A COMPARATIVE STUDY OF THE *IN VITRO* DISSOLUTION PROFILES OF COMMERCIALLY AVAILABLE CLARITHROMYCIN ORAL DOSAGE FORMS IN NAIROBI COUNTY, KENYA

A thesis submitted in partial fulfillment of requirements for the award of the degree of Master of Pharmacy in Pharmaceutical Analysis of the University of Nairobi

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DECLARATION

This research thesis is my original work and has not been submitted elsewhere for award of any degree.

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DECLARATION OF ORIGINALITY

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Title of work	A comparative study on the *in vitro* dissolution profiles of commercially available clarithromycin oral dosage forms in Nairobi County, Kenya

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DEDICATION

This work is dedicated to my family.

To my husband Ezra, my pillar whose push for tenacity drove this work to completion.

To our daughter Rehema, my cheerleader and study companion through the long nights in the study room.

To my brother Kepher and my parents Joyce Kwamboka and Zablon Manani for instilling in me the importance of hard work early in life.

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Above all, I thank God through whom the work has been accomplished.

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SYMBOLS AND ABBREVIATIONS

ACN   Acetonitrile

AIDS   Acquired Immunodeficiency Syndrome

ANOVA   Analysis of variance

API   Active Pharmaceutical Ingredient

BCS   Biopharmaceutics Classification System

BP   British Pharmacopoeia

C$_{18}$ Octadecyl silane

CBD   Central Business District

CDER   Centre for Drug Evaluation and Research

CLA   Clarithromycin

CTD   Common Technical Document

DARU   Drug Analysis and Research Unit

DECL   Decladinosyl clarithromycin

EMEA   European Medicines Agency

FTIR   Fourier Transform Infra Red

g/mol   Grams per mole

GMP	Good Manufacturing Practices
h	Hour
HIV	Human Immunodeficiency Virus
HK	9,12-hemiketal
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxypropyl methylcellulose
ID	Internal diameter
JP	Japanese Pharmacopoeia
KEML	Kenya Essential Medicines List
KNHF	Kenyatta National Hospital Formulary
L	Litre
LC	Liquid Chromatography
Log(K_{dec})	Logarithm of decomposition rate constant
M	Molar (concentration)
MAC	Mycobacterium avium complex
Min	Minutes
mL	Millilitre

mL/min	Millilitre per minute
MLS	Macrolide-lincosamide-streptogramin
μL	Microlitre
μ	Micron
N	Number of theoretical plates
ND	Not detected
nm	Nanometre
NQCL	National Quality Control Laboratory
NR	Not registered
ºC	Degrees centigrade
OIE	Office International des Epizooties
PPB	Pharmacy and Poisons Board
PV	Pharmacovigilance
QC	Quality Control
R	Registered
RNA	Ribonucleic acid
rpm	Rotations per minute

rRNA	Ribosomal ribonucleic acid
RRT	Relative Retention Time
RT	Retention Time
SST	System Suitability Test
SUPAC	Scale-up and post-approval changes
TOTAL CLA	Total Clarithromycins
tRNA	Transfer ribonucleic acid
UK	United Kingdom
US FDA	United States Food and Drug Administration
USP	United States Pharmacopoeia
UV	Ultra-violet
WHO	World Health Organization
w/v	Weight by volume

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ABSTRACT

Clairthromycin (6-O-methylerythromycin A) is a semi-synthetic macrolide derived from erythromycin A. It has improved acid stability, higher oral bioavailability and less gastrointestinal side effects than the naturally occurring macrolides. It is indicated in the treatment of upper respiratory tract infections due to *Streptococcus pyogenes, Haemophilus influenzae, Streptococcus pneumoniae, Haemophilus parainfluenzae, Mycoplasma pneumoniae* and *Chlamydia pneumoniae*. It is effective against skin infections due to *Staphylococcus aureus* and *Streptococcus pyogenes*. It is also used in the treatment of disseminated mycobacterial infections due to *Mycobacterium avium* and *Mycobacterium intracellulare*. It forms the cornerstone of treatment of MAC infections in HIV-AIDS and is an important component of triple therapy regimens for eradication of *H. pylori* in peptic ulcer disease and chronic gastritis.

Despite its improved acid stability compared to erythromycin, clarithromycin undergoes degradation to microbiologically inactive products when subjected to acidic conditions and low gastric pH. It also has poor aqueous solubility that is pH-dependent and a dissolution rate-limited absorption.

In the present study, a comparative dissolution profiling of oral clarithromycin products in Nairobi County, Kenya was carried out with a view to determining its stability in dissolution media of varying pH values. For this reason, dissolution profile tests were carried out at pH 1.2, 4.5 and 6.8 in order to mimic gastric and intestinal conditions. The tests were run for 60 and 90 minutes for clarithromycin tablets and suspensions respectively. The study was aimed at determining the pharmaceutical equivalence of generic clarithromycin oral preparations to the innovator products.

All products tested complied with pharmacopoeial assay specifications. They also complied with single-point dissolution specifications. However, significant differences were observed in their dissolution profiles. At pH 1.2, 50% (six out of twelve) of tablet products failed to meet the specifications for similarity factor in relation to the innovator product. At pH 4.5, 33% (four out of twelve) of tablet products were non-compliant while 50% non-compliance was noted at pH 6.8 with six out of the twelve tablet products failing to meet the specifications. None of the suspensions tested met the requirement for similarity factor in relation to the innovator suspension. Overall, only 25% (four out of sixteen) of all products tested complied with the specifications for similarity factor in relation to the innovator products.

The equivalence patterns observed in this study indicate that assay and single-point dissolution tests are not sufficient to demonstrate pharmaceutical equivalence between innovator products and their generic equivalents. As a minimum requirement, it would be necessary to carry out comparative dissolution profiling of generic products in order for them to be certified as interchangeable with the innovator products.

CHAPTER ONE

GENERAL INTRODUCTION

1.1   Macrolide Antibiotics

Macrolides constitute a large group of natural and semi-synthetic antibiotics characterised by macrocyclic lactone rings to which amino sugars are attached. In some macrolides a neutral sugar is attached in addition to the amino sugar. Other substituents attached to the lactone nucleus are keto, hydroxyl and alkyl groups. Naturally occurring macrolide antibiotics are produced by various strains of *Streptomyces* and *micromonospora* species. They were first discovered through serendipity in the 1940's and 1950's (Gartner *et al.* 2011; Taylor and Triggle 2007; Yamauchi *et al.* 2008).

Erythromycin is the prototype of the macrolide antibiotics. It was discovered in 1952 in the metabolic products of a strain of *Streptomyces erythreus*. It was the first macrolide to enter clinical use. Drug delivery problems and side effects resulting from acid instability of erythromycin prompted the design of semi-synthetic macrolides which were introduced into the market in the 1990's. These possessed higher potency, broader spectrum of activity, superior pharmacokinetic profiles and fewer gastrointestinal side effects compared to erythromycin (Abraham 2003; Alzolibani *et al.* 2012; Periti *et al.* 1992; Piotr 2011; Shinkai *et al.* 2008).

1.2   Classification of Macrolides

Naturally occurring macrolide antibiotics are classified according to the size of the lactone ring as 12-, 14- and 16-membered compounds respectively. Clinically useful macrolides are composed of 14-, 15- and 16-membered lactones. The 14-membered group is composed of the naturally occurring macrolides erythromycin and oleandomycin, as well as the semi-synthetic derivatives clarithromycin, dirithromycin, flurithromycin and roxithromycin (Abraham 2003; Shryock *et al.* 1998).

The 15-membered class comprises semi-synthetic ring-expanded macrolides, the azalides azithromycin and gamithromycin. Azithromycin is the most commercially successful semisynthetic macrolide for human use, while gamithromycin is clinically useful in veterinary medicine (EMEA 2010; Matijašić *et al.* 2012; Munic *et al.* 2011).

Macrolides with a 16-membered ring include naturally occurring products josamycin, spiramycin, kitasamycin, tylosin and midecamycin, as well as the semi-synthetic derivatives rokitamycin, miocamycin and tilmicosin. Whereas these 16-membered macrolides have achieved limited success in human medicine, they are useful in veterinary medicine. Their indications include treatment of *Mycoplasma* infections in pigs and poultry, hemorraghic digestive disease in pigs, liver abscesses and respiratory infections in cattle (Abraham 2003; EMEA 2010; OIE 2007; Shryock *et al.* 1998).

1.3   Antimicrobial Activity and Clinical Applications

Macrolide antibiotics are effective against both Gram positive and some Gram negative bacteria, as well as *Mycoplasma pneumoniae, Treponema pallidum, Bordetella pertussis,*

Chlamydia trachomatis, Chlamydophila pneumoniae, Legionella spp, Campylobacter spp and Borrelia spp (Chiba *et al.* 2010).

First-line indications for macrolides include the treatment of atypical community acquired pneumonia, eradication of *Helicobacter Pylori* as part of triple therapy, treatment of patients with chlamydial infections, legionella pneumonia and treatment of acute non-specific urethritis. They are also effective against skin, soft tissue and atypical mycobacterial infections. They are useful alternatives in cases of penicillin and cephalosporin allergies (Chiba *et al.* 2010; Steel *et al.* 2012).

In addition to their therapeutic efficacy as antimicrobials, macrolide antibiotics possess anti-inflammatory, immunomodulatory and antiparasitic activity. They are beneficial in the inflammatory conditions rosacea, psoriasis and bullous pemphigoid. They have also found use in chronic pulmonary diseases such as diffuse panbronchiolitis, cystic fibrosis, asthma and bronchiectasis. Erythromycin and clarithromycin are used in Japan for treatment of sinusitis and chronic obstructive pulmonary disease, while azithromycin has been adopted as an immunomodulatory agent for the treatment of cystic fibrosis and bronchiectasis (Alzolibani *et al.* 2012; Matijašić *et al.* 2012; Munić *et al.* 2011; Shinkai *et al.* 2008).

The success of macrolides as antibiotics and immunomodulators is due to their distinct pharmacokinetic properties. They highly accumulate in cells and tissues. Notably, azithromycin and clarithromycin achieve high and prolonged concentrations at the sites of infection with their concentrations in tissues often reaching 10- to 100-fold higher than those found in plasma (Matijašić *et al.* 2012; Steel *et al.* 2012).

1.4   Limitations of Naturally Occurring Macrolide Antibiotics

Bacterial resistance, instability in gastric acid, gastrointestinal side effects, low potency and a narrow spectrum of activity have been the major shortcomings of naturally occurring macrolide antibiotics. The C-3 cladinose sugar attached to erythromycin is believed to be responsible for the induction of macrolide resistance as well as efflux resistance (Ma and Nemoto 2002; Vekariya *et al.* 2011).

Erythromycin A (Figure 1.1) undergoes acid degradation in gastric acid due to internal ketalisation and decladinosylation reactions. Internal ketalisation involves the C-6 hydroxyl, C-9 keto and C-12 hydroxyl groups to form erythromycin A enol ether and anhydroerythromycin A. Decladinosylation leads to formation of erythralosamine. These transformations result in drug inactivation, gastrointestinal cramping, nausea and vomiting. Erythromycin A enol ether is responsible for the associated nausea and vomiting due to its gastrointestinal motor stimulating activity (Tsuzuki *et al.* 1989).
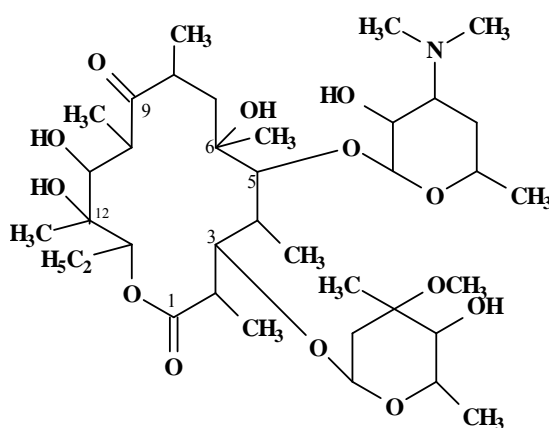
Figure 1.1: Chemical structure of erythromycin A

Erythromycin A also exhibits high intra- and inter-patient variabilities in bioavailability which threatens its effectiveness. Its bioavailability ranges from 15 - 45% with a mean bioavailability of 25%. In addition, erythromycin A has a short half life of 2 - 3 hours hence administered four times daily. It also has low potency and a narrow spectrum of activity (Abraham 2003; LeBel 1993; Taylor and Triggle 2007).

1.5   Semi-synthetic Macrolide Antibiotics

Chemical modifications aimed at overcoming the limitations of erythromycin A have included methylation, oximation, ring expansion and replacement of the C-3 cladinose sugar with keto groups. These modifications have yielded the semi-synthetic macrolides with increased acid stability, higher potency, extended spectrum of activity, higher gastrointestinal tolerability and superior pharmacokinetics than erythromycin A (Taylor and Triggle 2007; Zuckerman 2004).

Methylation of the C-6 hydroxyl group yielded clarithromycin (6-O-methylerythromycin A) that is more acid-stable than erythromycin (Figure 1.2). Halide substitution of the C-8 hydrogen is aimed at preventing the formation of erythromycin A enol ether. This has led to the development of flurithromycin (8-fluoro-erythromycin A) (Figure. 1.2) which is more potent than erythromycin against anaerobes and is used for treatment of lower respiratory tract infections ((Abraham 2003; Bariffi *et al.* 1994; Taylor and Triggle 2007).

Clarithromycin

Azithromycin

Roxithromycin

Flurithromycin

Cethromycin

Telithromycin

Fig. 1.2: Chemical structures of some semi-synthetic derivatives of erythromycin A

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Oximation and etherification at the C-9 keto group gives ether oximes with roxithromycin (Figure 1.2) giving the best pharmacokinetic profile in this class. Roxithromycin possesses greater acid-stability than erythromycin A.

Chemical modifications at the C-12 hydroxyl group have yielded cyclic acetals, carbonates and cyclic carbamates that are superior to erythromycin A. Erythromycin-11,12-methylene cyclic acetal possesses higher *in vitro* activity than erythromycin A. Erythromycin-11,12-carbonate has better pharmacokinetics than erythromycin A while the 11-deoxy-11-(carboxyamino)-6-O-methylerythromycin A 11,12-cyclic carbamates possess *in vitro* antibacterial activity comparable to that of clarithromycin (Abraham 2003; Lemke *et al.* 2008; Taylor and Triggle 2007).

Ring expansion produced the azalides, the most clinically useful drug being azithromycin (Figure 1.2). These are synthesized by Beckmann rearrangement of erythromycin-9-oxime with subsequent reduction of the intermediate imino ether and N-methylation to produce 15-membered derivatives. Azalides have better acid stability, longer half-lives and higher bioavailability than erythromycin (Abraham 2003; Lemke *et al.* 2008; Taylor and Triggle 2007).

Removal of the C-3 cladinose sugar of erythromycin A and the subsequent oxidation of the C-3 hydroxyl to a keto group promotes greater acid stability and prevents induction of macrolide resistance (Ma and Nemoto 2002; Xu *et al.* 2005; Zuckerman 2004). These substitutions have led to the development of ketolides that have greater acid stability, superior pharmacokinetics that allow once daily dose administration and better tissue penetration than erythromycin A. Ketolides exhibit good activity against Gram-positive aerobes and some Gram-negative aerobes including macrolide-resistant *Streptococcus*

pneumoniae. Additionally, they contain an 11, 12-cyclic carbamate linkage with attached arylalkyl groups which provide an alternative and more effective ribosomal binding site than the C3-cladinose moiety, thus compensating for loss of cladinose. These modifications impart a higher ribosomal binding affinity hence greater antibacterial potency than macrolides. Clinically used ketolides include telithromycin and cethromycin (Figure 1.2) (Douthwaite *et al.* 2000; Zhanel *et al*. 2002; Xu *et al.* 2005).

1.6   Clarithromycin

1.6.1   Chemistry

Clarithromycin is a second generation semi-synthetic macrolide antibiotic derived from erythromycin A by 6-O-methylation. It consists of a 14-membered macrocyclic lactone ring with cladinose and desosamine sugars linked via glycosidic bonds at positions 3 and 5 of the ring, respectively. The desosamine sugar has a β-glycosidic linkage to the lactone ring while cladinose is α-glycosidically linked. Chemically, clarithromycin is (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*S*,12*R*,13*S*,14*S*)-6-{[(2*S*,3*R*,4*S*,6*R*)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy}   -14-ethyl-12,13-dihydroxy-4-{[(2*R*,4*S*,5*S*,6*S*)-5-hydroxy   -4-methoxy-4,6-dimethyloxan-2-yl]oxy}-7   -methoxy-3,5,7,9,11,13-hexamethyl   -1-oxacyclotetradecane-2,10-dione (6-O-methylerythromycin A).

Its molecular formula is $C_{38}H_{69}NO_{13}$ and the molecular weight is 747.96 g/mol. It occurs as a white or almost white crystalline powder that is practically insoluble in water, soluble in methanol, acetonitrile, acetone and methylene chloride. It has a melting point

of 217 - 220 °C (Budavari *et al.* 1989; Morgan *et al.* 1991; PubChem; Watanabe *et al.* 1990).

1.6.2   Synthesis and Related Substances

Clarithromycin is synthesized by selective methylation of the C-6 hydroxyl group of erythromycin A (Figure 1.3). The initial steps in the synthetic process are aimed at protecting other reactive functional groups to allow for selective methylation. The first step is the conversion of the C-9 carbonyl to the oxime using hydroxylamine in acidic conditions. Protection of the oxime is achieved by the reaction with 1,1-di-isopropoxycyclohexane in the presence of a catalytic amount of pyridine to obtain a mixed acetal. The acetal is reacted with a silylating agent, hexamethyldisilazane in the presence of pyridine to protect the C-2' and C-4" hydroxyl groups. Methylation of the C-6 hydroxyl group is carried out using methyl iodide and potassium hydroxide in triethylamine. The final step is hydrolysis to remove the protecting groups and deoximation to C-9 keto. The acetal and 2′ and 4″ silanes deprotection is carried out in a solution of acetonitrile, water and 85% formic acid. Deoximation is accomplished by reaction with aqueous sodium metabisulfite so as to obtain clarithromycin (Kawashima *et al.* 1990; Ramón *et al.* 2003; Taylor and Triggle 2007; Watanabe *et al.* 1990; Wu 2000).
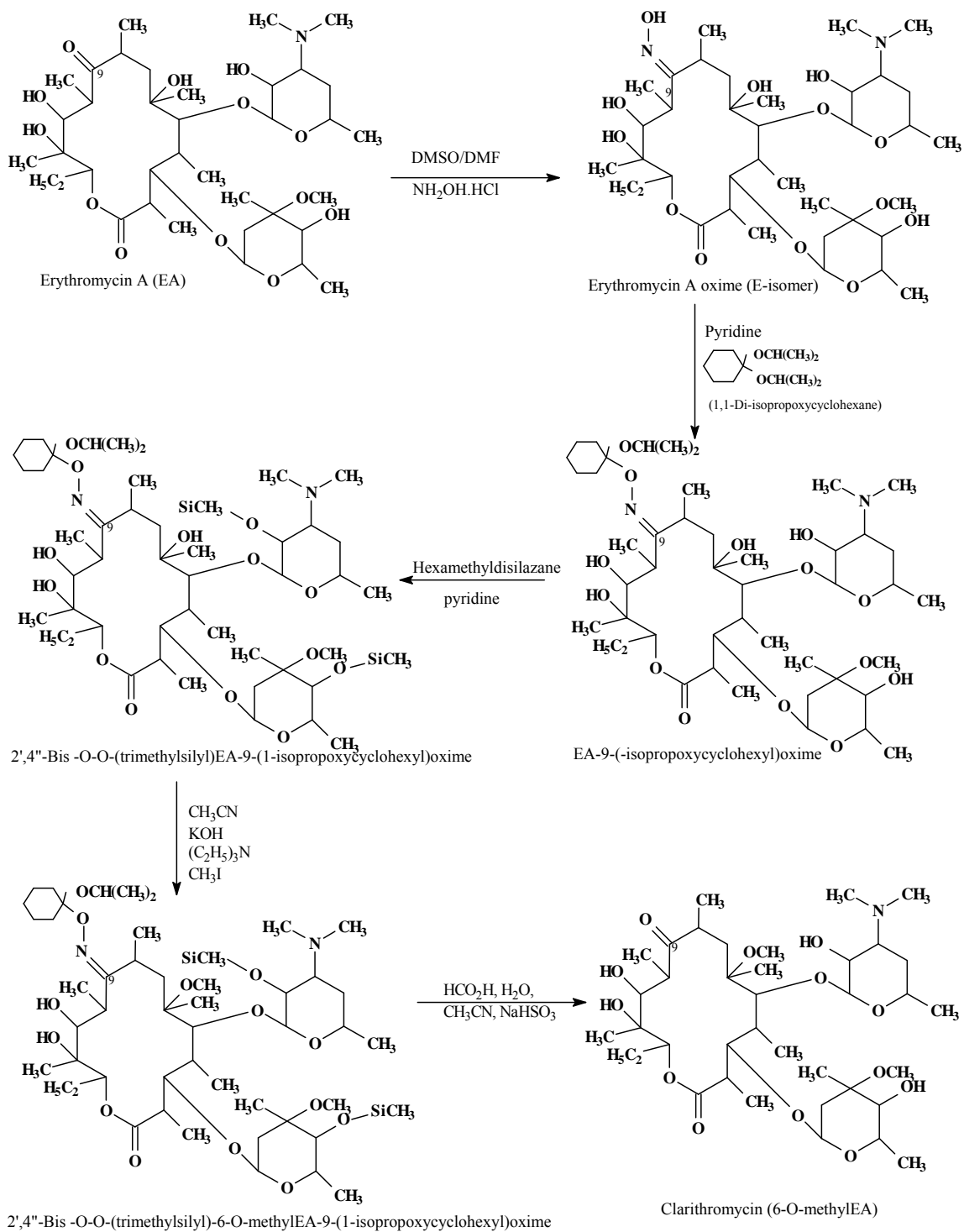
Figure 1.3: Synthesis of clarithromycin

Several related substances of clarithromycin arise during synthesis and degradation as synthetic intermediates, side-products and degradation products (Figure 1.4). Synthetic side products include N-demethylclarithromycin that arises from demethylation of the 3'-dimethylamino group on the desosamine sugar, 6,11-di-O-methylclarithromycin due to dimethylation of the C-6 and C-11 hydroxyl groups and 6,12-di-O-methylclarithromycin due to dimethylation of the hydroxyl groups at C-6 and C-12.

