QUALITY EVALUATION OF A SELECTION OF ANTIBIOTICS
DISTRIBUTED IN ACCRA (GHANA) AND LAGOS (NIGERIA)

THIS THESIS IS
Submitted to the

UNIVERSITY OF GHANA, LEGON

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MPhil CHEMISTRY DEGREE

BY

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DEPARTMENT OF CHEMISTRY
UNIVERSITY OF GHANA
LEGON
JULY 2013
DECLARATION

I, Mr Horatio Akpoviri Egbo, do solemnly declare that this report has never been submitted to any other University or Academic Institution for the purpose of obtaining an academic award. All the information in this work is based on observations made in my research undertaken under supervision.

Signature ______________________________ Date ________________________

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I am most grateful to my parents, all my siblings, uncle Lawrence Ohue, Mr Ajiboye, Dr Odedede Muarice, Mr Valentine Ojumah, and His Worship ACR Idise for their support.

May God Almighty bless you all and also those whom due to time and space I could not mention here. Amen.
DEDICATION

This work is dedicated to my parents, Ch. Barr. & Mrs T. E. O. Egbo, all my siblings and my friends. God bless you all. Amen.
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ABSTRACT

The persistent prevalence of Counterfeit and substandard drugs poses a clear and present danger globally. Its higher degree of occurrence and under-reporting in developing nations especially in Africa and Asia is of great concern. Antibiotics are amongst the most used and abused in developing countries and as such, the need for its constant surveillance cannot be over emphasised.

This study involved the assessment of the quality of three commonly used therapeutic groups of antibiotics; the macrolides (Azithromycin and Erythromycin) and lincosamides (Clindamycin). This involved the identification and quantification of the active pharmaceutical ingredient (API), and in vitro dissolution test. HPLC and in vitro dissolution tester instruments were used.

Based on the population density, level of industrialization and demand, past incidence of existence of poor quality drugs, and their socio-economic relationship, Accra (Ghana) and Lagos (Nigeria) were chosen for this survey. All the available brands of the three APIs making a total of 45 different brands of the antibiotics were sampled from the two countries. In Ghana, 31% of the drugs sampled were locally manufactured while 69% were imported. Nineteen (19%) of the drugs sampled in Nigeria were locally manufactured while most of the imported ones came from India with 53%. All the samples from Nigeria had their National Agency for Foods and Drugs Administration and Control (NAFDAC) registration number printed on their labels, while only one sample from Ghana had the registration number from Food and Drugs Authority (FDA) printed on its label.

This study revealed that all the samples contained the requisite API. On analysis of the results however, 73% of the total drugs sampled did not comply with the required amount of API
content in accordance with the US Pharmacopoeia standard, while 30% out of the 37 samples in vitro dissolution tested did not comply. Sixty-nine (69%) of drugs collected in Ghana and 75% of those collected in Nigeria did not comply with the API requisite pharmaceutical specification standard. Sixty eight (68%) of the Azithromycin, 67% of Clindamycin, and 86% of Erythromycin failed the API quantitative test as specified by USP. Seventy-five (75%) of the paediatric dosage form of Azithromycin samples assayed did not comply with the USP standard.

All the Azithromycin samples in vitro dissolution tested complied with the USP standard, while 36% of Erythromycin and 67% of Clindamycin complied with the in vitro test. Eighty (80%) of the Ghana samples complied with the in vitro dissolution test, while 67% of the Nigeria samples complied.

These findings suggest the existence of substandard antibiotics in Accra and Lagos. It is therefore recommended that the production companies should be better monitored to ensure the strict adherence to Standard Operating Procedures (SOP) and compliance with Good Manufacturing Practices (GMP) and Good Laboratory Practices (GLP). Furthermore, the need for continuous and effective monitoring of the influx, distribution and quality of drugs in our society through pharmacovigilance cannot be overemphasised.
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<tr>
<td>α</td>
<td>Selectivity</td>
</tr>
<tr>
<td>a</td>
<td>Adult</td>
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<tr>
<td>A</td>
<td>Azithromycin</td>
</tr>
<tr>
<td>ACT</td>
<td>Artesunate Combination Therapy</td>
</tr>
<tr>
<td>API</td>
<td>Active Pharmaceutical Ingredient</td>
</tr>
<tr>
<td>AMR</td>
<td>Antimicrobial Resistance</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the Curve</td>
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<tr>
<td>BCS</td>
<td>Biopharmaceutics Classification System</td>
</tr>
<tr>
<td>C</td>
<td>Clindamycin</td>
</tr>
<tr>
<td>CAD</td>
<td>Corona Aerosol Detection</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge Couple Device Cameras</td>
</tr>
<tr>
<td>cGMP</td>
<td>Current Good Manufacturing Practices</td>
</tr>
<tr>
<td>DAD</td>
<td>Diode Array Detection</td>
</tr>
<tr>
<td>DFID</td>
<td>Department for International Development</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl Sulfoxide</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
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<td>E</td>
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<tr>
<td>FDB</td>
<td>Food and Drugs Board</td>
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<td>FID</td>
<td>Flame Ionization Detection</td>
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<tr>
<td>G</td>
<td>Drugs bought in Ghana</td>
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<td>GC</td>
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<td>GDP</td>
<td>Good Distribution Practices</td>
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<td>Good Storage Practice</td>
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<tr>
<td>N</td>
<td>Drugs bought in Nigeria</td>
</tr>
<tr>
<td>NAFDAC</td>
<td>National Agency for Food and Drug Administration and Control</td>
</tr>
</tbody>
</table>
NDL  N-demethyllincomycin
NAG  Nacetyl glucosamine
NAM  Af-acetylmuramic acid
OOS  Out Of Specification

$p$  Paediatric
PE  Propionyl Erythromycin

$P_S$  Clindamycin peak area responses obtained from the standard solution
PSI  Pharmaceutical Security Institute
PSN  Pharmaceutical Society of Nigeria

$P_T$  Clindamycin peak area responses obtained from the test solution
PTC  Peptidyl Transferase Center

R  Resolution
SFFC  Spurious/Falsely-Labelled/ Falsified/Counterfeit
SON  Standards Organisation of Nigeria
SOP  Standard Operating Procedure
SUPAC  Scale-Up and Post-Approval Changes
THF  Tetrahydrofuran
TNF  Tumour Necrosis Factor
TLC  Thin Layer Chromatography

$t_R$  Retention Time
tRNA  Transfer Ribonucleic Acid

UNODC  UN Office on Drugs and Crime
USAID  United State Agency for International Development
US FDA  United States Food and Drug Agency
USP  United State Pharmacopoeia
UV  Ultra-Violet
UV-VIS  Ultraviolet-Visible
WCO  World Custom Organisation
WHO  World Health Organisation
WHPA  World Health Professions Alliance
$W_S$  Weight in grams of clindamycin hydrochloride taken in the standard
$W_t$  Weight in grams of clindamycin hydrochloride taken in test solution
CHAPTER ONE

INTRODUCTION

1.1 Study Background

The role and importance of pharmaceutical products to the well-being of humans cannot be emphasised enough. However, they have to be safe, efficacious, of acceptable quality and used rationally to produce the desired effect\(^1\).

A drug is of good quality when it meets the efficacy and safety standard claimed by the producers and as published in Pharmacopoeias, and if it also meets the specifications set for it by World Health Organisation (WHO) and other relevant authorities. The suitability of drugs for their intended use is determined by their efficiency, safety and their conformity to specifications regarding identity, purity, strength, and other characteristics\(^2,3\).

The quality of a pharmaceutical product is determined by the numerous activities which occur throughout its life cycle (i.e. from acquisition and handling of raw materials through manufacturing process, packaging, transportation to storage). Also involved are the quality of equipment used and manufacturing environment\(^4\).

Ensuring the safety, efficacy and quality of medicines throughout their lifecycle, their consistency in batches and even across every dose, lie in the strict adherence to Standard Operating Procedures (SOP), compliance with Current Good Manufacturing Practices (cGMP), Good Laboratory Practices (GLP), Good Distribution Practices (GDP) and Good Storage Practices (GSP). According to United States Pharmacopoeia (USP), the quality of
medicines is said to be poor when it does not meet official specifications for strength, quality, purity, packaging or labelling\(^4\text{-}^7\).

Poor quality medicines can be classified into three different main types: Counterfeit substandard, or degraded. These have been linked to chemical instability especially in tropical climates, poor quality assurance and control during manufacture, distribution, and storage; and counterfeiting\(^8\text{-}^{10}\).

According to the WHO, Spurious/Falsely-Labelled/Falsified/Counterfeit (SFFC) medicines (branded or generic) can be classified as “any medicines or pharmaceutical products that are deliberately and fraudulently mislabelled for identity and/or source”. Substandard pharmaceuticals also known as Out of Specification (OOS) products are those that are genuine and legally produced but fall outside the specifications or acceptance criteria established in product dossiers, drug master files, pharmacopoeias or by the manufacturer. A degraded medicine can be classified along with substandard medicine. However, they differ in that they might be originally of specification, but in the course of time naturally or catalysed by external factors, fall out of specification within its shelf life\(^5,^7,^{10},^{11},^{12}\).

Amongst these three groups of poor quality drugs, Counterfeit drugs attract most attention because it is the most serious and dangerous in terms of extent of distribution and effect. Counterfeiting of medicines often referred to as the second oldest profession dates back to the first century in Greece when drugs were first classified according to therapeutic uses by Dioscorides during which he also highlighted and warned of the dangers of consumption, as well as suggested ways of detecting them. It is recorded that fake cinchona bark and quinine date back to the 1600 and 1800s respectively. The increased rate and spread of medicine
adulteration in the mid-19th century led to the establishment of the Code of ethics for Pharmacists and guides on the detection of counterfeits in the United States of America\textsuperscript{4, 13}.

The factors responsible for pharmaceuticals being prone to counterfeiting and attractive candidates for illegal trade include its global usage and immense profit. The industry is estimated to worth about 75 billion dollars\textsuperscript{13}. The WHO also estimates the annual earnings from substandard or counterfeit drugs to be about 200 billion US Dollars\textsuperscript{14}. High value per unit nature, ease of transport, inexpensive means of production, avoidance of quality control and cGMP cost, and most importantly, the fact that the consumer cannot easily ascertain their quality and/or identify them are other contributing factors\textsuperscript{14, 15 – 18}.

The US Food and Drug Agency (US FDA) estimates that 10% of drugs in circulation worldwide are counterfeit while over 50% drug supply in some countries are fake\textsuperscript{19}. The WHO also estimates that 30% of drugs in circulation in developing countries are counterfeit\textsuperscript{20}. Results of basic quality tests conducted by the WHO on medicines in circulation in parts of Africa, Asia and Latin America showed that more than 40% of them are counterfeit\textsuperscript{21}.

As reported in Digital journal, the WHO and the Institute of Research Against Counterfeit Medicines (IRACM) in a warning about the health and safety of African populations declared that in an operation carried out in April 2013 in 23 African countries including Ghana and Nigeria, over 500 million doses of counterfeit medicines worth about 275 million US Dollars were intercepted\textsuperscript{22}. The drugs intercepted included Antibiotics, painkillers, anti-inflammatory drugs, medicines for high blood pressure and diabetes and food supplements\textsuperscript{22}. The Guardian newspaper of December 23, 2012 reported on the threat to lives in Africa and dangers posed
by the treatment of diseases such as malaria with counterfeit medicines imported from Asia. Some of these drugs are reported to possess no active pharmaceutical ingredient\textsuperscript{23}.

The prevalence of poor quality drugs continues to endanger and affect not only health, but also the socio-economic situation adversely. It is a constant embarrassment to healthcare providers and continues to erode the confidence of the public in healthcare delivery systems and the drug regulatory authorities. It contributes to morbidity, mortality, adverse drug reactions, drug resistance, and enormous financial consequences for pharmaceutical companies producing the genuine product\textsuperscript{10}.

The social and health effect of poor quality drugs on the society is enormous such that most people in the affected areas now resort to the old traditional/ancestral ways such as consultation of native doctors, occultism/spiritualism, consumption of unrefined toxic herbs, and other unorthodox means for alternative cure. This can be attributed to the fact that treatment failure, elongated or persistent illness resulting from ignorant consumption of poor quality drugs might be attributed to spiritual problems, and in their desperation for cure seek alternative means.

Drug counterfeiting is a grievous crime tantamount to mass murder hence has become a clear and present danger that urgently needs efficient strategies to combat. If not curbed, counterfeiting can result in more serious consequences such as in biochemical or medicinal warfare. Apart from the effects mentioned above, and the growing concern that monies derived from this sophisticated and organised crime may be geared toward funding terrorism or other social disruption, with the present world trend of terrorism, they can be deliberately produced and distributed to introduce poisons or toxic biological agents to targeted areas.
This may not be for monetary profit, but as an act of terrorism that can have slow, silent, and long-lasting deadly consequences\textsuperscript{4, 24}.

Literature reports show that medicines of all therapeutic categories (both branded and generic) in all regions of the world have been counterfeited. These include expensive lifestyle and anticancer medicines, antibiotics, medicines for hypertension and cholesterol-lowering medicines, hormones, steroids, and inexpensive generic versions of simple pain killers; antihistamines, blood glucose test strips and condoms\textsuperscript{18, 25}.

The varied health requirements and use of medicines in different countries is determined by various factors which include different burdens of disease, susceptibility (antibiotics), economic, ethnic, cultural and dietary factors, sanitary status and other environmental factors. In accordance with the economic theory of demand and supply, these factors in turn determine the most probable types of counterfeit /fake and substandard medicines present in a particular country\textsuperscript{26}.

Unlike the developed nations where life-style drugs such as antiallergic, endocrine agents (such as hormones and steroids), drugs for the treatment of chronic diseases such as anticancer and lipid-lowering drugs as well as drugs for the treatment of erectile dysfunction predominate, drugs for treatment of infectious diseases such as antibiotics, antiparasitic, and other antimicrobials are predominantly found in developing countries and these are the most counterfeited\textsuperscript{27}.

In Africa, due to lack of proper documentation in the health care system, reliable and current data on the prevalence of poor quality medicines and their effects cannot be obtained. Information on studies and surveys on fake, counterfeit and substandard drugs both published
and unpublished are currently obtained in reports from international organisations such as WHO and United Nations Children's Emergency Fund (UNICEF), non-governmental organizations, pharmaceutical companies, national medicine regulatory and enforcement authorities, ad hoc studies on specific geographical areas or therapeutic groups and occasional surveys \textsuperscript{18, 28}.

The high rate of infections in West African countries which has resulted in increased demand for antibiotics, antiretroviral, antiparasitic and anti-tuberculosis drugs has made these countries very vulnerable to the drug counterfeiting business. The chaotic nature of importation and distribution of pharmaceuticals, the porous nature of African borders, inadequate legislation and weak enforcement and penalty for counterfeiters, corruption and inefficient cooperation between stakeholders, and poor database on health related activities are amongst the contributing factors to the continued existence and circulation of counterfeit and substandard drugs in these countries \textsuperscript{27, 29 - 33}.

The WHO and the United States Pharmacopoeia (USP) joint study on the content and dissolution of anti-malarial and anti-tuberculosis drugs in six West African countries in 2010 revealed that anti-malarial and anti-tuberculosis products from Nigeria recorded the highest failure rate of over 70%, Ghana over 60% and Cameroon over 50%, Kenya and Tanzania had relatively low failure rates whilst Ethiopia had all its products passing the tests \textsuperscript{34}.

Literature and news reveal that recently, most studies and surveys on the quality of medicines in this part of the world have focused on antimalarial, anti-retroviral and anti-tuberculosis drugs with less attention paid to other antimicrobials used for other infectious diseases. Recently the Chemistry Department of the University of Ghana embarked on quality survey on antimalarial drugs distributed in Ghana, Togo and Malawi. Similar studies have been also
carried out in West Africa by other universities such as Kwame Nkrumah University of Science and Technology (KNUST) and the WHO\textsuperscript{34, 35}.

Not much attention has been paid to antibiotics and antibacterial agents in recent times, and this might imply that the extent of counterfeit and substandard drugs or medicines in this category circulating in the sub region may not be accurately known. Counterfeiters and poor quality drug manufacturers may take advantage and profit from this lack of pharmacovigilance. Furthermore, proper attention has not been paid to quality of paediatric medicines. According to an analysis of paediatric medication use conducted as part of Medco's drug trend study, over one in every four insured children in USA and about 30% of adolescents aged 10 to 19 took at least one prescription medicine to treat a chronic condition in 2009\textsuperscript{36}. According to the WHO, Counterfeit drugs kill an estimated 200 children a day\textsuperscript{37}. In Nigeria the NBC News reported that 84 children died between 2008 and 2009 from acute kidney failure after the consumption of diethylene glycol toxic solvent contained in fake teething syrup\textsuperscript{38}. According to literature, Ghana and Nigeria are said to have significant problems with substandard pharmaceuticals\textsuperscript{35}. As a result this study aims at conducting a survey in one principal city in each country.

Azithromycin, Erythromycin and Clindamycin antibiotic drugs were chosen for this study based on the fact that they are on the WHO List of Essential Medicines for Ghana and Nigeria\textsuperscript{39, 40} and also due to the availability of Reference standard. Oral interviews made in four major government hospitals in the Accra metropolis (Legon hospital, Achimota hospital, 37 Military hospital, and Korle-Bu Teaching Hospital) indicated them to be commonly prescribed antibiotics. Furthermore, quality studies have not been recently carried out on the chosen drugs in Nigeria and Ghana. An added factor is high rate of poor quality drugs
discovered in Nigeria and Ghana according to the WHO’s report on recent survey done in Sub-Saharan African countries\textsuperscript{41}. Identification and quantification of their active pharmaceutical ingredient as well as dissolution tests, using the HPLC and Dissolution techniques based on the US pharmacopoeia standards were used for this study.

1.2 Aims and Objectives

As poor quality drugs advance globally with focus on developing countries, coupled with the fact that the consumer cannot safely and easily ascertain product quality on his or her own; the need to increase the tempo of pharmacovigilance in our society becomes paramount. Thus it becomes necessary for concerned individuals/members of the public to complement the efforts of the governments, legitimate pharmaceutical companies, organizations concerned with health, trade, and security, in the continuous monitoring of the quality of drugs in circulation.

Ghana and Nigeria are members of Commonwealth Nations and both belong to the Economic Community of West African States (ECOWAS), and as such have political, economic and social relationships. As a result of this there is regular flow of goods (including drugs), services and population between these two countries. This is corroborated with the fact that one of the samples collected in Ghana had Nigeria’s NAFDAC registration number on its pack even though it was not manufactured in Nigeria.

The present study aims at the quality survey of commonly used antibiotics in Ghana and Nigeria using the WHO International Pharmacopoeia recommended standards, to determine if the sampled drugs comply with the individual governments’ regulatory and pharmacopoeia
specifications; and to give an insight of not only the quality status of antibiotics in Ghana and Nigeria, but of drugs generally in circulation.

The specific objectives of this study are:

- The quantitative check of the API of three commonly used antibiotics (Azithromycin, Erythromycin, and Clindamycin) in Ghana and Nigeria using HPLC methods.
- To use dissolution tests to further confirm the drugs’ quality in terms of their ability to release the Active Pharmaceutical Ingredient (API), if present and in the right quantity.
- To determine the existence of, and to estimate the degree of poor quality antibiotics in circulation
- To determine and compare the extent of the counterfeit and substandard drugs menace in the two countries.
- To contribute to the assurance of safety of medicines and thus promote pharmacovigilance activities in our society
- To provide information to the public and contribute to information exchange on drug safety in our society.
2.1 Poor Quality Medicines

Though the three main categories of poor quality medicines (degraded, substandard and counterfeit defined and introduced in chapter one are interwoven / or interrelated such that they are not easily distinguished, their differences are better appreciated from their characteristics. These are highlighted below:

2.1.1 Counterfeit Medicines

Some of the major identifying characteristics of this category are that, they are deliberately, illegally, and secretly produced with the intent to deceive. According to WHO’s confidential drug-related reports received between January 1999 and October 2000 from 20 countries, counterfeit drugs were categorised based on the possession of one or more of the following characteristics:

- Products without active ingredients,
- Products with incorrect quantities of active ingredients,
- Products with wrong ingredients,
- Products with correct quantities of active ingredients but with fake packaging
- Products which are mislabelled, and
- Products with high levels of impurities and contaminants

The National Agency for Food and Drug Administration and Control (NAFDAC) in Nigeria includes the following categories in addition to those mentioned by the WHO:

- drugs without the full name and address of the manufacturer,
• herbal preparations that are toxic, harmful, ineffective or mixed with orthodox medicine,

• expired drugs or drugs without expiry date, or expired and re-labelled drugs with the intention of extending their shelf-life, and

• drugs that are not certified and registered by NAFDAC\textsuperscript{43, 44}.

