INORGANIC PROFILING OF AMOXICILLIN DRUGS IN GHANA USING PROTON INDUCED X-RAY EMISSION (PIXE) ANALYSIS

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ABDUL-WAHAB ZURIKA

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DECLARATION

I, ABDUL-WAHAB ZURIKA, hereby declare that with the exception of references to other people's work which have been duly acknowledged, this thesis is the result of my own research work and no part of it has been presented for another degree at this University or elsewhere. I also declare that the preparation of this project was supervised in accordance with the guidelines of supervision of Thesis work laid down by the University of Ghana.

…….. ……

ABDUL-WAHAB ZURIKA Date

(Candidate)

…….. …….

REV. DR. S. AKOTO BAMFORD DR. JOSEPH B TANDOH

(PRINCIPAL SUPERVISOR) (CO-SUPERVISOR)

…… ……

Date Date

DEDICATION

This research work is dedicated to my parents, ABDUL-SALAM ABDUL-WAHAB & ABDUL-SALAM ZULFATU; my wife MEMUNATU; my children MUHAMMAD MUINUDEEN and MUBIN; and all my friends. May Allah richly bless you all.

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LIST OF ABBREVIATIONS

API	Active Pharmaceutical Ingredient
ATCC	Anatomical Therapeutic Chemical Classification System
BP	British Pharmacopoeia
cGMP	compliance with Current Good Manufacturing Practices
FDA	Food and Drugs Authority
GDP	Good Distribution Practices
GLP	Good Laboratory Practices
GSP	Good Storage Practices
GUPIX	Guelph Pixe
HPLC	High-Performance Liquid Chromatography
IRACM	Institute of Research Against Counterfeit Medicines
KNUST	Kwame Nkrumah University of Science and Technology
LC-MS	Liquid Chromatography-Mass Spectrometric
NAFDAC	National Agency for Food and Drug Administration and Control
NHIS	National Health Insurance Scheme
NIR	Near-Infrared Spectroscopy
NMRI	Nuclear Magnetic Resonance Imaging
OOS	Out of Specification
PIXE	Proton Induced X-ray Emission
SOP	Standard Operating Procedures
TLC	Thin Layer Chromatography
USAID	US Agency for International Development
USD	United States Dollar
USP	United States Pharmacopoeia
WWTP	Wastewater Treatment Plant
XRD	X-ray Diffraction

ABSTRACT

The increase of drug counterfeits and its unconscious use has become a major cause for concern to healthcare practitioners and relevant stakeholders. The occurrence of counterfeit or fake drugs is perceived to be a problem encountered in both developing and underdeveloped nations where Ghana is not an exemption. The lethal implications of counterfeit/fake medications are well understood to be a major challenge to the soundness of public health systems around the world, as well as a direct threat to our individual health and well-being. Sub-standard and counterfeit/fake drugs are a widespread problem in Ghana and the need to address it is eminent. The volume of drugs that require control, from the statutory organisations like Food and Drugs Authority of Ghana (FDA) and Ghana Standard Authority (GSA) is enormous, and hence the need to explore other faster analytical techniques to help control cannot be over emphasised. According to the World Health Organization (WHO), Antibiotics are the most counterfeited drugs and Amoxicillin ($C_{16}H_{19}N_3O_5S$) happened to be ranked first on the list. The most used and prescribed method for drug quality control analysis is the High-Performance Liquid Chromatography (HPLC) technique which accesses the quality of drugs from its Active Principal Ingredient (API) perspective. The main focus of this study is to harness additional analytical procedure to enhance the routine monitoring of the quality of some Amoxicillin drugs in Ghana from the inorganic constituent point of view. HPLC and the physical parameter tests were carried out on the samples analysed to help validate the interpretation of the inorganic element results from the PIXE technique. Two different local brands of amoxicillin and two imported amoxicillin brands were chosen for this study. A total 30 samples were analysed for this study including one (1) standard reference material (amoxicillin) acquired from a licenced pharmaceutical company in Ghana. Particle Induced X-ray Emission (PIXE)

analysis of the amoxicillin standard reference material revealed ten (10) elements which include Al, S, K, Ca, V, Cr, Mn, Fe, Ni, and Zn. Sulphur was identified as the major element in the standard reference material and recorded concentration value of 396,805 ppm. Samples from "Kwame Nkrumah Circle", a locally manufactured brand recorded the lowest sulphur concentrations of 251,745 ppm, compared to all drugs analysed using PIXE; imported amoxicillin (395,753 ppm) and "Okaishi" locally manufactured (384,710 ppm). Additionally, the elements Cr, Mn and Fe were found to be relatively higher in the "Kwame Nkrumah Circle" sample hence raising concerns over the quality of the "Kwame Nkrumah Circle" brand. When a physical parameter test was performed on all samples, all brands with the exception of "Kwame Nkrumah Circle" passed the disintegration and dissolution test deeming the "Kwame Nkrumah Circe" brand substandard. After an HPLC test was performed for all samples, it was discovered that all the drugs analysed passed the acceptance criteria (90-120%) for drugs set by the British Pharmacopeia; with the "Kwame Nkrumah Circle" brand recording a value of 97% relatively lower than all the other samples which range from 113 to 117%. Sulphur as an active principle ingredient was correlated with the other identified elements. Strong correlations with correlation coefficients within the range 0.9936 – 0.9978 were found with the elements Cr, Mn, and Fe. Hence the elements S, Cr, Mn, and Fe lend themselves as element signature for amoxicillin medical drug. This was done to discover element signatures that will facilitate differentiating between genuine and counterfeit amoxicillin drugs. Considering the simplicity in sample preparation, the non-distractive nature of PIXE analysis, and also fast turn out in producing results. PIXE offers a considerable advantage when analysing antibiotic drugs on a large scale.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

The proliferation of drug counterfeits and its oblivious use has become a major cause for concern to healthcare practitioners and relevant stakeholders. The purpose and significance of medicinal products and its awareness to individuals has not been emphasised adequately. These products have to be safe, fairly good quality and rationally used to induce the desired change (Geneva, 1999).

One-third (1/3) of the global population does not have adequate access to essential medicinal products (WHO, 2008). Also, in developing nations like India, where almost more than 40% of the population returns for services rendered is less than 1 USD per day, this makes it extremely challenging to purchase the expensive medicinal products by the general public (Bate et al., 2011). However, in such situations, the lawbreakers engage in spurious or false drugs business without difficulty since they provide the market with more affordable drugs.

A counterfeit/fake drug is a pharmaceutical product which is produced and sold with the intent to fraudulently portray its origin and genuineness. It may contain incorrect amounts of active components, may be inappropriately processed within the body or may contain components that are not on the brand, and is often sold with mistaken, incorrect, or fake parcelling and labelling (Gupta et al., 2012). Virtually all medicinal products are counterfeited or faked, but the most frequently faked or counterfeited pharmaceutical products in advanced countries were, costly lifestyle medicinal drugs, like pills for erectile dysfunction, antihistamines, steroids and hormones (Chika et al.,

2011) ; Gupta et al., 2012). All the same, in underdeveloped nations the most frequently faked or counterfeited pharmaceutical products are those which are used to treat life-threatening or impending situations such as HIV/AIDS, tuberculosis, malaria, cancer and, various antibiotics (Singhal et al., 2012).

The WHO in 985 first exposed the threat of counterfeit pharmaceutical products as becoming a widely known problem. But, since then this challenge has grown rapidly to a much considerable level with more than 10% of pharmaceutical drugs in the entire world being counterfeit/fake, and in some states the condition is even worse with up to about 50% of the medicinal product supply being counterfeit/fake (Gupta et al., 2012). The WHO also estimates that 30% of pharmaceutical products in circulation in developing nations are counterfeit/fake (GSMA, 2012). The outcome of the basic quality samples conducted by the WHO on pharmaceutical products in circulation in parts of Africa, Asia, and Latin America demonstrated that up to about 40% of them are fake/counterfeit (Mathur, 2011).

A pharmaceutical product is of high quality when it meets the effectiveness and safe keeping benchmark claimed by the manufacturers and as published in Pharmacopoeias, and if it also matches the specifications established for it by World Health Organisation (WHO) and other appropriate authorities. The suitability of medicinal products for their goal is determined by their effectiveness, safety and their accordance with specifications relating to identity, lack of harmful substance (purity), potency, and other features (Gorog, 2008 ; WHO, 2013).

However, the quality of pharmaceutical products is determined by the multiple of exercises which happen throughout its life cycle (i.e. from acquisition and handling of

raw resources or materials through the manufacturing process, packaging, transportation to storage). More so, some noted additions are the value of equipment used and manufacturing environment (Chioma Joy Onwuka, 2010).

Moreover, making sure of the safe keeping, effectiveness and value of pharmaceutical products throughout their lifecycle, the consistency in their batches and even over every amount of medicine taken (dose), rest in carefully obeying principles adherence to Standard Operating Procedures (SOP), compliance with Current Good Manufacturing Practices (cGMP), Good Laboratory Practices (GLP), Good Distribution Practices (GDP) and Good Storage Practices (GSP). According to United States Pharmacopoeia (USP), the quality of drugs is said to be poor when it does not meet official specifications for strength, quality, purity, packaging or labelling (European Agency, 2011; UNOPS, 2012).

Underdeveloped nations are not absolutely the victims of the problem but also serve as the seedbeds of fake or counterfeit pharmaceutical products with India and China being the biggest offenders globally (Bate R, 2007). An estimate by the European Commission portrayed India as the seedbeds of 75% of fake or counterfeit pharmaceutical products and according to one narration; the majority of the fake or counterfeit pharmaceutical products in Nigerian markets originated from India (Raufu, 2003).