Degradation products include clarithromycin N-oxide that arises due to oxidative degradation of clarithromycin, decladinosyl clarithromycin , 9,12-hemiketal and 10, 11-anhydroclarithromycin which are products of acid-catalysed degradation of clarithromycin.
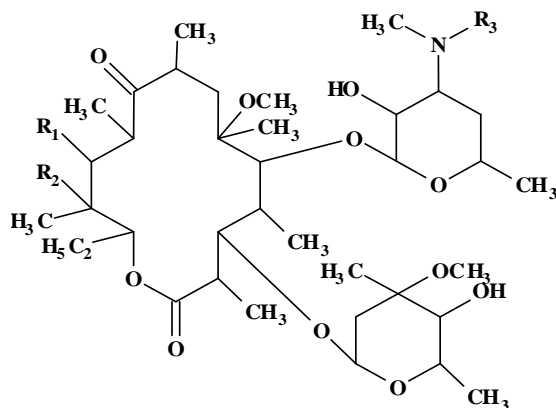
Name	R₁	R₂	R₃
Clarithromycin	OH	OH	CH₃
N-demethylclarithromycin	OH	OH	H
N-formylclarithromycin	OH	OH	CHO
6,11-di-O-methylclarithromycin	OCH₃	OH	CH₃
6,12-di-O-methylclarithromycin	OH	OCH₃	CH₃
Clarithromycin N-oxide	OH	OH	O; CH₃
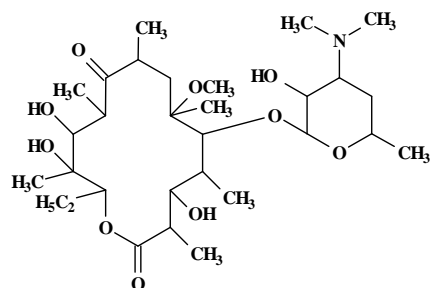
Decladinosylclarithromycin
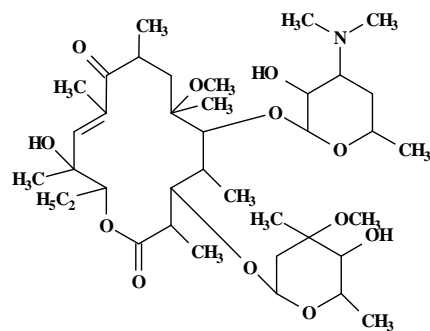
10,11-Anhydroclarithromycin

Figure 1.4: Chemical structures of clarithromycin and its related substances

9,12-Hemiketal

2-demethyl-2-(hydroxymethyl)-6-O-
methylerythromycin A

（Clarithromycin F）

2"-O-2-Carboxybenzoyl clarithromycin
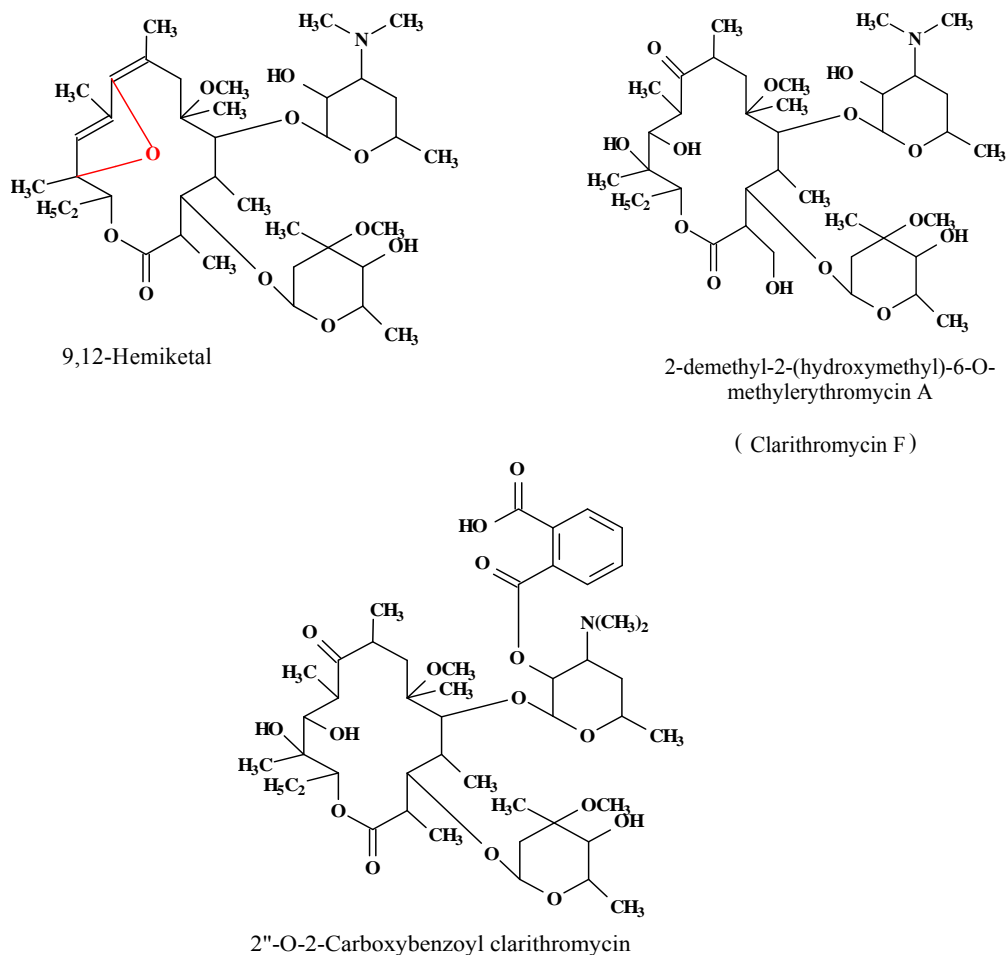
Figure 1.4 (continued): Structures of clarithromycin and its related substances

1.6.3   Pharmacokinetics

Clarithromycin has improved acid stability that results in higher oral bioavailability, a lower clearance rate and a higher volume of distribution. It also has reduced gastrointestinal intolerance due to 6-methoxy substitution that caps internal ketalization (Lemke *et al.* 2008; Taylor and Triggle 2007; Zuckerman 2004).

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1.6.3.1 Absorption and Distribution

Clarithromycin is rapidly and almost completely absorbed from the gastrointestinal tract. It has a systemic bioavailability of 50 - 55% after an oral dose due to extensive first pass metabolism. It is extensively bound to plasma protein. Peters and Clissold (1992) report 42 - 70% plasma protein binding at concentrations representative of those achieved clinically. It is widely distributed through the body, with an apparent volume of distribution of 126 - 306 L (Abduljalil *et al.* 2009; Peters and Clissold 1992; Sutar *et al.* 2011; Taylor and Triggle 2007).

Like other macrolides, clarithromycin is lipophilic and readily penetrates into fluids and tissues. Mean tissue concentrations are 2- to 20-fold greater than serum concentrations. It particularly accumulates in gastric tissues, lungs, tonsils, the gingiva, the sinus fluid, the middle ear fluid, the sputum, the prostate and the eye. The mechanisms by which clarithromycin accumulates in tissues include base-trapping of the drug in the acidic compartment of tissues, accumulation due to the motive force created by a pH gradient across lysosomal membranes and unidirectional penetration from blood to tissues. Unidirectional penetration is mediated by multidrug transporters found on cell membranes (Carryn *et al.* 2003; Langan and Bambeke 2010; Peters *et al.* 2011; Zuckerman 2004).

1.6.3.2 Metabolism and Excretion

Clarithromycin is extensively metabolised in the liver by the cytochrome P-450 3A4 (CYP3A4) enzymes into seven known metabolites. The major metabolic pathways are

C-14 hydroxylation, N-demethylation and hydrolysis of the cladinose sugar that occurs non-enzymatically (Figure 1.5).
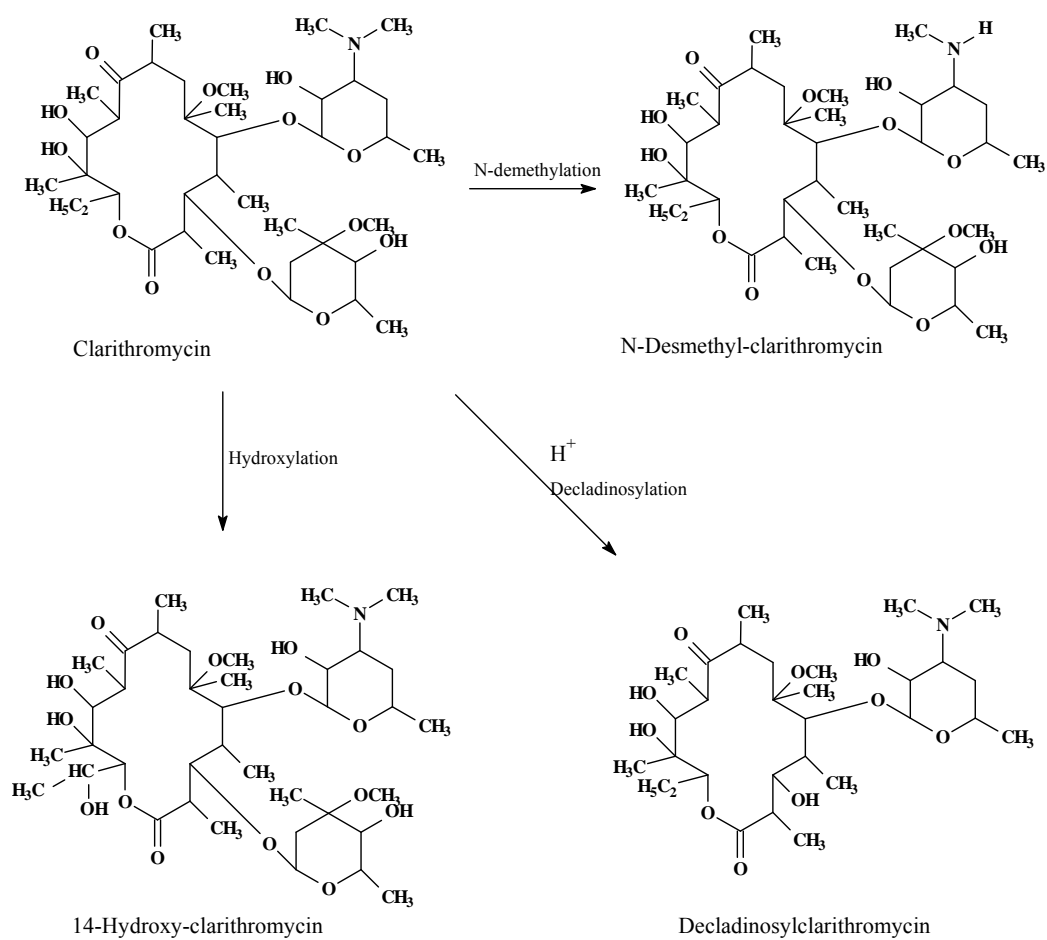
Figure 1.5: Major metabolic pathways of clarithromycin

Hydroxylation at C-14 is stereospecific, yielding the 14-hydroxy-(*R*)-epimer as the main metabolite that accounts for 20% of metabolites from the parent drug. The 14-hydroxyl

metabolite contributes significantly to the overall antimicrobial effect of clarithromycin. (Abduljalil *et al.* 2009; Davey 1991; Zuckerman 2004).

Other metabolites are *N*-desmethylclarithromycin, 14-(*S*)-hydroxyclarithromycin, 14-(*R*)-hydroxy/*N*-desmethylclarithromycin, 14-(*S*)-hydroxy/*N*-desmethylclarithromycin and *N,N*-didesmethylclarithromycin. The major known metabonate is decladinosyl clarithromycin (Rodrigues *et al.* 1997; Zuckerman 2004).

Clarithromycin is excreted in urine and into bile. Thirty to forty percent of an oral dose is excreted in urine either unchanged or as the active 14-hydroxy metabolite. The remainder is excreted into bile. Its elimination half-life is dose and time dependent and ranges from 2 to 4.8 h. Studies conducted by Davey (1991) indicate that approximately 18% of an oral dose of clarithromycin is recovered as the parent compound in urine while 4% is recovered as the parent compound in faeces (Abduljalil *et al.* 2009; Davey 1991; Traunmuller *et al.* 2007; Zuckerman 2004).

The presence of food does not have a clinically significant effect on the pharmacokinetic parameters of clarithromycin. It slightly delays the time to peak concentration but has no effect on its bioavailability (Peters *et al.* 2011; Kanatani *et al.* 1994).

1.6.4   Clinical Pharmacology

1.6.4.1 Mechanism of Action

Clarithromycin is both bacteriostatic and bactericidal depending on the species and the drug concentration. It exerts its antimicrobial activity by inhibiting RNA-dependent bacterial protein synthesis. It penetrates the bacterial cell wall and reversibly binds to bacterial ribosomes through interactions with nucleotides in domains II and V of 23S rRNA of the 50S subunit. The domain II interaction occurs between nucleotide A752 and the C3-cladinose moiety. This induces dissociation of tRNA from the ribosome during the elongation phase. As a result, RNA-dependent protein synthesis is suppressed, and bacterial growth is inhibited (Douthwaite *et al.* 2000; Farshchi *et al.* 2009; Pankey *et al.* 2004; Shinkai *et al.* 2008).

1.6.4.2 Spectrum of Activity

Clarithromycin possesses a wider spectrum of activity than erythromycin. It is active against Gram-positive and some Gram-negative aerobic bacteria, anaerobic bacteria and a variety of atypical microorganisms.

Susceptible Gram-positive bacteria include *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae* and *Streptococcus pyogenes*. Gram-negative aerobic bacteria with susceptibility to clarithromycin include *Haemophilus influenzae, Haemophilus parainfluenzae, Bordetella pertussis, Neisseria meningitidis* and *Moraxella catarrhalis*. Clarithromycin is also active against *Helicobacter Pylori*, a Gram-negative organism that survives in the deep layers of the gastric mucosa.

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Gram-positive anaerobes are more susceptible to clarithromycin than Gram-negative anaerobes. These include strains of *Propionibacterium acnes* and anaerobic diphtheroids. Susceptible atypical bacteria are *Mycobacteria*, *Ureaplasma, Toxoplasma*, *Borrelia*, *Chlamydia pneumoniae, Chlamydia trachomatis* and *Mycoplasma pneumoniae* (Farshchi *et al.* 2009; Hardy 1993; Leung *et al.* 2000; Pankey *et al.* 2004).

In addition to its antibacterial activity, clarithromycin causes immunomodulation and a decrease in corticosteroid metabolism. Studies have shown an improvement in the clinical symptoms of corticosteroid-dependent patients with asthma and a reduction in corticosteroid dosage when clarithromycin was co-administered with the corticosteroid. Subjects with Crohn's disease treated with clarithromycin demonstrated significant clinical improvement compared to the control group (Shinkai *et al.* 2008; Wales *et al.* 1999).

1.6.4.3 Clinical Indications

Clarithromycin is indicated for treatment of a wide range of infections caused by susceptible species of bacteria. It is effective against pharyngitis and tonsillitis due to *Streptococcus pyogenes* and acute maxillary sinusitis due to *Haemophilus influenzae, Moraxella catarrhalis* and *Streptococcus pneumoniae*. It is also indicated for acute exacerbation of chronic bronchitis due to *Haemophilus influenzae, Haemophilus parainfluenzae, Moraxella catarrhalis* and *Streptococcus pneumoniae*.

Other indications include pneumonia due to *Haemophilus influenzae, Mycoplasma pneumoniae, Streptococcus pneumoniae* and *Chlamydia pneumoniae*. It is effective against skin infections due to *Staphylococcus aureus* and *Streptococcus pyogenes*.

Clarithromycin is effective in the treatment of disseminated mycobacterial infections due to *Mycobacterium avium* and *Mycobacterium intracellulare.* It forms the cornerstone of treatment of MAC infections in HIV-AIDS patients (Hardy 1993; Park *et al.* 2009; Salem and Nuzgunes 2003).

Clarithromycin is a component of triple therapy regimens for eradication of *H. pylori* in peptic ulcer disease and chronic gastritis. It is combined with a proton pump inhibitor or ranitidine bismuth citrate and amoxicillin or metronidazole with eradication rates of more than 95% (Leung *et al.* 2000; Srinivasu *et al.* 2012).

1.6.5   Stability of Clarithromycin

Clarithromycin undergoes degradation when subjected to acidic, basic, oxidative conditions and light. It however is more stable than erythromycin in acidic conditions. The degradation products are microbiologically inactive.

Oxidation of clarithromycin with hydrogen peroxide solution produces clarithromycin N-oxide among other impurities. Photolytic degradation due to light and UV radiation yields the N-oxide and 2"-O-2 carboxybenzoyl clarithromycin while ozonolysis yields clarithromycin N-oxide and N-demethyl clarithromycin as the major products (Abuga *et al.* 2001; Lange *et al.* 2006; Srinivasu *et al.* 2012).

Alkaline hydrolysis of clarithromycin in sodium hydroxide or potassium hydrogen phosphate solutions yields 10,11-Anhydroclarithromycin and clarithromycin F (2-demethyl-2-(hydroxymethyl)-6-O-methylerythromycn A). (Abuga *et al.* 2001; Srinivasu *et al.* 2012).

Acid degradation of clarithromycin follows pseudo first order kinetics. The products of acid degradation are decladinosyl clarithromycin and the 9,12-hemiketal. Acid-catalysed decladinosylation is typical of α-glycosidic bonds (Abuga *et al.* 2001; Nakagawa *et al.* 1992; Srinivasu *et al.* 2012).

Experimental data presented by Erah *et al.* (1997), Fujiki *et al.* (2011) and Nakagawa *et al.* (1992) indicate that clarithromycin is stable at pH ≥ 3.0 with optimum stability being observed between pH 5.0 and 8.0. The decomposition kinetics indicate an approximately linear relationship between $\log(K_{dec})$ and pH.

In the study conducted by Fujiki *et al.* (2011), clarithromycin exhibited negligible decomposition at pH 3.0 with more than 90% active drug still available at 2 hours of incubation. At pH 2.0 and 30 minutes incubation, the level of active drug was found to be 75%. At pH 1.5 and 30 minutes incubation, only 30% active drug was available. No drug was detectable below pH 1.5 at 30 minutes incubation time. Erah *et al.* (1997) reported loss of 90.2% of clarithromycin from aqueous samples at pH 1.0 within one hour of the experiment.

These studies demonstrate the lability of clarithromycin to acid conditions. At pH levels < 3.0, a decrease in pH of one unit causes 50% decrease in the availability of active clarithromycin. A decrease in pH of 0.5 units causes up to 45% loss in the amount of active drug present.

1.7   BCS Classification of Clarithromycin

Efficacy of drugs is dependent on the availability of adequate active concentrations at the site of the desired pharmacodynamic effect. Drug absorption from oral dosage forms of clarithromycin depends on the release of clarithromycin API from the drug product, the dissolution of the API under physiological conditions and its permeability across the gastrointestinal tract membranes. Dissolution is, therefore, of primary importance and is a rate-limiting step in the absorption of clarithromycin since the drug is lipophilic (Alkhalidi *et al.* 2010; US FDA 1997; Nagabandi *et al.* 2010; Peters *et al.* 2011).

Drugs are classified based on their aqueous solubility and intestinal membrane permeability using a scientific framework, the Biopharmaceutics Classification System (BCS). The BCS takes into account the major factors that govern the rate and extent of drug absorption. These factors are dissolution, solubility, and intestinal permeability. According to the BCS, drug substances are categorised into four classes (Table 1.1) (US FDA 1997; US FDA 2012).

Table 1.1 Biopharmaceutics Classification System of Drugs

Class	Aqueous Solubility	Membrane Permeability
I	High	High
II	Low	High
III	High	Low
IV	Low	Low

Clarithromycin is a poorly soluble drug whose absorption is limited by the dissolution rate. Owing to this, clarithromycin tablets are classified as BCS class II while clarithromycin paediatric suspension is classified as BCS class IV. The suspension possesses low aqueous solubility and low membrane permeability.

Most paediatric oral preparations are developed for BCS Class I and III APIs because of their high solubility in water or gastrointestinal media. Despite its low solubility and permeability profile, clarithromycin suspension has been developed due its clinical importance in the management of atypical infections and its safety profile in children. Its formulation utilizes special techniques such as micronization, complexation with resins, use of cosolvents and surfactants to enhance solubilization (Castrellon *et al.* 2012; US FDA 2012; Lakum *et al.* 2013; Shahbaziniaz *et al.* 2013; Zhang *et al.* 2005).

Drugs in BCS Class II and IV often exhibit variable absorption due to various physicochemical, formulation and gastrointestinal factors that can affect their dissolution performance. Dissolution rate and solubility are identified as the principal limitations to their absorption and are therefore key factors for predicting precise human absorption (Shahbaziniaz *et al.* 2013; Takano *et al.* 2006; Zhang *et al.* 2005).

1.8   Effect of pH on Dissolution of Clarithromycin Formulations

Clarithromycin is practically insoluble in water and its solubility in aqueous medium is pH-dependent. The solubility pattern was studied at constant phosphate buffer concentrations of varying pH values. It was found that its solubility decreases with increasing pH and remains constant above pH 9.0 (Table 1.2) (Nakagawa *et al.* 1992; Salem and Nuzgunes 2003).

The relationship between pH of dissolution medium and dissolution rate of clarithromycin was determined in a study conducted by Ishii *et al.* (1995). It was found that the dissolution rate decreased as the pH increased and the dissolution obeyed first order kinetics (Table 1.2) (Ishii *et al.* 1995).

Table 1.2 Effect of pH on Solubility and Dissolution Rate of Clarithromycin

pH	Solubility (mg/mL)	Dissolution Rate (%)	
		10 minutes run time	30 minutes run time
2.4	11.8	-	-
3.0	-	98	100
5.0	-	68	98
5.4	7.5	-	-
6.0	-	30	72
6.5	3.7	17	50
7.0	2.4	-	-
7.4	<1	-	-

- = Data not available

The relationship between solubility and pH for a weak base drug is explained by the Henderson-Hasselbach equation:

$$pH = pK_a + \log(C_b/C_i)$$

where pK_a is the dissociation constant, C_i is the solubility of the dissociated molecule and C_b is the solubility of the undissociated molecule.

Clarithromycin is a weak base with pKa of 8.76. As the pH falls below 8.76, the concentration of the dissociated molecule increases and this increases the overall solubility of the drug due to increased ionisation (Nakagawa *et al.* 1992).

