra-abdominal infections. It is listed by the World Health Organisation and by the Ministry of Health Kenya as an essential drug. The number of ciprofloxacin tablet brands on the Kenyan market has increased in the recent years from different sources and currently as of 2014 there are 88 different brands registered by the Pharmacy and Poisons Board (PPB). The increase in the number of generic drug products from multiple sources has placed people and prescribers in a position of selecting one from among several seemingly pharmaceutically equivalent products. This poses a challenge to the quality regulatory bodies leading to the influx of counterfeits and substandard products. Therefore there is need to subject the different brands of the same drug to physicochemical tests to determine their pharmaceutical equivalence and the possibility of substituting one brand for another while achieving the same therapeutic effect. Hence, this work was done to determine the physicochemical properties of 20 brands of film coated ciprofloxacin tablets.

Study objectives

The general objective of this study was to determine the quality of commercially available ciprofloxacin tablets in Nairobi, Kenya. This was in order to determine the appropriateness of their interchangeability.

Methodology

In this study, 20 brands of ciprofloxacin hydrochloride 500 mg tablets were subjected to the standard physicochemical tests of identification, uniformity of weight, hardness, disintegration, dissolution and assay to assess their physical and chemical equivalence. The tests were performed using official methods described in the British Pharmacopoeia and United States Pharmacopoeia.

Results

The results of the generics were compared to those of the innovator brand with reference to the official standards. The retention time of the major peak of the sample solutions of all ciprofloxacin brands corresponded to that of the standard solution, as obtained in the assay. The retention times of the samples ranged from 5.1 to 5.2 min and corresponded to the standard's retention time at 5.2 min. All the brands complied with the compendial specification for uniformity of weight. And all the generics and the innovator brand were satisfactory for hardness.

The disintegration time for the innovator brand and the generics ranged from 0.5-23.5 min. All the brands were satisfactory for the disintegration time since they all disintegrated in less than 30 min. There was no direct correlation between tablet hardness and disintegration time. All the brands of ciprofloxacin tablets passed as per the USP specification. The highest percentage content was obtained for brand C013 (104.58 %), while the least drug content was obtained for brand C018 (90.38 %). Brands C009, C010, C013, C014, C015 and C017 contained more active pharmaceutical ingredient than the innovator brand.

At pH 1.2, most the brands of ciprofloxacin tablets studied released more than 85 % of the drug within 30 minutes except C004, C007 and C017, which had released only 82.48, 83.46 and 84.95 %, respectively. At pH 4.5, most the brands released more that 85 % of ciprofloxacin except brand C013, C015 and C016, which released 73,73, 80.44 and 81.57 %, respectively within 30 min. All the products failed to release the specified amount of ciprofloxacin of 85 % by USP (2014) within 30 min.

Conclusion

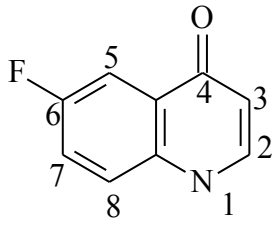
From the similarity factor f_2 calculation values, 10 of the 19 brands (52.63 %) can be said to be pharmaceutically equivalent with the innovator brand (IB), therefore they can be interchanged with the innovator brand.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 The fluoroquinolone antibiotics

Fluoroquinolones are a family of synthetic antibacterial agents with bactericidal activity. They are derivatives of quinolones and produced through structural modification by addition of the 7-piperazinyl group and a fluoride atom at position 6. Figure 1 below shows the general structure of fluoroquinolones.



6-Fluoro-4-quinolone

Figure 1: The basic structure of fluoroquinolones

Since their introduction in the late 1980s, there has been a great expansion in the usage of fluoroquinolones as antimicrobial agents in both hospital and community sectors due to their broad spectrum of activity and more suitable pharmacokinetic properties (Prabodh *et al*., 2009). Fluoroquinolones are also effective in other diseases, for example skin and respiratory infections. Because of their excellent safety and tolerability, they have become popular alternatives to penicillin and cephalosporin derivatives in the treatment of various infections since they have activity against Gram-positive and Gram-negative microorganisms (Wolfson and Hooper, 1989).

1.2 Classification of quinolones

Table 1 shows the different generations of quinolones in the current clinical use (Dana *et al*., 2000). They are classified based on their antibacterial activity on Gram-positive and Gram-negative organisms with the first generation being the narrowest and the subsequent ones having a wider spectrum of activity (Jason *et al.*, 2010).

First-generation agents include cinoxacin and nalidixic acid, which are the oldest and least often used quinolones. Because minimal serum levels are achieved, use of these drugs has been restricted to the treatment of uncomplicated urinary tract infections. Cinoxacin and nalidixic acid require more frequent dosing than the newer quinolones, and they are more susceptible to the development of bacterial resistance. These agents are not recommended for use in patients with poor renal function because of significantly decreased urine concentrations (Wolfson and Hooper, 1989).

Second generation quinolones have increased Gram-negative activity, as well as some Gram-positive and atypical pathogen coverage. Compared with first-generation drugs and considered as a group, these agents have broader clinical applications in the treatment of complicated urinary tract infections and pyelonephritis, sexually transmitted diseases, selected pneumonias and skin infections. Ciprofloxacin is the most potent fluoroquinolone against *Pseudomonas aeruginosa*. Because of its good penetration into bone, orally administered ciprofloxacin is a useful alternative to parenteral antibiotics for the treatment of osteomyelitis caused by susceptible organisms (Stein and Ensberg, 1998). Ciprofloxacin is the most commonly used fluoroquinolone and it has been routinely used for the treatment of urinary tract infections (UTI), respiratory tract infections, chronic prostatitis, cystic fibrosis, gonorrhea, prophylaxis of meningococcal meningitis, acute uncomplicated cystitis in women and in surgical prophylaxis.

Third-generation quinolones have expanded activity against Gram-positive organisms, particularly penicillin-sensitive and penicillin-resistant *Streptococcus pneumoniae* and atypical pathogens such as *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*. Although the third-generation quinolones retain broad Gram-negative coverage, they are less active than ciprofloxacin against *Pseudomonas* species (Stein and Ensberg, 1998).

Fourth generation quinolones are used for treating intra-abdominal infections. Trovafloxacin adds significant antimicrobial activity against anaerobes while maintaining the Gram-positive and Gram-negative activity of the third-generation quinolones. It also retains activity against *Pseudomonas* species comparable to that of ciprofloxacin (Brighty and Gootz, 1997).

Table 1: Classification of quinolone antibiotics

Classification	Examples	Antimicrobial spectrum	General clinical indications
First generation	Nalidixic acid Cinoxacin	Gram-negative organisms (but not *Pseudomonas* species)	Uncomplicated urinary tract infections
Second generation	Norfloxacin Lomefloxacin Enoxacin Ofloxacin Ciprofloxacin	Gram-negative organisms (including *Pseudomonas* species), some Gram-positive organisms (including *Staphylococcus aureus* but not Streptococcus pneumoniae) and some atypical pathogens	Uncomplicated and complicated urinary tract infections and pyelonephritis, sexually transmitted diseases, prostatitis, skin and soft tissue infections
Third generation	Levofloxacin Sparfloxacin Gatifloxacin,	Same as for second-generation agents plus expanded Gram-positive coverage (penicillin-sensitive and penicillin-resistant *S. pneumoniae*) and expanded activity against atypical pathogens	Acute exacerbations of chronic bronchitis, community-acquired pneumonia
Fourth generation	Trovafloxacin Sitafloxacin Prulifloxacin Clinafloxacin Moxifloxacin	Same as for third-generation agents plus broad anaerobic coverage	Same as for first, second and third generation agents (excluding complicated urinary tract infections and pyelonephritis) plus intra-abdominal infections, nosocomial pneumonia, pelvic infections

Dana *et al*., 2000.

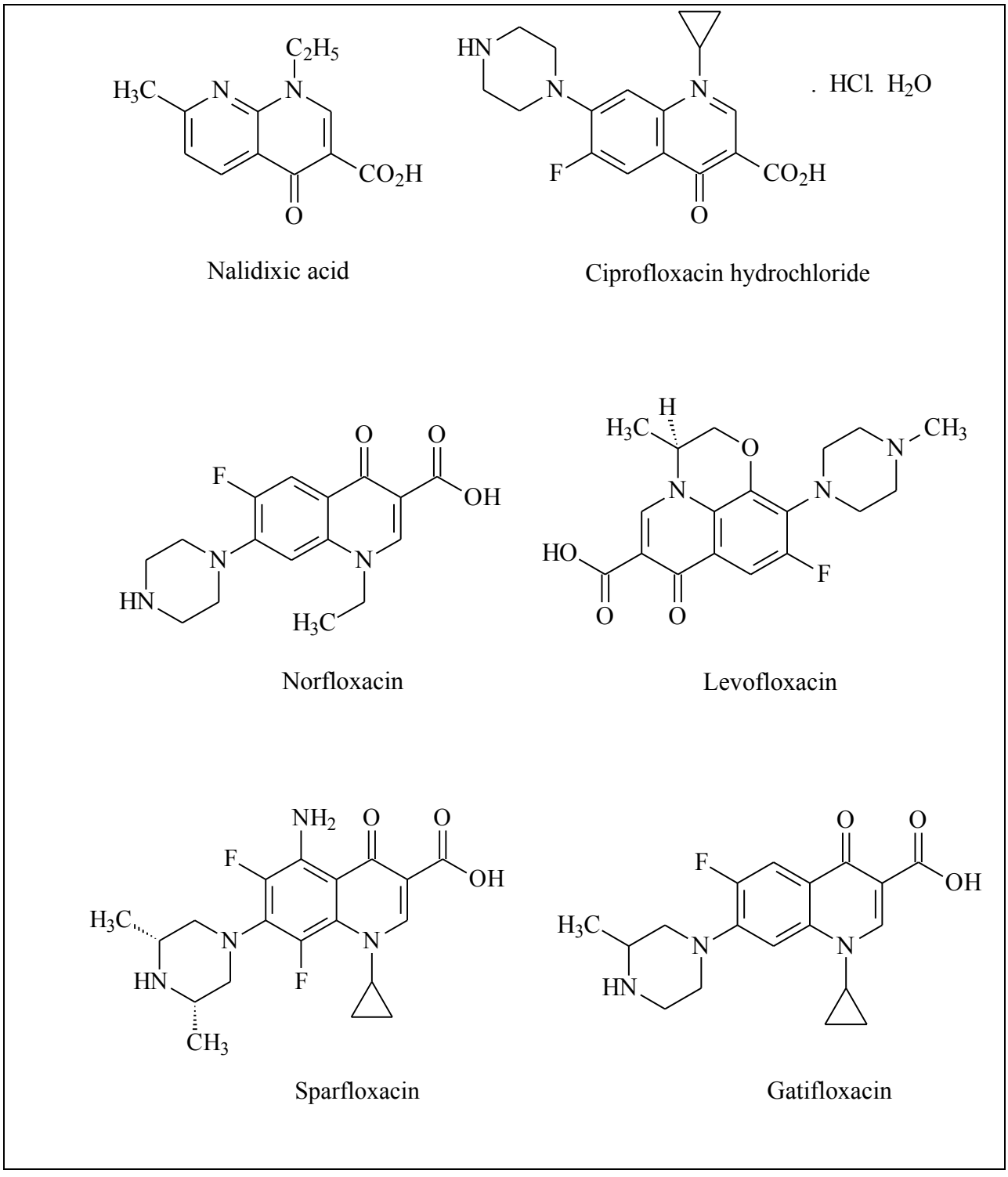


Figure 2: Chemical structures of some quinolones

1.3 Mechanism of action of fluoroquinolones

Fluoroquinolones enter the cells by porins, binding to and blocking the activity of deoxyribonucleic acid (DNA) gyrase and topoisomerase IV hence stopping replication, transcription, repair as well as recombination. The fluoroquinolones bind to the enzyme-DNA complex and form a stable ternary complex. The complex of drug, topoisomerase IV and DNA collides with the DNA replication complex and forms a physical barrier that blocks further progression of the replication fork. In addition, the drug, DNA gyrase and DNA complex blocks the passage of ribonucleic acid (RNA) polymerase and leads to the premature termination of transcription (Maruri *et al*., 2012).

In Gram-negative organisms like *Escherichia coli*, fluoroquinolones bind to DNA gyrase as a primary target and topoisomerase IV as the secondary target. In contrast, in Gram-positive organisms like *Staphylococcus aureus*, fluoroquinolones bind to topoisomerase IV as the primary target and DNA gyrase as the secondary target (Bolon, 2009). However, there are certain exceptions to this rule. For example, in *S. pneumoniae*, a Gram-positive organism, DNA gyrase was found to be more sensitive to fluoroquinolones than topoisomerase IV (Pan and Fisher, 1997). In *Mycobacterium tuberculosis,* DNA gyrase was found to be the novel target for fluoroquinolones (Poissy *et al*., 2010).

1.4 Antibacterial activity of fluoroquinolones

Fluoroquinolones exhibit similar antibacterial properties and *in vitro* testing showed that they act on a variety of Gram-positive and Gram-negative organisms (Rookaya *et al*., 2002). Various Gram-negative bacilli and cocci are also targeted and include *Neisseria gonorrhea, Pseudomonas aeruginosa, Aeromonas hydrophila, Haemophilus influenzae* and *Legionella pneumophila.* Fluoroquinolones can also be used against pathogens of the gastrointestinal tract like *E. coli, Salmonella* species, *Shigella* species*, Yersinia enterocolitica, Clostridium jejuni* and *Vibrio* species (Wolfson and Hooper, 1985). As some of the fluoroquinolones are eliminated by renal excretion, their concentration in the urine is very high and hence helps to manage a number of UTIs. They can also be used against pathogens that have developed multiple antibiotic resistances like methicillin resistant *S. aureus* and *β*-lactamase producing *N. gonorrhoea*. The only problem associated with this usage is the high risk of development of resistance to

fluoroquinolones (Steven *et al*., 2011). The most commonly targeted Gram-positive microorganisms are penicillin sensitive and resistant *S. pneumoniae, S. aureus, Streptococcus pyogenes* and *Streptococcus agalactiae* (Blondeau *et al*., 2000).

Fluoroquinolones are most active against *Pseudomonas* than any other bacterial species (Andriole, 1998). Ciprofloxacin is the most potent drug against *Pseudomanas aeroginosa*. The third and fourth generations of fluoroquinolones are active against *S. pneumonia*. In addition, the fourth generation fluoroquinolones, especially trovafloxacin, are lethal to anaerobic bacteria, namely *Actinomyces* species, *Bacteroides* species, *Bactriodes distasonis, Bacteroides thetaiotaomicron, Bacteroides fragilis, Clostridium perfringens,* and certain species of *Eubacterium, Lactobacillus* and *Peptostreptococci* (Andriole, 1998). Biowarfare microorganisms like strains of *Bacillus anthrax* and *Yersinia pestis* are also sensitive to fluoroquinolones (Powel *et al*., 2011). All generations of fluoroquinolones are active against Enterobacteriaceae.

1.5 Resistance

Resistance to fluoroquinolones develops in various ways. Firstly, it involves mutation of any one or both of the enzymes DNA gyrase and topoisomerase IV, resulting in decreased binding of the fluoroquinolones to the enzyme-DNA complex. If a mutation occurs on one enzyme only, the degree of resistance observed depends on the sensitivity of the enzyme targeted by the fluoroquinolones (Maruri *et al.,* 2012). A stepwise mutation is built up by spontaneous mutations in the genes *gyr* A*, gyr* B*, par* C and *par* E, as well as mutations leading to the increased expression of efflux pumps, such as mutations abolishing the expression of a transcriptional repressor for the pump (Marguerite, 2010). Initial mutation may not have a significant effect in the development of resistance but may be important for the occurrence of subsequent mutations that lead to higher levels of resistance. Such mutations, both single and stepwise, may lead to cross-resistance to other quinolones. Examples of mutations include *S. aureus* and *P. aeruginosa* that require only one mutation for sufficient degree of resistance. *Escherichia coli* on the other hand, requires more than one mutation to confer resistance, especially to the newer class of fluoroquinolones. In *S. aureus*, mutations were observed in Ser-80 to Tyr of *grl* A, which is a homologue of *par* C (Herin *et al.,* 2001).

The second method for the development of drug resistance involves the differential expression of efflux mechanisms whereby the energy-dependent efflux pumps, on recognizing an antibacterial compound as a potential substrate, force it out. The recognised compounds are usually hydrophilic. Over expression of the efflux pumps will lead to multi-drug resistance. For example, over expression of *Acr* AB*, Mdf* A and *Nor* E efflux genes in *E. coli* has been shown to contribute individually and simultaneously as a group to fluoroquinolone resistance (Marco *et al.,* 2009).

A third mechanism for resistance is due to the presence of a plasmid generally found in Gram-negative organisms that can be horizontally transferred. This plasmid encodes for plasmid-mediated quinolone resistance gene proteins (Qnr proteins) namely *qnr* A, *qnr* B and *qnr* S that belong to a pentapeptide repeat family and bind mainly to DNA gyrase, thus preventing the binding of the drug to the enzyme. They are also known to have structures similar to DNA and thus act as a substrate to DNA gyrase. These factors greatly reduce the action of fluoroquinolones (George *et al.,* 2006). A multiresistant plasmid (pMG252) with a wide host range that expresses quinolone resistance was isolated from *Klebsiella pneumoniae.* It leads to an 8- to 32-fold increase in the minimum inhibitory concentration (MIC) of fluoroquinolones (Rodriguez *et al.,* 2009). There has been no plasmid-mediated resistance observed in *M. tuberculosis*. It has been shown that the aminoglycoside inactivating enzymes, aminoglycoside acetyltransferase, can inactivate fluoroquinolones as well (Robiscek *et al*., 2006).