2.1.2 Substandard Medicines

Substandard medicines can be classified as those legally branded or generic products that do not meet official standards for identity, quality, purity, strength, packaging, and labelling. One of their defining characteristics is that they contain the correct APIs but deviate in amounts in excess of ± 15. These can result during production either deliberately (to maximize profit), due to non-compliance with SOP, cGMP, or due to equipment error or malfunctioning, or can result at any part of the medicine continuum due to mishandling. They can also result from degradation\textsuperscript{8, 45 - 47}

2.1.3 Degraded Medicines

Degraded medicines can be considered a subclass of substandard medicines in that they could have been manufactured to meet specification before being degraded. Degradation rate is related to the stability of the product which is dependent on several factors amongst which are: quality of the raw materials used, the storage and distribution conditions/facilities, and the environmental conditions (ambient temperature, humidity, and light) in which it is stored. Degradation can occur at any stage in the chain from acquisition of the raw material to consumption of the finished product by the patient. Poor storage conditions usually lead to the degradation of the agents making them lose their biological activities earlier before the
expiry dates on their labels. This is a challenging issue in developing countries (most of which have tropical climates), as poor storage conditions, high temperature and high humidity conditions generally enhance chemical degradation and may alter the biopharmaceutical properties of the drugs earlier than the expiry dates on their labels. Storage conditions of the medicine in the home by consumer can also be a contributing factor to degradation\textsuperscript{10,12}.

2.2 Human Sensory Identifiable Characteristics of Substandard/Counterfeit Drugs

Poor-quality packaging and /or mislabelled packages / falsified holograms, possession of strange smell, taste, or colour, low friability and irregular shaped tablets are amongst the usual visual characteristics used to identify counterfeits. High literacy and modern equipment have made counterfeiting so sophisticated that it is now almost impossible to detect counterfeits by visual inspection only. There has been improvement / perfection not only in mimicking the label, but also other parameters such as tablet shapes /size, and general appearance including tablet colour that can be identified without the use of equipment\textsuperscript{1,15,27,48}.

Substandard drugs most of the time are not easily identifiable by human sensory organs unless there are obvious (colour) changes in the appearance of the label or drug substance itself. It is difficult for consumers to make the identification as these are all relative parameters; to make sense out of them one has to have the knowledge of and be able to compare them with those of standard / genuine products. Under normal circumstances, most consumers are not privy to this information.
2.3 Causes of Poor Quality Drugs

The causes of poor quality drugs can be broadly grouped into two categories: natural causes (e.g. substandard/degraded drugs) and artificial/man-made and/or deliberate causes (e.g. counterfeit/fake/substandard drugs). Natural causes of poor quality drugs involve degradation of the drugs naturally with time. It is connected with the shelf-life of the drug and is determined by that of the production starting material such as the API or the excipients. Natural degradation can be influenced by the storage environmental condition (e.g. excessive decomposition of active ingredient as a result of high temperature and humidity, light, and etc.), poor packaging and etc. Poor storage conditions most likely increase the existence of degraded medicines in developing countries as the recommended storage temperature cannot be maintained due to lack of constant electric power\(^\text{10}\).

Artificial causes of poor quality drugs could either be deliberate and/or non-deliberate. These can result from the deliberate use of insufficient required amount of or low-quality API, lack of expertise, poor manufacturing practices, insufficient infrastructure, deliberate falsification, poor quality assurance and quality control, use of faulty and old machines, use of substandard materials, poor transportation and possible contamination by impurities\(^\text{46, 47}\).

2.4 Factors That Facilitate / influence The Prevalence of Counterfeit Drugs

Just as the type of drug in circulation vary in different regions in accordance with the Health requirements and the use of medicines, different burdens of disease, economic, ethnic, cultural and dietary factors, and the level of development of a system for the regulation of medicines; so also the factors that influence their continued existence and circulation though somewhat general but slightly vary between developed and developing countries.
The major factors that influence the prevalence of counterfeit/falsified medicines in developing countries including West Africa can be linked to a variety of causes amongst which are:

- It being a lucrative business\(^{10}\)
- The chaotic nature of most pharmaceutical distribution\(^{15}\)
- Weak and ineffective border control\(^{34}\)
- Economic globalisation and internet trading\(^{18}\)
- Corruption\(^{34}\)
- Involvement of too many intermediaries and non-professionals in the medicines business\(^{10, 31}\)
- Inadequate legislation, Weak enforcement and penal sanctions\(^{18}\)
- Lack of political will and commitment and Conflicts of interests\(^{18}\)
- Inefficient cooperation between stakeholders, and\(^{15}\)
- Irrational use of drugs\(^{15}\)
- Lack of awareness among health professionals and consumers\(^{18}\)

### 2.5 Effects of Poor Quality Medicines

The existence and administration of poor quality medicines have enormous health, economic and social effects. The gravity of the effects in Ghana and Nigeria as well as in most parts of the developing countries cannot be accurately assessed and quantified due to lack of proper surveillance and documentation of their health activities. The adverse consequences both on the individual patients and society in general can be identified. According to the International Policy Network, “Counterfeit drug incident around the world causes an estimated 700,000 deaths annually from malaria and tuberculosis alone\(^{33, 49}\). The impact of substandard and counterfeit drug on the society and individuals even though slow to be
noticed, is enormous, and the impact on stakeholders such as the pharmaceutical industries is immediate and drastic\textsuperscript{10, 15, 17, 48, 50}.

Administration of poor quality drug is believed to be one of the major causes of drug resistance. The drug resistant pathogen that evolves in one geographical region due to poor quality drug administration can be spread to another region through migration and free movement of persons (as in ECOWAS sub-region). Selection of drug-resistant pathogens will lead to increased morbidity, mortality and a significant economic burden on developing regions of the world\textsuperscript{10}.

2.6 Brief Review of the quality of drugs trends in Africa

Pharmaceutical crime poses a major threat to public health across the world and it poses grave danger to innocent victims who unknowingly consume the products of this crime\textsuperscript{51}. The current medicine situation caused by the persistence of poor quality drugs calls for continuous survey of drugs in circulation in our society. Measures to combat this menace have intensified across the globe and this is evident in the increased growth of pharmacovigilance in developing countries, especially in Africa where the number of countries with good pharmacovigilance has increased by about 78\% between 2000 and 2010. Nigeria and Ghana are among those that have responded to this challenge\textsuperscript{52}. Based on the WHO study carried out between 1999 and 2002, Antibiotics account for 28\% of global counterfeit medicines and are reported to be the most counterfeited therapeutic class of drugs\textsuperscript{53}. It is impossible to quantify the extent of the problem, but several incidences of the existence of poor quality drugs in West Africa have been reported. Several studies have been made on quality of drugs around the globe, though majority of them are on antimalarial
and anti-tuberculosis therapeutic class of drugs. Some studies carried out in African countries are highlighted below.

- In a 2013 publication, Dr Kei Robert et al. reported a study of the quality performance of Erythromycin from different manufactures in Kenya. Fifty samples were assayed using microbial method, disintegration time and friability to test their potency. It resulted that all the samples failed the microbial assay test\(^5^4\).

- A 2012 publication by Saba et al. reports a survey done in South Western Nigeria. The results show that the mean API concentration of all the commercial samples of Oxy-tetracycline (Veterinary use) analysed was below that which is claimed on the label by the manufacturers\(^5^5\).

- A survey done of 18 African countries including Ghana and Nigeria by Roger Bate et al. in 2012 revealed that 4.1% of the 1437 samples of Ciprofloxacin analysed failed the Minilab test. 11 of them failed visual test and these turned out to possess no requisite API\(^5^6\).

- A 2013 publication in BioPortfolio reveals that a total of 33 antibiotic eyedrops containing following substances: ciprofloxacin, levofloxacin and ofloxacin, gentamicin and tobramycin were analysed for quality. All samples were found to contain the requisite API however, 9 (27%) of the samples were under dosed and 10 (30%) were over dosed\(^5^7\).

- A 2010 publication by Getu et al. revealed that a quality survey of 6 brands of ciprofloxacin tablets distributed in Ethiopia resulted in all the samples passing the identity, disintegration, and dissolution tests except ‘Ciflox’ brand which failed the dissolution test\(^5^8\).

- In R. B Taylor et al. report of 2001, 76 (55%) out of the175 antibacterial samples from Nigeria analysed were not compliant with the British Pharmacopoeia standard in
terms of API content. 10 (27%) out of the 37 Amoxicillin, 28 (61%) out of the 48 Ampicillin, 11 (55%) out of the 20 Benzylpenicillin, 3 (33%) out of the 9 Cloxacillin, none out of the 3 Dapsone, 13 (68%) out of the 19 Doxycycline, and 31 (76%) out of the 41 Metronidazole samples were not compliant with British Pharmacopoeia standards in terms of API content.  

- Maike Tipke et al. publication of 2008 on quality of 77 antimalarial distributed in Burkina Faso revealed that 32 (42%) out of the 77 drug samples were found to be of poor quality. 28 samples failed the visual inspection, 9 samples were substandard, 4 samples showed poor disintegration, and one sample contained none of the stated active ingredient.

2.6.1 Poor Quality Drugs in Ghana

Ghana, one of the countries with high economic activities in West Africa is not left out in the situation of persistent occurrence of counterfeit and substandard drugs. Like Nigeria, frightening levels of occurrence of counterfeit and substandard drugs have been discovered and reported. According to the Food and Drugs Authority (FDA), the commonly counterfeited drugs in Ghana include antibiotics, anti-malarials, anti-diabetics, and aphrodisiacs. Also, fake local herbal products containing dangerous substances such as brake fluid, saw dust and turpentine have been discovered in Ghana. A 2013 publication of Ghana Business News reports an alert by FDA Ghana on circulation of fake drugs in Ghana. These drugs were identified as Mimet (ergometrine) tablets and Rox-Clav 625 tablets (a combination of amoxicillin and clavulanic acid).
It is on record that 35% of antimalarial drugs imported into Ghana in 2008 were counterfeit, and according to research conducted in that same year at Kwame Nkrumah University of Science and Technology (KNUST), 82% of sampled artesunate sold in pharmacies in Kumasi were substandard. Also two separate researches were conducted in 2010 and 2011 by the Chemistry Department of University of Ghana, Legon on antimalarial drugs in Ghana. That of 2011 showed that Artesunate API content in all ACTs (Artemisinin-based Combination Therapy) and monotherapies antimalarial drugs sampled were under-dosed while 87% of amodiaquine-containing drugs analysed met the International Pharmacopoeial (Ph. Int) standard in terms of API content. In 2008, thirteen key antimalarial medicines in Ghana were found to be substandard and/or counterfeit. This was discovered during the medicine monitoring cooperative program set up by Ghana Food and Drug Authority in collaboration with the US Pharmacopoeial Convention (USP) and the US Agency for International Development (USAID)\textsuperscript{63–65}.

Meanwhile, the FDA in association with other related agencies have not relented in their efforts to curb this menace in Ghana. In 2010, after laboratory analysis of anti-malaria drugs sampled from public and private hospitals, retail pharmacies, licenced chemical shops and wholesale facilities across the country, some batches including Metakelfin tablets, Artesunate tablets and Quinine sulphate discovered to be counterfeit were recalled. In the same year, substandard antibiotics including: Cipro-Dor (Ciprofloxacin Hydrochloride equivalent to 500mg Ciprofloxacin) and Clavu-Dor (Amoxicillin 500mg and Clavulanic Acid 125mg) tablets were recalled. In 2011, substandard Camoquin-Plus suspension manufactured by Pfizer was also recalled from circulation in Ghana\textsuperscript{66,67}.
2.6.2 Poor Quality Drugs in Nigeria

Counterfeit medicines were first discovered in Nigeria in 1968 when the authority of Crown Agents was deregulated as the sole importer/distributors of medicines. This led to the promulgation of the Food and Drug Decree No. 35 in 1974 and later the formation of The National Agency for Food and Drug Administration and Control (NAFDAC) in 1993 with one of its major objectives being monitoring the quality of drugs. The situation progressively worsened and by 2001, more than 40% of medicines in circulation nationwide were fake. There was a drop in the incident rate of counterfeit medicines in 2005 by 15.7% and by 2008 this unprecedentedly increased to 64%. This increase could be probably due to the sophistication and expertise of the counterfeiters\(^4,68\).

According to NAFDAC, in 2010 alone the agency destroyed fake drugs worth about 1.6 Billion Naira; while in 2011, the total amount of substandard drugs that were confiscated and subsequently destroyed was N627m. Also, in Awka, Anambra State, NAFDAC destroyed fake, expired and substandard drugs and cosmetics worth N279m in 2012\(^69\). Poor quality Erythromycin has been reported in Nigeria\(^10\).

Nigeria has a long history of counterfeit antibiotics. Table 1 below show a list of counterfeit antibiotics detected in Nigeria along with the year of discovery\(^70\).

<table>
<thead>
<tr>
<th>Year</th>
<th>Type of Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1988</td>
<td>Neomycin</td>
</tr>
<tr>
<td>1996</td>
<td>Ampicillin, Gentamycin, Tetracycline, Metronidazole, Ciprofloxacin, Erythromycin</td>
</tr>
<tr>
<td>1997</td>
<td>Amoxicillin</td>
</tr>
<tr>
<td>2000</td>
<td>Amoxicillin, Ampicillin + cloxacillin</td>
</tr>
<tr>
<td>2001</td>
<td>Amoxicillin, Ampicillin, Ampicillin + cloxacillin, Cloxacillin, Metronidazole,</td>
</tr>
<tr>
<td>Year</td>
<td>Antimicrobial Combination</td>
</tr>
<tr>
<td>------</td>
<td>----------------------------------------------</td>
</tr>
<tr>
<td>2002</td>
<td>Trimethoprim + sulfamethoxazole</td>
</tr>
<tr>
<td>2003</td>
<td>Ampicillin</td>
</tr>
<tr>
<td>2006</td>
<td>Amoxicillin</td>
</tr>
<tr>
<td>2009</td>
<td>Amoxicillin + clavulanic acid, Chloramphenicol</td>
</tr>
<tr>
<td>2010</td>
<td>Cefuroxime axetil, Amoxicillin + clavulanic acid, Ampicillin + cloxacillin</td>
</tr>
<tr>
<td>2011</td>
<td>Ciprofloxacin</td>
</tr>
</tbody>
</table>

The outcome of these studies coupled with the report of the study carried out by WHO in collaboration with USP mentioned earlier on page 6 confirms the existence of counterfeit and/or substandard drugs in Nigeria and also buttresses the hypothesis that its prevalence vary in different countries depending on demand, border porosity, ineffective monitoring and regulation of drug influx and distribution, lack of law enforcement and poor sources of drug.

### 2.7 Antibiotics’ Quality and their Demand

The use of antibiotics to fight against infection and the importance of their application in other medical technologies such as transplants, surgery and chemotherapy make them one of the most commonly prescribed in hospitals and in the world in general. Antibiotics constitute about 5% of the total global pharmaceutical sales and have a market currently worth about 31 billion US dollars. Furthermore, the vulnerability of children to infections is another factor for the high demand for antibiotics. This buttresses and explains the influx and circulation of antibiotics worldwide and it is not surprising that they are the most counterfeited therapeutic group in the WHO’s counterfeit drugs collated between 1999 and 2002 (Figure 1)\(^{18, 19, 71 - 75}\).
According to the Ghana FDA, “Antibiotics are the largest prescribed medication in the books of the Ghana Health Service, and the demand for them cannot be met on a consistent basis”\textsuperscript{76}. It is reported that Azithromycin is one of the most commonly used antibiotics, and one of the best-selling drugs worldwide, Erythromycin is among the well-established antibacterials frequently used in medical practice and the most frequently used macrolide for paediatric administration, and clindamycin now has renewed interest after clinical trials of successfully using it to treat over 500 patients of malaria infection in Africa, South America, and Asia between 1975 and 1994 proved its efficacy and safety\textsuperscript{77 - 79}.

\textbf{2.8 Antibiotics}

The word antibiotic derived from the Greek words anti (against) and bios (life) was first used by Selman Waksman and his collaborators in journal articles in 1942 to describe any substance produced by a microorganism that is antagonistic to the growth of other microorganisms in high dilution\textsuperscript{80}. An antibiotic can be defined in a broader sense as a microbial metabolite or synthetic structural analogue which, in small dosage regimens, inhibits the growth and survival of
microorganisms without any serious toxicity whatsoever to the parent host. It must therefore possess the following four cardinal characteristics:

- A product of metabolism
- Synthetic product produced as a structural analogue of a naturally occurring antibiotic.
- Antagonizes the growth and/or the survival of one or more species of microorganisms, and
- Is effective in low concentrations.

In everyday use, the word antibiotic denotes the subclass of antimicrobials that are active against bacteria\textsuperscript{81}, and will be deemed as such or interchangeably for the purpose of this study.

2.8.1 Classifications of Antibiotics

There are several classification schemes for antibiotics. They can be classified based on their effective range, their microbial origin, chemical structure, their mode of action or based on their method of synthesis. Based on effective range, they can be termed broad-spectrum (Tetracyclin), medium-spectrum (Erythromycin), or narrow spectrum (polymixins). Based on their mode of action, they can be classified as bactericidal (i.e. kill bacteria), and bacteriostatic (i.e. prevent bacteria growth), or they can also be classified based on their mechanisms/target site\textsuperscript{82}.

Also, based on their chemical structure, they can be classified as beta-lactam antibiotics (e.g. penicillin), Cephalosporins, Carbapenems, Monobactams, Aminoglycosides, tetracyclines and glycyclcyclines, Macrolides and ketolides, Bacitracins, Fluoroquinolones, polyene
antibiotics, nitro furan derivatives, and so on. And, based on microbial origin, they can be classified as penicillins - derived from fungi- whose names ends with the suffix –cillin, as in ampicillin; cephalosporins whose names begin with the prefix cef- or ceph; those from Streptomyces species whose names ends with the suffix –mycin as in streptomycin; and those from Micromonospora species whose names end with the suffix –micin as in gentamicin.  

2.8.2 Mechanism of Antibiotic Action

Chemotherapy is based on the exploitation of biochemical differences between the host and the parasite. The differences in molecular structural features between prokaryotic and eukaryotic cells form the bases of antibacterial action. The antibacterial drugs exert their effects at one of four fundamental structural components of bacteria which include: the bacterial cell wall, bacterial cell membrane, nucleoid, and the ribosomes. Antibiotic mechanisms of action include inhibition of cell wall synthesis, activation of enzymes that destroy the cell wall, increase of cell membrane permeability, and interfering with protein synthesis and nucleic acid metabolism. The five main mechanisms by which antibacterial agents act are:

- Inhibition of cell metabolism
- Inhibition of bacterial cell wall synthesis
- Interactions with the plasma membrane
- Disruption of protein synthesis: translation, and
- Inhibition of nucleic acid transcription and replication.
2.8.3 Antibiotic Resistance

Antimicrobial resistance is said to occur when a microbe is capable of growth and multiplication in the presence of an antimicrobial agent. Bacteria with the capability of replicating every 15-20 minutes, has excellent ability to adapt easily to the presence of antibiotics thereby developing resistance to drugs. This occurs by the acquisition of the necessary genetic information which they can easily pass across to others through several mechanisms. Bacteria develop several mechanisms to fight back against antibiotics and these include production of enzymes that bind to and deactivate antibiotics, expulsion of antibiotics through the use of expelling pumps, change of target sites, and the counter of drug action by synthesising excess of the enzymes targeted by antibiotics\textsuperscript{46, 77, 86, 87}.

2.9 Brief Review of the Antibiotic Groups under Study

The three antibiotics studied in this survey, Azithromycin, Erythromycin and Clindamycin belong to the Macrolide, and Lincosamide groups of antibiotics respectively. It is pertinent for the purpose of this study to have an insight of them.

2.9.1 Macrolide Antibiotics

The macrolides belong to the polyketide class of natural products whose activity is based on the presence of a large macrocyclic lactone ring (usually 14, 15, or 16-membered), to which is attached two sugar moieties (a neutral sugar, cladinose, and an amino sugar, desosamine). The sugar moieties are important for activity and that carrying the substituted amino-group is responsible for their overall weakly basic (pKa $\sim 8$) chemical character. Shown in figure 2 is the structure of macrolide molecule showing possible positions for its derivatives.
Since the discovery of its first member, the 14-membered macrolide Erythromycin in 1952, the macrolide group has attracted considerable interest and the attention of many researchers. This could be due to its broader spectrum of activities, its non-antibiotic therapeutic activities, its ease and inexpensive means of production, and the suitability of its backbone structure to modifications, thus enabling its use as a template for the production of several advanced macrolides and ketolides with broader spectrums of activity, improved pharmacokinetic properties, and therapeutic activities\(^{57, 77}\).

Apart from the clinically useful macrolide antibiotics consisting of a macrocyclic lactone ring containing 14, 15, or 16 atoms with sugars linked via glycosidic bonds, the macrolide families also comprise of the non-antibiotic macrolide group such as: tacrolimus, pimecrolimus, and sirolimus drugs used as immunosuppressants or immunomodulators, with similar activity to cyclosporine; and the toxic macrolides such as the mycolactones\(^ {88}\). This is summarized below:

1. The 14-membered ring macrolide comprises
• Erythromycin isolated by a group of Filipino scientist in 1949 from the metabolic products of a strain of *Streptomyces erythreus*.

• Clarithromycin invented in the 1970s by scientists of Taisho Pharmaceutical company in Japan and,

• Roxithromycin produced in 1987 by Hoechst Uclaf pharmaceutical company in Germany, and Dirithromycin.  

2. The 15-membered ring macrolide comprise of Azithromycin, the first of the azalide group of antibiotics discovered in 1970 by PLIVA pharmaceutical company based in Zagreb, Croatia and produced in 1980.

3. The 16-Membered Macrolide Antibiotics most of which are applied in veterinary medicine comprises: Carbomycin A, Josamycin, Kitasamycin, Midecamycine acetate, Spiramycin, Troleandomycin, and Tylosin.

