Multi-Analytical Approach for Profiling Some Essential Medical Drugs

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DECLARATION

I hereby declare that with the exception of references to other people's work which have duly been 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. I also declare that the preparation of this project was supervised in accordance with the guidelines of the supervision of Thesis work laid down by the University of Ghana.

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Date Date

DEDICATION

This research is dedicated to my parents and my brother (Manaf Abubakar) for their love and support and inspiration over the years.

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LIST OF ABBREVIATIONS

AMG	Arzneimittelgesetz (German Medicinal Products Act)
EMA	European Medicines Agency ("the Agency")
EU	European Union
IMPACT	International Medical Products Anti-Counterfeiting Taskforce
PRR	Proportional Reporting Ratio
PSUR	Periodic Safety Update Report
SOP	Standard Operating Procedures
UMC	Uppsala Monitoring Centre (The WHO Collaborating Centre for International Drug Monitoring)
US	United States
WHO	World Health Organization
ZL	Zentrallaboratorium Deutscher Apotheker e.V. (German Official Medicines Control Laboratory
API	Active Pharmaceutical Ingredient
HPLC	High Performance Liquid Chromatography
TLC	Thin Layer Chromatography
BP	British Pharmacopoeia
NHIS	National Health Insurance Scheme
USP	United States Pharmacopoeia
KNUST	Kwame Nkrumah University of Science and Technology

LC-MS	Liquid Chromatography / Mass Spectrometer
LC-NMR	Liquid Chromatography / Nuclear Magnetic Resonance

ABSTRACT

Counterfeit and substandard pharmaceutical drugs are chiefly rampant in developing countries due to inadequate analytical facilities and lack of regulatory oversight. The production of counterfeit or substandard drugs is broadly problematic. Underestimating it therefore leads to morbidity, mortality, drug resistance, introduction of toxic substances into the body and loss of confidence in health care systems. Medical drugs that are often counterfeited range from antimalarial drugs to antiretroviral drugs with antibiotics being counterfeited the most. This research work, therefore, aims at contributing towards the establishment of measures/processes for distinguishing between fake and genuine amoxicillin drugs. This was achieved by the identification and quantification of the Active Pharmaceutical Ingredient (API) and the excipients in the drug formulation. The major analytical techniques employed for this research work were Instrumental Neutron Activation Analysis (INAA), X-ray Powder Diffraction (XRD), High Performance Liquid Chromatography (HPLC) and in vitro Dissolution Test. The amoxicillin samples analyzed were the foreign generic amoxicillin purchased from Ernest Chemists pharmacy at East Legon, Accra, the National Health Insurance Scheme (NHIS) amoxicillin purchased at Fair Mile pharmacy at West Legon, Accra and the Suspected Fake amoxicillin purchased at Okaishi market. For the establishment of fingerprint for identification of substandard amoxicillin, INAA was used to qualitatively determine the short lived radionuclides (excipients) which then facilitated the correct identification of the API and the excipient phases in each of the amoxicillin groups. The phases identified were Amoxicillin Trihydrate as the excipient, Magnesium Stearate (hydrated) and Magnesium Stearate (anhydrous) as the excipients. For Quality control purposes, High Performance Liquid Chromatography approach and also, the in vitro

Dissolution test were conducted on each of the groups of amoxicillin samples. Before that, the Physical Parameter test on each revealed that, the British Pharmacopoeia (B.P ±7.5%) weight range for the Foreign Generic Amoxicillin was 0.6322 g – 0.7348 g, with the actual range being 0.6587 g – 0.7111 g. For the NHIS Amoxicillin, the B.P ±7.5% weight range was 0.4243 g – 0.4931 g with the actual range being 0.4313 g – 0.4824 g. The Suspected Fake Amoxicillin had a B.P ±7.5% weight range of 0.3733 g – 0.4339 g with its actual range being 0.3966 g – 0.4130 g. For the HPLC, The range of the percentage content of the API according to the 3rd edition of the British Pharmacopoeia is 92.5% - 110.0%. The Foreign Generic Amoxicillin contained 102.5% of the API, the NHIS Amoxicillin contained 99.8% of the API and the Suspected Fake Amoxicillin contained 98.7% of the API which all fall within the pharmacopoeia range. For the Dissolution Test, According to the 2014 edition of the British Pharmacopoeia, the threshold tolerance level is 80% below which a medical drug is rejected. The dissolution percentage range of the Foreign Generic Amoxicillin was 93.6% – 95.5%, that of the NHIS Amoxicillin was 87.5% – 92.0%, which were above the 80% tolerance level. But the dissolution percentage range of the Suspected Fake Amoxicillin was 55.7% – 57.2% which is far below the threshold tolerance level of 80% indicated. The Suspected Fake Amoxicillin was therefore found to be substandard. This research work has also shown that for quality control purposes, HPLC is a better tool but XRD in combination with INAA is a more effective tool for structural identification and phase quantification for the purposes of distinguishing between fake and genuine drugs. It also has faster analysis time, and simpler sample preparation.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 BACKGROUND

Various countries in the world have different understanding of what a counterfeit pharmaceutical drug is. It is, therefore, very challenging to find a single unified definition of a counterfeit drug globally, with each country coming out with its definition based on a wide range of possible drug product falsifications. These indicators include production of drugs ranging from those with no active pharmaceutical ingredient, little active pharmaceutical ingredient through to those with too much active pharmaceutical ingredient, deliberate or accidental production of drugs to contain toxic or potentially dangerous contaminants, forging of labels by changing expiry dates, trademarks, holograms or packaging information (Krüger, 2011).

There are a number of factors upon which the quality of medical drugs depends. These factors include storage conditions, formulation, the raw materials used, manufacturing process, equipment, technical knowhow for production and packaging of the product, transportation and manufacturing environment. It is, therefore, the duty of the drug manufacturers to set the Quality specifications which are then published in Pharmacopoeias (Onwuka, 2010).

According to the United States Pharmacopoeia (USP), a medical drug is of poor quality when it fails to meet official specifications for quality, purity, strength, labelling or packaging. The consistency in producing medical drugs is maintained by adhering strictly 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) in order to ensure the safety, efficacy and quality of the drugs throughout their lifecycle (Egbo, 2013).

The definition of a counterfeit drug according to the World Health Organization (WHO) is a drug which is deliberately and fraudulently mislabeled with respect to identity and/or source (WHO, 2003). Manufacturers of counterfeit drugs have so many ways of presenting counterfeit drugs. Both branded and generic drugs could be counterfeited thereby containing the right or wrong ingredients (i.e., low active ingredients, with no active ingredients or those with counterfeit packaging). The availability of counterfeit drugs in the market is a major source of worry for any country. The existence of a wide range of types of counterfeit drugs from the exact copy of a genuine product to the extreme case of a drug product with wrong active pharmaceutical ingredient in developing countries has been reported in so many articles (Demissie et al., 2012).

Pharmaceutical companies, health care providers, consumers and government regulatory agencies pay much attention to counterfeit drugs and active pharmaceutical ingredient. It has been reported that manufacturing of counterfeit or substandard drugs is an extensive problem and its under-recognition leads to morbidity, mortality, drug resistance, introduction of toxic substances into the body, and loss of confidence in health care systems (Sherma, 2007)

Among the specific cases reported by Mukhopadhyay (2007) were the confiscation of 5,000 counterfeit packets of a flu medication known as Tamiflu valued around $1,000,000 in 2006 in the United Kingdom; the deaths of 2,500 people in Niger after using counterfeit vaccines presented to them to fight a menengitis epidemic in 1995; the

deaths of 30 Indian infants after the consumption of cough syrup containing diethylene glycol in 1998; and the deaths of over 30 Cambodian citizens in 1999 after taking older and less potent antimalarial drugs that were packaged and sold as a very potent and expensive drug artesunate. Additionally, the diethylene glycol (a toxic industrial solvent) scams led to the deaths of children in Haiti and Nigeria (Jahnke, 2004).

A survey of counterfeit antimalarial drugs in Southeast Asia conducted in Burma, Lao PDR, Vietnam, Cambodia and Thailand revealed that there was no artesunate in the 188 tablet packs labeled "artesunate 53%" that were bought and analyzed, and out of the 44 mefloquin samples that were analyzed, 9% contained less than 10% of the expected amount of the active ingredient (Mukhopadhyay, 2007). According to the World Health Organization, antibiotic, malarial, tuberculosis (TB), and HIV/AIDS drugs are those that are counterfeited the most, with an estimate that 25% of pharmaceutical drugs in developing countries are fake, which in some areas are as high as 50% (Mukhopadhyay, 2007). The forecast made by the Center for Medicine in the Public Interest in the U.S.A indicates that the sales of fake medical drugs worldwide will reach $75,000,000,000 in 2010, representing a 95% increment from 2005 (Mukhopadhyay, 2007).

Counterfeit drugs' failure to treat or prevent the intended disease makes it unsafe, thereby causing harm to the patient. Resources are usually wasted on purchasing, inventory, transport and dispensing resulting in disastrous patient outcomes such as poisoning, disability and mortality. Antimicrobial resistance in contagious diseases such as tuberculosis is on the rise due to the use of fake medical drugs. For instance, fake antibiotics can change a tuberculosis case that could be treated within a few months at a less cheaper cost, say US$10, into a serious resistant tuberculosis that could take two

years to cure (i.e., about 100 times more expensive than the first-line medical drugs that are used for the treatment of non-resistant diseases) (WHO, 2000).

In order to assure patient's safety, medicinal products have to meet highest standards in terms of quality, effectiveness and safety, as indicated in the medicinal drug regulations. Implementation of a post marketing surveillance or supervision system to make sure that the product remains within the established risk-benefit balance is the responsibility of the marketing authorization holder. Therefore, the World Health Organization defines pharmacovigilance as "the science and activities that are related to the detecting, assessing, understanding and preventing adverse effects or for that matter any other problem relating to medicine" (Krüger, 2011).

In the past 25 years, the globalization and the explosion of free trade, as well as the ascending availability of medicines via internet, calls for a widening of the scope of pharmacovigilance. New hazards to public health have emerged in connection with the changing situation regarding the drug market. Some examples for these alarming developments are increasing self-medication practices, illegal sale of medicines, including drugs of abuse, over the internet and, widespread manufacture and sale of counterfeit and substandard medicines (Krüger, 2011).

A worldwide effort to combat the distribution and sale of counterfeit and illegal medicines online climaxed in "Operation Pangea III," resulting in the seizure of approximately one million illicit and counterfeit drugs worth around $2.6 million by the end of 2010 (Mackey and Liang, 2011).

Reports about counterfeit drugs, received by the World Health Organization, are in relation to the pharmaceutical drug categories antibiotics, hormones, analgesics, steroids and antihistamines (http://www.who.int/medicines/services/counterfeit/overview/en/).

From the previous incidents of counterfeit medical drugs, the extent of the problem and its impact on public health becomes increasing disturbing. The Nigerian National Agency for Food and Drug Control (NAFDAC) in 2002 reported that 60% of their medical drugs were counterfeited, substandard or with exhausted expiry dates (www.gphf.org/web/en/minilab/hintergrund_arzneimittelfaelschungen.html).

Both developing and developed countries face the problem of counterfeit drugs. About 20 million doses of Lipitor® were recalled in 2003 in the USA. Also, with regards to counterfeit Lipitor®, a full batch had to be recalled in the United Kingdom in 2005. Again, in 2005 the trading of counterfeit "lifestyle" drugs which include the anti-obesity medicines Reductil® and Xenical®, the smoking cessation drug Zyban®, the hair restorer Propecia® and the erectile dysfunction medicines Cialis® and Viagra® through the internet was exposed and those involved were rightly identified (Schweim et al., 2005).

A patient might be a bit fortunate in cases whereby the medical drug itself is not counterfeited, except the packaging. Even so, the economical harm can be immense. The effect on the product quality of the tampered drug is uncertain, which poses potential health risk to the patient with a recent case being the sale of HIV-medicines in Germany, in 2009. The HIV-medicines were set to be sold on the African market at a cheaper price by the pharmaceutical manufacturer and were therefore repackaged and sent back to Germany illegally. It was revealed that they were more than 10,000 packages of the HIV drugs worth around 6 Million Euros (Korzilius, 2011).

Measuring the quantity of the global morbidity and mortality of counterfeit medical dugs is extremely challenging since there have been no comprehensive studies to quantify this harm. Some of the literatures are obtained from local investigations with slight scientific public health inquiry in relation to the huge scale of this illegal activity. Estimates put the total loss of life to counterfeit pharmaceuticals between 500 000 and 1 million people each year (Kafchinski, 2009).

Counterfeit drugs can sequel to economical mischief as well as treatment failure or death. In spite of the fact that counterfeit drug cases in the legal supply chain in the developed countries is below 1% of the market value, it is projected to reach around 50% in the illegal supply chain. Incidents of counterfeit medical drugs are much higher in the developing countries with some having as much as 90% of the medicines on sale to be counterfeit (Schweim, 2010). These percentages show the extent of the illegal trade of counterfeit drugs along with a turnover of about 32 billion dollars every year, making it the most profitable business in the black market. Manufacturers of counterfeit drugs do not maintain cost intensive systems for quality assurance and Good Manufacturing Practices, making their cost of production considerably low. Measuring the cheap cost of production of counterfeit drugs against their high market value, the profit to be made is very huge. The risk of apprehension and prosecution in the trade of counterfeit medicines is very low whilst the profit is abnormally high. Also, the punishments offered to the few that are caught are not deterring enough

(www.who.int/medicines/services/counterfeit/overview).

It becomes very difficult for the manufacturers of counterfeit drugs to sneak into the various distribution channels of a country with the existence of an effective national drug regulatory authority to check the manufacture, importation, distribution and sale of medical drugs. Currently, just about 20% of the WHO Member States have effective drug regulations. The rest have drug regulations which are less effective or in some cases none which makes the quantity of counterfeit medical drugs extremely high in these countries. Many people in underdeveloped or developing countries cannot afford costly drugs and, therefore, result to buying cheap drugs. There is also a high demand of medical drugs that already exceeds the available original drug supply. All these factors contribute to the reasons why counterfeit drugs are very common in these countries. (http://www.who.int/medicines/services/counterfeit/overview/en/index1.html).

Zooming into the medical distribution problem in Ghana, the pharmaceutical distribution system of the private sector in the country (and any other place in the sub region) can be described as completely unordered, unpredictable and confusing. It is difficult to determine the number of intercessors involved in the pharmaceutical distribution, but is probably in 1000s. There can be very slight dubiety that this chaotic system very much affect the product security, availability and the ultimate price of pharmaceutical drugs and reduces the possibility of patients getting medical drugs as and when they need them. In view of this, the introduction of parallel pharmaceutical trade would probably make things worse. Local manufacturers of pharmaceutical drugs have serious problems in distributing pharmaceutical products across the sub-region due to lack of stringent rules and regulations, and the disorderly nature of the medical distribution supply chain.

As reported by the Ghana Food and Drugs Authority, Ghana has a 'relatively' secure retail pharmaceutical supply chain without a major problem with street trading of medical drugs, even though it happens in rural and peri-urban places to some extent. This to a large extent contributes to the wild spread of counterfeit pharmaceutical drugs. The International Medical Products Anti-Counterfeiting Taskforce (IMPACT) was formed in 2006 by the World Health Organization as a reaction to the heightened damage caused by counterfeit medical drugs. It was a collaboration involving pharmaceutical manufacturer associations, international organizations, and drug regulatory authorities. The main goal of the IMPACT is curbing the production and distribution of counterfeit medical drugs by establishing reconciliated networks worldwide between countries. The IMPACT together with the Interpol is currently working with three international operations to curb illegal trade of medical drugs via the Internet, known as Pangea I, II and III (Sucker-Sket, 2010).

Eight (8) countries in 2008 participated in the first operation. Inspection of pharmacies on the internet, internet service providers and mail services is done each time by the IMPACT, Interpol and the WHO in conjunction with the national drug regulatory authorities, customs and the police for a whole week. A large quantity of counterfeit and illegally supplied medical products could be confiscated resulting in the identification of a number of non-authorized internet pharmacies and closing down of many websites. Also, the Pangea operations is responsible for sensitizing the public on the problem of counterfeit drugs as well as the broad risk to the health of patients that they constitute (Sucker-Sket, 2009, 2010).

1.2 RESEARCH PROBLEM STATEMENT

Traditionally Ghana has relied on a 'cash and carry' system of health funding. The Ghana NHIS set up in 2005 covers as of September 2007 approximately 50% of the Ghana population. Ghana's National Health Insurance Scheme was introduced in 2004 following the passage of the Act of Parliament, Act 650 of 2003 and Legislative Instrument 1809, 2004. Health insurance was introduced because of several challenges that confronted health development including the failure of the out-of-pocket fee for service system to promote quality and access. Ghana also faces fake drug problems. This may be attributed to the facts that the National Health Insurance Scheme (NHIS) pays for only generic drugs which opens a door for the importation of generic drugs from various sources across the globe. Besides, there is very little quality control system in place for the importation of these generic drugs into the country. Also, one of the most popular analytical techniques used for drug analysis is the High Performance Liquid Chromatography technique. This technique can be used to identify and quantify the amount of API in a particular drug formulation and can therefore be used to determine whether a drug is genuine or substandard. However, due to its low sensitivity for certain compounds and volatile substances not being separated properly, there is the need to explore other techniques for drug analysis and fingerprinting.

1.3 OBJECTIVES

The main objective of this research work is to contribute towards the establishment of measures/processes for distinguishing between fake and genuine medical drugs.

1.3.1 Specific Objectives

- To compare analytical procedures for the quality control of imported generic drugs.
- To provide characteristic fingerprints that will facilitate the identification of fake drugs.

1.4 RELEVANCE AND JUSTIFICATION

The deadly implications of counterfeit drugs are well understood to be a central challenge to the integrity of public health systems around the globe, as well as a direct threat to our individual health and welfare. Effects of counterfeit drugs include morbidity, drug resistance, toxicity, etc. Generally, it is very hard to identify substandard drugs. It is relatively easy to trace the origin of a pharmaceutical drug provided it comes from a well-known international pharmaceutical company having a tracking system in place which is usually not the case. In fact, most health care providers in Ghana are only capable of affording medical drugs without an in-built security network. It is therefore very important to come out with a tool to analyze drugs to make sure they conform to the standards a genuine drug is supposed to adhere to. Hence this research seeks to address the problem of drug counterfeiting by providing an analytical tool for identifying fake

antibiotic drugs. Now that most people in Ghana rely on the NHIS which only pays for generic drugs, conducting this research work to help identify counterfeit medical drugs will be beneficial to the nation by helping to save the country a lot of money. For instance, fake antibiotics can change a tuberculosis case that could be treated within some few months at a less cheaper cost about US$10 into a serious resistant tuberculosis that could take two years to cure i.e. about 100 times more expensive than the first-line medical drugs that are used for the treatment of non-resistant diseases (WHO, 2000). In addition to the financial benefits, it will also save the doctors and nurses a lot of time to take care of other patients.

CHAPTER TWO

LITERATURE REVIEW

2.1 PHARMACEUTICAL DRUG

A pharmaceutical drug is defined as a chemical substance which is compound or 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 so many ways in which medications are classified. One of the most important divisions is between traditional small molecule drugs normally obtained from biopharmaceuticals, and chemical synthesis, including 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 to classify pharmaceutical drugs apart from their origin. The Anatomical Therapeutic Chemical Classification System also called the ATC system is an elaborate and popularly used classification system. The list of essential and key medical drugs is also kept by the World Health Organization. Discovery and development of medical drugs 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, drug pricing.

2.2 DEFINITION

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 diagnosis (Official Journal of the European Union, 2004).

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).

2.3 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 Classification System (ATC 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 ANTIBIOTICS

2.4.1 Beta-Lactam Antibiotics

2.4.1.1 Basic characteristics of Beta-lactam

Beta-lactam antibiotics are bactericidal drugs which subdue or inhibit by interference, the construction of bacterial cell wall by synthesizing peptidoglycan. Penicilinbinding proteins (PBPs) are the bacterial enzymes which are affected by beta-lactams. Various penicilinbinding proteins differ in their detail function, affinity and quantity for betalactams. Primarily, the consequence of beta-lactams is usually shown against multiplying bacteria which intensively construct their cell wall. However, in the absence of the peptidoglycan in the cell wall, beta-lactams are not very effective against microorganisms.

Pharmacokinetics

A lot of beta-lactam antibiotics are reactive to acid and as such decomposed with gastric juice. Also, there is some limitation with regards to the absorption of beta-lactams from the gastrointestinal tract. The bulk of beta-lactam antibiotics are produced in parenteral form only. In certain cases, the original drug is esterified so as to enhance absorption, and these esterified beta-lactam antibiotics should be taken with food. Usually, Beta-lactam antibiotics are circulated in the extracellular space. Even though there is restriction on the penetration across biological barriers, at times higher dosing can reverse it. Excretion of

majority of beta-lactam antibiotics are done through the kidneys with exceptions being cefoperazone, oxacillin and ceftriaxone. Beta-lactam antibiotics have short half-lives which vary from half an hour (in the case of penicillin, oxacillin and cephalotin) to between 2 to 2.5 hours with ceftriaxone having an exceptional long half-life of 8 hrs, allowing once a day administration.

Pharmacodynamics

The effectiveness of Beta-lactams depends on the "time above Minimal Inhibitory Concentration (MIC)". Dosing is always aimed at keeping the level of antibiotic above MIC at the infected area as long as possible. In situations whereby the infection is a mild one, the level of drug exceeds MIC for 40-50% of the dosage interval.

Undesirable effects

Beta-lactams are non-toxic and have adverse effects which are dependent on minimum concentration. In penicillins, for example, the degree of dosing is very high. Any allergy to penicillins or cephalosporins has to be established by examining the level of antibody in the blood. The genuine allergy to any penicillin antibiotic should be interpreted as allergy to all penicillins. On the other hand, the allergy to a cephalosporin antibiotic does not necessarily imply the allergy to all other cephalosporins. There is a possibility of cross-allergy between penicillins and cephalosporins but not frequent. It was estimated that between 5 to 10% is the probability of allergy to cephalosporins in patients that are allergic to penicillins. Hence, cephalosporins can cautiously be prescribed to patients

with history of a mild penicillin allergy also known as exanthema. But then, allergy to cephalosporins means greater probability of cross-allergy to penicillins. Beta-lactams can be used by women in advanced stage of pregnancy or breast-feeding women, as well as in newborn babies.

Disposal:

Beta-lactams are most effectively used for the treatment of acute infections in a well perfunded tissue, or for the treatment of sepsis. Part of the drugs are also suited for surgical prophylaxis.Dosing regularly is required in order to get the desirable effect. Enhancing the individual doses is important if penetration to the infected area is proving to be a problem.

A) Penicillins

There are four categories of penicillin namely:

1) Natural penicillins

Natural penicillins have narrow spectrum which contains gram-positive and gram-negative cocci (i.e. enterococci, pneumococci, meningococci, streptococci), gram-positive bands (L.monocytogenes and corynebacteria), spirochetes (Treponema sp., Borrelia sp., Leptospira sp.), and virtually all anaerobes (clostridial, Actinomyces, species and peptostreptococci,).

Benzylpenicillin or Penicillin G: This is not stable in gastric acid juice and it is suitable only when it to be administered intravenously.

Phenoxymetylpenicillin or penicillin V: It is stable in acid form and it is most effective when administered orally.

Procain-penicillin: It is available in depot form and it is to be administered intramuscularly, most often once a day.

Benzatinpenicillin: It is also available in depot form and creating stabile low level of antibiotic for 2-4 weeks. It is therefore utile for prophylaxis of streptococcal reinfections. The dosage spectrum of penicillin is extremely extensive i.e. 1 mill.U. to 40 mill.U. each day for adults with regards to the type of infection and how severe the infection is. For comparison 1,000.000 units equals 625 mg of penicillin. Streptococcal skin infections, pseudomembranous, tonsillitis, or animal bite and scratches are the typical cases requiring low-dose penicillin treatment. Patients with infective endocarditis which is caused by either enterococci or viridans streptococci), streptococcal, pneumococcal or meningococcal sepsis, clostridial wound infection are treated with high-dose.

2) Anti-staphylococcal penicillins:

They are not resistant to beta-lactamases which are produced by gram-negative microbes but are resistant to staphylococcal beta-lactamase. Since the outcome against gram-positive bacteria other than staphylococci is weaker as compared to penicillin G, the drugs have a spectrum which is limited in size.

Methicillin: It is only available in forms administered by means other than through the alimentary tract, as by intramuscular or intravenous injection. Eg: nafcillin, oxacillin, cloxacillin, dicloxacillin.

3) Aminopenicillins:

Aminopenicillin drugs are the ones with spectrum which is naturally marked by resemblanceto natural penicillin but just that they have extension against common gram-negative bacteria such as Shigella sp., Haemophilus influenza, Salmonella enterica,

Helicobacter pylori or Proteus mirabilis and Escherichia coli. They are therefore more effective than natural penicillin against enterococci and listeriae.

Ampicillin: This is the fundamental representative of the subgroup and it is suited for administration by means other than through the alimentary tract.

Amoxicillin: Amoxicillin has better adsorption after it has been administered orally (70-80%) compared to ampicillin (40-50%). 2 – 12 g of ampicillin is to be taken each day. Because of plasmiderelated production of beta-lactamase, a lot of strains of the afore-mentioned gram-negative bacteria have become resistant. New formulae were therefore made which contained the antibiotic plus a betalactamase inhibitor. The two combinations in use are to be administered orally and parenterally and they are:

ampicillin + sulbactam

amoxicillin + clavulanic acid

These combinations are very efficient against the afore-mentioned gram-negative microbacteria owing beta-lactamase as well as against Staphylococcus aureus. But then there is no need prescribing these antibiotics against enterococci, streptococci, or any other bacteria which do not produce beta-lactamase. Aminopenicillins are largely used in clinics with beta-lactamase inhibitor or without. They are presented in mesotitis and lower respiratory tract infections, bacterial sinusitis, purulent gynecological infections, urinary and hepatobiliary tract infections, and other community-obtained infections. 4) Penicillins are efficient against pseudomonads (and other gram-negative pathogens which are problematical due to natural resistance) e.g. **karbenicillin, ticarcillin, azlocillin, mezlocillin, piperacillin** (which are to be used parenteral only). According to the cultivation results, the administration of these drugs is in intensive care

infections whereby the only path to administer these drugs is intravenous. The third generation cephalosporins are most often chosen over these other drugs due lower costs. The combination of these antibiotics and beta-lactamase inhibitors were produced: **ticarcillin + clavulanic acid, piperacillin + tazobactam**

They have similar use as the basic drugs.

2.5 COUNTERFEIT DRUGS

Counterfeit pharmaceutical drugs as defined by the World Health Organization are "deliberately and fraudulently mislabeled with respect to identity and/or source". Brand name and generic pharmaceutical drugs are both capable of being counterfeited and that may consist of drugs having correct ingredients but with wrong packaging, having the wrong ingredients, not containing any active pharmaceutical ingredients, or not having sufficient active pharmaceutical ingredient". Pharmaceutical drug counterfeiting posing as a problem which needs serious attention was first brought up at the conference of experts organized by WHO on the topic "Rational use of the drugs" which took place in Nairobi in1985 (WHO, 2005). The Center for Medicines established in the United States made a projection that sales of illegal drugs would reach USD 75 billion in 2010, meaning a 92% increment since 2005. The World Health Organization (WHO) came out with estimations that out of the 1 million people who die because of malaria in Africa annually, 200,000 of them are as a result of the consumption of counterfeit anti-malarial drugs (WHO, 2003). It is said by Harris, Stevens and Morris (2009) that counterfeit drugs used for the treatment of malaria and tuberculosis result in the deaths of 700,000 people yearly in Africa. The health of the general public is at a growing risk due to a seemingly

growing global epidemic of the production and sale of counterfeit pharmaceutical drugs with a typical evidence being that, more than 50,000 people were given fake meningitis vaccination in Nigeria in 1995, leading to the deaths of 2,500 children (WHO, 2003). In Nigeria, Haiti and Bangladesh, it was estimated that 400 children died as a result of ingesting paracetamol (acetaminophen) syrup being counterfeit, manufactured with ethylene glycol, which is a toxic industrial solvent (O'Brien et al., 1998). Multiple fake drug consumption resulted in the death of about 192,000 in 2001 in the People's Republic of China (Fackler, 2002). It has been said that approximately 10% of all pharmaceutical products in the world are counterfeits which constitutes $32 billion each year yet only a fraction of the incidents regarding drug counterfeiting are reported to the appropriate bodies meaning the some estimations of counterfeit drug cases are likely to be wrong (Wolff et al., 2003 and Newton et al., 2002). It has also been found out that more than half of counterfeit medical drugs that are available worldwide have no active pharmaceutical ingredients or contain an entirely different active pharmaceutical ingredient than what is on the labeling with about 10% containing contaminants. From the late 1990s till date, counterfeit medical drugs that imitate those used to cure potentially-fatal tropical diseases, like malaria, have been found in greater numbers. According to Daviss (2005) and van Agtmael et al. (1999), one of the major health problems in a number of tropical countries especially in sub-Saharan Africa and Southeast Asia is Malaria. Between 300 and 500 million people in Asia and Africa contract malaria caused by Plasmodium falciparum yearly, resulting the death of 1.5 million people with most of them being children.

2.5.1 Characteristics of Substandard/Counterfeit Drugs

There are various forms of counterfeit pharmaceutical drugs. Some do not contain any of the active pharmaceutical ingredients (API) or may contain the API in harmful quantities. Preparation of others may be from sources that are unacceptable or may even be formulated differently, may contain excessively high amounts of impurities. The falsification can also be done based on the drug's outward packaging, shape, size, pill colour, and markings.