1.6 Adverse effects of fluoroquinolones

Most of the side effects of fluoroquinolones are mild to moderate like those related to the gastrointestinal tract or central nervous system. On some occasions the side effects can be serious like dysglycemia and hepatotoxicity (Murphy *et al*., 2012). Some fluoroquinolones like gatifloxacin tend to distort the sugar levels in the blood leading to hypoglycaemia and hyperglycaemia (Jiesheng *et al.,* 2001). Some fluoroquinolones prolong electrocardiographic QTc interval because they block human cardiac potassium channel. It has been reported that the C-5 substituent like a methyl group in sparfloxacin is responsible for prolonging the QTc interval by 14 ms and 11 ms, respectively (Briasoulis *et al.,* 2011). A prolonged QTc interval is indicative of arrhythmias and is considered a risk factor for sudden death. In different studies,

ciprofloxacin and levofloxacin were implicated in *Clostridium difficile* associated diarrhoea (Ozawa and Valadez, 2002). Sparfloxacin is associated with phototoxicity. It has been found that the reactive oxygen species, like superoxide anions, hydrogen peroxide and hydroxyl radicals produced by the ultra-violet (UV) illumination of fluoroquinolones lead to destruction of the target tissues such as the skin and mitochondria (Seto *et al.,* 2011). Fluoroquinolones also lead to toxicity of various organs such as liver, brain, central nervous system and the heart (Stahlmann and Lode, 2010). In addition to direct effects on the body, fluoroquinolones may also produce harmful effects by their interaction with other drugs such as theophylline, caffeine, methylxanthines, and tizanidine (Harder *et al.,* 1989). Fluoroquinolone administration is often discouraged in pregnant and breast-feeding women because they can easily traverse the placental barrier and get distributed in the fetus and are also secreted into breast milk. This may cause abortions as well as birth defects and arthropathy in the immature child (Polachek *et al.,* 2011).

1.7 Ciprofloxacin

1.7.1 Description

Ciprofloxacin was the first fluoroquinolone to be marketed. Bayer, a German-based drug and chemical company, discovered it in 1981. In 1987 ciprofloxacin under the brand name Cipro® was approved by the United States Food and Drug Administration (FDA) as the first oral broad-spectrum fluoroquinolone antibiotic. Absorption after oral administration follows zero-order kinetics and peak serum ciprofloxacin concentrations are reached in approximately 1 to 2 h. The volume of distribution is large with a steady-state range after oral or intravenous dosing of 1.74 to 5.0 L/kg reflecting penetration of the drug into most tissues. Its pharmacokinetic parameters allow for twice daily dosing with minimal side effects (Vance *et al.,* 1990).

Doses and strengths of ciprofloxacin hydrochloride are expressed in terms of its base (Martindale, 2014). The dose of ciprofloxacin (as hydrochloride) recommended by WHO is 250 mg. Tablets of ciprofloxacin hydrochloride are usually marketed as tablets containing 100 mg, 250 mg, 500 mg, 750 mg and 1000 mg (ciprofloxacin base equivalent) strengths. It is also present in flavoured syrups for pediatrics containing ciprofloxacin hydrochloride 5 mg/100 mL, eye/ear drops and in intravenous forms.

1.7.2 Chemistry

Ciprofloxacin hydrochloride is a monohydrochloride monohydrate salt of *1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid* with molecular formula $C_{17}H_{18}FN_3O_3.HCl.H_2O$ and molecular weight 385.82 amu (figure 1). It occurs as a faintly yellowish to light yellow crystals or crystalline powder. It is sparingly soluble to soluble in water; slightly soluble in acetic acid and in methyl alcohol; very slightly soluble in dehydrated alcohol; practically insoluble in acetone, in acetonitrile, in dichloromethane, in ethyl acetate, and in hexane (Martindale, 2014). It is acidic (pH between 3.0 and 4.5, in a solution 1 in 40) with a pK_a of 6.09 at 25 °C and a melting point of 255 - 257 °C. The pK_a of 6.09 belongs to the alkylamine nitrogen in the piperazinly group (Tornianen *et al*., 1996).

1.7.3 Synthesis

Several routes for the production of ciprofloxacin have been developed. One of the synthetic routes involves *β*-keto esterification of 2,4-dichloro-5-fluoroacetophenone with diethyl carbonate in presence of sodium hydride, then ethoxymethylenation, amination, cyclization, hydrolysis and piperazination (Dai *et al.,* 1992). In another synthetic route, the condensation of 2,4-dichloro-5-fluorobenzoyl chlorides with diethyl malonate by means of magnesium ethoxide in ether gives diethyl 2,4-dichloro-5-fluorobenzoylmalonate, which is partially hydrolyzed and decarboxylated with *p*-toluenesulfonic acid water yielding ethyl 2, 4-dichloro-5-fluorobenzoylacetate.

Condensation of ethyl 2, 4-dichloro-5-fluorobenzoylacetate with triethyl orthoformate in acetic anhydride with refluxing yields ethyl 2-(2,4-dichloro-5-fluorobenzoyl)-3-ethoxyacrylate, which is treated with cyclopropylamine in ethanol to give ethyl 2-(2,4-dichloro-5-fluorobenzoyl)-3-cyclopropylaminoacrylate. The cyclization of ethyl 2-(2,4-dichloro-5-fluorobenzoyl)-3-cyclopropylaminoacrylate with sodium hydride in refluxing dioxane yields 7-chloro-1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxoquinoline-3-carboxylic acid, which is finally condensed with piperazine in hot dimethyl sulfoxide yielding ciprofloxacin hydrochloride hydrate. This synthetic route is shown in figure 3 (Cen and Dai, 2001).

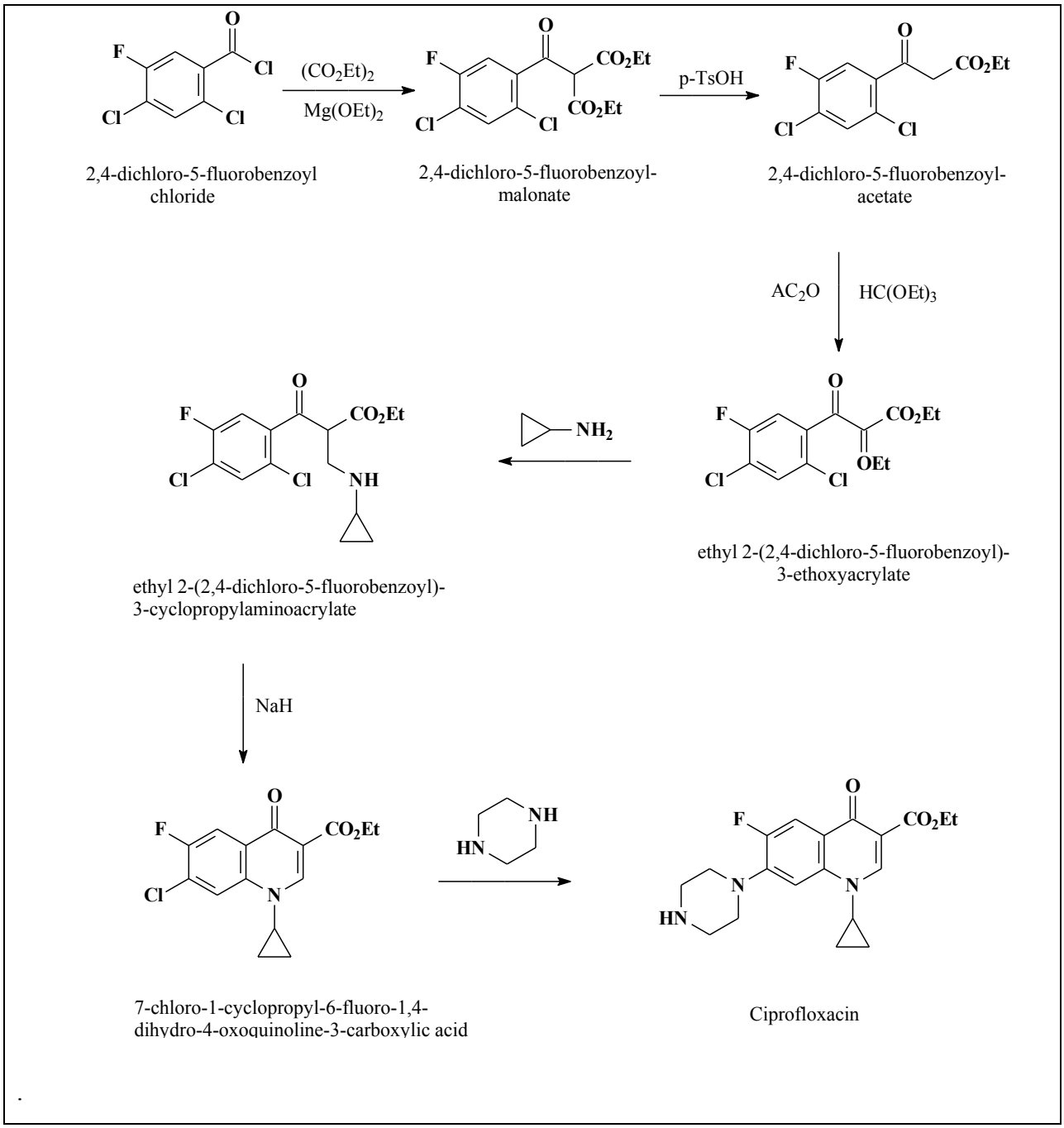


Figure 3: Synthetic scheme of ciprofloxacin

(Cen and Dai, 2001)

1.7.4 Stability

Ciprofloxacin is susceptible to photodegradation process, which may lead to reduction and/or loss of antibacterial activity and to induce phototoxicity as a side effect. It decomposes photochemically in aqueous solutions at acidic pH forming two major degradation products namely 7-[(2-aminoethyl) amino]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid and an aromatic amino-compound, 7-amino-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid (Torniainen *et al.,* 1997). Ciprofloxacin therefore should be strictly protected from all light during storage and administration.

The solubility of ciprofloxacin extremely depends on the pH value. The pK_a not only describes relative acid-base strengths of functional groups, but also allows for calculation of a given pH, the relative percentages of the ionized and un-ionized forms of the drug. It helps to predict relative water solubility, absorption and excretion for a given compound. At high pK_a values (low pH), ciprofloxacin is more soluble since it is in the protonated form. It is almost insoluble in water and alcohol. At pH 4-5 it shows the highest solubility (> 40 mg/mL). This corresponds to the hydrochloride form of ciprofloxacin, if the pH value is adjusted with hydrochloric acid. Ciprofloxacin is almost insoluble in the neutral pH range, while solubility increases with increasing pH value (approximately 30 mg/mL at pH 11). The stability of the dry substance of ciprofloxacin is very high at room temperature. Solutions in dialysis fluid (25 mg/L) are stable even after 42 days when stored at 37 °C (Mawhinney *et al.,* 1992).

The degradation products are formed during ozonation of secondary waste water effluent containing ciprofloxacin tracked via absorbance measurements. In a study by Nanaboina and colleagues (2012), twenty degradation products were elucidated for ciprofloxacin. The quinolone ring remains intact in the presence of *t*-butanol thus indicating that OH radicals can only oxidize this functional group while the piperazine ring was readily oxidized by molecular ozone. The cleavage of the quinolone moiety that resulted in several identified degradation products occurred *via* the attack by hydroxyl radicals on the carbon-carbon double bond adjacent to the carboxylic acid group.

1.7.5 Clinical pharmacology

Ciprofloxacin is particularly active against Gram-negative bacteria, including *Salmonella, Shigella, Campylobacter, Neisseria,* and *Pseudomonas*. It has only moderate activity against Gram-positive bacteria such as *S. pneumoniae* and *Enterococcus faecalis*. It should not be used for pneumococcal pneumonia because of increased resistance (Harwell and Brown, 2000) It is active against chlamydia and some mycobacteria. Most anaerobic organisms are not susceptible. Ciprofloxacin is approved for the treatment of 14 types of infections such as respiratory tract infections (but not for pneumococcal pneumonia), urinary-tract infections, infections of the gastro-intestinal system (including typhoid fever), bone and joint infections, gonorrhoea and septicaemia caused by sensitive organisms (Thoppil and Amin, 2000; Laurence *et al*., 2005; Li *et al*., 2007). It can be used alone or in combination with other antibacterial drugs in the empiric treatment of infections for which the bacterial pathogen has not been identified, including urinary tract infections and abdominal infections (Solomkin *et al*., 2010).

1.7.6 Pharmacokinetics

1.7.6.1 Absorption and distribution

Ciprofloxacin is rapidly and well absorbed from stomach and duodenum after oral administration. The absolute bioavailability varies between 55 to 88 % with no substantial loss by first pass metabolism (Lettieri *et al.,* 1991).

It demonstrates a linear increase in maximum concentration with increasing dosages. About 20-30 % of ciprofloxacin is protein-bound, and the drug is present in the plasma largely in a non-ionized form (Nakashima *et al.,* 1995). Ciprofloxacin is widely distributed into body tissues and fluids after intravenous administration. Highest concentrations of the drug generally are attained in bile, lungs, kidney, liver, gallbladder, uterus, seminal fluid, prostatic tissue and fluid, tonsils, endometrium, fallopian tubes, and ovaries. The large steady-state volume of distribution of 2-3 L/kg body weight shows that the drug penetrates tissues and fluids resulting in concentrations that substantially exceed those in serum. The drug is also distributed into adipose tissue, aqueous humor, bone, cartilage, fat, heart tissue (heart valves, myocardia), muscle, nasal and bronchial secretions, saliva, skin, sputum, and pleural, peritoneal, ascetic, blisters, lymphatic, and renal cyst fluid. Although the drug diffuses into the cerebral spinal fluid (CSF), concentrations in the

CSF are generally less than 10 % of peak serum concentrations. Drug levels in the aqueous and vitreous chambers of the eye are slightly lower than in serum (Lode *et al.,* 1987; Vance *et al.,* 1990).

1.7.6.2 Metabolism and excretion

Ciprofloxacin undergoes partial metabolism in the liver by modification of the piperazinyl group to four metabolites, which are desethylene ciprofloxacin, sulphociprofloxacin, oxociprofloxacin and formylciprofloxacin. They account for approximately 15 % of an oral dose and have antimicrobial activity, but are less active than unchanged ciprofloxacin (Vance-Bryan *at al.,* 1990). The serum elimination half-life of ciprofloxacin is about 3 to 4 h and the total clearance is approximately 35 L/h. About 50-70 % of the dose is excreted in the urine as unchanged drug. Approximately 15 % of a ciprofloxacin dose has been reported to be recovered in the faeces. This is because of elimination through the intestinal mucosa combined with biliary excretion. Two-thirds of a ciprofloxacin dose is eliminated by a combination of glomerular filtration and tubular secretion (Vance-Bryan *et al*., 1990).

1.8 Structure activity relationship

All fluoroquinolones have a basic 6-fluoro-4-quinolone structure with a fluorine atom at C-6 position as shown in figure 1. Differences between the various fluoroquinolones are usually due to various groups that are attached at positions 1, 5, 7 and 8 (Randal, 1999). The cyclopropyl group attached to the nitrogen atom (N-1) is responsible for the antibacterial property of ciprofloxacin (Wise *et al.,* 1983). It has been shown that a nitrogen group improves the pharmacokinetic properties, while on the other hand a sulphur atom at position 2 increases the antibacterial properties of the fluoroquinolones. At position 3, a carboxylic group is essential to form a link with the ketone group present at position 4. This interaction is essential for binding to DNA gyrase, one of the enzymes targeted by fluoroquinolones to bring about cell death (Schentag and Domagala, 1985). Replacement of the carboxylic acid group by a biosostere-fused isothiazolo ring aids in the understanding of the structure-activity relationship among the quinolones. The nitrogen atom of the ring mimics the function of the carboxylic acid group (Chu *et al.,* 1985).

The replacement of the ketone group at position 4 with other derivatives leads to inactive compounds. At position 5, substitutes of amino, nitro, halo or alkyl groups have been synthesised. An amino group at this position has been found to increase absorption and tissue distribution in the body (Domaga *et al.,* 1988). Various substitutions of H, F, Cl, Br, CH_3, SCH_3, $COCH_3$, CN and NO_2 groups can be made at the C-6 position. Of all these substitutions, the fluoro substituent increases activity the most by improving the binding ability and cell penetration of these fluoroquinolones such as in ciprofloxacin (Megan *et al.,* 1992).

The removal of the C-6 fluorine atom decreases ability to cause enzyme mediated DNA cleavage (Megan *et al.,* 1992). A piperazinyl group as in norfloxacin attached to C-7 position has been found to increase the antibacterial property, though other groups like 4-methylpiperazin-1-yl substituent as in perfloxacin and ofloxacin, piperazin-1-yl substituent as in ciprofloxacin and enofloxacin and 3-methylpiperazin-1-yl substituent as in lomefloxacin and temafloxacin have also been synthesized (Shafieea *et al.,* 2009). In addition to the piperazinyl group, a methyl group attached to it at the C-4 position increases its activity towards Gram-positive microorganisms. Substituent of halides or methoxy groups at position C-8 or the replacements of the C-8 carbon with nitrogen have been synthesized. Fluorine or methoxy group substituents at position 8, as in gatifloxacin and moxifloxacin, are more effective against resistant microorganisms than C-8 hydrogen (Hassan *et al.,* 2010).

1.9 LITERATURE REVIEW

1.9.1 Quality of drugs in the world

The availability of poor quality drugs - that is, medicinal products that are substandard, spurious, falsely labeled, falsified or counterfeit, as defined by the World Health Organization (WHO) has become an issue of public health concern. Such medicines can jeopardize patient safety, lead to treatment failure and to the development of drug resistance (WHO, 2012) and represent a waste of financial resources (Almuzaini e*t al*., 2013).

The problem of poor quality medicines is particularly widespread and potentially more devastating in low-income countries. It is therefore extremely important to strengthen such countries' capacity for quality control (QC) and quality assurance (QA) of medicines. However,

many low-income countries lack the resources to develop appropriate regulatory frameworks and the necessary QC and QA systems (USP Drug Quality and Information Program, 2009)

The report on pharmacopoeial quality of drugs supplied by Nigerian pharmacies published in 2001 revealed that 48 % of drug tested failed to comply with pharmacopoeial limits, which is the benchmark for assessing quality of pharmaceutical preparations (Taylor *et al.,* 2001). Another survey carried out in Tanzania on the quality of antimalarials in retail outlets also found a 12.2 % failure (Harparkash *et al*., 2008).