The needful qualities of medicinal products can be grouped into three distinct main brands: Counterfeit/fake, substandard or degraded. These have been linked to chemical anxiety particularly in tropical environments, poor quality assertion and control during

production, distribution, and storage; and counterfeiting (Theodore et al., 2007 ; American Chemical Society, 2012).

WHO (1999) states that, Counterfeit / Falsified / Falsely-Labelled/ Spurious (CFFS) medicinal products (branded or generic) can be termed as "any pharmaceutical products that are intentionally and fraudulently mislabelled for identity and/or source". Substandard medications which are also called Out of Specification (OOS) products are those that are genuine and legitimately produced but fall outside the specifications or acceptance criteria established in product dossiers, medications master files, pharmacopoeias, by the producer and manufacturer. A dissipated drug can be categorised along with the inferior drug. However, they vary in that they might be original of the specification, but in the course of time, they are naturally catalysed by foreign factors, which eventually fall out of specification within its shelf life (Hartigan-Go, 2007a ; WHO, 2013).

Generally, the substandard drug may not be thought of as being counterfeit if only it is not aimed at misleading but all counterfeit/fake medications are considered as substandard drugs (Reggi, 2007).

Malaria treatment in Africa has been under serious threats for several decades and intends to be controlled by substandard and fake antimalarial medication (Nordqvist, 2012). According to World Health Organization (WHO), 64% of antimalarial products imported into Nigeria in 2011 were found to be substandard and fake. The largest market for pharmaceutical products in Africa is Nigeria and up to about 70% of its drugs are from China and India. These two countries are major source of substandard and fakes medicines (Medicine, 2012).

Most antibiotics used for treatments in underdeveloped countries are counterfeit and substandard, the "old" antibiotics comparable is sulfamethoxazole, beta-lactams, trimethoprim, chloramphenicol, and tetracycline; these fake antibiotic drugs used affects the current generation (Wondemagegnehu, 2002).

The list of drugs stated by WHO in 2010, of which 47 were antibiotics, amoxicillin happened to be the first on the list with the highest fake active ingredient on earth (http://www.bdsp.ehesp.fr/Base). The class of antibiotics like erythromycin, amoxicillin, and doxycycline are mostly not genuine by the counterfeit market in India (Mayer et al, 2011).

The link between substandard/counterfeit drugs and antibiotic resistance is twofold. One, due to the rampant nature of counterfeit/fake medicinal products in the developing countries and to the knowledge that one has to keep heed to the drawbacks of invalid reports of antimicrobial wilfulness in an area where drug substandard/counterfeiting is far-reaching (Rozendaal, 2000).

The most faked/counterfeited pharmaceutical product is antibiotics, representing about 28% of the fake/counterfeit medicinal products globally. About 5% of fake/counterfeit antibiotic drugs are evaluated in general from the antibiotic market. There is no area that seems to be nursed from substandard/counterfeiting of antibiotics in the world. Nonetheless, antibiotics are in increasing demand in most of the under developed countries which pave way for counterfeiters to be badly captivating in the market, but the demand for counterfeit antibiotics in developed countries is very minimal. (http://www.sceincedirect.com/science/art) Consequently, South-East Asia accounts for about 78 % of the fake/ counterfeit antibiotics and the major countries of supply have the following percentages (Europe and North America: 9%; sub-Saharan Africa:

30%; South-East Asia: 44%; and the Saharan Africa: 17%) (Delepierre et al., 2012). Counterfeit antibiotics have been commonly used for years (beta-lactams: 50%; quinolones: 12%; macrolides, lincosamides, and synergistins: 1%; cyclins: 7%; and the rest recorded: 20%) (Delepierre et al., 2012). The greatest fake and substandard drug expressions are (77%) pertaining oral administration (, syrup, capsules, tablets) whiles injected drugs account for 17%, and, ointments and eye drop 6%. Antibiotics counterfeiting is somehow comparable to certain kinds of medicines (counterfeit packaging: 5%; poor quality: 24%; inaccurate Active ingredients: 7%; none Active ingredients: 43%; and an inadequate amount of Active Ingredients: 21%;) (International Narcotics Control Board, 2009). Besides the adverse results of patients, the resistance of bacteria to antibiotics is due to the chunk of fake and substandard pharmaceutical products which has a bigger impact on the individuals and the world at large. The combat against fake/counterfeit drugs globally has been in existence as long as 1985 (Delepierre et al., 2012).

According to Digital Journal, the Institute of Research Against Counterfeit Medicines (IRACM) and the World Health Organization (WHO) made a statement about the possible danger the health or well-being and safety of African population was made which indicated that in a mission carried out in April 2013 in twenty-three (23) African nations including Ghana and Nigeria, over 500 million doses of counterfeit or fake pharmaceutical drugs worth about 275 million US Dollars were interdicted (Journal, 2013).

The medicinal products interdicted included Antibiotics, painkillers, anti-inflammatory drugs, medications for high blood pressure and diabetes and food addendum/supplements (Journal, 2013). Also, the Guardian newspaper (Ghana) of December 23, 2012, reported an issue on the vulnerability of lives in Africa and the

risks posed by the individuals by the treatment of diseases like malaria with counterfeit/fake pharmaceutical products imported from Asia. Some of these pharmaceutical products are reported to have no active pharmaceutical elements and ingredients (G. News, 2012).

Counterfeit medications are of significant threat to global public health. More than 700,000 deaths were recorded in 2012 through the use of fake/counterfeit anti-tuberculosis and anti-malaria medicines. There is an immense global demand for drugs, which are usually much more costly than their counterfeit equivalents. That demand, in combination with the moderately low production cost of counterfeits and potentially poor regulation in undeveloped and developing countries, creates a huge market for fake or counterfeit drugs. This makes the counterfeit drug production and trading a highly profitable, and of low-risk business (WHO, 2006).

The WHO showed that anti-infective agents accounted for up to about one-third of all counterfeit therapeutics between 1999 and 2002; and more than a quarter of all the counterfeit therapeutics were antibiotics (Mohammad Irhimeh, 2013).

The application of fake anti-infectious medicines has catastrophic effects with even default in treatment, the resistance of antimicrobial development and in the most detrimental cases, toxic ingredients may lead to patient death. In some instances, the harmful ingredient contained in a counterfeit drug may cause allergic reactions (Wilson, 2009). In addition, the downstream effects also include macro economic pandemics, and loss of confidence from medical professionals in the medications they prescribe (Wertheimer, 2009).

Economic losses as a result of drug counterfeiting are enormous and appear to be increasing annually. In 2004 around US$ 32 billion was lost to fake and substandard

drugs patronage (WHO, 2006). This amount, however, increased to about US$ 40 billion in 2006 and was expected to exceed US$ 75 billion in 2010 (WHO, 2006; Bate & Boateng, 2007)). Quite a number of companies tumble as a result of numerous pharmaceutical companies being at a disadvantage of their deserved earnings due to the faithless matchup from this cruel offence (Akunyili, 2005).

According to Ghana's Food and Drugs Authority (FDA), about 70% of the country's pharmaceutical needs are imported. The inability to put on effective surveillance programme at the borders, airports and seaports has resulted in the influx of poor-quality pharmaceutical products whose quantities are even difficult to evaluate. Apart from the health consequences, such a heavy reliance on foreign pharmaceutical products also threatens to weaken the local pharmaceutical manufacturing companies. The automated media on many occasions provide anecdotal statements on the seizure of pharmaceutical products with compromised quality of the FDA (Guardian News, 2013 : Frimpong, 2014).

Two findings also show the presence of substandard and fake antibiotics on the Ghanaian market (Fadeyi et al., 2015).

The United States Pharmacopoeia (USP) and the WHO combined study on the certified and dissolution of antimalarial and anti-tuberculosis medications in six (6) West African countries in 2010 showed that anti-malarial and anti-tuberculosis drugs from Nigeria registered the biggest failure.

With estimation of over 70%, Ghana above 60% and Cameroon above 50%, Kenya and Tanzania had averagely low failure estimates whilst Ethiopia had all of its products appeared very good through the test (GhanaWeb, 2010).

Literature and reports show that lately, most researches and surveys on the value of pharmacological products in this part of the world have centred on antimalarial, antiretroviral and antituberculosis medications with little concentration given to other antimicrobials used for other infectious /contagious diseases. Not long ago the University of Ghana (Chemistry Department) embarked on a quality interview on antimalarial products distributed in Ghana, Togo, and Malawi. Comparable studies have also been accomplished in West Africa by other universities like Kwame Nkrumah University of Science and Technology (KNUST) and the World Health Organization (WHO) (GhanaWeb, 2010 ; Hess et al., 2010).

However, in Ghana the rate at which pharmaceutical stores operates pave way for fake and substandard drugs into the system. The models of data on antibiotics intake in Ghana is quite indeterminate and inadequate drug regulation, though antibiotics still remain the drug readily accessible in the health centres throughout the country and can easily be obtained without authorization from a qualified prescriber. More so, the general public finds it very demanding to purchase expensive drugs.

Donkor et al., (2012) shows that around 30% of the tertiary students in Accra take an antibiotic through their mouth. In addition to that 70% of persons who gave their response were self-medicated without any instructions

Coherently, amoxicillin, a wide spectrum antibiotic, about 50% of these antibiotics are acquired in many parts of Ghana without any prescription (Donkor et al., 2012).

Health care systems misplaced its self-esteem due to patient resistance in drugs, number of death recorded and the prevalence of fake and substandard drugs in the pharmaceutical industry.

People in underdeveloped countries are mostly at a disadvantage, and there are very little research and information on the counterfeits considering the magnitude of the problem which calls for analytical examination.

Most studies carried out have focused on the active principal ingredients of medicinal drug, which is from organic profile point of view. But however, this work seeks to pursue the investigations of inorganic profiling by providing an analytical tool for identifying fake amoxicillin antibiotic drugs

Some work has been initiated in the School of Nuclear and Allied Sciences (SNAS), but also focused on the organic ingredient of the Amoxicillin drug using NMRI and XRD.