Macrolides are primarily bacteriostatic, with potential for a time-dependent bactericidal action particularly at high concentrations. They are active on both gram-positive and, to a lesser extent, on gram-negative microorganisms. They are lipophilic in nature and are extensively distributed in body fluids and tissues. Their ion trapping and high lipid solubility generally often cause tissue concentrations to be higher than serum concentrations. The macrolides have proven to be well tolerated in the treatment of many infectious diseases. These include upper respiratory tract infections such as acute pharyngitis, lower respiratory tract infections and sexually-transmitted diseases such as uncomplicated urethritis or cervicitis, genital ulcer disease caused by primary or secondary syphilis. The versatility of the macrolides extends beyond their uses as antibiotics to other therapeutic activities including immune-modulating/anti-inflammatory effects. They are therefore used to treat non-bacteria-associated diseases, such as severe asthma, chronic obstructive pulmonary diseases and, more...
recently, cystic fibrosis. Some macrolides, due to the exhibition of peculiar pharmacokinetics resulting in a phenomenon known as the “delayed death phenotype”, proffers anti-parasitic activity, and are thus effective in treatment of malaria. Furthermore, some are reported to possess anti-cancer, and gastrointestinal motor stimulating activity.\cite{57,78,89-92}

2.9.1.1 Azithromycin

Azithromycin shown in figure 3 below is a subclass and the first representative of the second-generation of macrolides called Azilides. It is Semi-synthetically derived from Erythromycin A (EA), by the structural modification of the Erythromycin A basic frame. It is a white monohydrate crystalline salt with molecular formula of C$_{38}$H$_{72}$N$_2$O$_{12}$, molecular weight of 767.02. The systematic (IUPAC) name is (2R,3S,4R,5R,8R,10R, 11R,12S,13S,14R) -13-[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one. Its Solubility in water is 39 mg per mL (pH 7.4) at 37 °C. It has melting point range of 113 – 115°C, and pKa value of 7.9. It is very soluble in alcohols, acetone, chloroform, acetonitrile, and ethyl acetate and moderately soluble in ether, ethylene chloride, and amyl acetate.\cite{58,60,90}

![Figure 3: Structure of Azithromycin](image-url)
2.9.1.2 Erythromycin

Erythromycin is a slightly hygroscopic, crystalline, or powder like compound. It occurs as a slightly yellow almost odourless compound with a melting point range of 135 – 140°C, and pKa value of 8.8. Chemically, erythromycin A consists of three structural parts: a 14-membered poly-hydroxy-lactone macrolide skeleton (a 9-keto group), connected to two sugars, a neutral sugar and an amino sugar. The aglycone portion of the molecule, erythranolide is a 14-membered lactone ring. Two deoxysugars, D-desosamine and L-cladinose (CH3-O-3-mycarose) are attached to the lactone ring. The amino sugar, desosamine, is attached through a β-glycosidic linkage to the C-5 position of the lactone ring, and the second sugar, cladinose, which is unique to erythromycin, is attached via a β-glycosidic linkage to the C-3 position of the lactone ring.

Erythromycin, with molecular weight of about 733 Da, pKa of about 8.8 and chemical stability within the range of pH 3.0–10.5 has its basic character conferred to it by the tertiary amine of the desosamine sugar. Through this a number of acid salts of the antibiotic (semi-synthetic derivatives) have been synthesised. Erythromycins are a group of antibiotics comprising of erythromycin A (EA) as the main and active product. Other related compounds found in small amounts include erythromycin B (EB), erythromycin C (EC), erythromycin D (ED), erythromycin E (EE), and erythromycin F (EF). They have similar structure and nearly identical physiochemical properties, but have varying antibacterial activities. Erythromycin exists in various forms. The chemical structures of the major component of erythromycin base, erythromycin A (EA) is shown below in figure 4.

93, 97, 100, 101
2.9.1.3 Shortcomings of Erythromycin

Erythromycin is unstable in stomach acid environment. The acid sensitivity is due to the presence of a ketone and two alcohol groups which are acid-catalysed leading to the intramolecular formation of a ketal as shown in figure 5 below. One way of preventing this is to protect the hydroxyl groups; another way which is mostly applied is by coating the tablets to minimize this effect. The unpleasant taste is partially overcome with water insoluble dosage forms; this also reduces acid instability and gut cramp. Enteric coatings are beneficial in reducing these adverse effects as well

Apart from the general side effects and drug interactions common to macrolide class of antibiotics, Erythromycin has some shortcomings. It can extensively be used only on gram-positive bacteria and has very limited use against gram-negative bacteria owing to poor permeability of their outer membrane which is attributed to the action of efflux pumps. High
incidence of phlebitis and gastrointestinal adverse effect including nausea, vomiting and diarrhoea, have been consistently reported. Antimicrobial resistance by some strains including *P. aeruginosa* and *K. pneumoniae* to Erythromycin has also been reported. Resistances to erythromycin among methicillin-susceptible *S. aureus* (MSSA) and *Streptococcus pyogenes* has also been detected^102 - 107^.

### 2.9.1.4 Advantages of Azithromycin over Erythromycin

Azithromycin has good stability at low pH. Over the stomach pH range it is 300 times more stable than erythromycin because of the presence of the extra nitrogen atom. It has excellent tissue penetration, lower toxicity and a longer half-life, and higher oral bioavailability compared to Erythromycin. The structural modification of Azithromycin offers it numerous pharmacological advantages over its parent Erythromycin. Its unbound volume of distribution, unbound clearance, and apparent plasma elimination half-life has increased from 4.8 L Kg\(^{-1}\), 55 mL min\(^{-1}\) kg\(^{-1}\), and 3 hours in erythromycin to 62 L Kg\(^{-1}\), 18 mL min\(^{-1}\) kg\(^{-1}\), and 48 hours respectively in Azithromycin. The tissue distribution of azithromycin compared to the plasma or blood concentration may be 10-to 100-fold higher, compared to only 0.5- to 5-fold higher for erythromycin^60, 88, 91, 108, 109^.

Unlike Erythromycin and other macrolides, it has the ability to penetrate into eukaryotic and prokaryotic cells; and it is has activity against gram-negative bacteria. Its long elimination half-life, excellent tissue penetration and its ability to reach and maintain long optimum peak concentration, proffers its post-antibiotic effect and hence is able to exhibit anti-parasitic activity unlike erythromycin. Furthermore, apart from its comparatively lower gastrointestinal effects, it does not inhibit CYP3A4 as erythromycin does, and hence possesses lower drug interaction. Its comparatively better pharmacokinetic profile enables it
to move rapidly from blood to tissues. It remains in the tissue for prolonged periods at levels that are higher than the minimum inhibitory concentration for many common pathogens, this gives it a better dosage regime of once a day administration and hence better patient compliance than Erythromycin\textsuperscript{60, 91, 110 - 116}.

2.9.2 Lincosamides

The lincosamides are glycosidic antibiotics that contain an unusual sugar moiety, \(\alpha\)-amino-6,8-dideoxy-1-thio-D-erythro-\(\alpha\)-D-galactooctopyranoside (methylthiolincosamide) linked by an amide bond to the amino acid derivative \(\text{trans-N-methyl-4-n-propyl-L-proline (propylhygric acid)}\). Amongst its several hundred synthetic and semi-synthetic derivatives, including about 20 biosynthetic derivatives, only Lincomycin A, and its semi-synthetic derivative clindamycin are therapeutically used, with clindamycin being 4 to 16 times more active than lincomycin. This is probably due to amongst other reasons, their low bioactivity and/or high toxicity\textsuperscript{117}.

Lincosamides with the parent structure shown in figure 6 have so many closely related naturally occurring members in addition to lincomycin A, clindamycin, and lincomycin B (4’-depropyl-4-ethyllincomycin). Figure 6 below is the backbone structure of lincosamide showing possible positions for its derivatives. Some of the major members include 7-substituted-7-deoxylincomycins derivatives with substitutes at R1 and R2\textsuperscript{117}. 

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The first chemically classified member of the lincosamide group of antibiotics called Lincomycin, with a typical feature of 6,8-dideoxy-6-amino-octose lincosamine present in its molecule was discovered and isolated in 1962 from a soil actinomycete classified as *Streptomyces lincolnensis var. lincolnensis* in Lincoln, Nebraska. Lincomycin and clindamycin are clinically important antibiotics. According to literature, they inhibit most Gram-positive bacteria, the genera *Staphylococcus* and *Streptococcus* in particular. They are the first choice bacteriostatic antibiotics used in veterinary dermatology. At normal *in vivo* concentration, Lincosamides exhibit bacteriostatic activity, but at higher concentrations their effect may be bactericidal\textsuperscript{117}.

Clindamycin exists as the hydrochloride and produced by the fermentation of *Streptomyces lincolnensis* in a process which involves firstly, the separate synthesis of the two building units - the synthesis of aglycone propyl-L-proline from L-tyrosine, and the sugar moiety – methylthiolincosamide followed by their NDL synthetase catalysed condensation to N-demethyllincomycin (NDL). The final step is the N-methylation of the propylproline moiety of NDL \textsuperscript{97}.  

![Figure 6: Backbone structure of Lincosamides](image)
2.9.2.1 Clindamycin

Clindamycin is a drug of choice for several infectious diseases. It has replaced the parent compound therapeutically due to its broader antimicrobial spectrum, enhanced gastrointestinal absorption and activity against Gram-positive bacteria and reduced side effects. Its proven efficacy, safety and convenience of parenteral and oral administration in patients are also advantages. Furthermore, it is believed to be 4 to 16 times comparatively more active than the parent compound.\(^{79, 118-123}\)

Clindamycin (7-chloro-7-deoxylincomycin) is produced by replacing its hydroxyl group at 7-position with a chlorine atom, resulting in an inversion of the configuration. It is available in various dosage forms which include clindamycin hydrochloride (methyl 7-chloro-6, 7, 8-trideoxy-6-[(2S,4R)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-1-threo-D-galacto-octopyranoside monohydrochloride) for oral administration in capsules, clindamycin phosphate (Methyl-7-chloro-6, 7, 8-trideoxy-6-[[2S, 4R]-1-methyl-4-propylpyrrolidin-2yl] carbonyl amino]-1-thio-L-threo-a-D-galacto-octopyranoside 2 (dihydrogen phosphate)) for intramuscular or intravenous injection, and clindamycin palmitate for oral suspensions.\(^{79, 118, 124, 125}\)

The structures of Lincomycin, Clindamycin, and Clindamycin phosphate are shown in the figure 7 below.\(^{122}\)
Clindamycin is Antibacterial, anti-parasitic, anti-inflammatory. Its modes of action vary in accordance with its intended therapeutic activity. It may be bacteriostatic or bactericidal depending on the clinical situations, the organism, and drug concentration. It is capable of inhibiting bacterial proteins, toxins, enzymes, and cytokines once bacteria are inside the target tissue\textsuperscript{79, 118, 119, 126 - 129}.

2.10 Brief Review of Some Methods of Analysis Commonly Used For Drug Quality Assurance

Generally, the pharmacopoeia specifications represent part of the essential requirements for the overall quality control and assurance of pharmaceuticals that contribute to the safety and efficacy of medicines. It comprises a collection of recommended procedures and methods for analysis, and specifications for the determination of pharmaceutical substances, excipients and dosage forms; which include: Labelling, Basic Tests and Assays.

General methods of analysis that are commonly used in carrying out the tests and assays included in monographs standards, specifications and guidelines are categorised into Physical and Physicochemical Methods, Chemical methods, and Biological methods. Some of these include

- Spectrometry in the infrared (IR) region and Ultraviolet-visible (UV-VIS) regions,
• Chromatographic methods - High performance liquid chromatography (HPLC) and Thin Layer Chromatography (TLC),
• Chemical methods such as wet chemistry titrations (acid-base, complexometric, potentiometric, etc) and
• Biological methods such as microbiological assay of antibiotics ¹³⁰.

Highlighted below are some of the commonly used assay instrumental methods.

2.10.1 Spectrometric Methods

Spectrometric methods are the most commonly used methods in pharmaceutical analysis. They involve the absorption and emission of light and can be used for quantification and identification purposes. The quantitative analysis phenomenon is based on the degree to which light is absorbed, or the intensity of light that is emitted being related to the amount of an analyte present in the sample tested. This is in accordance with Beer Lambert’s basic law for spectroscopic quantitative analysis which shows how a sample concentration is related to a measure of radiation intensity in a spectrometer. It is more simply expressed as $A = abc$; where $A$ is called the absorbance, defined as: $A = \log_{10} \left( \frac{1}{T} \right)$ and $T$ represents Transmittance and $C =$ concentration. Qualitative analysis is based on the absorption signatures of the chemical compound ¹³¹,¹³².

Spectrometric methods of organic analysis involve molecules absorbing light due to the presence of chromophores (absorbing groups or functional groups) at maximum wavelengths unique to them. The intensities are proportional to the number and type of chromophores present in the molecule, and dependent on their proximity, configuration, and interaction ¹³³.
2.10.1.1 UV-VIS Molecular Spectrometric Methods

Ultraviolet–visible (UV-VIS) molecular spectrometric methods use light in the ultraviolet and visible regions of the electromagnetic spectrum for the analysis of molecular compounds and complex ions. The light energy absorbed results in electronic transitions from the ground level to an excited state occurring in the analyte molecules and complex ions\textsuperscript{131}.

In the UV-VIS range, absorption of light causes electronic and vibrational excitations. Relaxation of excited states back to the ground state may cause emission or luminescence, which are evaluated spectroscopically. Qualitative analysis (identification of unknowns and detection of impurities in known samples) is accomplished by comparing absorption or transmission spectra (molecular fingerprints) with known spectra. Quantitative analysis is accomplished with the use of Beer’s law. UV-VIS scanning spectrometers consist of a light source, a monochromator, a chopper (rotating sector mirror or rotating sector disc) to generate a sample and a reference beam as well as to recombine them, a sample and reference compartment, and a detector. Standard UV-VIS detectors are photomultipliers and silicon diodes and the recently developed Charge Couple Device Cameras (CCD) cameras\textsuperscript{131, 134}.

2.10.1.2 Infrared (IR) Spectrometry

Infrared spectrometry is one of the few analytical techniques that can be used to analyse any sample virtually in any state. Liquids, solutions, pastes, powders, films, fibres, gases and surfaces can all be examined with a judicious choice of sampling technique. The IR spectrum can be used as a type of “fingerprint” unique to a molecule, allowing the establishment of the presence or absence of many chemical functional groups such as phenyls and carbonyls. Also, Quantitative analyses of mixtures can be obtained from it\textsuperscript{131-135}. 
The most convenient identification process is the use of vibrational spectral databases to match the vibrational spectrum of the Analyte molecules. This can be done manually or can be much accelerated with the use of computerized search programs. A more chemical approach for qualitative/structural analysis is the evaluation of characteristic or group frequencies, which is based on the fact that some chemical groups exhibit very characteristic bands regardless of the kind of molecule in which they are included; and from the frequencies and intensities of the spectral bands it is possible to predict the kind of chemical groups present in the molecule, and how they are connected to other groups\textsuperscript{134}.

Quantitative analysis procedures using infrared spectrometry utilize Beer’s law. The baseline method involves the selection of an absorption band that is separated from the bands of other matrix components, then drawing a straight line tangent to the absorption band. The value of the absorbance, log \((P_0/P)\), is then plotted against concentration for a series of standard solutions, and the unknown concentration is determined from this calibration curve\textsuperscript{131, 133}.

The improved instrumentation has made it possible to develop a variety of new sensitive techniques in order to examine formerly intractable samples. The advent of refined optical systems and fast computational techniques has made the hyphenated combination of Gas Chromatography (GC) with Fourier-transform IR spectrometry the second most important structural identification tool after GC/mass spectrometry\textsuperscript{132, 135}.

2.10.2 High-Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography or High Pressure Liquid Chromatography commonly known as HPLC is an efficient and effective separation technique routinely used for both qualitative and quantitative analyses of environmental, pharmaceutical, industrial, forensic, clinical, and consumer product samples \textsuperscript{136}.  

In HPLC, a liquid sample, or a solid sample dissolved in a suitable solvent, is carried through a chromatographic column by a liquid mobile phase. Separation is determined by solute/stationary-phase interactions, such as: (liquid–solid adsorption, liquid–liquid partitioning, ion exchange and size exclusion), and by solute/mobile-phase interactions. Though its sole function is separation, its ability and ease of being hyphenated to other analytical devices such as Liquid Chromatography / Mass Spectrometer (LC-MS), Liquid Chromatography / Nuclear Magnetic Resonance (LC-NMR), makes it a major analytical tool applied at all stages of drug discovery, development, production in the modern pharmaceutical industry\textsuperscript{137}, and in post marketing quality surveys.

It differs from the normal column Chromatography in that the analyte and the mobile phase are pumped under high pressure through a column of more compactly packed small diameter stationary phase, thus resulting in more effective and comparatively quick separation. HPLC is classified into Normal-Phase and Reversed-Phase based on the type of column and mobile phase used; and the type used being determined by the nature of the analyte.

The mechanism of HPLC is based on either one or a combination of adsorption, partition, ion exchange or size exclusion, depending on the type of stationary phase used. The separation of the analyte is due to a complex retention mechanism involving the participation of the analyte in different processes either singly or a combination of two or more processes in the column which include ionization, solvation, and other secondary equilibria processes\textsuperscript{130, 137}. The apparatus consists of Solvent Reservoirs, pumping system, an injector, a chromatographic column, stationary and mobile phases, connecting tubing and fittings, a detector, and a data collection device (computer, integrator or recorder)\textsuperscript{130, 137}. 

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The separation output of a standard HPLC system is represented in the form of a chromatogram. Each specific analyte in the Chromatogram is represented by a peak which is normally symmetrical and resembles a typical Gaussian (normal distribution) curve. When using a UV-Vis detector, the resulting chromatogram is a plot of absorbance as a function of elution time\textsuperscript{136}.

Four major descriptors commonly used to characterize the chromatographic column, system, and particular separations through the chromatogram are (i) Retention Factor (k), (ii) Efficiency (N), (iii) Selectivity (α), and (iv) Resolution (R)\textsuperscript{137}. The Retention Time (tR), which is the distance of the peak maxima from the injection point expressed in time units is the most widely used descriptor used as an identifier (qualitative) for the given analyte on the chromatogram; while Area Under the Curve (AUC) is used in comparison with that of a reference as the parameter used for quantitative analysis.

2.10.2.1 HPLC Method Development

Method development plays a decisive role in the effective and efficient use of HPLC. In everyday method development practice, it is important to ensure the separation of target compounds, matrix components, and other impurities. A multitude of approaches can be applied to arrive at one that can give desired results. For Pharmaceutical uses, a good method should be able to separate the active pharmaceutical ingredient (API) from the drug product, degradation products, excipients, excipient degradation products, and any synthetic impurities that are present in the API\textsuperscript{137}.

Factors to consider when developing a method include properties of the analyte, detector, mobile phase, stationary phase, gradient considerations, and the maximum run time for analysis. The initial steps include, collecting as much information about the properties of the
analyte such as its structure, physical and chemical properties (including (pKa, log P), toxicity, purity, hygroscopicity, solubility, stability, and ionizability. These will determine the type of column, mobile phase, and even the type of detector to be used. These data may be available from the literature on similar compounds, or from past experience with similar compounds.

The screening of different columns is based on four distinct characteristics namely; Type (monolithic; porous; nonporous), Geometry (surface area, pore volume, pore diameter, particle size and shape, etc.); Surface chemistry (type of bonded ligands, bonding density, etc.); and Type of base material (silica, polymeric, zirconia, etc.). The most common columns used for initial method development include those comprised of C18, polar embedded/end-capped and/or phenyl bonded ligands. The quality and type of column chosen is dependent on the nature of the analytes (i.e. if acidic, basic, or neutral) and even on the chromatographic conditions used. The chemical nature of the ligands bonded on the surface of support material defines the main type of chemical interactions of the surface with eluent and analyte molecules.

The properties of a stationary phase are determined by the nature of the organosilane’s alkyl group. If R is a polar functional group, then the stationary phase will be polar. Examples of polar stationary phases include those for which R contains a cyano (–C2H4CN), diol (–C3H6OCH2CHOHCH2OH), or amino (–C3H6NH2) functional group. These are used in normal-phase chromatography with the mobile phase being a nonpolar or moderately polar solvent. In reverse-phase chromatography, the stationary phase is nonpolar and the mobile phase is polar. The most common nonpolar stationary phases use an organochlorosilane for which the R group is an n-octyl (C8) or n-octadecyl (C18) hydrocarbon chain.
Analytes that are labile (i.e., react with protic solvents) or that exhibit poor solubility in aqueous media are prime candidates for normal-phase chromatography. Normal phase is well-suited for the separation of isomers and diastereomers, as well as for separating compounds with saturated and unsaturated side chains. Choice of the mobile-phase and gradient conditions is dependent on the ionogenic nature of the analyte and the hydrophobicity of the analytes in the mixture respectively. These factors have impact on the analyte selectivity, especially for ionizable compounds.

Several indices have been developed to assist in selecting a mobile phase, the most useful of which is the polarity index. The elution order of solutes in HPLC is governed by polarity and the polarity can be adjusted by mixing together two or more of the mobile phases. In a normal-phase separation the least polar solute spends proportionally less time in the polar stationary phase and is the first solute to elute from the column, while in a reverse-phase separation the order of elution is reversed, with the most polar solute being the first to elute. Furthermore, the elution can be Isocratic where separation is done using a single mobile phase of fixed composition; or Gradient Elution where the composition of the mobile phase, other parameters, and hence polarity are changed with time.

