Profiling of Some Amoxicillin Drugs in Ghana Using Nuclear Magnetic Resonance Spectroscopy

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DECLARATION

I hereby declare that with the exception of references to other people’s work which have been duly acknowledged, this Thesis is the result of my own research work and no part of it has been presented for another degree in this University or elsewhere.

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(Candidate)

I hereby declare that the preparation of this project was supervised in accordance with guidelines of the supervision of Thesis work laid down by the University of Ghana.

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(CO-SUPERVISOR)
DEDICATION

This research work is dedicated to my parents and siblings for their support and encouragement over the years.
ACKNOWLEDGEMENTS

I would like to thank the Almighty God for granting me the strength and good health to embark on this academic exercise. I would also like to express my profound gratitude to my supervisor, Rev. Dr. Samuel Akoto Bamford, for his immense hard work, guidance and dedication in shaping of this thesis work. I am grateful to my supervisor, Prof. Aba BentilAndam, for her wisdom and guidance in supervising this research work. My appreciation also goes to Dr. Emmanuel Osei-Twum of the Department of Chemistry, University of Ghana for access and use of the NMR Laboratory and his invaluable assistance and support in the NMR analysis of this work. Finally I thank all the lecturers and students of the Department of Nuclear Science and Applications, Graduate School of Nuclear and Allied Sciences University of Ghana for their various contributions made towards this research work.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>API</td>
<td>Active Pharmaceutical Ingredients</td>
</tr>
<tr>
<td>BP</td>
<td>British Pharmacopoeia</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drugs Authority</td>
</tr>
<tr>
<td>GSA</td>
<td>Ghana Standard Authority</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>MS</td>
<td>Mass Spectrometry</td>
</tr>
<tr>
<td>NAFDAC</td>
<td>National Agency for Food and Drug Administration and Control</td>
</tr>
<tr>
<td>NHIS</td>
<td>National Health Insurance Scheme</td>
</tr>
<tr>
<td>NIR</td>
<td>Near Infra-red Spectroscopy</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance Spectroscopy</td>
</tr>
<tr>
<td>OOS</td>
<td>Out of Specification</td>
</tr>
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</table>
ABSTRACT

The prevalence of counterfeit drugs is seen as a problem faced in both developed and developing countries where Ghana is not an exception. Antibiotics are amongst the most counterfeit drugs in developing countries. What is less understood is that there are inadequate and ineffective quality control procedures in monitoring of drugs manufactured and imported into the country. This research work is aimed at contributing towards the development of routine analytical procedures that will facilitate distinguishing between fake and genuine amoxicillin drugs. This was accomplished by elaborating operating procedures for the analysis of specific antibiotic drug using nuclear magnetic resonance (NMR) spectroscopy and establishing the NMR profile of active principal ingredient (API) of amoxicillin drug and assessing the API in samples of amoxicillin drug purchased in Accra. Three brands of amoxicillin samples consisting of imported amoxicillin, National Health Insurance Scheme (NHIS) amoxicillin were purchased from a licensed pharmacy shop in Accra and amoxicillin purchased from Okaishie market were used for analysis. Standard amoxicillin known as amoxicillin trihydrate obtained from Ernest Chemist in Accra was also used analysed. The authenticity of the drugs was analysed using 1H and C-13 nuclear magnetic resonance spectroscopy. Upon analysis H-NMR and C-13 NMR profiles were obtained for the API (Amoxicillin Trihydrate) in amoxicillin. H NMR showed relatively higher sensitivities for the drug than C-13 NMR therefore analysis for the antibiotics was focused on H-NMR. After analysis amoxicillin trihydrate was identified as the API. A procedure suitable for NMR sample preparation of amoxicillin for NMR analysis was elaborated. Dimethyl sulfoxide was identified as a suitable solvent for the experiments. The samples were prepared by dissolving suitable quantities (10mg) of the drug in (1ml) of the chosen solvent. H-
NMR technique was used to provide an NMR profile for the Active Pharmaceutical Ingredient (API). With the profile it was possible to identify the presence of API in all the amoxicillin samples studied. There were differences in the number of hydrogen peaks and intensity values for the peaks. This therefore provided a means for identifying the different types of drugs using the structure of the API. NMR analysis requires small sample size as compared to other analytical techniques. Analysis is faster, does not require much time.
CHAPTER ONE

GENERAL INTRODUCTION

1.1 BACKGROUND

Among the numerous counterfeit products, counterfeit drugs have the potential of causing harm to the health of consumers. In order to regulate the production of pharmaceutical products, compliance with the highest quality and safety standards need to be ensured. Therefore all drugs need to undergo clinical trials before being marketed to test its efficiency and verify their quality. These measures are meant to work as a safety standard to guarantee the quality of drugs (UNICRI, 2012).

According to the 1992 World Health Organisation (WHO) definition, a counterfeit drug is a pharmaceutical product which is deliberately and fraudulently mislabelled with respect to identity and/or source. The WHO makes it clear that this definition is applicable to both branded and generic drugs which are made up of drugs with the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient active ingredients or with fake packaging (UNICRI, 2012).

Counterfeit drugs with its implications are understood to be a central challenge not only to the integrity of public health systems around the world but a direct threat to the welfare and health of an individual (Akinyadenu, 2013).

There are numerous factors which determine the quality of medicines including the raw materials used, manufacturing process, equipment, technical knowhow for production and packaging of the product, transportation and storage conditions.
Therefore there is the need for manufacturers of drugs to set up quality specifications (Onwuka, 2010).

Substandard drugs are also regarded as counterfeit drugs. A report by Pfizer in 2015 says substandard medicines may not meet the appropriate quality standards and may be harmful to the life of patients. Substandard medicines may not meet the demands of the registered formulation which include the intended amount of active ingredients, may contain unsafe amount of ingredients and impurities. Substandard medicines can also be as a result of improper manufacturing process, improper packaging, storage or transportation or distribution that does not stick to good manufacturing practices.

The threat of drug counterfeiting appears not to be seen due to the neglect it has suffered over the years from stakeholders involved in the pharmaceutical supply chain. It has therefore become a global challenge requiring deliberate collaboration in arresting this canker. Globally the types of counterfeit drugs depend on regional distributions.

The WHO has indicated that hormones and steroids are common targets in developed countries whilst in the developing countries counterfeit antibiotics, anti-malarial agents, anti-tuberculosis drugs, anti-retroviral agents, painkillers, hormones and steroids are common targets.

In Indonesia, in 2003, 55 counterfeit drugs sold in the market were discovered by the Indonesian Drug and Food Control Agency. Among them were amoxicillin and penicillin capsules. In 2008 more than $6.65 million of counterfeit drugs were seized by Interpol meant for the treatment of malaria, HIV, tuberculosis and other known infections in Southeast Asia and made 27 arrests (http://www.whpa.org).
Though Indian pharmaceutical industry plays a vital role in India’s economy by producing drugs, about 12 to 25% of drugs produced and sold in India are counterfeit. Various reasons account for why drug counterfeiting is expanding in India. These include poor pharmaceutical regulation, growing of pharmaceutical industry, high cost of drugs, value added tax, lack of public awareness, weak enforcement of legislations and flexibility in the current legal framework. Drug counterfeiting has become a moneymaking business such that consumers and physicians who prescribe the drugs are not able to differentiate between genuine drug and counterfeit drug (Verma et al, 2014).

Drug counterfeiting in Africa is gaining international recognition. World leaders have now realised that trading in counterfeit drug is not a problem only faced by a few developing countries but a global one which comes with its own consequences (Fenoff et al, 2009). According to WHO, a significant fraction of the world’s drug supply is counterfeit and a survey conducted estimates that 10 to 15% of the world drug supply are counterfeit, and more than 30% of all medicines sold in Africa and up to 70% of drugs sold in Nigeria (Fenoff et al, 2009). In Africa the three forms of drugs which are often counterfeited are malaria drug, HIV/AIDS drug and tuberculosis drug. In 2009, many children were killed in Nigeria because of poorly compounded chloroquine syrup. A total of 109 children also died in Nigeria in a different issue of paracetamol syrup which contained toxic ethylene glycol solvent instead of propylene glycol (Ubajaka et al, 2016).

An estimate made by WHO suggests that of the 1 million malaria deaths that occur in Africa annually, 200,000 are the result of counterfeit antimalarial drugs. Counterfeit tuberculosis and malaria drugs kill 700,000 people every year in Africa (Muthiani and Wanjau, 2012).
According to the United States Pharmacopeia Convention, substandard and counterfeit versions of anti-malaria medicines were found in Ghana through the Medicines Quality Monitoring Surveillance program implemented through the Food and Drugs Authority (FDA). The Food and Drugs Authority ordered the recalls for the medicines and disclosed the names of the pharmacies, clinics and hospitals where the medicines were found (http://www.usp.org). Counterfeit Coartem® was found in the year 2009 when an individual brought a suspicious sample to the attention of the Medicines Quality Monitoring program (http://www.usp.org). In Kumasi it was announced by the zonal office of the Food and Drugs Authority that counterfeit antimalarials (coartem) with batch no X0089 and M1200 found in the market contained no active ingredient (http://www.ghananewsagency.org).