Decreased Stability and Bioavailability

There have been so many reports with regards to the stability of some essential medical drugs in the tropical zones which are stored in various warehouses and in some wholesale pharmacies that may not be acceptable to ascertain the integrity of pharmaceutical drug products. Antibiotics such as ampicillin can start to degrade when transported or stored at temperatures above 25ºC as well as high humidity. On the contrary, other pharmaceutical drugs may be unaffected under such conditions with studies showing that high storage temperatures have no adverse effects on the composition of some antibiotics such as tetracyclines and penicillins. This indicates that the most probable cause of low quality pharmaceutical drugs is found to be during the manufacturing process (Kelesidis et al., 2007). Interactions can also take place when drugs are kept under humid conditions and at high temperatures resulting in reduced dissolution rate. Even though some substandard antibiotic drugs may have the right amount of active pharmaceutical ingredient, they may not have optimal activity due to decrement in bioavailability. Examples are co-trimoxazole, tetracyclines, metronidazole, chloroquine, mefloquine, pyrimethamine.

Decrement in the Concentration of the API

Poor manufacturing process or poor transport and storage conditions may be the cause of low amount of active pharmaceutical ingredient in antibiotic drugs. It was reported that decomposition was the cause of poor quality in some antibiotic drugs. But even so, it has been established that many antibiotic drugs may have optimal stability even under tropical conditions making poor manufacturing the chief cause of poor quality drugs. Lastly, low amount of active pharmaceutical ingredient can be as a result of diluting drugs with substances such as contaminated water or sugar (Reidenberg et al., 2001)

Changed Chemical Content

Counterfeit pharmaceutical drugs can be identified by changed odour since they compose of diluted active pharmaceutical ingredients or in some cases harmful additives. At times, the counterfeit drugs are identical to the genuine drug product chemically. Even so, most counterfeit pharmaceutical drugs contain the active pharmaceutical ingredients in harmful amounts. Tablets or capsulated drugs may sometimes contain a wrong antibiotic such as erythromycin powder in amoxicillin capsules, or can even contain worthless flour, powder or starch. Examples include neomycin eye drops and meningococcal vaccine manufactured using tap water, antimalarials and antibiotics with no active pharmaceutical ingredients and ampicillin made up of turmeric (Reidenberg et al., 2001).

2.5.2 Prevalence of Counterfeit Drugs in Ghana

There was an announcement from the zonal office of the Food and Drugs Authority (FDA) in Kumasi that fake antimalarial drugs i.e. Coartem with batch numbers X0089

and M1200 were obtained from the market. These drugs upon analysis were found to not contain any active pharmaceutical ingredient (www.ghananewsagency.org). This discovery was made when a private citizen brought a suspicious sample of the drugs to the attention of the Medicine Quality Monitoring program, that was enforced by the U.S. Pharmacopeia Drug Quality and Information Program and supported financially by the U.S. Agency for International Development (www.usp.org). 14 of 17 (82.4%) artesunate tablets that were purchased and used as samples from some pharmacies in Kumasi did not meet the European Pharmacopeia content requirements (http://www.bioline.org). The medicine quality monitoring program formed by Promoting the Quality of Medicines program and enforced by the Food and Drugs Authority (FDA) obtained 13 Substandard and counterfeit antimalarial drugs in Ghana. The medicines were therefore recalled by the FDA after which the names of the pharmacies, clinics, and hospitals where the medicines were obtained were publicized (www.usp.org). A two-day raid conducted by the police and other stakeholders in collaboration with the FDA resulted in the apprehension of 30 people who were found to be selling fake medicines. It was confirmed by the FDA that fake artesunate tablets with batch no. 080504 and manufacturer's address listed as Gulin Pharmaceutical Co. Ltd.) were obtained from the market (www.ghanaweb.com). A man was arrested by The Takoradi Police and Zonal Office of the FDA for selling fake drugs whereby the capsules were suspected to contain sawdust and sand (www.myjoyonline.com). The pharmaceutical company Pfizer was ordered by the FDA to recall some batches of Camoquin Plus Pediatric Suspension (amodiaquine and artesunate) that were found to be substandard. It was reported that the batches were manufactured at Pfizer's facility located in Dakar, Senegal (www.securingpharma.com).

In three districts of Ghana, 55 ergometrine samples and 46 oxytocin samples were bought from 69 formal points of sale and all the ergometrine samples failed to be within British Pharmacopeia specifications. Only 11 (26%) of the oxytocin samples fell within the BP specifications (www.bmjopen.bmj.com). Another antimalarial drug called Halfan in 2002 was faked in unknown amounts (Cockburn, 2005). Ghana went ahead with the implementation of innovative strategies to fight counterfeit pharmaceutical drugs which focus on consumer awareness upon recognizing the magnitude of the problem. A medication verification system was introduced in 2008 by the government which permits consumers who purchase pharmaceutical drugs to send an SMS message of a special number found on the medicine package to the drug manufacturer through a short code, and receive a reply within some few seconds verifying authenticity of the drug (http://www.irinnews.org). Samples of artesunate tablets that were sold in pharmacy shops in Kumasi were analyzed by researchers at the Kwame Nkrumah University of Science and Technology (KNUST) in 2008. Upon analysis, it was revealed that 82 percent of the drug samples were not in agreement with the European pharmacopeia guidelines (Ofori-Kawakye, 2008). In November 2009, the Chief Executive Officer of the Ghana's Food and Drugs Authority ordered the withdrawal of 22 batches of anti-malarial drugs from the Ghanaian drug market due to concerns about their quality (Opuni, 2009).

2.6 REVIEW OF ANALYTICAL METHODS

According to Hu et al. (2006), FCIS comprising of two colour reactions based on functional groups in molecules of macrolide antibiotics and two TLC methods were developed for screening of fake macrolide drugs in China which revealed that two lots of

capsules and one lot of granule had no active ingredients imitating erythromycin ethylsuccinate capsule and azithromycin granule, respectively, one lot of erythromycin tablets imitating roxithromycin tablets and two lots of meleumycin capsule imitating midecamycin capsule.

According to Prazuck et al. (2002), drug quantitative analysis of antimicrobials (benzathine benzylpenicillin, ceftriaxone, chlortetracycline, ciprofloxacin, TMP–SMX doxycycline and erythromycin) was performed with titrimetry and visible UV spectrophotometry. Qualitative analysis was performed with TLC. among the 21 different specialty products, only 3 displayed the official 'registered' label. Three drugs were expired and the expiration date was not available for six others. One product did not contain the active drug declared (chlortetracycline) and did not show any in vitro activity against bacteria. Seven of 21 products (33%) did not contain the stated dosage (one more than stated dosage and six less than stated dosage). The highest deficit observed was 48% in two products (co-trimoxazole and benzylpenicillin). The dosage was not available for five drugs. As a result, only 8 of 21 products (38%) did not contain the stated dosage of active drug. The pharmaceutical companies involved are Lombisin, Unicorn, China (chlortetracycline), Yong Fong, Myanmar (co-trimoxazole), China (benzylpenicillin), Helm Pharmaceutical GMBH, Hamburg, Germany (benzathine benzylpenicillin), Cadila Lab, Ahmedabad, India, Dr Reddy's Lab, Bollaram, India (ciprofloxacin), Remedica Ltd, Limassol, Cyprus (erythromycin and doxycycline), ICPA Lab Ltd, Bombay, India (TMP–SMX).

Three of the five (60%) capsule samples from dispensing points in Nigeria were found to be of lower quality than the officially prescribed standards of pharmaceutical quality. The

quality lapses observed were sufficient to bring about determinable differences in biological availability, according to Okeke and Lamikanra (2001).

366 samples of ampicillin (tablets and capsules) and tetracycline (tablets and capsules) were analyzed in Laos. According to Stenson et al. (1998), three tests were used: identity, assay and measurement of weight variation. The identity was confirmed by TLC, UV and colour reactions. Titrimetric, UV and HPLC methods were used for assay. 12 (3.3%) out of the 366 drugs contained no active ingredient, 42 (11.5%) had levels of active ingredient outside acceptable limits in assay, 128 (35.0%) had excessive weight variation and 4 (1.1%) were managed badly in the pharmacy, 67% of ampicillin samples and 38% of tetracycline had bad quality.

According to Basco (2004), a simple colour reaction test and semi-quantitative TLC analysis on antimalarials (chloroquine, quinine and SP) in Cameroon showed that, fifty (38%) of 133 chloroquine, 52 (74%) of 70 quinine and 10 (12%) of 81 antifolates had no active ingredient, an insufficient active ingredient, the wrong ingredient or unknown ingredient(s). The primary screening based on colour reaction showed that 42 (32%) of 133 chloroquine samples were counterfeit. Further analysis by TLC showed that 8 (9%) of 91 colour-positive samples contained,80% of the reference standard. The colour reaction suggested that 63 (90%) of 70 quinine samples contained a quinoline-type drug, the other 7 samples were clearly counterfeit with unknown ingredients. However, on further analysis, 45 of 63 colour-positive quinine samples were substandard. Of 78 SP samples, 10 (13%) had a negative colour reaction. There were no substandard medications of SP samples on further analysis of positive colour reaction.

A survey by Rozendaal (2001) in Cambodia on antimalarials (mefloquine, artesunate) indicated that most of the bottles with mefloquine tablets and about half of the artesunate blister packs sampled seemed to be fakes. For mefloquine and artesunate, two different varieties of fakes were found: a first-generation fake that was easy to distinguish from the genuine product and a second-generation fake that much more closely resembled the genuine product. A total of 242 drug vendors and pharmacies were mapped in 12 marketplaces, and 133, about half in each marketplace, were selected randomly for investigation. Fake artesunate was sold by 71% (86% sold the genuine product) and fake mefloquine by 60% (61% sold the genuine variety). The fakes were frequently preferred by patients and village health providers because of the lower price. Given their widespread use, the fake malaria drugs are probably a major cause of mortality andmorbidity due to malaria in Cambodia.

Commercially available drug formulations of the USP 24 dissolution tests and HPLC were used to analyze 33 samples of amoxicillin capsules, metronidazole tablets and SMX–TMP tablets in Rwanda and Tanzania according to Kayumba et al. (2004). At the time of purchase, the drug content of all the formulations were within the limits recommended by the USP 24, but after 6-month storage, the drug content of one sulfamethoxazole/trimethoprim was found to be substandard. Immediately after purchase, four formulations (three sulfamethoxazole/trimethoprim and one sulfadoxine/pyrimethamine combination) failed the USP 24 dissolution test. Except for three metronidazole, dissolution tests performed after 6 months of storage under simulated tropical conditions showed that drug release remained within the USP 24

recommended values. In total, 24% of the sampled formulations (8/33) failed the dissolution test.

50% of the antimalarials (primaquine) samples in the Amazonian region analyzed did not conform to the USP qualitative requirements and none conformed to the quantitative requirements. One of three samples analyzed by the BP qualitative criteria did not conform to those either. Chemical concentration of the active ingredient varied from 19% to 168% of the concentration indicated on the label, which made the total dose of primaquine received by the patients either insufficient or excess (toxic levels) according to Petralanda (1995).

A report by Taylor et al. (1995) on antimalarials (tablets and syrup formulations of chloroquine) revealed that three samples of chloroquine tablets (0%, 0%, 42%) contained 50% of the stated amount of active ingredient. The reason why BP requirements were not met is unknown. Decomposition is not likely to be a major factor (no large amounts of decomposition products found), poor quality assurance probably plays a part but the very small amounts found in some samples point to fraudulent manufacture or tampering.

A study by Egbo (2013) of the assessment of the quality of three commonly used therapeutic groups of antibiotics; the macrolides (Azithromycin and Erythromycin) and lincosamides (Clindamycin) involved the identification and quantification of the active pharmaceutical ingredient (API), and *in vitro* dissolution test. The 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 pediatric dosage form of Azithromycin samples assayed did not.

2.7 ANALYTICAL TECHNIQUES EMPLOYED FOR THIS WORK

2.7.1 NAA Methods

Activation analysis is a method for the determination of elements based upon the conversion of stable nuclei to other, mostly radioactive nuclei via nuclear reactions, and measurement of the reaction products. In neutron activation analysis (NAA) the nuclear reactions occur via bombarding the material to be analyzed with neutrons. The reaction products to be measured are either the radiation, released nearly instantaneously upon neutron capture; or, if the resulting new nuclei are radioactive, the induced radioactivity by which they decay. Only the latter mode will here be discussed since it is the most common way to perform NAA. All of the stable elements have properties suitable for production of radioactive isotopes albeit at different reaction rates. Each radionuclide is uniquely characterized by its decay constant – the probability for the nuclear decay in unit time – and the type and energy of the emitted radiation. Among the several types of radiation that can be emitted, gamma-radiation offers the best characteristics for the selective and simultaneous detection of radionuclides and thus of elements. The activation will result in a mixture of radioactivities, which can be analyzed for individual contributions by two approaches: (i) The resulting radioactive sample is decomposed, and through chemical separations it is divided into fractions with a few

elements each: Destructive or Radiochemical Neutron Activation Analysis (RNAA). (ii) The resulting radioactive sample is kept intact, and the elements are determined by taking advantage of the differences in decay rates via measurements at different decay intervals utilizing equipment with a high energy resolution: Non-destructive or Instrumental Neutron Activation Analysis (INAA). A procedure in INAA is characterized by:

(i) activation via irradiation with reactor neutrons

(ii) measurement of the gamma radiation after one or more decay times

(iii) interpretation of the resulting gamma-ray spectra in terms of radionuclides, associated elements and their mass fractions.

Activation

The activation with neutrons is, upon preparation of the test portion, the first stage in an INAA procedure. Its purpose is to convert some of the stable nuclei in radioactive nuclei emitting radiation that can be used for analytical purposes. Insight into the reactions that may take place during activation facilitates the identification of the relation between the observed radioactive nucleus, its target nucleus and associated element. Insight into the reaction rates is of importance for the quantitative analysis and for a priori estimates of the feasibility of an analysis. Each atomic nucleus can capture a neutron during irradiation, resulting in a nuclear reaction in which often the nuclear mass changes; immediately (typically $10 - 14$ s) after the capture excess energy in the form of photons and/or particles will be emitted. The newly formed nucleus may be unstable. If unstable after activation, it starts decaying to a stable state by the emission of radiation through one or more of the following processes: α-decay, β−-decay, electron capture, β+-decay,

or internal transition decay. In most cases γ and X- rays will be emitted too. The capture of a neutron by an atomic nucleus and the resulting reaction may be illustrated, in the case of a cobalt target nucleus, by.

$^{59}C0 + {}^{1}n \rightarrow {}^{60}Co + (prompt)\ \gamma - radiation$

The resulting 60Co nucleus is radioactive and decays (and thereby converts) by emission of β− radiation to excited nuclear levels of the stable 60Ni nucleus, followed by the emission of γ radiation from the internal transition of the nucleus from these excited levels to its ground state. Commonly, the reaction is written in the shorthand notation as $^{59}C0(^{1}n, \gamma)^{60}Co$ in which 'γ' refers to the prompt emitted radiation, not to the gamma radiation following the β− decay.

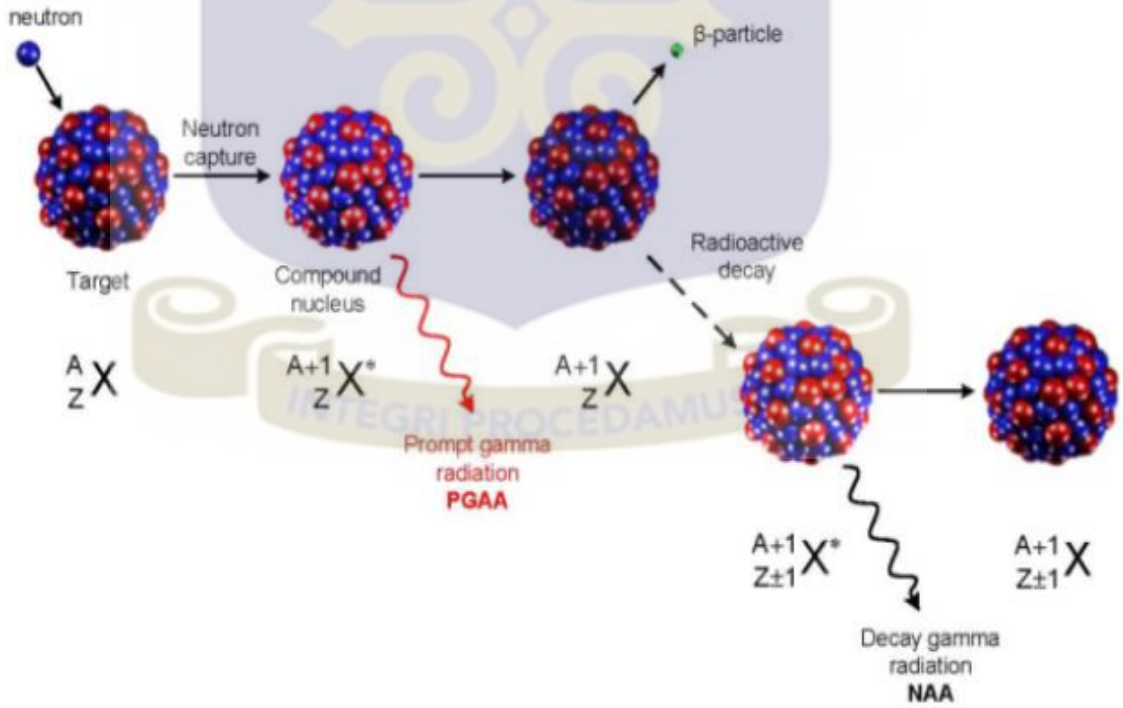

Fig. 2.1 Neutron Activation Analysis

Decay

Radioactive nuclei are instable and decay resulting in product(s) with mass(es) less than that of the parent. The decay involves emission of various types of ionizing radiation towards the ground state of a stable nucleus. Radioactive decay is a statistically random process; the probability that a given nucleus will decay in a certain time interval depends only on the time of observation. It is not possible to predict when a given nucleus will decay, but the decay characteristics can be described by the physical laws of radioactive decay, which are comparable to first order chemical kinetics. This is further elaborated in the paragraph on the derivation of the measurement equation. Decay schemes provide the details of the decay of radionuclides, such as, e.g., the energy levels and half-lives involved, the transitions, spins, multipolarities and branching ratios, types of radiation emitted, relative intensities, and conversion coefficients.

Measurement

The radioactivity induced is measured by the detection of the radiation, emitted during the decay of the radionuclide. In principle both the beta radiation from the nuclear transformation and the often following gamma radiation from the isomeric transition to the ground state can be used for this; moreover, the decay rate of the radionuclide can be measured. In NAA, nearly exclusively the (energy of the) gamma radiation is measured because of its higher penetrating power of this type of radiation, and the selectivity that can be obtained from distinct energies of the photons — differently from beta radiation which is a continuous energy distribution. The interaction of gamma- and X-radiation

with matter results, among others, in ionization processes and subsequent generation of electrical signals (currents) that can be detected and recorded.

Calibration

Calibration is based on the determination of the proportionality factors F that relate the net peak areas in the gamma-ray spectrum to the amounts of the elements present in the sample under given experimental conditions:

$$F = \frac{C}{m} \tag{2.1}$$

where

C net counts in the γ-ray peak of Eγ, dimensionless

m mass of the irradiated element, g

Direct comparator method

The unknown sample is irradiated together with a calibrator containing a known amount of the element(s) of interest. The calibrator is measured under the same conditions as the sample (sample-to detector distance, equivalent sample size and if possible equivalent in composition). From comparison of the net peak areas in the two measured spectra the mass of the element of interest can be calculated:

$$m_{x(unk)} = m_{x(cd)} . \frac{\left(\frac{C}{t_m . e^{-\lambda t_d} . (1-e^{-\lambda t_m})}\right)_{unk}}{\left(\frac{C}{t_m . e^{-\lambda t_d} . (1-e^{-\lambda t_m})}\right)_{cal}} \tag{2.2}$$

in which

$m_{x(unk)}$, $m_{x(cal)}$ =mass of the element of interest, in unknown sample and calibrator,

respectively; in (g)

In this procedure many of the experimental parameters — such as neutron fluence rate, cross section and photopeak efficiency cancel out at the calculation of the mass and the remaining parameters are all known. This calibration procedure is used if the highest degree of accuracy is required. The relative calibration on basis of element calibrators is not immediately suitable for laboratories aiming at the full multi-element powers of INAA. It takes considerable effort to prepare multi-element calibrators for all 70 elements measurable via NAA with adequate degree of accuracy in a volume closely matching the size and the shape of the samples. Single comparator method Multi-element INAA on basis of the relative calibration method is feasible when performed according to the principles of the single comparator method. Assuming stability in time of all relevant experimental conditions, calibrators for all elements are co-irradiated each in turn with the chosen single comparator element. Once the sensitivity for all elements relative to the comparator element has been determined (expressed as the so-called k-factor, see below), only the comparator element has to be used in routine measurements instead of individual calibrators for each element. The single comparator method for multi-element INAA was based on the ratio of proportionality factors of the element of interest and of the comparator element after correction for saturation, decay, counting and sample weights. Girardi et al. defined the k-factor for each element i as:

$$k_{i=} \frac{(M_a)_{i,cal} \gamma_{comp}\, \varepsilon_{comp}\, \theta_{comp}\, \sigma_{eff,comp}}{M_{a,comp} \gamma_{i,cal}\, \varepsilon_{i,cal}\, \theta_{i,calc}\, (\sigma_{eff})_{i,cal}} \qquad (2.3)$$

Masses for each element i then can be calculated from these Ki factors; for an element determined via a directly produced radionuclide the mass $m_{x(unk)}$ follows from:

$$m_{x(unk)} = m_{x(comp)} \cdot \frac{\left(\frac{C}{\left(1-e^{-\lambda t_i}\right).t_m.e^{-\lambda t_d}.(1-e^{-\lambda t_m})}\right)_{unk}}{\left(\frac{C}{\left(1-e^{-\lambda t_i}\right).t_m.e^{-\lambda t_d}.(1-e^{-\lambda t_m})}\right)_{cal}} . k_i \qquad (2.4)$$

with

$m_{x(comp)}$ mass of element x in comparator, in g. These experimentally determined k-factors are often more accurate than when calculated on basis of literature data as in the absolute calibration method. However, the k-factors are only valid for a specific detector, a specific counting geometry and irradiation facility, and remain valid only as long as the neutron fluence rate parameters of the irradiation facility remain stable. The single comparator method requires laborious calibrations in advance, and finally yield relatively (compared to the direct comparator method) higher uncertainties of the measured values. Moreover, it requires experimental determination of the photopeak efficiencies of the detector.

2.7.2 XRD Methods

X-ray diffraction (XRD) is a versatile, non-destructive analytical method to analyze material properties like phase composition, structure, texture and many more of powder samples, solid samples or even liquid samples. Identification of phases can be achieved by comparing X-ray diffraction pattern obtained from an unknown sample with patterns of a reference database. This process is very similar to the identification of finger prints in crime scene investigations. The most comprehensive database is maintained by the ICDD (International Centre of Diffraction Data). Alternatively, it is possible to build up a

reference database from experimental diffraction patterns of pure phases and/or patterns published in the scientific literature or derived from own measurements. Modern computer-controlled diffractometer systems like the PANalytical X'Pert Powder or Empyrean in combination with phase analysis software (e.g. HighScore) use automatic routines to measure and interpret the unique diffractograms produced by individual constituents in even highly complex mixtures (Martin and Detlef, 2013).

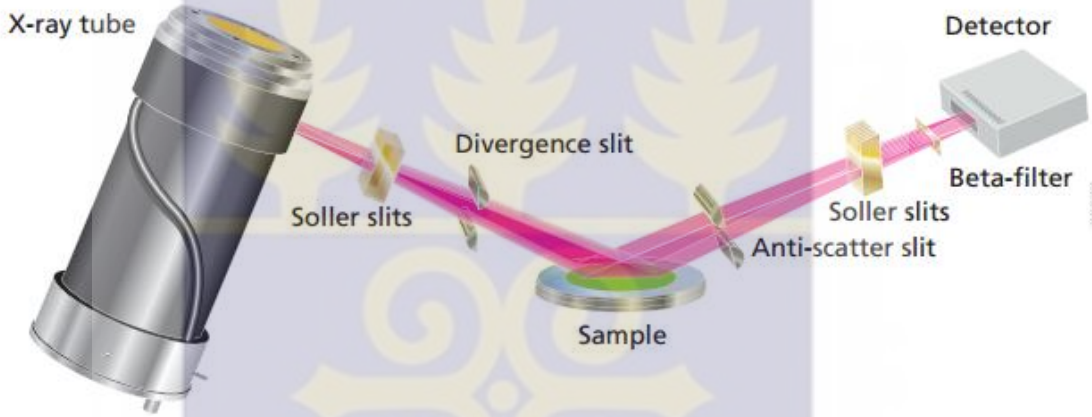

Fig. 2.2 Classical powder diffractometer setup in Bragg-Brentano geometry

Generation of X-rays

Bombarding a target of a suitable material (anode) with a focused electron

The maximum achievable X-ray intensity is limited by the maximum power, which is restricted by the cooling system of the stationary anode. These X-ray sources are called sealed X-ray tube. Modern designs use ceramic insulators instead of glass bodies to improve stability and lifetime.

The sealed X-ray tube

In a sealed X-ray tube, electrons are emitted by a hot filament, the cathode. The high voltage difference (U) between cathode and anode accelerates the electrons with a high

speed towards the anode material, resulting in a line image of the filament on the anode. The kinetic energy (E_{kin} = e.U) of the electrons is converted mainly into heat (99%) and X-ray radiation (1%). Hence the (back side of the) anode has to be cooled effectively.

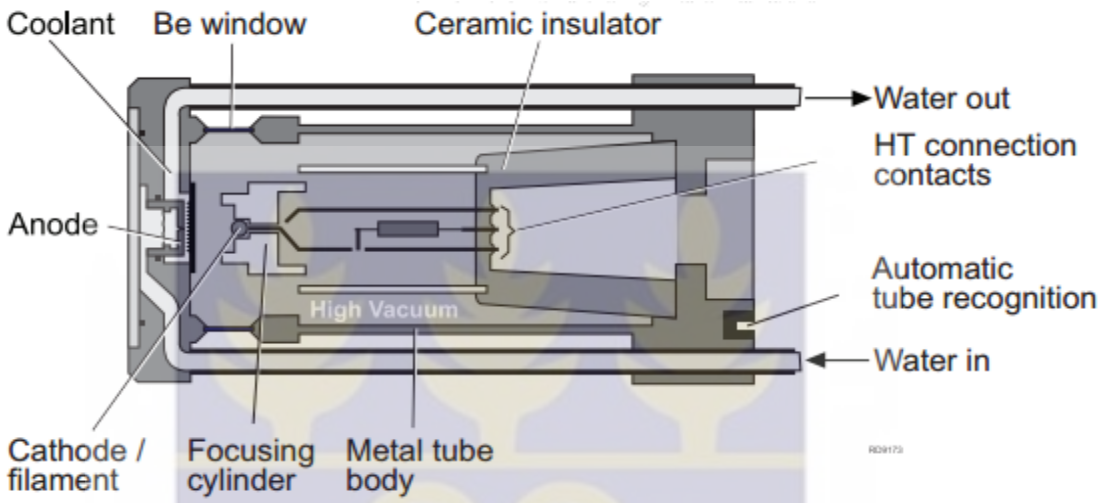

Fig. 2.3 Schematic drawing of ceramic x-ray tube (X'Pert Type)

Powder diffractogram

A powder diffractogram displays the scattered intensity versus the Bragg angle (2θ).It contains a number of peaks (reflections). The peaks are characterized by their position intensity and profile. The peaks and the background are the source of all information of the X-ray powder diffraction technique (Martin and Detlef, 2013).

.The position of the reflections: Bragg's law

As a consequence of the regular arrangement of the atoms in solid matter coherent scattering of X-rays at the atoms results in constructive interference at certain well-defined angles. This effect is similar to the well-known diffraction of visible light at gratings with a nm-scaled spacing close to the light's wavelength. A scaled spacing close

to the light's wavelength. A crystal can be seen as a three-dimensional grating with a spacing of a few Ångströms, and diffraction effects can be observed when the wavelength of the incoming X-ray photon is of similar size.

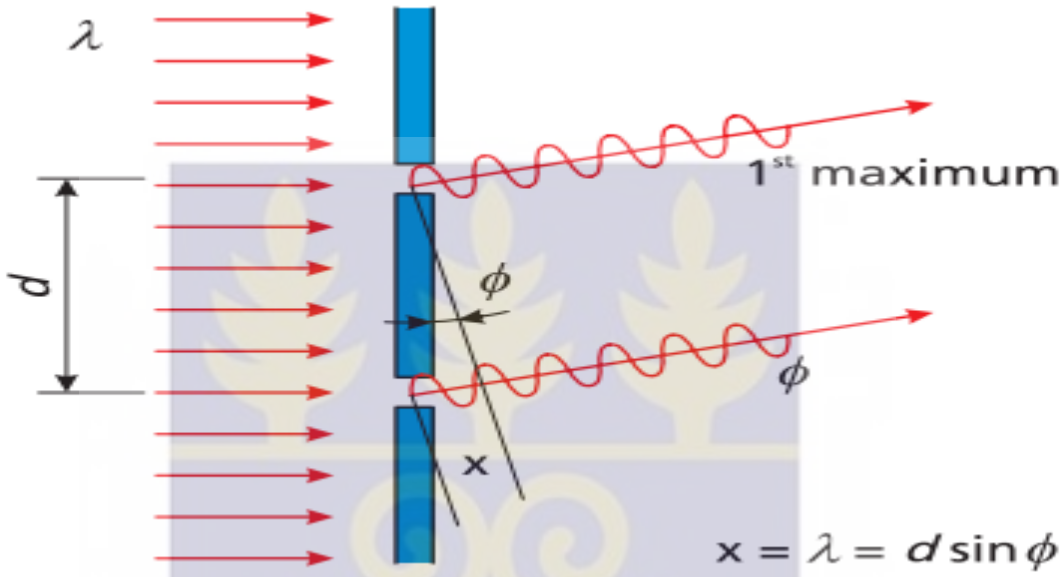

Fig. 2.4 Constructive interference at a grating (visible light)

Diffraction and interference of X-rays in a crystal have been described as reflections at the atomic planes of the crystal lattice. The positions of the reflections are calculated using the optical path difference 2s, with s=dsinθ, between two reflected rays at neighboring interplanar spacings. As in visible light optics, maxima are produced for integer multiples of λ.