A thorough search has been seriously active on how to unfold a smooth and fast system for ascertaining substandard drugs even though different approaches have been put in place in order to discover drugs that may be doubtable. The techniques/methods are classified from the easiest like simple thin layer chromatography (TLC) to more complex methods/techniques like Raman spectroscopy, liquid chromatography-mass spectrometric (LC-MS) and near-infrared spectroscopy (NIR) approaches.

1.2 PROBLEM STATEMENT

Substandard and counterfeit/fake drugs are a widespread problem in Ghana and the need to address it is eminent. The analytical technique employed by FDA and GSA are not comprehensive and focuses primarily on the organic components of the API, there is therefore the need to explore other supplementary and fast analytical techniques to help control these drugs. The current problems can be attributed to the rather few analytical tools to test for testing the required ingredients in drugs.

1.3 RESEARCH OBJECTIVES

1.3.1 Main Objective

- To harness additional analytical procedures/techniques that will contribute towards routine monitoring of the quality of amoxicillin drugs, on the Ghana market.

1.3.2 Specific Objectives

- To provide an inorganic profile of pharmaceutical constituent in selected branded and generic amoxicillin drugs on the Ghanaian Market, through identification of element constituents in drugs.
- To evolve element signatures that will facilitate differentiating between genuine and counterfeit amoxicillin drugs by factor analysis.

1.4 RELEVANCE AND JUSTIFICATION

The lethal implications of counterfeit/fake medications are well understood to be a major challenge to the soundness of public health systems around the world, as well as a direct threat to our individual health and well-being.

Most studies carried out to investigate the quality of antibiotics have focused on the active principal ingredients of the medicinal drug (organic profile view point), totally ignoring the inorganic components (the elemental composition). Elemental signatures in drugs can be beneficial or harmful to a consumer depending on its levels in the drug, the binders, colouring and sometimes the active ingredients of drugs can contain elemental signatures which can be useful for drug quality monitoring.

This work seeks to use the inorganic components of drugs to identify the quality of drugs. Ion beam techniques which present a faster and a more suitable alternative will be developed and validated for this purpose.

This additional procedure will assist the FDA and the GSA in quick monitoring of the quality of antibiotics on the market

NMRI and XRD analytical technique have been used for similar studies at the Graduate School of Nuclear and Allied Sciences.

1.5 SCOPE AND DEFINITION

The main focus of this study is to add an analytical method to the already existing ones in drug quality monitoring, which focuses on the inorganic elemental composition. Particle Induced X-ray Emission technique was the main tool used for this study and was validated by the HPLC method. The sample drugs used for this study were only limited to amoxicillin capsules purchased in some selected pharmacies and chemical shops market in Accra the capital city of Ghana. Five (5) different brands of amoxicillin capsules were taken into consideration.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

There are different kinds of techniques/methods used to analyse substandard and counterfeit pharmaceutical drugs. These include chemical tests such as dissolution and colorimetry methods, visual inspection comprehends product and packaging, tests for physical properties such as breakdown (disintegration), assays or deconstructions, reflectance spectroscopy, and refractive index. Distinguishable methods/techniques also include chromatography techniques such as TLC, HPLC and LC-MS, spectroscopic techniques such as NIR and Raman and mass spectrometry. Owing to the inadequate control system of the pharmaceutical products in the market and insufficient categorise regulations in developing countries, affordable, readily, safe and quick techniques/methods of analysing drugs is a necessity.

To detect substandard, falsified and counterfeit pharmaceutical drugs novel technologies are developed to adhere to this canker.

Therefore, new technologies including X-ray powder diffraction and near infrared spectroscopy methods have been progressively used for the detection of counterfeit/falsified antimicrobials drugs.

For medicinal or drug samples that have been confirmed to be a counterfeited or faked or falsified drug, isotope ratio MS, X-ray Diffraction (XRD), and nuclear magnetic resonance (NMR) are used to assist in finding the geographical root of the product of the spurious and falsified pharmaceutical products for forensic purposes. The proton induced x-ray emission (PIXE) is the considerable analytical method that is used in these research findings.

2.2 Poor Quality Medicines

Studies have indicated that there are three main classifications of poor quality drugs (substandard, fake/counterfeit and degraded), these, however, have been explained in Chapter one. Even though they are used in such an approach that they are not easily distinguished, their features go beyond regards in their distinctiveness. These features are listed and discussed below:

2.2.1 Substandard Medicines

Substandard drugs can be grouped as those legitimately generic or branded products that do not stumble the official standards for identity, quality, purity, strength, packing, and labelling. However, the precise quantity of APIs is the defining features of this classification since it exceeds by excess amounts of ± 15. This can be an issue in relating to methods of production which might be intentionally (to increase profit), non-deference to SOP, cGMP or due to apparatus error or malfunctioning, or can be developed at any aspect of the medicinal range due to mishandling. Degradation can also be a factor (Lukulay, 2009 ; American Chemical Society, 2012).

2.2.2 Degraded Medicines

Degraded drugs are considered to be a section of substandard drugs which could have been manufactured to meet the specification deteriorating chemically. The rate of degradation rate can be affiliated to the strength of the product which is decided by many factors, these include the qualities of the raw materials used, the storage facilities, the distribution conditions and the climate conditions (light, humidity and ambient temperature) in which the drugs are stored. Degradation can occur at any point in the

advancement of a medicinal product, from gaining raw material to consumption of the finished product by the patient. Poor storage at depots usually leads to the degradation of most drugs in which case the drugs begin to lose their biological activities earlier before the expiry dates on their labels. This is a difficult issue in the underdeveloped nations in the tropics where humidity and temperature are high throughout the year. This type of climate enhances chemical deterioration and can also dent the biopharmaceutical properties of the drugs. Depository conditions of drugs at home by consumers can also be a contributing factor to degradation (Theodore et al., 2007 ; Hartigan-Go, 2007b).

2.2.3 Counterfeit Medicines

The definition of what a counterfeit drug is, vary from one country to the other. This is because of how certain drugs are classified as counterfeit in some nations.

According to World Health Organization (WHO), ‘‘a counterfeit/fake drug is one which is deliberately and fraudulently mislabelled with respect to identity and/or source’’ (WHO, 2017).

The counterfeit/fake drug is applied to both generic and brand medications. Counterfeit drugs may include drugs with the wrong ingredients, without active ingredients, with incorrect quantities of active ingredients or with fake parcelling or packaging.

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

The levels at which substandard and counterfeit drugs are commonly sold to individuals and the world at large is very terrifying.

Pharmaceutical Authorities claim that some Indian drug manufacturers cut corners and make substandard drugs with no active ingredients for markets mostly in African countries.

The quality of unidentified drugs that were sold in countries like India, most Africa countries and five mid-income non-African were found, from the 1470 antibiotics and tuberculosis drug samples that claimed to be made from India, have 10.9% of that product failed the fundamental imposition of Active pharmaceutical ingredients (API) out of which 7% were substandard with failures due to incorrect API amount. These substandard were not erratically distributed, they were more probable to be found as unregistered products in Africa than in India or non-African countries.

Once this finding is wholesome for manufactures-drug fixed effects, one likely explanation is that Indian pharmaceutical firms and/or their export intermediaries do indeed distinguish pharmaceutical drug qualities that are in conformity with the destination of consumption (Roger et al., 2014).

The periodical crises in the availability of antimicrobials, which are comparable to fake and substandard cinchona in the 1600s and fake/counterfeit quinine in the 1800s, has been an enduring problem for poor quality pharmaceuticals throughout history. The challenge seems to have to thrive for the last past decades which is rather unfortunate, specifically causing pain and suffering to those searching medications that are prepared and sold online and patients that are unquestionable. The difficulties related to the combat in resistance to poor quality pharmaceutical products, substandard and counterfeit drugs, particularly with an emphasis on the analytical equipment obtained,

their performance comparatively and the workflows that are mandatory which is required for differentiating between certified, inferior, degraded and fake pharmaceutical products (Buckley, 2013).

The public health challenges that Poor-quality pharmaceuticals products pose a serious effect on the individual's welfare, most especially underdeveloped nations and their struggling economy which already have a significant influence relating to the medical treatment that is given to patients in hospitals, clinics and economic responsibility. Fake/Falsified medications are given serious care for the reason been that they are largely focused payable to the rate at which they are deliberately in control and accessible, but patients are prone to substandard medications for the reason that poor quality-control practices and manufacturing in the procedure of making of certified pharmaceutical products (either branded or generic).

2.3 MEDICINAL PRODUCT

The medicinal product which can surely be termed as the pharmaceutical drug is a medicine used to cure, diagnose, prevent and treat diseases.

European Union Directive (Directive 2011/62/EU) defined "medicinal product" “as any substance/combination of substances presented as having properties for treating and preventing disease in human beings; or any substance/combination of substances which may be used in/administered to human beings either with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological and metabolic action, or to making a medical diagnosis” (Official Journal of the European Union 2004 and Scholz, 2015).

The United States also have their definition of the drug as follows:

- A substance whose constituent is known by the official pharmacopoeia.
- An ingredient intended for practice/use in examining, healing, mitigation or less severe, treatment and inhibiting of disease.
- A substance (other than food) set to upset the structure or any function of the body.
- A material anticipated for use as an element of a drug but not a means, part and accessory of a device.
- Biological samples are involved within this definition which they are mostly and naturally covered by the same rules and regulations, but disparities happening concerning their manufacturing approaches (chemical procedures versus biological procedures) (www.fda.gov ; Standards, 2011).