Counterfeit drugs pose dangers to the health of an individual since they do not contain the expected amount of active ingredient and do not meet any standard requirements for quality, efficiency and safety. Patients are therefore exposed to a number of risks despite the presence of harmful substances, these drugs can be inactive and cause major unfavourable conditions and complications for patients (http://www.sanofi.com). Drug counterfeiting reduces trust in health care providers, and undermines the integrity of health systems. This may lead to patients not adhering to their medicines (Onwuka, 2010).

Counterfeiting can be harmful to the economy. This is due to the fact that illegal manufacturers who do not pay any import duties and sales tax of the medicines they sell compete with genuine manufacturers. The trade relations between countries do not progress as it may bring about trade limitations. Counterfeiting brings about profit reduction in developing countries by reducing motivations meant for further research.
and development. It also prevents foreign investors from coming into the country (Onwuka, 2010). It has been estimated by the Center for Medicines in the United States that the sale of counterfeit drugs reached 75 billion US dollars in 2010 which has led to an increase of 92% since 2005 (Muthiani and Wanjau, 2012).

Recent figure from the world Economic forum evaluates that counterfeit medicine sales are at approximately 200 billion US dollars which is the leading sector for unlawful trafficking (http://www.iracm.com).

Drug regulation covers a number of functions. Some of these functions include licensing, inspection of facilities of the manufacturer and channels of distribution, assessment of product and registering, quality control and drugs undergoing clinical trials. These measures must be put in place and acted upon for the effective protection of the consumer (Ratanawijitrasin et al, 2002).

In the developed counties, production and distribution of counterfeit drugs is not widely spread due to enhanced legislation, stronger institutions and a more efficient regulatory control. According to the WHO, countries which fall within the European Union (EU) and countries like United States of America, Canada, Australia, Japan and New Zealand have a lower proportion of counterfeit drugs of not more than 1% of the market value.

Nevertheless, in developing countries these regulations and control mechanisms are woefully inadequate. Hence, there exist a loss of public confidence in medicines and the health organization. Drugs purchased by consumers from pharmacy shops, street vendors and patent medicine stores are about 50%, where there is little control and the risk of the drug being counterfeit is high.
There are several methods used in the detection of counterfeit drugs which include simple thin layer chromatography (TLC), near infrared spectroscopy (NIR) and Liquid chromatography- mass spectrophometry (LC-MS) (Deisingh, 2004).

1.2 RESEARCH PROBLEM STATEMENT

Ghana as a country is faced with problems of counterfeit drugs which can be attributed to the fact that there is inadequate and ineffective quality control procedures in monitoring of drugs manufactured and imported into the country.

The statutory organizations like Food and Drug Authority (FDA) and the Ghana Standard Authority (GSA) do not have enough personnel and facilities to deal with the enormous volume of drugs that require control. There is the need to explore other optional analytical techniques and procedures for control of medical drugs.

1.3 OBJECTIVES

The main objective of this research work is aimed at developing a routine analytical procedure that will facilitate distinguishing between fake and genuine medical drugs.

1.3.1 Specific Objectives

1. To elaborate operating procedures for the analysis of amoxicillin using nuclear magnetic resonance spectroscopy.

2. To establish the Nuclear Magnetic Resonance profile for the active principal ingredient for amoxicillin drug, and assess the API in samples of amoxicillin drug purchased in Accra.
1.4 RELEVANCE AND JUSTIFICATION

The relevance of the study is seen in the fact that counterfeit drugs have implications of being a central challenge to the public health systems and consumers. This research work addresses the problem of drug counterfeiting by using nuclear magnetic resonance spectroscopy to obtain expected profile.

This work will give the consumer the assurance of the quality of amoxicillin on the market. It will also inform the manufacturer of the availability of another analytical technique that can be employed to assess quality of amoxicillin being manufactured. This study will help the international trade to monitor different generic drugs from different sources.
CHAPTER TWO

LITERATURE REVIEW

2.1 POOR QUALITY DRUGS

To determine the quality of drugs one must consider factors such as the raw materials used, manufacturing environment, formulation, manufacturing process, equipment, technical know for production and packaging of the product, transportation and storage conditions. The manufacturers set the quality specifications and are published in Pharmacopoeias. Poor quality drugs are drugs which do not meet the prescribed specifications for quality, strength, pureness, packaging or labelling (USP, 2010).

The quality of drugs is a major concern in many parts of the world as a result of an increase in counterfeiting of drugs. A survey conducted by Pfizer on counterfeiting of drugs suggests that about 61% of people believe that it presents a serious problem in their countries. In developing countries this problem is as serious to an extent that there is an increase in transmittable and chronic disease among other public health issues. Poor quality drugs can be classified as being counterfeit or substandard.

2.1.1 Counterfeit Drugs

Due to the nature of drug counterfeiting in some countries, the definition of counterfeit drugs varies from one country to the other. According to the World Health Organization (WHO), a counterfeit drug is one which is deliberately and fraudulently mislabelled with respect to identity and/or source.

Drug counterfeiting is applicable to both branded and generic drugs. Counterfeit drugs may include drugs with the correct ingredients or with the wrong ingredients, without
active ingredients, with incorrect quantities of active ingredients or with fake packaging.

The National Agency for Food and Drug Administration and Control (NAFDAC) of Nigeria defines counterfeit drugs as drugs with the same quantity of active ingredient as the genuine brands which are identical that are unlikely to produce the desired curative effects due to differences in their formulation and bioavailability when compared to the genuine brand (Onwuka, 2010).

2.1.2 Substandard Drugs
According to WHO, Substandard drugs are drugs that present an unintentionally incorrect package or that may have incorrect quantity of ingredients (UNICRI, 2012). Substandard drugs which is also known as “out of specification” (OOS) drugs are drugs produced by manufacturers who have the license to operate within the framework of national pharmaceutical regulatory standards. These drugs contain no active ingredients or the wrong amount of active ingredients. With substandard drugs the legitimate manufacturer is known and may arise as a result of unexpected and deliberate actions of the manufacturer (Christian et al, 2012).

2.2 PHARMACEUTICAL DRUG
A pharmaceutical drug is defined as a chemical substance which is formulated as one active pharmaceutical ingredient or in combination with other substance that is active pharmacologically which may be in a separate but packed in a single unit pack as combination product meant for external or internal part of the body, or
for use in the prevention of disease, medical diagnosis, treatment or cure (http://www.fda.gov).

There are several ways in which medications are classified. One of the most significant divisions is between traditional small molecule drugs normally obtained from biopharmaceuticals, and chemical synthesis, which include vaccines, recombinant proteins, blood products used for therapy (for example, IVIG), cell therapy (for instance, stem cell therapies), and gene therapy.

Therapeutic effects, route of administration, mode of action and pharmacological action or activity, and biological system affected, are all pharmacological properties used in the classification of pharmaceutical drugs apart from their origin. The Anatomical Therapeutic Chemical Classification System also called the ATC system is a detailed and notably used classification system. The list of important and key medical drugs is also kept by the World Health Organization. Medical drugs discovery and development are challengingly complex and expensive endeavours tackled by scientists, pharmaceutical companies and government agencies. Government agencies usually determine the drugs that can be on the market, the way the drugs can be marketed and; sometimes the pricing of drug.

### 2.3 CLASSIFICATION OF PHARMACEUTICAL DRUGS

The categories of pharmaceutical drugs according to their origin are:

i. Medical drug obtained from radioactive materials/substances.

ii. Medical drug derived from chemical and natural origin; i.e., drug that is obtained
from partial herbal and partial chemical synthesis. A typical example is steroidal
drugs.

iii. Medical drug that is obtained from chemical synthesis.

iv. Pharmaceutical drug which is obtained from animal origin: For example,
enzymes, hormones, etc.

v. Pharmaceutical drug that is obtained from microbial origin i.e. Antibiotics.

vi. Medical drug which is from natural origin; i.e., from mineral or herbal origin,
other medical drugs are of marine origin.

vii. Pharmaceutical drug that is derived using biotechnology genetic-engineering, with
a typical example being hybridoma technique.

One of the key classifications is between traditional small molecule drugs, usually
derived from chemical synthesis, and biologic medical products, including
recombinant proteins, vaccines, blood products used therapeutically (such as IVIG),
gene therapy, and cell therapy (for instance, stem cell therapies).