Mobile phases commonly used in reversed-phase HPLC are hydro-organic mixtures. The most common reversed-phase organic modifiers include methanol and acetonitrile and/or combinations of these two modifiers. Others include, Tetrahydrofuran (THF), Isopropyl Alcohol (IPA), and Dimethyl Suloxide (DMSO). The mobile phase can be modified by adjusting the pH. Based on the analyte pKa, buffers with good buffering capacity can be used to improve peak shape.
Choice of the detector is dependent on the properties of the analyte. The most common detector used in pharmaceutical analysis is UV (ultraviolet), which allows monitoring and continuous registration of the UV absorbance at a selected wavelength. Diode Array Detection (DAD) which span over a range of wavelengths are also used. However, the majority of reversed-phase and normal-phase HPLC method development in the pharmaceutical industry is carried out with UV detection.\textsuperscript{137}

Different types of detectors that can be hyphenated with HPLC include: ultraviolet (UV), fluorescence, electrochemical, light scattering, refractive index (RI), Flame Ionization Detection (FID), Evaporative Light Scattering Detection (ELSD), Corona Aerosol Detection (CAD), mass spectrometric (MS), NMR, and others.\textsuperscript{137}

\subsection*{2.10.3 In Vitro Dissolution Test}

Due to its high degree of patients’ compliance and ease of administration, oral route has become the most used route of administration. Studies have shown that oral drug delivery accounts for more than half the share of the global drug delivery market. On a 2008 publication on market insight it was estimated that at the end of 2012, sales of oral formulations in the United States alone would have reached $56.7 billion.\textsuperscript{138} The journey of an orally administered drug to its target site begins with its disintegration before dissolution. This is followed by its absorption in the gastrointestinal (GI) tract, more specifically, the small intestine; and then finally its elimination, mostly done by the kidney.\textsuperscript{138,139}

In addition to other quality checks, the use of in vitro dissolution and drug release test as a quality control tool greatly assists to validate quality surveillance checks on drugs in circulation. The importance of in vitro dissolution and drug release test in post marketing
drug quality surveillance cannot be overemphasised. Even if qualitative and quantitative compliances check out, the question of the API being fully released into the (GI) tract as expected still remains unanswered.

In order to be effective, the dissolution and release of a drug’s API in the GI tract is of utmost necessity. This is because absorption / permeability across the GI tract take place in soluble form and the therapeutic activity of a drug depends on the bioavailability of the API at the target site. This in turn depends on dissolution and release of the API from the drug. So many factors including API excipient interaction, and coating degradation that may occur during poor storage conditions could affect the release of the API under the physiological conditions in human GI tract. Dissolution from the dosage form is more of a product property. It involves mainly two steps: the liberation of the drug from the formulation matrix (disintegration) followed by the dissolution of the drug (solubilisation of the drug particles) in the liquid medium, which is dependent on factors which include: the physicochemical properties of the drug such as its chemical form (e.g., salt, free acid, free base) and physical form (e.g., amorphous or polymorph and primary particle size).  

Drug dissolution testing plays an important role in drug development, production, quality control and assurance. Because the conditions of the in vitro dissolution environment is set to mimic that of the physiologic condition of the GI tract, it can be used to predict / calculate in-vivo bioequivalence. Thus it can be used to waive bioequivalence requirements (biowaivers) for dosage forms. It is also used to predict or signal potential problems with in vivo bioavailability. In vitro dissolution has also found use in quality assurance and quality control. It is used to monitor batch-to-batch consistency and as also used as one of the criteria
for accepting sameness of drugs under *Scale-up and Post-Approval Changes (SUPAC)* 139, 140.

The *in vivo* behaviour (i.e. bioavailability/bioequivalence) of a drug is represented by the concentration-time profile in humans. This can be predicted using *in vitro–in vivo correlation* (IVIVC) either by a mathematical model which describes the relationship between *in vitro* and *in vivo* properties of a drug product, techniques based on de-convolution or convolution which may be obtained with the use of simple spread sheet software. *In vitro–in vivo correlation* (IVIVC) application ranges from drug and product development to their scale up and post-approval changes 139,141,142.

2.11 A Brief Review of HPLC Conditions Used For Analysis of the Drugs under Study

Various analytical methods like UV, HPLC, HPTLC and LC/MS have been reported for the determination of azithromycin, Erythromycin and Clindamycin in its tablet or capsule or suspension dosage forms either in single or in combination therapy. HPLC method was used for the analysis of the antibiotics in this study. The Tables 2, 3, and 4 below show a brief review of some HPLC conditions used in the analysis of Azithromycin, Erythromycin and Clindamycin respectively.
### Table 2: HPLC Conditions used for the analysis of Azithromycin

<table>
<thead>
<tr>
<th>Ref</th>
<th>Column Type</th>
<th>Mobile phase</th>
<th>Buffer</th>
<th>Flow Rate</th>
<th>Detector /WL</th>
<th>Temperature</th>
<th>Retention Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>USP 2010&lt;sup&gt;143&lt;/sup&gt;</td>
<td>4.6 mm x15-cm column that contains 3-µm packing L49 without the guard column.</td>
<td>KH₃PO₄ buffer: Acetonitrile (2130 : 870 ml v/v)</td>
<td>5.8g of KH₃PO₄ dissolved in 2130ml of water and adjusted to pH 11.0 with 6ml of 10N KOH</td>
<td>0.4 mL per minute</td>
<td>amperometric electrochemical detector</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>K.A. Shaikh, 2008&lt;sup&gt;144&lt;/sup&gt;</td>
<td>Waters Xterra RP18 (250nm×4.6mm, 5m)</td>
<td>acetonitrile–dipotassium phosphate (30mM) (50:50, v/v) (pH 9.0)</td>
<td></td>
<td>1.7 ml/min</td>
<td>at 215 nm</td>
<td>50°C</td>
<td>11.5 min</td>
</tr>
<tr>
<td>T. Ghari, 2012&lt;sup&gt;145&lt;/sup&gt;</td>
<td>C18 column, 5µm, 250 mm×4.6mm</td>
<td>An isocratic methanol/buffer mobile phase at the ratio of 90:10</td>
<td>0.02M Phosphate buffer at pH (8)</td>
<td>1.5ml/min</td>
<td>UV detection at 215 nm</td>
<td>50°C</td>
<td>6min.</td>
</tr>
<tr>
<td>Patricia Zubata, 2002&lt;sup&gt;146&lt;/sup&gt;</td>
<td>LiChroCART® 125×4.6 mm HPLC</td>
<td>mixture of buffer, acetonitrile and methanol (60:20:20) pH 8.0</td>
<td>2.88 g of ammonium phosphate Monoacid in 500 ml of water, 45.6 ml of a 10% solution of tetra-butylammonium phosphate in water was added made up to 1000 ml with water</td>
<td>1.0 ml/min, 215 nm</td>
<td>room temperature</td>
<td>roughly 5 min</td>
<td></td>
</tr>
<tr>
<td>Zi Yi Yang, 2009&lt;sup&gt;147&lt;/sup&gt;</td>
<td>Dikma Technologies Diamonsil C18 column, 5m 150mm×4.6mm</td>
<td>mixture ammonium dihydrogen phosphate (0.045 M):acetonitrile 47:15 (v/v)</td>
<td>0.002M sodium heptanesulfonate. the ammonium dihydrogen phosphate (0.045M) was adjusted to pH 3.0 by phosphoric acid.</td>
<td>UV detector operated at 210 nm</td>
<td>25 °C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sachin Kumar Singh et al., 2010&lt;sup&gt;148&lt;/sup&gt;</td>
<td>Gemini C-18 column (Phenomenex, 150×4.6 mm, 5 µm particle size)</td>
<td>Acetonitrile: 0.5 % Formic acid as mobile phase (Isocratic A: B = 40: 60 % v/v), (isocratic elution)</td>
<td>0.5% formic acid</td>
<td>1 ml/min</td>
<td>UV detector 215 nm</td>
<td></td>
<td>3.0mins.</td>
</tr>
<tr>
<td>Ref</td>
<td>Column Type</td>
<td>Mobile phase</td>
<td>Buffer</td>
<td>Flow Rate</td>
<td>Detector/WL</td>
<td>Temperature</td>
<td>Retention Time</td>
</tr>
<tr>
<td>-----</td>
<td>-------------</td>
<td>--------------</td>
<td>--------</td>
<td>-----------</td>
<td>-------------</td>
<td>-------------</td>
<td>--------------</td>
</tr>
<tr>
<td>L. Van den Bossche, 2010</td>
<td>Waters XTerra RP C18 column, 250mm×4.6mm i.d., 3.5m</td>
<td>Gradient elution: mobile phase Acetonitrile: 0.2MK$_2$HPO$_4$ buffer: H$_2$O. Mobile phase A=(35:5:60, v/v) and mobile phase B = (50:5:45, v/v).</td>
<td>0.2MK$_2$HPO$_4$, at pH 7.0</td>
<td>1.0 ml/min</td>
<td>UV detection at 210 nm</td>
<td>65 °C</td>
<td></td>
</tr>
<tr>
<td>H.K. Chepkwony, 2000</td>
<td>Hypersil BDS C 5 mm column</td>
<td>(A) 2-methyl-2-propanol–2-propanol–0.2 M phosphate buffer pH 7.5inwater (8.5:8.5:5:78); (B) 2-methyl-2-propanol–acetonitrile–0.2 M phosphate buffer pH 7.5–water (22:5:5:68)</td>
<td></td>
<td>1.0 ml/min</td>
<td>UV–Vis detector at 215 nm</td>
<td>30°C</td>
<td></td>
</tr>
<tr>
<td>Jacqueline W et</td>
<td>Astec C18 Polymeric, 5</td>
<td>0.02 M K2HPO4 buffer</td>
<td>0.02 M potassium</td>
<td>1</td>
<td>(UV)</td>
<td>50 °C</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: HPLC Conditions used for the analysis of Erythromycin
<table>
<thead>
<tr>
<th>Ref</th>
<th>Column Type</th>
<th>Mobile phase</th>
<th>Buffer</th>
<th>Flow Rate</th>
<th>Detector /WL</th>
<th>Temperature</th>
<th>Retention Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>(White D. et al., 2006)</td>
<td>Waters Xterra RP18 column (4.6mm×100 mm, 3.5m).</td>
<td>Gradient elution. Mobile phase A: 90:10 carbonate buffer: acetonitrile; mobile phase B: 20:80 carbonate buffer: acetonitrile.</td>
<td>10mM carbonate buffer with pH adjusted to 10.5 using concentrated hydrochloric acid.</td>
<td>1.0 mL/min</td>
<td>UV detection at 214 nm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japanese Pharmacopoeia, 2009</td>
<td>octadecylsilanized silica gel column (25 cm x 4.6 mm x 5 μm)</td>
<td>Buffer: Acetonitrile (55 : 45)</td>
<td>0.05 mol/L potassium dihydrogen phosphate TS adjusted to pH 7.5 with 8 mol/L potassium hydroxide TS</td>
<td>Flow rate adjusted to give Rt of 10 minutes</td>
<td>UV detector at 210 nm</td>
<td>25°C</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Chinese Pharmacopeia, 2008</td>
<td>GraceSmart™ C18, 5μm, 250 x 4.6mm</td>
<td>Methanol:Ammonium Dihydrogen Phosphate Solution (300:210)</td>
<td>2.88g Ammonium Dihydrogen Phosphate dissolve in 1000ml Water and adjust with Phosphoric Acid to pH3.0</td>
<td>1.0mL/min</td>
<td>UV at 214nm</td>
<td>30°C</td>
<td>4.866</td>
</tr>
<tr>
<td>Hui Zhou et al., 2006</td>
<td>reversed-phase C18 column (YMC-PACK ODS-A, 150mm ×4.6mm i.d., 5μm, 120Å”)</td>
<td>Acetonitrile: Phosphate buffer (50:50, v/v).</td>
<td>6.8 g of monobasic potassium phosphate dissolved in 1 l of water, and adjusted with 8M potassium hydroxide to pH 7.5</td>
<td>0.8 ml/min,</td>
<td>G1314A Variable-wavelength detector (VWD) at 210 nm.</td>
<td>25 °C</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4: HPLC Conditions used for the analysis of Clindamycin**
CHAPTER THREE

THE PRESENT STUDY

3.1 Introduction

The current surge in the availability of counterfeit and substandard drugs in developing countries has created a wave of drug screening with emphasis on anti-parasitics such as antimalarial, anti-retroviral and antimicrobial drugs. Literature reveals that, in recent times much attention has not been paid to the quality of the selected antibiotics under study that are circulating in Ghana and Nigeria. As a result, there is greater tendency for counterfeiters to target such drugs which are not in the spotlight. Genuine pharmaceutical companies, either in a bid to maximize profit or out of negligence, may also produce substandard products of these drugs.

Antibiotics are among the most commonly prescribed drugs in healthcare centres. They are frequently prescribed for acute respiratory tract infections (especially in children), acute diarrhoea and other gastrointestinal symptoms. They also compliment medical procedures such as surgery, chemotherapy and transplant which require antibiotics to ward off infection.

Due to their importance in primary healthcare delivery, the administration of counterfeit and / or substandard antibiotics poses a grave danger. Children will be most affected because their immune systems are not fully developed and therefore are prone to infections. Thus there is an urgent need for quality survey of this group of therapeutic agents.

In the present study, a selection of available brands of azithromycin, erythromycin and clindamycin distributed in two major cities in West Africa: Accra (Ghana) and Lagos
(Nigeria) were evaluated for their quality. The criteria for the selection of the products were based on: inclusion in the countries’ essential drugs, high consumption rate, therapeutic importance, and availability of reference standard.

The methods employed were HPLC for quantitative determination of the active pharmaceutical ingredient (API) for comparison with the manufacturer’s label claim and dissolution test to ascertain the bioavailability of these drugs when administered. The latter is a particularly very important quality parameter since it ultimately determines how much of the drugs taken become available to the target site for physiological action. The analyses were carried out in accordance with United States Pharmacopoeia specifications.

3.2 Sampling

Samples were bought from two of the major and most populated cities in West Africa (Lagos, Nigeria and Accra, Ghana). In Lagos, samples were collected from the Mushin Local Government Area, and in Accra, samples were collected around Legon and the central business area. All available brands of the three selected antibiotics - azithromycin, erythromycin and clindamycin - totalling 45 were collected and enough of each brand was purchased to accommodate the qualitative, quantitative and dissolution tests. All the products were obtained from the open market which included distributors, pharmacies, and licenced chemical stores in the respective countries. They were stored in a cold dry place as prescribed by the manufacturers to avoid any decomposition prior to the various analytical tests. Details of all the drugs purchased for this study are given in Appendix 1.
3.2.1 Categories of Samples Collected

A total of 45 different samples comprising 8 (18%) paediatric antibiotic dosage forms (oral suspension), 21 (47%) tablet and 16 (35%) capsules dosage forms were collected from Ghana and Nigeria. These consisted of 25 (56%) Azithromycin, 14 (31%) Erythromycin and 6 (13%) Clindamycin brands of antibiotics. The thirteen samples collected in Ghana comprised 8 Azithromycin, 4 Clindamycin and 1 Erythromycin brands. Twenty five brands of Azithromycin, 14 Erythromycin and 6 Clindamycin were sampled in Nigeria.

Only one brand of adult dosage form of Erythromycin was found in Ghana. All attempts to get other brands in both countries were unsuccessful, thus indicating that these could be the only brands available in circulation. The reason for this could not be ascertained but it could be as a result of the decisions of the drug regulatory bodies of the two countries on which antibiotic drugs they deem suitable for their country based on the difference in health requirements, burden of diseases, history and type of resistance present, susceptibility, economic, ethnic, cultural and dietary factors. The pictorial representations of these are shown in figures 8 – 13 below.

![Figure 8: Types of Antibiotics Sampled.](image)

![Figure 9: Percentage representation of type of antibiotic sampled.](image)
Figure 10: Samples’ dosage form composition.

The brands of the same APIs found in Ghana and Nigeria were quite different. More brands of these antibiotics were available in the Nigeria market as compared to Ghana. For instance only one brand of adult Erythromycin (locally produced) was found on the Ghanaian market, while 14 different brands were found in Nigeria. This could be as a result of the decisions of the drug regulatory bodies of the two countries on which antibiotic drugs are deemed suitable based on the differences in health requirements, burden of diseases. Quality and source of drug, history and type of resistance present, susceptibility, and economic, ethnic, cultural and dietary factors may have also contributed.

Zithromax oral suspension brand of Azithromycin was found in both countries. Though they were manufactured in different countries (the one in Ghana was from Pfizer Company in
Italy, while that from Nigeria was from India), the one collected in Ghana had NAFDAC registration number on its pack.

3.2.2 Origin / Sources of the samples

Lack of expertise and high cost of research equipment have hampered many countries, especially developing countries, from becoming self-sufficient in terms of meeting the medical and pharmaceutical requirements of their people. Hence there is over reliance on importation of drugs, even essential medicines by developing countries. The production of drugs locally has so many advantages over imported drugs since it makes it easier for drug regulatory authorities to enforce laws on good manufacturing practice (GMP) and strict adherence to pharmacopoeial standards through various quality control mechanisms like pharmacovigilance. Same cannot be said about imported drugs since most laboratories in developing countries who unfortunately are the worse sufferers from substandard and counterfeit drugs do neither have the expertise nor the sophisticated equipment to thoroughly access the quality of the diverse drugs imported into their countries on a regular basis.

Apart from the advantages of the possibilities of first hand GMP and post market monitoring of locally manufactured drugs, lower prices, availability and easy access to drug product are other added advantages. More importantly, counterfeiting and the existence of substandard drugs will be reduced as these factors will discourage counterfeiting. Furthermore, it will be easier for vigilant regulatory authorities to trace and stop local counterfeiters.

Thirty-one (31%) of the antibiotics sampled in Ghana were locally manufactured, while only 19% of those sampled in Nigeria were locally manufactured making Ghana a bit more self-dependent in terms of drug production compared to Nigeria. Importation of drugs into Ghana
according to the survey was England (15%) with a reputation of good quality drugs and imports from India accounting for 15%. On the other hand, India followed by China, both with poor reputation in quality drug manufacture were the highest source of the importation of drugs into Nigeria with 53% and 13% respectively, thus accounting for more than half of the drugs circulating in Nigeria. Ghana and Nigeria seem to have different sources of these drugs, but both have India and France as a common source. A summary of the sources of importation of drugs into the two countries is given in figures 14 – 17 below.

Figure 14: Chart showing Source of the Antibiotic drugs in Ghana.  Figure 15: Chart showing the Percentage of samples in Ghana by country of origin

Figure 16: Chart showing Source of the Antibiotic drugs in Nigeria. Figure 17: Chart showing Percentage of samples in Nigeria by country of origin
3.2.3 Registration Status and Source of Antibiotics Sampled

Out of the 45 antibiotics sampled (Ghana, 13 and Nigeria, 32), 34 (76%) had approval / registration numbers printed on their packs. All the samples from Nigeria had their registration numbers printed on their packs, while only 2 of those sampled in Ghana (one of them with NAFDAC registration number) had theirs printed on their packs. The registration status of those without these numbers could not be ascertained. Table 5 below shows a summary of the registration status of all the drugs purchased.

Table 5: Registration Status and Source of Antibiotics Sampled

<table>
<thead>
<tr>
<th>Country of collection</th>
<th>Total Number of Samples</th>
<th>No of Registered Samples</th>
<th>No of Un registd Samples</th>
<th>No of Imported Samples</th>
<th>No of locally manufactd Samples</th>
<th>No of locally manufactd Samples Registered</th>
<th>No of imported Samples Registered</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHANA</td>
<td>13</td>
<td>2</td>
<td>11</td>
<td>9</td>
<td>4</td>
<td>1</td>
<td>1*</td>
</tr>
<tr>
<td>NIGERIA</td>
<td>32</td>
<td>32</td>
<td>0</td>
<td>26</td>
<td>6</td>
<td>6</td>
<td>26</td>
</tr>
</tbody>
</table>

*Drug with Nigeria’s NAFDAC registration number

3.3 Analytical Techniques Used in the Study

Visual preliminary inspection was done to check for visible signs of counterfeit. Qualitative test for the presence of the requisite API was done using available colour reaction test or the HPLC retention time. HPLC method was used to assay of the API contents. In vitro dissolution test was used to determine the bioavailability of the drugs.

3.3.1 Visual Inspection

The packaging of the drug samples were examined for defects and possible traits of counterfeits. Though there were no standards available to compare with, all of them were perfectly packaged and the visible characteristics normally attributed to counterfeits were not

54
observed. 20 tablets / capsules of each drug, after examination for packaging for authenticity, were unpacked for further inspection. Each tablet, capsule, and suspension was examined visually for, shape, colour, and any evidence of deterioration or other physical disfiguration. They were all undamaged, smooth, and of uniform colour. The manufacture and expiration dates on the label of each sample were checked. From the labels, the approximate shelf-life of Azithromycin brand was 2 years, that of Erythromycin brand was 2 years, and Clindamycin was approximately 5 years.

3.3.2 Qualitative Test of the Antibiotic Drugs (Basic Test or Colour Reaction)

3.3.2.1 Erythromycin

The Erythromycin tablets under study consist of Erythromycin Stearate as their API. Colour reaction test in accordance with the 2013 International Pharmacopoeia identification test C method was used in the qualitative test of Erythromycin Stearate.\(^{130}\)

**Basic Test: Colour Reaction of Erythromycin Stearate**

Test C): A quantity of the tablet powder or well mixed oral suspension equivalent to about 10mg of Erythromycin stearate was weighed into a clean dry 50ml beaker. 2.0ml of acetone and then about 2.0ml of concentrated hydrochloric acid (approx. 420g/l) were added and then shaken for a while to allow a colour, and then about 2.0ml of chloroform was added.