Pharmaceutical drugs can be categorised into the distinctive configuration. Usually, one of the notable processes of dividing the product is between trivial culture and small molecule remedies, but in most cases this is acquired from chemosynthesis, and the products from biopharmaceuticals, these comprises substance found in foods (such as beans, milk, eggs, and meat) that is an important part of human diet (recombinant proteins), injectable substance (vaccines), blood merchandises and products recycled therapeutically (such as IVIG), a way of treating disorders and diseases (gene therapy), single cell manufactured by the body to combat disease (monoclonal antibodies) and cell psychoanalysis/therapy (for example, stem-cell therapies). The mode of exploitation, the way of administration, biological/organic makeup system, method affected and the therapeutic effects are all characteristics used to categorise pharmaceutical products other than their prototype (original source). The Anatomical

Therapeutic Chemical Classification System (ATC system) is among the detailed and widely used taxonomy system. The list of important pharmaceutical products is kept by the WHO (Lei Chen et al., 2014 ; Bergström et al., 2014).

Drug discovery and its developments are demanding composite and expensive endeavours that are shouldered by pharmaceutical companies, research scientists, and governments. In many parts of the countries, the agencies from government mostly regulate the kinds of drugs to be marketed, the methodologies of marketing the drugs, and in certain situations the cost of the drugs. However, there are lots of controversies on the issues of drug pricing and the mode of discarding used drugs (Sujata S Jayawant and Rajesh Balkrishnan, 2005).

2.3.1 Classification of Medicinal Product

The Medicinal/Pharmaceutical drug is classified based on their origins. The following are the origins of some of the medicinal product:

i. Drug obtained from natural source, for example, Herbal or plant or mineral origin,
ii. Drug acquired from chemical synthesis.
iii. Drug attained from an animal source, for instance, hormones, and enzymes.
iv. A drug derived from the microbial origin, Antibiotics.
v. Drug acquired by biotechnology genetic-engineering, hybridoma method for example.
vi. Drug captured from radioactive substances/materials.

vii. Drug from chemical and biochemical as well as a natural origin that is obtained from incomplete herbal and fractional chemical synthesis, example steroidal drugs (U.S. FDA, 2015).

Conversely, the key classifications are among the traditional trivial portion drugs, mostly copied from chemical synthesis, and biological therapeutic products, which is made of recombinant proteins, vaccines, blood products used therapeutically (such as IVIG), gene therapy, and cell therapy (for instance, stem cell therapies).

Pharmaceutical products are arranged in many different forms other than their source (origin) on the idea of some pharmacological properties which corresponding to the methods of action and their pharmacological doings such as by chemical properties, method of administration, biological/organic system affected, and the therapeutic effects.

Pharmaceutical drugs play a very pivotal role in the well-being and health of individuals are therefore sampled in their various classes of medicines which include:

i. Antipyretics: reducing malaise or fever (pyrexia/paresis)
ii. Analgesics: reducing ache/pain (painkillers)
iii. Antimalarial medicines/drugs: treating malaria
iv. Antibiotics: impeding germ development and growth
v. Antiseptics: anticipation/prevention of germ development and growth near scalds, cuts, and wounds
vi. Mood/Attitude stabilisers: lithium and valpromide
vii. Hormone replacements: Premarin

viii. Oral contraceptives: Enovid, "biphasic" pill or tablet, and "triphasic" pill//capsule

ix. Stimulants: amphetamine, methylphenidate,

x. Tranquillizers/Anaesthetics: meprobamate, chlorpromazine, reserpine, chlordiazepoxide, diazepam, and alprazolam

xi. Statins: lovastatin, pravastatin, and simvastatin (Spatz I et al., 2013).

2.4 ANTIBIOTICS

Antibiotics are particles that destroy or stop the development and growth of microorganisms, as well as both germs and mildews. Antibiotics that destroy or kill bacteria are called "bactericidal" and those that stop the development and growth of sbacteria are called "bacteriostatic" (Michael et al., 2010).

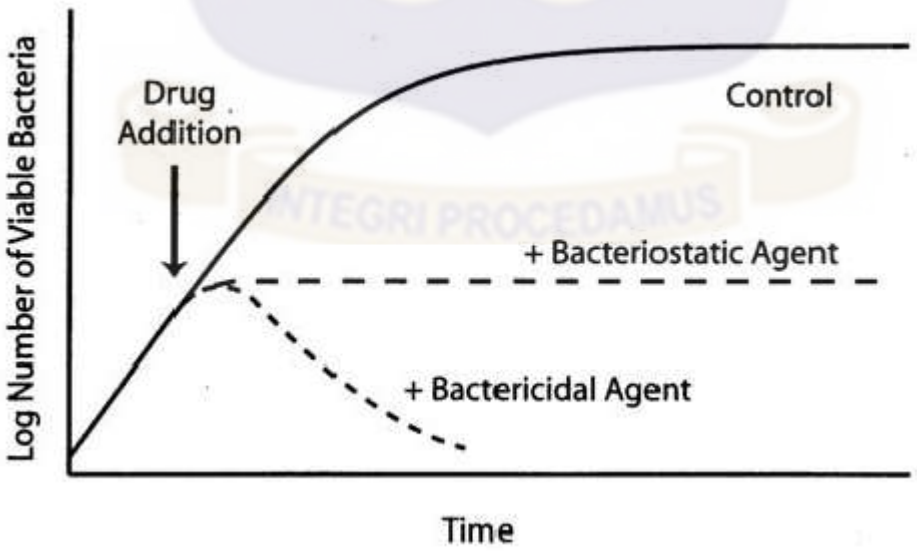

Figure 2.1 Effects of Bacteriostatic vrs Bactericidal Antibiotics on a Logarithmically Growing Bacterial Culture (From Scholar and Pratt [2000], with permission.)

2.4.1 Types of Bacteria

Danish scientist H. C. Gram devised a method of differentiating two categories of microbes based on their structural distinctiveness in their cell walls. In his test, bacteria that retain or continue to have the quartz violet peroxide do so because of the depth of peptidoglycan and are called Gram-positive bacteria. In contrast, Gram-negative bacteria that do not preserve the violet colourant are coloured red or pink. But by comparison, Gram-negative bacteria/microbes are more opposing against antibodies because of their impenetrable cell wall than Gram-positive. These bacteria have a variety of applications ranging from medical treatment to industrial use.

2.4.3 Gram-Negative Bacteria

Gram-negative bacteria cannot retain or continue to have the violet tint after the decolorization step; alcohol used in this stage shrinks the surface of the membrane of gram-negative cells making the cell wall more permeable and incapable of absorbing or maintaining the quartz violet stain. However, their peptidoglycan layer is more diluent and inserted between an inner cell membrane and bacterial outer membrane, enabling them to take up the counterstain (safranin or fuchsine) and appeared red or pink.

Despite their thicker peptidoglycan layer, gram-positive bacteria/microbes are more amenable to antibiotics than gram-negative, owing to the non-appearance of the outer sheath (Umeda et al.,1998 ; Madigan et al., 2006).

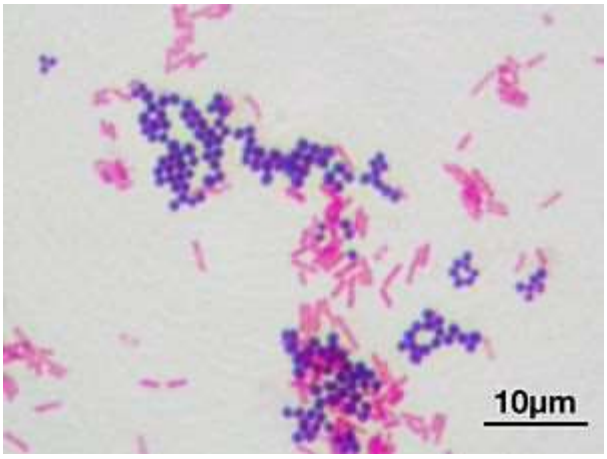

Figure 2. 2 : Violet stained Gram-positive cocci and pink stained gram-negative rod-shaped bacteria.

2.4.2 Gram-Positive Bacteria

Gram-positive bacteria/microbes are bacteria that exhibit a positive effect in the Gram tinge trail. Gram-positive bacteria/microbes take up the quartz violet tinge used in the trial and appeared to be purple colour when perceived through a microscope. This is the fact that the thick peptidoglycan layer in the bacterial/microbial cell wall preserves the pigment after being splashed away from the rest of the samples, in the decolourization stage of the trial (Umeda et al., 1998).

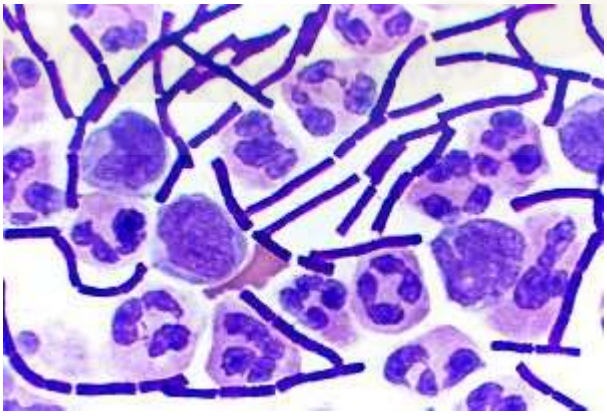

Figure 2.3: Rod-shaped gram-positive Bacillus anthracis bacteria in a cerebrospinal fluid sample stand out from round white blood cells.