Pharmaceutical drugs or medicines are classified in various other groups,
besides their origin, on the basis of pharmacological properties like mode of
action and their pharmacological action or activity, such as by chemical
properties, mode or route of administration, biological system affected, or
therapeutic effects. An elaborate and widely used classification system is the
Anatomical Therapeutic Chemical (ATC) Classification System. The World Health
Organization keeps a list of essential medicines (http://www.epgonline.org/class.cfm).
Medical drugs play a very crucial role so far as the well-being of humans is concerned and are therefore categorized according to various classes which include:

a) Antibiotic drugs: Medicines that subdue the growth of microbes or germs.

b) Analgesic drugs: Pain reduction medicine, also known as painkillers.

c) Mood stabilization drugs: valpromide, lithium, etc.

d) Antimalarial drugs: Drugs that treat malaria.

e) Hormone replacement drugs; e.g., Premarin.

f) Antiseptic drugs: Drugs which prevent the growth of germs around wounds, burns, and cuts.

g) Antipyretic drugs: Medicines that reduce fever (pyrexia/pyresis).

h) Statins: simvastatin, pravastatin and lovastatin.

i) Oral contraceptive drugs: "triphasic" pill, enovid, "biphasic" pill etc.

j) Tranquilizers: chlordiazepoxide, reserpine, meprobamate, chlorpromazine, etc.

k) Stimulants: methylphenidate.

2.4 MEDICAL DRUG

According to the European Union law, a medical drug can be defined as any substance or combination of substances presented as having properties for treating or preventing disease in human beings; or any substance or combination of substances which may be used in or administered to human beings either with a view to restoring, correcting or modifying physiological functions by exerting a
pharmacological, immunological or metabolic action, or to making a medical

The United States also defines a medical drug as:

- A substance recognized by an official pharmacopoeia or formulary.
- A substance intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease.
- A substance (other than food) intended to affect the structure or any function of the body.
- A substance intended for use as a component of a medicine but not a device or a component, part or accessory of a device.

Biological products are included within this definition and are generally covered by the same laws and regulations, but differences exist regarding their manufacturing processes (chemical process versus biological process) (http://www.fda.gov).

2.4.1 Brand-Name Drug

A brand-name drug is a drug that has the trade name and is protected by a patent ie: can be produced and sold only by the company holding the patent.

2.4.2 Generic Drug

A generic drug is a drug product that is comparable to a brand-name drug in dosage form, strength, quality, performance characteristics, intended use and are sold once the patent protection of a brand-name drug expires.
2.4.3 Characteristics of Substandard/Counterfeit Drugs

Counterfeit drug products are in many forms. Some contain active pharmaceutical ingredients in harmful quantities or contain no active pharmaceutical ingredient. Some of the preparations are from sources which are unacceptable or are differently formulated and may contain high amount of impurities such as packaging which makes the product a falsified one and can be seen in terms of the colour of pill, shape and size.

2.4.3.1 Reduced Stability and Bioavailability

There have been several reports on essential drugs stability under actual storage conditions in the tropics, warehouses and in some of the wholesale pharmacies which may not be acceptable for ensuring the integrity of pharmaceutical drug products. Antibiotics including ampicillin particularly may degrade during the transportation or storage at temperatures above 25 °C and high humidity. However there are other drugs which may not be affected under such conditions.

Studies have also shown that high storage temperatures do not affect adversely the composition of most antibiotics such as penicillins and tetracyclines and this indicates the most probable cause of low quality pharmaceutical drugs is found during the process of manufacturing (Kelesidis et al, 2007). When drugs are kept at high temperature and humidity, interactions may take place which reduces the dissolution rate. Though most antibiotics may contain the correct amount of active pharmaceutical ingredient (API), their activity will not be optimal as a result of reduced bioavailability. Some examples are methronidazole, pyrimethamine, chloroquine, tetracyclines, mefloquine and co-trimoxazole.
2.4.3.2 Decreased Concentration of Active Pharmaceutical Ingredient (API)

The low concentration of active pharmaceutical ingredient in antibiotics may be as a result of poor manufacturing practices which could be the result of poor transport and storage conditions. The cause of poor quality in some antibiotic drugs was decomposition though it has been revealed that many antibiotics may have high stability under tropical conditions which tends to make poor manufacturing of drugs the supreme cause of poor quality of drugs. The last but not the least, low amount of active pharmaceutical ingredient is as a result of diluting of drugs with substances like contaminated water or sugar (Kelesidis et al, 2007).

2.4.3.3 Varied Chemical Content

Counterfeit drugs can be detected through their varied odour, this is because they contain diluted active ingredients. In certain cases, counterfeit drugs are chemically identical to the genuine drug product which in turn makes them counterfeit generics but most counterfeit drugs contain harmful or inactive ingredients. Tablets or capsules may contain wrong antibiotic like erythromycin powder which are unworthy. Examples are neomycin eye drops and meningococcal vaccine which is made of tap water, ampicillin comprising of tumeric, antimalarials and antibiotics which contained no active ingredients (Kelesidis et al, 2007).

2.5 PREVALENCE OF COUNTERFEIT DRUGS IN GHANA

The United States Promoting the Quality of Medicines (PQM) program worked with the Ghana Food and Drugs Authority (FDA) in 2012 across all the ten regions of the country to sample and evaluate the quality of some key maternal health products. Samples picked from dispensing points like pharmacies, drug stores, and private
clinics showed that all ergometrine tablets failed the basic tests for quality and all oxytocin samples showed it has been wrongly formulated (http://www.myjoyonline.com).

It was also reported that a citizen of Bolgatanga in the Upper East region of Ghana had malaria and Fansider was prescribed for him, so he purchased it at a pharmacy in Bolgatanga. After taking the medicine he still did not feel well so he went to a health centre and a different course of treatment was given to him. It was announced in the newspaper that the owner of the pharmacy had been arrested by the Food and Drugs Authority which indicated that the Fansider he bought was fake (IPS 2015- Inter Press Service).

In 2008, artesunate tablets sold in Pharmacy shops in Kumasi were analysed by researchers of the Kwame Nkrumah University of Science and Technology. Qualitative and quantitative tests were carried out using colorimetric field method to determine the presence or absence of artesunate in the tablets meant to distinguish genuine artesunate from counterfeit ones. Qualitative tests performed on all the different brands of artesunate tablets resulted in the formation of a yellowish product of varying colour intensity. After quantitative analysis it was confirmed that the percentage artesunate content ranged from 47.9 to 99.9 %. Six (35.3%) of the tablets had artesunate content of 90% while eleven (64.7 %) had artesunate content less than90 % of the label claim. Also three (17.6 %) of the samples had artesunate content of 95 % while fourteen (82.4 %) was less than 95%. In all only 3 representing 17.6 % of the samples met the European Pharmacopoeia (Ph. Eur.) content requirements while 14 representing 82.4 % failed to meet the requirements (Ofori-Kwakye et al, 2008).
Prah et al. (2016) did a study to determine the existence of substandard and counterfeit artemether-lumefantrine (AL) tablets and suspension as well as artemether injection on Cape Coast market. Six brands of artemether-lumefantrine tablets, two brands of artemether-lumefantrine suspensions, and two brands of artemether injections were purchased from pharmacies in Cape Coast. The samples were analyzed for the content of active pharmaceutical ingredients using High Performance Liquid Chromatography (HPLC) and a variable wave detector. Quantitative tests performed on all the different brands of AL and artemether injections proved the existence of artemether and lumefantrine in the AL samples and artemether in the artemether injections. The percentage of artemether in the samples was found to be in the range of 98.04 to 102.82 in the AL tablet and suspension samples and for the artemether injections (AT). The artemether content was 99.92% in AT 1 and 98.17% in AT 2. The percentage of lumefantrine ranged from 98.70 to 111.87. AL 8 suspensions had the highest artemether content of 102.82%, whilst AL 5 tablet had the lowest but acceptable artemether content of 98.04%. AL 4 tablet recorded the lowest but acceptable lumefantrine content of 98.70% whilst the AL 8 suspension studied had the highest lumefantrine content of 111.87% which is above the maximum acceptable concentration of 110% (International Pharmacopoeia, 2003). Analysis done quantitatively using HPLC-VWD showed 7 (87.50%) of the artemether-lumefantrine samples passed and 1 (12.50%) failed the International Pharmacopoeia requirement which specifies that artemether-lumefantrine samples should contain not less than 90.00% and not more than 110.00% of the amount of artemether and lumefantrine stated on the label. One of the artemether-lumefantrine samples that did not meet the International Pharmacopoeia requirement had a higher than accepted lumefantrine content.
On the whole, artesunatelumefantrine samples manufactured outside Ghana were of similar quality as those produced in Ghana. The artemether content of the injection sample AT 1 was 99.92% and that of AT 2 was 98.17% of indicated amount on label. This means the samples met the International Pharmacopoeia requirement which states that artemether injection should contain not less than 95.0% and not more than 105.0% of the artemether stated on the label. The study did not identify any counterfeit drug but one of the artesunatelumefantrine drugs was proven to be substandard.

2.6 ANTIBIOTICS

Antibiotics are molecules that kill or stop the growth of micro-organisms which include both bacteria and fungi. Antibiotics that kill bacteria are known as bactericidal whilst those that stop the growth of bacteria are bacteriostatic.

2.6.1 Classes of Antibiotics

a. Beta-Lactam antibiotics: examples penicillins (eg amoxicillin), cephalosporins, carbapenems, monobactams etc.

b. Tetracycline example: tetracycline.

c. Macrolide example: erythromycin.

d. Aminoglycosides examples: genatamicin, tobramycin, amikacin.

e. Quinolones example: ciprofloxacin.

f. Cyclic peptides examples: vancomycin, streptogramins, polymyxins.
g. Linocosamides example: clindamycin.

h. Oxazolidinoes example: linezolid.

i. Salfa antibiotics example: sulfisoxazole.