1.9 Enhancing Stability and Dissolution of Clarithromycin

Different formulation approaches have been adopted singly or in combination in order to improve the stability and dissolution of clarithromycin oral dosage formulations. These include enteric coating, formulation of multiple unit dosage forms such as film-coated pellets, particle size reduction approaches such as micronization and nanonization, use of mucoadhesive drug carriers and wetting agents, among others.

Sutar *et al.* designed an oil-in-water micro emulsion formulation of clarithromycin. They used ethyl oleate and olive oil as the oil phases, phosphate buffer solution of pH 6.8 as the aqueous phase and different hydrophilic and lipophilic surfactants. This resulted in increased solubility of clarithromycin. The micro emulsion was evaluated for *in vitro* release characteristics in comparison with the unsolubilised drug at pH 1.2. At 30 minutes dissolution run time, 27% of active drug was released from the micro emulsion while the unsolubilised drug released 2% active clarithromycin within the same run time (Sutar *et al.* 2011).

Rajinikanth *et al.* prepared a floating-bioadhesive microsphere formulation of clarithromycin. They used carbopol 934P as a mucoadhesive polymer and ethylcellulose

as a matrix polymer. The formulation was evaluated for gastric stability and drug release characteristics. It was found that the floating-bioadhesive microspheres of clarithromycin had improved the gastric stability (Rajinikanth *et al.* 2008).

Lakum *et al.* prepared clarithromycin in a bioadhesive gastroretentive dosage form by use of bioadhesive polymers. The formulation had improved stability in gastric acid, improved release profile and extended duration of drug release (Lakum *et al.* 2013).

Particle size reduction approaches increase the surface area to volume ratio with resultant dissolution rate enhancement of clarithromycin. Shahbaziniaz *et al.* prepared clarithromycin nanoparticles from a ternary ground mixture in presence of sodium lauryl sulphate and polyvinyl pyrrolidone as co-grinding water soluble compounds. The resultant formulation had 75 - 100% of clarithromycin dissolving in 60 minutes, while only 35% of unsolubilised clarithromycin dissolved within the same period (Shahbaziniaz *et al.* 2008).

1.10   Comparative Dissolution Studies

1.10.1 Introduction

Comparative dissolution testing is a valuable tool in drug development and characterisation. In addition to serving as routine quality control tests, comparative dissolution tests have been used to support waivers for bioequivalence requirements, for approval of generic drug products and accepting product sameness under Scale-up and Post Approval (SUPAC)-related changes (Anand *et al.* 2011; US FDA 1997).

1.10.2 Specifications and Experimental Conditions

The Centre for Drug Evaluation and Research (CDER) at the United States Food and Drug Administration (US FDA) describes three categories of dissolution test specifications for immediate release products. These are single point specifications, two-point specifications and dissolution profile comparison.

Single and two-point specifications are sufficient to characterise drug products containing high solubility-high permeability substances. However, this is not suitable for characterization of low solubility products because such products have inherent different dissolution profiles. Consequently, they may comply with the point estimates, thereby giving an erroneous impression of pharmaceutical equivalence in dissolution characteristics. Dissolution profile comparison is recommended for such products, as it is more precise and discriminative than point estimates (US FDA 1997; Sathe *et al.* 1996; Yuksel *et al.* 2000).

Comparative dissolution profile testing of drugs is carried out in at least three dissolution media in order to study their stability and release characteristics in the different physiological conditions that they may be subjected to *in vivo*. The recommended dissolution media are 0.1 M HCl or buffer solution of pH 1.2 as well as buffer solutions of pH 4.5 and 6.8. Water can be used as an additional medium (Aiache *et al.* 1997; Anand *et al.* 2011; EMEA 2010; US FDA 2003; Kuwana 2007; Shah 2001).

1.10.3 Methods for Comparison of Dissolution Profile Data

The methods for the comparison of *in vitro* dissolution profiles can be classified into three groups:

 i. Methods based on analysis of variance (ANOVA)

 ii. Model-dependent methods

 iii. Model-independent methods.

ANOVA-based methods use univariate and multivariate approaches to quantify differences in dissolution percentages at each time point and among different products. Model-dependent methods include the cubic root law (Hixson and Crowell) mathematical model, the Weibull distribution model and the logistics (Rowlings) model for sigmoidal dissolution curves (Yuksel *et al.* 2000).

A simple model independent method proposed by Moore and Flanner (1996) uses fit factors to compare dissolution profile data of a pair of products under similar testing conditions. These fit factors directly compare the difference between percent drug dissolved per unit time for a test and a reference product. These factors are denoted f_1 (difference factor) and f_2 (similarity factor) (US FDA 1997; Saranadasa and Krishnamoorthy 2005; Sathe *et al.* 1996; Yuksel *et al.* 2000). Comparison of the dissolution profiles of clarithromycin can be satisfactorily carried out using the model-independent approaches.

The difference factor (f_1) is a measurement of the percent difference between two dissolution curves under comparison at each time point. It is a measure of the relative error between the two curves and is given by the formula:

$$f_1 = \{[\Sigma_{t=1}^{n} |R_t - T_t|] / [\Sigma_{t=1}^{n} R_t]\} \times 100$$

where, n is the number of testing time points; R_t is the average dissolution value of the reference product units at time t and T_t is the average dissolution value of the test product units at time t. Similarity of two dissolution curves is indicated by f_1 values of 0 - 15% (US FDA 1997; Hasan *et al.* 2007; Yuksel *et al.* 2000).

The similarity factor (f_2) is a measurement of the similarity in the percent dissolution between two dissolution curves. It is inversely proportional to the average squared difference between the two profiles. It is a logarithmic reciprocal square root transformation of the sum of squared error and is given by the formula:

$$f_2 = 50 \times \log \{[1 + (1/n) \Sigma_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \times 100\}$$

where, n is the number of testing time points; R_t is the average dissolution value of the reference product units at time t and T_t is the average dissolution value of the test product units at time t (US FDA 1997; Hasan *et al.* 2007; Shah 2001; Yuksel *et al.* 2000).

The proviso for evaluation for similarity is availability of data for six (6) or twelve (12) units of each product, availability of three or more dissolution time points, same conditions of testing for reference and test products and same dissolution time points for both profiles. As a further recommendation, it is suggested that only one measurement be considered after 85% dissolution of both products. (US FDA 1997; Hasan *et al.* 2007; Ochekpe *et al.* 2006).

The similarity factor has been adopted by the US FDA and the European Medicines Agency (EMEA) for dissolution profile comparison. When two dissolution profiles are identical, $f_2 = 100\%$. An average dissolution difference of 10% at all measured time

points results in an f_2 value of 50%. For this reason, the public standard for similarity of two dissolution profiles has been set at 50 - 100% (EMEA 2010; US FDA 1997; Shah 2001).

1.10.4 Comparative Dissolution Studies and Generic Prescribing

The *in vitro* dissolution test is important in characterization of drug product performance. It is useful for quality control and in the prediction of *in vivo* performance of pharmaceutical products.

Comparative *in vitro* dissolution testing of generic drugs versus innovator products serves as a tool to determine pharmaceutical equivalence of the two products. Two products are considered pharmaceutically equivalent if they contain the same amounts of API in the same dosage forms that meet the same or comparable standards. Determination of pharmaceutical equivalence serves as a surrogate for *in vivo* bioequivalence tests that are expensive and not readily undertaken by generic drug manufacturers. The *in vitro* dissolution test is therefore a useful surrogate for assessment of bioequivalence. It plays an important role in comparison of therapeutic performances of pharmaceutical products containing the same API and has for this reason gained importance since the inception of generic equivalents of innovator drugs as a cost-cutting measure in healthcare (Anand *et al.* 2001; US FDA 1997; Mastan *et al.* 2011; Shah 2001; Yuksel *et al.* 2000).

Establishment of bioequivalence is essential to interchangeability of drug products. Whereas pharmaceutical equivalence does not necessarily imply bioequivalence, it is an important determinant in establishing interchangeability. Theoretically, any generic drug

that is bioequivalent to its innovator counterpart may be interchanged with it. It is expected that the generic formulations have an equivalent clinical effect and safety profile to the innovator formulation. In settings where bioequivalence studies are not viable, comparative dissolution testing can be used to determine which products can be used interchangeably (Mastan *et al.* 2011; Ruiz *et al.* 2012).

Credible comparisons of clinical outcomes can be done using data obtained from comparative dissolution studies of a generic and innovator product, or between different generic products. By determination of pharmaceutical equivalence, comparative dissolution testing guides drug product substitution in clinical practice with guaranteed optimal therapeutic outcomes.

1.10.5 Dissolution Testing Methods for Clarithromycin

Different dissolution testing methods for the determination of clarithromycin API in tablet formulations are available. The USP (2014) describes a dissolution method that employs the use of sodium acetate buffer (pH 5.0), 900 mL as the dissolution medium equilibrated at 37 ^{o}C, USP paddle apparatus rotated at 50 rpm and dissolution time 30 minutes. Quantitation of dissolved clarithromycin is by HPLC with UV detection at 210 nm and a C_{18} reversed phase column (15 cm x 4.6 mm ID) thermostated at 50 ^{o}C. The mobile phase is composed of methanol-0.067 M monobasic potassium phosphate (13:7) at pH 4.0. The flow rate is 1 mL/min. The recommended sample injection volume is 20 - 50 µL (USP 2014a).

The BP (2012) monograph for dissolution testing of clarithromycin tablets recommends use of 900 mL of a solution containing 1000 volumes of a 1.361% w/v solution of sodium acetate and 350 volumes of 0.1 M acetic acid at pH 5.0 equilibrated at 37 ^{o}C as the dissolution medium, paddle apparatus rotated at 50 rpm and dissolution time 45 minutes. Quantitation of dissolved clarithromycin is by HPLC with UV detection at 210 nm and a C_{18} reversed phase column (150 mm x 4.6 mm ID) thermostated at 50 ^{o}C. The mobile phase comprises 35 volumes of 0.067 M potassium dihydrogen orthophosphate and 65 volumes of methanol adjusted to pH 4.0. It recommends isocratic elution at a flow rate of 1.5 mL per minute and a sample injection volume of 50 µL (BP 2012a).

The Japanese Pharmacopoeia specifies a dissolution method performed at 50 rpm using the Paddle method and 900 mL of 0.05 mol/L disodium hydrogen phosphate-citric acid buffer solution, pH 6.0 as the dissolution medium. Dissolution rates in 30 minutes of a 50 mg tablet and a 200 mg tablet shall not be less than 80% and 75%, respectively. Dissolved clarithromycin is quantified by HPLC with UV detection at 210 nm and a C_{18} column (150 mm x 4 mm ID) thermostated at 50 ^{o}C. The mobile phase consists of a mixture of diluted 0.2 mol/L potassium dihydrogen phosphate (1 in 3) and acetonitrile (13:7). It recommends adjustment of the flow rate such that the retention time of clarithromycin is about 8 minutes. The injection volume is specified as 10 µL (JP 15th edn).

Abuga *et al*. (2001) developed and validated a stability indicating HPLC method for the separation of clarithromycin and its related substances. The isocratic method employs a XTerra$^{®}$ reversed phase C_{18}, 5 µm (250 mm x 4.6 mm ID) column thermostated at 56 ^{o}C, with UV detection set at 205 nm. The mobile phase consists of acetonitrile-0.2 M

potassium phosphate buffer pH 6.80-water (40:3.5:56.5). This method is suitable for comparative dissolution profile testing of clarithromycin products because, unlike the afore-mentioned pharmacopoeial methods, it is capable of both identification and quantitation of degradation products of clarithromycin which may arise under different degradative conditions. The related substances can be identified using relative retention times in absence of chemical reference substances. It can, therefore, be used for monitoring of the stability of clarithromycin products as a function of time in different dissolution media.

A HPLC method for quantification of related substances in clarithromycin powder for an oral suspension dosage form has been developed and validated by Srinivasu *et al*. (2012). The method employs a reversed phase Purospher Star C_{18}, 3 μm (150 mm x 4.6 mm ID) column thermostated at 50 ^{o}C. The mobile phase consists of acetonitrile-0.035 M potassium dihydrogen orthophosphate at pH 4.4 with gradient elution and UV detection at 210 nm. The method, being stability-indicating, is suitable for comparative dissolution profile testing of clarithromycin. Identification of related substances can be done using relative retention times.

1.10.6 Post Market Surveillance Studies on Clarithromycin Products

A study conducted on the quality of eleven (11) generic clarithromycin products manufactured in Slovenia and Israel by Nightingale (2000a) found that 10% of the generic products did not contain the amount of clarithromycin claimed in the label. It was also found that 18% of the products released less drug than did the innovator product in the standard dissolution assay at pH 5.0 (Nightingale 2000a). Later the same year

Nightingale (2000b) reported on the quality of forty clarithromycin products from 13 countries in Latin America and Asia. The study revealed that many generic products were not equivalent to the innovator product. Out of the forty generic products tested, eight (20%) failed the assay test within the BP specified limits of 95 - 105%. Six out of eighteen (33%) products from Latin America failed the same assay limits. At pH 5.0, 70% (28 out of 40) of the products released less drug than did the innovator product in 30 minutes run time. One product failed to meet the dissolution specifications. Out of the generic products tested, 60% (24 out of 40) exceeded the 3% limit for total impurities in bulk drug specified by Abbott Laboratories. The limit for 6,11 di-O-methyl erythromycin A set at 0.8% was exceeded by 70% of the generic products tested (Nightingale 2000b).

A survey on the quality of 65 clarithromycin products from 18 countries in Latin America, Africa, Asia and Pacific region conducted in 2005 revealed that overall, 9% (6 of 65) of all generic products tested failed the assay test within limits of 95 - 105% label claim. It was also revealed that 17% (1 of 6) of products from Latin America, 8% (3 of 38) of products from the Asia, Africa, Pacific region, and 10% (2 of 21) of products from Europe did not contain the amount of clarithromycin drug content claimed in the label. At pH 5.0, 34% of the products released less drug than did the innovator product in 30 minutes. One generic failed to meet the dissolution specification with 68% of drug dissolved in 30 minutes. It was also found that 12 out of 65 (19%) of all the generic products tested exceeded the specified limit for total impurities in bulk drug (3%). This may point to unsatisfactory quality assurance measures in the manufacture and storage of clarithromycin bulk drug substance leading to degradation, or inadequate stability studies and quality control checks. The set limit for 6,11 di-O-methyl erythromycin A was exceeded by 30% (20 of 65) of all the products tested.

In all the three studies, it was concluded that the generic products are not equivalent to the innovator product. This raised concerns that clinical trial results achieved with the branded clarithromycin product (Abbott Laboratories Ltd.) should not be extrapolated to generic products.

1.11 Study Justification

The quality of pharmaceutical products is important in determining therapeutic outcomes. Compromise in quality leads to sub-optimal therapeutic outcomes and treatment failures with associated increase in morbidity, hospitalization costs, mortality and microbial resistance in the case with antibiotics. Although quality control tests are key tools in assessing the quality of drugs, pharmaceutical quality is an element of product design and cannot be imparted into a product through the compendial tests. Quality by design is therefore an essential part of the contemporary approach to pharmaceutical quality and has been embraced by the US FDA and other regulatory agencies (Issack 2001; Lionberger *et al.* 2008; McCurdy 2011; WHO 1999; WHO 2007).

The existence of poor quality drugs in circulation in many third world countries has been reported (Birhanu *et al.* 2013). The Drug Analysis and Research Unit (DARU) of the University of Nairobi, Kenya reported failure rates of 46% and 6.1% during the periods 1983 - 1986 and 2001 - 2005, respectively (Kibwage *et al.* 1992; Thoithi *et al.* 2008).

The Kenyan National Quality Control Laboratory analysis reports during the period 2002 - 2005 on the quality of antimalarials indicate failure rates of up to 46% (Chepkwony *et al.* 2007). A study carried out on anti-infectives sampled from Nigeria and Thailand

reported that 37% of the products were substandard with respect to pharmacopoeial specifications (Shakoor *et al.* 1997). Studies conducted on the dissolution profiles of registered sulphadoxine-pyrimethamine tablets in the Nigerian market revealed high variations in their release profiles with 41.7% of products failing to meet dissolution specifications despite complying with compedial limits for percentage label claim (Ochekpe *et al.* 2012).

Clarithromycin is listed in the Kenya Essential Medicines List where it is classified for use at hospital level where specialist diagnostic and medical care is available (KEML 2010). The Kenyatta National Hospital formulary also recognises clarithromycin as an essential drug (KNHF 2013). It is categorised as a level 2 prescription drug, suitable to be prescribed by medical officers. In addition, the WHO lists clarithromycin in its model list of essential drugs as an antimicrobial for use in combination regimens for eradication of *H. pylori* in adults. It also is an important component of drug combinations used in the treatment of MAC in HIV-AIDS patients (Kanizaj *et al.* 2009; Shahbaziniaz *et al.* 2013; Park *et al.* 2009; WHO 2013).

Clarithromycin tablets are classified as BCS class II and the suspension is in BCS class IV. These products have low aqueous and pH-dependent solubility and dissolution rate-limited absorption (Lakum *et al.* 2013; Shahbaziniaz *et al.* 2013; Zhang *et al.* 2005). In addition, previous studies have reported that clarithromycin undergoes rapid degradation at low pH values which exist in the stomach (Erah *et al.* 1997; Fujiki *et al.* 2011; Mordi *et al.* 2000) and may significantly alter the bioavailability of the API.

Use of generic drug products has increased in the recent past as a cost-saving measure in healthcare provision. Since generic drugs are intended to be interchangeable with

innovator products, they must satisfy the same standards of quality, efficacy and safety as those applicable to the innovator products and reasonable assurance must be provided that they are clinically interchangeable with nominally equivalent market products (Dunne *et al.* 2013; Meadows 2005; WHO 1996; WHO Generic drugs 2013).

Currently there are about forty nine (49) registered brands of clarithromycin oral formulations in the Kenyan market. Of these, twenty one (21) are 500 mg tablets, one (1) is a 500 mg capsule, nineteen (19) are 250 mg tablets, and eight (8) are clarithromycin suspensions (PPB 2012).

There are no bioequivalence centres in Kenya. Additionally, no published reports of bioequivalence studies on generic clarithromycin oral formulations in the Kenyan market exist and no comparative dissolution profile studies conducted to determine the stability and drug release characteristics of the generics have been reported. Such studies are necessary to demonstrate pharmaceutical equivalence of the generic products with respect to the innovator product Klacid® from Abbott Laboratories Ltd.

Compendial methods specify dissolution testing of clarithromycin formulations at pH > 4.5 (USP 2014a; BP 2012a; JP 15th edn). Since the stability of clarithromycin has been confirmed at such pH values (Erah *et al.* 1997; Mordi *et al.* 2000), the tests may provide no data to evaluate the stability of the formulations in gastric pH. The compendial dissolution tests do not provide sufficient proof of efficacy for generic drugs. Simulation of gastrointestinal conditions is essential to adequately predict the stability and *in vivo* behaviour of the formulations. The US FDA guidelines recommend that comparative studies be carried out in dissolution media of pH 1.0 to 6.8 (US FDA 1997).

Comparative dissolution testing for clarithromycin in the present work was carried out at pH 1.2, 4.5 and 6.8.

Comparison of similarity of dissolution profiles using the similarity factor was preferred. Studies indicate that the similarity factor is more sensitive for dissolution profile comparison than the difference factor. For this reason, the similarity factor has been adopted by the US FDA and is more widely used for dissolution profile studies (Anand *et al.* 2011; Flanner *et al.* 2001; Shah 2001; Yuksel *et al.* 2000).

The present study serves as a baseline survey on comparative dissolution profiling of clarithromycin oral formulations in the Kenyan market to provide data on stability and drug release characteristics of the generic products. The results obtained in this study will serve to inform regulatory authorities on the quality of products in the market as it relates to the pharmaceutical equivalence of clarithromycin products. They will also serve to guide prescribers on product quality with respect to stability in gastric pH after oral dosing. Thus, the study can serve as a basis for future studies on the quality of many other commercially available generic drug products.

1.12   Study Objectives

1.12.1 General Objective

To conduct comparative *in vitro* dissolution studies of oral clarithromycin products in Nairobi County.

1.12.2 Specific Objectives

The specific objectives are:

1. To conduct sampling of clarithromycin oral products available in pharmacy outlets within Nairobi County

2. To perform identification and assay tests on the clarithromycin products

3. To conduct comparative dissolution testing of the innovator and generic oral clarithromycin products

4. To determine pharmaceutical equivalence of the generic preparations to the innovator products.

CHAPTER TWO

EXPERIMENTAL

2.1   Introduction

The study on comparative dissolution profiles of clarithromycin products was carried out using a published stability-indicating HPLC method for separation of clarithromycin and related substances (Abuga *et al.* 2001). The method was verified and the chromatographic conditions optimized before application for sample analysis.

2.2   Reagents, Chemicals and Solvents

Analytical grade hydrochloric acid (Loba Chemie PVT Ltd., Mumbai, India), glacial acetic acid (Sigma-Aldrich Co., Steinheim, Germany), potassium dihydrogen orthophosphate (Merck PTY Ltd., Gauteng, South Africa) and sodium acetate trihydrate (RFCL Ltd., Faridabad, India) were used during the study. Dipotassium hydrogen phosphate and methanol HPLC-grade were from RFCL Ltd. (New Dheli, India) while sodium hydroxide pellets was a product of RFCL Ltd. (Gujarat, India).

Acetonitrile HPLC-grade was from Avantor Ltd. (Haryana, India). All aqueous solutions were prepared using purified water prepared using an Arium water system (Sartorius AG, Göttingen, Germany) consisting of a reverse osmosis module and an ultrafiltation module with a UV irradiation component.

2.3   Instrumentation

2.3.1   Dissolution Test Apparatus

A Labindia DS 8000 dissolution tester (Labindia Analytical Instruments Pvt. Ltd., Maharashtra, India) was used for dissolution experiments. It incorporated a clear acrylic water bath, a stirrer hood with paddle shafts, an automatic sampling unit and a control unit supported by a microcontroller software with a non-volatile memory for 15 methods. The water bath incorporated an immersion circulator with an in-built thermostat for temperature control, an external temperature sensor, a water level sensor and a lid with support for eight dissolution bowls. The stirrer hood was equipped with 8 paddle shafts fitted with USP apparatus 2 and a tablet dispenser with 8 conical shaped dissolution bowl lids. The automatic sampling unit consisted of 10 μ in-line filters, a bi-directional 12-channel peristaltic pump with tygon tubings, a microprocessor controlled sample collector and a sample tray capable of collecting 10 x 6 sets of samples. Polycarbonate dissolution vessels with a hemispherical bottom and a capacity of 1000 ml were used for the study.