It has been estimated that up to 15 % of all medicines sold across the world are substandard (Mohamed, 2007). Whilst counterfeit medicines are almost certainly substandard, it is not necessarily the case that all substandard medicines are considered to be counterfeit (Kelesidis *et al*., 2007). Indeed, to assume so would ignore those substandard medicines that occur as a result of negligence, human error or insufficient human and financial resources. In fact, there are many drugs that have been approved for use by the relevant drug regulatory authorities, hence considered "legitimate", which do not meet the necessary standards aimed at ensuring their quality, safety and efficacy in terms of patients' use like their appropriate conservation and transportation. In 2003, WHO reported that substandard drugs reported between 1999 and 2002 include analgesics and antipyretics (6 %), antimalarial (7 %), anti-asthma, anti-allergy (8 %), antibiotics (28 %), hormones and steroids (18 %) and other therapeutic categories (33 %). Thus, the aforementioned problems have resulted in a weak therapeutic efficiency and selection of resistance strains (Menkes, 1997; Newton *et al*., 2001; Taylor *et al*., 2001; Dora and Ijeoma, 2004).

A counterfeit medicine is one, which is deliberately and fraudulently mislabeled with respect to identity and/or source. Counterfeiting can apply to both branded and generic products and counterfeit products may include products with the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient active ingredients or with fake packaging (WHO, 2012). Counterfeits are not the genuine product in as much as they are not manufactured by the company that holds the relevant intellectual property rights for the pharmaceutical's chemical composition, packaging and labeling. Counterfeit trading in any

industry is usually linked with organized crime, under-regulated industries, and sometimes with corruption in business and politics. In this respect, pharmaceuticals are no different, with the caution that there is not much health threat posed by a fake handbag but taking a fake medicine can prove potentially rather dangerous (Kelesidis *et al*., 2007).

Counterfeit medicines are a major cause of morbidity, mortality and loss of public confidence in drugs and health structures. In wealthier countries the most frequently counterfeited medicines are new, expensive lifestyle medicines, such as hormones, steroids and antihistamines. In developing countries the most counterfeited medicines are those used to treat life-threatening conditions such as malaria, tuberculosis and HIV/AIDS (Cockburn *et al*., 2005; Dora and Ijeoma, 2004). In a WHO 2007 survey, 20 % of antimalarials and 28 % of antibiotic drugs failed quality specifications. Penicillins, tetracycline, co-trimoxazole and chloramphenicol are among the favored counterfeited antimicrobials in developing countries (Kelesidis *et al*., 2007).

The FDA estimates that counterfeits make up more than 10 % of the global medicines market and are present in both industrialized and developing countries. A WHO survey of counterfeit medicine reports from 20 countries from January 1999 to October 2000 found that 60 % of counterfeit medicine cases occurred in developing countries and 40 % in industrialized countries. Another study conducted by The Lancet concluded that up to 40 % of artesunate products (the best medicine to combat resistant malaria today) contain no active ingredients and therefore have no therapeutic benefits (WHO, 2007).

The WHO has been collecting reports of counterfeit pharmaceuticals since 1982. There were 771 reports from 1982 to 1999 and 46 more reports were received between 1999 and 2000 (WHO, 2007). Of the reports 60 % came from developing countries even though there is gross under-reporting. Approximately 30 % of the medicines on sale for consumption in many countries in Africa and parts of Asia and Latin America are counterfeit (Ahmad, 2006). China and India are known as the leading countries in counterfeit drugs' production and also the bulk active ingredients they produce are used for counterfeiting worldwide (Khan and Khan, 2007). For example, a study done in Cambodia 2001 showed that 38 % of antimalarials on sale in pharmacies did not have any active ingredients (Ahmad, 2006).

1.9.2 Quality of antibacterial drugs in Kenya

In Kenya, quality studies done over the past three decades indicate failure rates, which vary with the type of medicines, the manufactures and the country of origin. In these studies which involved analysis of hundreds of drugs over three decades, categories of greatest concern include antibiotics, antimalarial and anti-TB drugs. Counterfeits have been encountered on the Kenyan market and some have been detected during laboratory testing. These included antibiotics, antimalarials, antiretrovirals and corticosteroid skin creams. The Pharmacy and Poisons Board (PPB) has proceeded to take various measures, including public alerts through media outlets, impounding the medicines and arresting the perpetrators. Also PPB has adopted text message-based anticounterfeiting systems and endorsed the Sproxil solution (Phil, 2012)

During a 4-year period (January 1983 to December 1986), 418 requests for drug analysis were received in the Drug Analysis and Research Unit (DARU), Department of Pharmacy, University of Nairobi. Of the samples analysed, 70.8 % were from local manufacturers, 26.1 % were imported and 3.1 % were from undeclared sources. Failure to comply with test for quality, as set out in official compendia was observed at 45.8 % for locally manufactured drugs and 31.4 % for imported drug products (Kibwage *et al.,* 1992). During the period 1987 to 1990, DARU received and analysed 130 samples, 70 samples were of local origin while 60 were imports. A total of 101 samples satisfied requirements for quality (Mangera *et al*., 1992). The DARU received and analyzed 262 drug samples over a five-year period 1991 to 1995. Samples were obtained from regulatory authorities, local industry, non-Governmental organizations, Hospitals and private practitioners. The samples analyzed, constituted 59.4 % local and 40.6 % imported. Failure to comply with quality specifications, as set out in respective monographs was overall, 17.5 % representing 19.9 % of local and 14.2 % of imported products (Kibwage *et al*., 1999).

For instance in a study by Orwa *et al*. (2008), Caps Ampicillin 250 mg (7), Chloramphenical 250 mg (1); Tabs Pen V 250 mg (2), co-trimoxazole (7), Chlorpropamide 250 mg (4), Methyldopa 250 mg (2), Carbamazepine 250 mg (2), Phenytoin 50 mg (2), Phenobarbitone 30 mg (4); were sampled from the number of sources. Two samples of Phenytoin tabs failed to meet BP specifications for content. Two samples of chlorpropamide and 1 of carbamazepine tabs failed to meet dissolution test. Uniformity of weight test was not met by 3 samples of ampicillin capsules

and 1 each for chloramphenicol capsules, tablets of Penicillin V, phenobarbitone and co-trimoxazole samples manufactured by various industries.

In Kenya, there are a number of studies specifically on quality of antibiotic/antibacterial products. Amoxicillin products were evaluated for quality by liquid chromatography (LC) at DARU, University of Nairobi. Thirty-three of these were capsule formulations and 24 were dry suspensions. Three capsule formulations failed the limits on content. The amoxicillin content in one suspension product was below the limit, while in two other products it dropped below 80 % on storage at 25 °C for 7 days (Kamau *et al*., 2003). In yet another study by Thoithi *et al*. (2001), 20 capsules and 23 dry suspensions of ampicillin were evaluated for quality by LC at DARU. Four capsule formulations failed limits on content. The ampicillin content in 5 suspensions dropped below 80 % on storage, but had no correlation to decrease in chemical content.

A research study by Kibwage *et al*., (1991) evaluated metronidazole tablets products by different manufacturers on the Kenyan market. Three products with percent weight loss of 1.4, 11.08 and 14.93 failed the crucial friability test, for multidose packs. Two products failed the dissolution test releasing 46.8 % and 45.8 % of drug in 40 min. Drug release from tablet was found to vary between batches for one product. Another study by Orwa *et al*. (2008) assessed the quality of ampicillin capsules, chloramphenicol capsules, penicillin tablets and co-trimoxazole tablets manufactured by various industries and three samples of ampicillin capsules, one sample of chloramphenicol capsules, one sample of penicillin tablets and one sample of co-trimoxazole tablets failed the uniformity of weight test.

Generally, from the quality studies done in the last thirty years on the various medicines on in Kenya, there has been a high failure rate. Not all the studied drugs met the quality specifications. Other Investigations showed that products are marketed with declared active moiety different from that used in the manufacture. Properly conducted product development and adequately trained quality assurance personnel would alleviate such problems (Kibwage *et al*., 1988). Because some quality parameters of some drugs did not meet the required compendial

specifications, they cannot be used interchangeably with the innovator brands since they are not pharmaceutically equivalent.

1.9.3 Quality control tests for ciprofloxacin

Several methods for the analysis of ciprofloxacin hydrochloride tablets have been described in official pharmacopoeia and in published scientific papers. The pharmacopoeia, as a public tool, maintains quality of medicines by collecting the recommended procedures for analysis and specifications for the determination of pharmaceutical substances, excipients and dosage forms and, in most cases, consists of a general part (tests, methods and general requirements) and a specific part in the form of monographs for pharmaceutical substances. Some international used pharmacopoeias include; United States Pharmacopoeia (USP), British Pharmacopoeia (BP), International Pharmacopoeia (Ph.Int.), European Pharmacopoeia (Ph.Eur.), Indian Pharmacopoeia (IP), Chinese Pharmacopoeia (CP) and the Japanese Pharmacopoeia (JP). Among these, the USP and BP are the most commonly used in Kenya.

1.9.3.1 Identification test

The initial identification test is necessary to ensure that the product contains the relevant active ingredient. The BP (2012) describes an infra-red (IR) spectrophotometric method of transmittance at about 2000 cm^{-1} (5 μm) for the identification of the raw material. It also prescribes a thin layer chromatography (TLC) method that uses a silica gel F_{254} high-performance precoated plate, acetonitrile-13.5 M ammonia-dichloromethane-methanol (10:20:40:40) as the mobile phase and UV detection at 254 nm and 365 nm. On the other hand, the USP (2014) describes a TLC method that uses TLC plates coated with a 0.25 mm layer of silica gel mixture, methylene chloride-methanol-ammonium hydroxide-acetonitrile (4:4:2:1) as the mobile phase and a detection wavelength of 254 nm and 365 nm.

Several methods for the identification of ciprofloxacin based on the use of iron (III) have been reported in literature. Quinolone antimicrobials are known to bind several metal ions (Kara *et al*., 1991). The interaction between plasma and saliva ciprofloxacin hydrochloride and iron preparations which was investigated by Kara *et al*. (1991) led to the development of the reaction of iron (III) with ciprofloxacin hydrochloride and the exploration of the present method. Iron (III) was found to react instantaneously to form a highly stable coloured compound (Sultan and

Suliman, 1992). The reaction was found to be rapidly taking place through insertion of iron (III) into the active carboxylic acid and the adjacent keto group in the 3 and 4 positions, respectively, forming a six membered ring (Abulkibash *et al*., 2003). Sultan and Suliman (1992), Fratini and Schapoval (1996) and Bhowal and Das (1991) described spectrophotometric methods for analysis while Abulkibah *et al*. (2003) used an electrolytic potentiometric titration method without use of indicators.

1.9.3.2 Uniformity of weight determination

Uniformity of weight serves as a pointer to good manufacturing practices (GMP) as well as the amount of the active pharmaceutical ingredient (API) contained in the formulation. Weight uniformity test is therefore required to ensure that the drug content in each unit dose is distributed in a narrow range around the label strength. In oral dosage forms, any weight variation obviously reflects variation in the content of the active ingredient.

The tablet weight is routinely measured to ensure that a tablet contains the proper amount of drug (Banker and Anderson, 1986). High variability of dose may cause toxicity or insufficient therapeutic drug level (Akarawut *et al*., 2002). It also ensures that the tablets in each lot are within the appropriate size range (Odeniyi *et al*., 2003). Variation between tablet with respect to dose and weight must be reduced to a minimum. Uniformity of weight is an in-process test parameter that ensures consistency of dosage units during compression. Table 2 shows the limits of tablet weight variation as defined by the USP, BP and Ph. Int.

1.9.3.3 Hardness test

The hardness or the crushing strength assesses the ability of tablets to withstand handling during packing and transportation without fracturing, chipping or crumping (Rawlins, 1992). Tablet hardness is usually expressed as the force required to breakdown the tablet (Hanna, 1990). Hardness may affect other parameters like tablet friability and disintegration time and drug dissolution (Banker, 1974).

The tablet breaking force is dependent on the tablet geometry. If the tablet shape is not round, for example oblong shapes, the user has to carefully place the tablets into the instrument in a reproducible way (Sotax, 2013). Some tablet hardness testers available on the market use a constant loading rate for example 20 N per sec, others use a constant speed for example 1 mm

per second. Data collected using these two modes of operation cannot always be compared. The available tablet hardness testers can be operated in either constant speed or constant force mode. A force of 40 N is the minimum requirement of satisfactory for tablet hardness (Allen *et al*., 2004)

1.9.3.4 Disintegration test

The disintegration test determines whether tablets disintegrate into particles when in contact with gastrointestinal fluids. Disintegration time is of importance and is a rate-determining step in the process of drug absorption. Complete disintegration is the state in which any residue of the tablet except fragments of insoluble coating remaining on the screen of the test apparatus or adhering to the lower surface of the disk, if used, is a soft mass having no palpably firm core (USP, 2014). The type and amount of excipients used in tablet formulations such as type and concentration of disintegrant, as well as the manufacturing process such as the method of tablet granulation and compression pressure among others affect both the disintegration and dissolution parameters (Banker, 1974).

The BP (2012) describes use of an apparatus consisting of a basket-rack assembly, a 1 litre, low-form beaker, 149 ± 11 mm in height and having an inside diameter of 106 ± 9 mm for the immersion fluid, a thermostatic arrangement for heating the fluid between 35 °C and 39 °C, and a device for raising and lowering the basket in the immersion fluid at a constant frequency rate between 29 and 32 cycles per minute, through a distance of 55 ± 2 mm.

1.9.3.5 Dissolution test

Dissolution testing is a surrogate marker for bioequivalence testing. It is a practical and economic approach in developing countries where technology and resources are limited for *in vivo* studies (Shah, 2001). The dissolution test aims to ensure the availability of drug for absorption. Since the dissolution of drug is considered to be an essential step in the absorption process, the availability of drug for absorption from a dosage form largely depends on the drug dissolving in gastrointestinal fluids (Augsburge, 1974). It also predicts the *in vivo* bioavailability. The prediction of *in vivo* bioavailability of most oral drugs depends mostly on the *in vitro* dissolution studies as *in vitro* disintegration tests do not give *in vivo* correlation (Nwodo *et al*.,

2007). The rate of dissolution may be directly related to the efficacy of the product (Banker, 1974). Ideally, dissolution tests provide data to distinguish between good and bad products formulations and batches especially when operating conditions are optimal. Therefore substantial variations in the dissolution rate among same generics indicate deficiency in the entire drug formulation and the delivery system (Nwodo *et al*., 2007).

Under laboratory conditions, dissolution testing is the measurement of the proportion of drug dissolving in a stated time under standardized conditions *in vitro* (Armstrong, 1996). The BP (2012) specifies use of the Basket apparatus, Paddle apparatus, Reciprocating cylinder, or Flow-through cell in a suitable dissolution medium at 37 ± 0.5 °C and suitable pH. The USP (2014) specifies use of paddles (USP apparatus 2), employing UV absorption at a wavelength of 276 nm, 50 rpm and the dissolution medium at 37 ± 0.5 °C and suitable pH.

1.9.3.6 Assay

In this test, the amount of drug in the dosage form is determined. A number of units from a batch are selected at random and assay procedures are carried out then the results obtained must be within the prescribed percentage limits (Gupta, 1999). The aim of this assay is to assure the presence of the required amount of active ingredient. Pronounced variation could lead to ineffective therapeutic drug levels or overdosing which leads to toxicity (Akarawut *et al*., 2002).

The USP (2014) specifies a liquid chromatographic method with a mobile phase consisting of a degassed mixture of 0.025 M phosphoric acid, previously adjusted with triethylamine to a pH of 3.0 ± 0.1, and acetonitrile (87:13 % v/v) with a flow rate of 1.5 mL/min, 4.6 mm × 25 cm C_{18} reverse phase column that contains packing L1 with a spherical particle size of 3 µm or 5 µm, a pore size of 100 Å and 15 % carbon load operated at 30 ± 1 °C and a detection wavelength of 278 nm for the assay of ciprofloxacin hydrochloride tablets.

The BP (2012) prescribes a LC method with a mixture of acetonitrile 0.245 % w/v solution of orthophosphoric acid (13: 87 % v/v) the pH of which has been adjusted to 3.0 with triethylamine as the mobile phase with a flow rate of 1.5 mL/min, a 25 cm × 4.6 mm reverse phase column

packed with base-deactivated octadecylsilyl silica gel for chromatograph, maintained at 40 °C and a detection wavelength of 278 nm.

Assay of ciprofloxacin hydrochloride in raw material and in dosage forms has previously been achieved by several analytical techniques such as high performance liquid chromatography (HPLC) (Kassab *et al.,* 2005; Thoppil and Amin, 2000; Zotou and Miltiadou, 2002), and capillary electrophoresis where substances migrate through solutions in an electric field (Faria *et al*., 2008). Compared to other chromatographic techniques, such as TLC, HPLC is extremely quick and efficient. It uses a pump, rather than gravity, to force a liquid solvent through a solid adsorbent material, with different chemical components separating out as they move at different speeds. The process can be completed in roughly 10 to 30 minutes, and it delivers high resolution. It is accurate and highly reproducible. Because it is largely automated, basic HPLC runs can be performed with minimal training. Despite its advantages, HPLC can be costly, requiring large quantities of expensive organics. Techniques such as capillary electrophoresis can be cheaper and even quicker, especially for analysis under good manufacturing practice (GMP). Also, although it is relatively easy to use existing HPLC methods, it can be complex to troubleshoot problems or to develop new methods. This is largely because of the array of different modules, columns and mobile phase.

The UV-spectrophotometric method has also been used for the assay of ciprofloxacin hydrochloride single dosage forms and in two component mixtures (Basavaiah *et al*., 2006). Numerous visible spectrophotometric methods are found in the literature such as in studies by; Abdel-Gawad *et al*., (1998); Basavaiah *et al*., (2006); Bhowal and Das, (1991); El-Brashy *et al*., (2004a); El-Brashy *et al*., (2004b); Fratini and Schapoval, (1996); Nagaralli *et al*., (2002); Salem, (2005), Sastry *et al*., (1995) and (Salem *et al*., 2007; Salem, 2005). The Dibbern *et al*. (2002) method is also used for determination of ciprofloxacin hydrochloride in pure state and it based on using only 0.1 M sodium hydroxide and measure absorbance using UV-visible spectrophotometer at 271 nm and taking 885, 286 as the value of A (1 %, 1 cm). The method is simple, rapid, accurate, sensitive, easy to apply, and are more advantageous compared to official methods, which are laborious. Furthermore, they do not need costly instrumentation such as HPLC and capillary electrophoresis methods.