2.5 CLASSIFICATION OF ANTIBIOTICS

Antibiotics were originally well-defined as materials produced by one microorganism, which impedes the development and growth of other microorganisms. The introduction of synthetic approaches has however resulted in modifying the definition and antibiotic now referred as a substance or material produced by the presence of a microorganism or to a similar substance, which in low concentrations impedes the development and growth of other microorganisms (Szczepanowski et al., 2009).Antibiotics are one class of antimicrobials that are relatively harmless to the host. They are small molecules with a molecular weight less than 2000 g/mol (Kaiser, 2009). The class of antibiotics includes:

2.5.1 B-Lactams

B-Lactams are the type of antibiotics that play a specific role as bacteriostatic by impeding the bacterial peptidoglycan cell wall synthesis (Feldman, 2004) and (Hulscher et al., 2010). Common examples of B-Lactams are cephalosporins and penicillins and their antibiotics spectrum are narrow, which is immensely productive against the Gram-positive genera Streptococcus, Staphylococcus and Gonococcus (Woodhead et al., 2005). The b-lactam drugs are highlighted in all the four-member ring which includes penicillin and its by-products (penams), cephalosporins (cephems), monobactams and carbapenems. These have enzymatic and chemical hydrolysis which is cyclic, extremely sensitive and replicated. In the course of the reaction when the ring is broken, the hydrolyzed B-lactam medications precipitate turn to be an inactive produce. B-lactam antibiotics like penicillin undergo disintegration when placed under alkaline and acidic conditions, via the feeble nucleophiles reactions, such as water and metal ions (Simoens et al., 2006). The discretion for acid hydrolysis is the same as

penicillin which can be enzymatically hydrolyzed through b-lactam enzyme. The enzymes that are predominantly in bacteria are the B-lactamases, and they are formed through the multiple of species that inactivate the pharmacological significance of the b-lactam antibiotics (Moore et al., 2008).

Penicillins

There are four (4) classifications or subgroups of penicillins. These includes:

1) Natural penicillins

Natural penicillins comprises of slim or narrow spectrum which contain gram positive and negative cocci (streptococci, pneumococci, enterococci, meningococci), gram-positive bands (corynebacteria, L.monocytogenes), spirochetes (Leptospira sp. Treponema sp. Borelia sp.) and almost entirely of anaerobes (peptostreptococci, clostridial species, Actinomyces).

Penicillin G or benzylpenicillin: This is unstable or unsteady in gastric juice and it is simply appropriate when it to be administered or dispense intravenously.

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

Procaine-penicillin: This is accessible in a depot form and it is to be administered intramuscularly, preferably once daily. It an antibiotic that fights bacteria your body. (https://www.drugs.com/mtm/procaine-penicillin.html).

Benzathinepenicillin: This is also obtainable in the form of a depot and creating a stable low level of antibiotic for 2 to 4 weeks. Hence it is futile for prophylaxis of

streptococcal reinfections. The dosage spectrum of penicillin is extremely widespread that is 1 mill .U. to 40 mills .U. daily for an adult person depending on the type and magnitude of infection. For comparison, 1,000.000 units equal 625 mg of penicillin. Streptococcal skin infections, pseudomembranous, tonsillitis, or animal nibble/bite and abrasions/scratches are the typical cases requiring low-dose penicillin treatment. For a large or high dose treatment, it is given to patients with endocarditis infection which are generated or caused by (viridian streptococci or enterococci), streptococcal, pneumococcal or meningococcal sepsis, and clostridial spiral/wound infection. (https://en.wikipedia.org/wiki/Benzathine-benzylpenicillin)

2) Antistaphylococcal penicillins

These types of penicillins are resistant to staphylococcal beta-lactamase but not to other beta-lactamases which are obtained through the creation of gram-negative microbes.

As long as the effect or outcome against gram- positive bacteria other than staphylococci is less effective as related to penicillin G, the drugs have a spectrum which is determinate in size. (Chambers et al., 2005).

Methicillin

Examples of methicillin include cloxacillin, nafcillin, oxacillin, and dicloxacillin. This is necessitating by administering through intramuscular or arterial/intravenous injection.

Aminopenicillins

These are medications which have their spectrum comparable to that of natural penicillin with drawing out against common Gram-negative bacteria like Escherichia

coli, Salmonella enteric. They are more productive than natural penicillin against enterococci and listeria.

Ampicillin: This is a medication administered through intramuscular or arterial/intravenous injection and it is tremendously important as a representative of the subgroup. (Chambers et al., 2005).

Amoxicillin: This is an antibiotic medication with better absorption after oral administration than ampicillin (that is 70-80% as equated to 40-50%). A least 2 to 12 g should be taken every day.

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

ampicillin + sulbactam

amoxicillin + clavulanic acid

These combinations are operative in contrast to the above-mentioned gram-negative microbes due to beta-lactamase and against Staphylococcus aureus. Also, these antibiotics are not needed and there must not be any prescription against enterococci, streptococci or bacteria which do not produce beta-lactamase. Clinically aminopenicillins with or without an inhibitor are used. These are given in bacterial sinusitis, mesotitis and lower respiratory tract infections, urinary and hepatobiliary tract infections, purulent gynaecological infections and other community-acquired infections. (Idsoe et al., 1968).

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

ticarcillin + clavulanic acid, piperacillin + tazobactam

Their usage is very similar to that of the basic drugs. (Fossieck et at., 974).

2.5.2 Sulfonamides

They are also known as sulfa /sulphonamide/sulpha drugs. They are bacteriostatic machinery that synergistically impedes the two pathway expedient in bacterial folic acid synthesis (Marzo et al., 1998 ; Todar, 2002). The bedrock of several arrays of drugs is sulpha drugs/sulphonamide (Aksu et al., 2005). But in the biosynthesis of bacterial DNA, the purines and pyrimidines in all the living cell with folate derivatives are simply the essential cofactors. Consequently, the obstructions in the production of reduced folates and eventual fusion of nucleic acid, in turns affects bacterial growth by blocking the pathway of all living cell. The combination of trimethoprim and sulphonamides provide an actual treatment against the diverse infections from potential bacteria. Sulfonamides do not alter their form completely (metabolised) in the usage process but rather excrete through urine into the sewage, in which parts are seen as metabolites and partly as unaffected parent compounds (HC, 1992 ; Mansters PA et al., 2003). The N4-acetylated products are naturally lethargic and they are the major metabolites of sulphonamides entering the sewage, in a reportage during sewage treatment, the

transformations are reverse to the energetic parent compound (Skold, 2001). The supposed negative eradication of certain sulphonamide may be coming from this sensation (phenomenon), particularly sulfamethoxazole, which is throughout the entire biological waste water treatment (Tilles, 200 ; Gobel et al., 2005). The most constantly identified sulphonamides in municipal sewage are the sulfamethoxazole (Karthikeyan et al., 2006).

2.5.3 Trimethoprim (TMP)

TMP is also a form antibiotic (bacteriostatic) used mainly in the prophylaxis and treatment of bladder (urinary tract) infections. Travelling diarrhoea and ear infections are other diseases that TMP can cure. TMP belong to the category of chemotherapeutic agents which is referred to as dihydrofolate reductase inhibitors. The action of bacterial dihydrofolate reductase is due to the TMP interference in which the tetrahydrofolic acid synthesis has been impeded. Countries like Mexico, USA, and Croatia (Yang et al., 2004) have a document which says TMP has been reported to transpire in the raw sewage. Sulfamethoxazole is closely connected to the manifestation of trimethoprim in general forms (Brown et al., 2006). It was found out that two drugs were dispensed frequently in combination at a ratio 1:5 (Eichhorn et al., 2005) and was also described in the primary effluent of a WWTP given that the concentration of trimethoprim was almost four times smaller than the sulfamethoxazole, which is relatively consistent with the typical medication ratio.

2.5.4 Nitroimidazoles

From chemistry viewpoint, the nitroimidazole antibiotics can be alienated into classes corresponding to the locus of the nitro functional group. The imidazole by-product

(derivative) that contains a nitro group is the 4-nitroimidazole. The class of nitroimidazole antibiotics is made up of several by-products of nitroimidazole that has been combating parasitic and anaerobic bacteria infections (Mital, 2009). The antibiotics broad spectrum are in eight (8) different relationship from the tetracyclines, these are bacteriostatic and also vigorous against Gram negative and Gram positive bacteria (Todar, 2002). The protein synthesis impeder is the tetracyclines which are used in the microorganisms for binding 30 S ribosome and also used to block the access to aminoacyl-tRNA to the acceptor site on the mRNA-ribosome complex (Marzo et al., 1998a).

2.5.5 Macrolides

The gentry (class) of natural products (commonly antibiotics) whose exertion or activity stems from the existence of a macrolide ring, which consists of a big macrocyclic lactone ring in that one or more deoxy sugars, prevailing dopamine and cladinose, may be inclined. The lactone rings are conventionally 14, 15-16 membered. The polyketide is a class (genre) of natural products where macrolides belong. Macrolides antibiotics include Azithromycin, fidaxomicin, erythromycin, clarithromycin etc, but erythromycin is very active against many Gram-positive bacteria by binding reversibly to 50 S ribosomal submits and synthesis impedes in a microorganism (Giguère et al., 2006). The antimicrobial spectrum of the penicillin is slightly slim compared to macrolides and for that the substitute for patients with penicillin allergy are macrolides. The unchanged forms at excretion rate rates more than 60% indicates that macrolides are largely excreted into sewage after being administered. In Switzerland, the concentration of macrolides in raw sewage varies between 0.01 and 0.6 mgL (Lindberg

et al., 2005 ; Connel, 2003) while WWTP influent in the USA has as high as the 1.5mgL1 concentration of the macrolides (Mital, 2009).