2.6.2 Origin of Antibiotics

A lot of antibiotics including β-lactam antibiotics, tetracyclines, aminoglycosides, and macrolides were derived originally from natural sources and were further modified chemically to confer better properties on the drug. Also some relevant classes of antibiotics which include sulfa antibiotics, quinolones and oxazalidinoes are man-made and originated from synthetic chemical operations.

2.6.3 Beta-Lactam Antibiotics

2.6.3.1 Basic Characteristics of Beta-Lactam

Beta-Lactam antibiotics are bactericidal drugs which subdue the building of bacterial cell wall as a result of interference with the synthesis of peptidoglycan. Beta-lactams are affected by bacterial enzymes known as penicillin binding proteins (PBPs). There are various PBPs which differ in their detail function, quantity and affinity for beta lactams. The effect of Beta-lactams is usually expressed against multiplying bacteria whose cell wall are being built intensively.
2.6.3.1.1 Pharmacokinetics

Most beta-lactams decompose with gastric juice and readily reacts with an acid. In addition there is limitation in the absorption of beta-lactams antibiotics from the gastrointestinal tract. A large number of beta-lactams antibiotics are only prepared in parenteral form. In certain cases, esterification of the original drug is performed to aid in absorption. The beta-lactams esterified is administered with food. β-lactams are mostly spread in the extracellular space and there is restricted penetration across biological barriers which can sometimes be reversed with higher dosing. The excretion of majority of b-lactams antibiotics are through the kidneys with exceptions of oxacillin, cefoperazone and ceftriaxone. β-lactams antibiotics have short half-lives which changes from half an hour with antibiotics such as penicillin, oxacillin, and cephalotin to 2-2.5 hours. Ceftriaxone has a longer half-life of 8 hours which allows once daily administration.

2.6.3.1.2 Pharmacodynamics

The effect of beta-lactams depends on the time above Minimal Inhibitory Concentration (MIC). The dosing target is to keep the level of antibiotic above MIC at the place of infection. With situations pertaining to mild infections, the drug level exceeds MIC for 40-50% of the dosage interval.

2.6.3.1.3 Undesirable Effects

Beta-lactams antibiotics are not poisonous and have minimal concentration which is dependent on adverse effects. The degree of dosing is very high in penicillins. Any allergy to penicillins or cephalosporins ought to be proven with examination of the level of antibody in blood. The true allergy to any penicillin drug has to be understood
as allergy to all penicillins. Also the allergy to a cephaloprin does not essentially imply the allergy to all other cephalosporins. It is possible to have cross allergy between cephalosporins and penicillins but not frequent. There was an estimation of 5 to 10% probability of allergy to cephalosporins in patients who are allergic to penicillins. In view of this, it is necessary to give cephalosporins with care in patients with history of a mild penicillin allergy known as exanthema. Again, an allergy to cephalosprins means a high probability of cross-allergy to penicillins. β-lactams can be utilised by women in an advanced stage of pregnancy or in breast feeding women and the newly borns.

2.6.3.1.4 Disposal

Beta-lactams are best used for the treatment of acute infections in a well prefunded tissue or for the treatment of sepsis. Some of the drugs are suited for surgical prophylaxis. Subsequent dosing is needed to arrive at a strong effect. Enhancing the individual doses is necessary when penetrating the place of infection becomes a problem.

A) Penicillins

This group is divided into four subgroups which include

1) Natural penicillins

Natural penicillins are made up of narrow spectrum which contain gram positive and negative cocci (streptococci, pneumococci, enterococci, meningococci), gram-positive
bands (corynebacteria, L.monocytogenes), spirochetes (Leptospira sp. Treponema sp. Borelia sp.) and a lot of anaerobes (peptostreptococci, clostridial species, Actinomyces).

**Penicillin G or benzylpenicillin:** It is unstable in gastric juice and is only desirable for intravenous administration.

**Penicillin G or phenoxymethylpenicillin:** This is stable in the form of an acid and it is orally administered.

**Procain-penicillin:** It is in a form of a depot and it is preferably used for intramuscular administration once daily.

**Benzathinpenicillin:** It is usually in the form of a depot which creates a stable low level of antibiotic for 2 to 4 weeks. It is used for prophylaxis of streptococcal reinfections. The dosage spectrum of penicillin is extremely wide ranging from 1 mill .U. to 40 mill .U. daily for an adult person depending on the type and extent of infection. For the purposes of comparison 1,000,000 units equals 625 mg of penicillin. In a typical situation, the low-dose penicillin treatments are pseudomembranous tonsillitis, animal bite and scratches or streptococcal skin infections. For high dose treatment, it is given to patients with endocarditis infection which is caused by (viridians streptococci or enterococci), streptococcal, pneumococcal or meningococcal sepsis, and clostridial wound infection.

2) **Anti-staphylococcal penicillins**

These are resistant to staphylococcal beta-lactamase but not to other beta-lactamases which are produced by gram-negative microbes. The drugs have a very constrict
spectrum which is due to the effect against gram-positive bacteria other than staphylococci which is weaker as compared to penicillin G.

**Methicillin**

It requires administration through intramuscular or intravenous injection. Examples include cloxacillin, nafcillin, oxacillin and dicloxacillin.

3) **Aminopenicillins**

These are drugs which have their spectrum similar to that of natural penicillin with extension against common gram-negative bacteria like Escherichia coli, Salmonella enteric. They are more efficient than natural penicillin against enterocci and listeriae.

**Ampicillin**: It is a drug administered through intramuscular or intravenous injection and it is essentially representative of the subgroup.

**Amoxicillin**: It is an antibiotic drug with better adsorption after oral administration than ampicillin (that is 70-80% as compared to 40-50%). Two (2g) to Twelve (12g) is to be taken on a daily basis.

Since there have been plasmide-related production of beta-lactamase, strains of gram-negative bacteria is resistant. New formulae were made which contained an antibiotic together with an inhibitor of beta-lactamase. The administration of the drug was based on the two combinations present both for oral and parenteral which include

- ampicillin + sulbactum
- amoxicillin + culvulanic acid
These combinations are effective against above-mentioned gram-negative microbes due to beta-lactamase and against staphylococcus aureus. Also these antibiotics are not needed and there should not be any prescription against enterococci, streptococci or bacteria which do not produce beta-lactamase. Clinically aminopenicillins with or without an inhibitor are used. These are given in bacterial sinusitis, mesotitis and lower respiratory tract infections, urinary and hepatobiliary tract infections, purulent gynaecological infections and other community-acquired infections.

4) **Penicillins** effective against pseudomonads (and other problematic gram-negative pathogens owing natural resistance) examples are ticarcillin, karbenicillin, azlocillin, mezlocillin, piperacillin (it is only for parenteral use). These are drugs presented according to the results cultivated and in intensive care infections. It is administered intravenously. In most cases, the third generation cephalosporins are preferred to these drugs as a result of lower costs. Beta-lactamase inhibitors and these antibiotics were combined as follows:

\[ \text{ticarcillin} + \text{clavulanic acid}, \text{piperacilli} + \text{tazobactum} \]

Their usage is very similar to that of the basic drugs.

### 2.7 REVIEW OF ANALYTICAL TECHNIQUES USED IN THE ANALYSIS OF COUNTERFEIT PHARMACEUTICAL DRUGS

According to Nuhu (2011), Raman spectroscopy was used to analyse 50 artesunate anti-malarial tablets purchased from South East Asia. It was detected that the drugs contained minimum amount of active pharmaceutical ingredient whilst majority of the
tablets contained large amounts of various excipients, paracetamol and titanium dioxide.

According to (de Veij et al, 2008), detection of counterfeit Viagra was performed with Raman Spectroscopy. Analysis was done to differentiate genuine Viagra from counterfeit Viagra. A total of 18 tablets were analysed. From visual inspection the results showed that 3 of the tablets were counterfeit. Raman Spectroscopy proved that 9 tablets were counterfeit, though the tablets contained the API sildenafil citrate, it also contained less or other inactive compounds.

A survey conducted on 519 drugs in 3 African countries between 1991 and 1993 revealed that 77 (18%) of the drugs were found to be substandard. In Tanzania counterfeit ampicillin contained no active ingredient in the year 2000 (Akunyili, 2005).

According to the World Health Organisation (WHO), a total of 451 drug samples were analysed in Asia using Thin Layer Liquid Chromatography(TLC) and disintegration tests. Averagely failure rate of artesunate was 19.8%, quinine was 71.8%, mefloquine was 7.7%, chloroquine was 8.5%, tetracycline was 26.6%. The study revealed that only 22 samples of dihydroartemisinin and two samples of artemether passed the tests. 122 (27.1%) of the overall samples failed TLC and disintegration tests. Among the samples which failed testing, 100 were obtained from unlicensed or illegal drug outlets.