Titrimetric methods have also been used for assay. A simple and rapid differential electrolytic potentiometric titration method for the determination of ciprofloxacin has been developed (Abulkibash *et al*., 2003; Basavaiah *et al*., 2006). The work is based on the fast complexation reaction between iron (III) and ciprofloxacin in a ratio of 1:3, respectively, in sulfuric acid media of 0.09 mol dm^{-3}. Among the electrodes tested silver amalgam electrodes were found to be a suitable indicating system. By applying a current density of 0.5 mA cm^{-2} to these electrodes and using iron (III) solution of 0.097 mol dm^{-3} as a titrant, normal titration curves were obtained. The method was successfully applied for the determination of ciprofloxacin in drug formulations as low as 4.0 ppm . Advantageous of titrimetric methods includes high precision, robustness, inexpensiveness and simplicity of equipment. Disadvantages include large amount of sample and reagents used, non-selectivity and time consuming.

Ciprofloxacin is also assayed through atomic absorption spectrometry. The method depends on direct determination of the ions in the precipitate or indirect determination of the ions in the filtrate by atomic absorption spectroscopy. (Salem, 2005). Spectrophotometric methods are the most convenient technique because of their inherent simplicity, high sensitivity, low cost, and wide availability in quality control laboratories

Microbiological assay methods have also been used for the assay of ciprofloxacin (Adegbolagun *et al*., 2007). Microbiological methods are very sensitive compared to chemical methods. However microorganisms are not highly robust and their response is limited to a small concentration range.

A new electrochemical method has been described for the determination of ciprofloxacin hydrochloride based on the enhancement effect of an anionic surfactant, sodium dodecyl benzene sulfonate (Zhang and Wei, 2007). The method is advantageous because of low detection limits and it is cheap.

Some of these methods however have limitations of low sensitivity, require high drug concentrations, instability and some require drug extraction while others require heating (Nagaralli *et al*., 2002; Basavaiah *et al*., 2006).

1.10 Justification of the study

The increase in the number of generic drug products from multiple sources has placed people and prescribers in a position of selecting one from among several seemingly pharmaceutically equivalent products (Odeniyi *et al*., 2003). Prescriptions may be issued for drugs specifying only the chemical name, rather than a manufacturer's name; such a prescription can be filled with a drug of any brand meeting the standard specifications for strength, purity, quality and identity (Meredith, 2003). For the health care providers to use these brands interchangeably, the pharmaceutical equivalence of these brands has to be ascertained (Ngwuluka *et al*., 2009). Many of these products are inexpensive but with uncertainty about their quality (Nwodo *et al*., 2007).

Fluoroquinolones are among the most commonly prescribed antibiotics in outpatient and inpatient settings in the United States (Linder *et al*., 2005). Resistance to fluoroquinolones has increased markedly since their introduction for treatment of urinary tract infections (UTIs). Many studies worldwide reported a clear increase in ciprofloxacin resistance. For instance, in China, from 1998 to 2002 the incidence of ciprofloxacin resistance increased steadily from 46.6 % to 59.4 % (Shao *et al*., 2003) In Spain, it was 14.7 % (Kahlmeter, 2003) and in Bangladesh, it was 26.0 % (Iqbal *et al*; 1997). However, in previous studies in the Gaza Strip, the resistance to ciprofloxacin among all isolates in 2000 was 4.1 % and among *E. coli* was only 2.9 % (Astal, *et al*., 2002a) whereas, it increased to 11.3 % in 2002 (Astal, *et al*., 2002b). In past, fluoroquinolones showed excellent clinical activity against *Enterobacteriaceae* including *Klebsiella*, but the frequency of ciprofloxacin-resistant *Klebsiella pneumoniae* has increased worldwide in recent years (Neuhauser *et al*., 2003). In addition, fluoroquinolone exposure has been associated with colonization and infection with other healthcare-associated pathogens, including methicillin-resistant *S. aureus*, vancomycin-resistant enterococci and *Clostridium difficile* (Paterson, 2004; LeBlanc *et al*., 2006; Owens, 2008).

The number of ciprofloxacin tablet brands on the Kenyan market has increased in the recent years from different sources and currently as of 2013 there are 88 different brands registered by the PPB.

Different reports on comparative dissolution study of ciprofloxacin tablets of different countries have been published. Ngwuluka *et al*., (2009) evaluated six brands of ciprofloxacin 500 mg tablets available in Nigeria and found that only 3 brands (50 %) may be used interchangeably with their chosen 'innovator' brand. On the other hand Amit *et al*., (2010) evaluated six generic ciprofloxacin tablets, manufactured by different manufacturers in India and reported that all (100 %) generic ciprofloxacin tablets were bioequivalent with the chosen innovator brand. Again Soula *et al*., (2009) studied 10 brands of ciprofloxacin tablet available in Lebanese market and found significant variations among some brands in terms of hardness, disintegration and dissolution.

In order 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 (Chow, 1997). As such the need to ensure that the generic and branded drugs products are pharmaceutically equivalent cannot be overemphasized and the necessity to select one product from several generic drug products of the same active ingredients during the course of therapy is always a cause for concern to healthcare practitioners (Adegbolagun *et al*., *2007*).

Generic products are usually far cheaper than their branded versions as generic manufacturers do not have the investment costs for the development of a new drug (Nayak and Pal, 2010). Therefore in order to reduce the cost of medicines especially for the low-income group of developing countries, the WHO has continuously advocated the use of generic brands but this approach has not provided sufficient evidence for the substitution of one brand for another. The difference in cost between a branded and generic medicine may be as high as 90 % (WHO, 2004).

Antibiotics are among some of the commonly used pharmaceutical products. In addition the country lacks an effective mechanism to curb the influx of counterfeit and substandard pharmaceutical products. Quality control laboratories in conjunction with the drug regulatory bodies can use the bioequivalence studies to rapidly gauge the quality of such medications before market authorization, during post market surveillance and also in batch release testing.

The previous equivalence studies in Kenya on antibiotics have been done on co-trimoxazole, amoxicillin, ampicillin and metronidazole (Orwa *et al*., 2008; Kamau *at al*., 2003; Kibwage *et al*., 1991). Ciprofloxacin is one of the three-fluoroquinolone antibiotics in the EDL of Kenya and it's the most commonly used in the group. The use of one of the 88 brands of ciprofloxacin for the treatment of the prescribed cases is controversial as there is no conclusive data on their effectiveness. The aim of the study was to determine if the different brands are equivalent to the innovator.

1.11 Objectives

1.11. 1 General objective

The general objective of the study was to determine the quality of commercially available ciprofloxacin tablets in Nairobi, Kenya. This was in order to determine the appropriateness of their interchangeability.

1.11. 2 Specific objectives

1. To investigate quality of ciprofloxacin tablets in the Kenyan market for identity, uniformity of weight, hardness, disintegration and assay.
2. To determine comparative dissolution profiles of the generic and innovator ciprofloxacin tablets in Kenya.
3. To determine the pharmaceutical equivalence between all the brands of ciprofloxacin in Kenya.

CHAPTER TWO

EXPERIMENTAL

2.1 Materials

2.1.1 Reagents and solvents

Analytical grade sodium hydroxide, glacial acetic acid and triethylamine were from RFCL Ltd. (New Delhi, India) and sodium acetate was from RFCL Ltd. (Haryana, India). Potassium chloride was from Merck Pvt. Ltd. (Guateng, South Africa). Potassium dihydrogen orthophosphate was from BDH Laboratory supplies (Poole, England). Hydrochloric acid was from Loba Chemie Pvt. Ltd. (Mumbai, India) and phosphoric acid was from Sigma-Aldrich Co. (Steinheim, Germany). High performance liquid chromatography grade acetonitrile was purchased from Avantor performance materials Ltd., Haryana, India. Purified water was prepared using distillation by a Sartorius aurium water system that consists of a reverse osmosis module and an ultrafiltration module with a UV irradiation component (Sartorius Stedim Biotech GmbH, Doettigen, Germany).

2.1.2 Ciprofloxacin working standards

Ciprofloxacin hydrochloride working standards of potencies 93.7 and 93.3 % from Pharmathen Pharmaceutical Industry (Athens, Greece) and Saluntas Pharma (GmbH, Germany) were donated by National Quality Control Laboratory (NQCL), Nairobi, Kenya.

2.1.3 Ciprofloxacin hydrochloride tablets

Twenty brands of ciprofloxacin hydrochloride tablets including the innovator brand each with strength of 500 mg accounting for 30 % of the registered products on the market were used for the study. The innovator product used was of known quality, safety, available on the local market, approved by International Conference on Harmonisatoin (ICH) and prequalified by WHO. All the tablets used in the study were film coated. Samples were purchased randomly from selected retail and wholesale pharmacies located in the Central Business District of Nairobi City and tests were performed within the products expiration dates. The purchased tablets were

from the same batch for all the brands. The drug samples were purchased in their original package as supplied by the manufacturers and protected from direct sunlight. On collection, the different brands were coded and stored at 25 °C and 60 % relative humidity.

2.2 Equipment

2.2.1 Liquid chromatographic system

An Agilent 1200 infinity series HPLC system (Agilent Technologies, Deutschland, Germany) was used for assay. The system was supported by OpenLab software Version A.01.03 and equipped with a G1314B (S/N: DEAAU02529) Agilent 1260 Infinity Variable wavelength UV detector. A G1311C (S/N: DEAB04964) Agilent 1260 Infinity quaternary pump and a G1329B (S/N: DEAAC09791) autosampler were part of the HPLC system. The temperature was controlled using a G1316A (S/N: DEACN11218) column oven with a G1330B (S/N: DEBAK07088) Agilent 1260 Infinity thermostatted column compartment. All the mobile phase preparations were degassed using a DC-200H (S/N: 14791) MRC Ultrasonic Cleaner (MRC Lab Ltd, Holon, Israel).

2.2.2 Ultra-violet spectrophotometer

A double beam T90+ UV/VIS spectrophotometer supported by the UVWIN software Version 5.2.0 (PG Instruments, Leicestershire, UK) and quartz cuvettes of a path length of 1 cm was used to obtain the absorbances of ciprofloxacin hydrochloride tablets and ciprofloxacin working standards over the ultra-violet and visible region of the electromagnetic spectrum.

2.2.3 Weighing balance

A Shimadzu AUW220D semi-micro analytical electronic weighing balance (S/N: D450012073) (Shimadzu Corp., Kyoto, Japan) with a sensitivity of ± 0.1 mg was used for uniformity of weight, dissolution and assay.

2.2.4 Disintegration tester

An Erweka ZT3-1 (S/N: 68320) fitted with a thermostat (GmbH, Heusenstamn, Germany) was used for the disintegration testing in the study.

2.2.5 Dissolution tester

A Labindia DS 800 (S/N: DS10350916) dissolution tester fitted with a high precision multichannel pump and an automated sample collector (Labindia Instruments Pvt. Ltd, Mumbai, India) was used in the study.

2.2.6 Tablet hardness tester

A 2E/205 electronic tablet hardness tester (Dr. K. Schleuniger and Company, Solothurn, Switzerland) was used to determine hardness of ciprofloxacin tablets.

2.3 Procedures

Uniformity of weight, disintegration, dissolution and assay are compendial tests to assess the quality of tablet formulation of ciprofloxacin while hardness and friability are non-compendial tests. Identification, uniformity of weight, hardness, disintegration, dissolution and assay for the content of active ingredients were done as described in the British Pharmacopoeia (BP, 2012) or the United States Pharmacopoeia (USP, 2014).

2.3.1 Uniformity of weight determination

Twenty tablets from each of the twenty brands were taken at random and brushed off dust using a soft brush then weighed individually with an analytical balance by direct weighing. The average weights of tablets for each brand and the percentage deviation from the mean value were calculated. Not more than 2 of the individual masses should deviate from the average mass by more than the percentage deviation shown in table 2, and none should deviate by more than twice that percentage.

Table 2: Limits for tablet weight variation defined by some official monographs

Limit	Ph.Int/ BP	USP
± 10 %	80 mg or less	130 mg or less
± 7.5 %	More than 80 mg or less than 250 mg	130 mg to 324 mg
± 5 %	250 mg or more	More than 324 mg

BP: British Pharmacopoeia; Ph.Int: International pharmacopoeia; USP: United States Pharmacopoeia.

2.3.2 Hardness test

The tablets were placed between the jaws of the tablet hardness tester and oriented in the same way with respect to the direction of application of the force. Six tablets were randomly selected from each brand and the pressure at which each tablet crushed was recorded (USP, 2014).

2.3.3 Disintegration test

Using the BP (2012) disintegration test apparatus (Erweka ZT3-1), six tablets were individually placed in the basket then lowered in a 1-liter vessel containing distilled water and the water bath set at 37 °C. The disintegration time was recorded as the time taken for the tablets to go into solution completely through the sieve and no particles remained on the basket of the system.

2.3.4 Assay

High performance liquid chromatography method was used for the assay of the drug content according to the method outlined in the individual drug monograph of the United States Pharmacopoeia (USP, 2014).

2.3.4.1 Preparation of mobile phase

A mixture of 0.025 M phosphoric acid, previously adjusted with triethylamine to a pH of 3.0 ± 0.1 and acetonitrile HPLC grade (87:13) was prepared. It was filtered through 0.2 µm membrane filters and sonicated for 10 min.

2.3.4.2 Preparation of standard

Twenty milligrams (20.0 mg) of the reference standard was weighed accurately and dissolved in the mobile phase in a 50.0 mL volumetric. The solution was sonicated for 10 min and the flask was topped to the mark with the mobile phase. Twenty-five ml (25.0 mL) were pipetted to a 50.0 mL volumetric flask and then filled to the mark with the mobile phase. The working concentration was 0.20 mg/mL.

2.3.4.3 Preparation of sample

Twenty tablets were weighed and the average weight determined. The tablets were then crushed for 20 min to fine powder. An equivalent of 50.0 mg active ingredient was calculated and weighed into a 50.0 mL volumetric flask and topped to the mark with the mobile phase. Ten mL were pipetted into a 50.0 mL volumetric flask and topped to the mark with the mobile phase. The solution was then filtered and sonicated for 10 min. The working concentration was 0.20 mg/mL.

2.3.4.4 Chromatographic system

The chromatograph was equipped with a UV detector set at 278 nm and separation was achieved from a Symmetry® C_{18} 5µm (250 × 4.6 mm) column (Waters Corp., Massachusetts, U.S.A). The injection volumes were 10.0 µL and the flow rate was 1.50 mL/min. The column temperature was maintained at 30 ± 1 °C in a thermostat oven.

2.3.4.5 Procedure

Equal volumes of 10.0 µL of the standard and sample were injected into the HPLC system at 278 nm wavelength detection and the responses of the major peaks measured. The percentage content of ciprofloxacin in each of the 20 brands was calculated.

2.3.5 Identification test of active substance

The identification of ciprofloxacin hydrochloride was carried out using the retention times of the major peaks in the chromatograms of the standard preparations and the assay preparations. The assay was carried out by separately injecting equal volumes of 10.0 µL of the standard preparation and the assay preparation into the chromatograph.

2.3.6 Dissolution test

The dissolution test was undertaken using USP dissolution apparatus 2. Dissolution media were USP buffer solutions at pH 1.2 (hydrochloric acid solution), pH 4.5 (acetate buffer solution), and pH 6.8 (phosphate buffer solution). The dissolution medium was maintained at 37 ± 0.5 °C and the paddle rotated at 50 rpm. The dissolution vessels were filled with 900.0 mL of the dissolution media. In all the experiments, 5.0 mL of the dissolution sample was withdrawn from the vessels at 0, 5, 10, 15, 20, 30 and 45 min and equal volumes were replaced to maintain sink conditions.

The samples were filtered and assayed by a UV method using a UV/Visible spectrophotometer. One mL (1.0 mL) of each sample was pipetted to a 100.0 mL volumetric flask and then topped up to 100.0 mL with the dissolution media to obtain a concentration of 0.005 mg/mL that was compared to the same concentration of the standard solution. Absorbances of the samples and the standard were read and the percentage amount of drug released calculated. The dissolution profiles of the different brands of ciprofloxacin hydrochloride tablets were generated from the graph of the amount of ciprofloxacin hydrochloride released versus time.

2.4 Data analysis

The results of uniformity of weight, hardness, disintegration, dissolution and assay were tabulated. In addition, the dissolution profiles were graphically presented. To compare the dissolution profiles of the brands, a model independent approach of difference factor f_1 and similarity factor f_2 was employed. Difference factor f_1 is the percentage difference between two curves at each point and is a measurement of the relative error between the two curves. The similarity factor f_2 is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent dissolution between two curves. Difference factor f_1 and similarity factor f_2 were calculated by using the following formulas:

$f_1 = \{[\sum_{t=1}^{n}|R_t - T_t|]/[\sum_{t=1}^{n}R_t]\}x100$

$f_2 = 50 \text{ x } \log \{[1+(1/n) \sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5} x100\}$

Where n is the number of testing points, R_t is the average dissolution value of the reference product unit at time t, T_t is the average dissolution value of the test product at time t.

For two dissolution profiles to be considered similar and pharmaceutically equivalent, f_1 should be between 0 and 15 while f_2 should be between 50 and 100 (FDA, 1997).

CHAPTER THREE

RESULTS AND DISCUSSION

It is a general belief that only substances in solution form are transported across the intestinal wall and absorbed into the systemic circulation. For a tablet formulation to readily release its active ingredients effectively within the official specifications, a number of independent variables such as uniformity of weight, hardness, disintegration and assay that may affect its systemic activity must be assessed. These parameters were evaluated to ascertain the viability and suitability of the tablets for the dissolution studies. A summary of the results of uniformity of weight, hardness, disintegration, dissolution, retention time and assay of the 20 brands of ciprofloxacin tablets are shown in table 3.

3.1 Identification

The initial identification test is necessary to ensure that the product contains the needed active ingredient. The identity of the ciprofloxacin was confirmed by HPLC (USP, 2014). The retention time of the major peak of the sample solutions of all ciprofloxacin brands corresponded to that of the standard solution, as obtained in the assay. The retention times of the samples ranged from 5.1 to 5.2 min and corresponded to the standard's retention time at 5.2 min. Thus, all the samples examined for the identity of the active ingredient passed the identification test USP specification. The chromatograms of all the brands are shown in appendix 1 and 2.

3.2 Uniformity of weight

Uniformity of weight serves as a pointer to good manufacturing practice (GMP) as well as amount of the active pharmaceutical ingredient ciprofloxacin hydrochloride contained in the formulation. During manufacturing, it ensures that the tablets are within the appropriate particle size range and that there was uniformity in mixing and die filling.