It follows: $2d\sin\theta=n\lambda$ (Bragg's law) (2.5)

d interplanar spacing d_{hkl} (hkl: Miller indices)

θ Bragg angle θ_B

2θ angle between incident and reflected beam

n 'order' of the interference n = 1,2,3 ..., normally n = 1

(called: reflection of order n)

λ wavelength

The Detector system

Gas-filled proportional counters consist of a metal cylinder with an entrance window for X- ray photons. The cylinder is cross-sected by a thin wire attached to a high-voltage positive potential. The detector is filled with a noble gas, either argon or xenon. The incoming X-ray photon ionizes the atoms of the counting gas. The ionization electrons are accelerated to the wire and are ionizing further atoms (charge avalanche). At the counting wire an impulse with an amplitude proportional to the energy of the absorbed X-ray photon is generated and recorded by a pulse-height discriminator. This detector type is the optimum choice for standard wavelengths as Cu K_α, Co K_α, and Cr K_α radiation. The detector has a very low background and a good energy resolution.

Quantitative Phase Analysis

The fundamental condition of the quantitative phase analysis is that the intensity of the X-rays diffracted by a certain phase is proportional to its amount in the phase mixture. The following equations refer to the investigation of crystalline phases. The samples are powder mixtures in the sense of the statistical demands. The conditions for the application of the kinematic theory must be fulfilled. The intensity I_{hkl} of a reflection is given by (Martin and Detlef, 2013):

$$I_{hkl} = K_o a \left(\frac{|F_{hkl}|}{V_{uc}}\right)^2 HPL \qquad (2.6)$$

with

a absorption coefficient

H multiplicity factor

P polarisation factor

L Lorentz factor

Vuc volume of the unit cell

Ko constant for the equipment: $K_o = \frac{I_o e^4 \lambda^3 Q_o}{m_e^2 c^4}$ (2.7)

with

Io intensity of incident or primary beam

e elementary charge

λ used wavelength

Qo cross section of the incident beam

m_e mass of the electron

c light velocity

F_{hkl} structure amplitude: $$F_{hkl} = \sum f_j e^{-M} e^{2\pi i(hx_j+ky_j+lz_j)} \quad (2.8)$$

with

f_j scattering factor of each atom j

e^{-M} temperature factor

h,k,l Miller indices

x,y,z coordinates of the atoms

To the percentages by volume Vk of the individual phases k in a mixture applies their diffracted intensity I_k:

$$I_k = K_o R_k A_k v_k \quad (2.9)$$

Provided that $\sum v_k = 1$

The absorption coefficient Ak can be set ½μk , whereby the attenuation coefficient μk has to be possibly modified for a phase k in the mixture with other phases (then named μk').

2.7.3 HPLC METHODS

Mobile Phase and Reservoir

The type and composition of the mobile phase affects the separation of the components. Different solvents are used for different types of HPLC. For normal-phase HPLC, the solvent is usually nonpolar, and, in reverse-phase HPLC, the solvent is normally a

mixture of water and a polar organic solvent. The purity of solvents and inorganic salts used to make the mobile phase is paramount. A general rule of thumb is to use the highest purity of solvent that is available and practical depending on the particular application. The most common solvent reservoirs are as simple as glass bottles with tubing connecting them to the pump inlet.

Pumps

High-pressure pumps are needed to push the mobile phase through the packed stationary phase. A steady pump pressure (usually about 1000–2000 psi) is needed to ensure reproducibility and accuracy. Pumps are typically known to be robust, but adequate maintenance must be performed to maintain that characteristic. Inability to build pressure, high pressures or leakage could indicate that the pump is not functioning correctly. Proper maintenance of the pump system will minimize down time (Tom, 2004).

Isocratic versus Gradient Elution

Elution techniques are methods of pumping mobile phase through a column. In the isocratic method, the composition of the mobile phase remains constant, whereas in the gradient method the composition changes during the separation process. The isocratic method is the simplest technique and should be the first choice when developing a separation. Eluent gradients are usually generated by combining the pressurized flows from two pumps and changing their individual flow rates with an electronic controller or data system while maintaining the overall flow rate constant.

Injectors

The injector can be a single injection or an automated injection system. An injector for an HPLC system should provide injection of the liquid sample within the range of 0.1-100 mL of volume with high reproducibility and under high pressure (up to 4000 psi). For liquid chromatography, liquid samples can be directly injected and solid samples need only to be diluted in the appropriate solvent.

Columns

The column or stationary phase is the core of any chromatographic system. Columns are commercially available in different lengths, bore sizes and packing materials. The use of the correct combination of length and packing material in correlation with the appropriate mobile phase can assist in the most effective separation of a sample compound. A variety of column dimensions are available including preparative, normal-bore, micro- and mini-bore and capillary columns. Different column dimensions can be used for different types of separations and can utilize different packing materials and flow rates. The most widely used packing materials for HPLC separations are silica-based. The most popular material is octadecyl-silica (ODS-silica), which contains C18 coating, but materials with C1, C2, C4, C6, C8 and C22 coatings are also available. Miscellaneous chemical moieties bound to silica, as well as polymeric packing, are designed for purification of specific compounds. Other types of column packing materials include zirconia, polymer-based and monolithic columns. Theoretical plates relate chromatographic separation to the theory of distillation and are a measure of column efficiency. The number of theoretical plates (n) can be determined by the

following equation:

$$n = 16\left(\frac{t_{R1}}{w}\right)^2 \quad (2.10)$$

where t_{R1} is the total retention time and w is the band width of the peak. In general, LC columns are fairly durable with a long service life unless they are used in some manner that is intrinsically destructive. For example, with highly acidic or basic eluents or with continual injections of "dirty" biological or crude samples. Column degradation is inevitable, but column life can be prolonged with proper maintenance. Flushing a column with mobile phase of high elution strength following sample runs is essential. When a column is not in use, it is capped to prevent it from drying out. Particulate samples need to be filtered and when possible a guard column should be utilized. Column regeneration could instill some life into a column, but preventive maintenance is the key to preventing premature degradation (Tom, 2004).

Detectors

There are many different types of detectors that can be used for HPLC. The detector is used to sense the presence of a compound passing through and to provide an electronic signal to a data-acquisition device. The main types of detectors used in HPLC are refractive index (RI), ultraviolet (UV-Vis) and fluorescence, but there are also diode array, electrochemical and conductivity detectors. Each detector has its assets, limitations and sample types for which it is most effective. Most applications in drug analysis use detectors that respond to the absorption of ultraviolet radiation (or visible light) by the solute as it passes through the flow-cell inside the detector. The recent development of the so-called hyphenated techniques has improved the ability to separate and identify multiple entities within a mixture. These techniques include liquid chromatography-mass spectrometry (LC-MS), liquid chromatography-mass spectrometry-mass spectrometry

(LC-MSMS), liquid chromatography-infrared spectroscopy (LC-IR) and liquid chromatography-nuclear magnetic resonance (LCNMR). These techniques usually involve chromatographic separation followed by peak identification with a traditional detector such as UV, combined with further identification of the compound with the MS, IR or NMR.

Data Acquisition/Display Systems

Since the detector signal is electronic, use of modern data acquisition techniques can aid in the signal analysis. The data acquisition system of most HPLC systems is a computer. The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret. Other more advanced features can also be applied to a chromatographic system. These features include computer-controlled automatic injectors, multi-pump gradient controllers and sample fraction collectors.

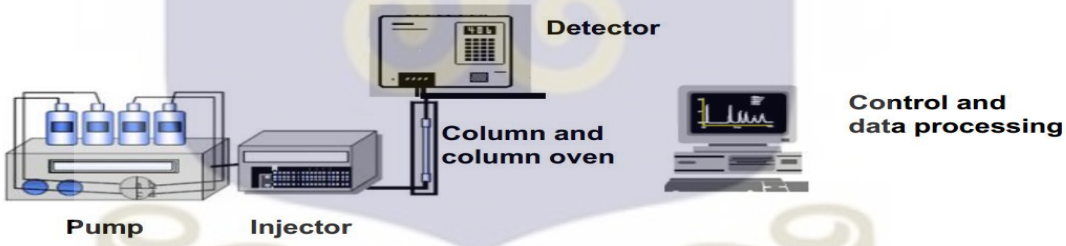

Fig. 2.5 A typical HPLC setup

Quantitative Analysis

The quantification methods incorporated in HPLC are borrowed mostly from gas chromatography methods. The basic theory for quantitation involves the measurement of peak height or peak area. To determine the concentration (conc.) of a compound, the peak area or height is plotted versus the concentration of the substance. For peaks that are well resolved, both peak height and area are proportional to the concentration. Three different

calibration methods, each with its own benefits and limitations, can be utilized in quantitative analysis: external standard (std.), internal standard and the standard addition method (Tom, 2004).

External Standard

The external standard method is the simplest of the methods. The accuracy of this method is dependent on the reproducibility of the injection volume. To perform this method, standard solutions of known concentrations of the compound of interest are prepared with one standard that is similar in concentration to the unknown. A fixed amount of sample is injected. Peak height or area is then plotted versus the concentration for each compound. The plot should be linear and go through the origin. The concentration of the unknown is then determined according to the following formula:

$$Conc._{unknown} = \frac{Area_{unknown}}{Area_{known}} \; x \; Conc._{known} \qquad (2.11)$$

CHAPTER THREE

MATERIALS AND METHOD

3.1 INTRODUCTION

A number of methods/techniques can be used to analyze/detect counterfeit pharmaceutical drugs which include inspection, assays, dissolution, colorimetric methods and chromatography techniques such as HPLC, TLC, and Mass Spectrometry. Some other analytical techniques such as dissociating tests, visual control, and simple colour reaction tests are done for the purpose of exposing only rough forgeries. Due to lack of systematic control of the pharmaceutical market and the absence of specific regulations in developing countries, easy, fast, reliable and cheap methods of drug analysis are essential. Therefore, new technologies including X-ray powder diffraction and near infrared spectroscopy methods have been progressively used for the detection of counterfeit antimicrobials with the latest addition being Instrumental Neutron Activation Analysis (INAA).

3.2 SAMPLING

3.2.1 Standard materials

Validation of the analytical procedures requires the use of certified materials, called standards. The standard reference material (amoxicillin trihydrate) for validation of the HPLC method was obtained from a certified pharmaceutical company in Accra, Ghana. Again, the Reference database by the International Center for Diffraction Data (ICDD) was used for the validation of the XRD method whiles 2012 International Plant-

Analytical Exchange First Quarter Sample Two (121IPE2), 2013 International Plant-Analytical Exchange First Quarter Sample Four (131IPE4) and Peach Leaves were used for the INAA calibration and validation. This was very necessary so as to help conduct accurately both the qualitative and the quantitative analysis of both the API and the excipients.

3.2.2 Brand-name drugs:

A brand-name drug is a drug that has a trade name and is protected by a patent i.e. can be produced and sold only by the company holding the patent. The brand-name drug to be used was amoxil produced by Bechem but due to its unavailability, it was replaced by an equally good generic drug from the United Kingdom called Amoxicillin (capsules) by Meidrich at Enerst Chemist in Accra. The quantity purchased for analysis was 10 g.

3.2.3 Generic drugs

A generic drug is a drug product that is comparable to a brand-name drug in dosage form, strength, quality and performance characteristics, intended use and are offered for sale when the patent protection for a brand-name drug expires. 10 g of the National Health Insurance Scheme Amoxicillin capsules produced by Ernest chemist was purchased from a licensed pharmacy shop in Accra and analyzed for the purpose of this work.

3.2.4 Suspected Fake Drugs:

Amoxicillin samples that were suspected to be fake were purchased from an unlicensed and illegal drug seller at Okaishi market in Accra to be analyzed and compared to the above listed groups to make out the difference. A quantity of 10 g was purchased for analysis.

3.3 NAA METHODOLOGY

Sample Preparation

Three biomedical samples were analyzed using Instrumental Neutron Activation Analysis (INAA). The three biomedical samples were the Foreign Generic Amoxicillin, NHIS Amoxicillin and Suspected Fake Amoxicillin. A triplicate of each drug sample was made by weighing 250 mg of each sample group as well as the same quantity of the standard reference materials (121IPE2 and 131IPE4) on polyethylene bags. The polyethylene bags were heat-sealed with a soldering bit after being wrapped and then placed in separate irradiation capsules of volume, 7 mL. The irradiation capsules were each stuffed with cotton wool to prevent any movement. The irradiation capsules were also heat-sealed before irradiation.

Irradiation

The irradiation capsules were sent into the reactor for irradiation through the pneumatic system of the reactor in one of the inner irradiation sites of the GHARR-1 operating at air pressure of 0.25 psi, at this site the thermal neutron flux is 5.0×10^{12} n/cm^{2}s with a norminal power of 15 kW. The samples were irradiated for 30 s, delayed for 10 s and then counted for 400 s for short lived radionuclides.

Counting

After allowing the samples to decay for 10 s, the samples were counted for gamma activity using an N-type High Purity Germanium detector with model number, GMX 40P4 with the diameter of the active area being 63 mm manufactured by Canberra Industries. The resolution of the detector is 1.8 eV (FWHM) at a biased voltage of 3000

V and its relative efficiency to NaI detector is 25%. The detector, having 0.50 mm Beryllium window thickness was connected to an Ortec Emcaplus multichannel analyzer for qualitative and quantitative analysis using a Microsoft based software known as MAESTRO (Model A 65-B 32, version 6.05).

3.4 XRD METHODOLOGY

Sample Preparation

The upper part of the sample holder is firmly fixed to a backloader. 500 mg of the Foreign Generic, NHIS and the Suspected Fake Amoxicillin samples were each poured on a spoon/spatula and then carefully sprinkled in the depression of the sample holder till it is filled up. A sample press was then used to compress the sample to get a nice pellet of the powder. A small knife was used to scrape the surface of the compressed sample to obtain a flat and more even surface. The bottom part of the sample holder was now fixed on top of the sample to close it. The sample holder was detached from the backloader and then put in the sample chamber of the XRD setup for irradiation.

Irradiation and Analysis

PanAnalytical X-ray diffractometer setup manufactured in the Netherlands on 22-11-2012 with serial number, 110090201 00000918 and typen, 9430 068 0002 located at the Physics department of the University of Ghana, Legon for irradiation. The setup was equipped with a vertical goniometer in the Bragg-Brentano focusing geometry. The

operating conditions for X-ray generation were set at 45kV and 40mA using the PanAnalytical Software called Data collector software after which the sample is irradiated at room temperature for 15 mins with Cu-$K_{\alpha 1}$ radiation of wavelength, λ=1.54056 Å. The data were collected by step-scan modes in a θ-2θ range between 10° and 80° with step-size of 0.05° and step time of 0.6 seconds. A diffractogram was then obtained showing peaks of different intensities at specific 2 theta angles which is characteristic for each crystalline substance, and its crystal structure is represented in the position and the intensity of the reflections. The qualitative and quantitative phase analysis are done using a PanAnalytical evaluation software called HighScore Plus. The phase analysis started with a visual inspection of the diffractogram (peak positions, intensities, peak widths and background shape) after which a peak search algorithm was started to get the positions and both absolute and relative maximum intensities of the reflections. The strongest line was normalized to 100%. Better intensities and peak positions were obtained after a profile fitting step was applied for a correct phase analysis. With the aid of elements detected using the NAA procedure, the API and the excepients were identified.

3.5 DISINTEGRATION TEST

The apparatus is relatively simple. It consists of a basket rack holding six plastic tubes open at the top and bottom with the bottom covered with 10 mesh screen. First, a capsule and then a disc were placed in each tube. The rack was immersed in a water bath of

temperature 37°C which moved up and down at a constant rate until the last capsule disintegrated and fell through the mesh. The time was then recorded.

3.6 HPLC METHODOLOGY

Sample Preparation

Mobile phase A was prepared by mixing 1 volume of acetonitrile with 99 volumes of a pH 5.0 buffer solution prepared by adding 2M sodium hydroxide to 250 mL of 0.2M potassium dihydrogen orthophosphate until the pH reaches 5.0 after which sufficient water was added to produce 1000 mL. Mobile phase B was also prepared by mixing 20 volumes of acetonitrile with 80 volumes of the pH 5.0 buffer solution.8 volumes of mobile phase B was then mixed with 92 volumes of mobile phase A to produce the ultimate solution/mobile phase used for the analysis. This analysis was done using isocratic elution. A mass corresponding to 0.25 g of amoxicillin of each sample was taken and diluted with 100 mL of the diluents prepared (mobile phase A) to form a solution with concentration of [0.25%w/v].

HPLC Assay

Vials filled with each of the sample solutions were first placed in the injector module of the Agilent 1260 infinity HPLC setup at the manufacturing division of Ernest Chemists limited, Tema with the chromatographic conditions already set and the mobile phase ready for the process to begin. 10 µL of the liquid sample was introduced into the flow stream of the mobile phase by the injector (auto sampler) to begin a chromatographic process. The column's stationary phase made up of octadecylsilyl silica gel separated the

sample components of interest using various physical and chemical parameters. The Diode Array detector provided an output to a computer that resulted in the liquid chromatograms obtained.

3.7 DISSOLUTION METHOD

Each vessel of the dissolution bath was first filled with 900 mL of water and heated to 37°C. For the capsules labelled 250 mg, two of them were put in each dissolution basket whereas for the capsules labelled 500mg, only one was put in each dissolution basket. The dissolution baskets were then lowered completely in each vessel after which the process was started. After 60 minutes 50ml of the vessel contents was withdrawn with a syringe and filtered into a beaker. 5 ml of the filtrate was pipetted into a 25 mL bottle after which the bottle was topped up with the diluents to make a solution of concentration [0.0111%w/v].

For the standard, 0.1 g of amoxicillin trihydrate corresponding to a mass 0.1151 g was dissolved in a 100 mL bottle with the solvent being the diluents. 2.77 mL of the solution was pipetted into a 25 mL bottle and topped up with the diluents to make a solution of concentration [0.0111%w/v].

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

4.2 Neutron Activation Analysis

The most important component of a pharmaceutical drug formulation is the Active Pharmaceutical Ingredient (API) which is sometimes referred to as the Active Substance. A drug is usually prepared or formulated to contain lubricants, fillers, flavors, disintegrates, binders and coloured pigments. In order to make drugs which are in tablet form very pleasing to the eye for easy ingestion, coloured pigments are used. They can also be used to differentiate between different dosage strengths of the same drug. To make the pills and tablets easily chewable for children and mask unpleasant smells, various sugars and waxes can be used. In some cases, buffers might be added to control the pH so that the tablet will be able to dissolve in the stomach lining or intestines in a controlled manner. These substances or materials together are known as excipients. More often than not, excipients make up the bulk contents of any formulation whereas the Active Pharmaceutical Ingredient is usually in small concentrations. Virtually all pharmaceutical drug formulations contain numerous excipients, with each having a specific function in the drug delivery. Since the active ingredient (API) is usually present in small concentrations, the excipient must be analyzed first, followed by the analysis of the API.

In this work, the identification of the bulk component of the excipient (i.e., the inorganic component) was done with INAA using GHARR-1 facility for short-lived radionuclides. The identification of the four major elements (i.e., magnesium, aluminium, chlorine and

calcium) was done by identifying the spectra peaks and assigning corresponding radio-nuclides and, therefore, the elements present in the foreign generic amoxicillin, NHIS amoxicillin as well as the suspected fake amoxicillin. The various elements identified as well as their concentrations are listed in Table 4.1.

The gamma spectra for NHIS amoxicillin, suspected fake amoxicillin and foreign generic amoxicillin are shown in figures 4.1 and 4.2, figures 4.3 and 4.4, and figures 4.5 and 4.6 respectively.

Figure 4.7 was obtained as a result of superimposing the first spectrum of each group of the amoxicillin samples with sample codes 114IPE2s for the foreign generic amoxicillin, AF1 for the suspected fake amoxicillin and AO1 for the local generic amoxicillin respectively. By visual inspection, all the three have the same spectral pattern, indicating that for the short-lived irradiation, the same elements (magnesium, chlorine, aluminium and calcium) were present. One major difference is the concentration of each element in each of the samples. It is quite clear from that the foreign generic amoxicillin has the highest concentrations of all the four elements (Fig. 4.7; Table 4.1). Another obvious difference has to do with the background of each of the spectra with the foreign generic amoxicillin sample having the highest background, followed closely by the local generic amoxicillin and then the suspected fake amoxicillin. The difference in background may be due to noise from the electronic setup of the equipment as well as precision in measuring the exact amount of each of the samples required for analysis.

Table 4.1: Concentrations of Short-lived Radionuclides (mg/kg)

	NHIS (Local) Generic Amoxicillin	Suspected Fake Amoxicillin
Mg	6042.5	2992
Al	422.1	2516
Cl	2101.5	310.9
Ca	3044.5	2458