Kahsay et al (2010) used TLC to determine the active pharmaceutical ingredient in ciprofloxacin tablets. Upon analysis the samples passed the British Pharmacopoeia and United States Pharmacopoeia specifications.
Analysis of two anti-tuberculosis drugs (isoniazid and rifampicin) were done using TLC and disintegration test to determine the API. 713 samples were analysed. Upon analysis 9.1% (65/713) of the sampled drugs failed quality control tests. The rate of failure was 16.6% in Africa, 10.1% in India and 3.9% in other middle income countries. For non registered products the failure rate was 28.6% and 4.4% for registered products. The products from Africa had the highest percentage of failing products with the highest amounts of API (70.4%), (51.9%) in India and 27.3% in other middle income countries. These products can be classified as substandard rather than counterfeit. 11(44%) of the 25 registered products were counterfeit as they did not contain any API or had suspect packaging. The non-registered product failures were (17.5%) 7/40 were counterfeit (Bate et al, 2013).

According to Shakoor et al (1997) samples of chloroquine and antibacterial from Nigeria and Thailand were analyzed using high performance liquid chromatography (HPLC). The results showed that 36% of drugs in Nigeria and 40% of drug samples from Thailand contained active ingredients which are outside the British Pharmacopoeia limits. 2 chloroquine drug samples and 1 amoxicillin sample from Nigeria and 3 chloroquine samples from Thailand contained no active ingredient.

Fadeyi et al (2015) analysed brands of amoxicillin and co-trimoxazole purchased in Ghana, Nigeria and the United Kingdom using German Pharma Health Fund (GPHA) Minilab® and HPLC. After analysis it was revealed that all samples of amoxicillin except the one purchased from Nigeria were compliant with the content analysis test. 1 co-trimoxazole tablet complied with the content analysis test whilst 14 samples of co-trimoxazole purchased in Ghana and Nigeria did not comply with the content analysis test.
Deconink et al (2013) used nuclear magnetic resonance spectroscopy for the characterization of 14 different artesunate preparations. Upon analysis the results revealed that only 5 of the preparations contained active pharmaceutical ingredient.

According to Habyalimana et al (2015) NMR was used in the analysis of quinine tablets. It was confirmed that fake quinine tablets corresponded with standard metronidazole but did not correspond with quinine sulphate. It was again confirmed that metronidazole was found in suspected quinine tablets. Analytical techniques including Raman Spectroscopy, Mass Spectrometry and High Performance Liquid Chromatography confirmed there was no API in quinine sulphate and the fake quinine.

Raman Spectrometry, semi-quantitative thin-layer chromatography, disintegration tests and near-infrared spectroscopy were used to determine the concentration of active pharmaceutical ingredient and excipients of antimalarial, antibiotic and antimycobacterial drugs. After analysis the results revealed that 15% of the samples failed TLC test, 13% of the sample failed disintegration test, 47% of the samples analysed failed Raman Spectroscopy and 41% of the samples analysed failed Near Infrared (NIR) spectroscopy tests. Overall, 49% of the samples passed all the four tests whilst 10% of the tested samples failed all tests. For NIR and Raman Spectroscopy, 9 samples had different results (Bate et al, 2009).

According to Hall et al. (2016), liquid chromatography together with mass spectrometry was used to measure the amount of API in artesunate tablet. Colorimetric Fast Red TR test was also used to confirm the packaging of drugs and classification of tablets as being genuine, substandard or fake. The results showed that the mean artesunate content of artesunate tablets that tested positive in the Fast Red
TR colorimetric test was 55 mg whilst 1 tablet contained 21 mg of artesunate was considered as substandard because it did not meet the quality specifications set by the legitimate manufacturer of the drug. Out of 34 artesunate samples, 23 did not contain the required API but with the fast red dye result and LC-MS results there was 100% agreement. Raman spectroscopy was used to identify the presence of calcium carbonate as excipient in 9 of 23 samples.

High Performance Liquid Chromatography (HPLC) was used in the analysis of 96 samples of chloroquine and selected antibacterials from Nigeria and Thailand to determine the presence related drug impurities and measure the Active Pharmaceutical Ingredient (API). The results indicated that 36% of samples from Nigeria and 40% of samples from Thailand contained API outside the British Pharmacopoeia limits. 6 of the substandard preparations did not contain any API. These include 2 chloroquine samples from Nigeria and 3 chloroquine samples from Thailand (Shakoor et al 2007).

Vredenbegt et al. (2006), used near infrared spectroscopy (NIRS) in the analysis of Viagra® tablet, Counterfeit Viagra tablets and imitations of Viagra. In all 103 samples were analysed and it was revealed that NIR identified 44 samples out of the 48 imitation of Viagra as counterfeit tablets. 4 of the samples were not distinguished from the genuine Viagra. Out of the 103 samples analysed 99 samples contained API, 2 contained no API due to pharmacological substance. Chemical analysis also showed the presence of other substances.

According to Fadeyi et al. (2015), the quality of amoxicillin and co-trimoxazole from Ghana, Nigeria and United Kingdom were analysed using German Pharma Health Fund (GPHF) Minilab which is made up of 4 tests including visual inspection,
tablet/capsule disintegration test, colorimetric tests and thin layer chromatography (TLC). After analysis, the visual inspection confirmed most brands of antibiotics identified as generics were produced in Ghana, China, India, Ireland and Nigeria. The colorimetric test indicated that 15 samples of co-trimoxazole passed the test whilst 2 samples from Ghana and Nigeria failed the TLC test. HPLC content analysis and dissolution testing revealed that all the capsules of amoxicillin with the exception of 1 bought in Nigeria were compliant with the content analysis test. 14 samples of co-trimazole bought in Ghana and Nigeria did not conform to the test. 1 tablet of co-trimazole obtained from United Kingdom was compliant with the content analysis test.

Vijaykadga et al. (2006), investigated the quality of antimalarial drugs (artesunate, chloroquine, mefloquine, quinine, sulfadoxine/ pyrimethamine (S/P) and tetracycline) obtained from 4 selected provinces of Thai. The drug analysis was done using simple disintegration test and semi-quantitative thin layer chromatography. Out of the 369 samples collected for analysis, 53 samples of all the artesunate collected for analysis passed visual/physical examination. 15.4% samples tested with HPLC were substandard, these drugs had expiry dates of 1 to 2 months prior to the date of spoilage. Out of 86 samples of chloroquine tested, 9 representing (10.5%) of the samples failed disintegration test in the GPHF-Minilab® and 2(11.1%) of 18 samples were substandard. 9 of the samples collected for analysis at the National Drug Analysis Laboratory (NL) showed 100% agreement between GPHF-Minilab® (TLC) and HPLC results. A total of 88 quinine samples were analysed, 4(4.5%) failed the disintegration test with GPHA-Minilab®. 5(29.4%) of quinine tablets were substandard with disintegration time greater than 30 minutes. 13 samples of sulfadoxine/pyrimethamine analysed were either not substandard or counterfeit. 93
tetracycline samples analysed showed (1.1%) failure in disintegration test with GPHF-Minilab®. 19 Samples analysed at NL confirmed with HPLC test and none of the samples tested was substandard.

Drug formulations of the USP 24, were analysed using dissolution tests and HPLC. 33 samples of amoxicillin capsules, metronidazole tablets and SMX-TMP tablets in Tanzania and Rwanda were analysed. At the time the drugs were purchased the drug content of all the formulations were within the USP-24 limits but after storage of 6 months. The drug content of one sulfamethoxazole/trimethoprim was found to be substandard. Just after purchasing the drugs, four formulations(one sulfadoxine/pyrimethamine combination and three sulfamethoxazole/trimethoprim) failed the USP 24 dissolution test. Three mehronidazole drugs passed dissolution tests though it was performed after 6 months of storage under simulated tropical conditions. The drug remained within the USP-24 limits. In total (8/33) representing 24% of the sampled formulations failed the dissolution test (Kayumba et al, 2004).

According to Osei-Safo et al. (2014), 132 aretemisinin-based medicines distributed in Ghana and Togo were analysed using colorimetric tests, semi-quantitative thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) tests to identify the presence of Active Pharmaceutical Ingredient (API) and the amount of API present in the drug. The results revealed that visual inspection did not show any form of false labelling but 1 oral artesunate monotherapy which was part of samples from Ghana was slightly powdery. 46% of drugs from both Ghana and Togo were not registered. Ghana had(79.3%) of the samples as unregistered while Togo had 21.6% of drug samples as unregistered. The basic tests showed that all antimalarial medicines analysed contained the required amount of API with the exception of a slightly powdery oral artesunate monotherapy from Ghana which completely lacked
API.HPLC test showed that 83.7% of ACTs and 57.9% of the artemisinin-based monotherapies did not comply the International Pharmacopoeia requirements which was due to insufficient API content. With Artemether and Artemether/ Lumefantrine samples, 22 samples representing (73.3%) and 25 samples representing (83.3%) failed HPLC and SQ-TLC tests. Drugs which were not registered recorded a failure rate of (84.7%) and the registered ones recorded failure rate of (70.8%).