Table 3: Summary of quality control test results of ciprofloxacin tablet brands

Brand Code	Average Uniformity of Weight (mg) ± SD	% Deviation from the mean weight	Hardness (N) (Average ± SD)	Disintegration Time (min)	Retention Time (min)	Assay (%) (RSD)
C001	777.16 ± 7.31	-2.12 – 2.24	141.83 ± 13.20	1.5	5.2	96.80 (0.81)
C002	899.08 ± 24.45	-4.69 – 6.10	188.33 ± 10.71	12.0	5.2	98.44 (1.51)
C003	770.75 ±10.21	-2.03 – 3.16	176.67 ± 19.25	0.8	5.1	94.83 (1.07)
C004	743.65 ± 11.31	-2.70 – 1.94	193.17 ± 6.88	1.5	5.2	97.26 (0.35)
C005	635.78 ± 5.93	-1.24 – 2.58	119.33 ± 16.81	3.0	5.2	98.20 (0.35)
C006	739.52 ± 14.29	-5.10 – 3.67	187.67 ± 8.98	2.2	5.2	95.86 (0.57)
C007	783.13 ± 7.89	-1.71 – 1.81	180.17 ± 6.74	0.5	5.1	97.26 (0.48)
C008	740.94 ± 9.91	-3.01 – 1.90	190.50 ± 5.92	2.7	5.1	97.21 (0.28)
C009	694.67 ± 6.66	-1.52 – 1.60	145.83 ± 16.59	2.3	5.1	99.37 (0.72)
C010	679.70 ± 6.02	-2.07 – 1.54	164.50 ± 19.88	1.2	5.2	98.78 (0.21)
C011	969.85 ± 9.62	-1.96 – 1.59	158.17 ± 12.16	1.2	5.2	97.03 (0.81)
C012	643.05 ± 10.54	-2.36 – 4.68	148.00 ± 23.45	6.0	5.2	94.45 (0.37)
C013	823.01 ± 15.56	-4.10 – 3.24	109.17 ± 7.60	23.5	5.1	104.58 (0.56)
C014	827.13 ± 5.42	-1.28 – 1.16	189.00 ± 8.07	3.5	5.1	99.59 (1.83)
C015	1033.80 ± 19.80	-7.18 – 2.42	166.17 ± 13.44	3.2	5.2	99.18 (0.65)
C016	1064.30 ± 25.50	-2.71 – 4.90	144.17 ± 6.85	1.0	5.2	95.49 (0.42)
C017	745.43 ± 10.37	-2.98 – 1.79	186.67 ± 8.45	1.3	5.2	98.62 (1.67)
C018	692.22 ± 24.10	-6.66 – 5.95	62.00 ± 14.81	6.5	5.1	90.38 (1.50)
C019	730.57 ± 8.59	-5.10 – 3.67	142.33 ± 5.28	1.3	5.2	94.97 (1.06)
IB	762.49 ± 436	-1.36 – 1.18	176.67 ± 9.27	0.5	5.2	98.57 (0.34)

This is especially for reproducibility of the product, which is very essential for mass production of any product. Weight uniformity test is also required to assure that the drug content in each unit

dose is distributed in a narrow range around the label strength. If the drug substance forms the greater part of the oral solid dosage form, any weight variation obviously reflects variation in the content of active ingredient. The results of weight uniformity test are depicted in table 3. All the brands complied with the compendial specification for uniformity of weight which states that for tablets weighing more than 250 mg (film coated tablets), weights of not more than 2 tablets should differ from the average weight by more than 5 % and also not more than one weight should deviate by twice that percentage (BP, 2012). The results thus indicate that all the products possess acceptable uniformity of weight as per the pharmacopoeia limit; therefore the different brands are pharmaceutically equivalent to the innovator brand in terms of weight uniformity. The results obtained agree with a previous study in Nigeria by Muaz *et al.* (2009).

3.3 Hardness

Hardness is a non-compendial test. The hardness or crushing strength assesses the ability of tablets to withstand handling without fracturing or chipping. It can also influence other parameters such as friability and disintegration. The friability test however was not done because it is specified for uncoated tablets (USP, 2014; BP, 2012) and all the tablets used in the study were film coated. The harder a tablet, the less friable and the more time it takes to disintegrate. Brand C018 required the least amount of pressure (62 N) to break. A force of about 40 N is the minimum requirement for a satisfactory tablet (Allen *et al*., 2004). Therefore all the tablets were satisfactory for hardness as depicted in table 3.

3.4 Disintegration test

Different formulation factors are known to affect results of disintegration test. The disintegration test measures the time required for a tablet to disintegrate into particles when in contact with gastrointestinal fluids. This is a necessary condition and could be the rate-determining step in the process of drug absorption. The type and amount of excipients used in tablet formulation as well as the manufacturing process are all known to affect both the disintegration and dissolution parameters. All brands of the ciprofloxacin tablets passed the pharmacopoeia (BP, 2012) standard which stipulates a disintegration time of not more than 30 min for coated tablets. There was a wide range in the disintegration time as observed in table 3. Both brand C007 and innovator brand (IB) disintegrated in the least amount of time (0.5 min) while brand C013 took

the most time at 23.5 min. Harder tablets are expected to take more time to disintegrate as compared to softer ones. This however was not the case as observed in table 3. The innovator brand for example had a breaking force of 176.67 N yet it disintegrates in half a min as compared to brand C018 with a breaking force of 62 N and it disintegrated in 6.5 min. This is possible depending on the type or levels of the levels of the binder and the disintegrant used and in the formulations. All the disintegration times had fallen within the acceptable range. These coupled with the excellent crushing strength observed suggest that there is a good balance between mechanical strength and release properties.

3.5 Assay

The aim of the assay was to assure the presence of the required amount of active ingredient. Significant variations could lead to ineffective therapeutic drug levels or overdosing that may lead to toxicity (Akarawut *et al*., 2002). Ciprofloxacin tablets should contain not less than 90.0 % and not more than 110.0 % of the stated amount (USP, 2014). All the brands of ciprofloxacin tablets passed as per the USP specification. The highest percentage content was obtained for brand C013 (104.58 %), while the least drug content was obtained for brand C018 (90.38 %). Brands C009, C010, C013, C014, C015 and C017 contain more active pharmaceutical ingredient than the innovator brand implying they were formulated well. Statistical comparison for drug content indicates that within 95 % confidence interval, there is no significant difference in the drug content among the different brands ($p < 0.05$).

3.6 *In vitro* drug release of ciprofloxacin tablets

Products with different formulations, different inactive ingredients, and different formulation design may have different dissolution profiles or release characteristics and therefore may have different bioavailability. In the present study, the dissolution profiles of the twenty products were tested according to the method described in USP (2014). It is stated that the amount of ciprofloxacin released within 30 minutes is not less than 85 % of the stated amount. The results of the dissolution studies are graphically represented in table 5, figures 4–10 and in appendix 5-7. All the dissolution data obtained was based on the actual drug content of the tablets as calculated from the assay results. Drug release from the various brands in the study was found to be pH dependent. This may be due to the pH dependent solubility of ciprofloxacin. Ciprofloxacin

exhibits a "U" shaped pH-solubility profile with high solubility at pH values below 5 and above 10, and minimum solubility near the isoelectric point which is close to neutral (Olivera *et al*., 2011).

Table 4: Dissolution of ciprofloxacin tablet brands at 30 minutes

Brand Code	% Dissolution at at 30 minutes (n = 6, RSD)			Compliance
	pH 1.2	pH 4.5	pH 6.8	
C001	95.81 (3.55)	96.62 (2.06)	32.16 (35.03)	Complies at pH 1.2, 4.5
C002	90.69 (2.13)	87.97 (2.88)	4.38 (56.50)	Complies at pH 1.2, 4.5
C003	92.17 (2.50)	93.22 (3.24)	5.02 (68.61)	Complies at pH 1.2, 4.5
C004	82.48 (8.62)	94.38 (1.72)	47.39 (12.35)	Complies at pH 1.2, 4.5
C005	90.96 (5.19)	94.85 (1.52)	38.32 (3.79)	Complies at pH 1.2, 4.5
C006	93.35 (2.48)	97.02 (1.41)	51.50 (44.46)	Complies at pH 1.2, 4.5
C007	83.46 (2.01)	96.51 (2.57)	55.41 (14.97)	Complies at pH 1.2, 4.5
C008	98.35 (2.78)	97.24 (1.81)	47.72 (15.67)	Complies at pH 1.2, 4.5
C009	94.73 (0.95)	97.46 (1.70)	29.24 (11.14)	Complies at pH 1.2, 4.5
C010	88.81 (3.59)	96.24 (0.88)	46.09 (17.56)	Complies at pH 1.2, 4.5
C011	97.20 (0.95)	91.38 (3.48)	10.46 (17.59)	Complies at pH 1.2, 4.5
C012	77.68 (6.24)	94.32 (2.27)	7.06 (45.29)	Complies at pH 4.5
C013	101.37 (2.55)	73.73 (4.25)	1.65 (43.38)	Complies at pH 1.2
C014	94.02 (1.54)	95.68 (2.24)	1.75 (30.64)	Complies at pH 1.2, 4.5
C015	52.85 (12.35)	80.44 (10.41)	7.14 (32.64)	Does not comply
C016	79.32 (17.35)	81.57 (8.12)	21.41 (33.07)	Does not comply
C017	84.95 (3.37)	94.50 (0.64)	59.02(11.67)	Complies at pH 4.5
C018	88.65 (3.41)	88.24 (5.33)	7.57 (82.46)	Complies at pH 1.2, 4.5
C019	91.31 (3.19)	95.41 (1.53)	43.45 (3.22)	Complies at pH 1.2, 4.5
IB	91.48 (1.30)	94.83 (2.30)	35.33 (25.03)	Complies at pH 1.2, 4.5

3.6 .1 *In vitro* drug release of ciprofloxacin tablets at pH 1.2

At pH 1.2, all the brands of ciprofloxacin tablets studied released more than 85 % of the drug within 30 minutes except C004, C007 and C017, which had released only 82.48, 83.46 and 84.95 %, respectively. Hence brands C004, C007 and C017 did not comply with the USP (2014) tolerance limits of the released amount within 30 min. Brand C015 released the least amount of drug (82.48 %) while brand C013 released the greatest amount (104.58 %). The innovator brand (IB) released 91.48 % of the drug within 30 min. From the results obtained, brands C001, C003, C006, C008, C009, C011, C013 and C014 released more drug (95.81, 92.17, 93.35, 98.35, 97.20, 101.37 and 94.02 %) than the innovator brand, which released 91.48 %. This implies brands C001, C003, C006, C008, C009, C011, C013 and C014 dissolve better than the innovator brand. Although brands C002, C005, C010, C012, C015, C016, C018 and C019 met compendial specifications of 85 %, the innovator product is a better product. Brands C002, C005, C010, C012, C015, C016, C018 and C019 were comparable to the innovator product.

Figure 4 and show the dissolution profiles of all the generic brands and the innovator brand of ciprofloxacin tablets at pH 1.2. Figure 5 shows dissolution profiles of brands C004, C007, C012, C015 and the innovator brand IB. Brands C004, C007, C016 and C017 follow a similar dissolution pattern like that of the innovator brand. Brands C012 and C015 however follow a different pattern. The cumulative amount of ciprofloxacin released by brands C012 and C015 is lower than that of the innovator product from 0 to 45 min. A 20 min, the innovator brand had release over 85 % (85.21 %) and it continued to release a greater amount of drug compared to the rest of the brands.

Figure 6 shows the dissolution profiles of brands C001, C008, C009, C011, C013, C014 and the innovator brand. Generally the dissolution profiles of the different generic brands are similar to that of the innovator brand. At 30 min, all the generic brands released a greater amount of ciprofloxacin than the innovator brand. Brand C009 however released 95.53 % of ciprofloxacin while the innovator brand released 96.09 %. Brands C001, C008, C009, C011, C013 and C014 generally have better drug release properties than the innovator product.

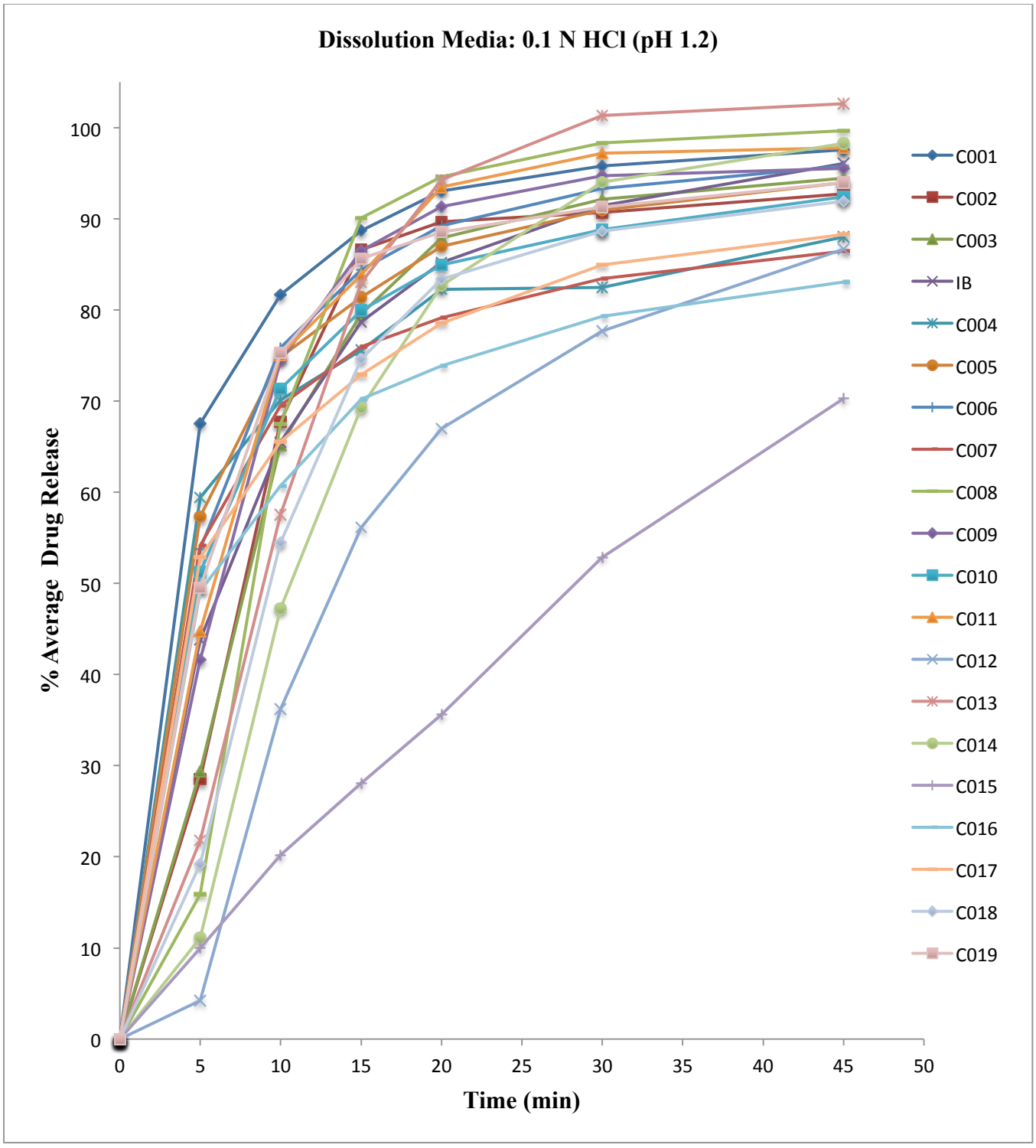


Figure 4: Dissolution profiles of all generic and the innovator brand of ciprofloxacin tablets in pH 1.2

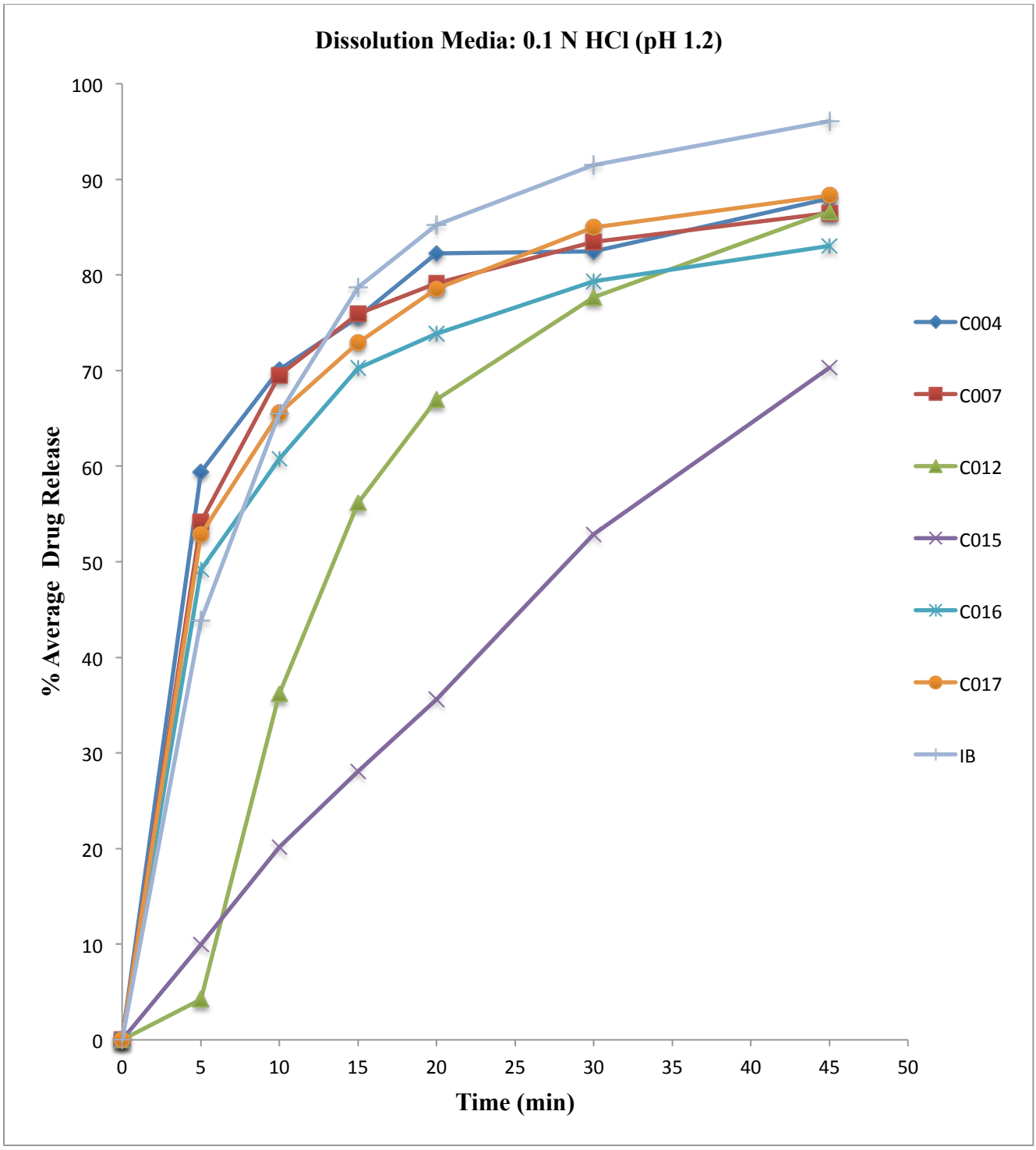


Figure 5: Dissolution profiles of brands C004, C007, C012, C015, C016, C017 and the innovator brand IB at pH 1.2