2.3.2   Liquid Chromatographic System

A Shimadzu Prominence high performance liquid chromatographic system (Shimadzu Corp., Kyoto, Japan) was used for chromatographic experiments. It consisted of a CBM-20A Prominence communications bus module, a SPD-M20A Prominence UV/Visible photo diode array detector equipped with a deuterium lamp for ultraviolet applications and a tungsten lamp for the visible region, a LC-20AD Prominence liquid chromatography solvent delivery system with a dual-plunger tandem-flow solvent

delivery module and a SIL-20AC Prominence auto sampler. The temperature was controlled using a CTO-20AC Prominence column oven with a block heating type thermostatic chamber and a preheater system. The LC system was controlled by LCSolutions Software Ver 1.22, SP1.

All mobile phase preparations were degassed using a compressed helium degasser (Air Products and Chemicals Inc., Dubai, United Arab Emirates) or a MRC DC 200H ultrasonic bath (MRC Ltd., Holon, Israel). A Waters XTerra RP18, 5 μm, 250 x 4.6 mm ID chromatography column (Waters Corp., Wexford, Ireland) was used as the stationary phase.

2.3.3 pH Meter

All pH measurements were carried out using an ADWA 1000 bench pH/mV and temperature meter (Adwa Hungary Kft., Szeged, Hungary). It consisted of an A1131B refillable pH electrode with a BNC connector, an A7662 stainless steel temperature probe, an electrode holder and a 12 V direct current power adapter.

2.3.4 Infra-Red Spectrophotometer

The Infra-red spectrum of clarithromycin working standard was determined using a Shimadzu IRPrestige 21 Fourier Transform Infra-Red (FTIR) spectrophotometer (Shimadzu Corp., Kyoto, Japan) supported by IRSolution Software Ver. 1.3. Sample discs for recording the spectrum were prepared using spectroscopic grade potassium

bromide (E. Merck, Darmstadt, Germany) and a manually operated hydraulic pellet press (Perking Elmer GmbH, Uberlingen, Germany).

2.3.5   Ultra-Violet Spectrophotometer

The ultra-violet absorption spectrum for clarithromycin working standard was recorded using a double beam T90+ UV/VIS spectrometer controlled via a computer using UVWIN spectrophotometer software version 5.2.0. (PG Instruments, Leicestershire, United Kingdom) over a 10 mm path length using quartz cuvettes.

2.4   Samples and Chemical Reference Substances

Clarithromycin tablets, capsules and suspensions from different manufacturers were used in the study. The samples were obtained from different private retail outlets within Nairobi County. Clarithromycin USP primary chemical reference standard (98.8% w/w) and clarithromycin working standard (96.7% w/w) were provided by NQCL (Hospital Road, Nairobi, Kenya).

2.5   Sampling

The study population was defined as all clarithromycin suspensions 125 mg/5 mL, clarithromycin 500 mg tablets and capsules brands in circulation within Nairobi County. The sampling time frame was set as January to March 2014 to allow comprehensive sampling of products across the County. The list of registered clarithromycin products

was obtained from the PPB website. Preliminary spot checks were conducted on selected outlets in different regions in Nairobi County to determine the stocking patterns of clarithromycin oral products whereof a list of available brands and locations of the stockists was prepared.

These spot checks revealed uneven distribution of clarithromycin products across the County with stocking patterns mimicking the economic capacity of the local population. Pharmacy outlets in some regions did not stock any clarithromycin products while in other areas one brand was consistently found in the outlets, probably due to product differentiation in the market leading to popularity of a few brands over time. For this reason, disproportional sampling was applied during the purchase of samples. The samples purchased were checked off the sampling list as sampling progressed.

A total of sixteen (16) samples were obtained from Nairobi County against a study population of twenty four (24) registered products including the comparator products. Of these, eleven (11) were tablet formulations (500 mg), one (1) was a capsule formulation (500 mg) and four (4) were dry granules for suspensions (125 mg/5mL). These were obtained from eleven (11) retail pharmacy outlets across the County (Table 2.1) translating to a stoking rate of 67% based on the study population of 24 products. Two (2) samples were obtained from Bordon, Hampshire, England for comparison purposes. The sample size for tablets and capsules was 60 units whereas that of suspensions was 500 mL in various commercial packages. The samples were coded as C1 to C18 and their batch numbers, manufacture and expiry dates recorded (Table 2.2). The innovator comparator products Klacid® tablets 500 mg and Klacid® suspension 50 mL (Abbott Laboratories S.A. Pty. Ltd., Johannesburg, South Africa) were coded C12 and C16

respectively. The samples were stored at room temperature in a lockable cabinet until use.

Table 2.1: Sampling Patterns in Pharmacies in Nairobi County

Region	Number of pharmacy outlets	Number of samples
CBD and Racecourse	4	7
Kibra	1	1
Kamukunji	1	1
Dagoretti	1	3
Roysambu and Kahawa West	2	1
Embakasi	2	3

Table 2.2: Sample Particulars of Clarithromycin Products

Sample Code	Dosage Form	Batch Number	Manufacture Date	Expiry Date	Registration Status
C1	Tablet	16B12006	Nov 2012	Oct 2015	R
C2	Tablet	P06821	Sept 2012	Aug 2015	R
C3	Tablet	21200	Oct 2012	Sept 2014	R
C4	Capsule	H2008/20203/1 85/121001A	Oct 2012	Oct 2015	R
C5	Tablet	TE-3893	Mar 2012	Feb 2015	R
C6	Tablet	320833	Aug 2013	Jul 2016	R
C7	Tablet	CATP-07	Oct 2012	Sept 2015	R
C8	Tablet	CWNH0034	Jul 2013	Jun 2016	R
C9	Tablet	098F02	Apr 2013	Apr 2016	R
C10	Tablet	B004	Nov 2013	Oct 2016	R
C11	Tablet	Y22952	Oct 2012	Sept 2015	R
C12	Tablet	26247TF04	Feb 2013	Jan 2015	R
C13	Suspension	EM447	Jan 2013	Dec 2015	R
C14	Suspension	30928	Aug 2013	Jul 2016	R
C15	Suspension	944D02	Aug 2013	Aug 2015	R
C16	Suspension	27320TF02	Mar 2013	Feb 2015	R
C17	Tablet	6041916	-	Oct 2016	NR[*]
C18	Suspension	6040583	-	Sept 2016	NR[*]

- = Date of manufacture not available.

R = Registered by the PPB.

NR[*] = Not registered by the PPB. These are innovator products in the UK market; therefore not in circulation in the Kenyan market.

Out of the sixteen (16) products obtained from pharmacy outlets within Nairobi County, three (C2, C6 and C7) were not listed as registered on the PPB website. Upon enquiry from the PPB, it was confirmed that the three products were registered. Products C6 and C7 being registered in 2013 while C2 was registered in 2012.

2.6 Preparation of Mobile Phases

The mobile phase consisted of acetonitrile-0.2 M potassium phosphate buffer, pH 6.80-water (40:3.5:56.5, v/v/v). Phosphate buffer was prepared by mixing equimolar solutions (0.2 M) of KH_2PO_4 and K_2HPO_4 under magnetic stirring to a pH of 6.80. An amount of buffer corresponding to 3.5% v/v was mixed with 56.5% v/v water in a measuring cylinder and added to 40% v/v acetonitrile, separately measured. The mobile phase was mixed and degassed by helium sparging for 3 minutes or sonication for 30 - 60 minutes in absence of the helium degassing system.

2.7 Analysis of Clarithromycin Working Standard

2.7.1 Ultra Violet Spectroscopy

The UV absorption spectrum for clarithromycin working standard was scanned over a range of 190 - 230 nm. For this purpose, a solution of 1 mg/mL clarithromycin was prepared in acetonitrile. The spectrum obtained (Appendix 1) was consistent with those reported in literature (USP MC 2012). Clarithromycin lacks a chromophore in the

lactone ring hence significant UV absorbance is obtained only at wavelengths below 210 nm.

2.7.2   Infra Red Spectroscopy

The infrared absorption spectra were recorded over the range 4000 - 400 cm^{-1}. A 1 mm thick potassium bromide disc containing about 1% w/w of clarithromycin working standard was used. The spectrum obtained (Appendix 2) was compared with that of clarithromycin primary standard similarly prepared (Appendix 3) and with values published in literature (BP 2012c; USP MC 2012). The principal absorption bands were in concordance with literature values and were comparable with those of the primary standard.

2.7.3   Potency of Working Standard

The potency of clarithromycin working standard was determined by HPLC as prescribed in the USP (USP 2014b). Solutions of 0.7 mg/mL were prepared for both clarithromycin USP primary standard and clarithromycin working standard in acetonitrile-water (2:3). They were subjected to assay using an injection volume of 50 μL and detection wavelength 205 nm. The integrated peak areas were used to calculate the % potency using MS Excel® spreadsheets (Microsoft Corporation, Redmond, WA, USA. The potency of the working standard was found to be 96.7% w/w.

2.8   Method Verification and Optimization

Preliminary studies were conducted to determine the optimum chromatographic conditions for sample assay and dissolution. The factors investigated were: detection wavelength, standard and sample concentrations, injection volumes, mobile phase flow rate and optimum quenching conditions for dissolution samples at pH 1.2.

2.8.1   Detection Wavelength

The method of analysis prescribed a detection wavelength of 205 nm which is prone to matrix interferences. Studies were carried out at 205 and 210 nm to determine the wavelength of higher sensitivity and less background interference. Equal concentrations of clarithromycin working standards were prepared and subjected to assay at detection wavelengths of 205 and 210 nm. The resultant peak areas and column efficiency parameters were compared to determine the suitable detection wavelength (Table 2.3). At similar concentrations, the peak areas at 205 nm were 67% higher than those obtained at 210 nm. Due to higher sensitivity, 205 nm was chosen as the optimal detection wavelength for both assay and dissolution.

2.8.2   Standard Concentrations and Injection Volumes

A standard stock solution of concentration of 1 mg/mL was prepared and assayed at injection volumes of 10, 20 and 50 μL to investigate the effect of increased injection volumes on peak area and parameters. It was observed that higher injection volumes produced proportionately higher peak areas (Table 2.3).

Table 2.3: Method Optimization Variables

Chromatographic Conditions	Variations		Results	
Detection wavelength	**Std Conc. (mg/mL)**	**λ (nm)**	**Peak Area**	**N**
	0.125	205	802555	9211
	0.125	210	477840	9278
Injection volume	**Std Conc. (mg/mL)**	**Volume (μL)**	**Peak Area and Shape**	**N**
	1	10	878315	12418
	1	20	1160186	10027
	1	50	2997722	9862
	0.125	50	231467	9496
	0.125	100	477840	9278
	0.25	50	483495	9424
Mobile phase flow rate	**(mL/min)**	**Baseline**	**Run time (Min)**	**Column Pressure (Bars)**
	1	Stable	22	100
	1.5	Stable	12	130 - 135
	2.0	Stable	10	> 180

Column: XTerra® C_{18} 5μ (250 × 4.6 mm ID). Column temperature: 56 °C. Mobile phase: acetonitrile-0.2 M phosphate buffer, pH 6.80-water (40:3.5:56.5%, v/v/v).

The stock solution was diluted to 0.25 and 0.125 mg/mL and injected at volumes of 50 and 100 μL. The resultant peak areas, peak shapes and number of theoretical plates (N) were compared to study the effect of increasing the sample concentration versus that of increasing the sample volume injected (Table 2.3). It was observed that increasing

sample concentration resulted in higher N values and was therefore preferred to increasing injection volume.

2.8.3 Degassing of Mobile Phases and Flow Rate

Mobile phase preparations were degassed by helium sparging and sonication while monitoring the stability of baseline. It was observed that degassing by helium sparging for 3 minutes produced more stable baseline compared to sonication for 30 minutes hence more effective.

Chromatographic runs were carried out at flow rates of 1, 1.5 and 2 mL/min to compare baseline stability, column back pressures and chromatographic run times (Table 2.3). The optimal flow rate established was 1.5 mL/min.

2.8.4 Quenching Conditions for Dissolution Samples

Instability of clarithromycin in acidic media has been reported in literature (Abuga *et al.* 2001; Erah *et al.* 1997; Srinivasu *et al.* 2012). For this reason, dissolution at pH 1.2 would lead to degradation thus yielding inconsistent results. Quenching by an acid-base reaction in each vial immediately after sampling was therefore necessary. The optimal quenching volume was investigated by mixing 10 mL of dissolution sample with 0.2 M NaOH and determining the pH of the mixture (Table 2.4). The end point was taken as sudden change in pH from 1.2 to 10.4. The optimal volume of NaOH required to quench the acidic reaction at pH 1.2 was found to be 3 mL.

Table 2.4: Volumes of 0.2 M NaOH for Quenching at pH 1.2

Sample Volume (mL)	Average Volume of 0.2 M NaOH (mL) (n = 3)	Average pH Value (n = 3)
	0	1.2
	1.0	2.2
	1.5	2.4
10	2.0	2.9
	2.5	3.6
	2.8	3.8
	2.9	3.9
	3.0	10.4

n = number of replicate trials.

2.9   Standard Solutions

For all assays, the standard solution (1 mg/mL) was prepared by dissolving 25 mg clarithromycin working standard in 6 mL acetonitrile and making up to 25 mL with mobile phase. The same procedure was used to prepare working standards for tablet dissolution (0.56 mg/mL) corresponding to the sample concentrations. The dissolution medium was used in place of mobile phase, except in the case of dissolution at pH 1.2 where acetonitrile-water (1:4) was used as diluent in order to prevent acidic degradation. Standard solutions (0.278 mg/mL) for dissolution of suspensions were prepared corresponding to 250 mg samples in dissolution volumes of 900 mL.

2.10   System Suitability Test

Clarithromycin working standard was subjected to assay by HPLC in pentaplicate at the start of chromatographic runs for each sample using identical run times, mobile phase flow rates, standard concentrations and injection volumes to those of the sample. This was carried out in all cases for both assay and dissolution. The peak areas, peak asymmetry, retention times and number of theoretical plates were recorded. The system suitability (SST) data was computed using MS Excel® spreadsheets (Microsoft Corporation, Redmond, WA, USA) and used to calculate the mean and relative standard deviation in order to ascertain that the requirements for precision were met as prescribed in the USP (USP 2014d).

2.11   Analysis of Samples

Samples were analyzed under the optimum chromatographic conditions of: a mobile phase consisting of acetonitrile-0.2 M phosphate buffer, pH 6.80-water (40:3.5:56.5, v/v/v) delivered at a flow rate of 1.5 mL/min through an XTerra reversed phase C_{18}, 5 µm (250 mm x 4.6 mm ID) column maintained at 56 ^{o}C and a detection wavelength of 205 nm.

2.11.1 Assay Test

Uniformity of weight for 20 tablets was determined in accordance to BP specifications (BP 2012b). All the 20 tablets were crushed to fine powder and powder equivalent to 200 mg clarithromycin weighed into a 100 mL volumetric flask. It was dissolved in

acetonitrile with the aid of mechanical shaking for 45 minutes. Aliquots of 25 mL were pipetted into 50 mL volumetric flasks, made to volume with mobile phase and filtered through 0.45 μ filters into 1.5 mL HPLC vials and subjected to chromatographic analysis.

Clarithromycin granules for suspension were reconstituted using water as per the label instructions. The contents of three bottles were mixed in a beaker for measurement of relative density and pH. Clarithromycin was extracted using the procedure prescribed in the USP (USP 2014c). For this purpose, weights equivalent to 125 mg clarithromycin were transferred into 50 mL volumetric flasks, mixed with 20 mL of 0.067 M dibasic potassium phosphate and extracted for 30 minutes by mechanical shaking. They were made to volume with methanol and extracted for a further 30 minutes. Dilutions of these solutions were made by pipetting 20 mL portions into 50 mL volumetric flasks and making to volume with mobile phase. The solutions were filtered through 0.45 μ filters into 1.5 mL HPLC vials prior to HPLC analysis.

Assay was performed by injection of 50 μL of 1 mg/mL standard and sample preparations in triplicate. The run times encountered were 12 - 30 minutes. Quantitation of clarithromycin API was carried out by comparison of peak areas of standard and assay preparations computed using MS Excel spreadsheets.

2.11.2 Dissolution Test

2.11.2.1 Sample Preparation

Six (6) tablets of the same batch were individually weighed into a numbered tablet dispenser and their masses recorded before dissolution. Suspensions were reconstituted as per the label instructions whereof the contents of three bottles were transferred to separate 100 mL beakers. Replicate samples of 10 mL were pipetted from each beaker under stirring conditions and transferred into graduated sample holders. The samples were transferred into the dissolution vessels and the weights determined by difference.

2.11.2.2 Dissolution of Samples

Dissolution runs were carried out using USP apparatus 2, 900 mL of dissolution media, bath temperature of 37.5 ^{o}C, bowl temperature of 37.0 ^{o}C, stirrer depth of 25 mm, stirring rate of 50 rpm, operating mode auto sampling with sampling volume 10 mL and equivalent volume replenishing after every sampling to maintain same sink conditions. The dissolution media used were 0.1 M HCl, pH 1.2, 0.1 M acetate buffer, pH 4.5 and 0.2 M phosphate buffer, pH 6.8. It was established through preliminary runs that dissolution for tablets plateaued at 60 minutes, while that of suspensions was slow and thus plateaued at about 90 minutes. The dissolution run times were therefore selected as 60 and 90 minutes for tablets and suspensions respectively. The sampling time points for tablets were 0, 5, 10, 15, 30, 45 and 60 minutes while those of suspensions were 0, 10, 20, 30, 45, 60 and 90 minutes.

Dissolution media were prepared as per USP specifications (USP 2014e). Phosphate buffer, pH 6.8 was prepared by combining portions of 0.2 M solutions of KH_2PO_4 and NaOH under magnetic stirring to the desired pH. Acetate buffer, pH 4.5 was prepared using glacial acetic acid and sodium acetate trihydrate solutions in three steps. In the first step, 0.1 M acetic acid solution was prepared by diluting 5.7 mL of 99.8 % glacial acetic acid to 1000 mL with water. The second step entailed dissolving $CH_3COONa.3H_2O$ (13.61 g) in water and making it to 1000 mL to prepare a 0.1 M solution. The acetic acid and sodium acetate trihydrate solutions were mixed in the ratio 8:6 to obtain a pH of 4.5.

The 0.1 M HCl medium was prepared by diluting 8.5 mL of concentrated HCl with water and adjusting the pH to 1.2 using NaOH solution. Sampling vials at this pH were prefilled with 3 mL of 0.2 M NaOH for quenching the acidic degradation reaction. Suspension samples at pH 4.5 and 6.8 were filtered using 0.45 μ filters before assay by HPLC. The injection volume for all dissolution samples was 100 μL.

2.12   Dissolution Profiles

MS Excel® (Microsoft Corporation, Redmond, WA, USA) program was used for data analysis. At pH 4.5 and 6.8, tabulations of average percent drug dissolved at every time point were made for each product and the data used to construct plots of percent drug dissolved as a function of time. A comparative tabulation of dissolution percentages for all products was constructed and used to plot comparative dissolution profiles for the products on the same chart.

Dissolution at pH 1.2 occurred with concurrent degradation to decladinosyl clarithromycin. For this reason, the dissolution data computed for each product comprised three components: percentage API dissolved, percentage decladinosyl clarithromycin produced and total clarithromycins released. In computing the total clarithromycins, the areas of decladinosyl clarithromycin were corrected using normalisation factors reported in literature (Abuga 2000). The respective curves were plotted on the same chart for comparison purposes. Comparative tables and plots for all products based on total clarithromycins were also constructed.

2.13 Similarity Factors (f_2)

Comparison of dissolution profiles was carried out by calculation of the similarity factor. Two sets of f_2 calculations were made at pH 1.2 based on clarithromycin API and total clarithromycins. At all the three pH values, f_2 values were computed based on 6 dissolution time points excluding time zero. A comparative tabulation of f_2 values for all samples at all pH values was also made to determine pharmaceutical equivalence to the innovator comparator product.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1   Introduction

The clarithromycin samples were subjected to assay and dissolution profile analysis under the optimum chromatographic conditions. The purpose of assay was to assess the samples for compliance with pharmacopoeial limits for content.

3.2   Assay of Samples

All tablet and capsule samples complied with the specifications for uniformity of weight while all the suspensions tested complied with the USP limits for pH (4.0 - 5.4) when reconstituted as per the label instructions. The relative density and pH results are shown in Table 3.1.

Table 3.1: Relative Density and pH for Clarithromycin Suspensions

Sample Code	Mean Relative Density (n = 3)	Mean pH (n = 3)
C13	1.295	4.75
C14	1.294	4.76
C15	1.348	4.47
C16	1.308	5.02
C18	1.312	5.05

n = number of pH and relative density measurements.

The assay results obtained are shown in Table 3.2. Samples C1 - C12 and C17 were tablets while C13 - C16 and C18 were suspensions. The USP limits for clarithromycin content are 90.0% - 110.0% and 90.0 % - 115.0% of the labelled amount for tablets and suspensions respectively.

All tablet samples complied with the USP assay limits for clarithromycin. The API assay for all generic products (C1 - C11) was 98.4 - 102.1%. The assay for the innovator comparator product, C12, was 105.9% while it was 103.5% for C17, the innovator product in the UK market. These assay values were consistent for the two products and higher than those obtained for generics, and could possibly be due to overages.

The five (5) suspension products tested complied with the USP assay limits for clarithromycin. The assay ranged between 99.5% and 110.0% with the innovator products C16 and C18 having the highest values (Table 3.2). These were notably higher than for tablets and could be overages.

Table 3.2: Assay Results for Clarithromycin Samples

Sample Code	Tablets / Capsules													Suspensions				
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C17	C13	C14	C15	C16	C18
Average% Content **n = 9** **[RSD]**	100.9 [0.87]	100.2 [1.40]	100.1 [0.56]	102.1 [0.33]	99.2 [0.39]	99.3 [1.61]	98.7 [0.38]	101.5 [1.44]	102.0 [1.13]	98.8 [0.53]	98.4 [0.39]	105.9 [0.97]	103.5 [0.27]	107.8 [1.16]	108.8 [0.88]	99.5 [1.31]	109.6 [1.85]	110.1 [0.84]

n = number of replicate injections; RSD = relative standard deviation.