Artemisinim-derivative drugs (tablets, capsules, dry suspensions and injections) containing either artemeter, arteether and artesunate from Kenya and Democratic Republic of Congo were analysed using HPLC-UV methods to determine the content of active ingredients. Out of 24 drugs analysed, 15(62.5%) met the European Pharmacopoeia requirement content of 95-105% of API. 7 samples were underdosed and two were slightly overdosed. Artesunate was found to be the API in 57% of underdosed samples with arteether having 77% of lower API content. Two-thirds (2/3) of the dry powder suspensions were either substandard or fake. The tablets were up to 23% out of range (Atemnkeng et al, 2006).

According to Hetzel et al. (2014), Validated HPLC was used to analyse for the API content in antimalarial drugs and selected antibiotics (amoxicillin, artesunate, chloroquine diphosphate, doxycycline, primaquine, diphosphate, sulphadoxine and pyrimethamine) in Papua New Guinea. Out of 360 samples analysed 37 failed the content analysis test. 2 samples of quinine failed chemical content analysis test due to excess amount of API while 35 samples contained less amount of API. Among all the samples tested some amount of API was determined. Samples which failed the content analysis test included 29(78.4%) of primaquine, 3(4.3%) of amodiaquine, 2 (6.3%) of quinine, 1(4.5%) of artemeter, 1(1.8%) of sulphadoxinepyrimethamine and 1(4.3%) of amoxicillin capsule sample. Primaquine failed the content analysis test
containing 70.7% API, failed amodiquine sample contained 45.2% API. 2 quinine samples failed the test containing 106.5% and 105.2% API.

Paracetamol and cotrimaxazole tablets in Malawi were analysed using HPLC based on Pharmacopoeia Standards and spectrophotometric methods in order to determine the existence and extent of substandard drugs in Malawi. The results revealed that 50% of samples were not registered as of 30th June 2009 which was made up of 6 paracetamol samples and 5 co-trimoxazole samples. All the paracetamol and co-trimoxazole tablets complied with identification of active pharmaceutical ingredient as defined by British Pharmacopoeia (BP) and United States Pharmacopoeia (USP) 32. Based on the individual tablet weight uniformity, only 1 product failed to meet the required standard which represents 5%. The content API in the paracetamol samples ranged from 94.57-98% hence falling short of the acceptance limit for paracetamol of 95-105% in the BP. 10 paracetamol brands met the required standard with only 1 paracetamol tablet formulation failing to meet the standard content of active ingredient which represents 9.1% of paracetamol samples. The content of sulfamethoxazole in the samples ranged from 91.04-107.3% while Trimethoprim was 65.57-110.13%. The accepted range for both sulfamethoxazole and Trimethoprim in BP 2007 is 92.5-107.5%. 5 of the co-trimazole brands failed to meet the required standards which represent 45.5% of co-trimoxazole. The combination of paracetamol and cotrimoxazole samples showed that 6 tablet formulations failed to meet the BP 2007 standards of API content representing 27.3% (Khuluza, 2014).

The quality of 45 samples of antibiotics (Azithromycin, Erythromycin and Clindamycin) from Accra and Lagos were analysed with HPLC to know the content of API. Dissolution test were done for bioavailability. Out of 45 samples analysed, the results revealed that HPLC test for API content showed 12(27%) were compliant with
USP requirements. They include 4 out of 13 (27%) samples collected from Accra and 8 out of the 32 (25%) samples from Lagos 8(32%) Azithromycin, 2(14%) Erythromycin and 2(33%) Clindamycin. For Clindamycin and Azithromycin samples there were excess amounts of API which accounted for non-compliance. Samples of Azithromycin showed good dissolution profiles while 36% of Erthromycin and 67% of Clindamycin were compliant with in-vitro dissolution test (Osei-Safo et al, 2016).

A survey conducted by Rozendaal (2001) in Cambodia antimalarials (artesunate, mefloquine) indicated that majority of bottles of mefloquine tablets and about half of artesunate blister packs sampled were detected to be fake. For artesunate and mefloquine, two different varieties were found: a first generation fake which was easily distinguished from the genuine product and a second generation fake which was exactly as the genuine product. A total of 242 vendors and pharmacies were mapped out in 12 market places and 133 almost half in each of the market place were randomly selected for investigation. Fake artesunate was sold by 71% (86% sold genuine product) and fake mefloquine was sold by 60% (61% sold genuine variety). The fake products were mostly preferred by patients and health care providers in the village because of the reduced price. Given the widespread usage fake malaria drugs are likely a major cause of morbidity and mortality as a result of malaria in Cambodia.

2.8 ANALYTICAL TECHNIQUE EMPLOYED FOR THIS WORK

2.8.1 NMR Methods

Nuclear magnetic resonance (NMR) spectroscopy is a non-destructive analytical technique used in elucidating the structure of an organic compound. It gives information on the environment in which the nuclei of the atoms are found in
molecules and compounds. NMR also makes it possible for direct observation of hydrogen and carbon compounds of a molecule.

2.8.1.1 Principle behind NMR

The principle behind NMR is that many nuclei have spin and if the number of protons and neutrons is odd (example: 1H and 13C) the nucleus exhibits a magnetic field meaning it is NMR active. However, if the number of protons and neutrons is even the nucleus does not exhibits a magnetic field and hence cannot be studied by NMR. The nuclear spins move randomly in the absence of an applied magnetic field. When placed in an external magnetic field, the nuclear spins either align with or against the external magnetic field. Those that align with the magnetic field are in lower energy state and those that align against the magnetic field are in higher energy state. The difference in energy between the aligned and opposed to the external magnetic field depends on the strength of the external magnetic field. For the nucleus to change to a higher energy state energy must be supplied. Therefore the energy absorbed corresponds to radiofrequencies and depends on the environment of the nucleus.

Figure 2.1: Nuclear spin orientations
\[ \Delta E = \frac{\gamma hB}{2\pi} \]  

(2.1)

Where \( \gamma \) = gyromagnetic ratio, \( h \) = Plank’s constant, \( B \) = magnetic field strength

2.8.1.2 NMR Instrumentation

The sample of the compound is dissolved in a deuterated solvent and put in a very strong magnetic field. The sample is then irradiated with a short pulse of radiofrequency disturbing the equilibrium balance between the two energy levels. This causes some of the nuclei to absorb the energy, hence promoting to a higher energy level. A radio receiver is used to detect the energy given out when the nuclei fall back to a lower energy level. After numerous computations the results are displayed as intensity against frequency.

Figure 2.2: A typical NMR set up
2.8.1.2.1 1-H NMR

1-H NMR also known as proton NMR is a technique which identifies the different types of hydrogen atoms present in a compound. The structure of molecules is also determined by analysing the energy required for the nuclear to spin flip. The energy required to spin flip gives detailed information on the conditions of the nucleus field strength. This information gives an insight into the chemical structure of the molecule. 1-H NMR gives information about the different signals in the spectrum, the position of signals (chemical shift), splitting pattern of the signals and the intensity of signals (integration).

Number of Signals is used to denote identical protons found in the same environment give rise to a signal. They experience the same magnetic force and create overlapping signals on the spectrum. The number of equivalent protons can be determined in the molecule by looking at the number of signals in its H- NMR spectrum.

Position of Signals (Chemical shift) in an NMR spectrum is based on how far they are from the signal of the reference compound. This information gives the kind of proton or protons that are responsible for the signal. Tetra methyl silane (TMS) is at the zero position on the left of the spectrum and as it moves towards the left the ppm values becomes larger.

Chemical Shift determines the location of an NMR signal along the radiofrequency axis of the spectrum. It is measured relative to a reference compound and is measured in parts per million. Chemical shifts are sensitive to the chemical environment of a nucleus.
Factors that cause chemical shift

Electron Density is the measure of the probability of an electron being present at a specific location. High electron density produces lower chemical shift, and the lower the electron density the higher the chemical shift. Additionally, high electron density of neighbouring atoms can also cause shielding of the nucleus from an external magnetic field. If there is little or no electron density in any nearby atoms or groups the nucleus can be deshielded. Shielding comes about when the nucleus experiences a weaker magnetic field around it. This is caused by other atoms getting in the way of the nucleus and the magnetic field, or the nucleus itself having a low spin flip energy. Due to the weaker magnetic field it experiences, a nucleus with more shielding will have a lower ppm and therefore lie on the right side of chemical shift scale.

Deshielding occur when the nucleus experiences a higher magnetic field around it. This is due to its proximity to a strong magnetic field or having itself a high spin flip energy. Because of its high magnetic field, a more deshielded nucleus will have a higher ppm and therefore lie on the left side of the chemical shift scale.

Electronegativity: hydrogen atoms that are normally attached to more electronegative atoms experience higher chemical shifts. Electronegative atoms remove electrons
from electron cloud which decreases their density resulting in less shielding. The electronegative atoms then deshield the proton causing it to have a higher chemical shift.

The reference compound used in NMR is Tetraemethysilane (TMS). It is used as the reference compound because it can be removed easily from the sample by evaporation due to its volatile properties.

Intensity of NMR signals (Integration) comes out with the structure of a molecule from 1H NMR. Because the number of the radio wave energy is proportional to the peak area in the 1H NMR graph, measurements of these areas gives the relative intensities of 1H NMR signals.