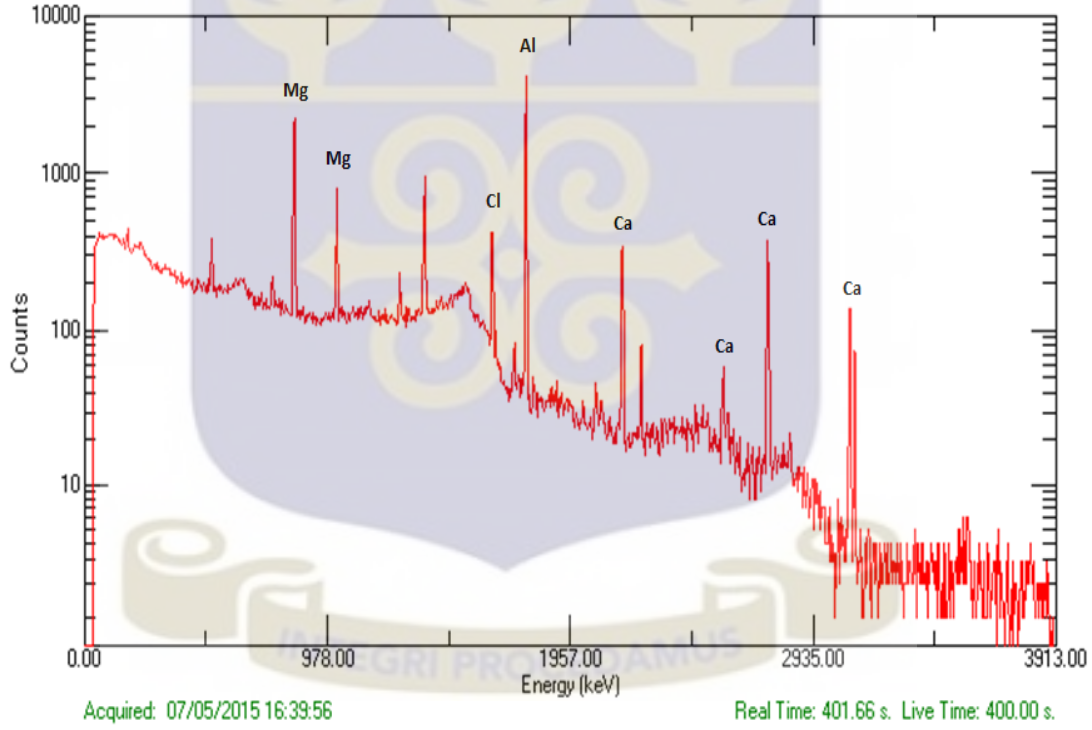

Figure 4.1: Gamma Spectrum for NHIS Amoxicillin

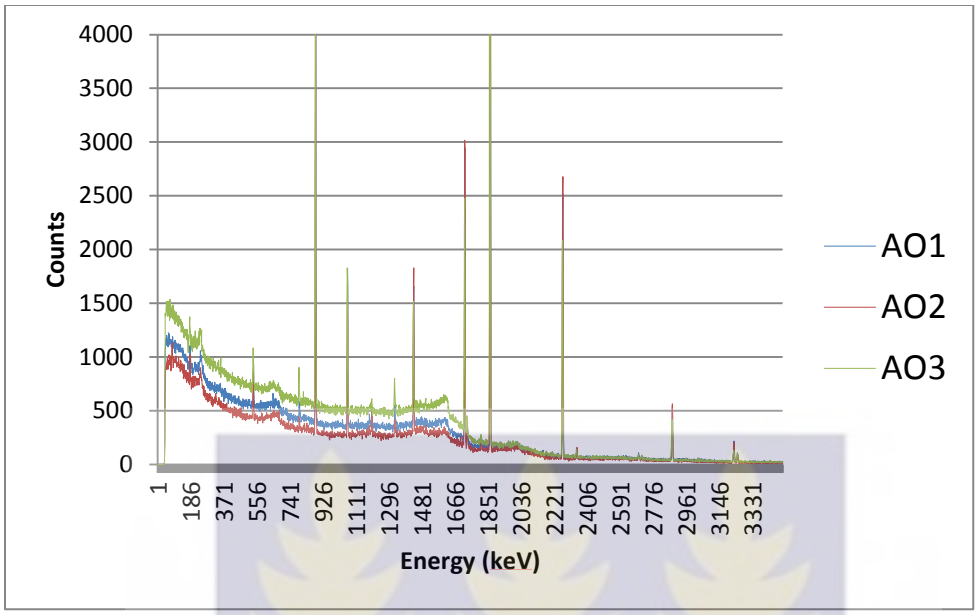

Figure 4.2: Gamma Spectra for NHIS Amoxicillin

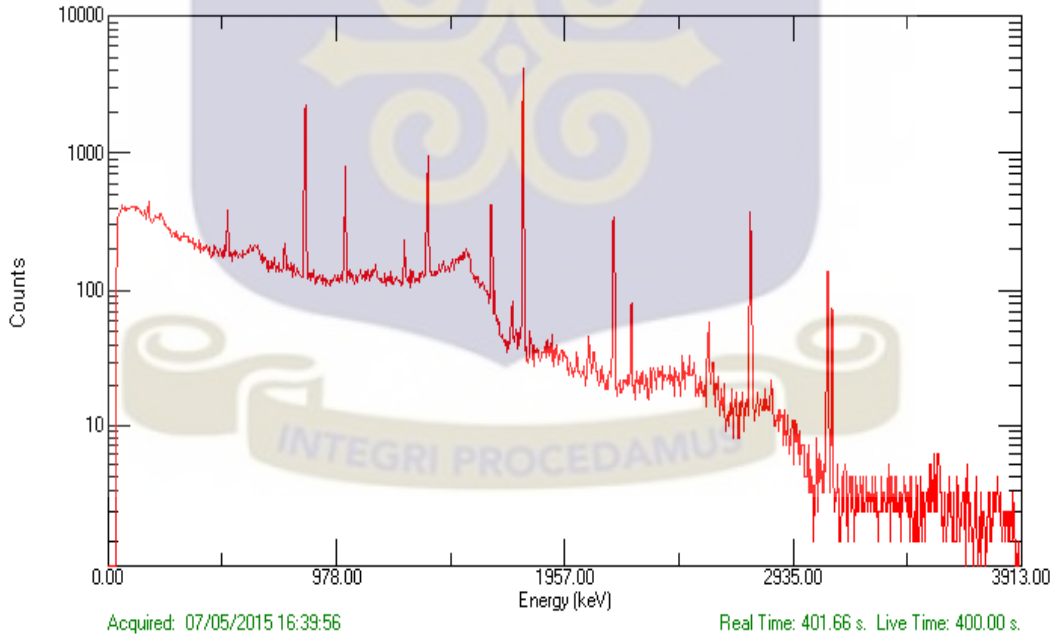

Figure 4.3: Gamma Spectrum for Suspicious fake Amoxicillin

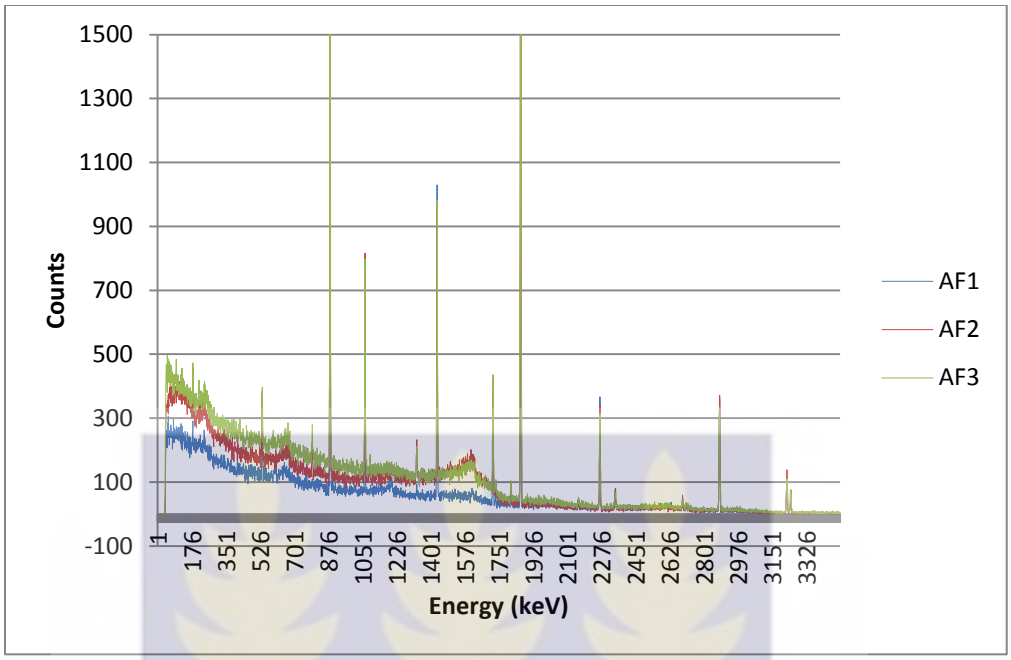

Figure 4.4: Gamma Spectra for Suspected Fake Amoxicillin

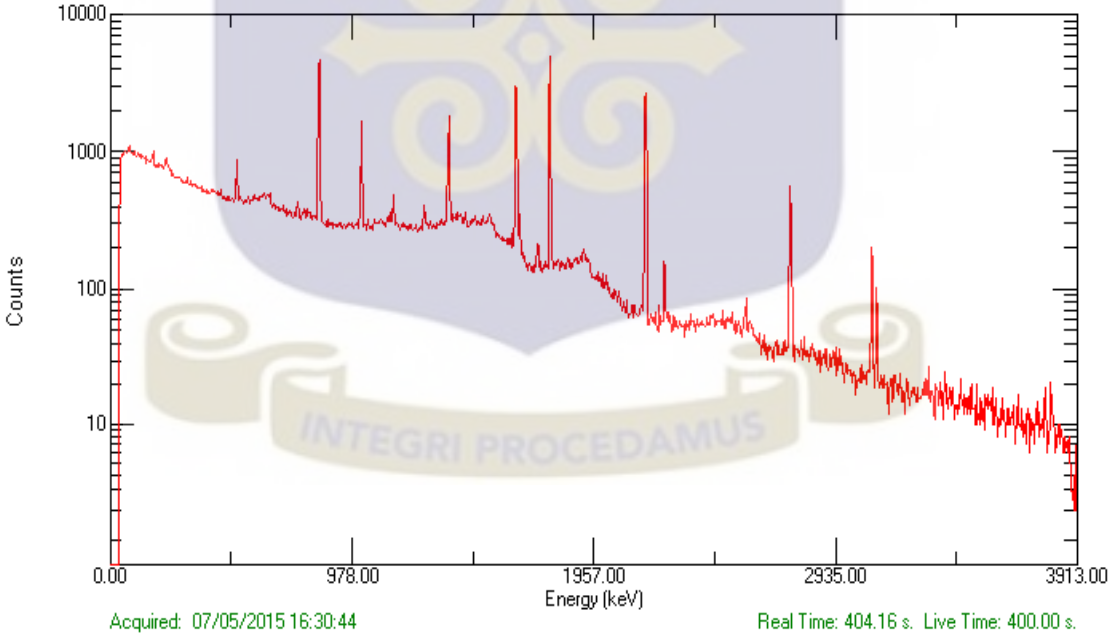

Figure 4.5: Gamma Spectrum for Imported Amoxicillin

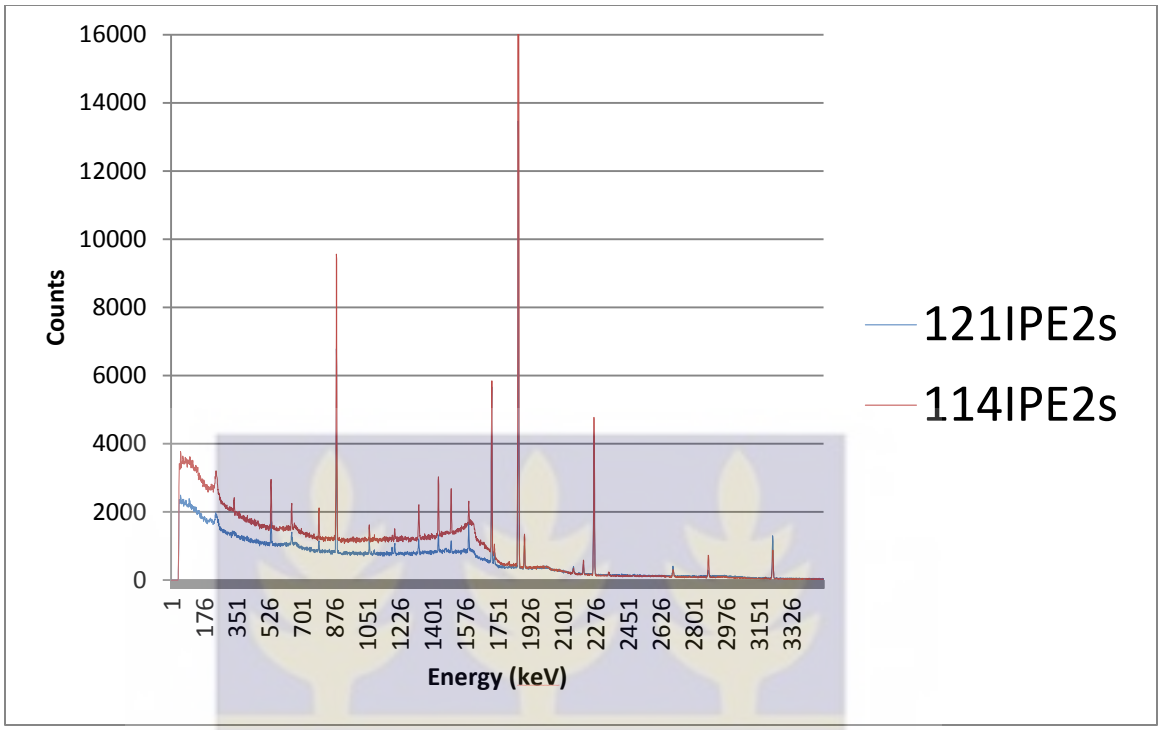

Figure 4.6: Gamma Spectra for Foreign Generic Amoxicillin

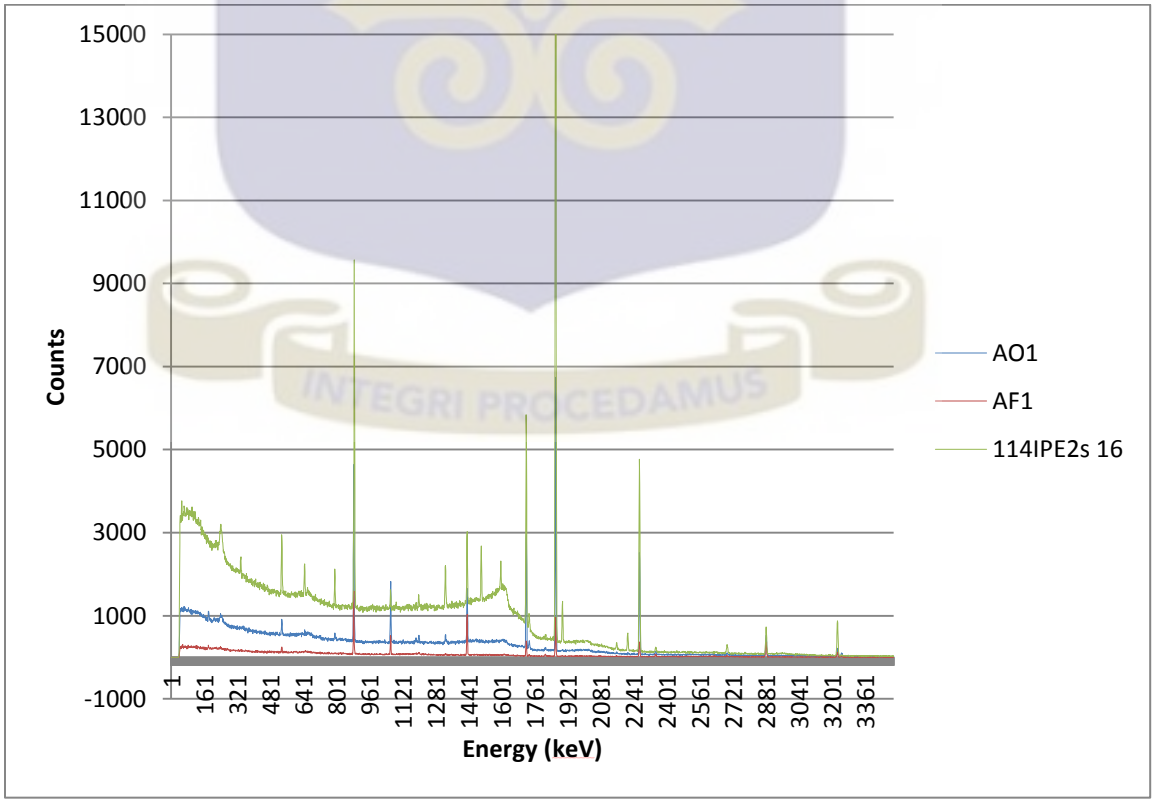

Figure 4.7: An Overlap of the Three Gamma Spectra

4.3 X-RAY POWDER DIFFRACTION

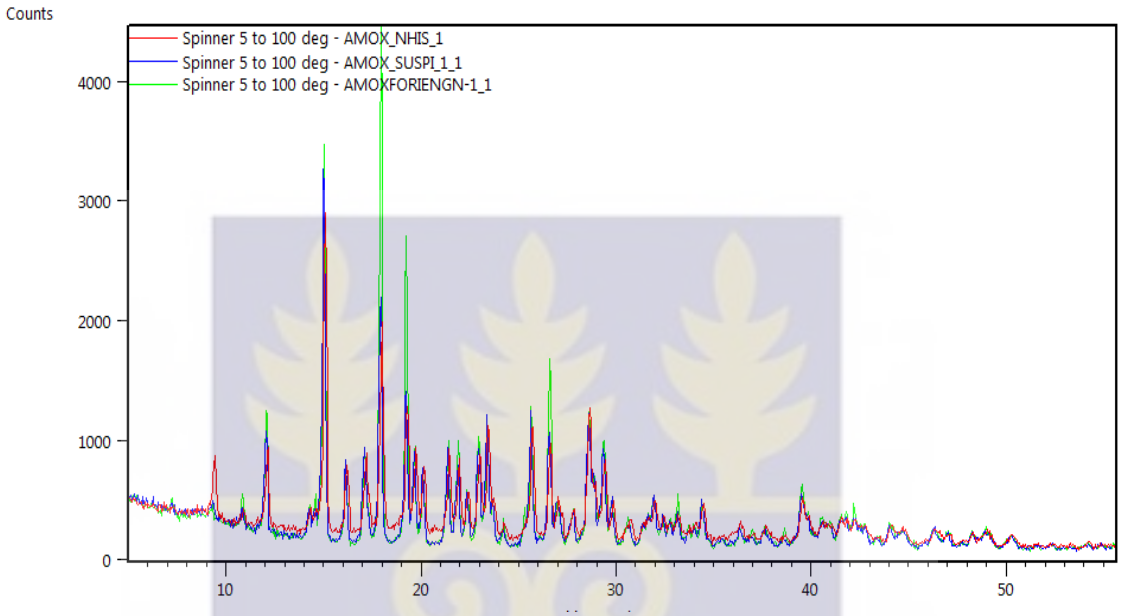

Figure 4.8: An Overlap of the Three Diffractograms for the Three Groups of Amoxicillin

All the high intensity peaks observed in the diffractograms of the foreign generic amoxicillin, NHIS amoxicillin and suspected fake amoxicillin were to be compared whilst also trying to derive some important parameters such as lattice spacing (Å) and relative intensities (I/I_0). Each diffractogram was to be characterized by the interplanar d-spacing (Å) and the relative intensities (I/I_0) of the outstanding and most resolved peaks in the pattern under the Brag-Brentano system. Structure identification in a diffractogram is usually based on the position of peaks and their relative intensities. The characteristic set of d-spacings generated in a typical x-ray scan provides a unique "fingerprint" of the drug molecule present in the sample. When properly interpreted, by comparing with pure drug as reference, this "fingerprint" allows for identification and also quantification of the

various phases present in the sample. The complete spectrum obtained as well as the segregation of the spectrum into various phases identified in the diffractogram are shown below:

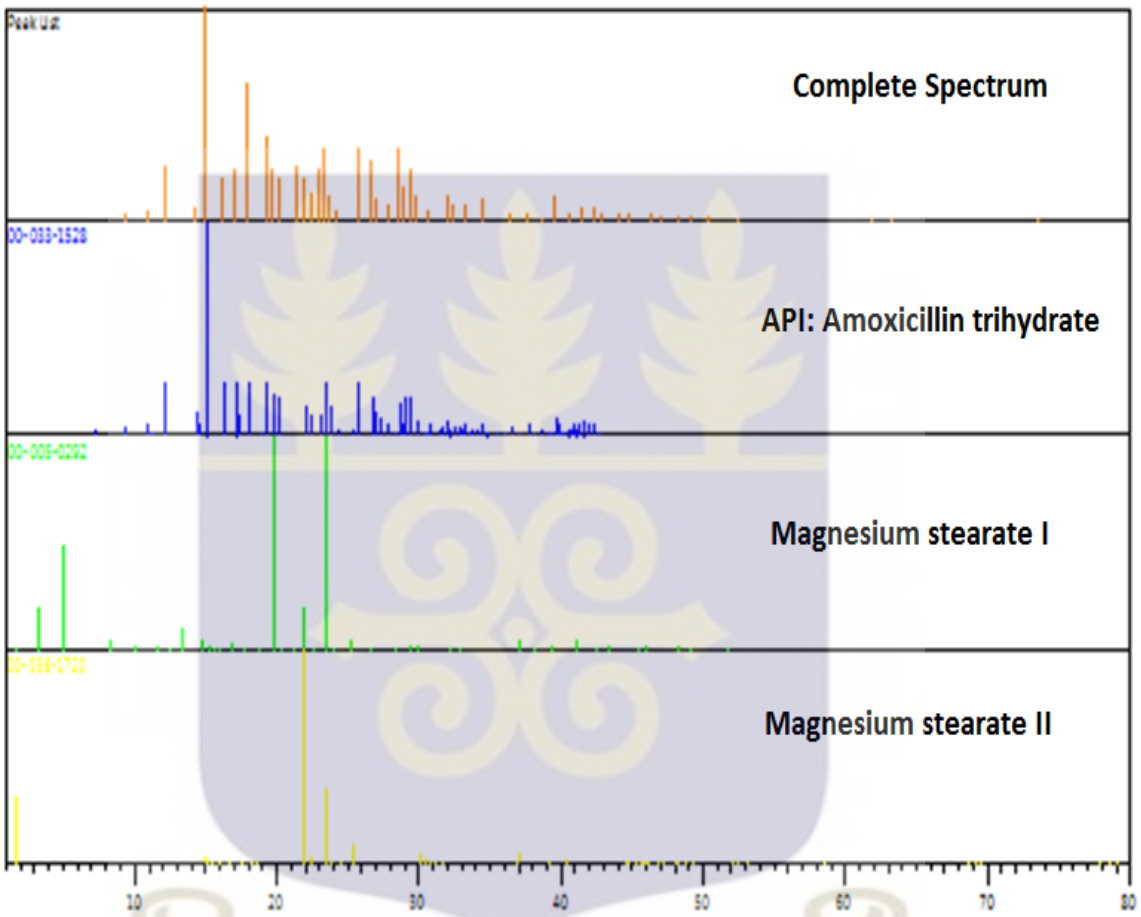

Fig. 4.9 The Three Identified Phases In The Diffractogram

From the above set of spectra, the phases identified in the Foreign Generic, NHIS and the Suspected Fake amoxicillin samples were Amoxicillin trihydrate (API), Magnesium stearate (hydrated) and Magnesium stearate (anhydrous). Most of the characteristic peaks in the diffractograms were generally prominent and sharp, and hence measurement of the angles and therefore, d-values was accurate. Proper sample preparation helped attain exact peak positions for the phase identification of both the API and the excipients. The

XRD patterns (peak positions) of the foreign generic, NHIS and the suspected fake amoxicillin indicate that they all have the same crystal structure even by visual inspection. The sharp prominent peaks also indicate that all the samples were highly crystalline and the problem of preferred orientation is minimal. Comparing the prominent peaks in the diffractograms, it is quite clear that the foreign generic amoxicillin has the highest intensities and therefore, the highest concentrations in those phases.

All the samples were subjected to various kinds of tests. First, the three categories of samples underwent visual inspection. It was then followed by the physical parameter tests and the disintegration test to determine the disintegration time, the assay was then done using HPLC. The dissolution test was the last to be done.

4.4 VISUAL INSPECTION TEST

All the three groups of amoxicillin capsules were examined in theirs blister packs and also out of it to check for possible flaws or defects or any signs associated with counterfeit drugs. All the groups of samples were packaged nicely in their blister packs without any signs of counterfeiting. The product names, manufacturing dates, expiry dates and the batch numbers were all labeled on them. The drugs were put to further test by unpacking them from the blisters. None of the capsules showed any signs of deterioration or disfiguration. All the capsules have the same shape with shiny and uniform coloration.

4.5 PHYSICAL PARAMETER TEST

In this test, the physical parameter measured was the mass in which the mass of 10 capsules together was measured using an electronic balance, after which the mass of the empty shells of the ten capsules was measured. The mass of each capsule was also measured as well as the mass of each empty shell.

In the case of the foreign generic amoxicillin labeled 500 mg on each capsule, the mass of the 10 capsules was found to be 6.8345 g. The average mass was therefore 0.6835 g. The empty shells together were weighed as 0.9656 g with the mass of the content being 5.8689 g. The average mass of the content was 0.5869 g. Therefore the B.P ±7.5% weight range was 0.6322 g – 0.7348 g which means none of the mass of the capsules fell outside the range with the actual range being 0.6587 g – 0.7111 g.

For the NHIS generic amoxicillin labeled 250mg on each capsule, the mass of the 10 capsules was found to be 4.5870 g. The average mass was therefore 0.4587 g. The empty shells together were weighed as 0.7591 g with the mass of the content being 3.8279 g. The average mass of the content was0.3828 g. According to the third edition of the British Pharmacopoeia 2014, the mass of the sample should fall within the range of ±7.5% of the average mass of the capsules. Therefore the B.P ±7.5% weight range was 0.4243 g – 0.4931 g which means none of the mass of the capsules fell outside the range. The actual range was 0.4313 g – 0.4824 g.

For the suspected fake amoxicillin labeled 250mg on each capsule, the mass of the 10 capsules was found to be 4.0355 g. The average mass was therefore 0.4036 g. The empty shells together were weighed as 0.7993 g with the mass of the content being 3.2362 g.

The average mass of the content was0.3236 g. Therefore the B.P ±7.5% weight range was 0.3733 g – 0.4339 g which means none of the mass of the capsules fell outside the range. The actual range was 0.3966 g – 0.4130 g.

Table 4.2 shows the mass variations (in grammes) of the capsules as well as the empty shells of the foreign generic amoxicillin, NHIS generic amoxicillin and suspected fake amoxicillin.

Table 4.2: Mass Variation (g) for Amoxicillin Drugs

Foreign Generic		NHIS		Suspected Fake	
Capsules	Shells	Capsules	Shells	Capsules	Shells
0.6670	0.0940	0.4771	0.0777	0.3977	0.0783
0.6923	0.0968	0.4690	0.0765	0.4130	0.0795
0.6890	0.0974	0.4553	0.0767	0.4019	0.0776
0.6810	0.0938	0.4619	0.0769	0.4041	0.0804
0.6587	0.0982	0.4621	0.0761	0.4069	0.0804
0.7111	0.0975	0.4438	0.0759	0.4017	0.0797
0.6800	0.0973	0.4564	0.0740	0.4090	0.0808
0.6832	0.0966	0.4313	0.0767	0.3966	0.0834
0.6944	0.0969	0.4466	0.0778	0.4026	0.0780
0.6800	0.0970	0.4824	0.0711	0.3999	0.0781

4.6 CAPSULE DISINTEGRATION TEST

The capsule disintegration test was used as a quality assurance measure and in this test, the parameter that was measured was the disintegration time, which is the time required for a dosage form to break up into granules of specified size under carefully specified conditions. The temperature of the water bath was kept constant at 37°C to simulate the condition in the stomach. All the samples from the three groups had the same disintegration time of 4 mins which falls even below the threshold time of the British Pharmacopoeia 2014.

4.7 High Performance Liquid Chromatography Assay

The next test conducted on the three groups of samples of antibiotics drugs was the assay using HPLC method according to the instructions of the third edition of the British Pharmacopoeia 2014, vol. V, pg V – A357.

Table 4.3: Chromatographic Conditions

Column Type	Agilent Prep C18 stainless steel packed with octadecylsilyl silica gel for chromatography (5 µm)
Column dimension	25 cm × 4.6 mm
Mobile Phase Elution	isocratic elution
flow rate	1.5 mL per minute
Temperature	28 °C
detection wavelength	254 nm
Injection Volume	10 µL
Pressure	58.7 bar – 58.9 bar

4.7.1 HPLC Assay Results for NHIS Amoxicillin

Sample:

0.25 g of Amoxicillin = 0.3828 g

Mass of sample taken = 0.3830 g

Standard:

0.1 g of Amoxicillin base = 0.115 g

0.25 g of Amoxicillin base = (0.25 g)/(0.1 g) x 0.115 g = 0.2875 g

But percentage purity of the standard = 99.2%

Therefore, the amount of standard to be used for analysis = (100%)/(99.2%) x 0.2875 g = 0.2898 g

The actual amount of standard taken = 0.2899 g

Dilution:

0.25g of amoxicillin trihydrate corresponding to 0.2899 g of the standard was diluted in 100 mL of diluents to obtain a solution of concentration of [0.25%w/v]. Also, 0.25g of amoxicillin trihydrate corresponding to 0.3830 g of the sample was diluted in 100 mL of the diluents to obtain a solution of concentration of [0.25%w/v]

Average area under curve for standard = 2578.7 mAU*s

Average area under curve for sample = 2574.3 mAU*s

Therefore percentage content of amoxicillin trihydrate contained in the NHIS amoxicillin capsule = (2578.7 mAU*s)/(2574.3 mAU*s) x 100%

= 99.8%

The range of the percentage content according to the 3rd edition of the British Pharmacopoeia is 92.5% - 110.0%. Therefore, the NHIS falls within the pharmacopoeia range.

4.7.2 HPLC Assay Results for Suspected Fake Amoxicillin

Sample:

0.25 g of Amoxicillin = 0.3236 g

Mass of sample taken = 0.3840 g

Standard:

0.1 g of Amoxicillin base = 0.115 g

0.25 g of Amoxicillin base = (0.25 g)/(0.1 g) x 0.115 g = 0.2875 g

But percentage purity of the standard = 99.2%

Therefore, the amount of standard to be used for analysis = (100%)/(99.2%) x 0.2875 g = 0.2898 g

The actual amount of standard taken = 0.2899 g

Dilution:

0.25g of amoxicillin trihydrate corresponding to 0.2899 g of the standard was diluted in 100 mL of the diluents to obtain a solution of concentration of [0.25%w/v]. Also, 0.25g of amoxicillin trihydrate corresponding to 0.0.3286 g of the sample was diluted in 100 mL of diluents to obtain a solution of concentration of [0.25%w/v]

Average area under curve for standard = 2578.7 mAU*s

Average area under curve for sample = 2544.1 mAU*s

Therefore percentage content of amoxicillin trihydrate contained in the NHIS amoxicillin capsule

= (2578.7 mAU*s)/(2544.1 mAU*s) x 100%

= 98.7%

The range of the percentage content according to the 3rd edition of the British Pharmacopoeia is 92.5% - 110.0%. Therefore, the suspected fake amoxicillin falls within the pharmacopoeia range.

4.7.2 HPLC Assay Results for Foreign Generic Amoxicillin

Sample:

0.5 g of Amoxicillin = 0.5869 g

0.25 g of Amoxicillin = 0.2935 g

Mass of sample taken = 0.2950 g

Standard:

0.1 g of Amoxicillin base = 0.115 g

0.25 g of Amoxicillin base = (0.25 g)/(0.1 g) x 0.115 g = 0.2875 g

But percentage purity of the standard = 99.2%

Therefore, the amount of standard to be used for analysis = (100%)/(99.2%) x 0.2875 g = 0.2898 g

The actual amount of standard taken = 0.2899 g

Dilution:

0.25g of amoxicillin trihydrate corresponding to 0.2899 g of the standard was diluted in 100 mL of the diluents to obtain a solution of concentration of [0.25%w/v]. Also, 0.25g of amoxicillin trihydrate corresponding to 0.2950 g of the sample was diluted in 100 mL of the diluents to obtain a solution of concentration of [0.25%w/v]

Average area under curve for standard = 2578.7 mAU*s

Average area under curve for sample = 2646.0 mAU*s

Therefore percentage content of amoxicillin trihydrate contained in the NHIS amoxicillin capsule = (2646.0 mAU*s)/(2578.7 mAU*s) x 100%

= 102.5%

The range of the percentage content according to the 3rd edition of the British Pharmacopoeia is 92.5% - 110.0%. Therefore, the Foreign generic amoxicillin falls within the pharmacopoeia range.

4.8 DISSOLUTION TEST

To determine how bioavailable the drugs will be after a period of time in the system, the in vitro dissolution test was also conducted on the Foreign generic, NHIS and the Suspected fake amoxicillin.

4.8.1 Dissolution Results for Foreign Generic Amoxicillin

The procedures set out by the British Pharmacopoeia were followed in which six capsules of the foreign generic sample with each containing 500 mg amoxicillin trihydrate corresponding to a capsule mass of 0.6835 g were each put in the dissolution basket. The dissolution process was started with each vessel filled with 900 mL of water. After 60 minutes 50ml of the vessel contents was withdrawn with a syringe and filtered into a beaker. 5 ml of the filtrate was pipetted into a 25 mL bottle after which the bottle was topped up with the diluents to make a solution of concentration [0.0111%w/v].

For the standard, 0.1 g of amoxicillin trihydrate corresponding to a mass 0.1151 g was dissolved in a 100 mL bottle with the solvent being diluents. 2.77 mL of the solution was pipetted into a 25 mL bottle and topped up with diluents to make a solution of concentration [0.0111%w/v].

Table 4.5 shows the dissolution percentage of each of the samples put in the dissolution baskets.

Table 4.4: Dissolution conditions

Apparatus	Basket
Medium	Water, 900 mL
Blade's speed	75 rpm
Blade's distance from bottom of vessel	25 ± 2 mm
Temperature	37 ± 0.5 °C
detection wavelength	254 nm
Time	60 mins

Table 4.5: Dissolution Percentage of Foreign Generic Amoxicillin

Vessel Number	Area Under Curve (mAU*s)	Average Area Under Curve (mAU*s)	Dissolution Percentage
1	1138.2 1133.3 1127.6	1135.8	94.2%
2	1129.7	1128.7	93.6%
3	1138.7 1138.8	1138.8	94.4%
4	1154.9 1154.3	1154.6	95.7%
5	1162.6 1163.6	1163.1	96.5%
6	1159.4 1158.7	1159.1	96.1%
Standard	1206.0 1205.8	1205.9	

Dissolution percentage = $\frac{\text{Average Area (standard)}}{\text{Average Area (sample)}}\ x\ 100\%$

According to the 2014 edition of the British Pharmacopoeia, the threshold tolerance level is 80% below which a medical drug is rejected. The dissolution percentage range from the above table is 93.6% - 95.5% which is above the 80% tolerance level.

4.8.2 Dissolution Results for NHIS Amoxicillin

Twelve capsules of the NHIS sample with each containing 250 mg amoxicillin trihydrate corresponding to a capsule mass of 0.4587 g were used for the test. In each dissolution basket, two capsules were used totaling 500 mg amoxicillin trihydrate. The dissolution process was started with each vessel filled with 900 mL of water. After 60 minutes 50ml of the vessel contents was withdrawn with a syringe and filtered into a beaker. 5 ml of the filtrate was pipetted into a 25 mL bottle after which the bottle was topped up with the diluents to make a solution of concentration [0.0111%w/v].

For the standard, 0.1 g of amoxicillin trihydrate corresponding to a mass 0.1151 g was dissolved in a 100 mL bottle with the solvent being diluents. 2.77 mL of the solution was pipetted into a 25 mL bottle and topped up with diluents to make a solution of concentration [0.0111%w/v].

Table 4.6 shows the dissolution percentage of each of the samples put in the dissolution baskets.

Table 4.6: Dissolution Percentage of NHIS Amoxicillin

Vessel Number	Area Under Curve (mAU*s)	Average Area Under Curve (mAU*s)	Dissolution Percentage
1	1107.9 1111.0	1109.5	92.0%
2	1096.0 1095.0	1095.5	90.8%
3	1086.7 1087.5	1087.1	90.1%
4	1054.4 1055.8	1055.2	87.5%
5	1055.3 10.57.9	1056.6	87.6%
6	1057.9 1056.7	1057.3	87.7%
Standard	1206.0 1205.8	1205.9	

The dissolution percentage range from the above table is 87.5% - 92.0% which falls above the threshold tolerance level of 80% indicated in the 3rd edition of the British Pharmacopoeia, 2014.

4.8.3 Dissolution Results for Suspected Fake Amoxicillin

Twelve capsules of the suspected fake sample with each containing 250 mg amoxicillin trihydrate corresponding to a capsule mass of 0.4036 g were used for the test. In each

dissolution basket, two capsules were used totaling 500 mg amoxicillin trihydrate. The dissolution process was started with each vessel filled with 900 mL of water. After 60 minutes 50ml of the vessel contents was withdrawn with a syringe and filtered into a beaker. 5 ml of the filtrate was pipetted into a 25 mL bottle after which the bottle was topped up with the diluents to make a solution of concentration [0.0111%w/v].

For the standard, 0.1 g of amoxicillin trihydrate corresponding to a mass 0.1151 g was dissolved in a 100 mL bottle with the solvent being the diluents. 2.77 mL of the solution was pipetted into a 25 mL bottle and topped up with diluents to make a solution of concentration [0.0111%w/v].

Table 4.7 shows the dissolution percentage of each of the samples put in the dissolution baskets.

The dissolution percentage range from the above table is 55.7% - 57.2% which is far below the threshold tolerance level of 80% indicated in the 3rd edition of the British Pharmacopoeia, 2014.