Chromatographic Conditions:

Column: XTerra® C$_{18}$ 5µ (250 × 4.6 mm ID). Column temperature: 56 °C. Mobile phase: acetonitrile-0.2 M phosphate buffer, pH 6.80-water (40:3.5:56.5%, v/v/v). Flow rate: 1.5 mL/min. Detection: 205 nm. Diluent: acetonitrile-water (1:3). Concentration: 1 mg/mL. Injection volume: 50 µL. C12 - Reference sample for tablets. C16 - Reference sample for suspensions.

3.3 Dissolution of Samples

The samples were subjected to dissolution profile tests at pH 1.2, 4.5 and 6.8 as per the US FDA guidelines (US FDA 1997).

3.3.1 pH 1.2

Clarithromycin is unstable in acidic media. It degrades to form decladinosyl clarithromycin and 9,12-hemiketal. Dissolution at pH 1.2 is thus expected to proceed with concomitant degradation.

3.3.1.1 Degradation of Clarithromycin in Acidic Medium

Degradation of clarithromycin in acidic medium was studied by assay of clarithromycin working standard dissolved in aqueous solutions at pH 1.4 and 1.2 over a suitable period. The samples were incubated at 37 ^{o}C to mimic the body temperature.

pH 1.4

Degradation studies at pH 1.4 were done to confirm stability data reported by Abuga (2000). For this purpose, 10.0 mg of clarithromycin working standard was dissolved in 4.0 mL of acetonitrile before adding 1.0 ml of water to make a 2 mg/mL solution. This solution was diluted to 10 mL using 5 mL of 0.2 M H_3PO_4 solution. The concentration of the resulting solution was 0.1 M H_3PO_4 containing 1 mg/mL clarithromycin. Vials filled with this solution were kept in an oven maintained at 37°C and chromatographed over a

period of nineteen (19) hours. The peak areas were used to determine the degradation kinetics of clarithromycin at this pH.

pH 1.2

Clarithromycin working standard was subjected to degradation at pH 1.2 to mimic the actual sample dissolution conditions in acidic medium. The degradation study was carried out by incubating a 1 mg/mL solution of clarithromycin in 0.1 M HCl (pH 1.2) at 37 $^{\circ}$C and chromatographic analysis over a period of six (6) hours. The solution was prepared by dissolving 25.0 mg of clarithromycin working standard in 10.0 mL of acetonitrile before adding 2.5 ml of water to make a 2 mg/mL solution. This was made to 25 mL using 12.5 mL of 0.2 M HCl solution to yield 0.1 M HCl solution containing 1 mg/mL clarithromycin. The generated peak areas were plotted as a function of time to determine the degradation kinetics.

At both pH values, the related substances arising due to degradation were identified using relative retention time (RRT) values computed from the experimental data. The RRT values were found to be consistent with those reported in literature. During quantitation, the peak areas of the related substances were normalized by dividing their values with their respective response factors reported by Abuga (2000).

Three peaks were observed in both degradation conditions as shown in Tables 3.3 and 3.4. These were identified as clarithromycin API, decladinosyl clarithromycin and 9,12-hemiketal (Figure 3.1).
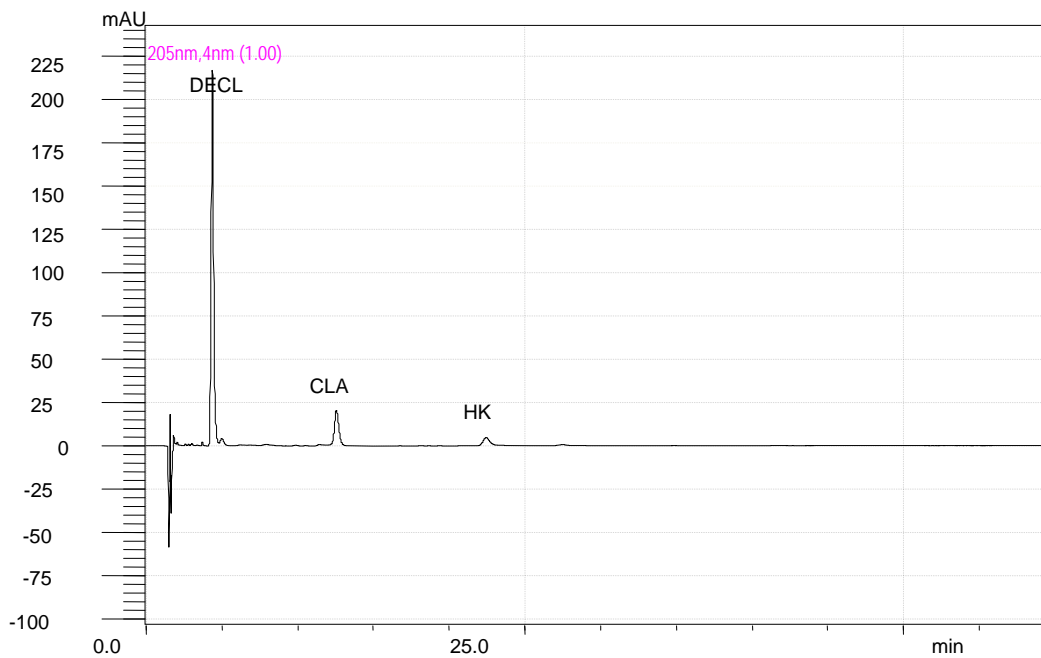
Fig. 3.1: Typical assay chromatogram for clarithromycin working standard in 0.1 M HCl, pH 1.2 at 110 minutes

Column: XTerra® C$_{18}$ 5μ (250 × 4.6 mm ID). Column temperature: 56 °C. Mobile phase: acetonitrile-0.2 M phosphate buffer, pH 6.80-water (40:3.5:56.5%, v/v/v). Flow rate: 1.5 mL/min. Detection: 205 nm. Concentration: 1 mg/mL. Injection volume: 100 μL. CLA - Clarithromycin. DECL - Decladinosyl clarithromycin. HK - 9,12-hemiketal.

Table 3.3: Acid Degradation of Clarithromycin Standard in 0.1 M H$_3$PO$_4$, pH 1.4

Time (Min)	Decladinosyl clarithromycin			Clarithromycin API			9,12-hemiketal		
	RT (Min)	Peak Area x 10^{-4}	RRT	RT (Min)	Peak Area x 10^{-4}	RRT	RT (Min)	Peak Area x 10^{-4}	RRT
60.5	4.39	118.638	0.28	12.61	268.3567	1.00	ND	ND	ND
121.0	4.38	179.1708	0.28	12.60	216.5863	1.00	22.60	1.0745	1.88
181.5	4.38	216.5068	0.28	12.57	174.2583	1.00	22.56	2.5463	1.88
242.0	4.38	272.2019	0.28	12.58	140.6399	1.00	22.56	3.8546	1.88
302.5	4.37	189.5031	0.28	12.56	103.7753	1.00	22.51	5.5455	1.88
363.0	4.36	208.3282	0.28	12.55	82.7774	1.00	22.48	7.6287	1.88
423.5	4.36	223.3919	0.28	12.55	65.8902	1.00	22.46	8.7348	1.88
494.0	4.37	235.6054	0.28	12.56	52.2946	1.00	22.45	12.3606	1.87
554.5	4.37	245.7701	0.28	12.55	40.9015	1.00	22.43	14.7978	1.87
615.0	4.36	253.9047	0.28	12.53	32.747	1.00	22.39	17.1686	1.87
675.5	4.35	259.5746	0.28	12.52	26.3318	1.00	22.37	19.5526	1.87
746.0	4.36	263.6391	0.28	12.53	21.1044	1.00	22.39	22.674	1.87
806.5	4.37	267.2103	0.28	12.55	16.915	1.00	22.39	25.2417	1.87
867.0	4.36	270.504	0.28	12.55	13.5669	1.00	22.38	27.7777	1.87
927.5	4.37	273.3931	0.28	12.56	10.8572	1.00	22.38	30.5233	1.87
988.0	4.37	274.6216	0.28	12.57	8.6365	1.00	22.39	33.1501	1.87
1048.5	4.38	276.7757	0.28	12.62	6.9222	1.00	22.45	36.2996	1.86

ND = Not detected. RT = Retention time in minutes. RRT = Relative retention time.

Table 3.4: Acid Degradation of Clarithromycin Standard in 0.1 M HCl, pH 1.2

Time (Min)	Decladinosylclarithromycin			Clarithromycin			9, 12-hemiketal		
	RT (Min)	Peak Area x 10^{-4}	RRT	RT (Min)	Peak Area x 10^{-4}	RRT	RT (Min)	Peak Area x 10^{-4}	RRT
10.2	4.81	60.5045	0.29	13.75	274.9404	1.00	ND	ND	ND
30.7	4.80	134.0124	0.29	13.67	175.8441	1.00	ND	ND	ND
51.2	4.80	193.862	0.29	13.58	110.5661	1.00	ND	ND	ND
71.7	4.77	226.451	0.29	13.51	70.0682	1.00	ND	ND	ND
92.2	4.79	253.9582	0.29	13.54	43.6118	1.00	ND	ND	ND
132.7	4.76	287.5258	0.29	13.47	18.8856	1.00	23.54	20.3924	1.82
163.2	4.76	294.5449	0.29	13.47	15.5107	1.00	23.56	27.1815	1.82
193.7	4.78	300.3398	0.29	13.54	8.3656	1.00	23.67	34.9073	1.82

ND = Not detected. RT = Retention time in minutes. RRT = Relative retention time

In both pH 1.4 and 1.2, clarithromycin API depicted a time dependent decrease in peak area while that of decladinosyl clarithromycin and 9,12-hemiketal increased. The degradation data fitted into a pseudo-first order kinetics model according to the rate equation;

$$A_t = A_o e^{-kt}$$

and the log transformation to the linear equation;

$$\ln A_t = \ln A_o - kt$$

where A_t is the peak area at time t, A_o is the initial peak area and k is the rate constant. The degradation kinetics parameters are shown in Table 3.5.

Table 3.5: Acid Degradation Parameters for Clarithromycin Standard

Degradation Parameters	pH 1.4	pH 1.2
Linear equation ($\ln A_t = \ln A_o - kt$)	y = -0.0037x + 15.007	y = -0.0186x + 14.892
r^2 value	0.999	0.992
Half-life (min)	187.3	37.3
Rate constant, k (min^{-1})	3.7×10^{-3}	18.6×10^{-3}
% API degradation in 60 min	18.7	64.2

The acid degradation parameters indicate faster degradation in 0.1 M HCl, pH 1.2 than 0.1 M H_3PO_4, pH 1.4. At pH 1.2, approximately 64% of API is degraded within 1 hour whereas the degradation at pH 1.4 is 18% (Table 3.5). Since the gastric residence time for clarithromycin is 0.5 - 2 hrs it is necessary for the API to be protected during product

design to ensure availability of intact API at intestinal absorption sites. This may be achieved through enteric coating of the tablets and granules for suspensions.

3.3.1.2 Dissolution of Clarithromycin Tablets and Capsules

All tablets and capsules were subjected to dissolution runs for 60 minutes with six (6) sampling points. At pH 1.2, the samples were quenched with 0.2 M NaOH immediately after sampling in order to terminate the acidic degradation reaction.

The results for dissolution and degradation profiles of all samples are shown in Table 3.6 while graphical presentations of the same are shown in Figures 3.3 - 3.6. The dissolution profile of the comparator product C12 has been presented alongside each set of graphs for comparison purposes. Figures 3.3 and 3.4 are graphs of products that met the requirements for similarity factor. In Figure 3.5 are graphs of products that failed to meet the specifications for f_2 due to higher percentage dissolution while Figure 3.6 shows the products that are non-equivalent to C12 due to lower release percentages. A graphical presentation of the dissolution profiles of all samples based on total clarithromycins is shown in Figure 3.7. Similarity factors were calculated based on both clarithromycin API and total clarithromycins released for each sample (Table 3.7) and the f_2 values obtained based on total clarithromycins plotted on a column chart (Figure 3.8).

All decladinosyl clarithromycin peak areas were normalised by dividing their values by a response factor of 1.0060 (Abuga 2000). A typical chromatogram obtained after dissolution at pH 1.2 for 30 minutes is shown in Figure 3.2. It shows degradation of clarithromycin to form decladinosyl clarithromycin in acidic media. The peaks of

clarithromycin and its degradation products were however resolved and well separated from excipient peaks.
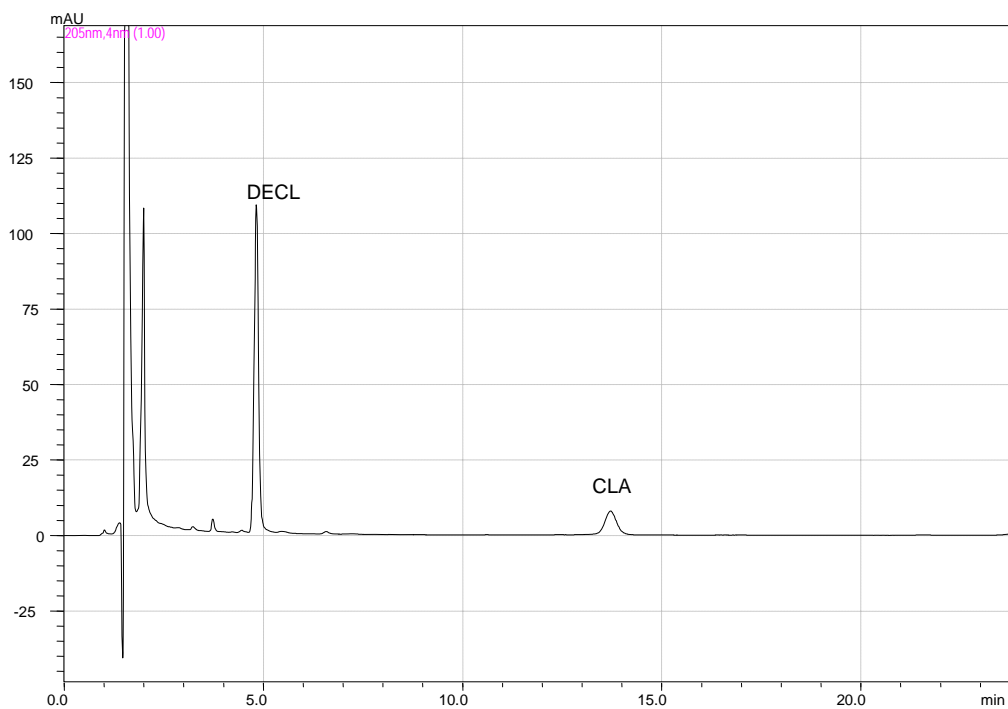
Fig. 3.2: Typical chromatogram for clarithromycin dissolution at pH 1.2, 30 minutes run time

Column: XTerra® C_{18} 5μ (250 × 4.6 mm ID). Column temperature: 56 °C. Mobile phase: acetonitrile-0.2 M phosphate buffer, pH 6.80-water (40:3.5:56.5%, v/v/v). Flow rate: 1.5 mL/min. Detection: 205 nm. Concentration: 0.43 mg/mL. Injection volume: 100 μL. CLA - Clarithromycin. DECL - Decladinosyl clarithromycin.

Table 3.6: Dissolution and Degradation Profiles of Clarithromycin Tablets and Capsules at pH 1.2

DISSOLUTION TIME (MIN)		AVERAGE % DRUG RELEASED (n = 6)												
		Sample Codes												
		C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C11**	**C12**	**C17**
5	CLA	8.41	14.08	1.43	5.16	37.64	2.29	2.61	3.77	0.88	53.90	5.39	8.97	11.70
	DECL	3.78	5.24	0.83	1.48	13.23	1.38	1.17	1.20	2.01	10.02	5.51	2.39	2.17
	TOTAL CLA	12.19	19.32	2.26	6.64	50.87	3.67	3.78	4.97	2.88	63.92	10.90	11.36	13.87
10	CLA	8.59	18.32	2.58	11.45	27.06	4.02	3.51	6.90	2.77	40.21	7.09	12.20	14.47
	DECL	8.94	11.43	1.16	5.37	25.42	3.60	1.63	4.60	5.03	30.46	5.00	6.48	4.18
	TOTAL CLA	17.53	29.75	3.74	16.82	52.48	7.62	5.14	11.50	7.80	70.67	12.09	18.67	18.65
15	CLA	7.58	23.16	4.25	11.88	18.48	5.60	3.78	7.96	2.43	20.99	7.93	11.89	17.33
	DECL	13.56	20.45	2.56	11.80	33.79	7.03	2.93	8.96	8.95	40.03	8.79	11.53	8.91
	TOTAL CLA	21.14	44.06	6.81	23.68	52.27	12.63	6.71	16.92	11.38	61.02	16.72	23.42	26.24
30	CLA	5.15	6.77	5.28	7.02	5.80	5.87	2.69	5.76	3.53	3.26	6.79	8.61	9.76
	DECL	25.17	38.53	8.97	23.00	46.82	18.24	10.57	20.25	18.78	51.48	20.06	24.50	20.77
	TOTAL CLA	30.32	45.31	14.25	30.02	52.62	24.12	13.26	26.01	22.31	54.74	26.85	33.12	30.53
45	CLA	3.83	1.81	5.45	2.99	2.14	5.37	3.16	3.84	2.11	0.48	5.67	6.10	6.04
	DECL	32.98	44.75	15.85	35.90	49.81	27.43	18.17	28.49	21.86	53.17	28.76	34.12	30.26
	TOTAL CLA	36.81	46.56	21.30	38.90	51.95	32.80	21.33	32.33	23.97	53.65	34.43	40.22	36.30
60	CLA	2.99	0.72	5.52	2.91	1.07	4.23	2.59	2.72	2.00	0.03	4.37	5.59	3.43
	DECL	38.21	43.39	23.04	33.90	49.35	40.40	18.20	35.44	25.45	54.10	36.56	40.44	35.27
	TOTAL CLA	41.20	44.11	28.55	36.81	50.42	44.63	20.79	38.16	27.45	54.13	40.93	46.04	38.70

n = number of dissolution replicates.
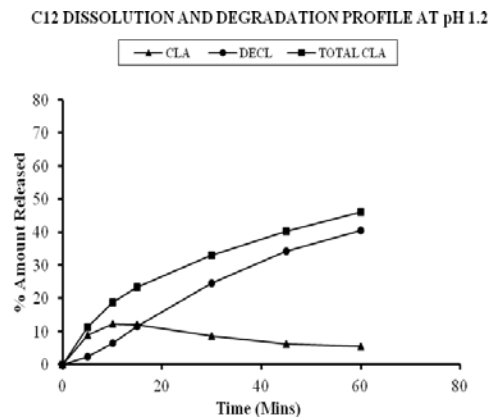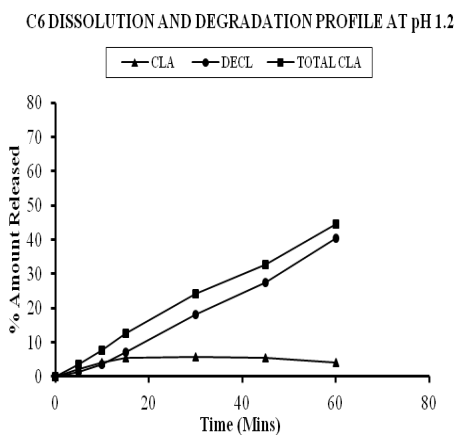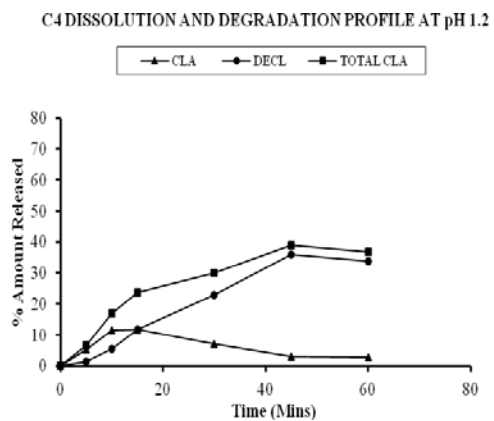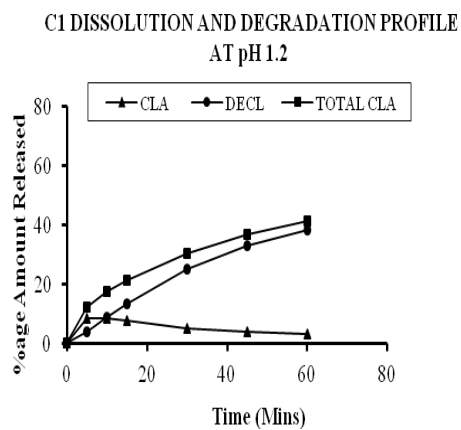
Figure 3.3: Dissolution and degradation profiles of product C12 and the equivalent products C1, C4 and C6 at pH 1.2