Splitting takes place when the spin of the nucleus affects that of a chemically different nucleus on an adjacent atom. In $^1$H NMR the protons are chemically different in nature from each other interact magnetically if they are separated by two or more bonds.

3.3.1.1.2 Carbon-13 NMR

If a nucleus has an odd atomic number or odd mass number it experiences nuclear magnetic resonance. Carbon-12 which is the most ample source of carbon has its atomic number being even and mass number also even, it therefore makes the nuclei NMR inactive. Carbon-13 isotope has an odd mass number and can be studied by NMR.

Carbon-13 NMR is an analytical technique which deduces information about the carbon atoms in a molecule. It gives information about the environment of the carbon atoms in a molecule and helps to confirm the structure of a molecule. Carbon-13
NMR gives information about the number of signals, the signal splitting and the chemical shift.

The number of signals give how many different or the set of equivalent carbons in the molecule.

In C-13 NMR splitting is not observed due to the low probability of two carbons which are adjacent. Signal splitting gives information about how many types of hydrogen are attached to each carbon.

The chemical shift gives information about the environment of each carbon. C-13 has its own chemical shift and can identify the number of different carbons in symmetry.

With carbon 13 NMR the peaks are not integrated to detect the relative number of carbons in the compound, the peak areas depend on the number of hydrogen attached to the carbon and not the relative number of carbons that causes a signal.
CHAPTER THREE

MATERIALS AND METHOD

3.1 INTRODUCTION

Various methods can be used in detecting counterfeit drugs which include visual inspection of the package and label, dissolution, chromatographic techniques like high performance liquid chromatography, liquid chromatography, thin layer chromatography and gas chromatography. Since drug counterfeiting has become more advanced additional techniques are needed in order to detect counterfeit drugs from genuine ones. These techniques include x-ray powder diffraction, near infra-red spectroscopy and nuclear magnetic resonance spectroscopy.

3.2 SAMPLING

3.2.1 Standard Materials

In order to validate analytical processes it is essential to use certified materials called standards. The reference material known as amoxicillin trihydrate obtained from Ernest Chemist in Accra was used for the validation of the NMR methods.

3.2.2 Brand-Name Drug

10g of amoxicillin produced by Bristol in the United Kingdom was purchased in a licensed pharmacy shop in Accra.

3.2.3 Generic Drug

10g of generic amoxicillin drug, manufactured in Ghana, and endorsed by the National Health Insurance Scheme was purchased from a licensed pharmacy shop in Accra.
3.2.4 Suspected Fake Drugs

Amoxicillin drug samples suspected to be fake were purchased from an unlicensed drug seller at Okaishie market in Accra to undergo analysis. A quantity of 10g was purchased for the analysis.

3.3 NMR METHODOLOGY (EXPERIMENTAL)

3.3.1 Sample Preparation

Three types of amoxicillin drug samples were analysed using NMR. These three drug samples were Foreign Amoxicillin, NHIS endorsed Amoxicillin and the Suspected Fake Amoxicillin. A triplicate of each drug sample was made by weighing 10mg of each capsule and was dissolved in 1mL of deuterated dimethylsulfoxide (DMSO). The sample was thoroughly mixed. The solution was transferred into an NMR tube using a pipette. Finally the samples were labelled and submitted for NMR analysis. \(^1\)H NMR and \(^{13}\)C NMR spectra were recorded on a 500MHz Bruker Avance spectrometer (Alexie et al, 2009).

3.3.2 NMR Analysis

A 500MHz Bruker Avance NMR Spectrometer located in the Chemistry Department at the University of Ghana, Legon was used for the analysis. The Spectrometer has an operator console including the host computer, monitor, keyboard and optional BSMS-Pad (Bruker Smart Magnet System).

After the sample was prepared, the glass tube containing the sample to be analysed was held in a plastic spinner. The sample and the spinner were inserted into a strong magnetic field. The nuclear spins of the atoms in the sample aligned either with or
against the external magnetic field. The spin which aligned with the external magnetic field is lower in energy and the spin which aligned against the external magnetic field is at higher energy state. The alignment was temporarily disturbed with a radio frequency pulse at 500MHz frequency for 10μs which caused the atoms to absorb energy hence radiating it. The Free Induction Decay (FID) in time domain was converted to a frequency domain spectrum by application of a Fourier transformation or other mathematical transformation. The exact frequency at which this absorption and emission occurred identified the kind of atom, provided information about nearby atoms and indicated how much of each was present.

Data acquisition and processing was implemented using the Bruker software XwinNMR. Spectrum plotting was also done with the Bruker software Xwinplot.
CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance Spectroscopy was used in the analysis of standard amoxicillin, imported amoxicillin, NHIS endorsed amoxicillin and amoxicillin bought from the open market. 1H NMR and C 13 NMR were used in identifying the hydrogen and carbon atoms in amoxicillin sample. The results obtained were compared to that of the standard drug.

Figure 4.1 Proton NMR Spectrum for the Standard Amoxicillin
The spectrum of the standard proton NMR amoxicillin contains 9 peaks which mean there are 9 different hydrogen atoms in the sample. The peaks between 1.4 and 1.6 ppm are attributed to methyl groups. The aromatic protons occurred between 6.7 and 7.3 ppm. From the structure of amoxicillin there are 19 hydrogen atoms making up the compound which exceeds the hydrogen atoms in the standard amoxicillin spectrum. This is attributed to the fact some of the hydrogen atoms in the structure are chemically equivalent, therefore making them appear as a single peak.

Figure 4.2 Proton NMR Spectrum of NHIS Amoxicillin

The spectrum of the standard proton NMR amoxicillin contains 9 peaks which mean there are 9 different hydrogens in the sample. The peaks between 1.4 and 1.6 ppm are attributed to methyl groups. The aromatic protons occurred between 6.7 and 7.3 ppm.
The spectrum of NHIS amoxicillin has peaks which exceeds that of the standard proton NMR amoxicillin. This is due to the presence of organic compounds which are excipients.

Figure 4.3 Proton NMR for Imported Amoxicillin

The spectrum for the proton NMR of the imported amoxicillin has 9 proton peaks as that of the standard proton amoxicillin which confirms the structure of the amoxicillin drug. The chemical shift values which indicate the type of hydrogen contained in the compound are the same as the standard amoxicillin. The imported amoxicillin does not contain any impurities.
Figure 4.4 Proton NMR Spectrum for Amoxicillin from the Open Market

The Spectrum for the proton NMR of amoxicillin bought from the open market relative intensities shows the number hydrogen atoms in the amoxicillin compound. From the structure of amoxicillin there are 19 hydrogen atoms in the compound but amoxicillin from the open market contains organic compounds which may are excipients.
In the C-13 NMR Spectrum of standard amoxicillin there are 13 peaks which imply there are 13 different types of carbon atoms in the compound. The peaks with chemical shift between 169 and 173 ppm are due to carbon in a carbon-oxygen double bond. The peaks with chemical shift between 115 and 129 ppm are carbons with alkene groups. Chemical shift at 129 ppm is due to carbon at either end of carbon-carbon double bond. The peak at 67 ppm is as a result of a carbon which is singly bonded to oxygen. Finally the peak at 27 ppm is due to carbon in a methyl group.
Figure 4.6 Carbon-13 NMR Spectrum of 500mg Amoxicillin from the Open Market

Carbon-13 NMR spectrum of amoxicillin from the open market is made up of 13 peaks which mean there are 13 different types of carbon in the sample. The chemical shift values indicate that there are alkenes and carbons with double bonds in the sample. When compared with the standard amoxicillin the number of peaks is exactly the same and the chemical shifts are also the same. This suggests that the amoxicillin from the open market fits that of the standard amoxicillin and can therefore confirm the structure of amoxicillin.
Figure 4.7 Carbon-13 NMR Spectrum of 250mg Amoxicillin from the Open Market

The spectrum above is a carbon-13 NMR spectrum of 250mg amoxicillin bought from the open market. In this spectrum there are 13 carbon peaks present of which those with chemical shift values between 26.59 and 31.37 are carbon-carbon single bond. The peaks that appeared at 56.27 to 67.22 ppm are carbon-oxygen singly bonded atoms present in the sample. The sample also contains alkenes, these occurred at 115.31 to 115.65 ppm. Chemical shift ranges of carbon atoms absorbed between 127.70-129.90 ppm suggests they are aromatics. The last but not the least the sample contains carbon in a carbon oxygen bond which occurred at 169.15-170.94 ppm.
RESULTS: STRUCTURE OF AMOXICILLIN

Figure 4.8 Structure of amoxicillin tri-hydrate (API)
From the structure of the amoxicillin tri-hydrate shown in Figure 4.8 the stochiometric formula indicates that there are nineteen (19) atoms of hydrogen. Nevertheless, the H-NMR spectra shows nine peaks since some of the hydrogen atoms are deemed to be structurally equivalent. The number of expected equivalent hydrogen atoms agrees with the number of peaks observed in the H-NMR spectrum for the standard amoxicillin.
COMPARISON OF NMR SPECTRA