Table 4.7: Dissolution percentage of Foreign Generic Amoxicillin

Vessel Number	Area Under Curve (mAU*s)	Average Area Under Curve (mAU*s)	Dissolution Percentage
1	674.4 668.0	671.2	55.7%
2	686.5 686.4	686.5	56.9%
3	689.9 688.8	689.4	57.2%
4	675.4 677.0	676.2	56.1%
5	686.8 687.5	687.2	57.0%
6	689.5 688.5	689.0	57.1%
Standard	1206.0 1205.8	1205.9	

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

Three analytical techniques have been employed in the drug analysis study. X-ray Powder Diffraction (XRD) was used for the analysis of the Active Pharmaceutical Ingredient (API) and the Excipients. Instrumental Neutron Activation Analysis (INAA) was also used for qualitative and quantitative analysis of the Excipients only whereas High Performance Liquid Chromatography (HPLC) was employed for analyzing both the API and Excipient.

Direct comparison of quantitative results of the analytical techniques could not be carried out. This is due to the absence of Amoxicillin reference data with relative intensities in the XRD's reference database. Nevertheless, the results obtained from the XRD identified the following phases in all the Amoxicillin antibiotic drugs studied: Amoxicillin trihydrate (API), Magnesium stearate (hydrated), Magnesium stearate (anhydrous). From the XRD spectrum shown above, there existed differences in intensity values for some of the peaks. INAA identified Magnesium, which facilitated the correct identification of the excipient phases in the antibiotic drugs with HPLC confirming and validating the API content in all the antibiotic drug samples that were analyzed.

However, the suspected fake amoxicillin sample failed the Dissolution Test, and is deemed to be substandard. The NHIS amoxicillin, as well as the foreign generic amoxicillin were found to meet the standards expected of an Amoxicillin antibiotic drug according to the 2014 edition of the British Pharmacopoeia whilst suspected fake amoxicillin was found to be substandard.

Therefore, for quality control purposes, HPLC is a better tool but XRD in combination with INAA is a more effective tool for structural identification and phase quantification for the purposes of distinguishing between fake and genuine drugs. It also has faster analysis time, and simpler sample preparation.

5.2 RECOMMENDATIONS:

- Further studies on other types of antibiotics should be considered.
- There should be regular and consistent monitoring of antibiotics on the market by the Food and Drugs Authority and other regulatory bodies in Ghana.
- The surveillance and monitoring systems at the borders as well as the habours should be intensified.
- Further studies should consider the quantitative aspect of the XRD to be able to determine the concentrations of the API and the excipient phases in pharmaceutical drugs.

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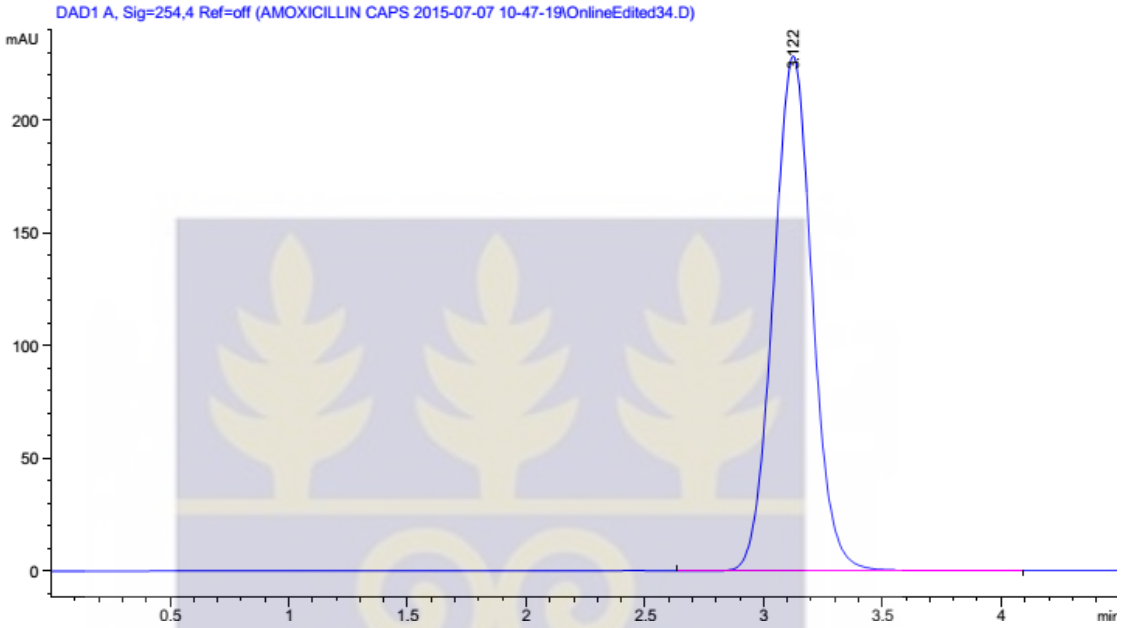
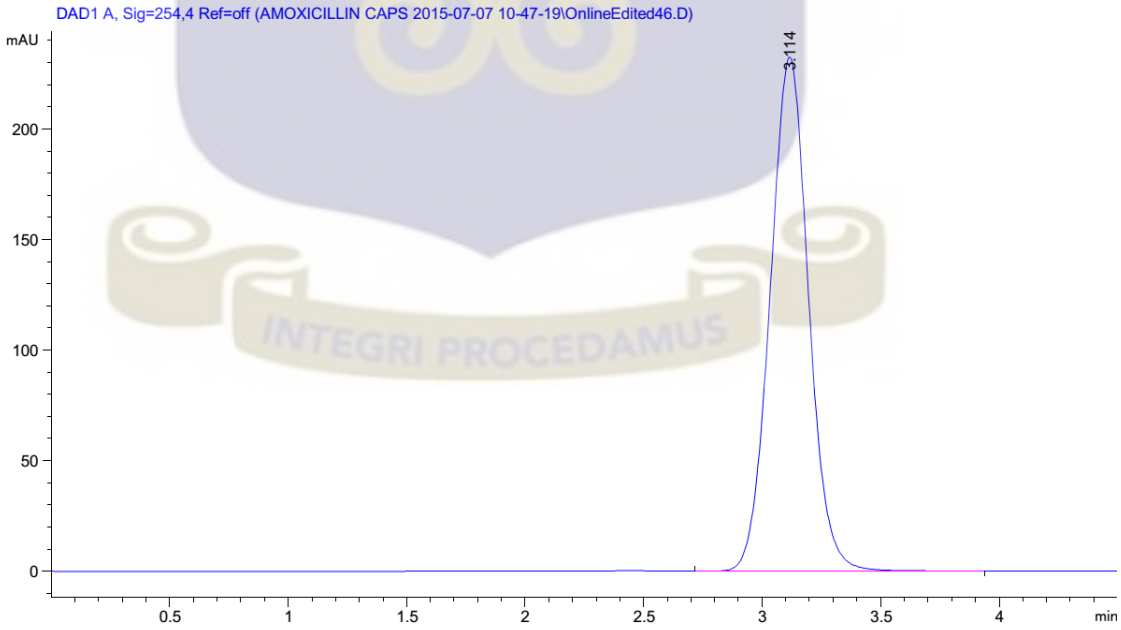
APPENDICES

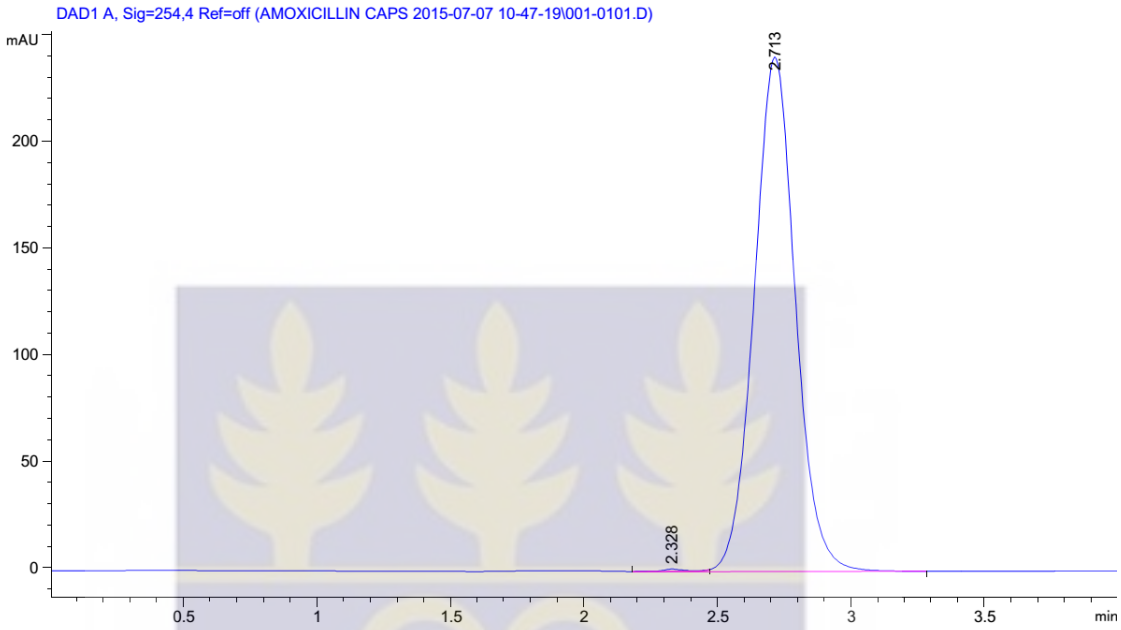
APPENDIX A1: HPLC Chromatogram For A moxicillin Standard



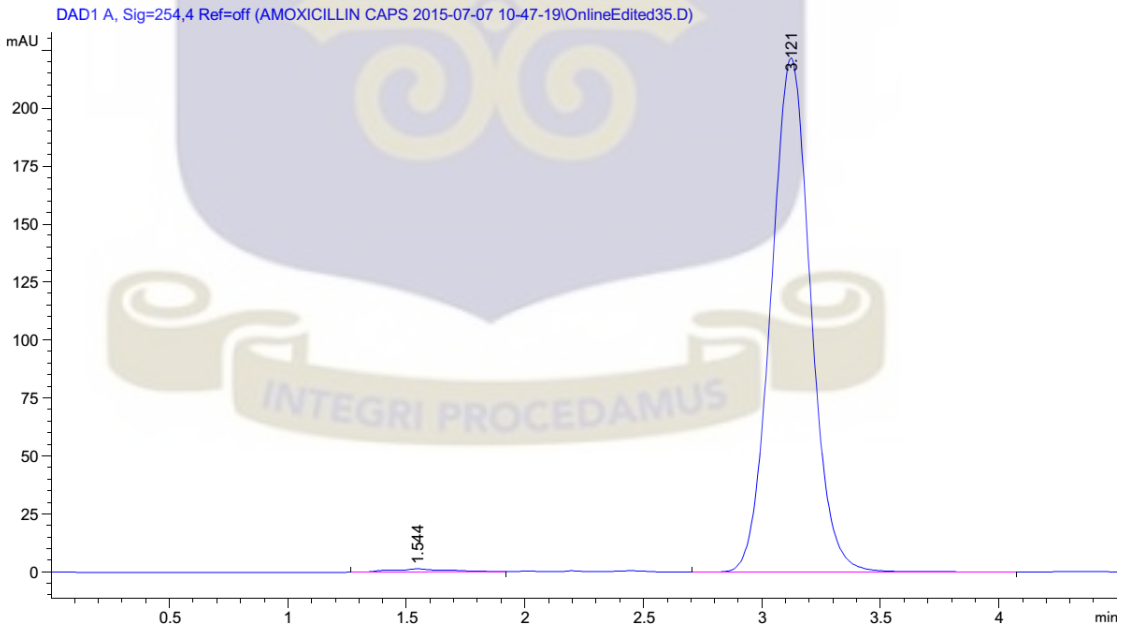
APPENDIX A2: HPLC Chromatogram For Amoxicillin Standard



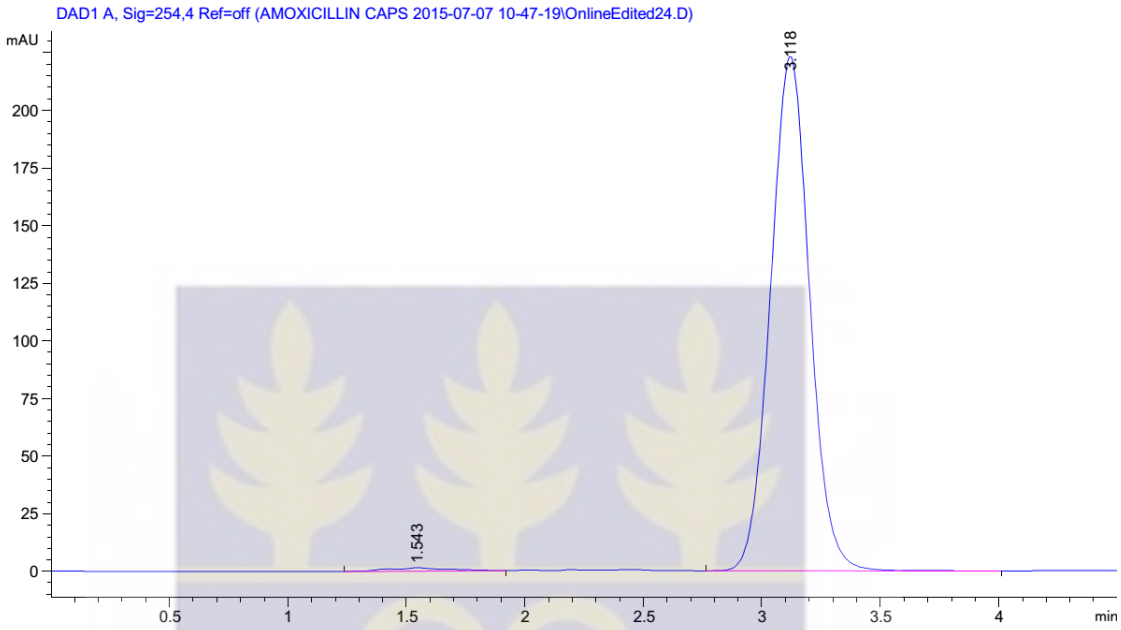
APPENDIX A3: HPLC Chromatogram For Amoxicillin Standard



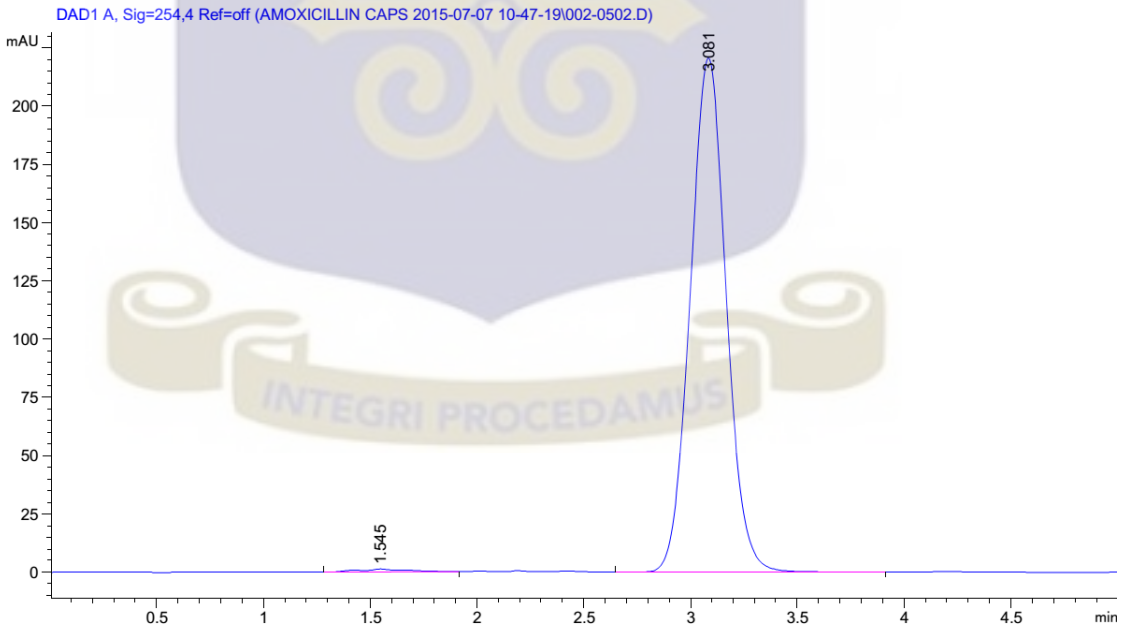
APPENDIX B1: HPLC Chromatogram For NHIS Amoxicillin



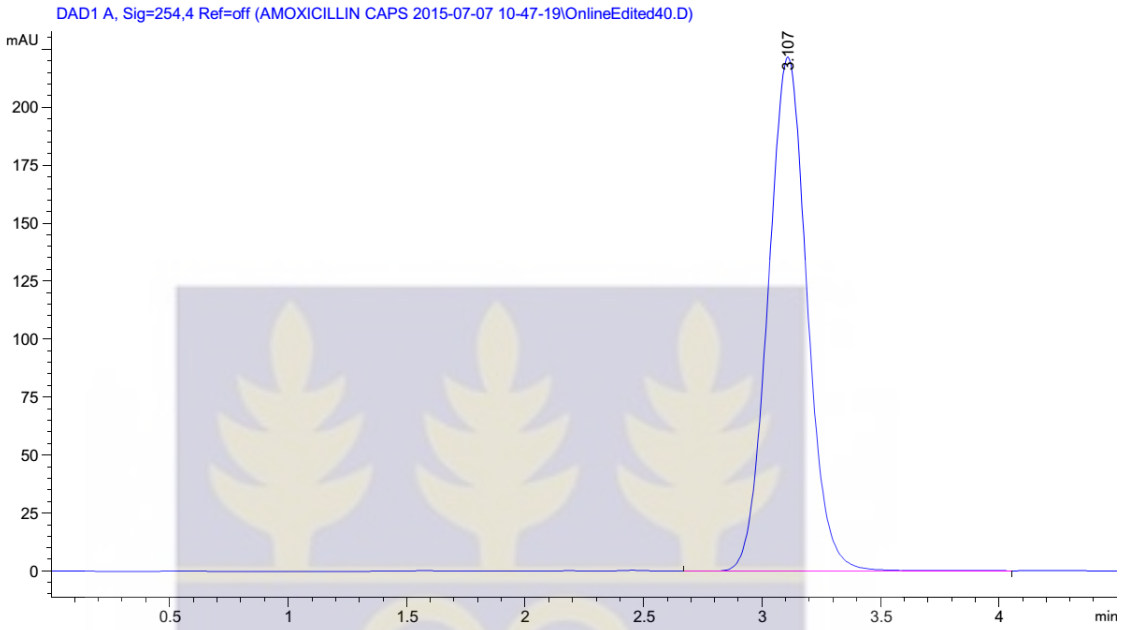
APPENDIX B2: HPLC Chromatogram For NHIS Amoxicillin



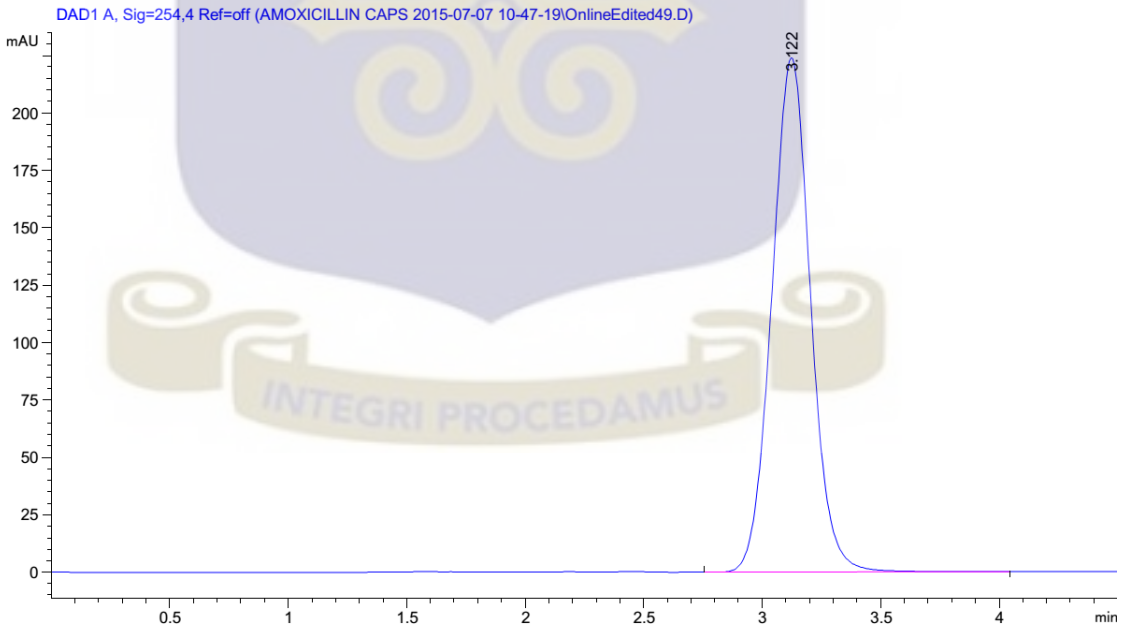
APPENDIX B3: HPLC Chromatogram For NHIS Amoxicillin



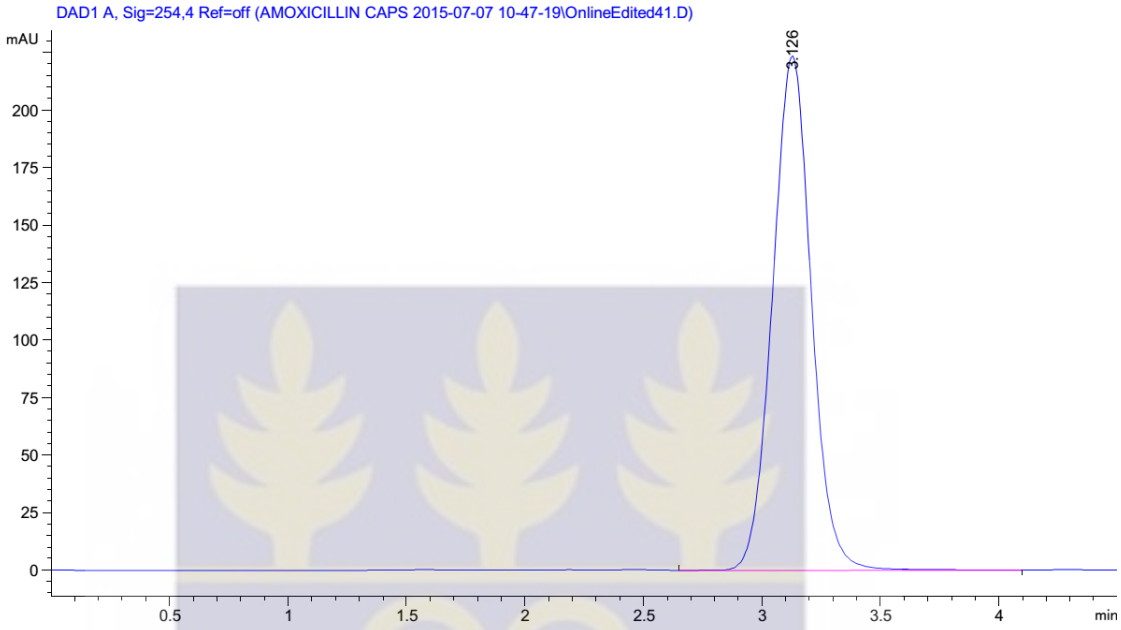
APPENDIX C1: HPLC Chromatogram For Suspected Fake Amoxicillin



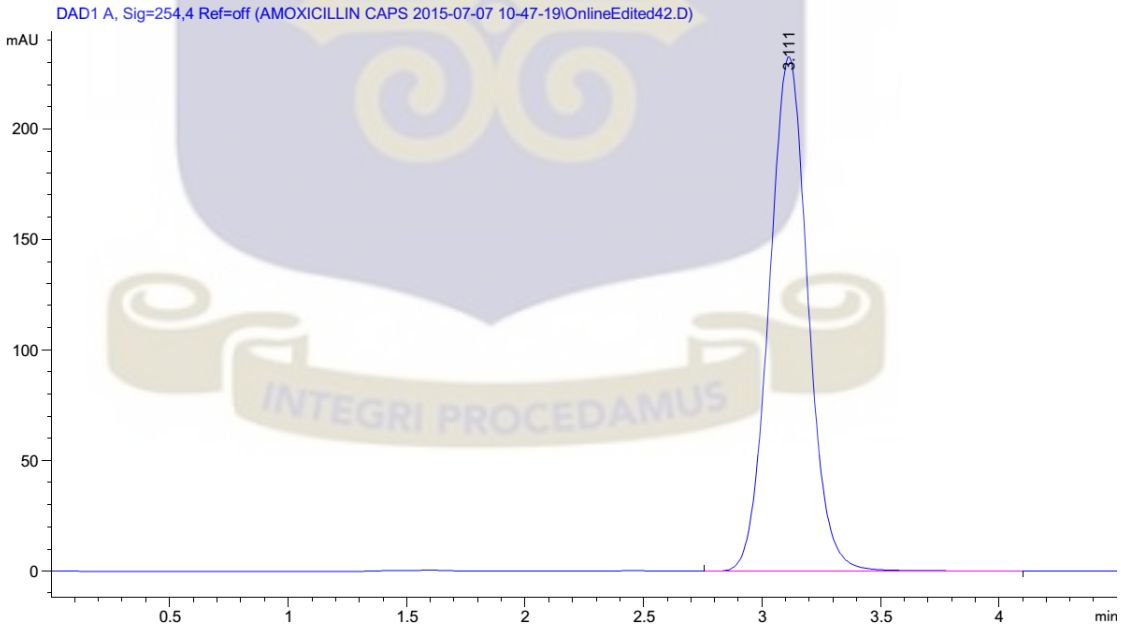
APPENDIX C2: HPLC Chromatogram For Suspected Fake Amoxicillin



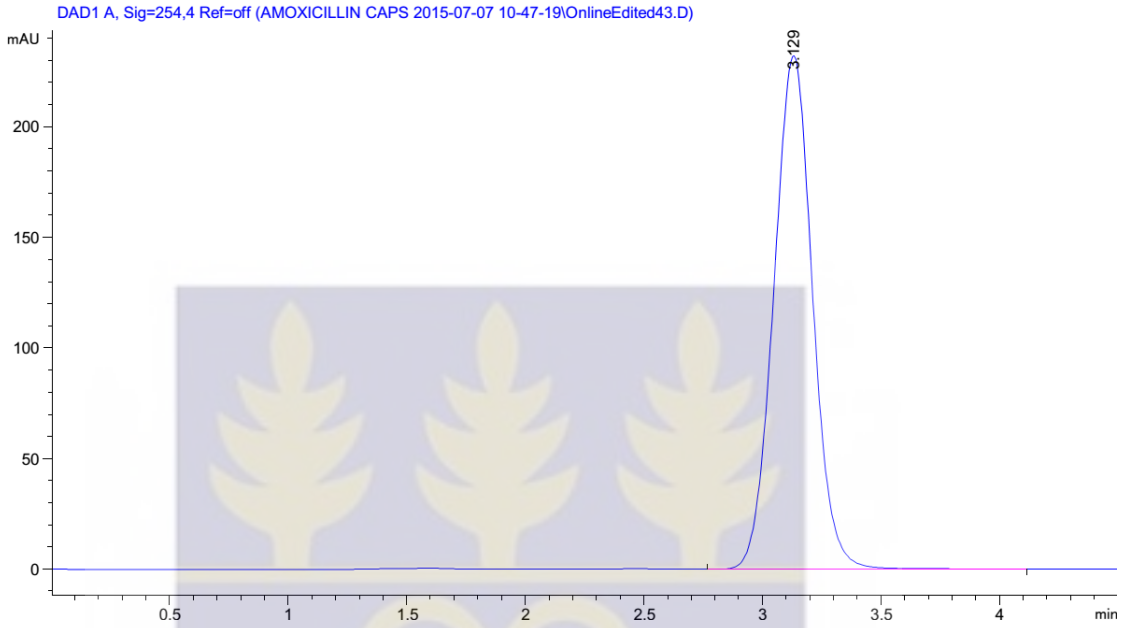
APPENDIX C3: HPLC Chromatogram For Suspected Fake Amoxicillin



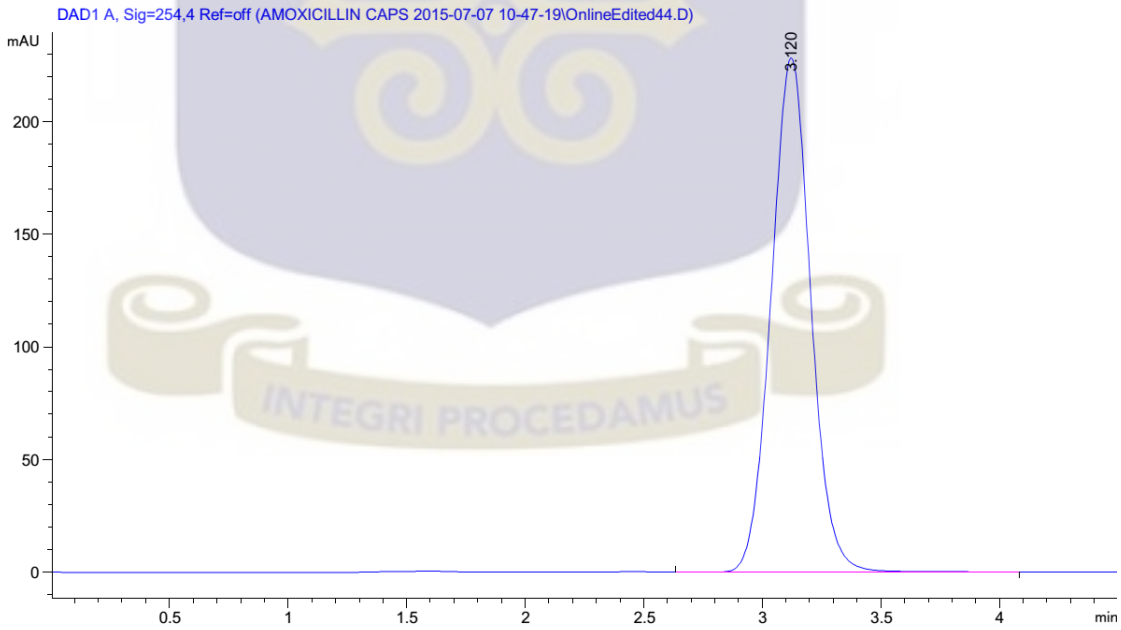
APPENDIX D1: HPLC Chromatogram For Foreign Generic Amoxicillin