**Expected observation:**

A colour change from clear colourless solution through pale orange colour to red or red-violet colour on addition of 2.0ml acetone and 2.0ml of hydrochloric acid, and then formation of violet coloured layer on addition of chloroform.
**Actual observation:**

The colourless solution formed upon addition of acetone changes to orange and then to red colour upon addition of hydrochloric acid, and then violet bottom layer is formed upon addition of chloroform.

**Comment:**

All the Erythromycin samples tested positive for the presence of Erythromycin stearate. This is shown in the table 6 below.

**Table 6:** Result of basic test of Erythromycin Stearate in Erythromycin drug samples.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Test C Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEa 1</td>
<td>+</td>
</tr>
<tr>
<td>NEa 1</td>
<td>+</td>
</tr>
<tr>
<td>NEa 2</td>
<td>+</td>
</tr>
<tr>
<td>NEa 3</td>
<td>+</td>
</tr>
<tr>
<td>NEa 4</td>
<td>+</td>
</tr>
<tr>
<td>NEa 5</td>
<td>+</td>
</tr>
<tr>
<td>NEa 6</td>
<td>+</td>
</tr>
<tr>
<td>NEa 7</td>
<td>+</td>
</tr>
<tr>
<td>NEa 9</td>
<td>+</td>
</tr>
<tr>
<td>NEa 10</td>
<td>+</td>
</tr>
<tr>
<td>NEa 11</td>
<td>+</td>
</tr>
<tr>
<td>NEa 12</td>
<td>+</td>
</tr>
<tr>
<td>NEa 13</td>
<td>+</td>
</tr>
<tr>
<td>NEa 14</td>
<td>+</td>
</tr>
</tbody>
</table>

**3.3.2.2 Azithromycin**

No colour reaction / dye test for Azithromycin was found in US Pharmacopoeia, British Pharmacopoeia and the International Pharmacopoeia, neither was any found in literature. But in accordance with Part 452, Volume 5 of the 1998 amended code of Federal Regulations of macrolide antibiotic drugs for human use, and the US Pharmacopoeia, Azithromycin was identified by comparing the retention time of the azithromycin peak in the chromatogram of the azithromycin samples’ assays with that of the working standard.
The retention time of all the samples were compared with that of the standard and they were approximately correspondent to that of the standard. As shown in Table 7 below, the retention time of the working Standard is 7.395 minutes, and the highest deviation from this was that of Sample NAa8 (1.126). This deviation could be to the interaction of the excipients present with the column. The variance in the retention times of the samples could be due to differences in properties of the drugs, and column usage. The variance in $R_t$ could also be due to variance in the excipient components in the drugs produced by different manufacturers.\textsuperscript{162}

**Table 7: Retention time of Azithromycin Samples and Deviation from Standard**

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Av. Rt (mins)</th>
<th>Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAa 1</td>
<td>7.15</td>
<td>0.245</td>
</tr>
<tr>
<td>GAa 2</td>
<td>6.93</td>
<td>0.465</td>
</tr>
<tr>
<td>GAa 3</td>
<td>7.172</td>
<td>0.223</td>
</tr>
<tr>
<td>GAa 4</td>
<td>6.775</td>
<td>0.62</td>
</tr>
<tr>
<td>GAa 5</td>
<td>6.874</td>
<td>0.521</td>
</tr>
<tr>
<td>NAa 1</td>
<td>7.617</td>
<td>-0.222</td>
</tr>
<tr>
<td>NAa 2</td>
<td>7.238</td>
<td>0.157</td>
</tr>
<tr>
<td>NAa 3</td>
<td>7.112</td>
<td>0.283</td>
</tr>
<tr>
<td>NAa 4</td>
<td>7.683</td>
<td>-0.288</td>
</tr>
<tr>
<td>NAa 5</td>
<td>7.598</td>
<td>-0.203</td>
</tr>
<tr>
<td>NAa 6</td>
<td>7.781</td>
<td>-0.386</td>
</tr>
<tr>
<td>NAa 7</td>
<td>7.427</td>
<td>-0.032</td>
</tr>
<tr>
<td>NAa 8</td>
<td>8.521</td>
<td>-1.126</td>
</tr>
<tr>
<td>NAa 9</td>
<td>7.396</td>
<td>-0.001</td>
</tr>
<tr>
<td>NAa 10</td>
<td>7.208</td>
<td>0.187</td>
</tr>
<tr>
<td>NAa 11</td>
<td>7.17</td>
<td>0.225</td>
</tr>
<tr>
<td>NAa 12</td>
<td>7.309</td>
<td>0.086</td>
</tr>
<tr>
<td>NAp 1</td>
<td>8.27</td>
<td>-0.875</td>
</tr>
<tr>
<td>NAp 2</td>
<td>7.449</td>
<td>-0.054</td>
</tr>
<tr>
<td>NAp 3</td>
<td>7.526</td>
<td>-0.131</td>
</tr>
<tr>
<td>NAp 4</td>
<td>7.204</td>
<td>0.191</td>
</tr>
<tr>
<td>NAp 5</td>
<td>7.379</td>
<td>0.016</td>
</tr>
<tr>
<td>GAp 1</td>
<td>7.366</td>
<td>0.029</td>
</tr>
<tr>
<td>GAp 2</td>
<td>7.366</td>
<td>0.029</td>
</tr>
<tr>
<td>GAp 3</td>
<td>7.147</td>
<td>0.248</td>
</tr>
</tbody>
</table>
3.3.2.3 Clindamycin

The correspondence of the HPLC retention time of the samples with that of the working standard was used to confirm the presence of the Clindamycin API in the samples. This is shown in Table 8 below. The average Retention time of the working Standard is 7.2425 minutes, and the highest deviation from this was that of Sample NEa9 (0.5415). This deviation could be due to the interaction of the excipients present with the column. The variance in the retention times of the samples could be attributed to the same reasons mentioned for Azithromycin above.

Table 8: Retention time of Clindamycin Samples and Deviation from Standard

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Av. Rt (mins)</th>
<th>Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCa1</td>
<td>7.364</td>
<td>0.1215</td>
</tr>
<tr>
<td>GCa2</td>
<td>7.235</td>
<td>-0.0075</td>
</tr>
<tr>
<td>GCa3</td>
<td>7.784</td>
<td>0.5415</td>
</tr>
<tr>
<td>GCa4</td>
<td>7.533</td>
<td>0.2905</td>
</tr>
<tr>
<td>NCa1</td>
<td>7.646</td>
<td>0.4035</td>
</tr>
<tr>
<td>NCa2</td>
<td>7.386</td>
<td>0.1435</td>
</tr>
</tbody>
</table>

3.3.3 HPLC Assay of the Antibiotic drugs

All the 45 samples of antibiotics drugs were assayed using HPLC method. The assay was carried out using a method that was modified from the 2010 US pharmacopoeia methods and those found in literature.

3.3.3.1 HPLC assay of Azithromycin Tablet, Capsules and Oral Suspension

A method for the assay of Azithromycin has been described in the USP 2010\textsuperscript{143} Several HPLC assay methods of Azithromycin assay have also been reported, some of which have
are shown in Table 3 in chapter two. The experimental conditions for the HPLC determination of Azithromycin used in this study were developed by modification of the method described by K. A Shaikh et al. The literature method was chosen based on the availability of the described column. The same mobile phase was used but the composition ratio and the pH were modified to get an improved shape of the chromatographic peak. The change in mobile composition could have been necessitated due to the difference in the HPLC machine used. The pH was reduced to safeguard the column. Presented in Table 9 below are the details of the changes. A typical chromatogram of blank (consisting only diluent used), and that of a preparation containing about 0.5 mg/ml of Azithromycin are shown in figures 18 and 19 respectively.

**Table 9: Experimental conditions for the HPLC determination of Azithromycin**

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>USP Method</th>
<th>K.A. Shaikh’s Method</th>
<th>Modified Method</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Column</strong></td>
<td>γ-Alumina or Zirchrom-PBD (4.6 x 250mm; 3μm)</td>
<td>Waters Xterra RP18 (250mm×4.6mm, 5μm)</td>
<td>Xterra MS C18 5 μm 150 x 4.6 mm</td>
</tr>
<tr>
<td><strong>Mobile Phase</strong></td>
<td>14mM Potassium phosphate; 20mM potassium hydroxide; 29% acetonitrile (pH 11)</td>
<td>acetonitrile–dipotassium phosphate (30mM) (50:50, v/v) (pH 9.0)</td>
<td>40 volume of 0.05M K2HPO4 buffer : 60 volume of Acetonitrile (pH 8.2)</td>
</tr>
<tr>
<td><strong>Injection Volume</strong></td>
<td>50μL</td>
<td>50 μl of 0.05mg/ml</td>
<td></td>
</tr>
<tr>
<td><strong>Flow rate</strong></td>
<td>1.0mL/min</td>
<td>1.7 ml/min</td>
<td>1 ml/min</td>
</tr>
<tr>
<td><strong>Wavelength of detection</strong></td>
<td>dual coulometric electrodes (5010A): E1=+500; E2=+800mV (vs. Pd*)</td>
<td>at 215 nm</td>
<td>Diode Array Detector (DAD): 210 nm</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td>50 °C</td>
<td>30° C</td>
<td></td>
</tr>
<tr>
<td><strong>Average Retention time</strong></td>
<td>10.25 min</td>
<td>11.5 min</td>
<td>7.995 min.</td>
</tr>
</tbody>
</table>
Figure 18: Chromatogram of Blank Azithromycin HPLC Assay

HPLC assay of Azithromycin tablets

The chromatogram of one of the samples (GAa1) is shown in figure 19 below.

Figure 19: Chromatogram of Azithromycin sample GAa1

HPLC assay of Azithromycin Capsules

The chromatogram of one of the samples (GAa2) is shown in figure 20 below.
HPLC assay of Azithromycin Oral Suspension

The chromatogram of one of the samples (NAp2) is shown in figure 21 below.

3.3.3.2 HPLC assay of Erythromycin Tablets

The official USP 2010 assay method for Erythromycin Stearate tablet is microbial assay\textsuperscript{163}. Though HPLC method is not the USP official method of assay of Erythromycin, the HPLC is a very versatile tool for quantitative determination of API in dosage forms and bulk samples.
and hence a trial was experimented after consultation of published literature. Some of the HPLC methods and their conditions are shown in Table 3 in chapter two. The method used is the same for the assay of Azithromycin, bearing in mind the structural similarity between Azithromycin and Erythromycin. Details of these are given in the Table 10 below. A typical chromatogram of blank (consisting only diluent used), and that of preparation containing about 1.0mg/ml of Erythromycin are shown in figures 22 and 23 respectively.

**Table 10: Experimental conditions for the HPLC determination of Erythromycin**

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>K.A. Shaikh’s Method</th>
<th>Modified Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Waters Xterra RP18 (250mm×4.6mm, 5μm)</td>
<td>Xterra MS C18 5μm 150 x 4.6 mm</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>acetonitrile-dipotassium phosphate (30mM) (50:50, v/v) (pH 9.0)</td>
<td>40 volume of 0.05M K₂HPO₄ buffer : 60 volume of Acetonitrile (pH 8.2)</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>50 μl of 1.0mg/ml</td>
<td>1 ml/min</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.7 ml/min</td>
<td>1 ml/min</td>
</tr>
<tr>
<td>Wavelength of detection</td>
<td>at 215 nm</td>
<td>Diode Array Detector (DAD): 210 nm</td>
</tr>
<tr>
<td>Temperature</td>
<td>50 °C</td>
<td>30 °C</td>
</tr>
<tr>
<td>Av. Retention time</td>
<td>11.5 min</td>
<td>3.285 min.</td>
</tr>
</tbody>
</table>

![Figure 22: Chromatogram of Blank for Erythromycin HPLC Assay](http://ugspace.ug.edu.gh)
Figure 23: Chromatogram of a preparation containing about 1.0 mg/ml of Erythromycin

### 3.3.3.3 HPLC assay of Clindamycin Hydrochloride Capsule

HPLC assay of Clindamycin Hydrochloride has been described by the Japanese Pharmacopoeia\(^{164}\). The conditions along with others from literature are stated in Table 4 in chapter two. The experimental condition for the HPLC determination of Clindamycin Hydrochloride in the Clindamycin samples was developed with reference to the Japanese Pharmacopoeia. Details of these are given in the Table 11 below. The reduced retention time could be as a result of the difference in temperature, the column and the type of HPLC machine used. A typical chromatogram of preparation containing about 1.0mg/ml of Clindamycin is shown in figure 24.

**Table 11: Experimental conditions for the HPLC determination of Clindamycin**

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Japanese Pharmacopoeia Method</th>
<th>Modified Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>octadecylsilanized silica gel column (25 cm x 4.6 mm x 5μm)</td>
<td>ODX SEC 250 X 4.6 mm</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>0.05M KH(_2)PO(_4) buffer : Acetonitrile (55 : 45) at pH 7.5</td>
<td>55 volumes of 0.025M KH(_2)PO(_4) buffer : 45 volume of Acetonitrile at pH 7.5</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>30 mL</td>
<td>25 μl of 1.0mg/ml</td>
</tr>
<tr>
<td>Flow rate</td>
<td>flow rate adjusted to give Rt of 10 minutes</td>
<td>1 ml/min</td>
</tr>
<tr>
<td>Wavelength of detection</td>
<td>UV at 210nm</td>
<td>Diode Array Detector (DAD) 210nm</td>
</tr>
<tr>
<td>Temperature</td>
<td>25°C</td>
<td>30°C</td>
</tr>
<tr>
<td>Average Retention time</td>
<td>10 minutes</td>
<td>7.205 min.</td>
</tr>
</tbody>
</table>
3.4 Calculations of the API Content of Drugs after HPLC Assay

3.4.1 Preparation of Calibration Curve for azithromycin

A calibration curve for Azithromycin was prepared using Azithromycin Reference standard (RS) batch number A2095A10, and % purity of 95.71 (acquired From Anuh Pharma Ltd). Concentrations of 0.3542, 0.4048, 0.4554, 0.5060, 0.5566, 0.6072 and 0.6578mg/ml of the RS were prepared. 50μl was injected into the HPLC machine and their respective Areas Under the Curve (AUC) were recorded. Each sample was analysed in 6 replicates and the average of the AUC and their respective standard deviation were calculated. The average Area under the curve of the chromatogram peak of the six replicates was plotted against their respective concentrations to give a graph of linear calibration. The equation of the calibration was generated by the Microsoft Excel 2010 software. The concentrations of the various API of the samples were extrapolated / calculated from this equation. The calibration curve for Azithromycin is shown in Figure 25, and the calculation of the API content of one of its sample is given below.
Figure 25: Calibration curve for Azithromycin

Sample Calculation: Azithromycin (GAa₁)

Average mass of 10 tablets = 3.6375g

Therefore mass of 1 tablet = 0.36375g = 363.75mg

Label claim of Azithromycin API per tablet = 250mg

Therefore, 250mg is contained in 363.75mg of tablet powder

Therefore supposed amount of API used for analysis = 5mg

Therefore 5mg of API will be contained in \( \frac{363.75mg}{250mg} \times 5mg = 7.275mg \) of tablet powder

Therefore 7.275 mg of tablet powder was weighed and dissolved in 10ml of diluent for analysis.

From the equation given by the calibration curve using Microsoft Excel 2010, the concentration of Azithromycin API in the sample assayed can be calculated as follows:

Equation of calibration curve: \( Y = 4160X + 35.22 \)  \( \text{Eqn. 1} \)

Where: \( Y = \text{AUC (Area under the curve)} \); and \( X = \text{Concentration} \)

Therefore \( X = \frac{AUC - 35.22}{4160} \)  \( \text{Eqn. 2} \)

Average AUC of Sample = 2023.29

Therefore Conc. of API in sample = \( \frac{2023.2887 - 35.22}{4160} = 0.4779\text{mg/ml} \)
But volume of sample prepared = 10ml
Therefore the amount of the original sample prepared = 10ml x 0.4779mg/ml = 4.779mg
The percentage of the actual quantity of API in sample as compared with the expected = \[ \frac{4.779mg}{5.0mg} \times 100 = 95.58\% \]
Therefore the actual mass of API contained in drug sample = \[ \frac{95.58}{100} \times 250mg = 238.95mg \]
Though this value is lower than that stated on the label, sample GAa1 is compliant in accordance with USP 2010 which states that Azithromycin tablet should contain not less than 90% and not more than 110.0% of the labelled amount of Azithromycin API.
This calculation was similarly done on the other samples assayed and the results are shown in Tables 12 – 14 below.

3.5  

*In vitro* Dissolution Test of Antibiotics

The *in vitro* dissolution tests of the Antibiotic samples were carried out in accordance with the 2010 USP methods.

3.5.1  Preparation of Test Solution

The 2010 USP recommended method of test solution preparation gave peak areas that are in the background in the chromatogram. This made quantification difficult as the peak areas were difficult to integrate. To remedy this problem, the recommended method was modified. The test solution was prepared as described on page 103 in chapter 4.

An aliquot of the dissolution sample was withdrawn from the vessel after the stipulated time and filtered immediately. After serial dilution of a volume to obtain an equivalent of 0.2777mg/ml concentration of the API, 50μl of this was injected into the HPLC for analysis.
3.5.2 Assay of Drug API Released

The quantitation of the drug API released was done with HPLC. The method used was the same as that used for their respective drug API assays.

3.5.2.1 In vitro Dissolution Test of Azithromycin Tablets

The official 2010 USP method for Azithromycin tablet dissolution was used for the in vitro dissolution test of the Azithromycin tablet samples. Paddle (USP Type 2) apparatus was used under the conditions shown below:

Medium: 900 ml 0.1 M Disodium phosphate (Na₂HPO₄) buffer at pH 6.0

Speed: 75 rpm

Time: 30 minutes

Temperature: 37 ± 0.5°C

Blade’s distance from bottom of vessel: 25 ± 2mm

The dissolved API content was assayed using the same HPLC condition that was used for the assay of Azithromycin tablet dosage form. A typical chromatogram of 50μl of 0.2777mg/ml of Azithromycin tablet sample (GAa4) is shown in figure 26 below.

Figure 26: Chromatogram of Azithromycin tablet dissolution sample
3.5.2.2 *In vitro* Dissolution Test of Azithromycin Capsules

The official 2010 USP method for Azithromycin capsules dissolution was used for the *in vitro* dissolution test of the Azithromycin capsule samples. Paddle (USP Type 2) apparatus was used under the conditions shown below:

*Medium*: 0.1 M of 900ml Disodium phosphate (Na$_2$HPO$_4$) buffer at pH 6.0 mixed with trypsin

*Speed*: 100 RPM

*Time*: 45 minutes

*Temperature*: 37 ± 0.5 °C

*Blade’s distance from bottom of vessel*: 25 ± 2mm

The dissolved API content was assayed using the same HPLC condition used for the assay of Azithromycin tablet dosage form. A typical chromatogram of 50μl of 0.2777mg/ml of Azithromycin tablet sample (NAa$_3$) is shown in figure 27.

![Chromatogram of 50μl of 0.2777mg/ml of Azithromycin capsule Dissolution sample (GAA4)](image)

Figure 27: Chromatogram of Azithromycin capsule dissolution sample
3.5.2.3 *In vitro* Dissolution Test of Erythromycin Tablets

The official 2010 USP method for Erythromycin Stearate tablets dissolution was used for the *in vitro* dissolution test\(^\text{209}\). Paddle (USP Type 2) apparatus was used under the conditions shown below:

*Medium:* 900ml 0.05 M of disodium hydrogen phosphate (Na\(_2\)HPO\(_4\)) at pH 6.8.

*Speed:* 100 RPM

*Time:* 120 minutes

*Temperature:* 37 ± 0.5 °C

*Blade’s distance from bottom of vessel:* 25 ± 2mm

The dissolved API content was assayed using the same HPLC condition that was used for the assay of Erythromycin Stearate tablet dosage form. A typical chromatogram of 50μl of 0.5556mg/ml of Erythromycin tablet sample (NEa\(_2\)) is shown in figure 28 below.

![Chromatogram of Erythromycin Stearate sample](image)

Figure 28: Chromatogram of Erythromycin Stearate sample
3.5.2.4 *In vitro* dissolution test of Clindamycin Hydrochloride Capsules

The official USP29 method for Clindamycin Hydrochloride Capsules dissolution was used for the *in vitro* dissolution test ⁶⁶. Basket (USP Type 1) apparatus was used under the conditions shown below:

*Medium:* 900 ml of 0.039M Disodium phosphate (Na₂HPO₄) buffer at pH 6.8

*Speed:* 100 RPM

*Time:* 30 minutes

*Temperature:* 37 ± 0.5 °C

*Blade’s distance from bottom of vessel:* 25 ± 2mm

The dissolved API content was assayed using the same HPLC condition that was used for the assay of Clindamycin Hydrochloride Capsule dosage form. A typical chromatogram of 50μl of 0.1667mg/ml of Clindamycin Hydrochloride Capsules sample (GC₄) is shown in figure 29 below.