2.5.6 Fluoroquinolone

Fluoroquinolone includes ofloxacin (Floxin), ciprofloxacin (Cipro), norfloxacin (Noroxin), lomefloxacin (Factive), moxifloxacin (Avelox) and levofloxacin (Levaquin) which are used to treat illnesses like urinary tract (bladder) and respiratory infections. Fluoroquinolones are the class of antibiotics that are very effective in many different kinds of Gram-positive and Gram-negative bacteria (Lo¨ffler et al 2003). The purpose of the DNA production is impeded by essential enzyme performed by these antibiotics (Marzo, 1998b). In countries like USA, Australia, Sweden, Canada, Mexico, China, and Italy the happening of fluoroquinolones in WWTP effluents has been significantly reported (Nakazawa et al., 1995 ; Singh et al., 1998 ; Brooks et al., 2003). In the development of screening twelve (12) human antibiotics in five WWTPs in Sweden, above the analytical quantification limits which is the fluoroquinolones was established to be the most repeatedly detected antibiotics (Lindberg et al., 2005). In the study of fluoroquinolones, 97% of ciprofloxacin and norfloxacin and 50% ofloxacin were detected of the analysed samples.

2.5.7 Tetracycline

Tetracycline, which is also among the class of antibiotics is a comprehensive spectrum antibiotic with its general usefulness been reduced or concentrated with the inception of antibiotic resistance. That is made by the Streptomyces genus of Actinobacteria designated for use against many bacterial/microbial infections. Four (tetra)

hydrocarbon rings (-cycl-) derivation (ine) is the origin of the name. They are termed as a subclass of polyketides once they have an octa hydro tetracene-2-carboxamide skeleton (Laxminarayan, 2003). It is a protein synthesis impeder. Infections that are mostly treated with tetracyclines are a respiratory tract, urinary tract (bladder) and the intestine, chlamydia is also another infection that can be pickled with tetracycline. But the current most common use is the treatment of averagely severe rosacea and acne (tetracycline, oxytetracycline, doxycycline or minocycline) and also reducing the figure of deaths from cholera is one its historical importance. Protein synthesis is impeded by blocking the attachment of charged aminoacyl-tRNA. Which leads to the prevention of introducing new amino acids to the nascent peptide chain (Marzo et al., 1998b).But upon the removal of the drug, the action is still inhibitory and reversible. Resistance to the tetracycline fallouts from variations in permeability of the microbial/infectious cell envelope. The drug has been concentrated from the environment indicates that it is not ready to break the cell which occurs in most susceptible cells. In resistant cells, the drug is not actively carried out into the cell or breaks it so rapidly that inhibitory concentrations are not maintained. This is frequently plasmid-controlled. (Perez et al., 2005).

2.6 The Undesirable Consequences of Antibiotics

The unfavourable outcomes of Beta-lactams are decided on the least amount of concentration and also do not contain any venomous substance. For instance, the aggregate of substance in penicillin is at its ascendancy. The level of constituent produced by the body to combat disease (antibody) in the blood is demonstrated by questioning the allergies of cephalosporins or penicillins. The real allergy to any penicillin medication has to be explained as allergic to all penicillins. However, the allergy to a cephalosporin antibiotic does not predictably means that the allergy to all

distinctive cephalosporins. It is often not frequent, but the cross-allergy among cephalosporins and penicillins are a possibility. Furthermore, the allergy to cephalosporins in patients that are allergic to penicillins was evaluated to fall between the ranges of 5 to 10% as the probability. For this motive, cephalosporins can carefully be prescribed to patients with the record of past activities as a moderate penicillin allergy, which is referred to as exanthema. Also, the allergy to cephalosporins simply signifies that there is a huge probability of cross-allergy to penicillins. Newly born babies, breastfeeding women or pregnant women in developed stage can be ordered to use b-lactams. (Neu, 1969).

2.7 DISPOSAL OF ANTIBIOTICS

Beta-lactams are the utmost producing result used for the management of acute infections in a well-prefunded tissue or for the management of illness caused by an infection or contagion in a part of the body (sepsis).

Some quota of the medications is also suited for surgical prophylaxis. Dosing in a regular basis is needed in a directive to arrive at a desirable effect. Improving the individual doses requires serious attention if penetration to the place of infection becomes a problem. (Neu, 1969).

2.8 POOR QUALITY MEDICAMENTS IN GHANA

In West Africa, Ghana one of the countries with high economic activities but is not exempted from the continuing incident of fake/counterfeit and substandard medications. Suchlike Nigeria, the scaring levels of an incident of fake/counterfeit and substandard medications haven been ascertained and noted. According to the Food and Drugs Authority (FDA), the most ordinary faked or counterfeited medications in Ghana

are antibiotics, anti-malaria, anti-diabetics, and aphrodisiacs. More so, counterfeit local herbal products made of dangerous materials like brake fluid, sawdust and turpentine have been found or discovered in Ghana (Ghana Business News 2012). A 2013 publication of Ghana Business News reports an observation by FDA Ghana on the circulation of counterfeit medications in the country. These medications were identified as Mimet (ergometrine) tablets and Rox-Clav 625 tablets (a combination of amoxicillin and clavulanic acid) (GBN, 2013).

In 2008, it has been recorded that 35% of antimalarial medications imported in Ghana were fake and according to the research taken in the same year at Kwame Nkrumah University of Science and Technology (KNUST), 82% of artesunate sampled sold in Kumasi pharmacies were substandard.

However, two different studies were carried out in 2010 and 2011 by the Chemistry Department of University of Ghana, Legon on antimalarial medications in Ghana. The results of 2011 show that artesunate API content in all Artemisinin-based Combination Therapy (ACTs) and monotherapies antimalarial medications sampled were under-dosed while 87% of amodiaquine-containing medications analysed met the International Pharmacopoeia (Ph.Int) standard in terms of API content.

Thirteen (13) key antimalarial medications were found to be substandard and/or counterfeit or fake in Ghana 2008. This was revealed during the medicine monitoring cooperative program set up by Ghana FDA in collaboration with the US Pharmacopoeia Convention (USP) and the US Agency for International Development (USAID) (Viasat News, 2009 ; Phys.org, 2010).

Additionally, the FDA in collaboration with other affiliated department has not surrendered in their pursuit to control this life-threatening in Ghana. A group was recalled in 2010, after laboratory analysis of anti-malaria medications sampled from the

public and private hospitals, licensed pharmaceutical stores, retail pharmacies and wholesale facilities across the nation, batches like Quinine sulphate, Metakelfin tablets, and Artesunate tablets were all found to be counterfeit/fake. Substandard or Spurious antibiotics like Clavu-Dor (Amoxicillin 500 mg and Clavulanic Acid 125 mg) and Cipro-Dor (Ciprofloxacin Hydrochloride equivalent to 500 mg Ciprofloxacin) tablets were all recalled in the same year.

In 2011, it was also recalled that spurious or substandard Camoquin-Plus suspension manufactured by Pfizer were too much in Ghana (G. B. News, 2010 ; GNA, 2010).

A report by the Pharmacy Council of Ghana (PCG) states that there is a sale of fake malaria pharmaceutical products in the country. It was also found that during the routine checks of Food and Drugs Authority (Eastern and Volta Zonal Office branched) Artesunate tablets that were bought from Narrow Chemical Store was observed to have no any active pharmaceutical ingredients after the laboratory test (Owusu, 2011).

The issue of the fake Artesunate tablets in the market was made known to the public by FDA. Rox-Clav (Amoxicillin and Clavulanic) 652 tablets is a counterfeit antibiotic, with batch number BRT115 was also bought from the market but when tested it was realised it does not contain any Clavulanic acid, which deems the product fake.

"A combination of Amoxicillin and Clavulanic Acid is used in the treatment of severe infections, therefore, the absence of the Clavulanic acid makes the product dangerous because if such a counterfeit medicine is administered to a patient, it can lead to serious complications with fatal consequences" (Opuni, 2013).

2.9 REVIEW OF ANALYTICAL TECHNIQUE/METHOD

A report from South East (SE) Asia indicates that the prevalence and the regularities of counterfeit/fake drugs in most developing, as well as the underdeveloped countries, has been supposedly increased for the last decade. In SE Asia the antimalarial artesunate which is a vital drug has been counterfeited/faked on a voluminous scale. Raman spectroscopy was used for the ascertaining of counterfeit artesunate tablets, this was due to its fast and reliable screening technique. Fifty (50) artesunate tablets were purchased and examined using Raman spectroscopy. The spectroscopic technique was able to segregate between the genuine and fake/counterfeit artesunate and also distinguish the components of the counterfeit drugs. These, however, contained no determine points of artesunate, but rather made up of mostly starch, calcite ($CaCO_3$), and paracetamol (4-acetaminophen). In one particular circumstance an amalgamation of rutile (TiO_2) and artesunate was detected (Marleen de Veij et al., 2007).

Raman spectroscopy has been an instrumental method used repeatedly in the careful study of suspected fake/counterfeit product. Most of the pharmaceutical companies, as well as the forensic analysis, performed dissimilar kinds of analysis by using Raman spectroscopy (McCreery, 2005).

The vibrational spectroscopic knowledge acquired from a forensic selection using Raman spectroscopy is commendatory to the infrared (IR) spectroscopic facts that may be captured for an equal forensic selection. Although, the essential instrumental and sampling advantages of Raman versus IR spectroscopy in some circumstances, makes Raman outdistance IR in the examining of counterfeit/fake and suspect adulterated medicinal products (McCreery, 2005).

However, Raman spectroscopy provides distinct facts on the identification of analytes, the qualities of sample matrices, and molecular spectroscopic information useful in the structural explanation of unknowns (McCreery, 2005). The method is fast and when fused with sample preparation methodologies (i.e., micro-extractions, fraction collections, small particle analysis), a greater quantum of facts can be attained from a single fraction of forensic validation.

Pharmaceutical studies by using Raman spectroscopy is becoming a routinely approved approach, as demonstrated by the rising number of research papers that are published in this field. Raman spectroscopy has been used for the identification of active substances in pharmaceuticals, their qualitative and quantitative analysis, the characterization of different crystalline forms and even for online watching of the medicinal integrating process (Marleen de Veij et al., 2007). In addition to that, the identification of unlawful drugs (cocaine, heroin, and MDMA) that are undesirable with one or more cutting instruments that have been offered. More so, seized tablets can easily be identified using Raman spectroscopy (Marleen de Veij et al., 2007).