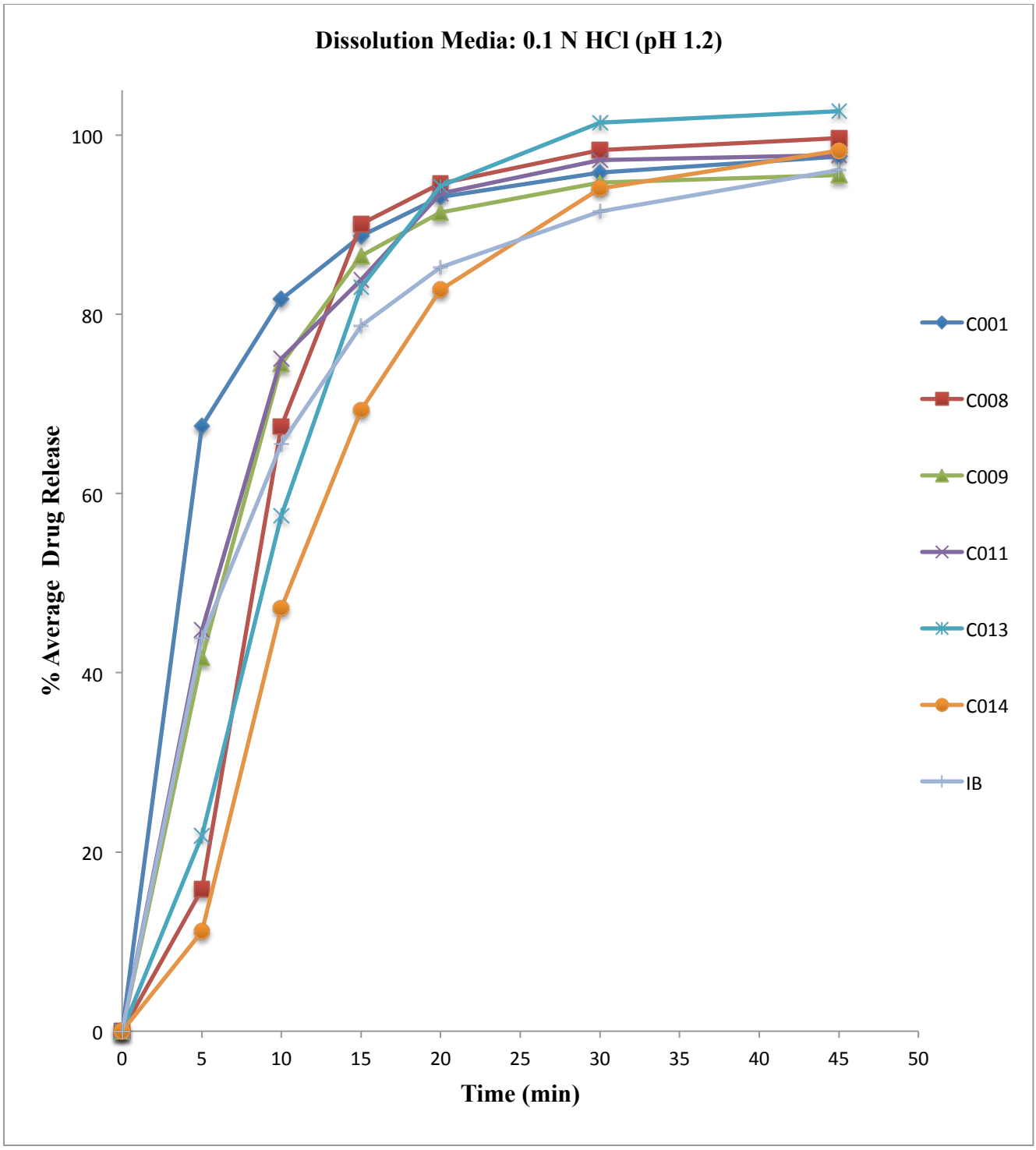


Figure 6: Dissolution profiles of brands C001, C008, C009, C011, C013, C014 and the innovator brand IB at pH 1.2

3.6 .2 *In vitro* drug release of ciprofloxacin tablets at pH 4.5

At pH 4.5, most of the brands released more that 85 % of ciprofloxacin except brand C013, C015 and C016, which released 73,73, 80.44 and 81.57 %, respectively within 30 min. Brands C001, C0012, C003, C004, C005, C006, C007, C008, C009, C010, C011, C012, C014, C017, C018, C019 and the innovator brand meet the USP (2014) specification of dissolution. Within 30 min, the innovator brand released 94.83 % of ciprofloxacin compared to brands C001, C005, C006, C007, C008, C009. C010, C014 and C019, which had released greater amounts (96.62, 94.85, 97.02, 96.51, 97.24, 97.46, 96.24, 95.68 and 95.41 %, respectively). Brands C001, C005, C006, C007, C008, C009. C010, C014 and C019, therefore have better release properties than the innovator brand.

Figure 7 shows the dissolution profiles of all generic ciprofloxacin brands and the innovator brand at pH 4.5. Generally most of the brands rapidly dissolve as compared to pH 1.2. Brand C005 for instance released 86.85 % of ciprofloxacin within 5 min. Figure 8 shows the dissolution profiles of brands C002, C013, C015, C016, C018 and the innovator product at pH 4.5. The amount of ciprofloxacin released by the innovator brand is greater than that of brands C002, C013, C016 and C018 from 5 min to 30 min. At 45 min, brand C015 released 98.13 % while the innovator brand rereleased 96.46 %. From the dissolution profiles, the innovator brand has better release properties than brands C002, C013, C015, C016 and C018.

The dissolution profiles of brands C001, C006, C007, C008 and C009 are similar to that of the innovator brands from 0 to 45 min. Within 30 min however, the innovator brand released 94.83 % of ciprofloxacin while brands C001, C006, C007 C008 and C009 had released 96.62, 97.02, 96.51, 97.24 and 97.46 %, respectively.

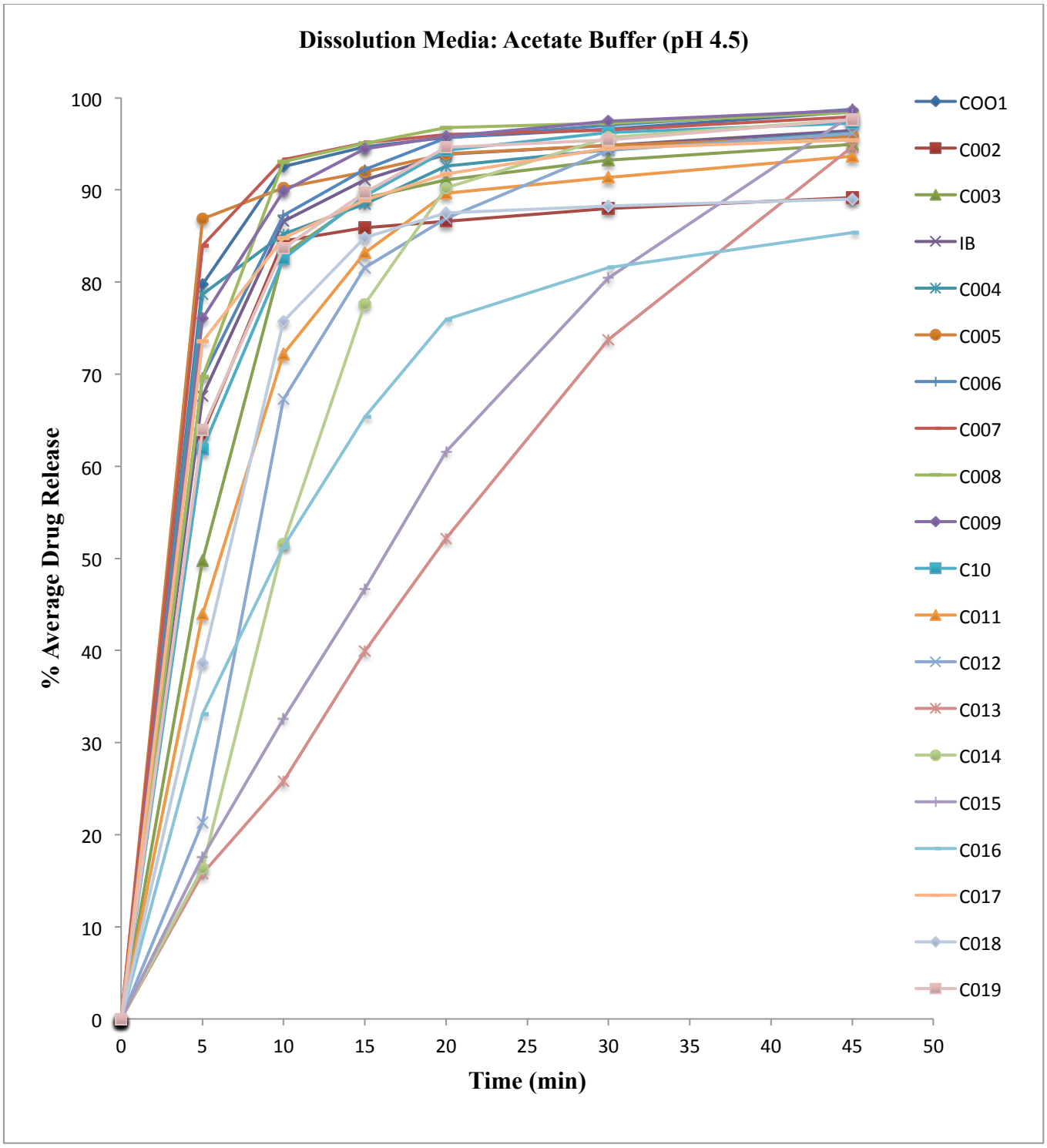


Figure 7: Dissolution profiles of all generic and the innovator brand of ciprofloxacin tablets in pH 4.5

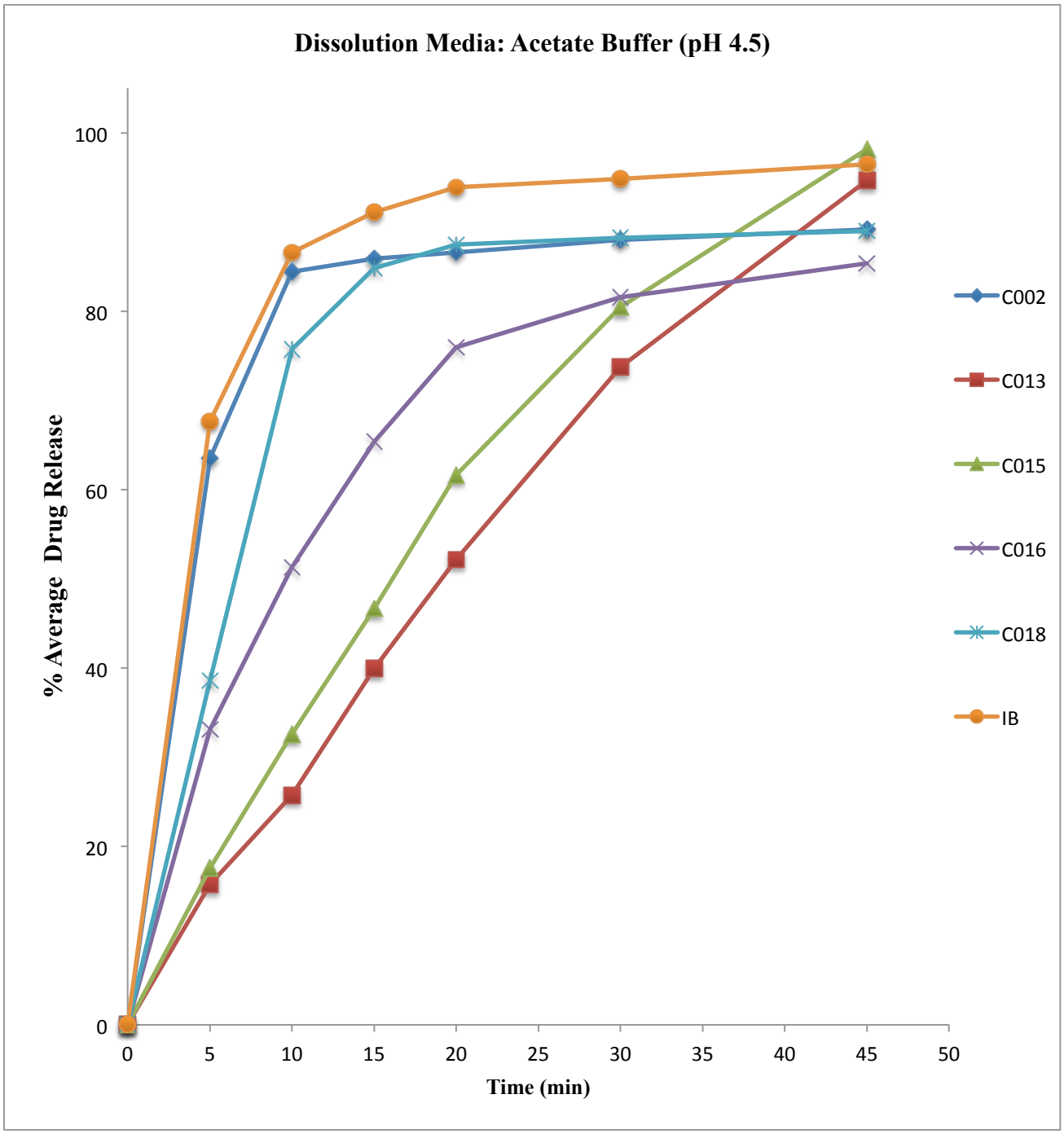


Figure 8: Dissolution profiles of brands C002, C013, C015, C016, C018 and the innovator brand IB at pH 4.5

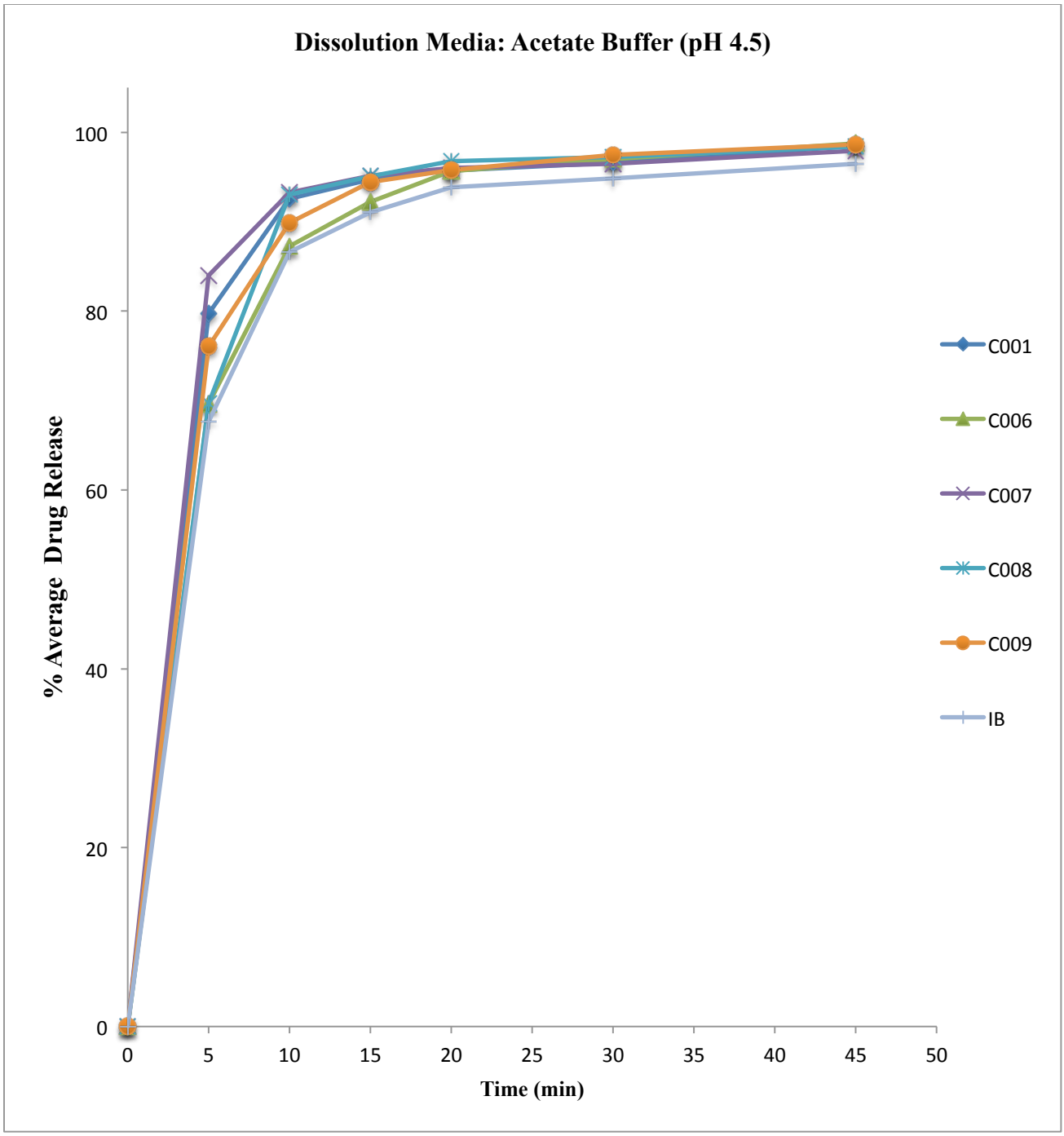


Figure 9: Dissolution profiles of brands C001, C006, C007, C008, C009 and the innovator brand IB at pH 4.5

3.6 .3 *In vitro* drug release of ciprofloxacin tablets at pH 6.8

All the products failed to release the specified amount of ciprofloxacin of 85 % by USP (2014) within 30 min. Brands C013 and C014 showed the least amount of released drug at 1.65 and 1.75 % within 30 min. Brands C006, C007 and C017 released 51.50, 55.41 and 59.02 % of ciprofloxacin as compared to the innovator brand, which only released 35.33 %. Although all the products failed to release the require amount of 85 % as specified by the USP brands C006, C076 and C017 had better release properties as compared to the innovator brand.