C8 DISSOLUTION AND DEGRADATION PROFILE AT pH 1.2
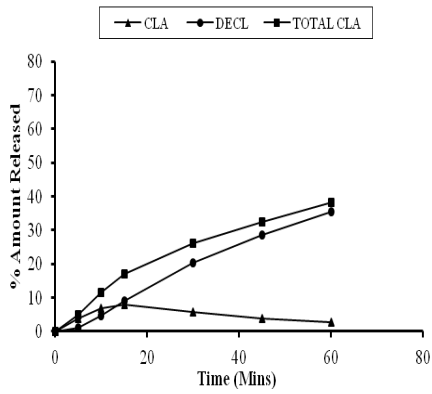
C11 DISSOLUTION AND DEGRADATION PROFILE AT pH 1.2
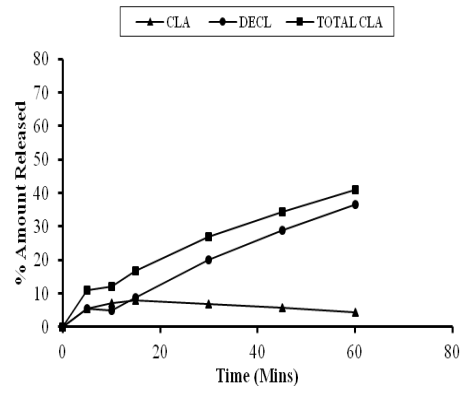
C17 DISSOLUTION AND DEGRADATION PROFILE AT pH 1.2
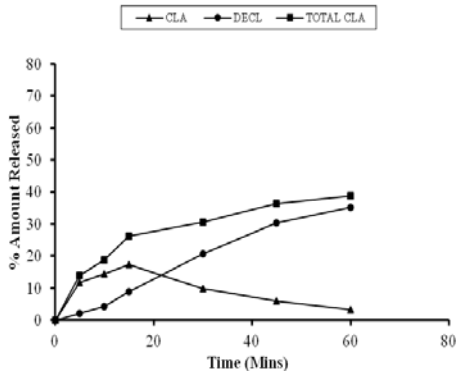
C12 DISSOLUTION AND DEGRADATION PROFILE AT pH 1.2
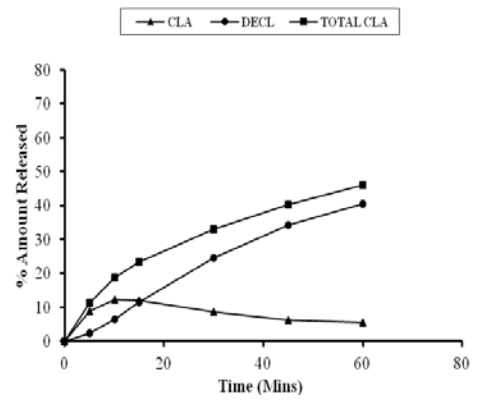
Figure 3.4: Dissolution and degradation profiles of product C12 and the equivalent products C8, C11 and C17 at pH 1.2
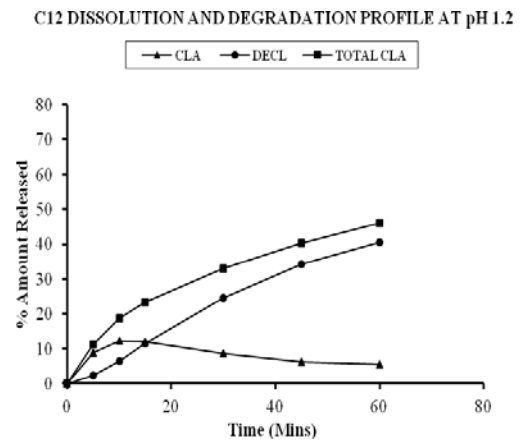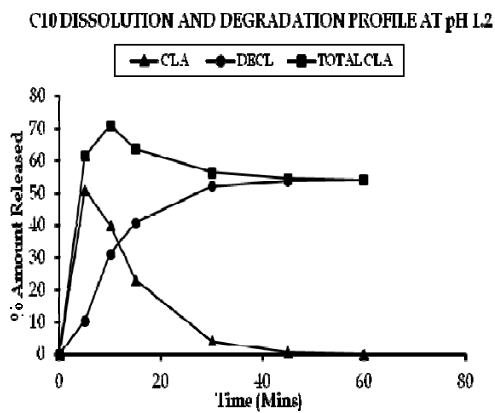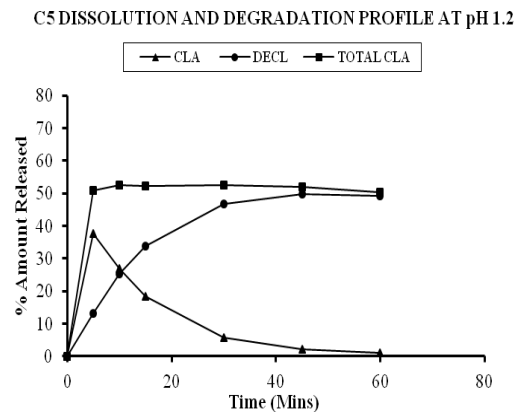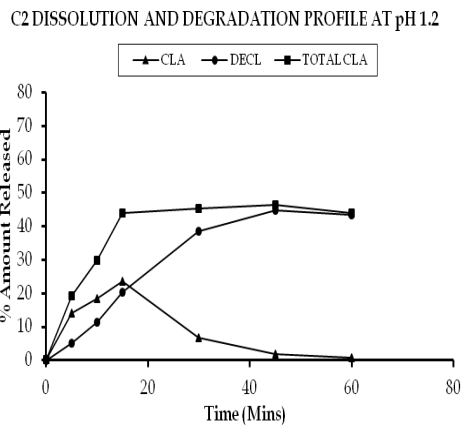
Figure 3.5: Dissolution and degradation profiles of product C12 and the non-equivalent products C2, C5 and C10 at pH 1.2

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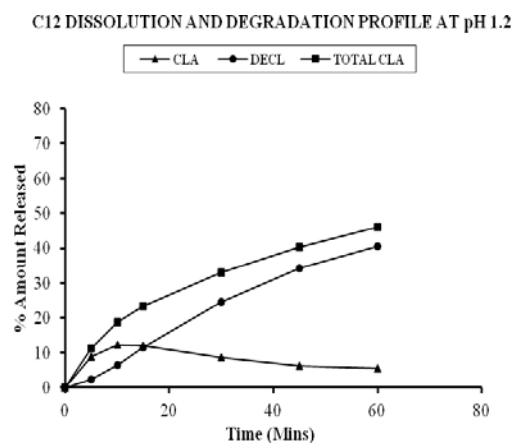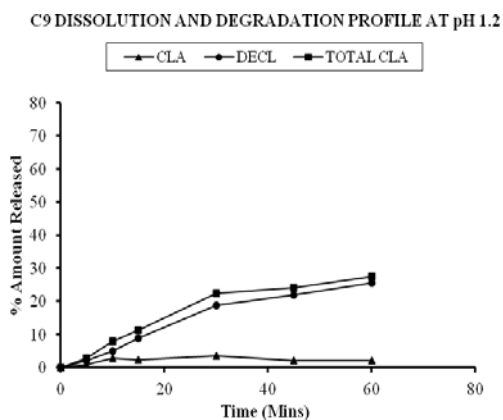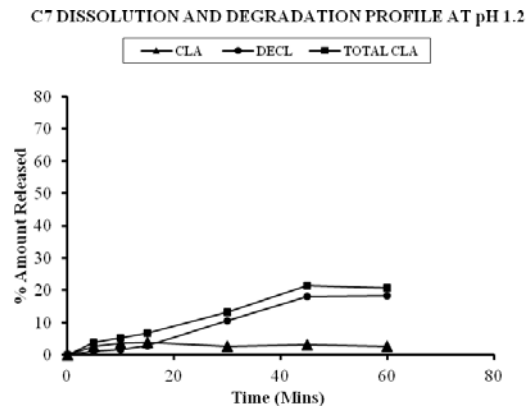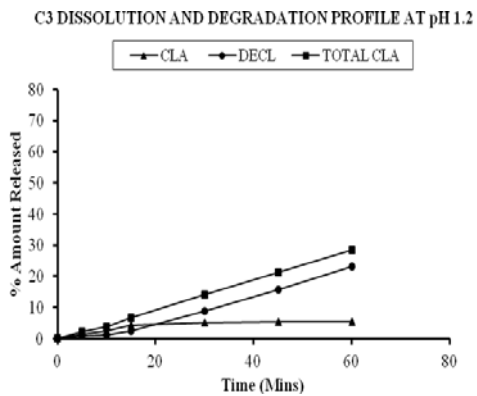
Figure 3.6: Dissolution and degradation profiles of product C12 and the non-equivalent products C3, C7 and C9 at pH 1.2
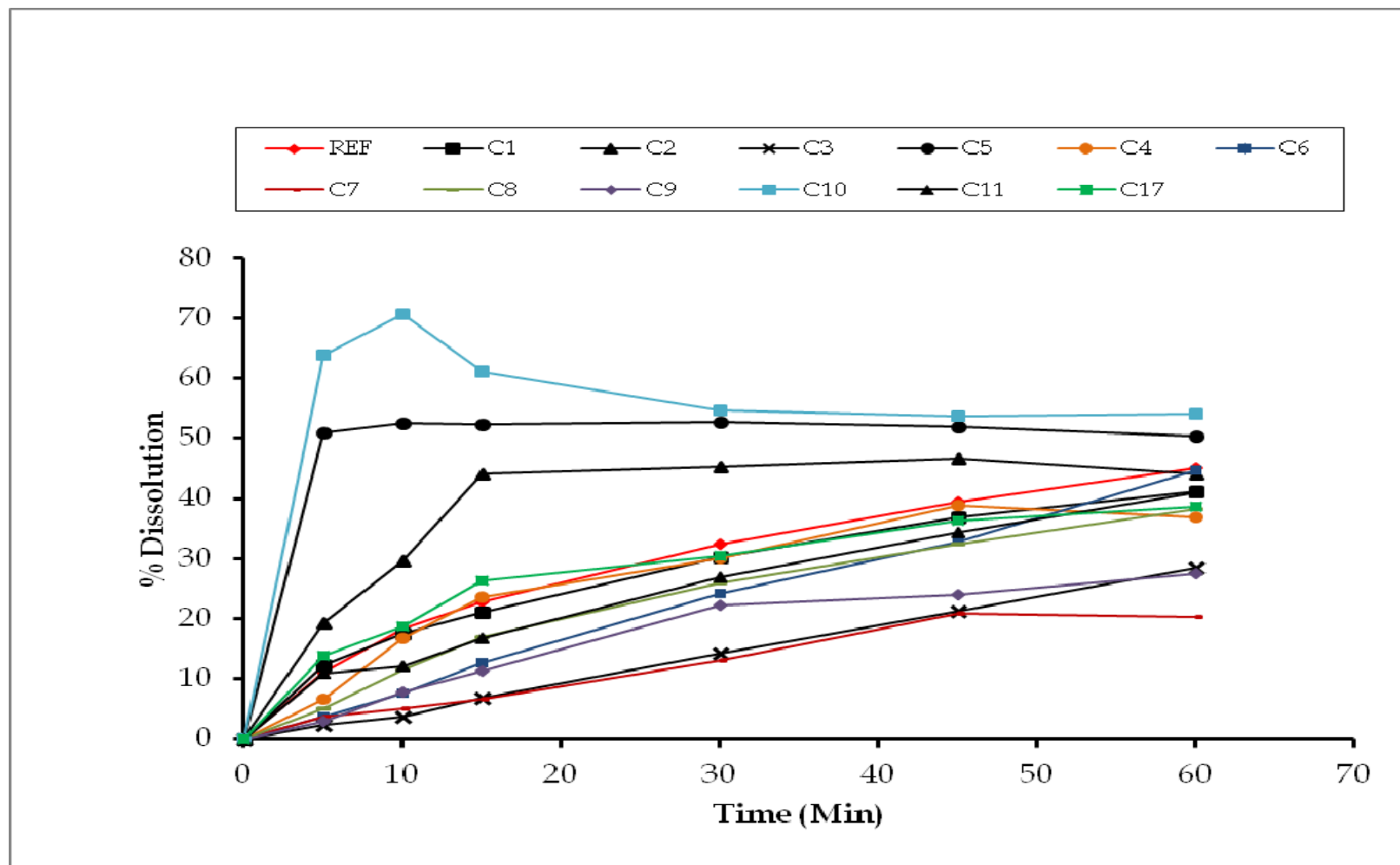
Fig. 3.7: Comparative dissolution profiles of clarithromycin tablets and capsules based on total clarithromycins at pH 1.2

Table 3.7: Similarity (f_2) Factors for Clarithromycin Tablets and Capsules at pH 1.2

Sample Code		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C17
f_2 Value (%)	Based on clarithromycin API	75.9	59.0	62.0	80.3	42.9	64.5	60.4	70.7	58.0	32.5	75.2	100.0	75.1
	Based on total clarithromycins	80.4	46.0	40.2	69.2	28.8	54.6	37.9	58.9	44.8	22.2	64.7	100.0	72.2
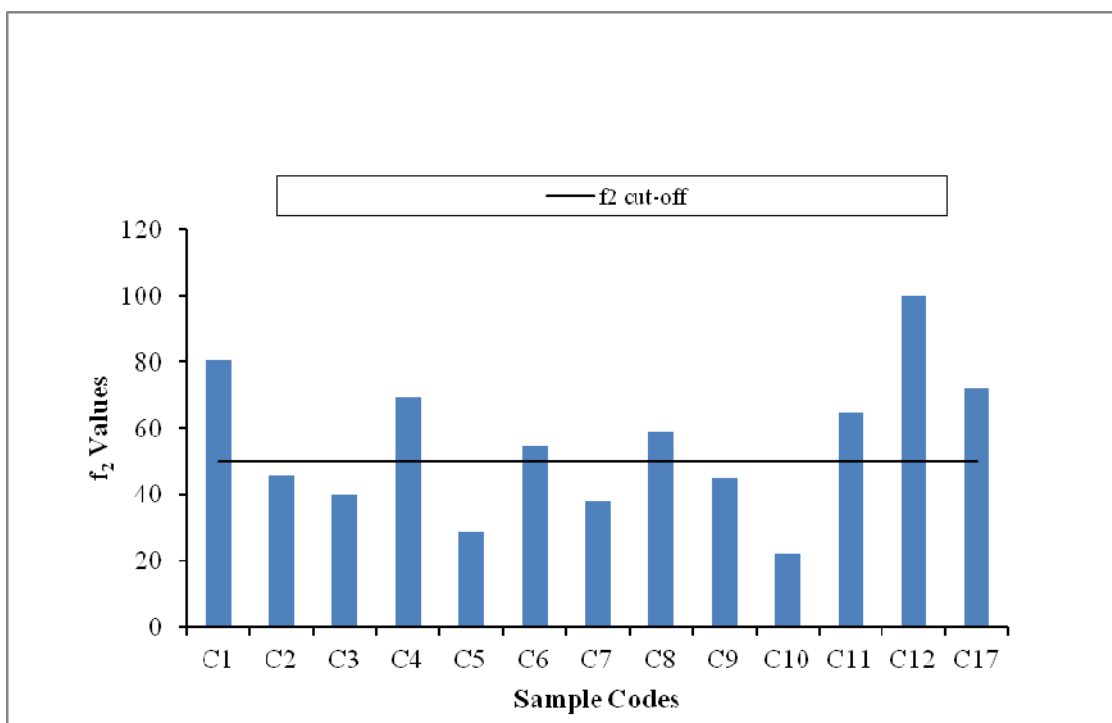
Fig. 3.8: Comparative similarity factors for clarithromycin tablets and capsules at pH 1.2

Dissolution of clarithromycin at pH 1.2 occurred with concomitant degradation into decladinosyl clarithromycin. Quenching 10 mL samples with 3 mL 0.2 M NaOH terminated the reactions and ensured consistency of results. The innovator product C12 released 46% total clarithromycins within 60 minutes dissolution time where the major component was decladinosyl clarithromycin (40%) while 6% was clarithromycin API. Generic products whose dissolution and degradation profiles differed by a margin of ≤ 10% at each time point from C12 were found to be pharmaceutically equivalent with it ($f_2 \geq 50$) as per the US FDA guidelines (US FDA 1997).

Based on total clarithromycins, six (6) out of twelve (12) tablet products were found to be equivalent to C12 translating to a 50% success rate. These were C1, C4, C6, C8, C11

and C17 (Figures 3.3 and 3.4). Products C2, C5 and C10 were non-equivalent to C12 on account of higher percentage total clarithromycins dissolved (Figure 3.5) while C3, C7 and C9 were non-equivalent due to lower percentage dissolution than C12 (Figure 3.6). Although the percentage dissolution of C2 at 60 minutes was comparable to C12 (44%), it failed to meet the criteria for similarity due to higher dissolution rate with 44% drug dissolving in 15 minutes compared to 23% for C12 within the same time.

Three generic products released over 45% total clarithromycins within 30 minutes dissolution run time. Products C10 and C5 released 70% and 52% total clarithromycins respectively in the first 10 minutes while C2 released 45% in 30 minutes. In both C5 and C10, clarithromycin degradation proceeded fast producing 45 - 50% decladinosyl clarithromycin within 30 minutes and generating multiple additional peaks that were not quantified as the method had been optimised for quantitation of clarithromycin and decladinosyl clarithromycin. For this reason, computation of total clarithromycins was based on only the API and decladinosyl clarithromycin thereby yielding a downward trend brought about by the minor degradation products.

Products C3, C7 and C9 had remarkably low dissolution percentages ranging between 20 - 29% indicating significant formulation differences between these and the innovator product. It was however not clear whether the low dissolution was due to enteric coating or other formulation approaches.

The acidic pH 1.2 mimics gastric conditions. Studies show that intragastric pH ranges from 0.5 - 2 in adults, 1.5 - 3 in children and 3 - 4 in neonates before food while postprandial pH values rise to around 4. The sick state may increase gastric acidity further with a pH range of below 2 (Merki 1988; Nagita 1996). Given that the gastric

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residence time of clarithromycin is 0.5 - 2 hrs, it is evident from the observed degradation patterns that little drug will be available for intestinal absorption. This raises concerns about API bioavailability and hence efficacy of the clarithromycin products whose f_2 values were below 50.

The similarity factor patterns reflect differences in formulation processes and possibly variations in the quality assurance systems among different manufacturers. It is notable that product design impacts significantly on pharmaceutical equivalence. Based on the results obtained for dissolution profiles at pH 1.2, about half of the products were found to be pharmaceutically equivalent to the innovator product.

3.3.1.3 Dissolution of Clarithromycin Suspensions

Sampling was carried out under stirring conditions for suspensions so as to ensure homogeneity. Formulation factors that could impact on the uniformity of dosage include viscosity and the quality and quantity of suspending agents incorporated as this determines the rate of sedimentation of the reconstituted suspension.

Less than 2% drug was released from suspensions at pH 1.2. In the majority of samples no peaks for clarithromycin or related substances were detected. This indicates that all the suspensions tested were composed of enteric coated granules, thus offering sufficient protection to clarithromycin API from acid degradation in the low gastric pH.

3.3.2 pH 4.5

3.3.2.1 Dissolution of Clarithromycin Tablets and Capsules

The stability of clarithromycin in pH > 3.0 has been documented (Erah *et al.* 1997; Fujiki *et al.* 2011). Quenching was therefore not necessary for dissolution samples at pH 4.5. The average percentage dissolution for the sexaplicate samples were computed from peak areas. The dissolution percentages were plotted as a function of dissolution time. A comparative dissolution tabulation for all samples was made (Table 3.8) and dissolution profile graphs constructed on the same chart (Figure 3.9). The similarity factors computed are shown in Table 3.9 and comparative column charts in Figure 3.10.

Table 3.8 Comparative Dissolution Profiles for Clarithromycin Tablets and Capsules at pH 4.5

Time (Min)	% Clarithromycin Dissolved												
	C12	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C11**	**C17**
5	88.82	86.91	34.56	77.06	2.93	75.45	67.53	23.40	90.62	46.87	73.71	90.57	93.65
10	91.60	91.23	63.32	82.74	30.43	94.19	93.94	57.86	95.85	65.30	85.81	90.67	94.87
15	92.92	92.54	88.80	84.27	58.14	94.86	94.61	73.47	96.88	82.15	87.73	90.64	95.17
30	93.12	93.20	94.10	86.46	80.76	95.25	94.99	88.46	97.27	91.31	89.57	90.66	95.22
45	93.31	93.81	94.78	86.86	83.44	95.37	95.39	96.52	97.40	91.79	90.59	90.88	95.56
60	93.68	94.48	94.87	87.24	85.34	95.64	95.77	97.75	98.44	91.42	90.16	90.91	95.78

C12 - Reference sample
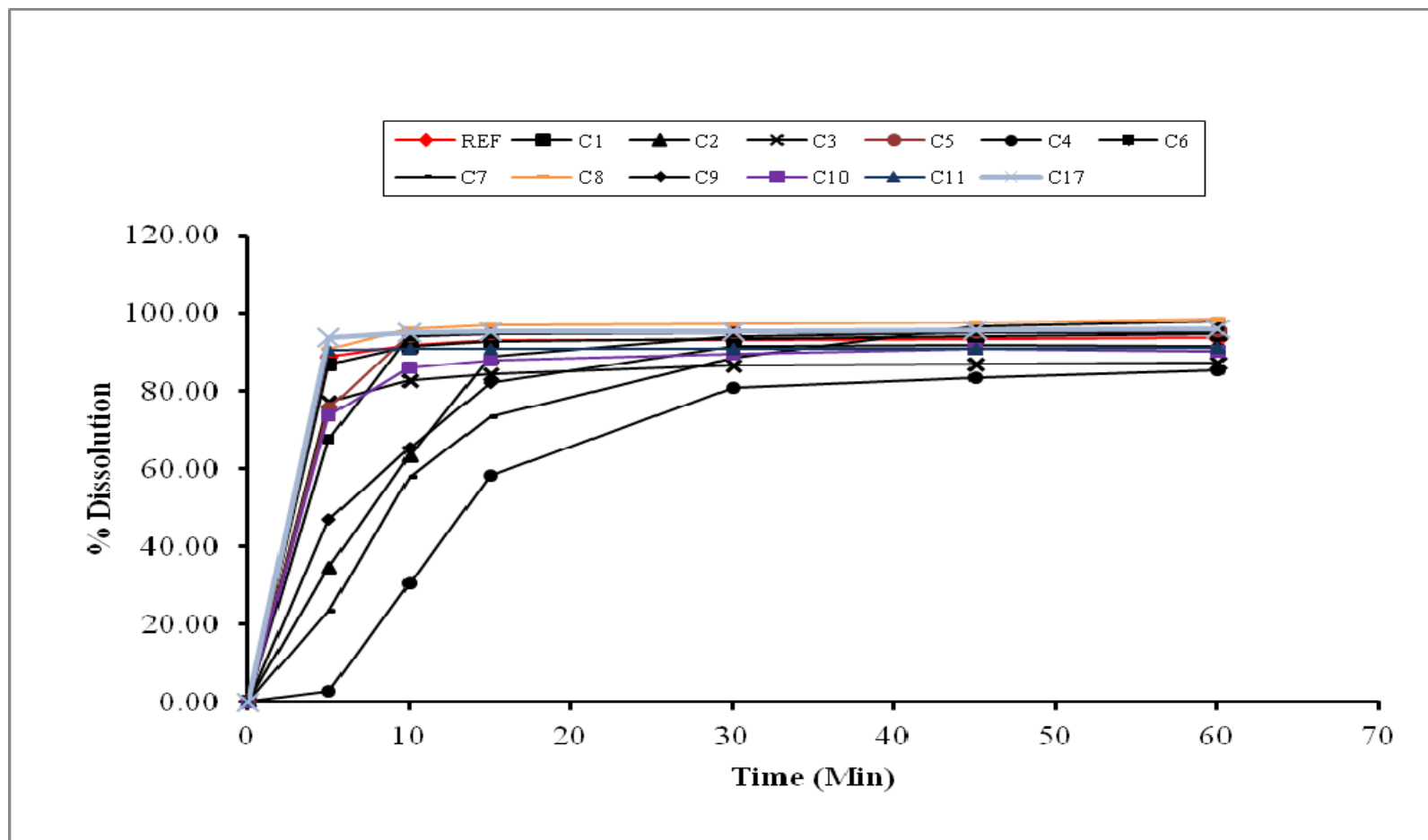
Fig. 3.9: Comparative dissolution profiles for clarithromycin tablets and capsules at pH 4.5