In the investigation of suspect fake/counterfeit medicinal dosage forms, Raman spectroscopy impact has significant advantages over other instrumental techniques. Raman allows for the qualitative identification of both API and excipients used to manufacture the product (PV, 1985 ; Bugay, 2015). It should be well-known, at a similar period, that in some instances medicinal excipients exhibit a weak or feeble Raman response and/or may exhibit a large amount of fluorescence. For this reason, both FT-IR and Raman spectroscopies are used at the FCC when determining the excipients used in suspected counterfeit/fake dosage formulations.

Furthermore, the drawbacks using any vibrational spectroscopic technique such as Raman and FT-IR in the inquiry of suspected counterfeit/fake of the quantity of a medicine (dosage) forms are unsatisfactorily for the sampling of the tablet coating and its core. But not similar to gas or liquid chromatographic methods, Raman spectroscopy contributes for no front-end separation of the specific constituents in the tablet preparation.

Deconinck et al., (2013) stated that fourteen (14) different artesunate preparations were characterised by using nuclear magnetic resonance spectroscopy (NMR). However, the results indicate that upon analysis only five (5) from the fourteen (14) preparations contained the active pharmaceutical ingredient.

Also, Malet-Martino et al., (2015) revealed that the studies on Levitra (vardenafil), Cialis (tadalfil) and Viagra (sildenafil) were also used by1H NMR. After the analysis the results uncovered that twenty-six (26) were accurate, eight (8) contained unforeseen API and six (6) of the quantity of medicine (dosage) was inaccurate from the forty (40) formulations that were analysed.

According to Habyalimana et al., (2015), quinine tablets were also analysed by using NMR. The results confirmed that counterfeit/fake quinine tablets corresponded with standard metronidazole but did not correspond with quinine sulphate. It was also confirmed that metronidazole was found in suspected quinine tablets. Analytical methods including Raman Spectroscopy, Mass Spectrometry, and High-Performance

Liquid Chromatography confirmed there was no API in quinine sulphate and the counterfeit/fake quinine.

However, it can be clearly stated that some of the desirable qualities or features of NMR are its sample preparation is very easy, quick in response and also it is non-destructive. Notwithstanding that the NMR drawbacks are less sensitive in techniques when comparing it with Mass spectrometry (MS) which makes it needs much larger samples for analysis.

Moreover, it is also expensive in that the Liquid NMR needs the usage of deuterated solvents. Conventionally the sample is analysed as a solution using a 5- or 3-mm NMR tube which is dependent on the NMR probe, which demands ca. 500 or 150 µl, respectively, of deuterated solvents. The appreciation in solvent requirements for LC–NMR makes this method highly expensive (Elipe, 2003).

A review conducted on 519 drugs in 3 African nations between 1991 and 1993 uncovered that 77 (18%) of the drugs were found to be substandard and counterfeited. In Tanzania also, counterfeit and substandard ampicillin was found to contain no active constituent in the year 2000 (Akunyili, 2005).

A report from Falconi et al. (2002) states that some medicinal quantitative examination of antimicrobials (benzathine benzylpenicillin, ceftriaxone, chlortetracycline, ciprofloxacin, TMP–SMX doxycycline and erythromycin) were carried out with titrimetry and apparent UV spectrophotometry. Qualitative analysis was carried out with TLC, among the twenty-one (21) distinct speciality products, only three (3) exhibited the official ‘registered’ label. Three (3) products were expired whiles six (6) other samples expiry date was not available. However, one of the product did not

contain any of the active medicine declared (chlortetracycline) and also did not show off any in vitro activity against bacteria. Seven (7) of twenty-one (21) products (33%) did not contain the quantified dosage (one more than the quantified dosage and six less than the quantified dosage). The highest inadequacy observed was 48% in two products (cotrimoxazole and benzylpenicillin). The dosage was not made available for five (5) drugs. As an outcome, only eight (8) of twenty-one (21) products (38%) did not contain the quantified dosage of the active drug. The pharmaceutical companies involved are Lombisin, Unicorn, China (chlortetracycline), Yong Fong, Myanmar (cotrimoxazole), 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). (Theodore et al., 2007).

Furthermore, High-Performance Liquid Chromatography (HPLC) was used in the research of ninety-six (96) samples of chloroquine and the preferred antibacterials from Nigeria and Thailand to determine the presence related drug impurities and also calculate the Active Pharmaceutical Ingredient (API). The results signify that 36% of samples from Nigeria and 40% of samples from Thailand contained API outside the British Pharmacopoeia ranges. Six (6) of the substandard or spurious preparations did not contain any API. These include two (2) chloroquine samples from Nigeria and three (3) chloroquine samples from Thailand (Taylor et al., 1997).

Thin Layer Chromatography (TLC) was used to evaluate the active pharmaceutical constituent in ciprofloxacin tablets. However, upon analysis, the samples passed the British Pharmacopoeia and United States Pharmacopoeia specifications (Awot et al., 2010).

A survey also explained that a total of four hundred and fifty-one (451) drug samples were analysed in Asia using TLC and disintegration tests. The average inaccuracy rate of artesunate was 19.8%, quinine was 71.8%, mefloquine was 7.7%, chloroquine was 8.5%, tetracycline was 26.6%. The research shows that only 22 samples of dihydroartemisinin and two samples of artemether passed the trials or tests. 122 (27.1%) of the overall samples failed TLC and disintegration tests. However, amid the samples which failed testing, 100 were acquired from illegitimate or unlicensed drug stores (Tsuyuoka et al., 2006).

Three hundred and four (304) antimalarial products were analysed in other to control the amount of the active ingredient and dissolution profile as per USP monographs. At instances where no unauthorised monograph was accessible, only the actual quantity of the active ingredient present was determined. Collectively 12.2% of the samples were established to be substandard and fake. This number was comprised of 13.4% of antifolate antimalarials (SP), 23.8% of quinine tablets and 7.5% of amodiaquine formulations. However, all artemisinin formulations samples contained the specified amount of active ingredient when analysed by employing HPLC (Goodman et al., 2008).

2.10 PARTICLE INDUCED X-RAY EMISSION AS A TOOL FOR ELEMENTAL ANALYSIS

PIXE is a simple technique which is based on the fact that when an electron is expelled, that is ejected from the inner orbit of an atom, an electron from a higher shell say the L-shell will drop into the lower shell, for instance, the K-shell to occupy the vacancy

created (left behind). The energy variation between the K and the L-shells results in the emission of an x-ray photon which is equal in energy between the two shells. When charged particles, like helium (He)-ions or proton, are made to collide/impinge on a sample, electrons will be ejected/expelled from the inner orbit of an atom.

The principle of this technique is to excite the atoms of the substance to be analysed by bombarding the sample with sufficiently energetic X-rays or charged particles. The photons derived from electromagnetic radiation and the charged particles (He) respectively are produced by the ionisation of an electron from the inner orbit (shell), this is simply because in coulomb-interaction for a situation like PIXE occurs due to ionisation.

Electrons are removed from the specimen's atom in the process of interaction, this leads to the frequent replacement of electrons from the outer orbit to the inner shell by filling the vacancy created. The distribution of electrons in the ionised atom is then out of equilibrium and within an extremely short time ($\sim 10^{-15}\ s$) returns to the normal state, by transitions of electrons from outer to inner shells. When an outer-shell electron occupies a vacancy, it, however, loses a specific quantum of energy to occupy the closer shell of more binding energy.

This quantity of energy is readily projected by the principles of Quantum physics (quantum theory or quantum mechanics) and usually, much of the energy is emitted in the form of X-rays. Here an atom loses some potential energy between L-shell and K-shell respectively whenever a transfer of an electron occurred. The phenomenon of x-ray emission occurred due to the liberating of an x-ray photon. Hence, the process is term X-ray emission, when X-ray photon is released. This energy appears as a photon (in this case a Kα photon) whose energy resulted in a change between the binding energies of the filled outer shell and the vacant inner-shell. The photoelectron

production is caused by the ejection of an inner-shell electron, which occurs in every normal process of emission (Verma, 2007).

However, 'the final energy E of the particle is dependent on the charge state of the ion (q), the initial extraction voltage V_i and the final voltage V_t (terminal)' as illustrated by the equation:

$$E = V_i + (q+1)V_t \quad (1)$$

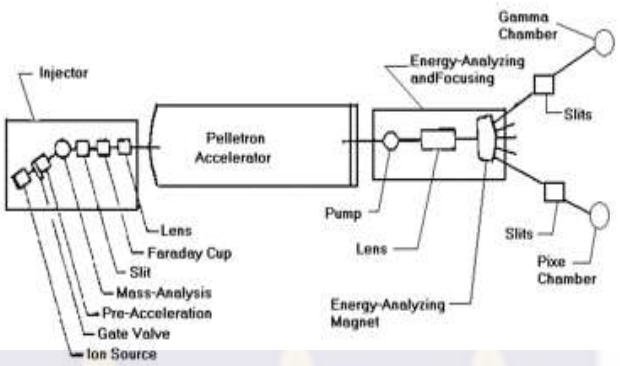

Figure 2. 4: A schematic diagram of the pelletron accelerator

2.10.1 Ion Sources

The negative or positive ion beam is produced by the ion source from neutral atoms. The injector system introduces this ion beam in the accelerator at an energy of about 100 keV.