From figure 10, the dissolution profiles of all the generic brands are similar in shape to that of the innovator brand. Brands C001, C002, C003, C009, C011, C012, C013, C014, C015, C016 and C018 released a lower amount of drug cumulatively from 5 min up to 45 min. Brands C004, C005, C006, C007, C008, C010, C017 and C019 released a greater amount than the innovator brand cumulatively from 5 min to 45 min.

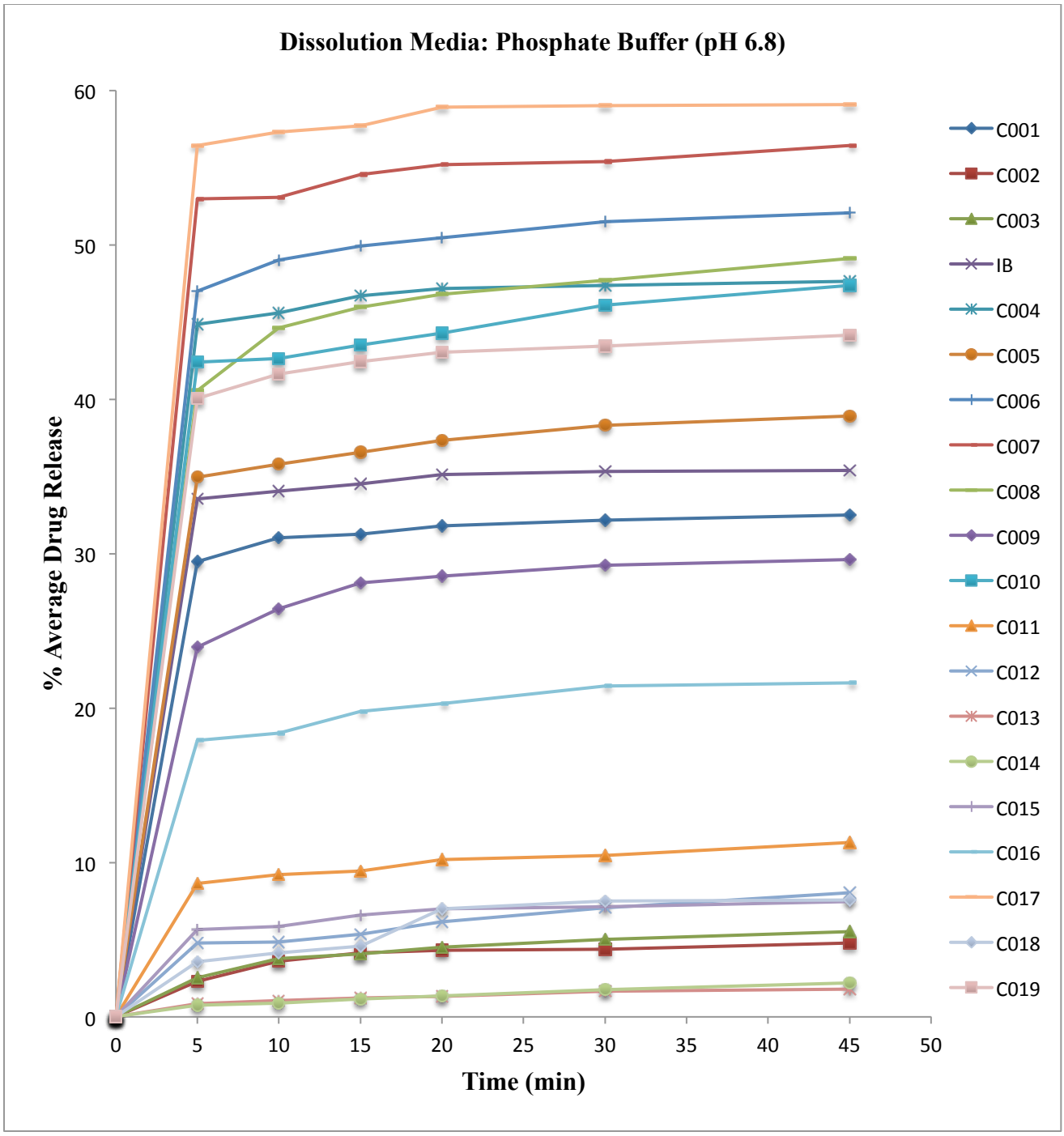


Figure 10: Dissolution profiles of all generic and the innovator brand of ciprofloxacin tablets in pH 6.8

Ciprofloxacin is highly soluble at pH 1.2 and 4.5; therefore a higher dissolution was obtained in the two media. However it has limited solubility at pH 6.8, so the 35.4 % dissolution of the innovator brand is justified in case of the phosphate buffer (pH 6.8).

Since ciprofloxacin is a zwitterionic drug and is probably absorbed by passive diffusion, intestinal pH changes suggest that rapid absorption may occur in the duodenum and proximal jejunum, whereas absorption may decrease in the distal portion of the intestine (Tartaglione *et al*., 1986). It contains multiple functional groups with acid-base properties, and therefore possesses both acidic and basic character. It contains a secondary alkyl amine, two tertiary arylamines (aniline-like amines), and a carboxylic acid. The two aryl amines are weakly basic and, therefore do not contribute significantly to the acid-base properties of ciprofloxacin under physiological conditions. Depending on the pH of the physiological environment, the ciprofloxacin molecule will either accept a proton (secondary alkylamine); donate a proton (carboxylic acid), or both. Thus it is described as amphoteric in nature. The proportion of the un-ionized form present (and thus the drug's ability to cross a membrane) is determined by the environmental pH and the drug's pK_a (acid dissociation constant). The pK_a is the pH at which concentrations of ionized and un-ionized forms are equal. When the pH is lower than the pK_a, the un-ionized form of a weak acid predominates, but the ionized form of a weak base predominates.

At pH 1.2 and 4.5 in the stomach and duodenum, only one of the functional groups (the alkylamine) is significantly ionized. The pH is less than the pK_a and therefore the protonated form of the drug predominates. And more of the drug is available for absorption. At pH 6.8, the carboxylic acid group is deprotonated and the pH is greater than the pK_a. Most of the drug is in basic form hence not available for absorption. This therefore indicates that ciprofloxacin is mainly absorbed from the upper part of the gastrointestinal tract after oral administration.

Asharaful did a similar study in India in 2012. Dissolution study was done in hydrochloric acid solution (pH 1.2), acetate buffer (pH 4.5) and phosphate buffer (pH 6.8). Five brands were compared to the innovator band and the dissolution profiles showed no significant variability and the results indicated that all generic ciprofloxacin tablets included in this investigation were bioequivalent with the innovator brand. Although the current study was done under similar pH values, the results obtained differed from Ashraful's.

3.7 Pharmaceutical equivalence

In order to compare the dissolution profiles of the various brands, a model independent approach of the difference factor f_1 and similarity factor f_2 was employed (FDA, 1997) with the 6 sampling time points (5, 10, 15, 20, 30 and 45 min) included in the calculations. Table 5 shows f_1 and f_2 values of the different brands in respect of the innovator brand as calculated for the dissolution data obtained for the different dissolution media. In the f_2 calculation, only one measurement is generally considered after the comparator product has reached 85 % dissolution as observed in the hydrochloric acid buffer (pH 1.2) and acetate buffer (pH 4.5).

3.7.1 Pharmaceutical equivalence at pH 1.2

At pH 1.2, the f_2 values of C002, C003, C004, C005, C006, C007, C008, C009, C010, C011, C016, C017 and C019 brands are more than 50 therefore, they are pharmaceutically equivalent to the innovator brand. The corresponding f_1 values are all below 15 confirming their pharmaceutical equivalence.

3.7.2 Pharmaceutical equivalence at pH 4.5

In the dissolution media at pH 4.5, the f_2 values of C001, C002, C003, C004, C005, C006, C007, C008, C009, C010, C017 and C019 brands are above 50 therefore, they are pharmaceutically equivalent to the innovator brand. The f_2 and f_1 values of brands C001, C002, C003, C004, C005, C006, C007, C009, C010, C017 and C019 at pH 1.2 and pH 4.5 are above 50 and below 15, respectively therefore they are considered to be pharmaceutically equivalent with the innovator brand.

3.7.3 Pharmaceutical equivalence at pH 6.8

At 45 min, the amount of ciprofloxacin released for all the brands and IB was below 85 %. Therefore the difference factor f_1 and similarity factor f_2 are not applicable for the dissolution data obtained from the phosphate buffer (pH 6.8) due to lower drug release. At pH 6.8 therefore, no ciprofloxacin generic brand is pharmaceutically equivalent to the innovator brand.

Table 5: Calculated difference factor (f_1) and similarity factor (f_2) of the generic ciprofloxacin tablets

Brand Code	pH 1.2		pH 4.5		pH 6.8		Compliance
	f_2	f_1	f_2	f_1	f_2	f_1	
C001	44.34	13.79	61.31	5.15	73.07	9.48	Complies at pH 4.5
C002	56.15	1.05	61.54	6.20	25.60	88.68	Complies at pH 1.2, 4.5
C003	60.55	2.65	55.68	5.54	25.82	87.77	Complies at pH 1.2, 4.5
C004	53.54	0.65	65.90	0.96	46.14	34.32	Complies at pH 1.2, 4.5
C005	57.94	5.34	54.68	4.37	79.00	6.69	Complies at pH 1.2, 4.5
C006	58.90	6.87	84.50	1.89	40.64	44.21	Complies at pH 1.2, 4.5
C007	56.51	2.64	55.67	6.62	34.97	57.53	Compliant at pH 1.2, 4.5
C008	42.93	1.73	71.11	3.73	47.21	32.12	Complies at pH 4.5
C009	61.92	5.07	68.04	4.10	57.10	20.26	Complies at pH 1.2, 4.5
C010	67.68	1.72	74.63	1.70	50.32	28.06	Complies at pH 1.2, 4.5
C011	60.50	6.78	45.91	10.65	30.26	71.53	Complies at pH 1.2
C012	30.67	28.86	33.83	15.60	27.14	82.58	Does not comply
C013	46.91	0.03	18.23	43.06	23.83	96.21	Does not comply
C014	39.91	12.64	29.24	19.11	23.86	96.11	Does not comply
C015	19.04	52.92	21.02	36.47	27.59	80.92	Does not comply
C016	50.46	9.65	30.16	25.99	41.46	42.59	Complies at pH 1.2
C017	58.73	3.84	76.07	0.30	31.50	67.57	Complies at pH 1.2, 4.5
C018	47.17	10.48	42.98	12.55	26.90	83.48	Does not comply
C019	62.07	5.09	81.79	1.03	55.14	22.50	Complies at pH 1.2, 4.5

Products with different formulations, different inactive ingredients and different formulation design may have different dissolution profiles or the release characteristics and therefore may have different bioavailability. In the study, the dissolution profiles of the 20 brands were tested according to the method described in USP (2014), which states that the amount of ciprofloxacin released within 30 min should not be less than 85 % of the stated amount.

Difference factor (f_1) and similarity factor (f_2) were used to calculate pharmaceutical equivalence of five brands ciprofloxacin tablets in India. The f_1 and f_2 values were below 15 and above 50, respectively, hence the different brands were pharmaceutically equivalent to the innovator brand (Ashraful e*t al*., 2012). Nine of the nineteen brands studied however, were not pharmaceutically equivalent to the innovator brand. In Nigeria, Ngwuluka *et al*. (2009) carried out a similar study on ciprofloxacin tablets. Three of the six studies ciprofloxacin brands (50 %) were not pharmaceutically equivalent with the innovator brand; therefore they may not be used interchangeably. The results obtained were in agreement with the current study where by almost 50 % (47.37 %) of the studied ciprofloxacin brands were not pharmaceutically equivalent to the innovator brand.

CHAPTER FOUR

CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

The study attempted to evaluate the quality of ciprofloxacin tablets of 20 brands on the Kenyan market. The physicochemical evaluation showed the entire ciprofloxacin tablet brands tested for identity, uniformity of weight, hardness, disintegration and assay complied with the pharmacopoeial specifications described in the BP (2012) and USP (2014). There was no direct correlation between tablet hardness, disintegration time and dissolution. In general, all the 20 brands in the study showed differences in their drug release *in vitro* which could also result in differences in their bioavailability *in vivo*. However, *in vitro* testing only predicts the *in vivo* bioavailability and bioequivalence of oral solid dosage forms. It does not exclusively indicate the *in vivo* performance of the drug.

At the pH of 1.2, six (6) brands namely C004, C007, C012, C015, C016 and C017 failed to release the stated amount of ciprofloxacin while three (3) brands C013, C015 and C016 failed to release the stated amount of the drug in 30 min at pH 4.5. This implies there is a significant difference in drug release among the brands. From the similarity factor f_2 calculation values, 10 of the 19 brands (52.63 %), namely C002, C003, C004, C005, C006, C007, C009, C010, C017 and C019 at pH 1.2 and 4.5 are said to be equivalent with the innovator brand IB, therefore they can be interchanged with the innovator brand. At pH 6.8, none of the ciprofloxacin brands if pharmaceutically equivalent to the innovator brand since the f_1 and f_2 values are considered only if the percentage dissolution has reached 85 % within 30 min. All the tablet brands released less than 85 % of ciprofloxacin within 45 min.

The importance of dissolution as a quality control tool for predicting the *in vivo* performance of a drug product is significantly enhanced if an *in vitro-in vivo* relationship is established (Sathe *et al*., 2001).

4.2 Recommendations

To justify the specification limits of the *in vivo* release characteristics of the drug, further studies should be done to establish the meaningful correlation between *in vitro* and *in vivo* bioavailability parameters. Hence the safety, quality efficacy of essential drugs in the region should be continuously monitored through post market surveillance.