Table 3.9: Similarity (f_2) Factors for Clarithromycin Tablets and Capsules at pH 4.5

Sample Code	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C17
f_2 Value (%)	93.6	30.0	53.7	16.9	61.5	52.4	25.3	69.5	34.2	56.6	80.9	100.0	75.2
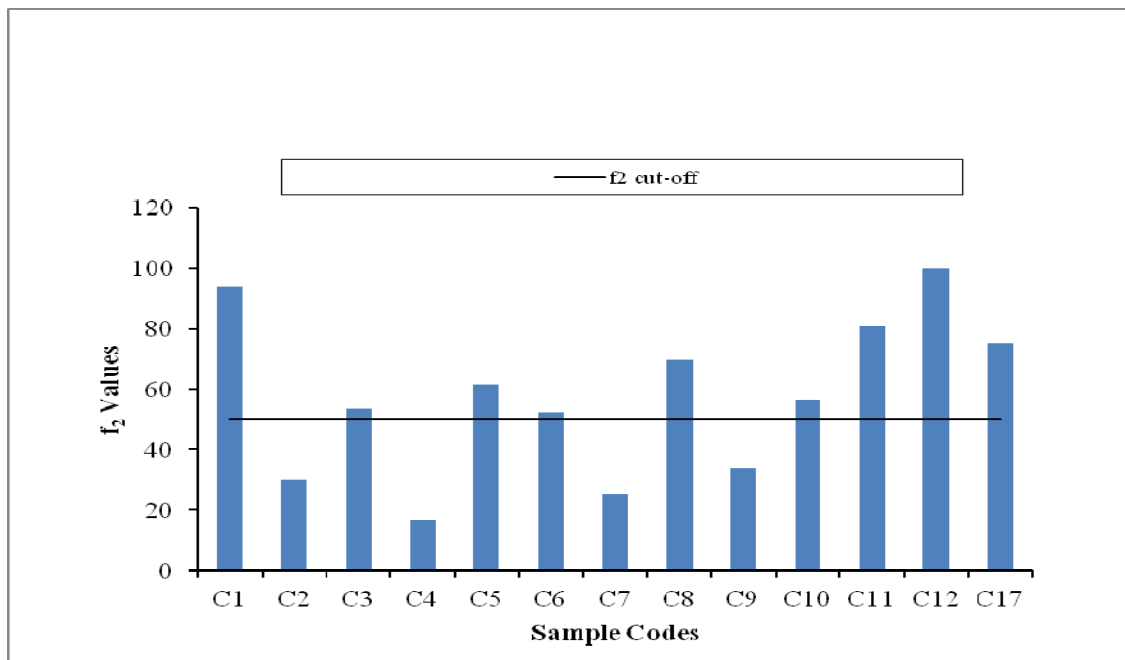
Fig. 3.10: Comparative similarity factors for clarithromycin tablets and capsules at pH 4.5

All tablet samples released $\geq$ 80% drug within 30 minutes of dissolution. The highest release rate was 97% (C8) while the lowest was 80% (C4). That notwithstanding, 4 products (C2, C4, C7 and C9) were found to be pharmaceutically non-equivalent to the innovator product C12 translating to 33% of all products tested. The inequivalence in all these samples arose due to slow release rates at earlier dissolution time points. At 10 minutes run time, the percentage dissolution for C12 was 92% while it was 63% for C2, 30% for C4, 58% for C7 and 65% for C9. At 60 minutes the percentage dissolution for C2 was similar to that of the comparator product C12 while it was higher for C7 and C9. The assay values for the four samples were 98.7 - 102.1%. The dissolution results therefore point to a variability of product design components applied in the manufacture of the products giving rise to differences in drug release characteristics when the assay content is comparable to that of the innovator product.

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3.3.2.2 Dissolution of Clarithromycin Suspensions

There was minimal drug release at pH 4.5. In three samples C14, C15 and C18 no drug release was detectable by chromatography within the 90 minutes dissolution run time. Product C16 had a release rate of 1.87% at 45 minutes while C13 released about 1% at 90 minutes, probably due to defective granules. This shows that the enteric coating materials used do not dissolve at pH 4.5 hence no drug release.

3.3.3   pH 6.8

Chromatography of dissolution samples under the optimum conditions yielded rising column pressures and peak shifting. Troubleshooting investigations implicated the hydroxypropyl methylcellulose based polymers used for enteric coating of clarithromycin granules. The effect of filtration on chromatographic performance was therefore investigated with a view to optimising sample treatment procedures.

3.3.3.1 Effect of Sample Microfiltration

At pH 6.8, dissolution samples filtered using only 10 μ dissolution in-line filters resulted in broad matrix peaks, peak shifting, rising chromatographic column pressures and chromatograph shut down. In contrast, samples filtered through both the in-line and 0.45 μ filters resulted in stable column pressures, reduced matrix peaks and consistent sample peaks. The same observation was made for assay preparations for both tablets and suspensions. Figure 3.11 illustrates the typical chromatograms obtained using microfiltered samples in both instances.
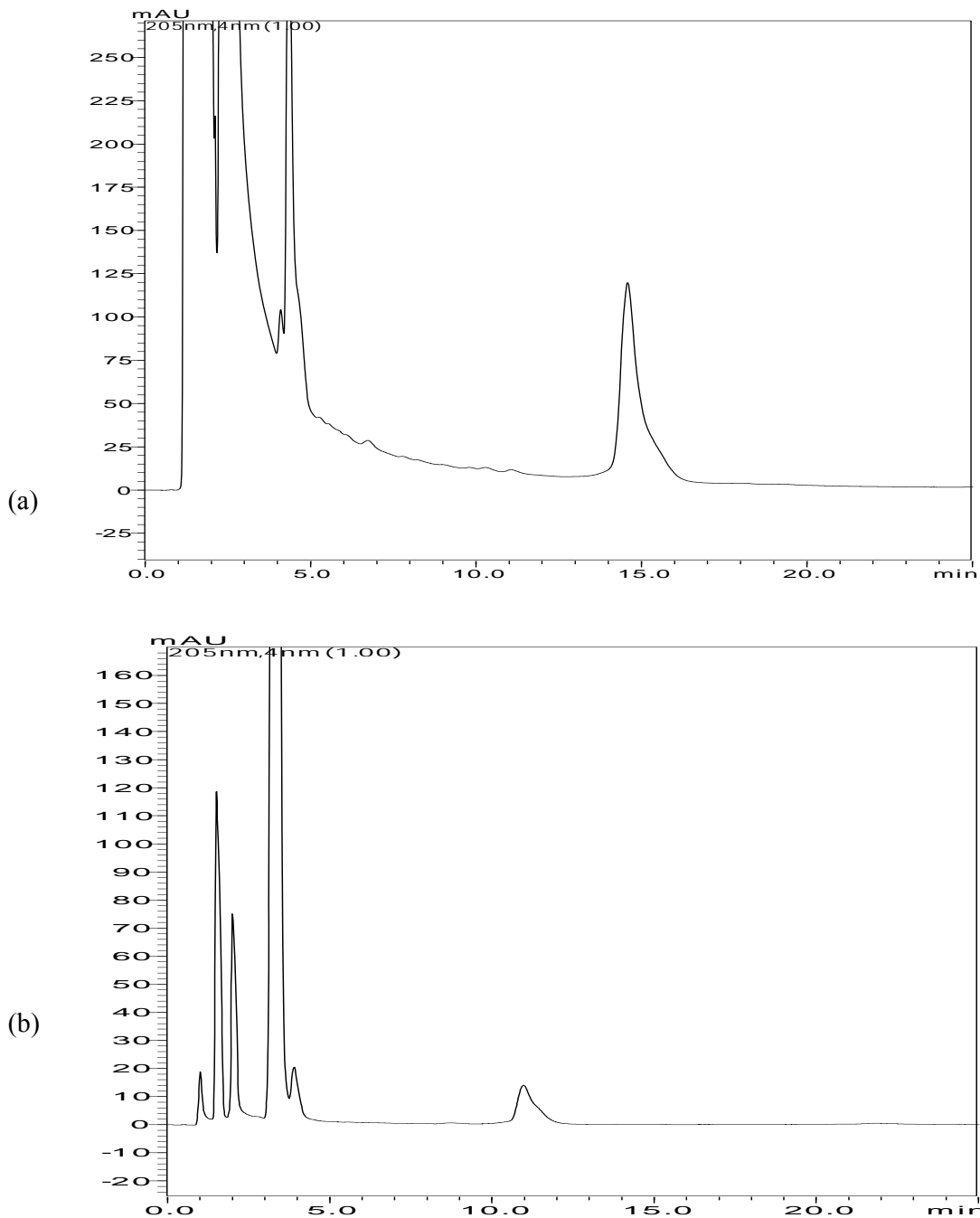
Fig. 3.11: Chromatograms for dissolution of clarithromycin suspensions at pH 6.8, 60 min run time

(a) - Chromatogram of suspension filtered through 10 μ filters only.

(b)-Chromatogram of suspension filtered through 10 μ and 0.45 μ filters.

Column: XTerra® C_{18} 5μ (250 × 4.6 mm ID). Column temperature: 56 °C. Mobile phase: acetonitrile-0.2 M phosphate buffer, pH 6.80-water (40:3.5:56.5%, v/v/v). Flow rate: 1.5 mL/min. Detection: 205 nm. Concentration: 0.28 mg/mL. Injection volume: 100 μL.

Rising column pressures were due to the enteric coating HPMC-phthalate polymer that dissolves in buffer at alkaline pH and has significant absorbance at 205 nm. Apparently, the solubilised polymer passed through 10 μ in-line filters but was significantly retained by the 0.45 μ filters resulting in consistency of sample peaks.

3.3.3.2 Dissolution of Clarithromycin Tablets

Dissolution runs were carried out in sexaplicates of one batch of each product. Dissolution conditions and sampling time points were maintained as those for pH 1.2 and 4.5. Tabulations of average percentage dissolution at each time point were made for each sample and the percentages plotted as a function of time. Comparative dissolution tables and plots for all samples (Table 3.10 and Figure 3.12) were created and similarity factors computed for comparison purposes (Table 3.11). Further, the similarity factors were presented in column charts (Fig. 3.13) for ease in comparison.

Table 3.10 Comparative Dissolution Profiles for Clarithromycin Tablets and Capsules at pH 6.8

Time (Min)	% Clarithromycin Dissolved												
	C12	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C17
5	29.02	30.34	26.43	16.99	0.04	18.19	29.50	6.35	22.32	11.47	34.18	40.94	34.44
10	46.97	40.55	42.11	27.18	3.39	27.02	44.49	16.22	34.95	23.80	47.53	52.83	46.21
15	56.71	45.64	49.13	34.56	19.16	32.74	51.00	23.58	42.07	33.44	56.96	58.27	51.73
30	66.41	54.16	57.82	45.51	44.35	42.57	60.70	37.02	53.53	46.96	68.28	66.66	58.53
45	71.87	57.86	62.02	51.30	53.73	48.25	65.19	44.92	58.23	52.65	71.99	69.44	60.87
60	74.02	60.14	64.75	54.19	58.36	50.03	67.89	50.22	61.50	56.17	72.43	70.66	62.75

C12 - Reference sample
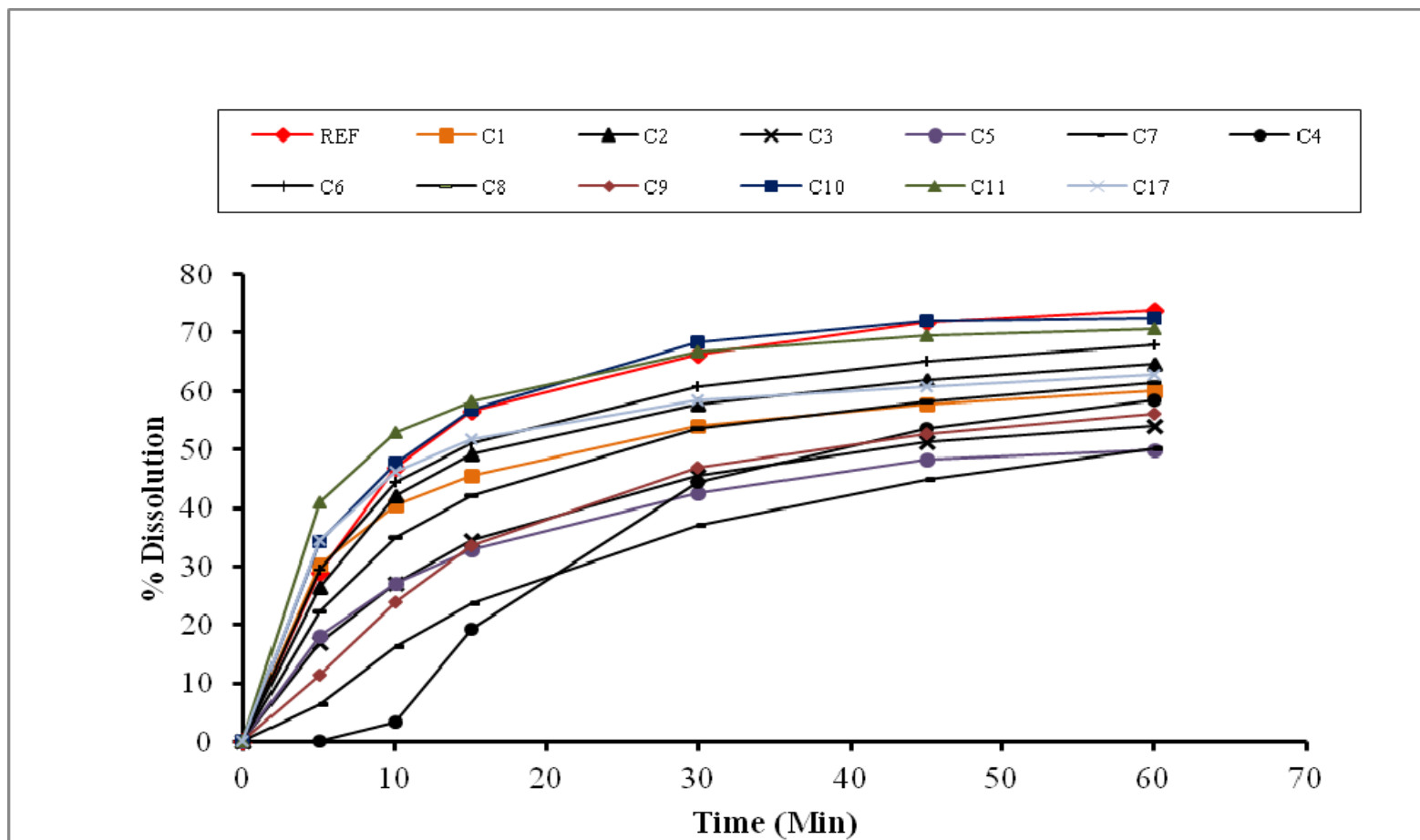
Fig. 3.12: Dissolution profiles for clarithromycin tablets and capsules at pH 6.8

Table 3.11: Similarity (f_2) Factors for Clarithromycin Tablets and Capsules at pH 6.8

Sample Code	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C17
f_2 Value (%)	54.8	55.8	35.5	26.5	33.3	64.4	27.6	45.4	34.7	79.7	61.8	100.0	55.2
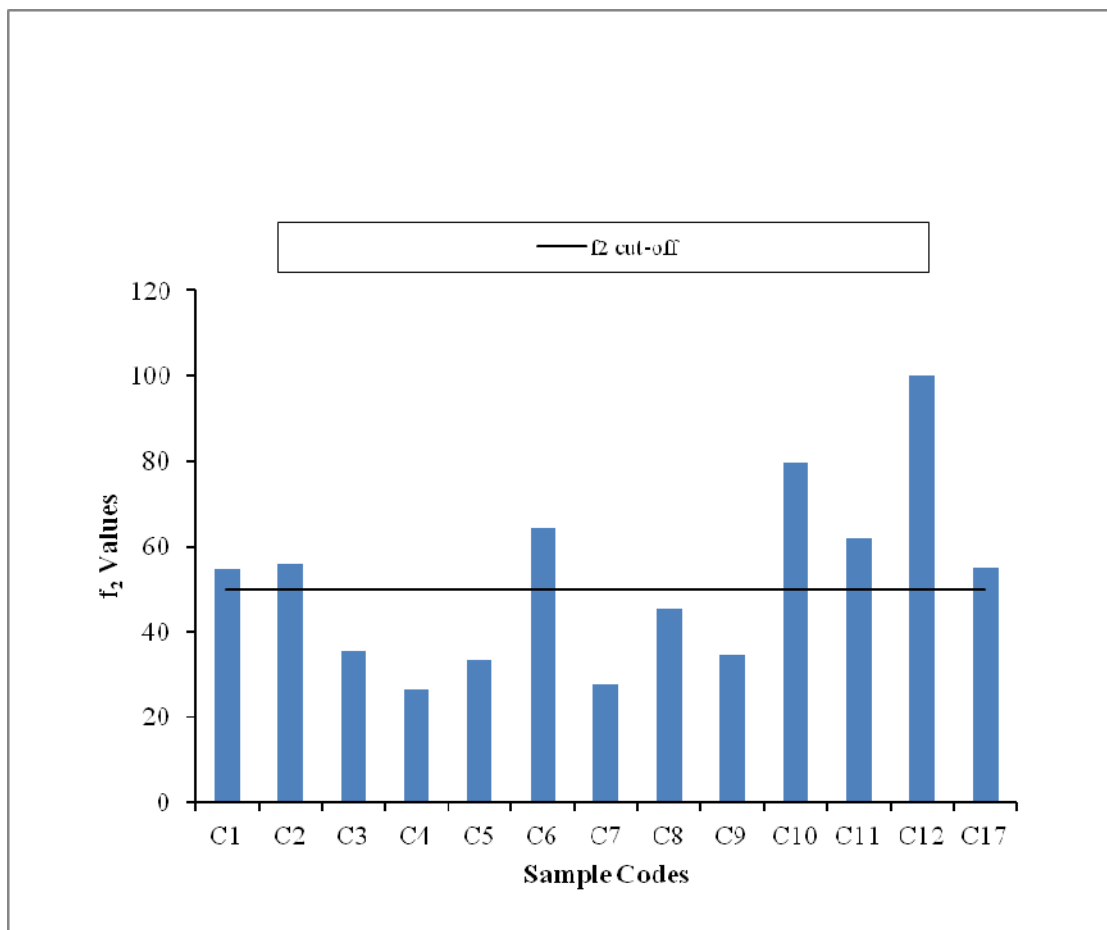
Fig. 3.13: Comparative f₂ factors for clarithromycin tablets and capsules at pH 6.8

The solubility of clarithromycin decreases with increasing pH. Out of the twelve (12) tablet products tested, six (6) met the requirements for similarity factor in relation to the comparator product C12 translating to a 50% compliance rate. Products C3, C4, C5, C7 and C8 were non-equivalent to C12 due to lower dissolution percentages. Products C5 and C7 had the lowest dissolution percentages (50%) in comparison with C12 (74%) at 60 minutes.

Systemic bioavailability of clarithromycin is dependent on intestinal absorption of the API. The low dissolution percentages therefore may indicate a possibility of sub-optimal drug

levels reaching systemic circulation. The high rates of pharmaceutical inequivalence observed may also raise concerns about consistency of therapeutic results obtained when the different generic products in the market are used.

3.3.3.3 Dissolution of Clarithromycin Suspensions

Significant drug dissolution from suspensions was observed at pH 6.8 (Table 3.12 and Figure 3.14). None of the suspensions tested met the requirements for similarity factor in relation to the innovator suspension C16. Products C13 and C18 were non-equivalent due to higher drug release rates than C16 while C14 and C15 had very low release rates. The similarity factors are shown in Table 3.13.

Table 3.12: Dissolution Profiles of Clarithromycin Suspensions at pH 6.8

Time (Min)	Percentage Drug Dissolved				
	C16	C13	C14	C15	C18
0	0	0.22	0.68	0	0.08
10	11.38	14.05	0.57	2.14	11.69
20	36.08	41.85	0.81	1.21	24.83
30	63.35	51.7	2.25	1.27	55.5
45	65.40	67.09	5.21	2.84	75.58
60	68.64	83.99	14.90	8.04	82.39
90	66.94	95.35	14.90	21.60	94.81

C16 - Reference sample
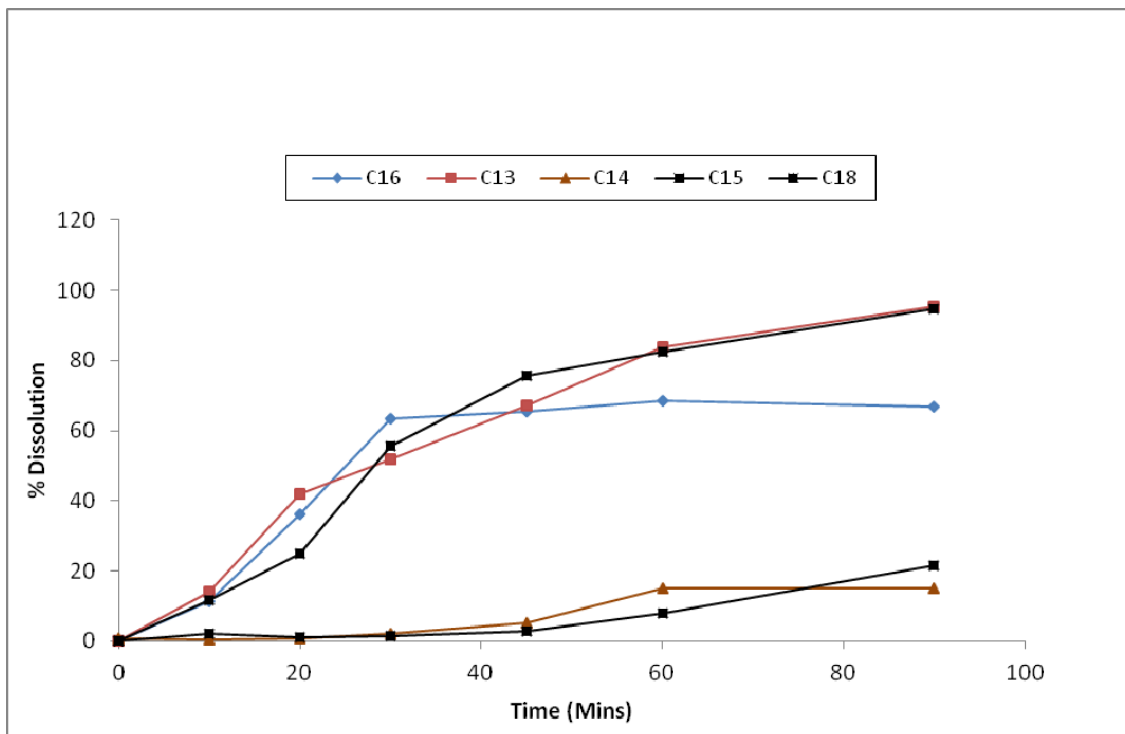
Fig. 3.14: Comparative dissolution profiles for clarithromycin suspensions at pH 6.8

Table 3.13: Similarity Factors for Clarithromycin Suspensions at pH 6.8

Sample Code	C13	C14	C15	C16	C18
f_2 Value (%)	42.4	14.3	15.2	100.0	42.0

Products C16 and C18 are from the same manufacturer but the manufacturing sites are different. They are thus expected to be equivalent which was not the case in the experiments carried out, raising concerns about standardisation of manufacturing sites as per the SUPAC

guidelines (CDER 1995). Notably, products C14 and C15 yielded minimal drug release over the dissolution period which raises valid concerns about their efficacy.