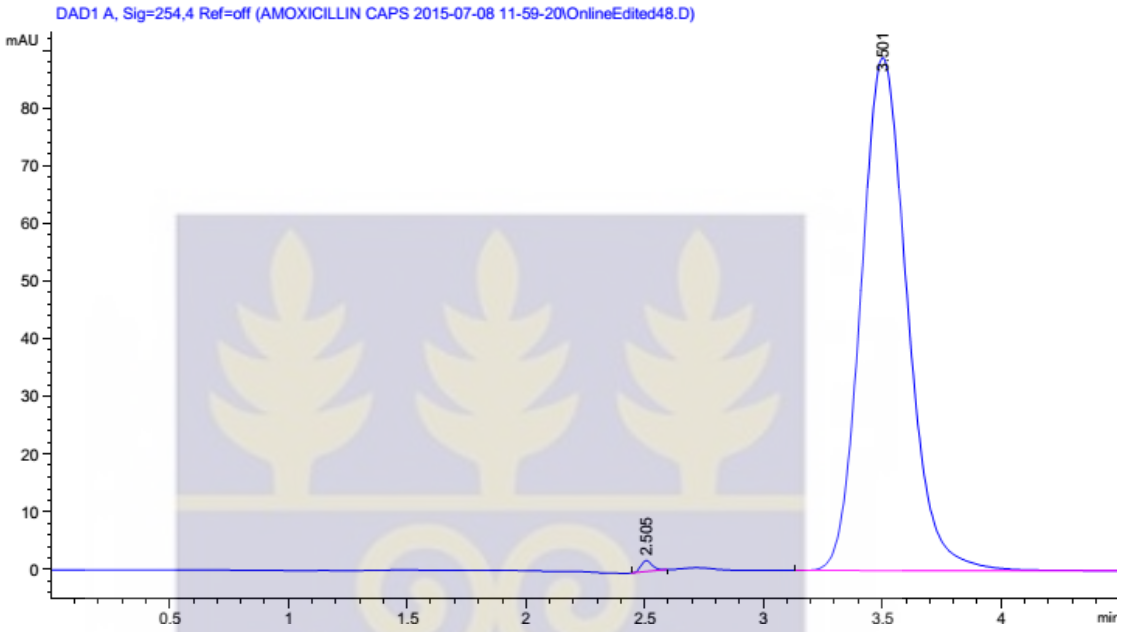
APPENDIX D2: HPLC Chromatogram For Foreign Generic Amoxicillin



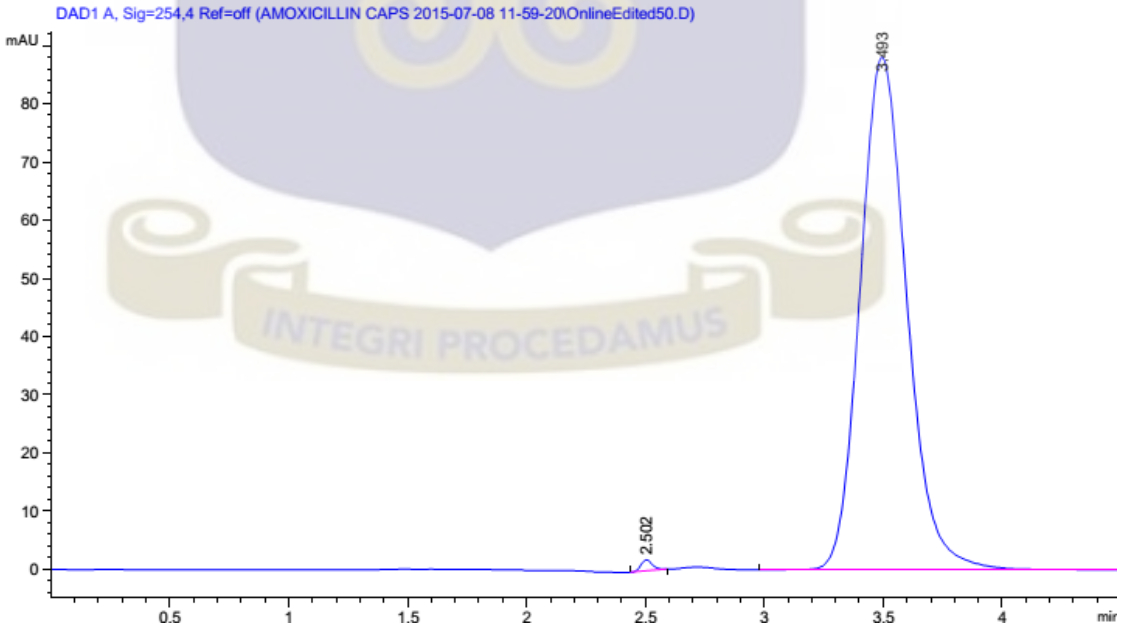
APPENDIX D3: HPLC Chromatogram For Foreign Generic Amoxicillin



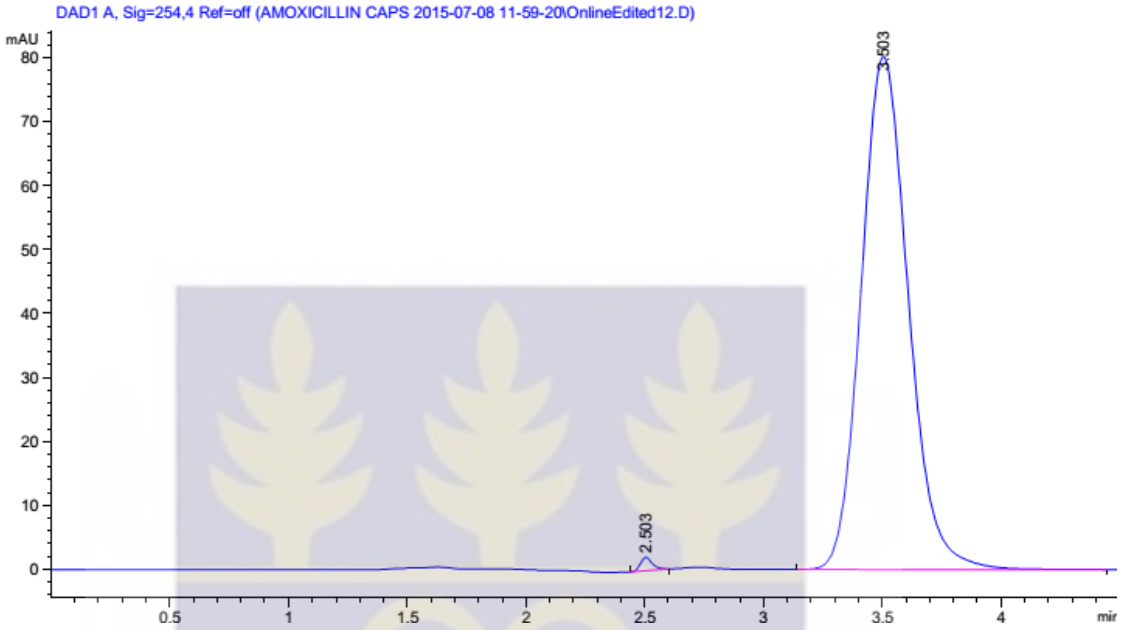
APPENDIX E1: Dissolution Chromatogram For Standard Amoxicillin



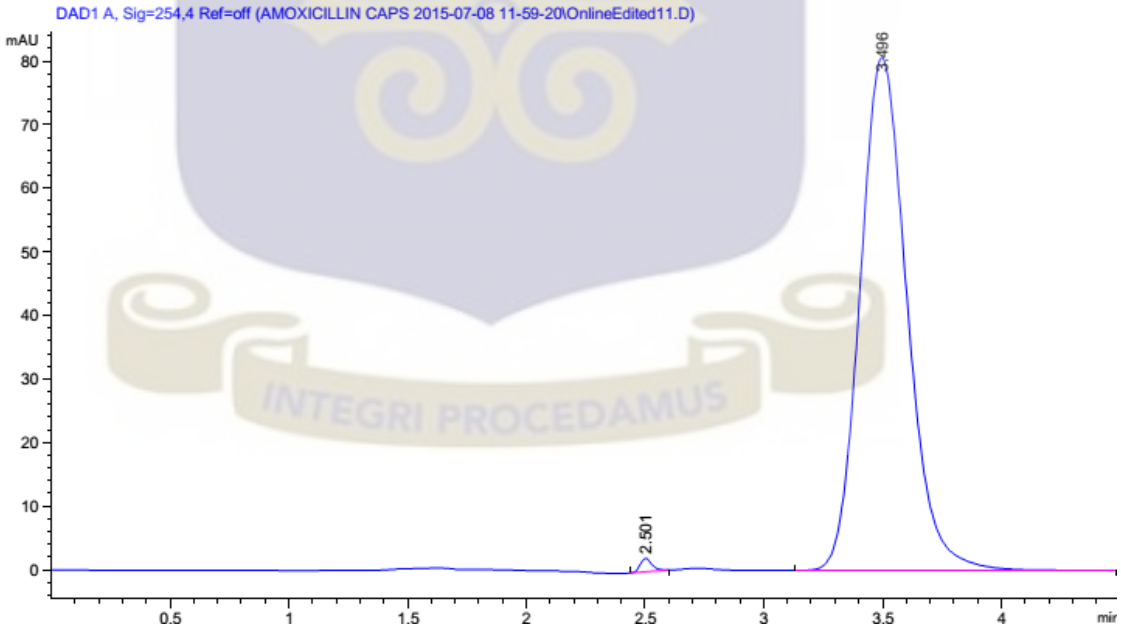
APPENDIX E2: Dissolution Chromatogram For Standard Amoxicillin



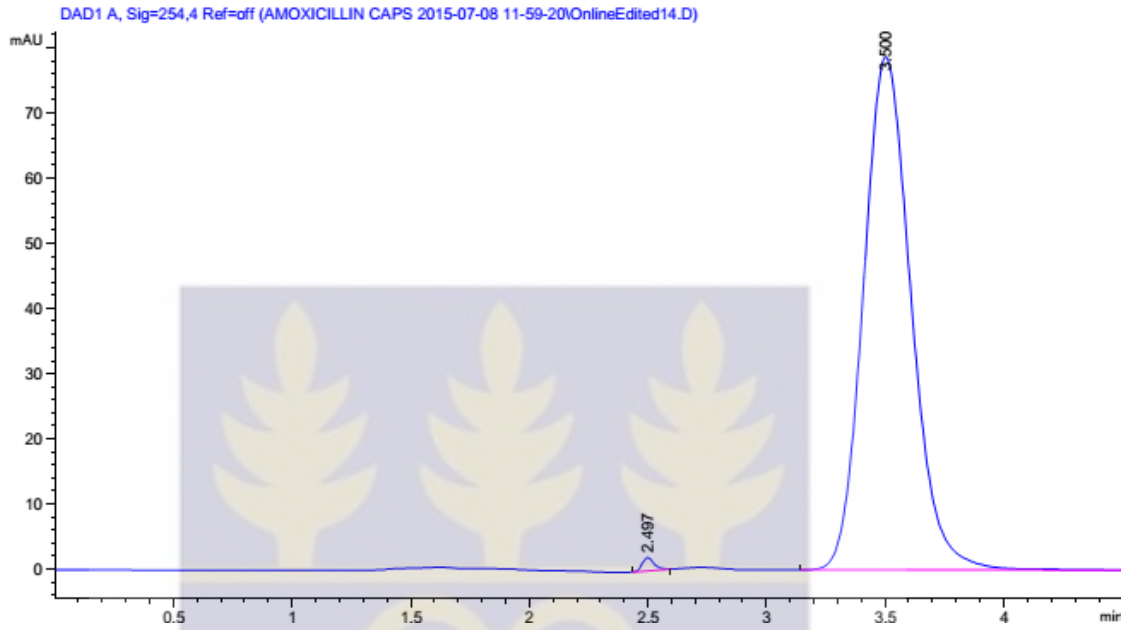
APPENDIX F1: Dissolution Chromatogram For NHIS Amoxicillin (VESSEL 1)



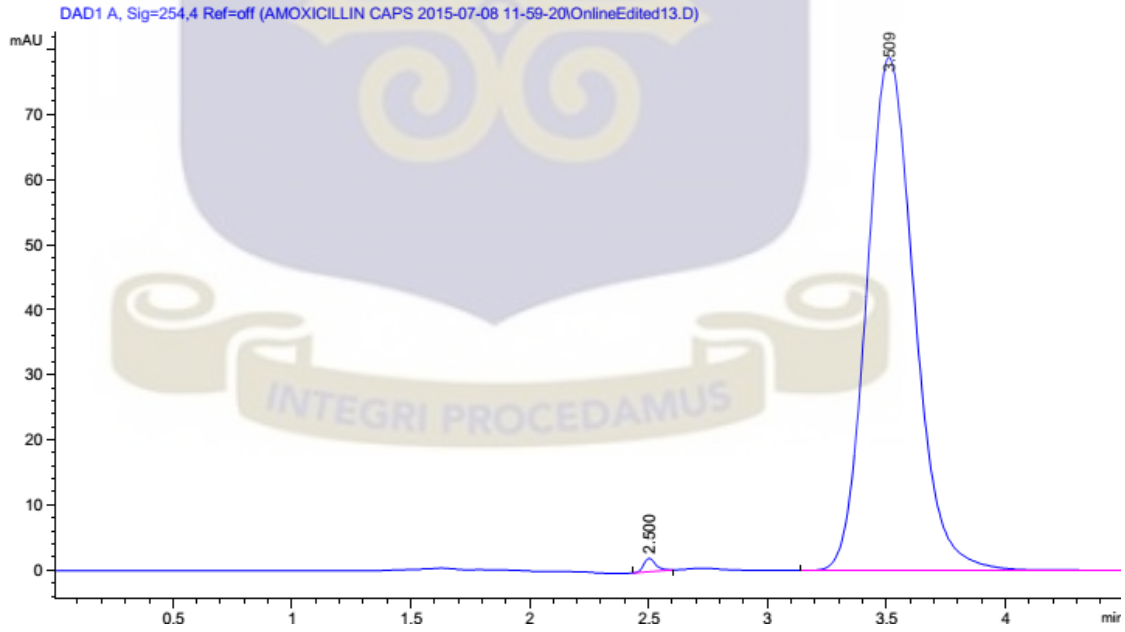
APPENDIX F2: Dissolution Chromatogram For NHIS Amoxicillin (VESSEL 1)



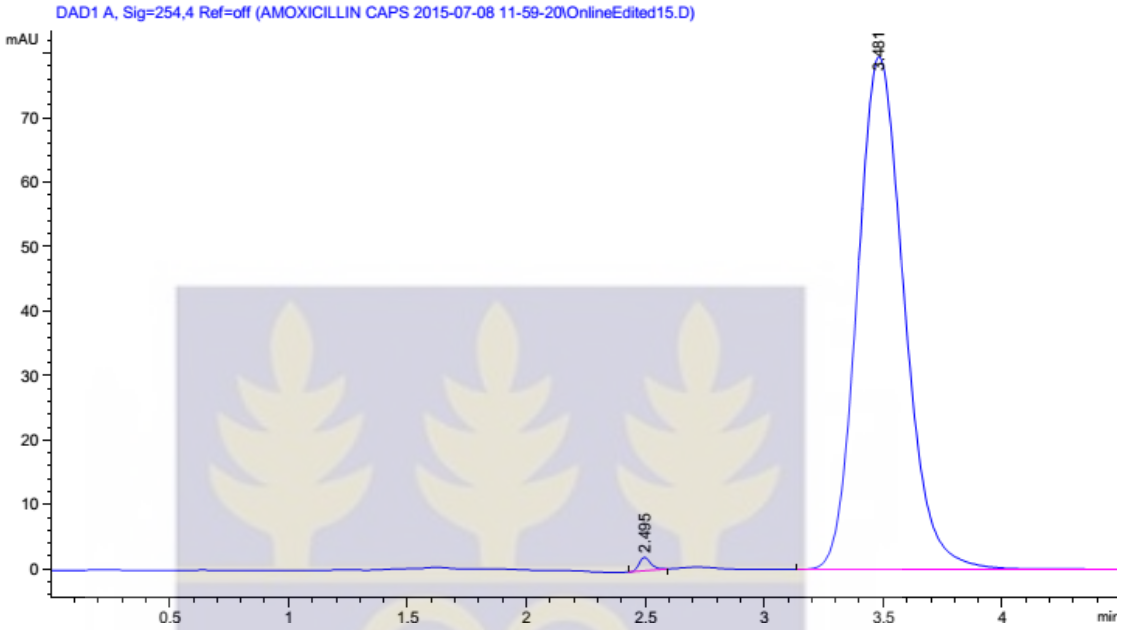
APPENDIX G1: Dissolution Chromatogram For NHIS Amoxicillin (VESSEL 2)



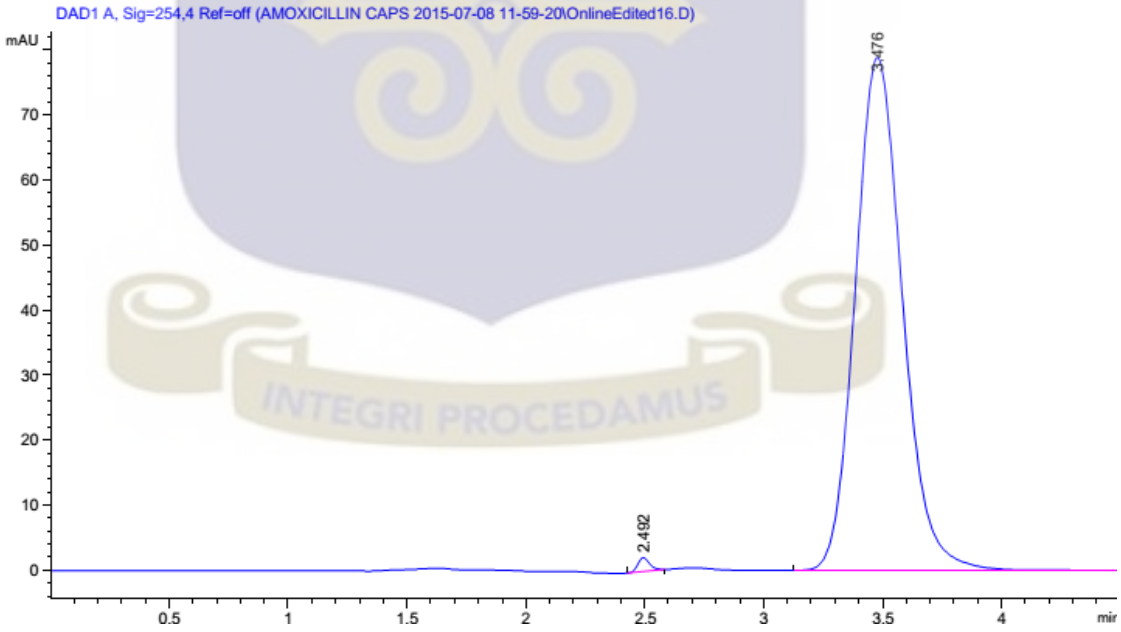
APPENDIX G2: Dissolution Chromatogram For NHIS Amoxicillin (VESSEL 2)



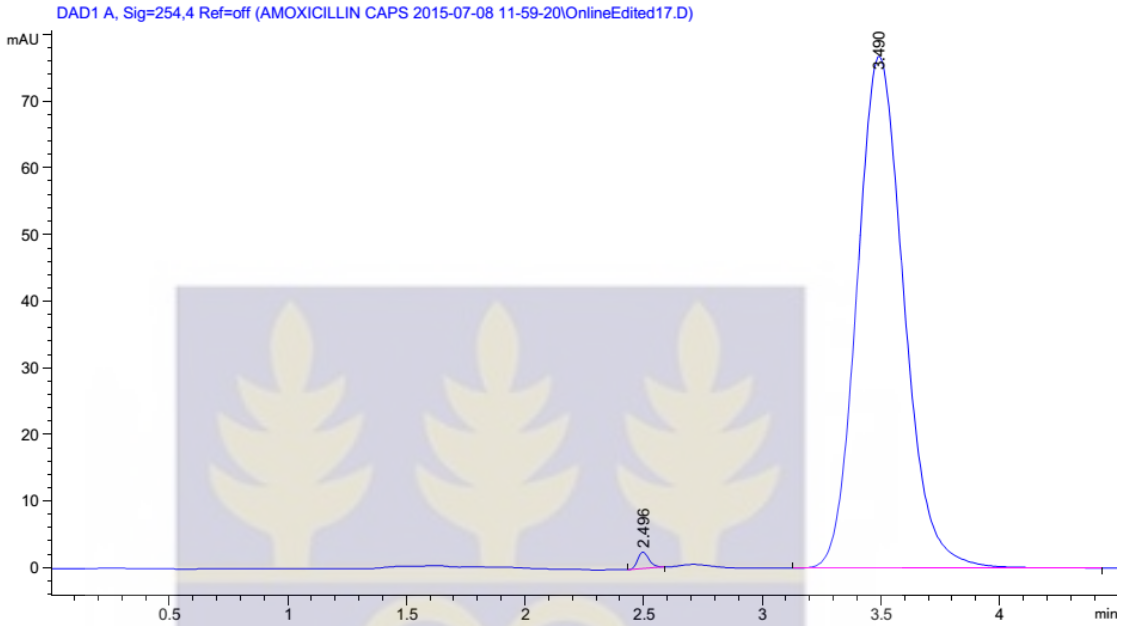
APPENDIX H1: Dissolution Chromatogram For NHIS Amoxicillin (VESSEL 3)



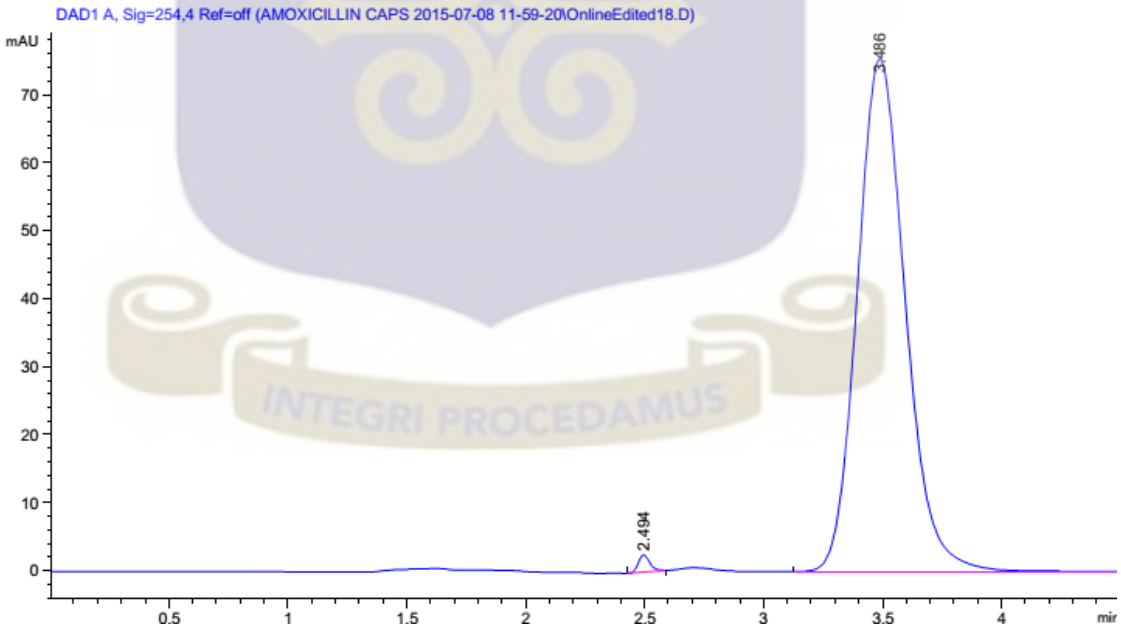
APPENDIX H2: Dissolution Chromatogram For NHIS Amoxicillin (VESSEL 3)



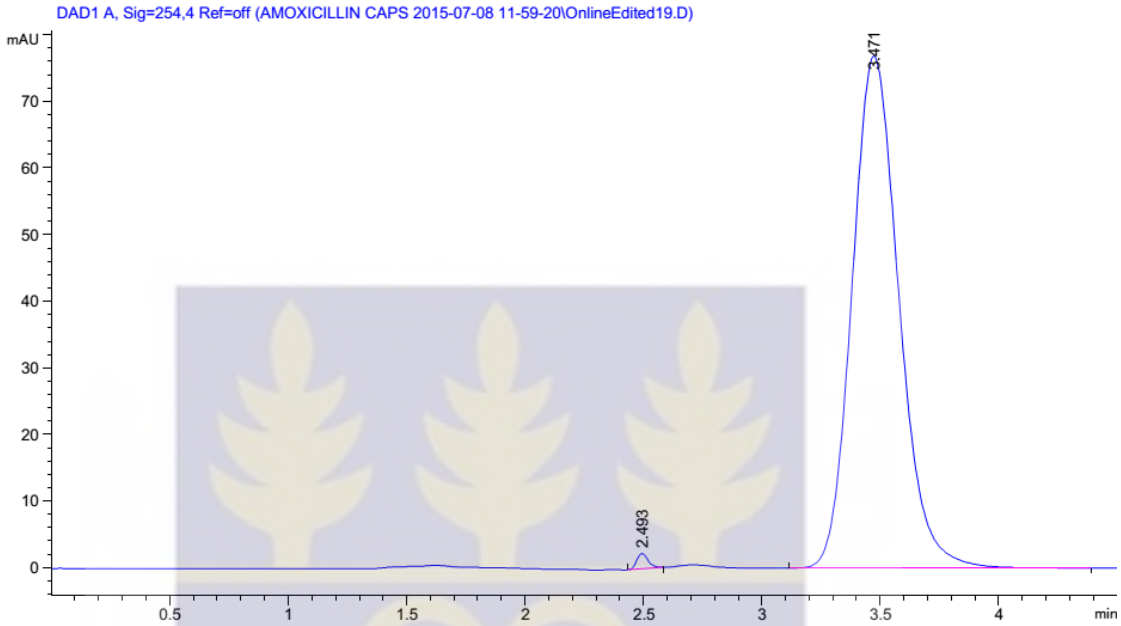
APPENDIX I1: Dissolution Chromatogram for NHIS Amoxicillin (VESSEL 4)



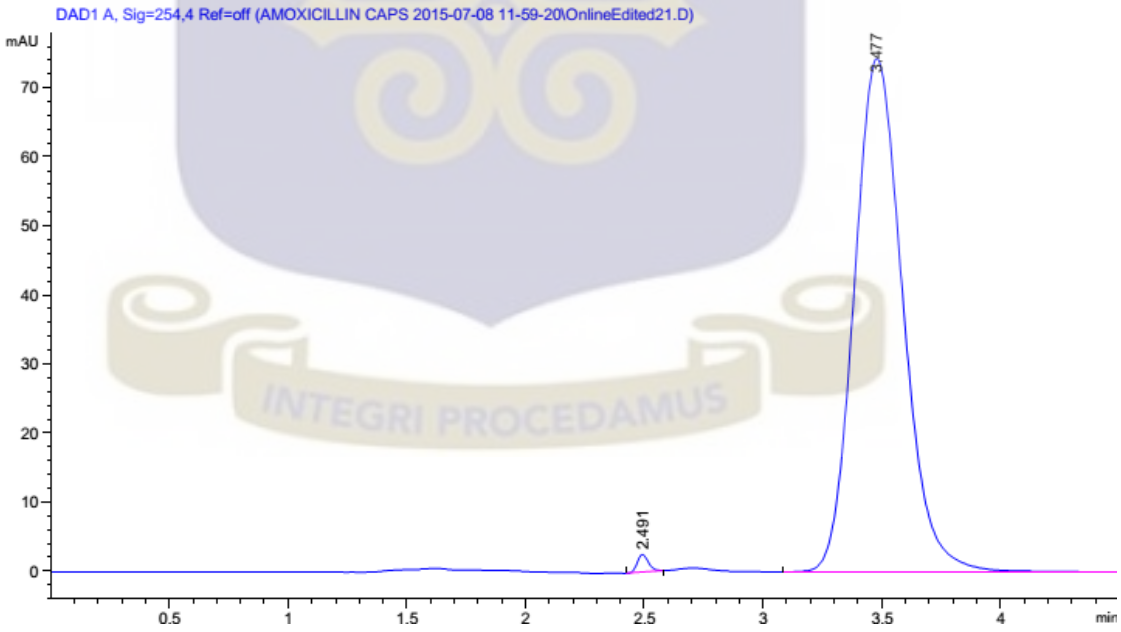
APPENDIX I2: Dissolution Chromatogram For NHIS Amoxicillin (VESSEL 4)