![Chromatogram of Clindamycin Hydrochloride sample](image)

**Figure 29:** Chromatogram of Clindamycin Hydrochloride sample

*Sample Calculation of Percentage Drug Release: Azithromycin (NA₄)*

Label claim of mass of API in a unit dose = 250mg
Volume of medium dissolved in = 900ml

Expected concentration of API in a unit dose = \( \frac{250\text{mg}}{900\text{ml}} = 0.2777\text{mg/ml} \)

The percentage drug release of a unit dose can be calculated using the formula below

% drug release = \( \frac{P_t \times W_S \times \text{Potency of standard}}{P_S \times W_t} \)

Where:

- \( P_t \) = Area under the Curve AUC of Test solution
- \( W_S \) = Concentration of Standard
- \( P_S \) = Area under the Curve AUC of the standard used
- \( W_t \) = Concentration of test solution

From the analytical data obtained

- AUC of Test obtained = 1043.526
- Concentration of test used = 0.2777mg/ml
- AUC of Standard obtained = 1034.73157
- Concentration of Standard used = 0.278mg/ml
- Potency/purity of Standard = 95.92

Therefore % drug release of sample = \( \frac{1043.526 \times 0.278\text{mg/ml} \times 95.92}{1034.73157 \times 0.2777\text{mg/ml}} = 96.8397\% \)

This calculation was similarly done on the other in vitro dissolution tested samples and the results are shown in Tables 15 – 17 below.

### 3.6 Discussion of HPLC Assay Results of Antibiotics Sampled

The data acquired from the antibiotics assayed were analysed based on the 2010 USP standard. Based on the HPLC assay analysis, the antibiotics were grouped into two
categories. Those that complied with the USP standard were tagged compliant (C), while those that did not comply were tagged as non-compliant (NC).

3.6.1 Assay Result of Total Azithromycin Sampled

The criterion for compliance was based on the USP range of Azithromycin tablets or capsules containing not less than 90.0 % and not more than 110.0 % of the labelled amount. A total of 25 samples obtained from Ghana and Nigeria consisting of 7 (28%) tablets, 10 (40%) capsules and 8 (32%) oral suspension (paediatrics) were assayed. A total of 8 brands of Azithromycin sampled in Ghana comprised of 2 tablets, 3 capsules and 3 oral suspension dosage forms, while the total of 17 brands of Azithromycin antibiotics sampled in Nigeria comprised 5 tablets, 7 capsules, and 5 oral suspension dosage forms. These are depicted as in figures 8 and 9 above.

The HPLC results as shown in Table 12 indicate that out of the total 25 samples of Azithromycin, 8 (32 %) of them were compliant. Five (71%) of the tablets, 1 (10%) of the Capsules, and 2 (25%) of the Oral suspension dosage forms were compliant; these are depicted in figures 36 - 40 below. Out of those sampled in Ghana, 37% of them were compliant, while 29% of those sampled in Nigeria were compliant. These are depicted in figures 30 – 32 below.

Table 12: HPLC Assay Results of Azithromycin

<table>
<thead>
<tr>
<th>Code</th>
<th>Manufacturer’s Label Claim (mg)</th>
<th>HPLC determination of composition of Azithromycin dosage forms in % and mg quantities. (n=6)</th>
<th>Remark based on HPLC results</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAA a1</td>
<td>250</td>
<td>94.62 ± 0.02 236.54</td>
<td>C</td>
</tr>
<tr>
<td>GAA a2</td>
<td>250</td>
<td>120.53 ± 0.03 301.32</td>
<td>NC</td>
</tr>
<tr>
<td>GAA a3</td>
<td>250</td>
<td>129.16 ± 0.05 322.90</td>
<td>NC</td>
</tr>
<tr>
<td>Sample</td>
<td>Concentration</td>
<td>Result</td>
<td>Compliance</td>
</tr>
<tr>
<td>---------</td>
<td>---------------</td>
<td>-----------</td>
<td>------------</td>
</tr>
<tr>
<td>GAa 4</td>
<td>250</td>
<td>96.03 ± 0.04</td>
<td>240.06</td>
</tr>
<tr>
<td>GAa 5</td>
<td>250</td>
<td>121.91 ± 0.06</td>
<td>304.79</td>
</tr>
<tr>
<td>NAA 1</td>
<td>250</td>
<td>103.92 ± 0.05</td>
<td>259.80</td>
</tr>
<tr>
<td>NAA 2</td>
<td>250</td>
<td>103.36 ± 0.04</td>
<td>258.41</td>
</tr>
<tr>
<td>NAA 3</td>
<td>500</td>
<td>127.99 ± 0.03</td>
<td>639.96</td>
</tr>
<tr>
<td>NAA 4</td>
<td>500</td>
<td>129.10 ± 0.06</td>
<td>645.49</td>
</tr>
<tr>
<td>NAA 5</td>
<td>500</td>
<td>121.45 ± 0.03</td>
<td>607.25</td>
</tr>
<tr>
<td>NAA 6</td>
<td>250</td>
<td>97.83 ± 0.05</td>
<td>244.57</td>
</tr>
<tr>
<td>NAA 7</td>
<td>250</td>
<td>125.33 ± 0.04</td>
<td>313.32</td>
</tr>
<tr>
<td>NAA 8</td>
<td>250</td>
<td>143.73 ± 0.05</td>
<td>359.31</td>
</tr>
<tr>
<td>NAA 9</td>
<td>250</td>
<td>122.38 ± 0.06</td>
<td>305.94</td>
</tr>
<tr>
<td>NAA 10</td>
<td>250</td>
<td>135.56 ± 0.03</td>
<td>338.90</td>
</tr>
<tr>
<td>NAA 11</td>
<td>500</td>
<td>127.72 ± 0.05</td>
<td>638.59</td>
</tr>
<tr>
<td>NAA 12</td>
<td>250</td>
<td>109.85 ± 0.02</td>
<td>274.61</td>
</tr>
<tr>
<td>NAP 1</td>
<td>200</td>
<td>162.36 ± 0.03</td>
<td>324.72</td>
</tr>
<tr>
<td>NAP 2</td>
<td>200</td>
<td>162.94 ± 0.07</td>
<td>325.89</td>
</tr>
<tr>
<td>NAP 3</td>
<td>200</td>
<td>152.25 ± 0.05</td>
<td>304.50</td>
</tr>
<tr>
<td>NAP 4</td>
<td>200</td>
<td>107.33 ± 0.03</td>
<td>214.66</td>
</tr>
<tr>
<td>NAP 5</td>
<td>200</td>
<td>132.82 ± 0.06</td>
<td>265.64</td>
</tr>
<tr>
<td>GAP 1</td>
<td>200</td>
<td>159.16 ± 0.03</td>
<td>318.33</td>
</tr>
<tr>
<td>GAP 2</td>
<td>200</td>
<td>105.82 ± 0.04</td>
<td>211.63</td>
</tr>
<tr>
<td>GAP 3</td>
<td>200</td>
<td>137.42 ± 0.04</td>
<td>274.84</td>
</tr>
</tbody>
</table>

Figure 30: Azithromycin assay result chart.

Figure 31: Ghana Azithromycin assay results

Figure 32: Nigeria Azithromycin assay result
3.6.2 Assay Result of Total Erythromycin Sampled

The criterion for compliance for Erythromycin was based on the 2010 USP range of Erythromycin Stearate tablets containing not less than 90.0 % and not more than 120.0 % of the labelled amount. A total of 14 tablets dosage forms of the Erythromycin samples comprising 1 from Ghana and 13 from Nigeria were assayed. The results are shown in Table 13 below.

Table 13: HPLC Assay Results of Erythromycin

<table>
<thead>
<tr>
<th>Code</th>
<th>Manufacturer’s Label Claim</th>
<th>HPLC determination of composition of Erythromycin Stearate dosage forms in % and mg quantities. (n=6)</th>
<th>Remark based on HPLC results</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEa 1</td>
<td>250</td>
<td>47.82 ± 0.01 119.55</td>
<td>NC</td>
</tr>
<tr>
<td>NEa 1</td>
<td>500</td>
<td>55.46 ± 0.09 277.3</td>
<td>NC</td>
</tr>
<tr>
<td>NEa 2</td>
<td>500</td>
<td>84.92 ± 0.001 424.6</td>
<td>NC</td>
</tr>
<tr>
<td>NEa 3</td>
<td>500</td>
<td>83.92 ± 0.11 419.6</td>
<td>NC</td>
</tr>
<tr>
<td>NEa 4</td>
<td>500</td>
<td>59.83 ± 0.02 299.15</td>
<td>NC</td>
</tr>
<tr>
<td>NEa 5</td>
<td>500</td>
<td>60.22 ± 0.03 301.1</td>
<td>NC</td>
</tr>
<tr>
<td>NEa 6</td>
<td>500</td>
<td>79.15 ± 0.01 395.75</td>
<td>NC</td>
</tr>
<tr>
<td>NEa 7</td>
<td>500</td>
<td>93.44 ± 0.23 467.2</td>
<td>C</td>
</tr>
<tr>
<td>NEa 9</td>
<td>500</td>
<td>83.08 ± 0.06 415.4</td>
<td>NC</td>
</tr>
<tr>
<td>NEa 10</td>
<td>250</td>
<td>65.32 ± 0.003 163.3</td>
<td>NC</td>
</tr>
<tr>
<td>NEa 11</td>
<td>500</td>
<td>85.33 ± 0.03 426.65</td>
<td>NC</td>
</tr>
<tr>
<td>NEa 12</td>
<td>500</td>
<td>88.74 ± 0.01 443.7</td>
<td>NC</td>
</tr>
<tr>
<td>NEa 13</td>
<td>500</td>
<td>107.02 ± 0.01 535.1</td>
<td>C</td>
</tr>
<tr>
<td>NEa 14</td>
<td>500</td>
<td>72.52 ± 1.13 362.6</td>
<td>NC</td>
</tr>
</tbody>
</table>

The HPLC results show that 12 (86%) out of the 14 Erythromycin (tablets) samples were not compliant. All the noncompliant ones had their API below the lower limit. The only brand of Erythromycin from Ghana and 11 (85%) out of the 13 samples from Nigeria did not comply with the USP standard of API content. These are depicted in the figures 33 - 35 below.
3.6.3 Assay Result of Total Clindamycin Sampled

The criterion for compliance was based on the USP range of Clindamycin Hydrochloride capsules containing not less than 90.0 % and not more than 120.0 % of the labelled amount.

A total of 6 Clindamycin samples (all in capsule dosage form) comprising 4 from Ghana and 2 from Nigeria were assayed. The HPLC results showed that out of the total 6 samples of Clindamycin assayed, 2 (33 %) of them were compliant, one from Ghana and one from Nigeria. The results are shown in Table 14 and depicted in figures 36 – 38 below.
Table 14: HPLC Assay Results of Clindamycin

<table>
<thead>
<tr>
<th>Code</th>
<th>Manufacturer’s Label Claim (mg)</th>
<th>HPLC determination of composition of Azithromycin dosage forms in % and mg quantities. (n=6)</th>
<th>Remark based on HPLC results</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCa1</td>
<td>150</td>
<td>139.22 ± 0.01</td>
<td>208.83</td>
</tr>
<tr>
<td>GCa2</td>
<td>150</td>
<td>142.81 ± 0.01</td>
<td>214.22</td>
</tr>
<tr>
<td>GCa3</td>
<td>150</td>
<td>84.66 ± 0.003</td>
<td>201.99</td>
</tr>
<tr>
<td>GCa4</td>
<td>150</td>
<td>91.15 ± 0.10</td>
<td>136.73</td>
</tr>
<tr>
<td>NCa1</td>
<td>300</td>
<td>101.15 ± 0.08</td>
<td>303.45</td>
</tr>
<tr>
<td>NCa2</td>
<td>150</td>
<td>134.01 ± 0.2</td>
<td>201.01</td>
</tr>
</tbody>
</table>

Figure 36: Percentage compliance of Clindamycin assay

Figure 37: Percentage compliance of Ghana's Clindamycin assay.

Figure 38: Percentage compliance of Nigeria's Clindamycin assay
3.6.4 Assay Result of Total Samples

Out of the total 45 samples assayed for API content, 12 (27%) were compliant. Four out of the 13 samples from Ghana and 8 out of the 32 samples from Nigeria complied with the USP standard. This is depicted in figure 39 below.

![Assay Result of Total Samples](image)

Figure 39: Percentage Compliance of Samples to HPLC Assay

3.6.5 Quantitative Assay Result of Samples collected from Ghana

A total of 13 samples acquired from Ghana were assayed and 4 (31%) were compliant. Of these, 3 brands of Azithromycin, 1 brand of Clindamycin complied; and the only Erythromycin did not comply. Three (30%) out of the 10 adult antibiotics from Ghana assayed were compliant, while 1 (33%) out of the 3 Ghana paediatrics antibiotics assayed complied. These are depicted in figure 40.

![Ghana's Samples Quantitative Assay Results](image)

Figure 40: Percentage assay compliance of Ghana's samples
3.6.6 Quantitative Assay Results of Samples from Nigeria

A total of 32 samples acquired from Nigeria were assayed 8 (25%) were compliant. Seven (26%) out of the 27 adult antibiotics from Nigeria assayed were compliant, while 1 (20%) out of the 5 paediatrics antibiotics from Nigeria assayed complied. These are depicted in figure 41 below. The failure rates in Nigeria as observed here is above the ranges of between 32 % and 48 % ranges previously observed in Nigeria as reported by Chioma Joy in 2010.  

Figure 41: Percentage compliance of antibiotics sampled in Nigeria.

3.6.7 Quantitative Result of Total Paediatric Antibiotics Assayed

Eight brands of Azithromycin paediatric (oral suspension) dosage forms were assayed. As depicted in figure 42, only 2 (25%), one each from Ghana and Nigeria complied. Though no published literature on quality survey of paediatric Azithromycin or paediatric antibiotics in general was available to compare, it can be inferred though not conclusively from this result that the quality rate is poor.
3.7 **In vitro Dissolution Test**

*In vitro* dissolution test was done to determine the drug release as a further quality check, and to determine compliance of the antibiotics with the pharmacopoeia standard. The rate and extent to which the active pharmaceutical ingredient (API) of a drug is released from the drug’s excipients to become available for absorption is critical to a drug’s efficacy. A drug might contain the required amount of active ingredient but might fail to release it adequately in the GI tract environment.

*In vitro* dissolution test was done on the tablet and capsule dosage forms of the samples. A total of 37 different brands of antibiotic drugs of the three APIs sampled from Ghana and Nigeria were *in vitro* dissolution tested. These comprised 17 brands of Azithromycin, 14 different brands of Erythromycin and 6 brands of Clindamycin.

### 3.7.1 **In vitro Dissolution test Results of Azithromycin**

The criterion for compliance was based on the USP standard which states that for Azithromycin tablets, not less than 80 % of the labelled amount is dissolved in 30 minutes, and that for capsules is not less than 75 % of the labelled amount is dissolved in 45 minutes.

![Assay Result of Total Paediatric Dosage Form Sampled](image)

Figure 42: Percentage compliance of Total paediatric dose of antibiotics assayed
17 samples comprising of 7 tablets and 10 capsules dosage forms were analysed for \textit{in vitro} dissolution. Out of these, 5 comprising of 2 tablets and 3 capsules were sampled in Ghana, while 12 comprising of 5 tablets and 7 capsules were sampled in Nigeria. The \textit{in vitro} dissolution results show that the total 17 (100\%) brands of Azithromycin sampled were compliant. The results are shown in Table 15 below.

\textbf{Table 15: Azithromycin Dissolution Result}  
Percentage drug release of Active Pharmaceutical Ingredient of Azithromycin in \textit{In vitro} Dissolution test in comparison with USP requirements. Not less than 80 \% of the labelled amount of Azithromycin (C$_{38}$H$_{72}$N$_{2}$O$_{12}$) tablets should dissolve in 30 minutes and not less than 75 \% of labelled amount of Azithromycin capsule should dissolve in 45 minutes

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Dosage Form</th>
<th>% drug Release</th>
<th>Remark based on HPLC Assay of dissolved API</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NAa1</td>
<td>96.84 ± 3</td>
<td>C</td>
</tr>
<tr>
<td>2</td>
<td>NAa2</td>
<td>107.94 ± 0.3</td>
<td>C</td>
</tr>
<tr>
<td>3</td>
<td>NAa3</td>
<td>83.37 ± 0.5</td>
<td>C</td>
</tr>
<tr>
<td>4</td>
<td>NAa4</td>
<td>110.23 ± 1</td>
<td>C</td>
</tr>
<tr>
<td>5</td>
<td>NAa5</td>
<td>112.53 ± 0.9</td>
<td>C</td>
</tr>
<tr>
<td>6</td>
<td>NAa6</td>
<td>94.31 ± 0.6</td>
<td>C</td>
</tr>
<tr>
<td>7</td>
<td>NAa7</td>
<td>96.02 ± 0.4</td>
<td>C</td>
</tr>
<tr>
<td>8</td>
<td>NAa8</td>
<td>94.00 ± 1</td>
<td>C</td>
</tr>
<tr>
<td>9</td>
<td>NAa9</td>
<td>88.15 ± 0.5</td>
<td>C</td>
</tr>
<tr>
<td>10</td>
<td>NAa10</td>
<td>95.38 ± 0.4</td>
<td>C</td>
</tr>
<tr>
<td>11</td>
<td>NAa11</td>
<td>86.58 ± 5</td>
<td>C</td>
</tr>
<tr>
<td>12</td>
<td>NAa12</td>
<td>108.29 ± 0.5</td>
<td>C</td>
</tr>
<tr>
<td>13</td>
<td>GAa1</td>
<td>100.44 ± 0.4</td>
<td>C</td>
</tr>
<tr>
<td>14</td>
<td>GAa2</td>
<td>105.27 ± 0.8</td>
<td>C</td>
</tr>
<tr>
<td>15</td>
<td>GAa3</td>
<td>116.09 ± 4</td>
<td>C</td>
</tr>
<tr>
<td>16</td>
<td>GAa4</td>
<td>113.93 ± 0.4</td>
<td>C</td>
</tr>
<tr>
<td>17</td>
<td>GAa5</td>
<td>93.64 ± 0.8</td>
<td>C</td>
</tr>
</tbody>
</table>

\textbf{3.7.2 \textit{In vitro} Dissolution Results of Erythromycin}  
The criterion for compliance was based on the USP standard which states that for Erythromycin Stearate tablets, not less than 75 \% of the labelled amount is dissolved in 120 minutes. Fourteen (14) samples all of which are in tablet dosage forms were analysed for \textit{in vitro} dissolution. Out of these, 1 was sampled in Ghana, while the remaining 13 were sampled in Nigeria. The \textit{in vitro} dissolution results show that out of the total 14 samples of
Erythromycin analysed for *in vitro* dissolution; 5 (36%) of them were compliant. Only one sample from Ghana was analysed for *in vitro* dissolution test and it did not comply. The results are shown in Table 16 below.

### Table 16: Erythromycin Dissolution Result

Percentage drug release of Active Pharmaceutical Ingredient of Erythromycin in *In vitro* Dissolution test in comparison with USP requirements. Not less than 75 % of labelled amount of Erythromycin Stearate (C\textsubscript{37} H\textsubscript{87} O\textsubscript{13}) should dissolve in 120 minutes.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Dosage Form</th>
<th>% drug Release</th>
<th>Remark based on HPLC Assay of dissolved API</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 NEa1</td>
<td>Tablet</td>
<td>53.18 ± 4</td>
<td>NC</td>
</tr>
<tr>
<td>2 NEa2</td>
<td>Tablet</td>
<td>74.61 ± 4</td>
<td>NC</td>
</tr>
<tr>
<td>3 NEa3</td>
<td>Tablet</td>
<td>85.96 ± 2</td>
<td>C</td>
</tr>
<tr>
<td>4 NEa4</td>
<td>Tablet</td>
<td>53.07 ± 3</td>
<td>NC</td>
</tr>
<tr>
<td>5 NEa5</td>
<td>Tablet</td>
<td>74.98 ± 2</td>
<td>NC</td>
</tr>
<tr>
<td>6 NEa6</td>
<td>Tablet</td>
<td>68.68 ± 2</td>
<td>NC</td>
</tr>
<tr>
<td>7 NEa7</td>
<td>Tablet</td>
<td>96.49 ± 1</td>
<td>C</td>
</tr>
<tr>
<td>8 NEa9</td>
<td>Tablet</td>
<td>67.20 ± 0.6</td>
<td>NC</td>
</tr>
<tr>
<td>9 NEa10</td>
<td>Tablet</td>
<td>95.03 ± 2</td>
<td>C</td>
</tr>
<tr>
<td>10 NEa11</td>
<td>Tablet</td>
<td>83.82 ± 0.3</td>
<td>C</td>
</tr>
<tr>
<td>11 NEa12</td>
<td>Tablet</td>
<td>103.46 ± 1.0</td>
<td>C</td>
</tr>
<tr>
<td>12 NEa13</td>
<td>Tablet</td>
<td>64.51 ± 0.9</td>
<td>NC</td>
</tr>
<tr>
<td>13 NEa14</td>
<td>Tablet</td>
<td>65.30 ± 0.3</td>
<td>NC</td>
</tr>
<tr>
<td>14 GEa1</td>
<td>Tablet</td>
<td>53.16 ± 5</td>
<td>NC</td>
</tr>
</tbody>
</table>

#### 3.7.3 *In vitro* Dissolution Results of Clindamycin

The criterion for compliance was based on the USP standard which states that for Clindamycin Hydrochloride capsules, not less than 80 % of the labelled amount is dissolved in 30 minutes. 6 Clindamycin capsules, 4 sampled in Ghana and 2 sampled in Nigeria were analysed for *in vitro* dissolution test. The *in vitro* dissolution results show that a total of 4 (67%) samples were compliant. 3 (75%) out of the 4 sampled in Ghana and 1(50%) out of the 2 sampled in Nigeria complied. The results are shown in Table 17 below.
Table 17: Clindamycin Dissolution Result

Percentage drug release of Active Pharmaceutical Ingredient of Clindamycin in \textit{In vitro} Dissolution test in comparison with USP requirements. Not less than 80\% of labelled amount of Clindamycin hydrochloride ($C_{18}H_{33}ClN_2O_5S$) should dissolve in 30 minutes

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Dosage Form</th>
<th>% drug Release</th>
<th>Remark based on HPLC Assay of dissolved API</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GCa1 Capsules</td>
<td>85.30 ± 2</td>
<td>C</td>
</tr>
<tr>
<td>2</td>
<td>GCa2 Capsules</td>
<td>83.04 ± 2</td>
<td>C</td>
</tr>
<tr>
<td>3</td>
<td>GCa3 Capsules</td>
<td>81.21 ± 2</td>
<td>C</td>
</tr>
<tr>
<td>4</td>
<td>GCa4 Capsules</td>
<td>74.06 ± 5</td>
<td>NC</td>
</tr>
<tr>
<td>5</td>
<td>NCa Capsules</td>
<td>85.41 ± 5</td>
<td>C</td>
</tr>
<tr>
<td>6</td>
<td>NCa2 Capsules</td>
<td>79.95 ± 2</td>
<td>NC</td>
</tr>
</tbody>
</table>

3.7.4 \textit{In vitro} Dissolution Quality Status of Total Antibiotics Assayed

A total of 37 samples comprising of all the tablets and capsules dosage forms were \textit{in vitro} dissolution tested. 10 of the samples analysed were collected in Ghana while 27 were collected from Nigeria. A total of 26 (70\%) of the samples \textit{in vitro} dissolution tested were compliant. The overall results of the \textit{in vitro} dissolution test show that the compliance rate observed in Ghana (80\%) is higher than that observed in Nigeria (67\%). These are depicted in figures 43 – 45 below.