Figure 4.10 Proton NMR Spectra of Amoxicillin (STANDARD/OPEN MARKET)

Figure 4.11 Proton NMR Spectra of Amoxicillin (STANDARD/IMPORTED)
Figure 4.12 Proton NMR Spectra of Amoxicillin (STANDARD/NHIS) Samples

Figure 4.13 Proton NMR Spectra of Amoxicillin Samples (ALL SAMPLES)
Figure 4.14 Overall spectra of the standard, imported, NHIS and open market amoxicillin
Table 4.1: $^1$H NMR Spectral Data of NHIS Amoxicillin

<table>
<thead>
<tr>
<th>Chemical Shift Values (δ in ppm)</th>
<th>Number of Protons</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.242-1.508</td>
<td>3</td>
</tr>
<tr>
<td>2.513</td>
<td>1</td>
</tr>
<tr>
<td>3.321-3.637</td>
<td>7</td>
</tr>
<tr>
<td>4.010-4.185</td>
<td>2</td>
</tr>
<tr>
<td>4.761-4.902</td>
<td>2</td>
</tr>
<tr>
<td>5.348-5.444</td>
<td>2</td>
</tr>
<tr>
<td>6.732-6.755</td>
<td>1</td>
</tr>
<tr>
<td>7.224-7.246</td>
<td>1</td>
</tr>
<tr>
<td>8.924</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 4.2: $^1$H NMR Spectral Data of Imported Amoxicillin

<table>
<thead>
<tr>
<th>Chemical Shift Values (δ in ppm)</th>
<th>Number of Protons</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.857-1.504</td>
<td>5</td>
</tr>
<tr>
<td>2.507</td>
<td>1</td>
</tr>
<tr>
<td>4.006</td>
<td>1</td>
</tr>
<tr>
<td>4.761</td>
<td>1</td>
</tr>
<tr>
<td>5.348-5.438</td>
<td>2</td>
</tr>
<tr>
<td>7.247-7.224</td>
<td>2</td>
</tr>
<tr>
<td>8.915</td>
<td>1</td>
</tr>
</tbody>
</table>
### Table 4.3: $^{13}$C NMR Spectral Data of Standard Amoxicillin

<table>
<thead>
<tr>
<th>Chemical Shift Values ($\delta$ in ppm)</th>
<th>Number of Carbons</th>
</tr>
</thead>
<tbody>
<tr>
<td>27.45-31.35</td>
<td>2</td>
</tr>
<tr>
<td>56.31-57.98</td>
<td>2</td>
</tr>
<tr>
<td>64.55-67.24</td>
<td>2</td>
</tr>
<tr>
<td>72.81</td>
<td>1</td>
</tr>
<tr>
<td>115.58</td>
<td>1</td>
</tr>
<tr>
<td>127.72-129.05</td>
<td>2</td>
</tr>
<tr>
<td>158.01</td>
<td>1</td>
</tr>
<tr>
<td>169.76-173.44</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table 4.4: $^{13}$C NMR Spectral Data of 500mg Amoxicillin from the Open Market

<table>
<thead>
<tr>
<th>Chemical Shift Values ($\delta$ in ppm)</th>
<th>Number of Carbons</th>
</tr>
</thead>
<tbody>
<tr>
<td>27.47-31.36</td>
<td>2</td>
</tr>
<tr>
<td>56.27-57.96</td>
<td>2</td>
</tr>
<tr>
<td>67.56-67.22</td>
<td>2</td>
</tr>
<tr>
<td>72.90</td>
<td>1</td>
</tr>
<tr>
<td>115.59</td>
<td>1</td>
</tr>
<tr>
<td>127.61-129.06</td>
<td>1</td>
</tr>
<tr>
<td>158.04</td>
<td>1</td>
</tr>
<tr>
<td>169.83-173.42</td>
<td>3</td>
</tr>
</tbody>
</table>
Table 4.5: $^{13}$C NMR Spectral Data of 250mg Amoxicillin from the Open Market

<table>
<thead>
<tr>
<th>Chemical Shift Values (δ in ppm)</th>
<th>Number of Carbons</th>
</tr>
</thead>
<tbody>
<tr>
<td>26.59-31.37</td>
<td>2</td>
</tr>
<tr>
<td>56.27-58.91</td>
<td>2</td>
</tr>
<tr>
<td>64.57-67.22</td>
<td>2</td>
</tr>
<tr>
<td>72.89</td>
<td>1</td>
</tr>
<tr>
<td>115.31-115.65</td>
<td>1</td>
</tr>
<tr>
<td>127.60-129.90</td>
<td>1</td>
</tr>
<tr>
<td>157.37-158.33</td>
<td>1</td>
</tr>
<tr>
<td>169.15-170.94</td>
<td>3</td>
</tr>
</tbody>
</table>

4.2 DISCUSSION

NMR was used in the analysis of amoxicillin samples in order to assess the presence of active principal ingredient, and the ability to differentiate between the different types of samples. $^1$H NMR and $^{13}$C NMR were used to identify the hydrogen and carbon atoms in the sample. The sensitivity of the $^1$H NMR approach was found to be relatively higher than that of the $^{13}$C NMR. Consequently, focus was placed on the $^1$H NMR for the study. From the structure of amoxicillin 19 hydrogen atoms can be identified but the spectrum for the standard proton NMR has 9 peaks indicating the presence of 9 structurally equivalent hydrogen atoms in the spectrum. This is so because some of the hydrogen atoms appeared as a single peak due to the chemical environment in which they exist.
The chemical shift value can be used to explain the structural equivalences.

Based on the structure of amoxicillin the hydrogen atoms designated with the letter “a” are equivalent because they are basically found in the same chemical environment (RCHO) and can be seen as a single peak. The hydrogen atoms labelled “b” are also found in the different but equivalent environment (Aromatic-H), and therefore can also be seen as one peak. Similarly, the hydrogen atoms labelled with the letter “g” are from equivalent environment since they are not bonded neither to a carbon nor nitrogen, they are also observed as a single peak. The hydrogen atoms in the methyl group, labelled “i”, are chemically in the same environment since each is attached to a carbon atom it is observed as a single peak. This accounts for the 9 peaks seen in the proton NMR spectrum for the standard amoxicillin.

If the proton NMR spectrum of the standard amoxicillin is compared to that of the NHIS spectrum it is observed that it contains 14 peaks. The occurrence of the nine peaks in the NHIS spectrum at identical positions as that of the standard confirms the presence of the amoxicillin trihydrate (the API) in the locally manufactured drug. The
extra peaks can be attributed to other organic composition in the drug due to the excipient component of the locally manufactured drug. The proton NMR spectrum for imported amoxicillin has 9 peaks in positions as that of the standard amoxicillin. This also confirms the presence of the API in the imported drug as well. The spectrum for the open market amoxicillin contains 11 peaks with 9 of them occurring in the same positions as that of the standard spectrum, hence indicating the presence of the API.
CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSION

Nuclear magnetic resonance spectroscopy (NMR) was used as an analytical technique in analysis of amoxicillin drug. H NMR and C-13 NMR were used as an effective tool for the structural identification of the active principal ingredient (amoxicillin trihydrate) in different types of amoxicillin antibiotic drugs purchased in Accra, Ghana.

H NMR and C-13 NMR profiles were obtained for the Active Principal Ingredient (Amoxicillin Trihydrate) in Amoxicillin Antibiotic medical drug samples. H NMR showed relatively higher sensitivities for the drug than C-13 NMR. Consequently analysis for the antibiotics was focused on H NMR.

A suitable solvent was determined after experimentation with available solvents. Dimethyl sulfoxide was chosen, and the samples were prepared by dissolving suitable quantities (10 mg) of the drug in 1 ml of the chosen solvent.

The results obtained from the NMR analysis provided an NMR profile for the API using the H-NMR technique. With this profile it was possible to identify the presence of API in all the amoxicillin samples analysed. There, however, existed differences in number of hydrogen peaks, and intensity values for some of the peaks. This therefore provides a means for identifying the different types of medical drug samples using the structure of their API. Consequently the NMR technique can be used to identify sources of origin of amoxicillin drug. Even though the results indicated the presence
of API in all the samples, it requires quantitative-NMR to be able to assess the quality of the API detected.

A procedure suitable for sample preparation of amoxicillin for NMR analysis was elaborated. Small sample size was required as compared to other analytical techniques which made the analysis faster. Hence, NMR will offer rapid analysis in facilitating the differentiation between fake and genuine amoxicillin drugs.

5.2 RECOMMENDATIONS

This work focused primarily on identification and semi-quantitative analysis looking at peak intensities. It is therefore recommended that future research should consider using NMR to quantitatively detect the content of Active Principal Ingredient (API) in the amoxicillin. In addition the scope of future research should be extended to cover other commonly prescribed antibiotic drugs. Attention was paid primarily to the organic profile (active principal ingredient) of the amoxicillin drug. For complete profiling of the amoxicillin drug it is also necessary to consider the excipient component as well as the inorganic profile of the drug with suitable analytical techniques.
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