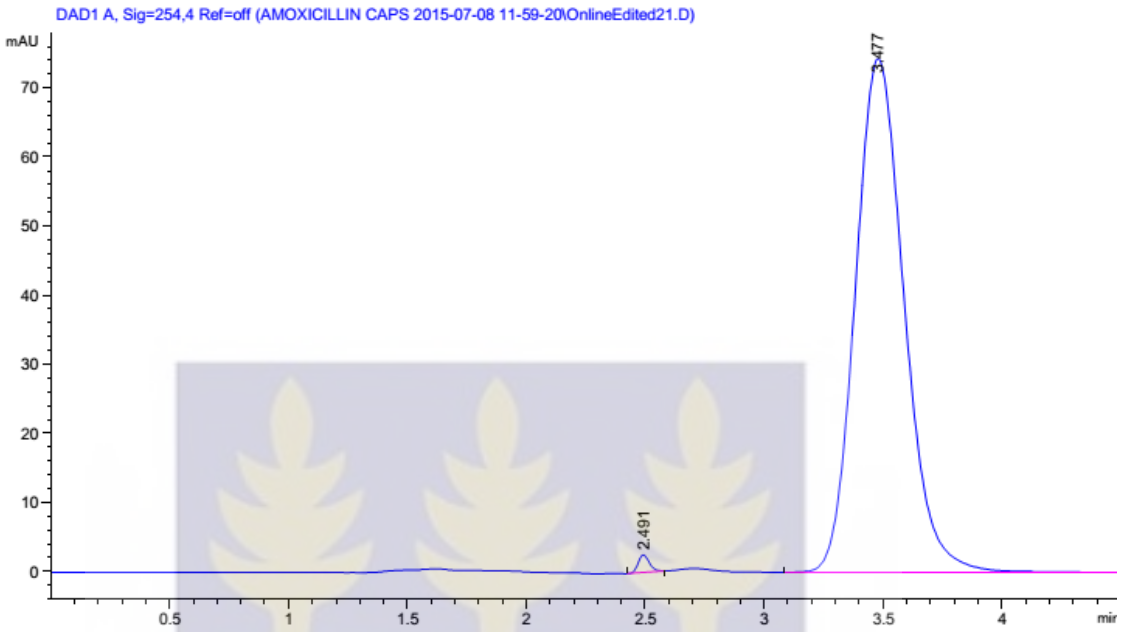
APPENDIX J1: Dissolution Chromatogram For NHIS Amoxicillin (VESSEL 5)



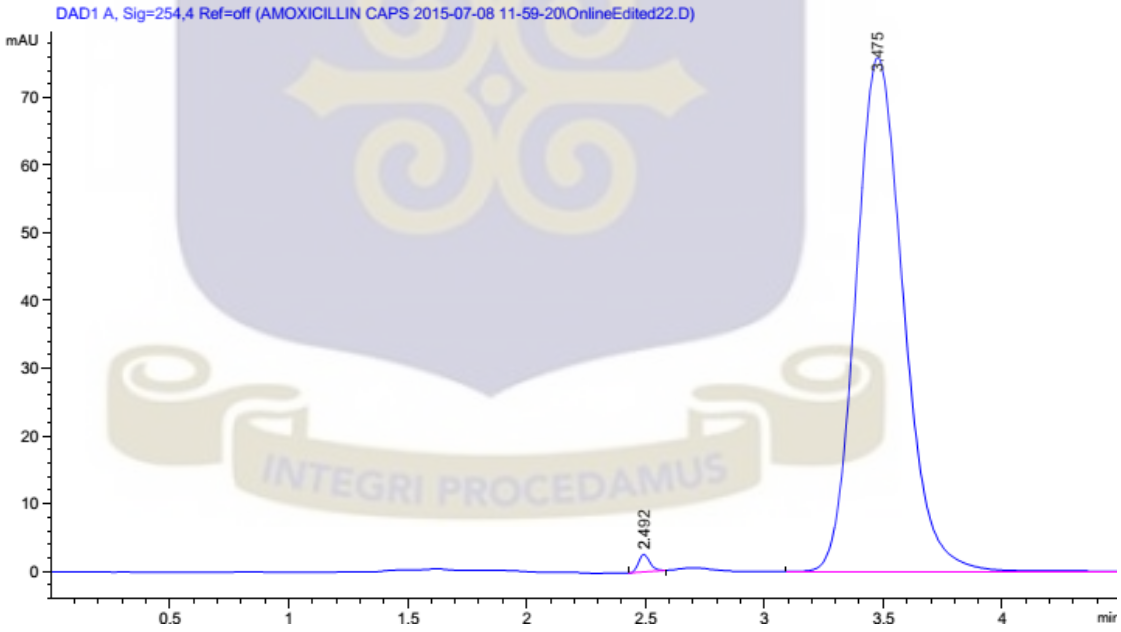
APPENDIX J2: Dissolution Chromatogram For NHIS Amoxicillin (VESSEL 5)



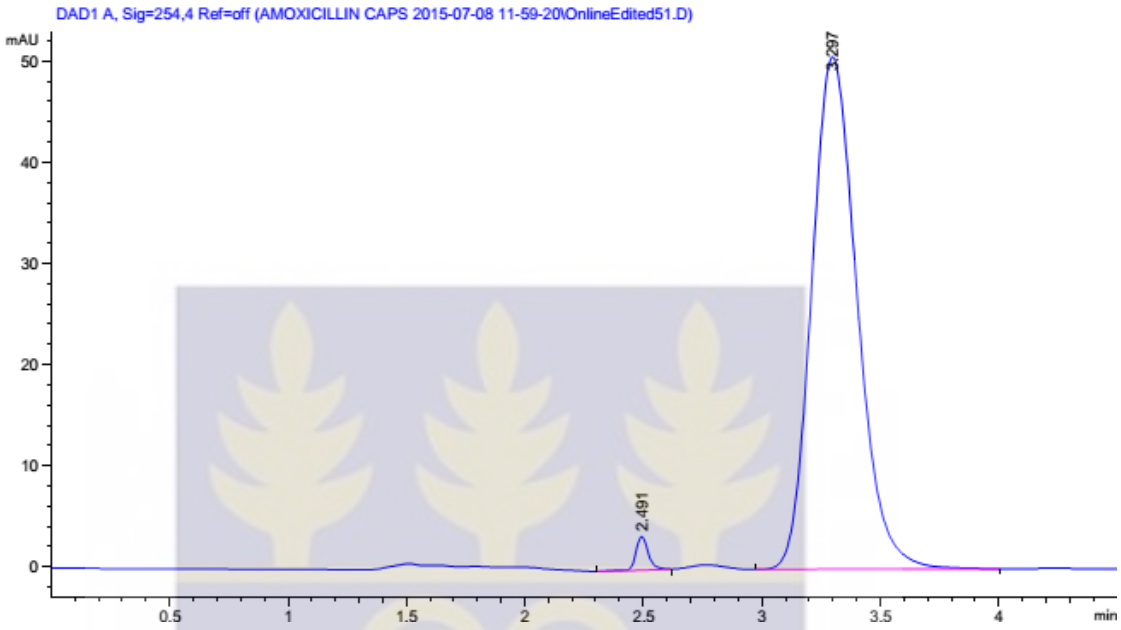
APPENDIX K1: Dissolution Chromatogram For NHIS Amoxicillin (VESSEL 6)



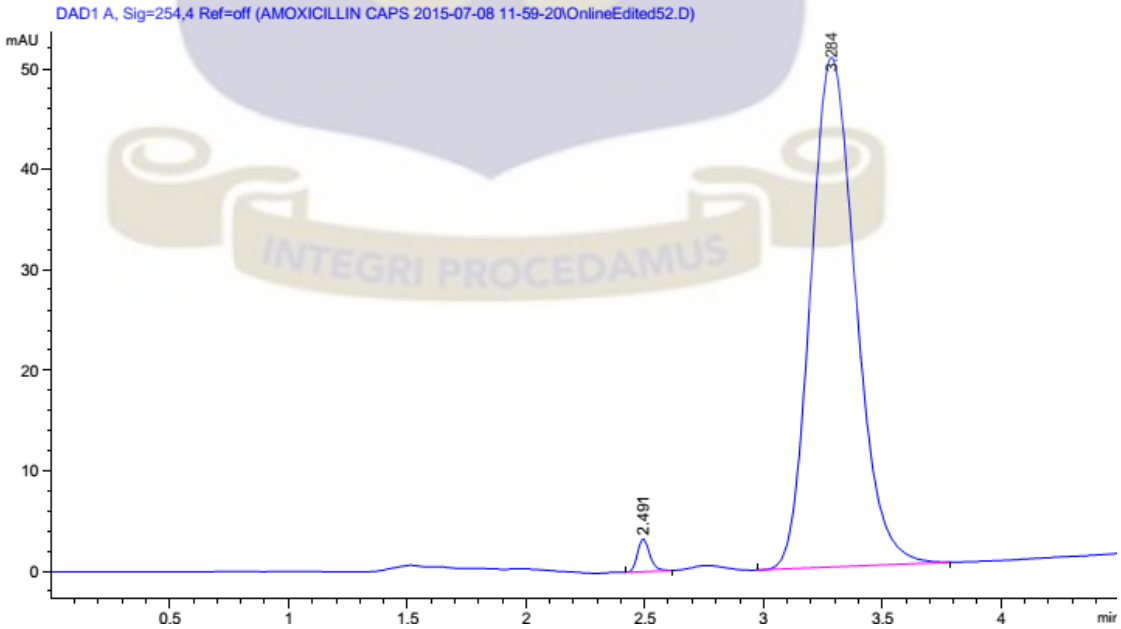
APPENDIX K2: Dissolution Chromatogram For NHIS Amoxicillin (VESSEL 6)



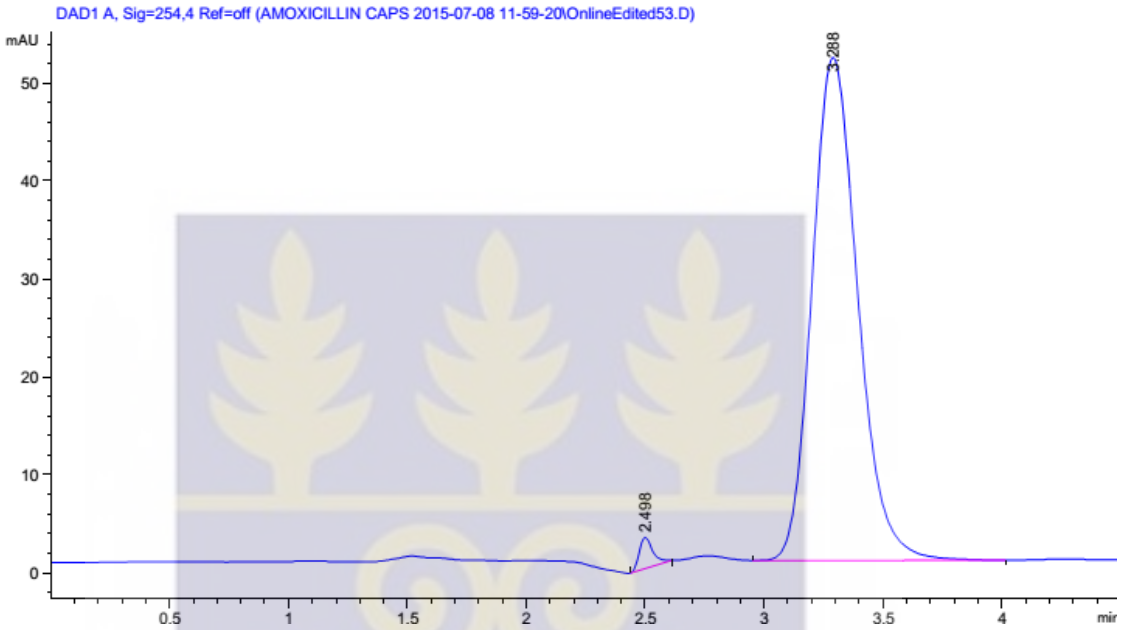
APPENDIX L1: Dissolution Chromatogram For Suspected Fake Amoxicillin (VESSEL 1)



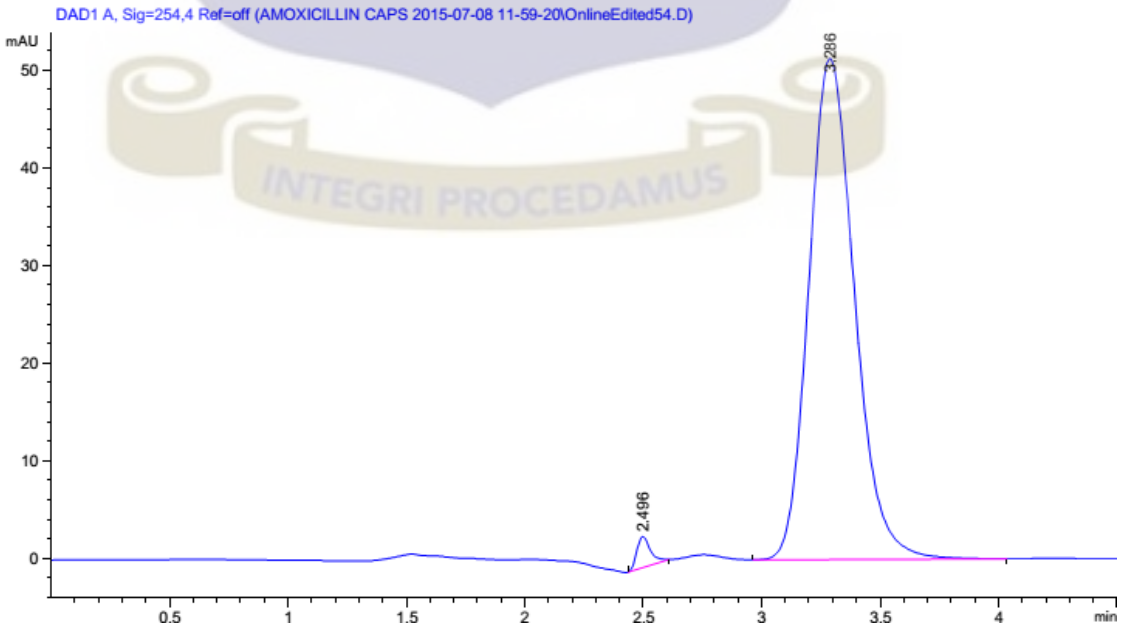
APPENDIX L2: Dissolution Chromatogram For Suspected Fake Amoxicillin (VESSEL 1)



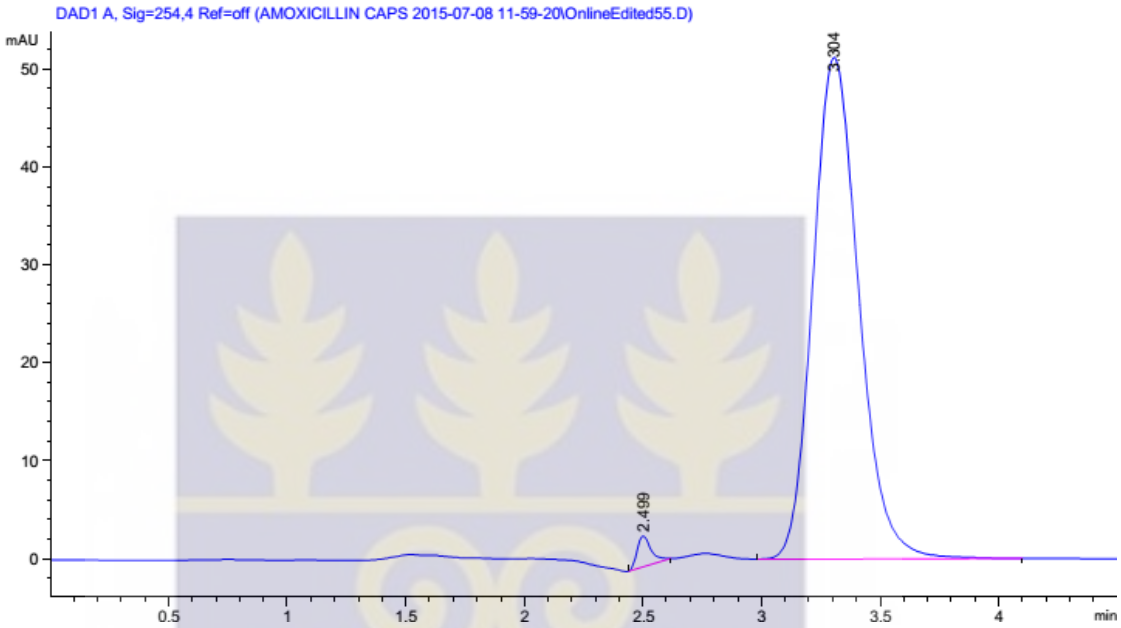
APPENDIX M1: Dissolution Chromatogram For Suspected Fake Amoxicillin (VESSEL 2)



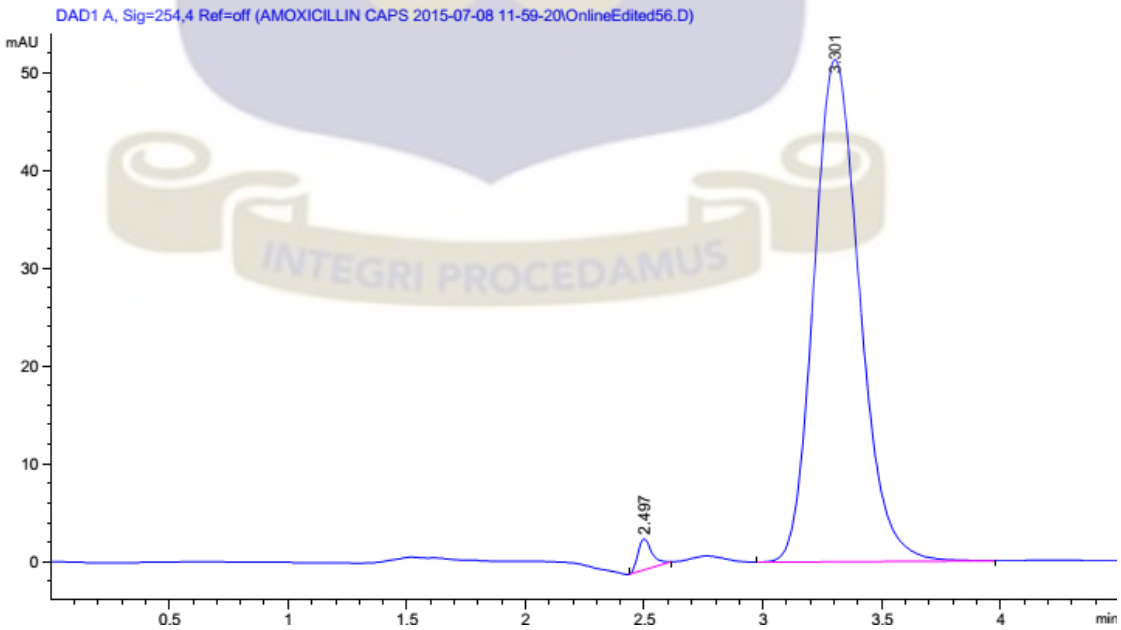
APPENDIX M2: Dissolution Chromatogram For Suspected Fake Amoxicillin (VESSEL 2)



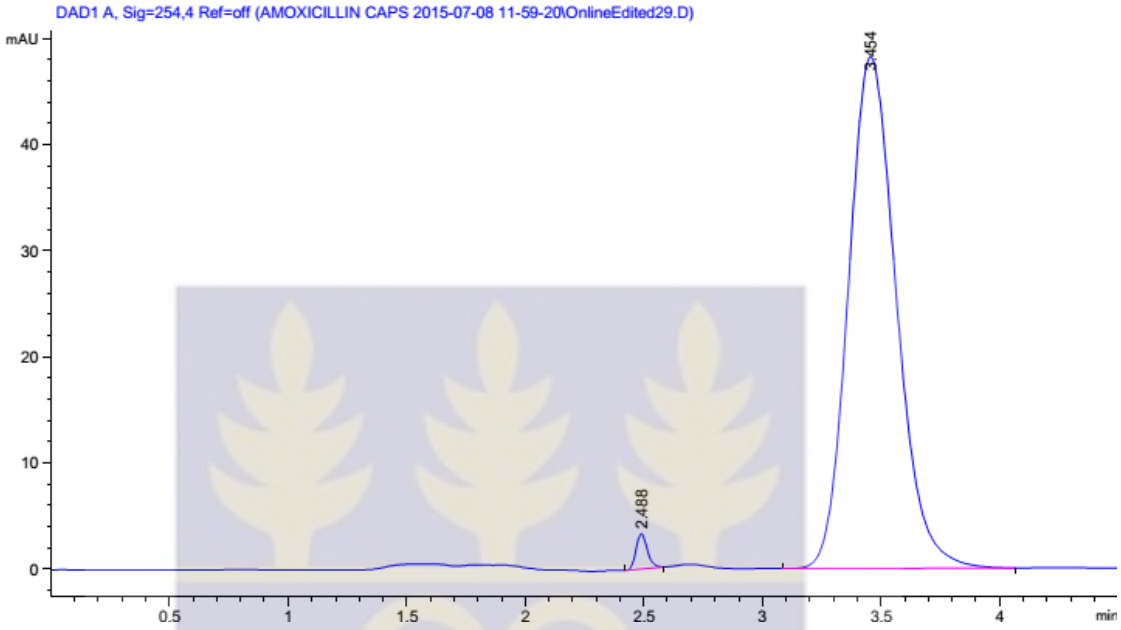
APPENDIX N1: Dissolution Chromatogram For Suspected Fake Amoxicillin (VESSEL 3)



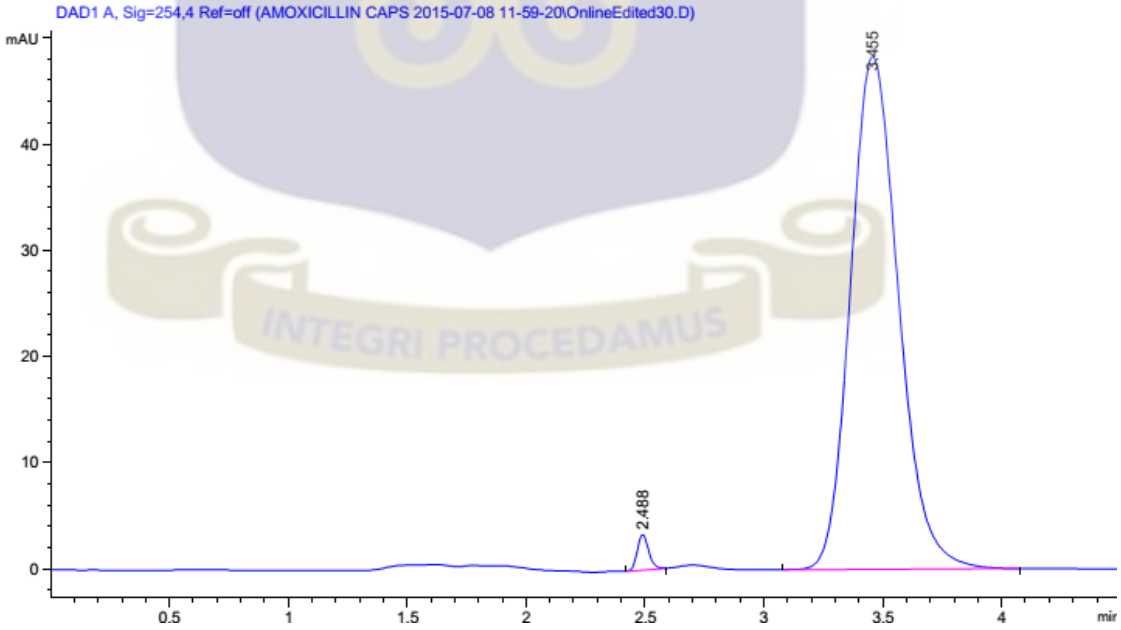
APPENDIX N2: Dissolution Chromatogram For Suspected Fake Amoxicillin (VESSEL 3)



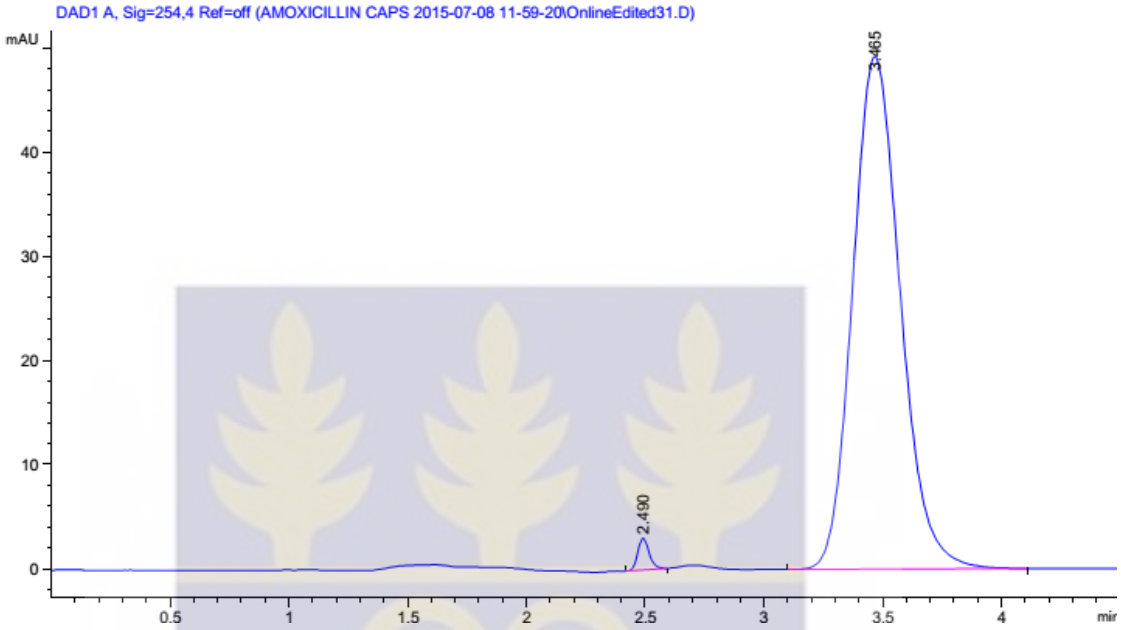
APPENDIX O1: Dissolution Chromatogram For Suspected Fake Amoxicillin (VESSEL 4)



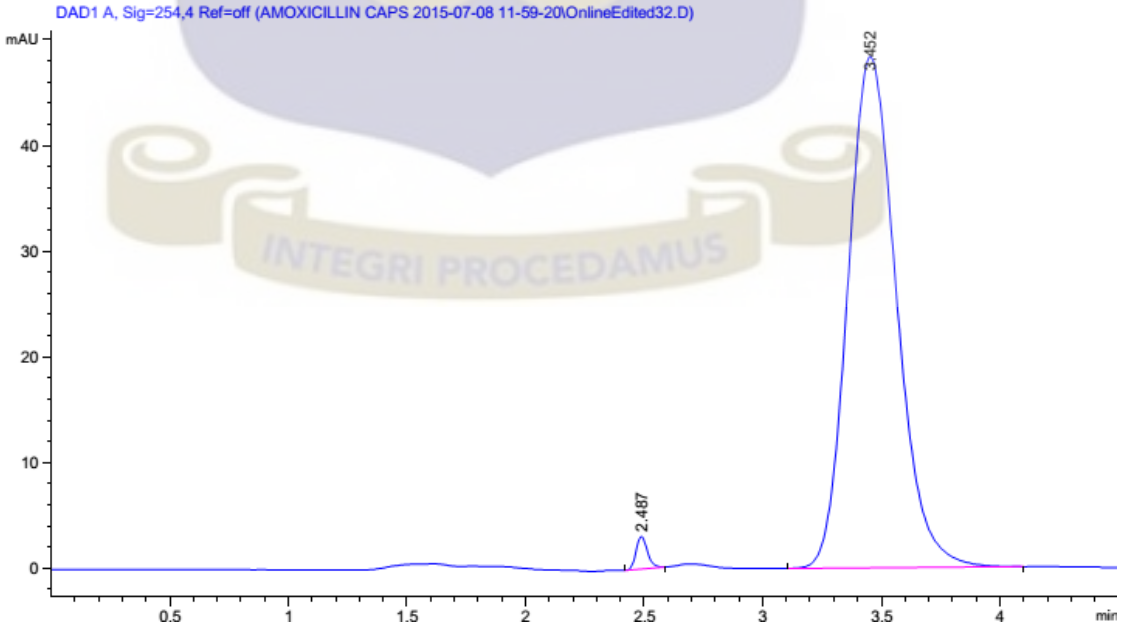
APPENDIX O2: Dissolution Chromatogram For Suspected Fake Amoxicillin (VESSEL 4)



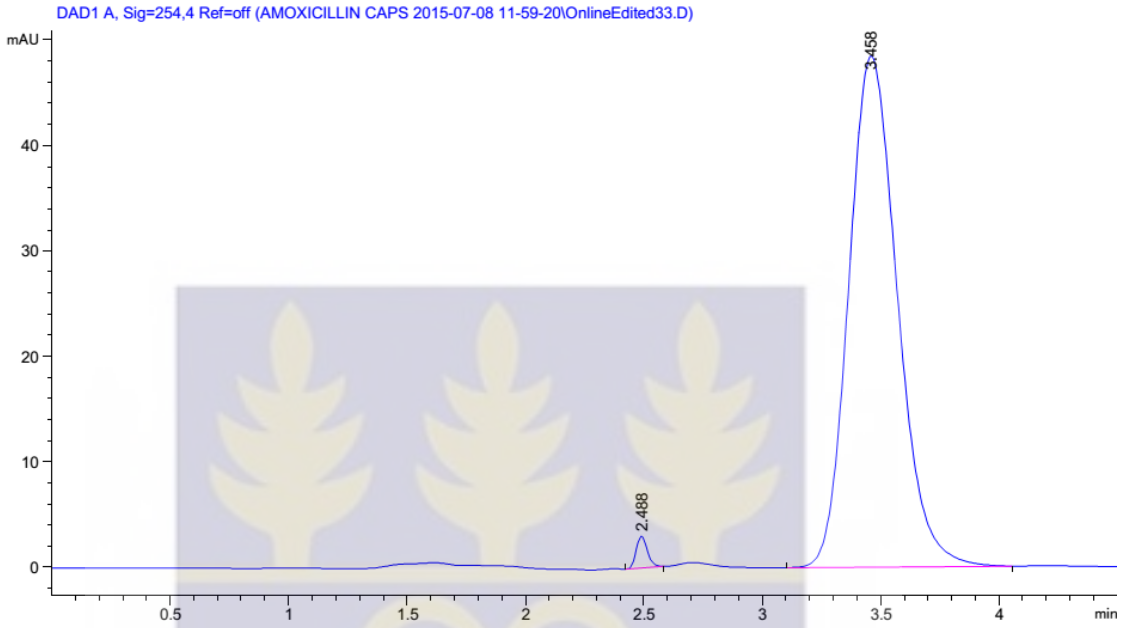
APPENDIX P1: Dissolution Chromatogram For Suspected Fake Amoxicillin (VESSEL 5)