Figure 43: Percentage compliance of total sample \textit{in vitro} dissolution tested.  
Figure 44: \textit{In vitro} Percentage compliance of samples categorised according to API
These failures could be attributed to manufacturing error (machine or human) which include: non consistent or fluctuation in pressure of the tablet compressing machine thus compressing the tablets / caplets too hard; bulk density / tapped density of powder, powder fineness, hardness and friability which is the tendency of the tablets to powder. Factors that can affect the hardness of a tablet include: excess compressive force, amount of binder, and granulation in preparing the tablets. It can also be attributed to storage during which changes such as: degradation, influence of excipients’ interaction over time with the API can occur. Furthermore, the analytical abilities of the analyst can also affect the outcome of the test.

3.8 Result Analysis of Samples Subjected to Both API Assay and In vitro Dissolution Test

Out of the total 45 samples analysed, 37 were analysed for both API content and in vitro drug release. These comprised of 10 samples from Ghana and 27 from Nigeria. The Ghana samples comprised of 5, 1, and 4 Azithromycin, Erythromycin and Clindamycin respectively. Those from Nigeria comprised of 12, 13, and 2 Azithromycin, Erythromycin and Clindamycin respectively.
A total of 10 (27%) samples passed both tests. This comprised of 8, 1 and 1 brand of Azithromycin, Erythromycin and Clindamycin respectively. Out of the 10 Ghana samples analysed, 3 (30%) of them passed both API content and *in vitro* dissolution test, all of them were Azithromycin. A total of 7 (26%) samples complied with both API content and *in vitro* dissolution tests out of the 27 samples from Nigeria subjected to both tests. This is comprised of 5, 1 and 1 Azithromycin, Clindamycin and Erythromycin samples respectively. These are shown in figures 46 and 47.

### 3.9 *In vitro* Dissolution Results of Samples from Ghana

A total of 10 different brands of antibiotic samples comprising of 3 tablets and 7 capsules dosage forms from Ghana were analysed for *in vitro* dissolution. This comprises 1 brand of Erythromycin (tablet), 5 brands of Azithromycin (2 tablets and 3 capsules), and 4 brands of Clindamycin (all capsules). Eight (80%) out of the 10 Ghana samples analysed complied, Of these, all the brands (100%) of Azithromycin; 3 (75%) out of the 4 brands of Clindamycin complied; the only Erythromycin brand did not comply. No recently published literature on *in vitro* dissolution test of drugs in Ghana was found, but the dissolution compliance rate
observed in the antibiotics from Ghana that were studied is higher than that reported in the antimalarial study by WHO in 2003\textsuperscript{42}.

### 3.10 \textit{In vitro} Dissolution Results of Samples from Nigeria

A total of 27 different brands of antibiotic samples from Nigeria were analysed for \textit{in vitro} dissolution. This comprises 13 brand of Erythromycin (tablet), 12 brands of Azithromycin (5 tablets and 7 capsules), and 2 brands of Clindamycin (all capsules).

As depicted in figure 58 below, 18 (67\%) out of the 27 antibiotics sampled in Nigeria complied with the \textit{in vitro} test. Of these, all the brands (100\%) of Azithromycin; 1 (50\%) brand of Clindamycin, 5 (38\%) of Erythromycin brand complied. No recent published literature on \textit{in vitro} dissolution test of drugs in Nigeria was found, but the dissolution compliance rate observed in the antibiotics from Ghana studied is higher than that of Nigeria.

![In-vitro Dissolution Result of Nigeria Samples](image)

Figure 48: Percentage compliance of \textit{in vitro} dissolution of antibiotics sampled in Nigeria.

### 3.11 Analysis of Result by Country of Origin

The drugs sampled from both countries under survey originated from different countries including the country sampled. The countries of origin are classified into Ghana, Nigeria,
India, China, and Others. This classification is based on a country producing brands of three and above. Those that produced below three are classified as others. The countries classified as others are shown in the complete table classifying the drugs according to country in Tables 18 and 19. As shown in figure 49, out of the 45 brands sampled, 4 (9%), 6 (13%), 19 (42%), 4 (9%), and 12 (27%) originated from Ghana, Nigeria, India, China, and Others respectively.

<table>
<thead>
<tr>
<th>Table 18: Classification of Ghana Samples by Country of Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Country of Origin</strong></td>
</tr>
<tr>
<td>Ghana</td>
</tr>
<tr>
<td>India</td>
</tr>
<tr>
<td>Others</td>
</tr>
<tr>
<td>England</td>
</tr>
<tr>
<td>Romania</td>
</tr>
<tr>
<td>Bangladesh</td>
</tr>
<tr>
<td>Italy</td>
</tr>
<tr>
<td>Surrey</td>
</tr>
<tr>
<td>TOTAL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 19: Classification of Nigeria Samples by Country of Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Country of Origin</strong></td>
</tr>
<tr>
<td>Nigeria</td>
</tr>
<tr>
<td>India</td>
</tr>
<tr>
<td>China</td>
</tr>
<tr>
<td>Others</td>
</tr>
<tr>
<td>Sankarda</td>
</tr>
<tr>
<td>Ecuador</td>
</tr>
<tr>
<td>Malaysia</td>
</tr>
<tr>
<td>Pakistan</td>
</tr>
<tr>
<td>France</td>
</tr>
<tr>
<td>TOTAL</td>
</tr>
</tbody>
</table>

Figure 49: Percentage No of Samples Categorised by Country of Origin
3.12 Assay Result Analysis by Country of Origin

Out of the 4 samples that originated from Ghana, 1 (25%) complied. Six samples originated from Nigeria out of which 3 (50%) complied. Nineteen samples originated from India out of which 5 (26%) complied. Four samples originated from China and 1 (25%) complied. While 2 (17%) out of the 12 sampled from the other countries (Others) complied. These results are shown in figure 50.

![Assay Compliance by Drug's Country of Origin](image.png)

Figure 50: Assay Compliance by Drug's Country of Origin

3.13 In vitro Dissolution Result Analysis by Country of Origin

Out of the 37 samples in vitro dissolution tested, 4 (11%), 6 (16%), 16 (43%), 4 (11%), and 7 (19%) originated from Ghana, Nigeria, India, China, and Others. Out of the 4 samples that originated from Ghana, 3 (75%) complied. Six samples originated from Nigeria out of which 5 (83%) complied. Sixteen of the samples originated from India out of which 12 (75%) complied. Four samples originated from China and 2 (50%) complied. While 4 (75%) out of the 7 sampled from the other countries (Others) complied. These results are shown in figures 51 and 52 below.
3.14 Assay Analysis of Paediatric Samples Result by Country of Origin

Out of the total 8 paediatric antibiotic sampled, 3 of them originated from India while the rest originated from the countries classified as others. A total of 2 samples complied, one from each group. One (33%) of the 3 sample originating from India complied, while 1 (20%) of the 5 samples originating from Bangladesh amongst the others complied. These are depicted in figure 53 below.

3.15 Conclusion

This study was done to assess the quality of some commonly used antibiotics in Ghana and Nigeria. The use of the two countries as a case study was based on their socio-economic similarities. All 45 samples collected contained the requisite API as claimed in the label. Seventy-three (73) per cent of the total drugs sampled did not comply with the required
amount of API content in accordance with the US Pharmacopoeia standard. Most of the failures were due to the API content being above accepted level. Eight (8) of the samples were paediatric dosage forms and out of this number, 6 did not comply with the API standard (above accepted level). \textit{In vitro} dissolution test carried out to determine the release of the API gave 70\% of the drugs complying with USP Standard. However, only ten samples passed both API content and dissolution test meaning that the majority of the samples failed API content but passed the dissolution test. The poor bioavailability of the drugs’ API could be attributed to a number of factors including hardness of a tablet that may occur during the manufacturing process, API excipient interaction and coating degradation that may occur during poor storage conditions.

From visual inspection of the packaging it can be assumed that none of the samples was counterfeit since no sign of adulteration or mislabelling was observed. However, since only 10 out of the 45 samples studied passed both tests, the findings suggest the existence of poor quality brands of azithromycin, erythromycin and clindamycin in Accra and Lagos. New information is provided by this work due to the fact that not much work has been done on the antibiotics chosen in the study area. This could improve the monitoring and enforcement procedures of drug regulatory bodies.
CHAPTER FOUR

EXPERIMENTAL

4.1 Qualitative Test of the Antibiotic Drugs

4.1.1 Erythromycin

A quantity of powdered tablets equivalent to 10mg of Erythromycin Stearate was accurately weighed into a 50ml beaker; 2.0ml of acetone R and 2.0ml of concentrated Hydrochloric acid were added and shaken. An orange coloured liquid which later changed to violet-red was produced. On addition of 2.0ml of chloroform R and shaking, a violet coloured chloroform layer was separated out. This confirmed the presence of Erythromycin.

4.1.2 Azithromycin

HPLC Retention time as recommended by the 2010 USP was used for the quality determination of Azithromycin API in the Azithromycin drug samples. The Retention time of the peak of Azithromycin in the drug sample in the chromatogram was compared with that of the reference standard.

4.1.3 Clindamycin

HPLC Retention time as recommended by the USP was used for the quality determination of Clindamycin hydrochloride API in the Clindamycin drug samples. The Retention time of the peak Clindamycin hydrochloride in the drug sample in the chromatogram was compared with that of the reference standard.
4.2 HPLC Test Methods for the Antibiotic Drugs

HPLC Equipment brand: Agilent Technologies 1200 series equipped with a quaternary pump, an auto-sampler and Diode Array Detector (DAD) was used for the assay. The chromatographic conditions used for the three APIs have already been shown in Chapter three.

4.2.1 HPLC Assay Method of Azithromycin

4.2.1.1 Buffer preparation

8.7 g of dipotassium hydrogen phosphate was dissolved in 990 ml of HPLC grade water. The pH was adjusted to 8.2 with 80 % phosphoric acid, after which more water was added to the 1000ml mark.

4.2.1.2 Preparation of azithromycin Standard solution

50.2 mg of Azithromycin working standard was accurately weighed into a 50 ml volumetric flask and dissolved in the diluent (refer to chromatographic condition of Azithromycin) to obtain a solution having a known concentration of 1.004 mg/ml. RS concentrations of 0.3514 mg/ml, 0.4016 mg/ml, 0.4518 mg/ml, 0.5020 mg/ml, 0.5522 mg/ml, 0.6024 mg/ml and 0.6526 mg/ml were obtained by diluting 3.5ml, 4.0 ml, 4.5 ml, 5.0 ml, 5.5 ml, 6.0 ml, and 6.5 ml and making it to a final volume of 10 ml with the diluent.
4.2.1.3 Linearity Azithromycin

The averages of the Area under the curve (AUC) of six replicates of each concentration were calculated and were plotted against their respective concentration in a graph. The slope, the intercept, and the correlation coefficient ($r^2$) were calculated accordingly using Microsoft excel 2010. The equation of the straight line was deduced as $\text{AUC} = mC + b$;

$$\text{AUC} = \text{Area under the curve of the chromatographic peak of Azithromycin}$$

$m =$ Slope of the straight line, and

$b =$ intercept on the AUC (y axis)

$C =$ concentration of analyte

The calibration curve as shown in figure 54 was drawn for the Azithromycin drug API. The tables 20 and 21 below show the concentrations and corresponding AUCs, and the calibration curve for Azithromycin API.

**Table 20**: Concentration of the Azithromycin RS and corresponding AUCs

<table>
<thead>
<tr>
<th>ID NO</th>
<th>Conc. (mg/ml)</th>
<th>AUC</th>
<th>Average AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3514</td>
<td>1437.923</td>
<td>1437.7642</td>
</tr>
<tr>
<td>2</td>
<td>0.4016</td>
<td>1631.506</td>
<td>1631.3353</td>
</tr>
<tr>
<td>3</td>
<td>0.4518</td>
<td>1846.974</td>
<td>1846.7936</td>
</tr>
<tr>
<td>4</td>
<td>0.5020</td>
<td>2037.905</td>
<td>2037.6702</td>
</tr>
<tr>
<td>5</td>
<td>0.5522</td>
<td>2271.856</td>
<td>2271.2253</td>
</tr>
<tr>
<td>6</td>
<td>0.6024</td>
<td>2445.253</td>
<td>2445.6033</td>
</tr>
<tr>
<td>7</td>
<td>0.6526</td>
<td>2703.572</td>
<td>2702.7321</td>
</tr>
</tbody>
</table>

**Table 21**: Calibration Curve data of Azithromycin

<table>
<thead>
<tr>
<th>Conc.(mg/ml)</th>
<th>SD</th>
<th>RSD</th>
<th>AUC ± RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3514</td>
<td>0.381041109</td>
<td>0.026502336</td>
<td>1437.76426 ± 0.0265</td>
</tr>
<tr>
<td>0.4016</td>
<td>0.403806607</td>
<td>0.024753133</td>
<td>1631.33534 ± 0.0248</td>
</tr>
<tr>
<td>0.4518</td>
<td>0.390679122</td>
<td>0.021154455</td>
<td>1846.793615 ± 0.0212</td>
</tr>
<tr>
<td>0.5020</td>
<td>0.380612508</td>
<td>0.018678807</td>
<td>2037.670287 ± 0.0187</td>
</tr>
<tr>
<td>0.5522</td>
<td>0.779426381</td>
<td>0.03431744</td>
<td>2271.225312 ± 0.0343</td>
</tr>
<tr>
<td>0.6024</td>
<td>0.505032806</td>
<td>0.020650642</td>
<td>2445.60338 ± 0.0207</td>
</tr>
<tr>
<td>0.6526</td>
<td>1.32455292</td>
<td>0.049007925</td>
<td>2702.732103 ± 0.0490</td>
</tr>
</tbody>
</table>
4.2.2 HPLC Assay Method of Erythromycin

4.2.2.1 Buffer preparation

8.7 g of dipotassium hydrogen phosphate was dissolved in 990 ml of HPLC grade water. The pH was adjusted to 8.2 with 80 % phosphoric acid.

4.2.2.2 Preparation of Standard solution

100 mg of Erythromycin working standard batch number=EB003007 and LOT M1E251 with % purity of 89.34 acquired from the same source as Azithromycin standard was accurately weighed into a 10 ml volumetric flask. This was then dissolved in the diluent (refer to chromatographic condition of Erythromycin) to obtain a solution having a known concentration of 10 mg/ml. RS concentrations of 0.7 mg/ml, 0.8 mg/ml, 0.9 mg/ml, 1.0 mg/ml, 1.1 mg/ml, 1.2 mg/ml and 1.3 mg/ml were obtained by diluting 0.7ml, 0.8 ml, 0.9 ml, 1.0 ml, 1.1 ml, 1.2 ml, and 1.3 ml and making it to a final volume of 10 ml with the diluent.

4.2.2.3 Preparation of Test solution of Erythromycin

Ten (10) dosage units of Erythromycin tablets were accurately weighed into a clean dry porcelain mortar and ground until finely powdered. Quantities equivalent to 10 mg of the
Erythromycin API of the dosage form were accurately weighed into a clean dry 10 ml volumetric flask and was sonicated for about 15 minutes after which it was made to mark with the diluent. The resulting solution was then filtered through a 0.45 μm filter discarding a few millilitres.

4.2.2.4 Linearity of Erythromycin

The averages of the Area under the curve (AUC) of six replicates of each concentration were calculated and were plotted against their respective concentration in a graph. The slope, the intercept, and the correlation coefficient \(r^2\) were calculated accordingly using Microsoft excel 2010. The equation of the straight line was deduced as \(AUC = mC + b\);

\[
AUC = \text{Area under the curve of the chromatographic peak of Erythromycin}
\]

\[
m = \text{Slope of the straight line, and}
\]

\[
b = \text{intercept on the AUC (y axis)}
\]

\[
C = \text{Concentration}
\]

The calibration curve as shown in figure 55 was drawn for the Erythromycin drug API. Tables 22 and 23 below show the concentrations and corresponding AUCs, and the calibration curve for Erythromycin API.

**Table 22: Concentration of the Erythromycin RS and corresponding AUCs**

<table>
<thead>
<tr>
<th>ID NO</th>
<th>Conc. (mg/ml)</th>
<th>AUC 1</th>
<th>AUC 2</th>
<th>AUC 3</th>
<th>AUC 4</th>
<th>AUC 5</th>
<th>AUC 6</th>
<th>Average AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.7</td>
<td>2428.932</td>
<td>2429.210</td>
<td>2428.254</td>
<td>2419.932</td>
<td>2428.589</td>
<td>2429.012</td>
<td>2427.3221</td>
</tr>
<tr>
<td>2</td>
<td>0.8</td>
<td>2771.985</td>
<td>2759.824</td>
<td>2721.523</td>
<td>2699.995</td>
<td>2702.032</td>
<td>2771.895</td>
<td>2737.8758</td>
</tr>
<tr>
<td>3</td>
<td>0.9</td>
<td>3057.316</td>
<td>3077.895</td>
<td>3057.265</td>
<td>3048.311</td>
<td>3094.001</td>
<td>3039.953</td>
<td>3062.4571</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>3300.468</td>
<td>3340.656</td>
<td>3309.958</td>
<td>3300.889</td>
<td>3340.001</td>
<td>3338.952</td>
<td>3321.8214</td>
</tr>
<tr>
<td>5</td>
<td>1.1</td>
<td>3693.702</td>
<td>3689.982</td>
<td>3692.985</td>
<td>3693.520</td>
<td>3690.652</td>
<td>3693.652</td>
<td>3692.4157</td>
</tr>
<tr>
<td>6</td>
<td>1.2</td>
<td>4103.824</td>
<td>4075.710</td>
<td>4103.652</td>
<td>4111.824</td>
<td>4011.824</td>
<td>4065.254</td>
<td>4078.6818</td>
</tr>
<tr>
<td>7</td>
<td>1.3</td>
<td>4308.339</td>
<td>4302.339</td>
<td>4306.258</td>
<td>4308.256</td>
<td>4309.029</td>
<td>4308.000</td>
<td>4307.0373</td>
</tr>
</tbody>
</table>
Table 23: Calibration Curve data of Erythromycin

<table>
<thead>
<tr>
<th>Conc.(mg/ml)</th>
<th>SD</th>
<th>RSD</th>
<th>AUC ± RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7</td>
<td>4</td>
<td>0.1</td>
<td>2427.32 ± 0.1</td>
</tr>
<tr>
<td>0.8</td>
<td>34</td>
<td>1</td>
<td>2737.88 ± 1</td>
</tr>
<tr>
<td>0.9</td>
<td>20</td>
<td>0.7</td>
<td>3062.46 ± 0.7</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>0.6</td>
<td>3321.82 ± 0.6</td>
</tr>
<tr>
<td>1.1</td>
<td>2</td>
<td>0.04</td>
<td>3692.42 ± 0.05</td>
</tr>
<tr>
<td>1.2</td>
<td>37</td>
<td>0.9</td>
<td>4078.68 ± 0.9</td>
</tr>
<tr>
<td>1.3</td>
<td>2</td>
<td>0.06</td>
<td>4307.04 ± 0.06</td>
</tr>
</tbody>
</table>

Figure 55: Showing calibration Curve of Erythromycin

4.2.3 HPLC Assay Methods of Clindamycin

4.2.3.1 Buffer preparation

3.4 g of potassium dihydrogen phosphate dissolved in 500 ml of HPLC grade water. The pH adjusted to 7.5 with potassium hydroxide.