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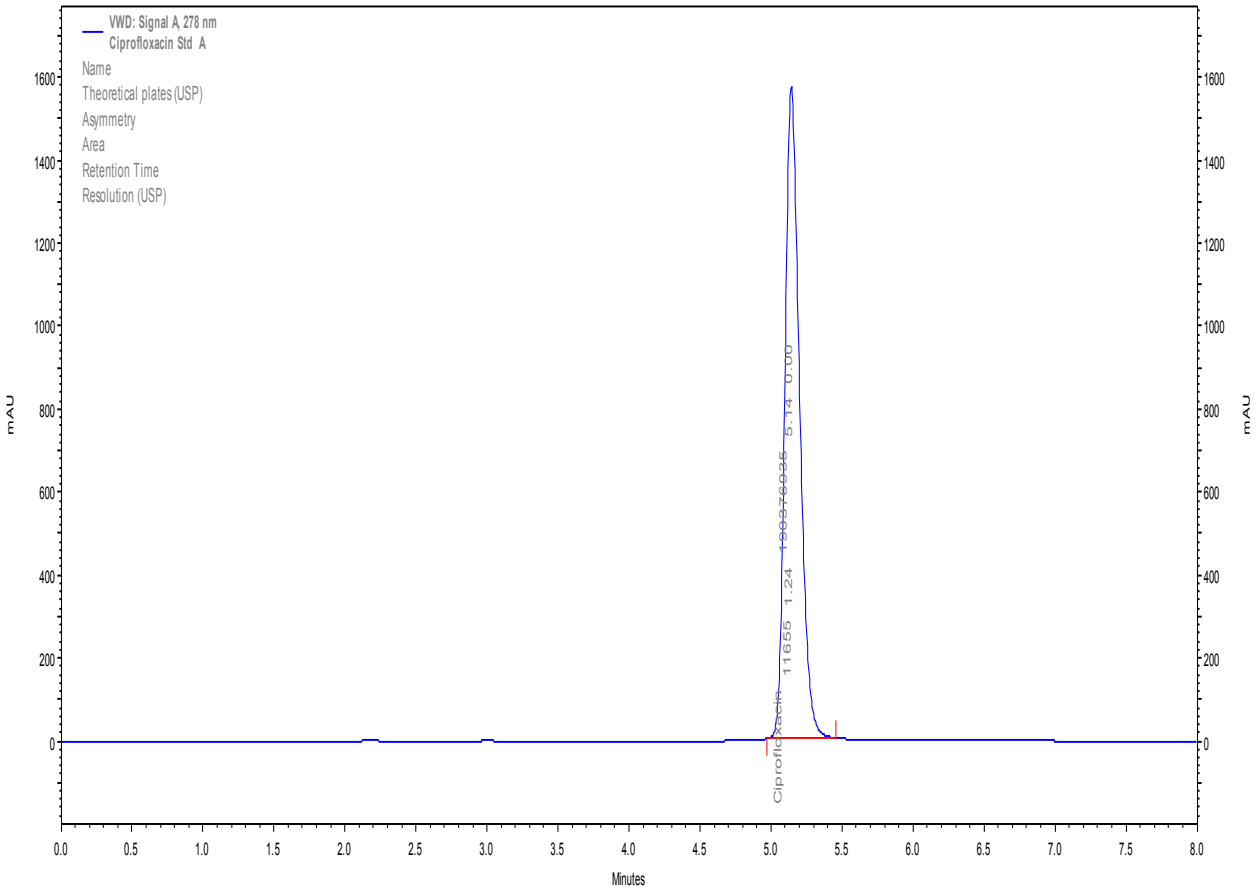
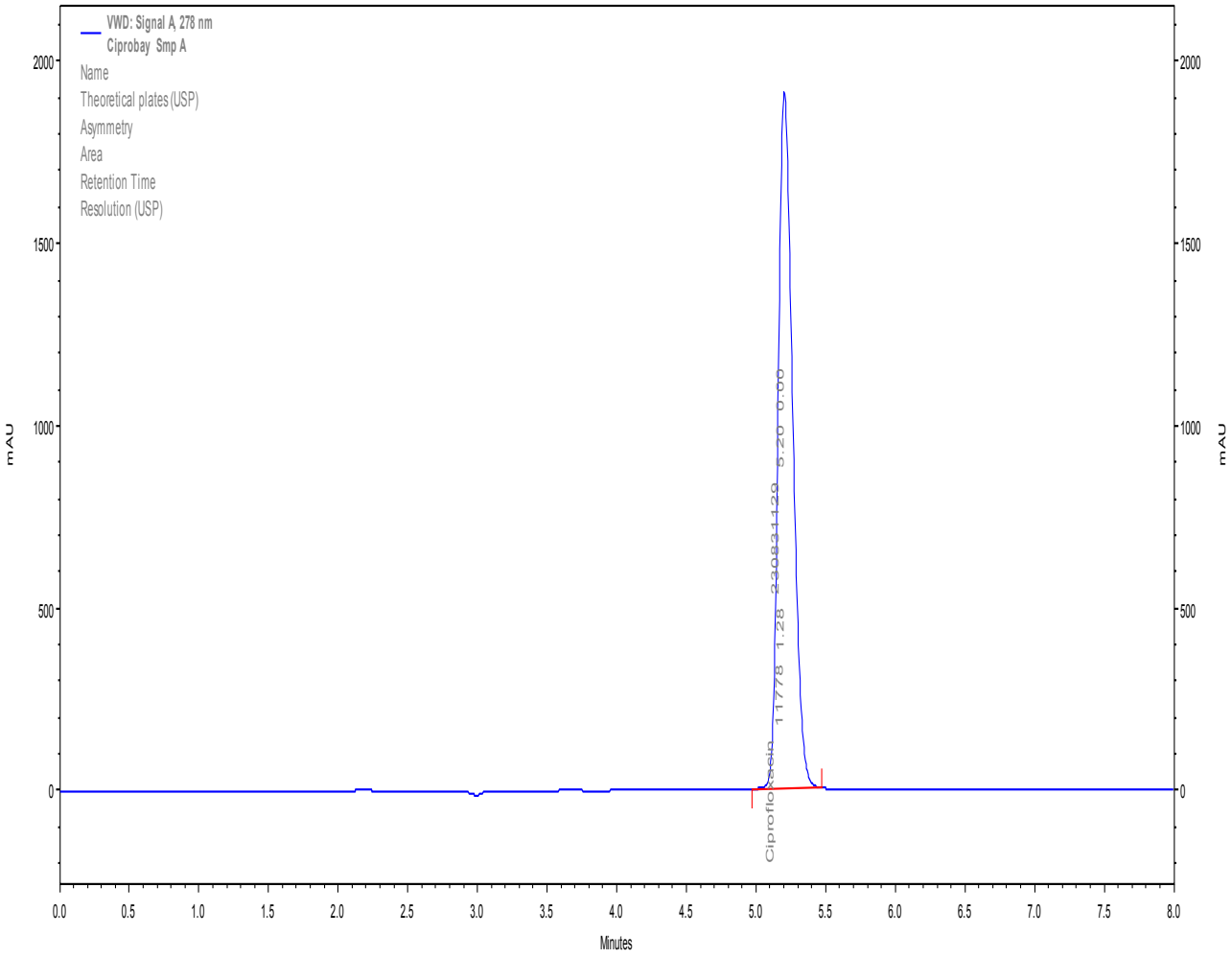
APPENDICES



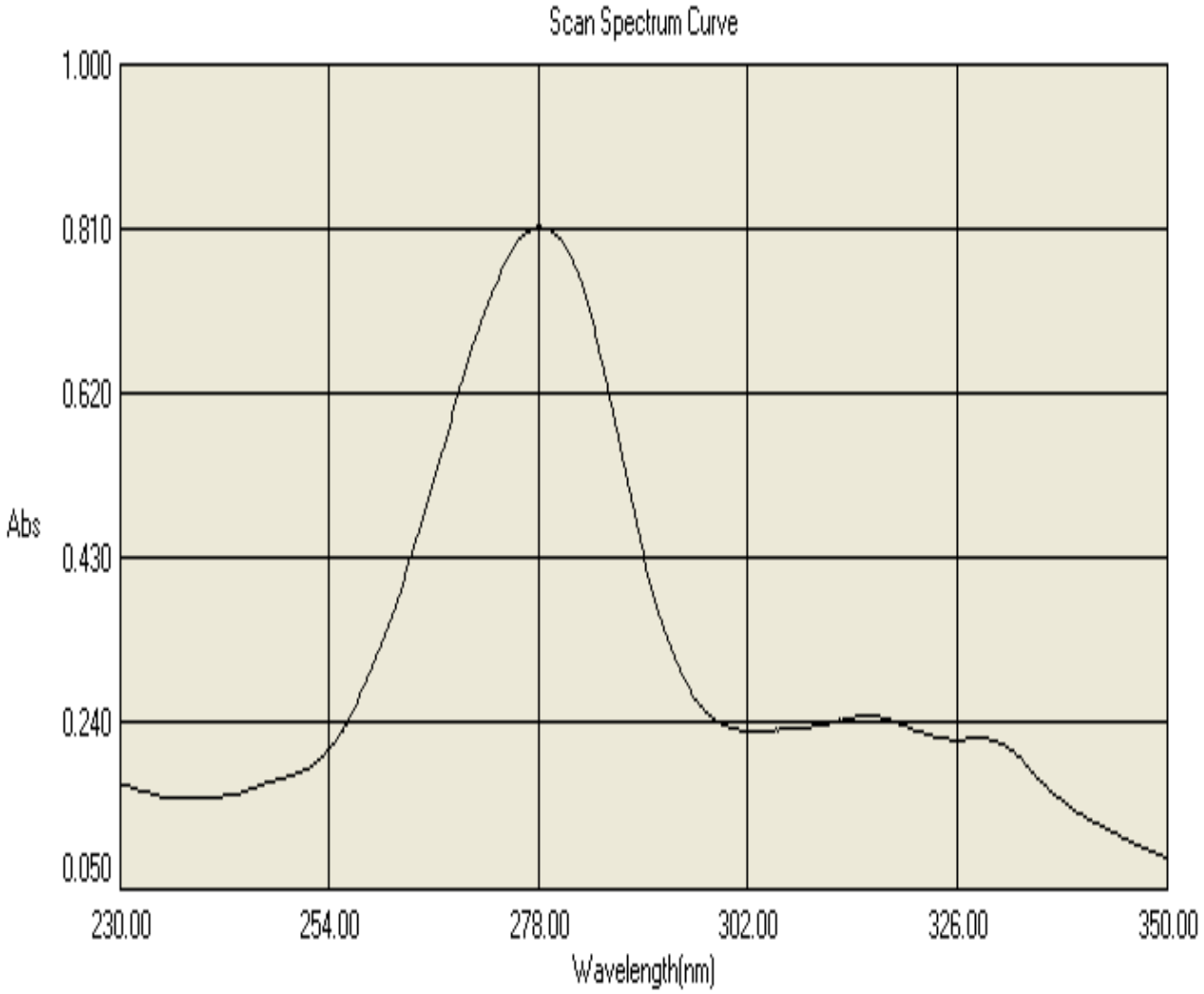
Appendix 1: Typical chromatogram of ciprofloxacin working standard

UV detector set at 278 nm and separation was achieved from a Symmetry® C_{18} 5µm (250 × 4.6 mm) column. The injection volumes were 10.0 µL and the flow rate was 1.50 mL/min. The column temperature was maintained at 30 ± 1 °C in a thermostat oven.



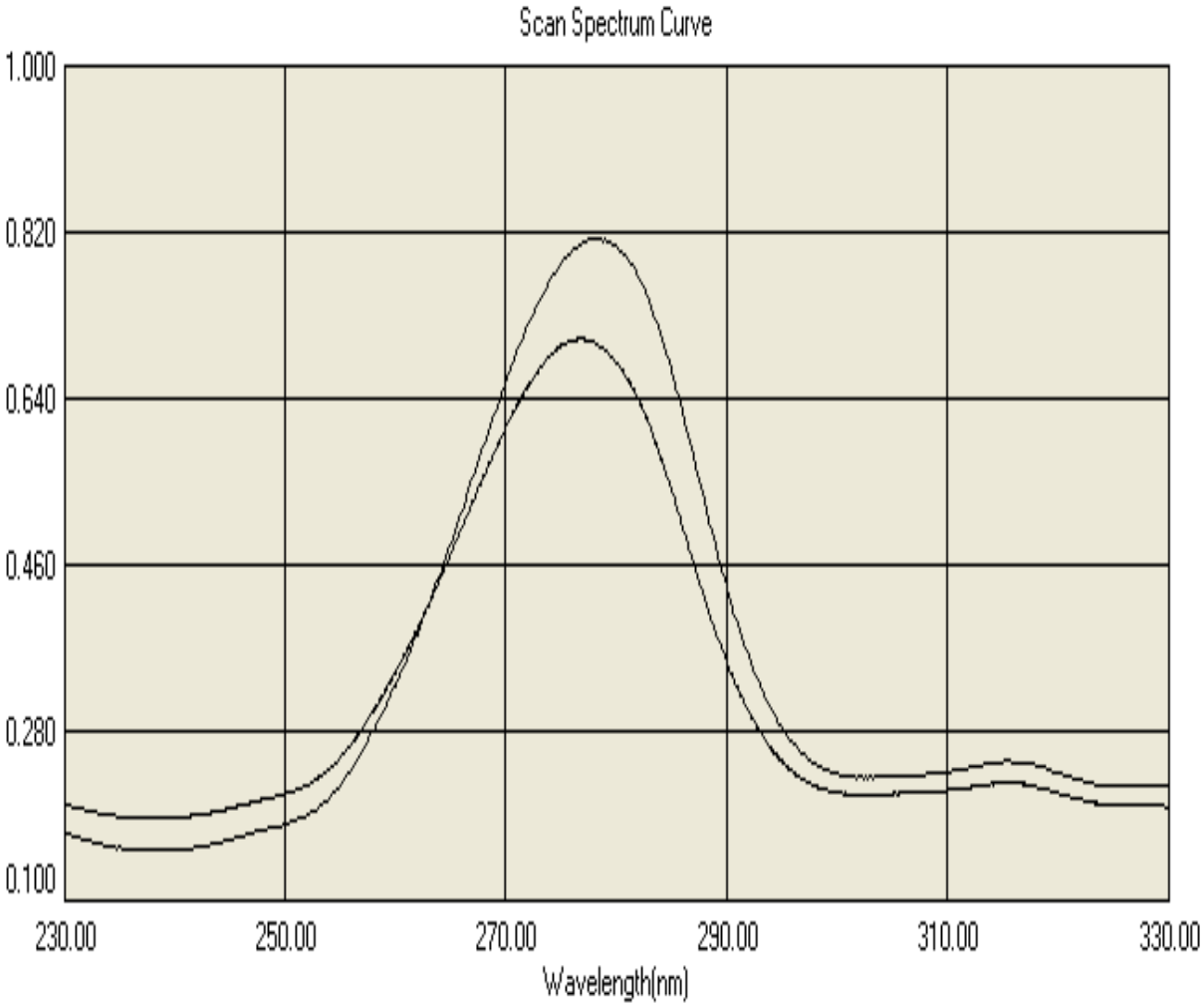
Appendix 2: Typical chromatogram of the innovator brand (IB)

UV detector set at 278 nm and separation was achieved from a Symmetry® C_{18} 5µm (250 × 4.6 mm) column. The injection volumes were 10.0 µL and the flow rate was 1.50 mL/min. The column temperature was maintained at 30 ± 1 °C in a thermostat oven.



Appendix 3: UV spectrum of ciprofloxacin working standard

UV detection at 278 nm, concentration of solution was 0.005 mg/mL



Appendix 4: UV spectrum of ciprofloxacin working standard and innovator brand (IB)

UV detection at 278 nm, concentration of solution was 0.005 mg/mL

Appendix 5: Uniformity of weight

Brand Code	% Deviation from the mean ± SD	% Deviation from the mean weight	Number of tablets within BP range	Number of tablets outside the BP range
C001	777.16 ± 7.31	-2.12 – 2.24	20	0
C002	899.08 ± 24.45	-4.69 – 6.10	18	2
C003	770.75 ±10.21	-2.03 – 3.16	20	0
C004	743.65 ± 11.31	-2.70 – 1.94	20	0
C005	635.78 ± 5.93	-1.24 – 2.58	20	0
C006	739.52 ± 14.29	-5.10 – 3.67	20	0
C007	783.13 ± 7.89	-1.71 – 1.81	20	0
C008	740.94 ± 9.91	-3.01 – 1.90	20	0
C009	694.67 ± 6.66	-1.52 – 1.60	20	0
C010	679.70 ± 6.02	-2.07 – 1.54	20	0
C011	969.85 ± 9.62	-1.96 – 1.59	20	0
C012	643.05 ± 10.54	-2.36 – 4.68	20	0
C013	823.01 ± 15. 56	-4.10 – 3.24	20	0
C014	827.13 ± 5.42	-1.28 – 1.16	20	0
C015	1033.82 ± 19.80	-7.18 – 2.42	20	0
C016	1064.30 ± 25.50	-2.71 – 4.90	20	0
C017	745.43 ± 10.37	-2.98 – 1.79	20	0
C018	692.22 ± 24.10	-6.66 – 5.95	19	1
C019	730.57 ± 8.59	-5.10 – 3.67	20	0
IB	762.49 ± 436	-1.36 – 1.18	20	0

Appendix 6: Tablet of percentage dissolution at pH 1.2

Brand Code	Time (min)					
	5	**10**	**15**	**20**	**30**	**45**
C001	67.53	81.67	88.74	93.07	95.81	97.58
C002	28.53	67.77	86.61	89.69	90.69	92.73
C003	29.37	65.17	79.60	87.89	92.17	94.47
C004	59.40	70.11	75.60	82.27	82.48	88.02
C005	57.30	74.84	81.38	87.01	90.96	93.99
C006	53.77	75.86	84.44	89.27	93.35	95.83
C007	54.16	69.51	75.95	79.15	83.46	86.47
C008	15.87	67.47	90.10	94.61	98.35	99.68
C009	41.65	74.42	86.52	91.37	94.73	95.53
C010	51.27	71.37	79.98	84.94	88.81	92.40
C011	44.77	75.04	83.85	93.48	97.20	97.78
C012	4.22	36.18	56.15	66.98	77.68	86.65
C013	21.82	57.53	83.02	94.32	101.37	102.64
C014	11.12	47.20	69.30	82.71	94.02	98.27
C015	9.96	20.16	28.10	35.60	52.85	70.29
C016	49.17	60.73	70.24	73.88	79.32	83.06
C017	52.88	65.33	72.93	78.54	84.95	88.31
C018	19.23	54.46	74.63	83.39	88.65	91.95
C019	49.51	75.29	85.66	88.54	91.31	94.00
IB	43.86	65.50	78.72	85.21	91.48	96.09

Appendix 7: Tablet of percentage dissolution at pH 4.5

Brand Code	Time (min)					
	5	**10**	**15**	**20**	**30**	**45**
C001	79.74	92.56	94.70	95.69	96.62	98.46
C002	63.50	84.43	85.89	86/61	87.97	89.19
C003	49.75	82.95	89.13	91.09	93.22	94.95
C004	78.68	85.22	88.47	92.59	94.38	96.24
C005	86.85	90.26	91.97	93.92	94.85	95.78
C006	69.65	87.24	92.20	95.64	97.02	98.77
C007	83.98	93.26	95.12	96.01	96.51	97.94
C008	69.70	93.06	95.06	96.77	97.24	98.41
C009	76.02	89.87	94.46	95.80	97.46	98.63
C010	61.85	82.52	89.30	94.30	96.24	97.25
C011	43.95	72.20	83.14	89.66	91.38	93.67
C012	21.31	67.29	81.58	86.9	94.32	96.11
C013	15.77	25.76	39.94	52.19	73.73	94.67
C014	16.33	51.60	77.62	90.22	95.68	97.64
C015	17.58	32.58	46.64	61.62	80.44	98.13
C016	33.10	51.26	65.36	75.95	81.57	85.36
C017	73.51	84.80	88.87	91.75	94.50	95.43
C018	38.61	75.73	84.84	87.49	88.24	89.01
C019	63.97	83.69	89.68	94.61	95.41	97.63
IB	67.63	86.61	91.07	93.87	94.83	96.46

Appendix 8: Tablet of percentage dissolution at pH 6.8

Brand Code	Time (min)					
	5	**10**	**15**	**20**	**30**	**45**
C001	29.48	31.03	31.28	31.81	32.16	32.51
C002	2.32	3.61	4.13	4.32	4.38	4.79
C003	2.54	3.76	4.07	4.53	5.02	5.51
C004	44.85	45.60	46.72	47.18	47.39	46.64
C005	34.95	35.78	36.58	37.35	38.32	38.93
C006	47.00	49.00	49.91	50.47	51.50	52.06
C007	52.99	53.07	54.55	55.20	55.41	56.43
C008	40.56	44.63	45.97	46.81	47.72	49.11
C009	23.94	26.42	28.10	28.53	29.24	29.62
C010	42.41	42.64	43.53	44.30	46.09	47.38
C011	8.63	9.22	9.43	10.18	10.46	11.29
C012	4.78	4.84	5.36	6.16	7.06	8.04
C013	.84	1.06	1.22	1.31	1.65	1.81
C014	0.76	0.87	1.16	1.37	1.75	2.18
C015	5.64	5.84	6.59	6.99	7.14	7.48
C016	17.90	18.37	19.77	20.30	21.41	21.65
C017	56.44	57.33	57.70	58.93	59.02	59.10
C018	3.57	4.15	4.58	6.99	7.51	7.57
C019	40.06	41.65	42.44	43.04	43.45	44.15
IB	33.55	34.07	34.52	35.13	35.33	36.39