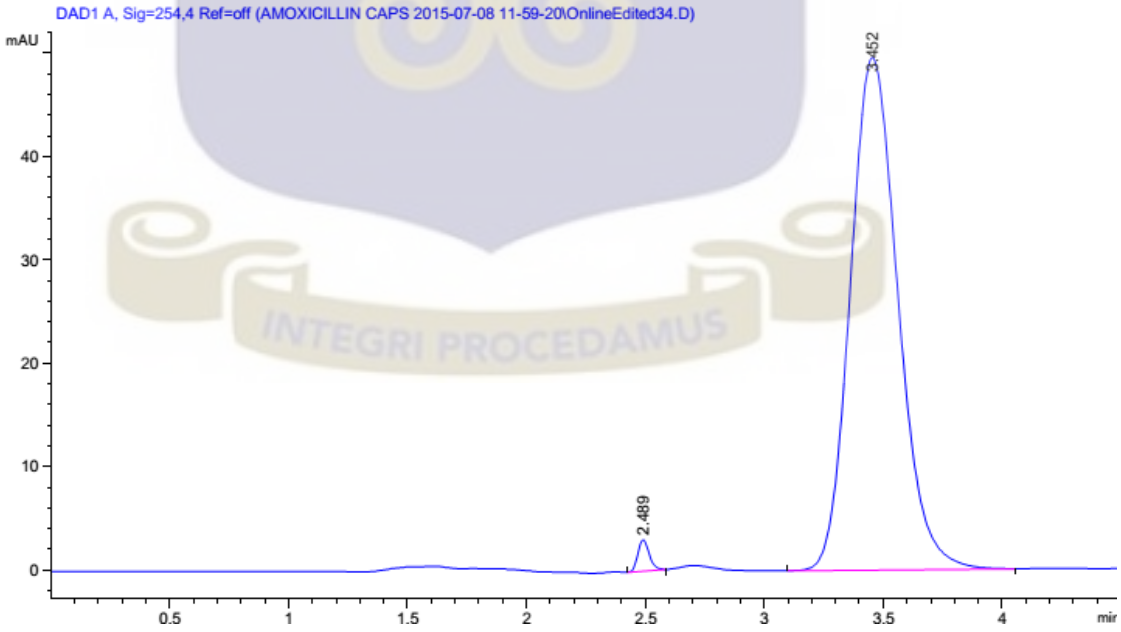
APPENDIX P2: Dissolution Chromatogram For Suspected Fake Amoxicillin (VESSEL 5)



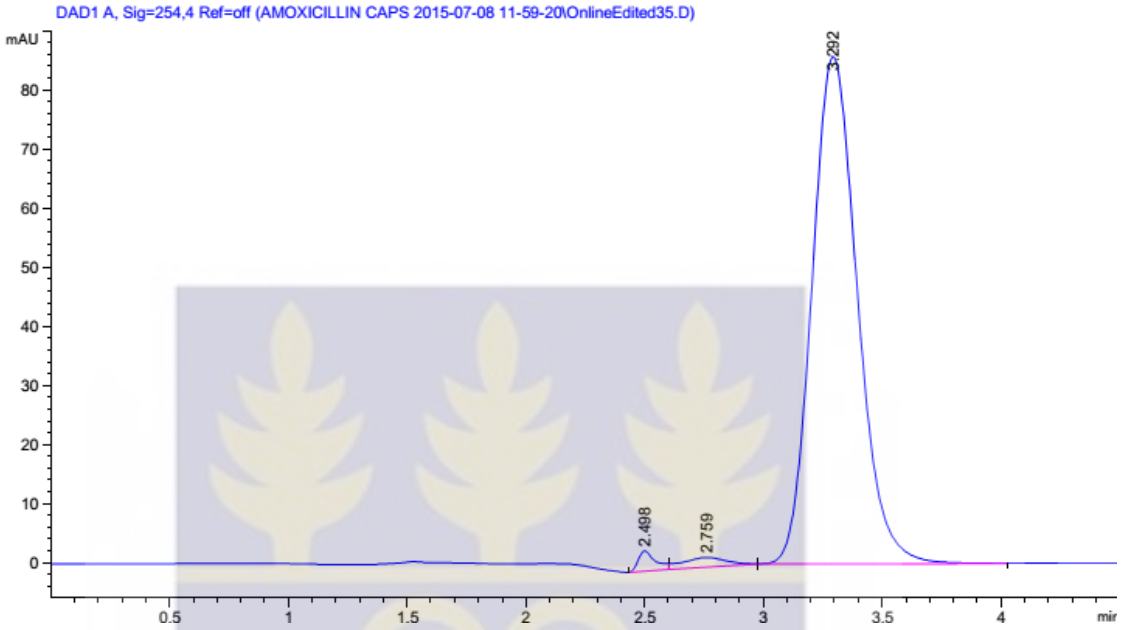
APPENDIX Q1: Dissolution Chromatogram For Suspected Fake Amoxicillin (VESSEL 6)



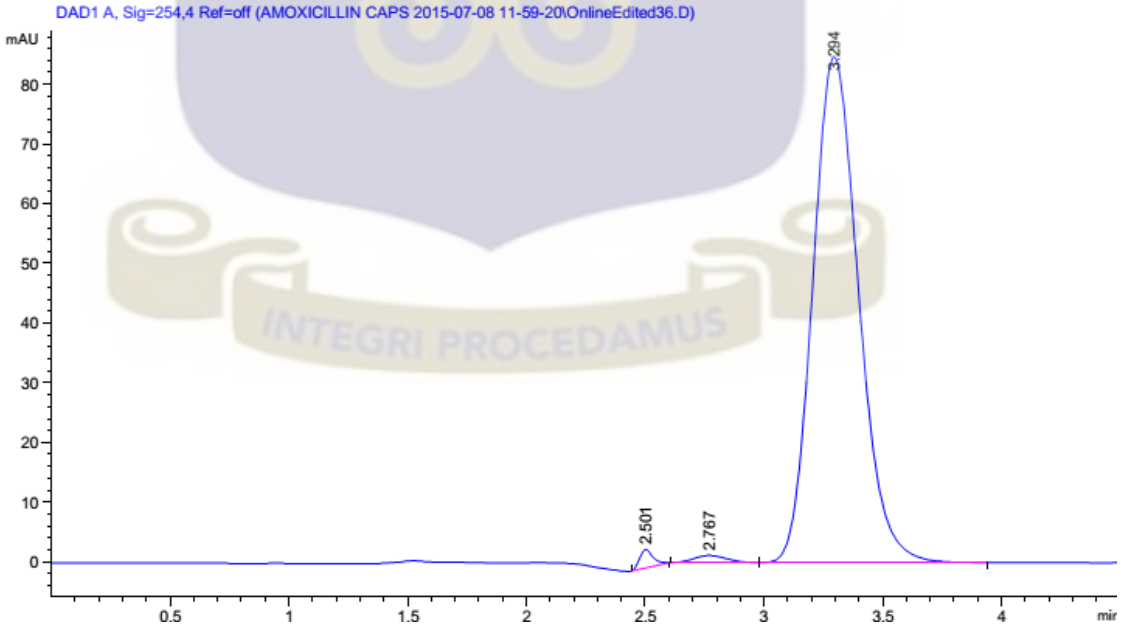
APPENDIX Q2: Dissolution Chromatogram For Suspected Fake Amoxicillin (VESSEL 6)



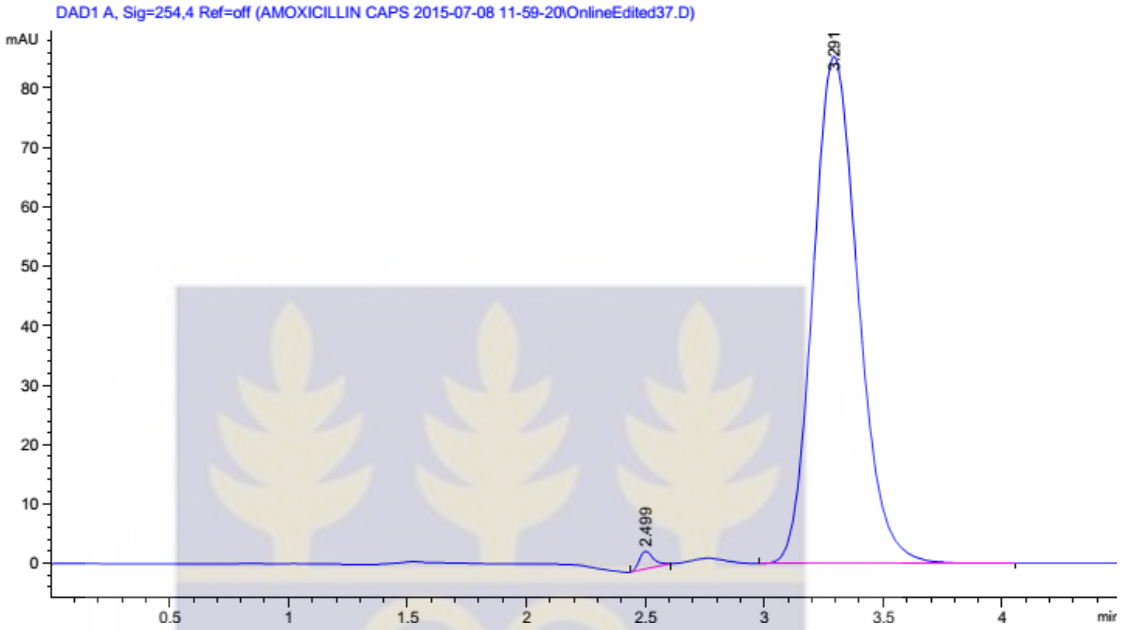
APPENDIX R1: Dissolution Chromatogram For Foreign Generic Amoxicillin (VESSEL 1)



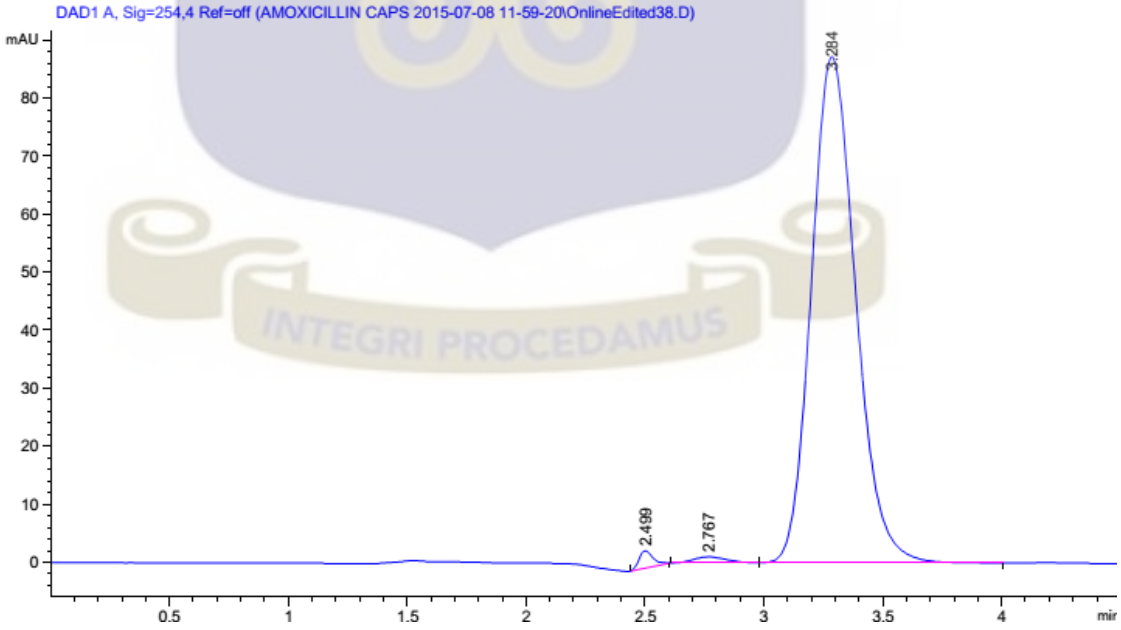
APPENDIX R2: Dissolution Chromatogram For Foreign Generic Amoxicillin (VESSEL 1)



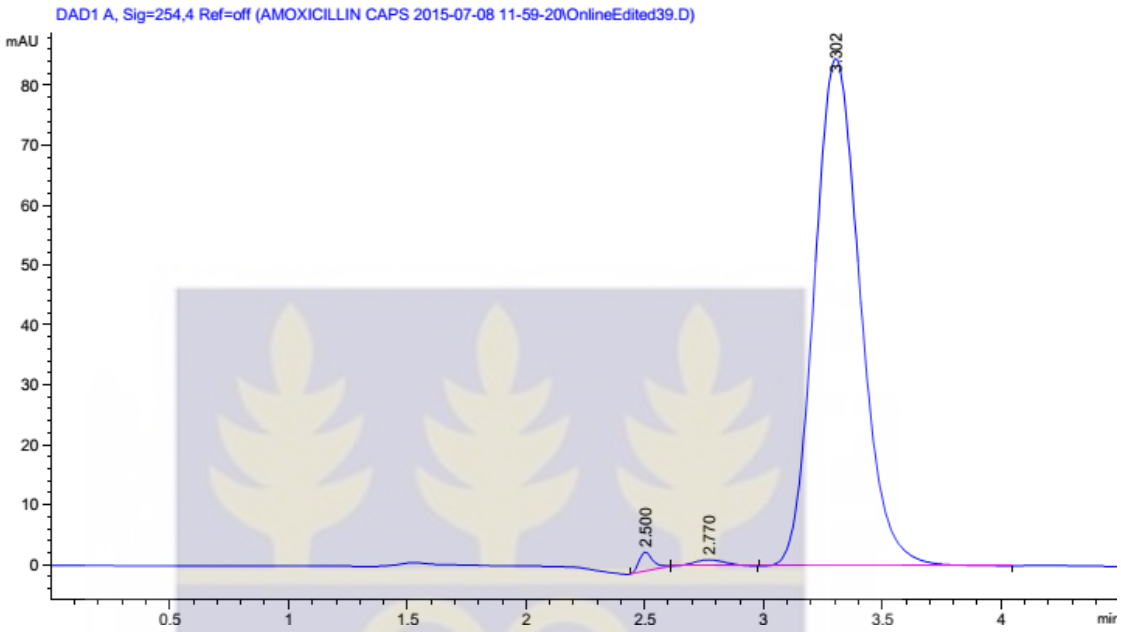
APPENDIX S1: Dissolution Chromatogram For Foreign Generic Amoxicillin (VESSEL 2)



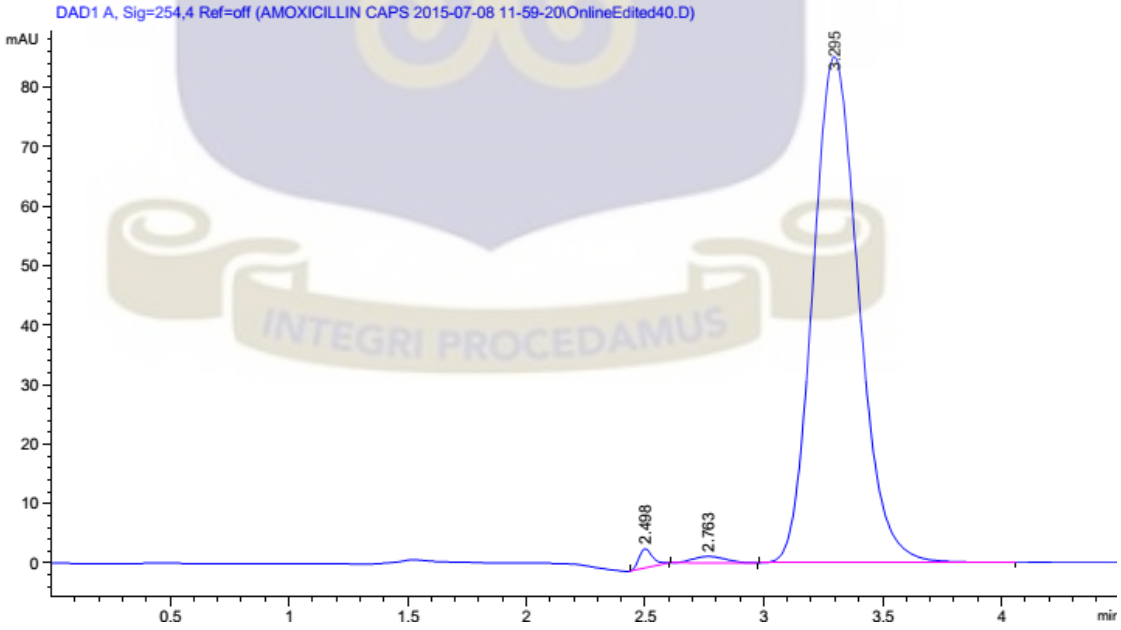
APPENDIX S2: Dissolution Chromatogram For Foreign Generic Amoxicillin (VESSEL 2)



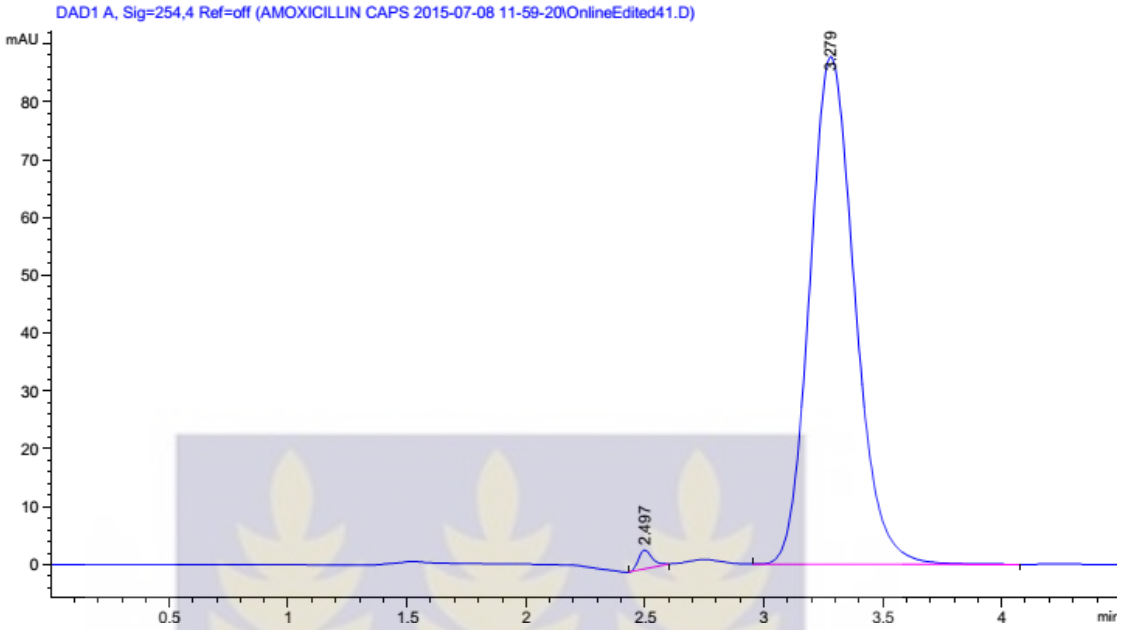
APPENDIX T1: Dissolution Chromatogram For Foreign Generic Amoxicillin (VESSEL 3)



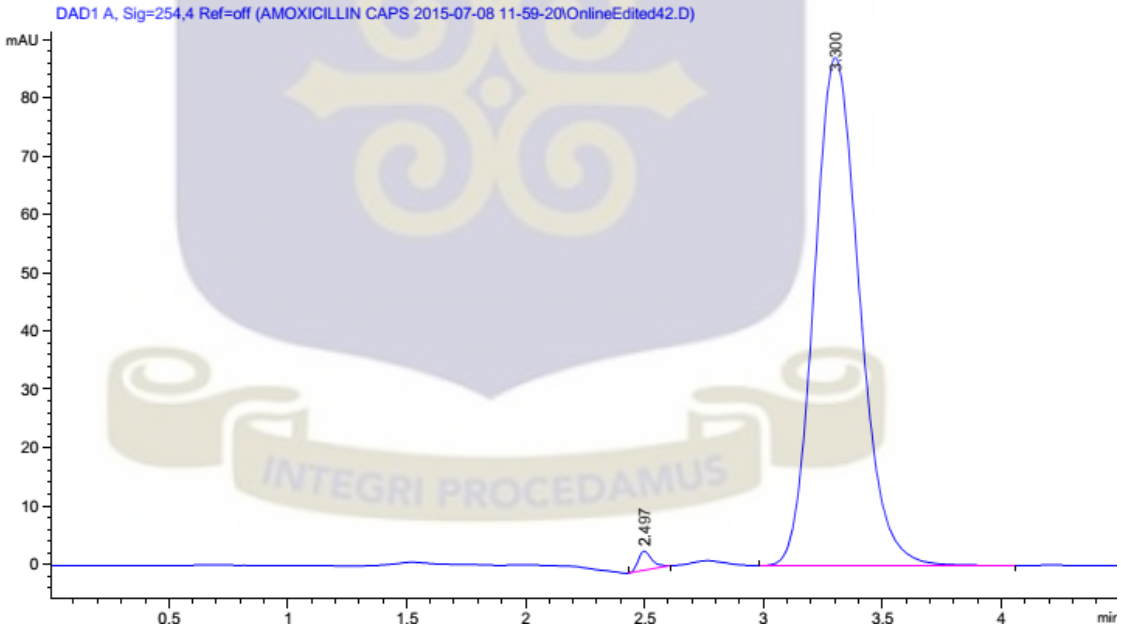
APPENDIX T2: Dissolution Chromatogram For Foreign Generic Amoxicillin (VESSEL 3)



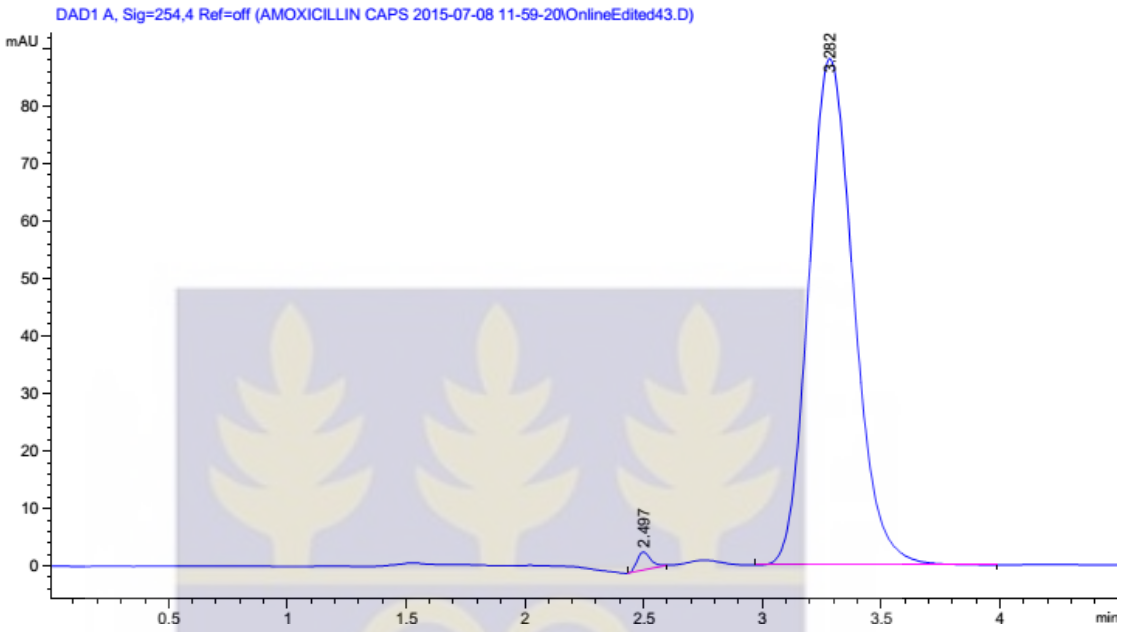
APPENDIX U1: Dissolution Chromatogram For Foreign Generic Amoxicillin (VESSEL 4)



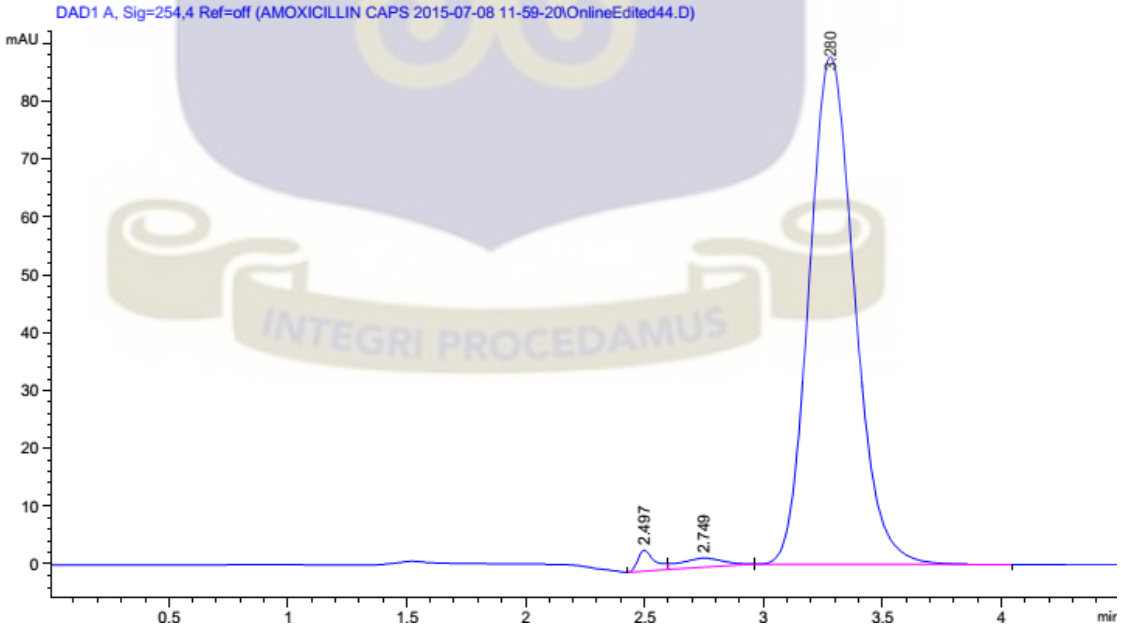
APPENDIX U2: Dissolution Chromatogram For Foreign Generic Amoxicillin (VESSEL 4)



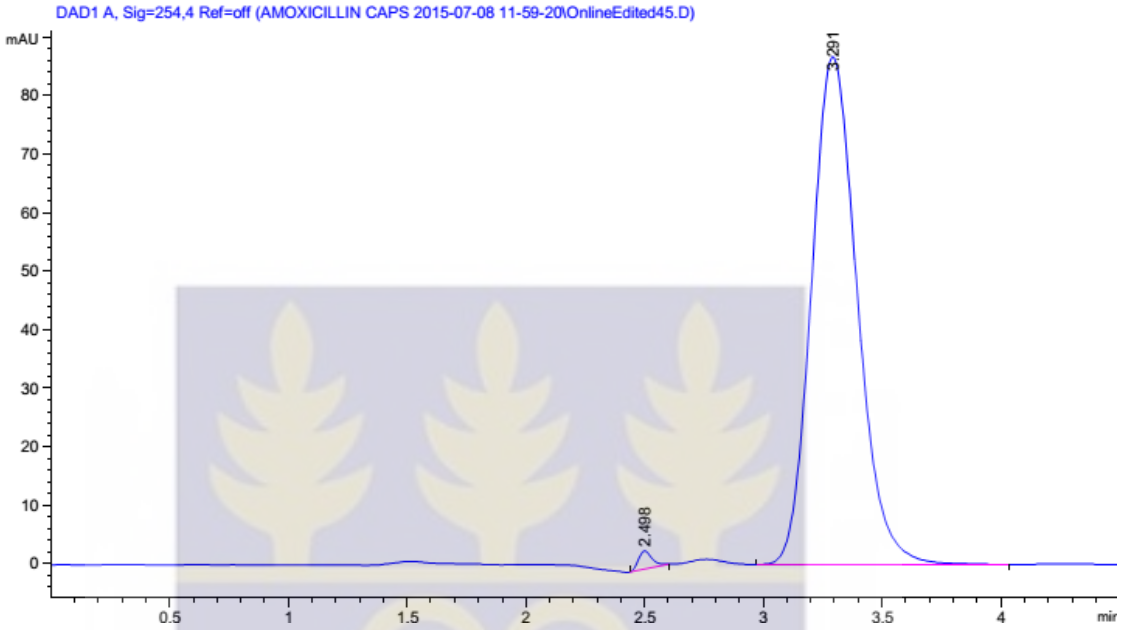
APPENDIX V1: Dissolution Chromatogram For Foreign Generic Amoxicillin (VESSEL 5)



APPENDIX V2: Dissolution Chromatogram For Foreign Generic Amoxicillin (VESSEL 5)



APPENDIX W1: Dissolution Chromatogram For Foreign Generic Amoxicillin (VESSEL 6)



APPENDIX W2: Dissolution Chromatogram For Foreign Generic Amoxicillin (VESSEL 6)