4.2.3.2 Preparation of Standard solution:

Clindamycin HCL standard, Batch No. 0032009086 with % Purity of 89.39 was obtained from the same source as that of Azithromycin. 100 mg of Clindamycin working standard was accurately weighed into a 10 ml volumetric flask and dissolved in the diluent to obtain a solution having a known concentration of 10 mg/ml. RS concentrations of 0.7 mg/ml, 0.8 mg/ml, 0.9 mg/ml, 1.0 mg/ml, 1.1 mg/ml, 1.2 mg/ml and 1.3 mg/ml were obtained by
diluting 0.7ml, 0.8 ml, 0.9 ml, 1.0 ml, 1.1 ml, 1.2 ml, and 1.3 ml and making it to a final volume of 10 ml with the diluent as stated in the chromatographic conditions for Clindamycin assay.

4.2.3.3 Preparation of Test solution of Clindamycin

Ten (10) dosage units of Clindamycin capsules were accurately weighed into a clean dry porcelain mortar and ground until finely powdered. Quantities equivalent to 10 mg of the Clindamycin API of the dosage form were accurately weighed into a clean dry 10 ml volumetric flask and was sonicated for about 15 minutes after which it was made to the mark with the diluent. The resulting solution was then filtered through a 0.45 μm filter discarding a few millilitres

4.2.3.4 Linearity of Clindamycin

The averages of the Area under the curve (AUC) of six replicates of each concentration were calculated and were plotted against their respective concentration in a graph. The slope, the intercept, and the correlation coefficient (r²) were calculated accordingly using Microsoft excel 2010. The equation of the straight line was deduced as AUC = mC + b;

AUC = Area under the curve of the chromatographic peak of Clindamycin
m = Slope of the straight line, and
b = intercept on the AUC (y axis)
C = concentration

The calibration curve shown in figure 56 was drawn for the Clindamycin drug API. The tables 24 and 25 below shows the concentrations and corresponding AUCs, and the calibration curve for Clindamycin API.
Table 24: Concentration of the Clindamycin RS and corresponding AUCs

<table>
<thead>
<tr>
<th>ID NO</th>
<th>Conc. (mg/ml)</th>
<th>AUC 1</th>
<th>AUC 2</th>
<th>AUC 3</th>
<th>AUC 4</th>
<th>AUC 5</th>
<th>AUC 6</th>
<th>Average AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.7</td>
<td>3919.524</td>
<td>3919.856</td>
<td>3920.004</td>
<td>3918.998</td>
<td>3917.999</td>
<td>3919.001</td>
<td>3919.2306</td>
</tr>
<tr>
<td>2</td>
<td>0.8</td>
<td>4417.766</td>
<td>4417.524</td>
<td>4420.766</td>
<td>4417.581</td>
<td>4410.125</td>
<td>4413.012</td>
<td>4416.1292</td>
</tr>
<tr>
<td>3</td>
<td>0.9</td>
<td>5072.808</td>
<td>5054.814</td>
<td>5054.814</td>
<td>5072.808</td>
<td>5056.958</td>
<td>5100.024</td>
<td>5068.7048</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>5607.425</td>
<td>5607.124</td>
<td>5604.102</td>
<td>5602.581</td>
<td>5610.125</td>
<td>5614.973</td>
<td>5603.9377</td>
</tr>
<tr>
<td>5</td>
<td>1.1</td>
<td>6135.076</td>
<td>6145.104</td>
<td>6135.076</td>
<td>6135.076</td>
<td>6135.076</td>
<td>6135.076</td>
<td>6136.7479</td>
</tr>
<tr>
<td>6</td>
<td>1.2</td>
<td>6616.001</td>
<td>6614.458</td>
<td>6614.654</td>
<td>6615.003</td>
<td>6614.25</td>
<td>6614.973</td>
<td>6614.8908</td>
</tr>
</tbody>
</table>

Table 25: Calibration Curve data of Clindamycin

<table>
<thead>
<tr>
<th>Conc.(mg/ml)</th>
<th>SD</th>
<th>RSD</th>
<th>AUC ± RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7</td>
<td>0.734606805</td>
<td>0.018743648</td>
<td>3919.231±0.02</td>
</tr>
<tr>
<td>0.8</td>
<td>3.84685115</td>
<td>0.087109115</td>
<td>4416.129±0.09</td>
</tr>
<tr>
<td>0.9</td>
<td>17.5413033</td>
<td>0.346070718</td>
<td>5068.705±0.3</td>
</tr>
<tr>
<td>1</td>
<td>6.467915866</td>
<td>0.115417339</td>
<td>5603.938±0.1</td>
</tr>
<tr>
<td>1.1</td>
<td>4.093913857</td>
<td>0.066711455</td>
<td>6136.748±0.07</td>
</tr>
<tr>
<td>1.2</td>
<td>0.616397599</td>
<td>0.009318334</td>
<td>6614.891±0.01</td>
</tr>
</tbody>
</table>

Figure 56: Showing calibration Curve of Clindamycin

4.3 *In vitro* Dissolution Test Methods for the Antibiotic Drugs

The *in vitro* dissolution test of the antibiotic samples in tablets and capsules dosage forms were carried out in accordance with the US pharmacopoeia standards using the Vanguard Pharmaceutical Machinery LID – 8 Dissolution Tester. Both Apparatus 1 (Basket Apparatus) and 2 (Paddle Apparatus) were used in this study depending on the dosage form. The HPLC
methods used for their respective assays were used in the quantification of the dissolved API after the stipulated time.

The machine was set up with each of the six stirrers calibrated for the proper required height from the base of the vessel. Then the six containers were each filled with the 900 ml of the required buffer medium as recommended in their individual monographs. The machine was then programmed at the required time, temperature, and stirrer rotation as stipulated in their individual monographs and then left to run to acclimatise. The machine was deemed acclimatised when the temperature of the buffer medium in all the vessels were up to mark 37 ± 0.5 °C. The stirrer was then stopped and each of the dosage units was added at staggered times of 30 seconds apart to the six vessels to allow for step withdrawal. The machine was then started with the timer set. At the stipulated time after the machine was stopped, 10 ml of sample were withdrawn at the centre of each vessel, filtered using 0.45μm filter paper; and then taken for immediate HPLC quantification analysis.

4.3.1 In vitro dissolution test of Azithromycin

4.3.1.1 Dissolution conditions

Apparatus: Paddle (USP Type 2) with different buffer and time conditions stated under their respective conditions below were used for the in vitro dissolution test of the tablet and capsule dosage forms of Azithromycin.

4.3.1.2 Preparation of Dissolution Medium (0.1 M dibasic Sodium phosphate)

14.196 g of dibasic sodium phosphate was accurately weighed and transferred quantitatively into a clean dry 1000 ml volumetric flask. 800 ml of deionized water was added to the
content of the flask, and the flask shaken for about 5 minutes. The pH was adjusted to 6.8 with about 40 mL of hydrochloric acid, after which more deionized water was added to the mark.

4.3.1.3 Dissolution conditions for Azithromycin Capsules

Medium: The dissolution vessels were each filled with 900ml Disodium phosphate buffer of pH 6.0 mixed with trypsin

Speed: 100 RPM

Time: 45 minutes

Temperature: 37 ± 0.5 °C

Blade’s distance from bottom of vessel: 25 ± 2mm

4.3.1.4 Preparation of Dissolution Medium: (0.1 M dibasic Sodium phosphate + Trypsin)

14.196 g of dibasic sodium phosphate was accurately weighed and transferred quantitatively into a clean dry 1000 ml volumetric flask. 800 ml deionized water was added to the content of the flask, and the flask shaken for about 5 minutes. The pH was adjusted with about 40mL of hydrochloric acid to a pH of 6.0, after which 100 mg of trypsin was added and then more deionized water added to the mark.

4.3.1.5 Preparation of test solution

Each of the 6 dissolution vessels was filled to 900 ml mark with the dissolution medium. The temperature was monitored to ensure that each of the vessels agreed with the content of the
bath. One dosage unit was carefully transferred into each basket and the test run as per the pre-set conditions (75RPM and 100RPM for tablet and capsule respectively). 10ml of the samples were withdrawn using the sample canula. The solutions were collected, filtered and each labelled as T1, T2, T3, T4, T5, and T6 respectively.

4.3.1.6 Conditions for HPLC Quantification of Azithromycin In vitro Dissolution Test

- Column Type: Xterra MS C18 5 μm 150 x 4.6 mm
- Flow Rate: 1 ml/min
- Injection Volume: 50 μl
- Mobile Phase: 40 volumes of buffer : 60 volume of Acetonitrile
- Wavelength of detection: Diode Array Detector (DAD): 210 nm
- Diluent: 40 volumes of buffer : 60 volume of Acetonitrile
- Temperature: 30º C

4.3.1.7 Preparation of buffer solution

8.7 g of dipotassium hydrogen phosphate was accurately weighed into a 1000 ml volumetric flask and dissolved with about 980 ml of HPLC grade water. The pH was adjusted to 8.2 with 80 % phosphoric acid, after with more the water was added to the mark.

4.3.1.8 Preparation of Test solution of the Dissolved Azithromycin API for quantitation

An aliquot of the dissolution sample was withdrawn from the vessel after the stipulated time and filtered immediately. Using serial dilution with the diluent a volume equivalent to a
concentration of 0.2777 mg/ml was prepared and 50 µl of this was injected into the HPLC for analysis. The percentage drug release was calculated as shown in the example in Chapter three.

4.3.2 *In vitro* dissolution test of Erythromycin Stearate

4.3.2.1 Dissolution conditions

Apparatus Required: Paddle (USP Type 2) was used for the *in vitro* dissolution test of the tablet dosage form of Erythromycin.

Medium: The dissolution vessels were each filled with 900 ml 0.05 M of disodium hydrogen phosphate of pH 6.8.

Speed: 100 RPM

Time: 120 minutes

Temperature: 37 ± 0.5 °C

Blade’s distance from bottom of vessel: 25 ± 2mm

4.3.2.2 Preparation of Dissolution Medium: (0.05 M of disodium hydrogen phosphate)

5.53 g of disodium hydrogen phosphate was accurately weighed and transferred quantitatively into a clean dry 1000 ml volumetric flask. 800 ml of deionized water was added to the content of the flask, and the flask shaken for about 5 minutes. The pH was adjusted to 6.8 with about 40 mL of hydrochloric acid, after which more deionized water was added to the mark.


**4.3.2.3 Preparation of test solution**

Each of the 6 dissolution vessels was filled to 900 ml mark with the dissolution medium. The temperature was monitored to ensure that each of vessels agreed with the content of the bath. One tablet was carefully transferred into each basket and the test run as per the pre-set conditions (100RPM). 10ml of the samples were withdrawn using the sample canula. The solutions were collected, filtered and each labelled as T1, T2, T3, T4, T5, and T6 respectively.

**4.3.2.4 Conditions for HPLC Quantification of Erythromycin *In vitro* Dissolution Test**

- Column Type: Xterra MS C18 5 μ m 150 x 4.6 mm
- Flow Rate: 1 ml/min
- Injection Volume: 50μl
- Mobile Phase: 40 volumes of buffer : 60 volume of Acetonitrile
- Wavelength of detection: Diode Array Detector (DAD): 210 nm
- Diluent: 40 volumes of buffer : 60 volume of Acetonitrile
- Temperature: 30° C

**4.3.2.5 Preparation of buffer solution**

8.7 g of dipotassium hydrogen phosphate was accurately weighed into a 1000 ml volumetric flask and dissolved with about 980 ml of HPLC grade water. The pH was adjusted to 8.2 with 80 % phosphoric acid, after with more the water was added to the mark.
4.3.2.6 Preparation of Test solution of the Dissolved Erythromycin API for quantitation

An aliquot of the dissolution sample was withdrawn from the vessel after the stipulated time and filtered immediately. Using serial dilution with the diluent a volume equivalent to a concentration of 0.5556mg/ml was prepared and 50μl of this was injected into the HPLC for analysis.

4.3.3  *In vitro* dissolution test of Clindamycin

4.3.3.1 Dissolution conditions

Apparatus Required: Basket (USP Type 1) was used for the *in vitro* dissolution test of the capsule dosage form of Clindamycin.

Medium: The dissolution vessels were each filled with 900ml Disodium phosphate buffer of pH 6.8

Speed: 100 RPM

Time: 30 minutes

Temperature: 37 ± 0.5 °C

Blade’s distance from bottom of vessel: 25 ± 2mm

4.3.3.2 Preparation of Dissolution Medium

5.53 g of disodium hydrogen phosphate was accurately weighed and transferred quantitatively into a clean dry 1000 ml volumetric flask. 800 ml of deionized water was added to the content of the flask, and the flask shaken for about 5 minutes. The pH was adjusted to 6.8 with about 40 mL of hydrochloric acid, after which more deionized water was added to the mark.
4.3.3.3 Preparation of test solution

Each of the 6 dissolution vessels was filled to 900 ml mark with the dissolution medium. The temperature was monitored to ensure that each of vessels agree with the content of the bath. One capsule was carefully transferred into each basket and the test run as per the pre-set conditions (100RPM). 10ml of the samples were withdrawn using the sample canula. The solutions were collected, filtered and each labelled as T1, T2, T3, T4, T5, and T6 respectively.

4.3.3.4 Conditions for HPLC Quantification of Clindamycin in Vitro Dissolution Test

- Column Type: kromasil 5μm C8 100A 150 x 4.6 mm
- Flow Rate: 1 ml/min
- Injection Volume: 25μl
- Mobile Phase: 40 volumes of buffer : 60 volume of Acetonitrile
- Wavelength of detection: Diode Array Detector (DAD): 210 nm
- Diluent: 110 buffer : 90 Acetonitrile
- Temperature: 30°C

4.3.3.5 Preparation of buffer solution

3.4 g of potassium dihydrogen phosphate was weighed into a 500ml volumetric flask and dissolved with about 490 ml of HPLC grade water. The pH adjusted to 7.5 with potassium hydroxide, after with more water was added to the mark.
4.3.3.6 Preparation of Test solution of the Dissolved Clindamycin API for quantitation

An aliquot of the dissolution sample was withdrawn from the vessel after the stipulated time and filtered immediately. Using serial dilution with the diluent a volume equivalent to a concentration of 0.1667mg/ml was prepared and 50μl of this was injected into the HPLC for analysis.
# APPENDIX

Appendix 1: List of Drug Samples

<table>
<thead>
<tr>
<th>S/N</th>
<th>CODE</th>
<th>NAME OF DRUG</th>
<th>DOSAGE FORM</th>
<th>ACTIVE INGREDIENT</th>
<th>BATCH NO</th>
<th>MANUFACTURER</th>
<th>MAN – EXP. DATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GAa1</td>
<td>AZIFAST – 250</td>
<td>Tablets</td>
<td>Azithromycin</td>
<td>R51002 R</td>
<td>Ipcia Laboratories Ltd. India</td>
<td>03/11 – 03/13</td>
</tr>
<tr>
<td>2</td>
<td>GAa2</td>
<td>AZIRON - 250</td>
<td>Capsules</td>
<td>Azithromycin</td>
<td>RC 109</td>
<td>ROXIN Ghana Ltd. Ghana</td>
<td>09/11 – 08/13</td>
</tr>
<tr>
<td>3</td>
<td>GAa3</td>
<td>AZIMAX - 250</td>
<td>Capsules</td>
<td>Azithromycin</td>
<td>210022</td>
<td>Medreich Plc. London</td>
<td>Jun 11 – Jun 14</td>
</tr>
<tr>
<td>4</td>
<td>GAp1</td>
<td>AZOMAX</td>
<td>Oral Suspension</td>
<td>Azithromycin</td>
<td>CH1841</td>
<td>Sandoz S.R.L. Romania</td>
<td>Mar 12 – Mar 14</td>
</tr>
<tr>
<td>5</td>
<td>GAa4</td>
<td>AZITHROMYCIN-250</td>
<td>Tablets</td>
<td>Azithromycin</td>
<td>MD2002</td>
<td>Danadamas, Ghana</td>
<td>Jan 12 – Dec 14</td>
</tr>
<tr>
<td>6</td>
<td>GAp2</td>
<td>BEXYMClN</td>
<td>Oral Suspension</td>
<td>Azithromycin</td>
<td>SVA 293</td>
<td>**Beximco Pharm. Ltd, Tong. Bangladesh</td>
<td>Jan 12 – Jan 14</td>
</tr>
<tr>
<td>7</td>
<td>GAp3</td>
<td>ZITHROMAX</td>
<td>Oral Suspension</td>
<td>Azithromycin</td>
<td>130400</td>
<td>Pfizer, Italy</td>
<td>Oct 11 – Oct 13</td>
</tr>
<tr>
<td>8</td>
<td>GAa5</td>
<td>ZYMAX – 250</td>
<td>Capsules</td>
<td>Azithromycin</td>
<td>0107K</td>
<td>Ernest Chemist Ltd, Ghana</td>
<td>Jul 10 – Jul 13</td>
</tr>
<tr>
<td>9</td>
<td>GEa3</td>
<td>Enamycin – 250</td>
<td>Tablets</td>
<td>Erythromycin</td>
<td>0303L</td>
<td>Ernest Chemist Ltd. Ghana</td>
<td>03/11 – 03/16</td>
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<td>05/11 – 05/14</td>
</tr>
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<td>Clidamycin – 150mg</td>
<td>Capsules</td>
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<td>12</td>
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<td>13</td>
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**SAMPLES FROM NIGERIA**

**AZITHROMYCIN**

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**CLINDAMYCIN**

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**G = Ghana ≡ Drugs bought in Accra, Ghana**

**N = Nigeria ≡ Drugs bought in Lagos, Nigeria**

**A = Azithromycin**

**E = Erythromycin**

**C = Clindamycin**

**a = Adult**

**p = paediatric**

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University of Ghana          http://ugspace.ug.edu.gh
Appendix 11: Chromatograms of HPLC Assay Analysis
Chromatograms of six replicate injections of a test preparation containing 0.5060mg/ml of Azithromycin RS

1st Injection

2nd Injection

3rd Injection

4th Injection
Chromatograms of six replicate injections of a preparation containing 0.5mg/ml Azithromycin tablet sample GAa2

1st Injection

2nd Injection

5th Injection

6th Injection
Chromatograms of six replicate injections of a preparation containing 0.5mg/ml Azithromycin Capsules sample NAa3

1st Injection

2nd Injection

3rd Injection

4th Injection
Chromatograms of six replicate injections of a preparation containing 0.5mg/ml Azithromycin Oral Suspension sample Nap1

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<th>Type</th>
<th>Width [min]</th>
<th>Area [mAU*s]</th>
<th>Height [mAU]</th>
<th>Area %</th>
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<tr>
<td>1st Injection</td>
<td>7.112</td>
<td>0.3910</td>
<td>2726.32511</td>
<td>116.22971</td>
<td>100.0000</td>
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</table>

<table>
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<th>Type</th>
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<tbody>
<tr>
<td>2nd Injection</td>
<td>8.270</td>
<td>0.4672</td>
<td>3493.60107</td>
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<td>5th Injection</td>
<td>7.112</td>
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<td>0.3910</td>
<td>2725.23150</td>
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<td>2nd Injection</td>
<td>8.270</td>
<td>0.4672</td>
<td>3490.62340</td>
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Chromatograms of six replicate injections of a test preparation containing 1.0mg/ml of Erythromycin RS
Chromatograms of six replicate injections of a preparation containing 1.0mg/ml Erythromycin tablet sample GEa1
3rd Injection

4th Injection

5th Injection

6th Injection

Peak RetTime Type Width Area Height Area
# [min] [min] [mAU*s] [mAU] %
1 3.446 BBA 0.2752 2474.53252 135.42558 100.0000

Totals:
2474.53252 135.42558

Peak RetTime Type Width Area Height Area
# [min] [min] [mAU*s] [mAU] %
1 3.456 BBA 0.2752 2474.34523 135.42558 100.0000

Totals:
2474.34523 135.42558

Peak RetTime Type Width Area Height Area
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1 3.471 BBA 0.2752 2473.08104 135.42558 100.0000

Totals:
2473.08104 135.42558

Peak RetTime Type Width Area Height Area
# [min] [min] [mAU*s] [mAU] %
1 3.455 BBA 0.2752 2474.56773 135.42558 100.0000

Totals:
2474.56773 135.42558

116
Chromatograms of six replicate injections of a test preparation containing 1.0mg/ml of Clindamycin RS

1st Injection

2nd Injection

3rd Injection

4th Injection
Chromatograms of six replicate injections of a preparation containing 1.0mg/ml Clindamycin Hydrochloride capsule sample GCa4
Appendix 111: Chromatograms of *In vitro* Dissolution Assay Analysis

Chromatograms of six injections of an *in vitro* dissolution test preparation containing 0.2777mg/ml Azithromycin tablet Sample GAa1

1st Injection

2nd Injection

3rd Injection

4th Injection
Chromatograms of six injections of an in vitro dissolution test preparation containing 0.2777mg/ml Azithromycin Capsule Sample NAa3
3rd Injection

4th Injection

5th Injection

6th Injection
Chromatograms of six injections of an in vitro dissolution test preparation containing 0.5556mg/ml Erythromycin Stearate Sample NEa9

1st Injection

2nd Injection

3rd Injection

4th Injection
Chromatograms of six injections of an in vitro dissolution test preparation containing 0.1667 mg/ml Clindamycin Hydrochloride Sample GCa4.
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