3.3.4   Comparative Evaluation for Similarity Factor for all Clarithromycin Samples

A summary of f_2 data for all analysed samples is presented in Table 3.14. The f_2 values computed for pH 1.2 are based on total clarithromycins in order to allow for comparisons of the results at pH 4.5 and 6.8 and to simulate physiological pharmacokinetics.

Overall, 4 (25%) out of the 16 products met the acceptance criteria for similarity factor, f_2 in relation to the innovator products C12 and C16. Notably, products C7 and C9 consistently failed to meet the f_2 acceptance criteria at all the three pH values. The results indicate that majority of clarithromycin generic products in the market may not be pharmaceutically equivalent to their innovator counterparts. Such products are distinguished as unacceptable by the US FDA guidelines (US FDA 1997), and may require bioequivalence studies to justify their circulation and use. The US FDA guidelines recommend that for such products whose f_2 < 50, guidance should be sought from CDER to determine whether an *in vivo* study is appropriate (US FDA 2003).

Table 3.14: Similarity Factors for Clarithromycin Samples

Sample Code	Dosage Form	f_2 Values (%)		
		pH 1.2	pH 4.5	pH 6.8
C1	Tablet	80.4	93.6	54.8
C2	Tablet	46.0	30.0	55.8
C3	Tablet	40.2	53.7	35.5
C4	Capsule	69.2	16.9	26.5
C5	Tablet	28.8	61.5	33.3
C6	Tablet	54.6	52.4	64.4
C7	Tablet	37.9	25.3	27.6
C8	Tablet	58.9	69.5	45.4
C9	Tablet	44.8	34.2	34.7
C10	Tablet	22.2	56.6	79.7
C11	Tablet	64.7	80.9	61.8
C12	Tablet	100.0	100.0	100.0
C13	Suspension	ND	ND	42.4
C14	Suspension	ND	ND	14.3
C15	Suspension	ND	ND	15.2
C16	Suspension	ND	ND	100.0
C17	Tablet	72.2	75.2	55.2
C18	Suspension	ND	ND	42.0

ND - Not Detected

CHAPTER FOUR

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATION

4.1   General Discussion

In this study, comparisons of dissolution profiles of clarithromycin oral formulations were made between fourteen (14) generic products coded C1 - C11 and C13 - C15 and four (4) innovator products coded C12, C16 - C18. Comparison of the dissolution profiles was carried out by calculation of the similarity factor, f_2. The criteria for similarity was taken as an f_2 value of 50 - 100 for both tablets and suspensions.

The study was carried out at pH 1.2, 4.5 and 6.8. At pH 1.2, 50% (six out of twelve) of tablet products tested (C2, C3, C5, C7, C9 and C10) failed to meet the specifications for similarity factor in relation to the innovator product. In products C2, C5 and C10, degradation of dissolved clarithromycin proceeded fast producing over 40% of the degradation products within 60 minutes. Given that the gastric residence time of clarithromycin is 0.5 - 2 hrs, it is evident that intestinal concentration of the API may be lower leading to lower bioavailability and this raises concerns about the efficacy of the products. All suspension products tested were enteric coated and therefore yielded insignificant dissolution.

At pH 4.5, 33% (four out of twelve) of the tablet products tested did not comply with the specifications for similarity factor. These were C2, C4, C7 and C9. It is notable that at this pH, the tablet products tested released $\geq$ 80% drug within 30 minutes. These results are comparable to the USP single-point dissolution specifications of releasing $\geq$ 80% (Q) at 30 minutes dissolution run time (USP 2014f). The single point dissolution studies at 30 minutes

specified in the pharmacopoeia do not, however, take into account gastric and intestinal residence times of clarithromycin. The drug has a gastric residence time of 0.5 - 2 hours and intestinal residence time estimated at 3 - 6 hours (Chandira *et al.* 2009). In addition, clarithromycin has low solubility that is pH-dependent. Single point studies may therefore lead to erroneous conclusions of pharmaceutical equivalence of the different products. Dissolution profile comparison has proved to be more discriminative and capable of providing more insight into the stability and bioavailability of clarithromycin API as a function of time in the different gastrointestinal conditions.(US FDA 1997; Sathe *et al.* 1996; Yuksel *et al.* 2000).

The rate of non-compliance with respect to f_2 at pH 6.8 was 50% with six (6) out of the twelve (12) products tested failing to meet specifications for f_2. Overall, only 25% (four out of sixteen) of products tested met the specifications for similarity factor in relation to the innovator products at all the three pH values.

Variability in product performance with respect to f_2 cut -off was observed at the different pH values used in this study for products C2, C8 and C10. Product C2 failed to meet the specifications for similarity factor at pH 1.2 ($f_2 = 46.0$) due to higher drug release than the comparator product. It also failed to meet the same specifications at pH 4.5 ($f_2 = 30.0$) while it complied at pH 6.8 ($f_2 = 55.8$). Although sufficient amounts of API are released at pH 6.8, it may lack physiological efficacy due to significant API degradation in gastric acid before it reaches intestinal sites for absorption. Product C8 met the specifications at pH 1.2 ($f_2 = 58.9$) and 4.5 ($f_2 = 69.5$) but failed at pH 6.8 ($f_2 = 45.4$) where dissolution is critical for absorption. Product C10 failed to meet similarity factor specifications at pH 1.2 ($f_2 = 22.2$) but complied at pH 4.5 ($f_2 = 56.6$) and 6.8 ($f_2 = 79.7$), and would therefore not be regarded to be efficacious due to degradation in gastric juice.

Comparisons were made between products from the same manufacturer licensed in two markets, Kenya and the UK. The tablet products studied were Klacid® (C12) and Klaricid® (C17) whereby C12 was used as the comparator product. At pH 1.2, the f_2 value for C17 was 72 indicating a difference of $\leq 5\%$ in the amount of drug released at each time point between the two products. At pH 4.5 the f_2 value was 75, which meets the expectations given that the products are manufactured in different sites. At pH 6.8 however, the f_2 value was 55. Although the specification for similarity factor was met, the f_2 value was lower than would be expected for products from the same manufacturer. Significant differences were also noted between the innovator suspension products Klacid® (C16) and Klaricid® (C18) with respect to their API dissolution profile. The f_2 value for C18 was 42 which is lower than the set specification for similarity factor.

These results point to a possibility of differences in innovator products arising from change in manufacturing sites. Although inherent differences are likely to occur due to change in site, they should not be so significant as those observed from these experiments. This emphasizes the need for manufacturers to adhere to GMP in order to ensure equivalence of products manufactured in extra sites and consistency in product quality. In addition, the US FDA and CDER guidelines recommend that the manufacturer avails sufficient data to evaluate SUPAC-related changes including change of manufacturing site before granting approvals for the extra sites (CDER 1995; US FDA 1997). Based on these findings, products C12 and C16 were taken as the reference samples for tablets and suspensions formulations, respectively.

Notably, products C14 and C15 yielded minimal API release over the dissolution period. This observation raises valid concerns because clarithromycin is an important antibiotic with indications for atypical infections whereby treatment failure is likely to have disastrous outcomes. In addition, the suspensions are designed for paediatrics, a delicate cohort where

treatment failures may lead to increased morbidity and mortality more easily than adult populations. Also, consumption of products of sub-optimal quality may contribute to development of microbial resistance to clarithromycin with possible MLS cross-resistance to other macrolides, lincosamides and streptogramin B antibiotics (Douthwaite *et al.* 2000).

An overage was discernible in assay results for the innovator tablet products C12, C17 and the suspensions C13, C14, C16 and C18. However, no overages were noted for all generic tablets products.

The extreme variations in the API release profiles for clarithromycin suspensions reflect differences in the quality of enteric coating. This could be due to differences in the source and quality of coating HPMC-based polymers, formulation factors like the coating process, relative composition of the content of the polymers and other excipients.

Generally, the similarity factor patterns observed in this study indicate that assay and single-point dissolution tests are not sufficient to prove efficacy or pharmaceutical equivalence of the products tested. Lack of comparative dissolution data for pharmaceutical equivalence and subsequently, bioequivalence raises questions of product quality. This impacts on efficacy of the products raising further concerns about the effect of sub-therapeutic outcomes and repercussions of treatment failures especially for antimicrobial drugs.

Drug regulatory authorities are key to controlling the quality of products in circulation in any market. The Conference of Experts on the Rational Use of Drugs, held in Nairobi in 1985, and WHO's Revised Drug Strategy, adopted by the World Health Assembly in May 1986, identified effective functioning of national drug regulation and control systems as a vital means to assure safety and quality of medicines (WHO 2007). The Pharmacy and Poisons Board (PPB) is the regulatory body responsible for approvals and granting of market

authorization of drugs in Kenya. This includes determining the requirements and content of drug registration dossiers as per the Common Technical Document (CTD) guidelines, dossier review, quality control (QC) tests and good manufacturing practices (GMP) inspections. After market authorization, the PPB is responsible for conducting post-marketing surveillance through its pharmacovigilance programme with a view to ensuring consistent good quality products in circulation. The pharmacovigilance (PV) programme must therefore be effective, sustained and targeted with clear regulatory actions on non-compliant products. The success of the PV programme also depends on sufficient manpower with the necessary education, training and experience to perform the PV functions. The PPB thus plays a key role in assuring the quality of drug products circulating in the Kenyan market.

4.2   Conclusions

In the present study, significant differences were observed in the dissolution profiles of the clarithromycin products tested. While all products complied with assay specifications, majority of generic products tested did not comply with the specifications for similarity factor f_2 in relation to the innovator product under the 3 pH values used.

The results obtained from this study can be extrapolated to the wider Kenyan market. This is due to the central role Nairobi County plays in the pharmaceutical market. The city harbours many pharmaceutical manufacturing industries and acts as a centre of distribution for imported drugs. In addition, the sub-counties in Nairobi mirror the economic capacities of the Kenyan population, which in turn affects stocking patterns for the drug products.

A significant percentage of generic products in the market may not be pharmaceutically equivalent to their innovator counterparts. As such, results of clinical studies conducted on the innovator product may not necessarily be applicable to generic products. Consequently, the generic products in the Kenyan market may not be interchangeable with the innovator product and their efficacy may also not be comparable to that of innovator drugs.

4.3   Recommendation

Results of assays and single-point dissolution tests should not be taken as proof of pharmaceutical equivalence, product quality, safety and efficacy. *In vitro* dissolution profile data for generic drug products should be included in routine QC and post-market surveillance tests in order to demonstrate consistent pharmaceutical equivalence to the innovator products.

In addition, stringent GMP inspections should be consistently conducted by the national drug regulatory authority, the PPB to ensure adherence to quality standards during the manufacture and storage of pharmaceutical products. As a further measure, post-market surveillance activities by the PPB should be regular and sustained as a tool for determining the consistency of good quality products in circulation. These measures are important steps in curbing sub-optimal therapeutic outcomes, treatment failures and microbial resistance incidences resulting from exposure to substandard therapeutic agents and will ensure patients get benefit from the generic drug products.

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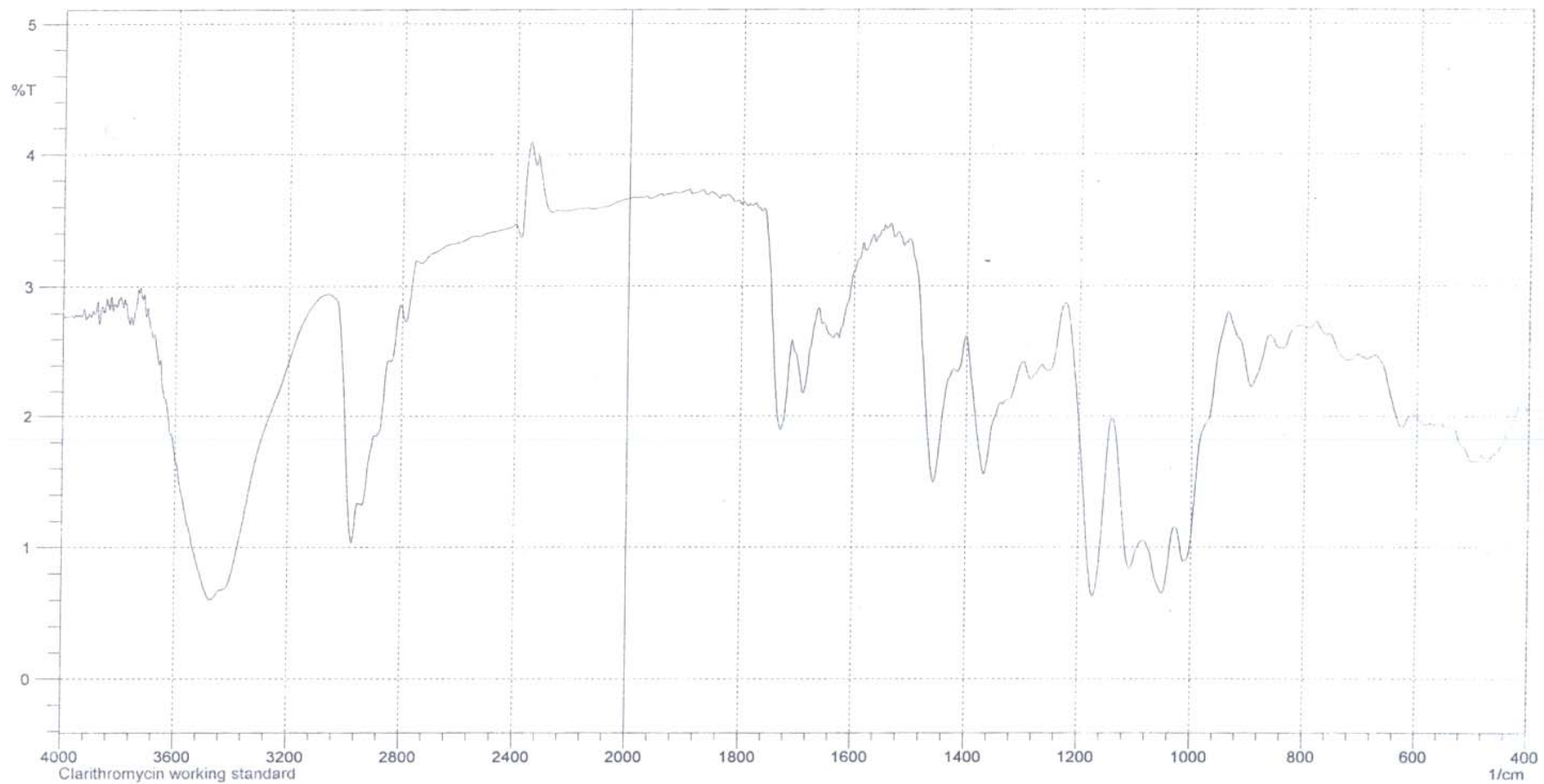
APPENDICES
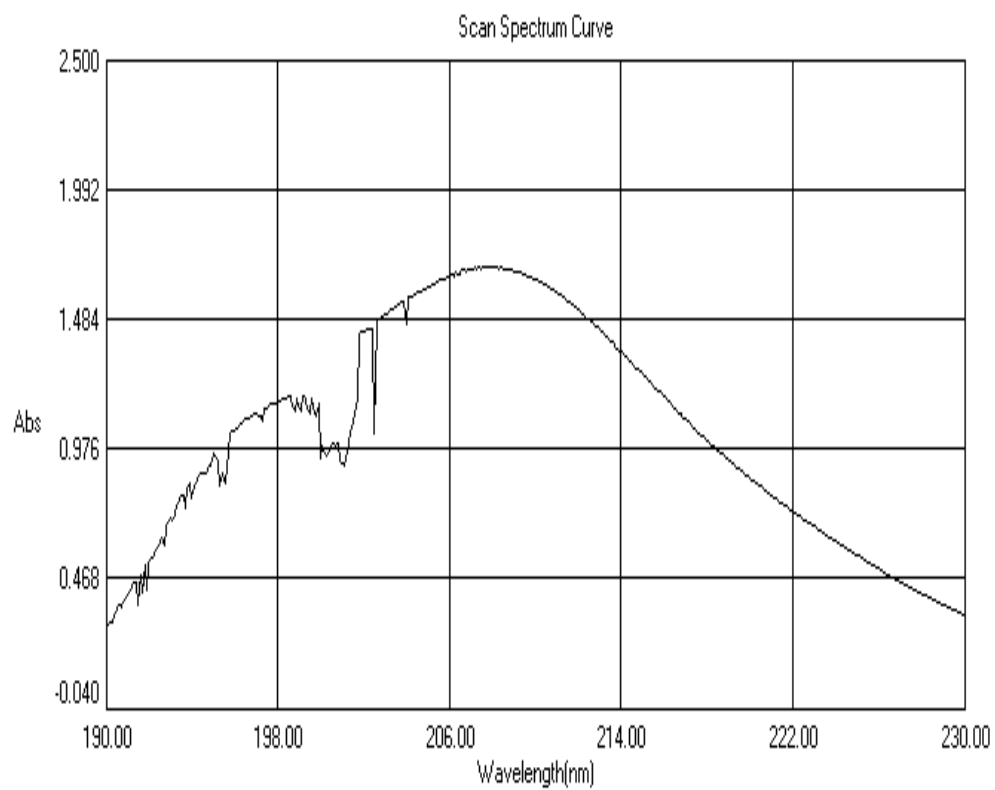
Appendix 1: UV spectrum of 1 mg/mL clarithromycin working standard in 100% acetonitrile
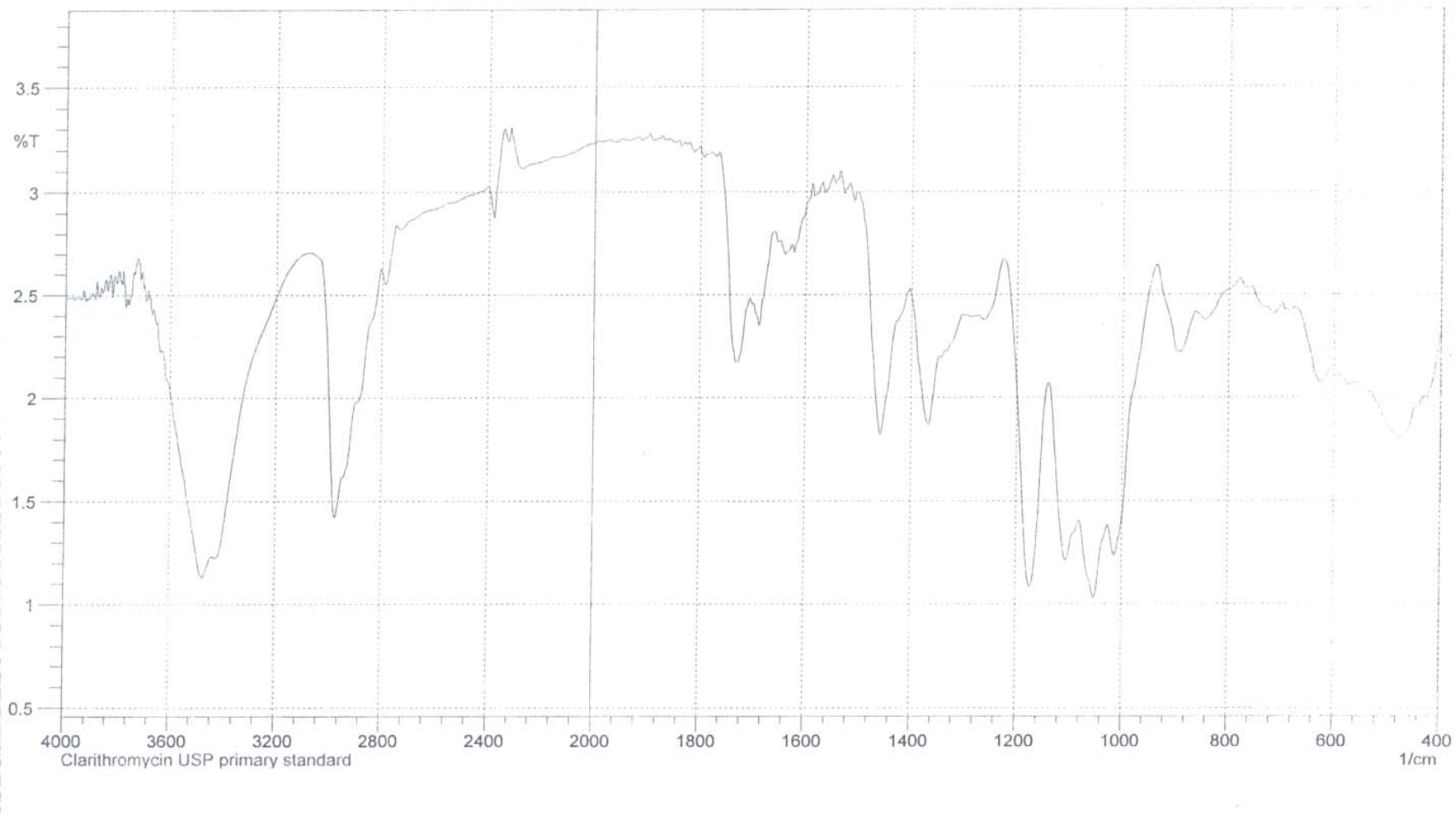
Appendix 2: FTIR spectrum of clarithromycin USP primary standard (KBr Disk)

Appendix 3: FTIR spectrum of clarithromycin working standard (KBr Disk)