The energy that is transmitted into the inner-shell electrons is simply coming from ion beams which are typically proton beams, ionise atoms with subsequent X-ray de-excitation. The ion source in an accelerator system is a device which serves the purpose of producing the incident ions, focusing, and accelerating these ions as a uniform and narrow beam**.**

The production of positive ions or negative ions (electron beam) from a single system were due to the reversible polarity of the single-ended pelletron availability. However, the released He or H gas is introduced through the gas inlet with electromagnetic energy of radio frequency (RF) of 100 MHz this enables the production of $H^+, He^+, He^{++}$and

other ions that are discarded into the quartz bottle. 2KV (for H) or 6KV (for He) is the potential difference applied through the quartz bottle, this leads to the production of ions out into the charge exchanger. It is through Rubdium (Rb) vapor that charges of $H^{+} or\ He^{+}$passes and pick up at collision an extra negative charge. The accelerator continue to receive He^{-} and H^{-} charges through the beam (Verma, 2007).

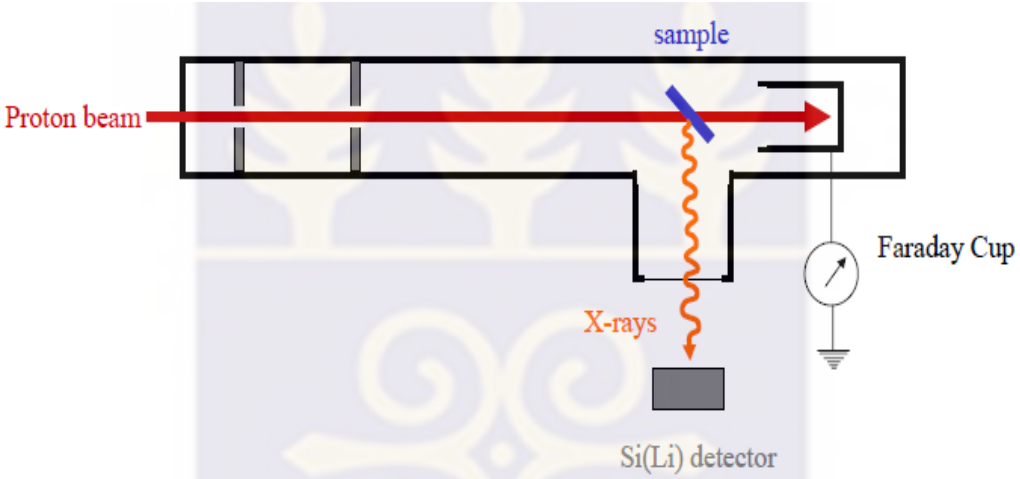

Figure 2. 5 : A diagram of proton beam to Faraday cup

2.10.2 Injection of Negative ion

The injector magnet guides the negative ions that are pre-accelerated from the ion source into the entrance of the accelerator. The focusing of the ion beam on the slit is done by the injecting or magnet through the profile monitor of the beam. The tandem accelerator is also widely used by the semiconductor industry. The stripper's main function is to strip electron in a gas channel when negative ions are introduced to a terminal of high voltage at a positive potential where the negative ions are changed to positive ions in the tandem accelerator. The positive ions are made to move reverse

very fast to the ground potential. Behind the 'injector magnet' is where the slit and the Faraday cup are placed in other to quantify the beam flux that is introduced in the accelerator and also to separate the unwanted ions (Verma, 2007).

2.10.3 Main Accelerator Tube

In the pelletron accelerator, the columns that are accelerating on each adjacent of the terminal is established in the main accelerator. The high voltage terminal is the core part of the tube, while the tank housed the accelerator chain. However, inside the tank comprised of (high energy column, terminal, low energy column, accelerating rings, pellet, and inductor) and the negative ion from the accelerated beam of electrons is stripped with the help of the stripper channel inside the terminal of high voltage. The stripper that is used in the accelerator is either the one with a low-pressure gas in a slim canal or the one with a tiny carbon foil of (2-5 $\mu g/cm^2$) (Instrumentation for PIXE and RBS, 2000). More so, 1.1 MV is applied from the right side of the photo to the terminal whiles from left side of the photo to the terminal is bled with nitrogen gas in the added electron is pull off from another collision of charge exchange.

Hence, the acceleration of the terminal back down 1.1 MV to the left side a result of positive particles leads the production of tandem acceleration.

2.10.4 Steering and Quadrupole magnets

All experiments connected to the accelerator cannot be performed at a single port, however, the accelerated ion beam has an amount of ports provided from the accelerator. The quadrupole magnet refined this task with the support of the switching magnet. Although, the beam is being focused into a particular port with the help of the

quadrupole magnet, which still needs some vertical or horizontal minor adjustment. More so, the electrostatic beam stirrers adjust the beam a little in the vertical and horizontal direction. The steering magnets are also the momentum filter which is the separation of charged particles that is based on their energy or momentum. The magnetic field allows charged particle to through it as the component of its velocity is perpendicular to the magnetic field, when this happened the charge experiences a force which moves in the direction of the applied force.

More so, the ion beam and its accurate focusing transmission loss are minimised as the beam profile monitors, a number of beam stirrers, Faraday cups, and quadrupole lenses are used through the beam transport system, high and low energy. As the sample electrical conductivity is sufficient the proton beam concentration is measured on the target directly.

That is the proton feels a force given by $F = qv \times B$ (2)

This, however, makes the particles travel in a circle of radius r due to the centripetal force it feels. $F_B = F_C \rightarrow q v_p B = \frac{m_p v_p^2}{r}$ (3)

Once the charges leave the magnetic field the force vanishes and they continue in a straight line toward the scattering chambers (Scott, 2014).

2.10.5 Samples, Target Holder, and Scattering Chamber

The ion beam strikes the target at the evacuated area where it is enclosed by the scattering chamber. The ion beam and the target angle is measured based on the calibrated chamber and these targets are placed at 45° to the direction of the beam. Behind the target is where the housed the Faraday cup at the far end of the chamber

window. From the chambers' centre is where the target ladder is placed. All experiments are carried in the chamber, as the beam enters from the right side and Faraday cup to the very left, target characteristic X-ray is collected from the Si(Li) detector that is placed at 90°. The Si(Li) detector main purpose is to detect the x-ray of the target (Verma, 2007).

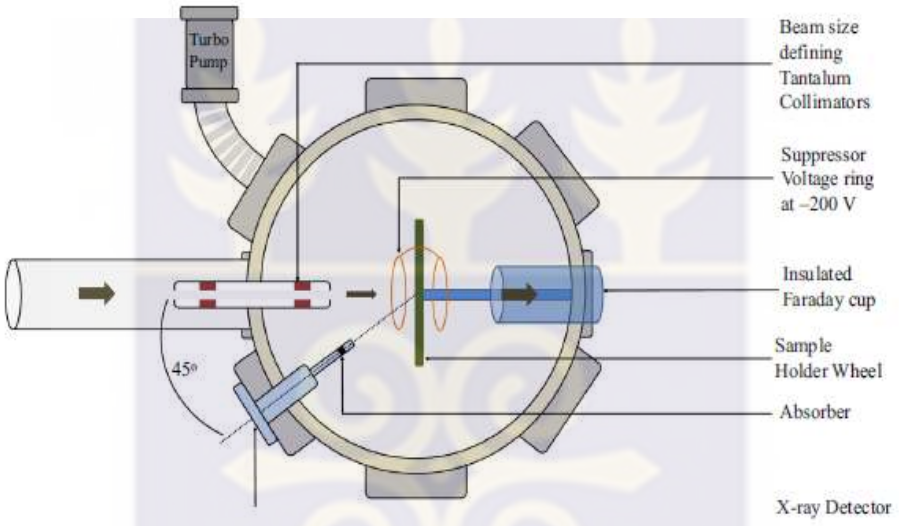

Figure 2. 6 : Schematic diagram of the new PIXE chamber(Md.T.Hassan et al, 2015).

2.10.6 Detection System

The Silicon Drift Detector (SDD) or Si(Li) detects the x-rays energies in the PIXE analysis. The energy dispersive system is made up of the preamplifier (which is used to optimise the detector connected to the amplifier's output) and the amplifier (which is used to amplify the signal after pulse shaping). In this process, the signal coming from

the amplifier is served into the analogue to digital converter (ADC), in which through the CAMAC interface the signal transmitted to the memory of the on-line computer. The analysis of the X-ray spectrum that is carried from the computer is simply observed on the multichannel analyser (MCA) terminal.

However, from the table, the spectrum of the components of Kα, and Kβ are related to the standard X-ray fluorescence lines. Once the background is subtracted the net counts involves in dissimilar regions of interests (ROIs) plus dissimilar peaks are calculated. As both sides of the channel numbers are taken, the average counts on the flat portion of the peak can be evaluated using the background counts. In using the multichannel analyser to quiz the composite spectrum (which contains several corresponding peaks), three parameters have to be used, these include; full-width at half maximum (FWHM), energy (*E*), resolution, full-width at half maximum (FWHM) with the adjusted area in definite limitations. All the same, during fit/fitting the entire spectrum/scale connects the channel number to the energy of the overall energy calibration coefficients for might well be optimised. Likewise, the probable detector followed an equation in which the x-ray peaks FWHM detected are not independent, below is the equation:

$$FWHM = A + B^{*}\sqrt{E} \qquad (4)$$

Hence, the XRF peaks amplitude may not even be a free parameter, for instance, one is likely to resolve the fraction of the Kα by forcing the Kβ peak. Mostly, such limitations permit one to fit corresponding Kα/Kβ peaks with much-improved precision. In 1994, Antolak and Bench developed PIXE spectrum analysis for the Livermore package that is PIXEF (for PIXE-fit). The Dirac-Hartree-Slater evaluations/computations of Scofield are, however, solved openly through the total photoelectric cross-sections and the subshell (Hubbell, 2006). These K-shell or L-subshell are more acquired from the

analytical functional fit with ionisation cross-sections of dependent energy. Fig. 2.9 shows a diagram of typical PIXE spectrum.

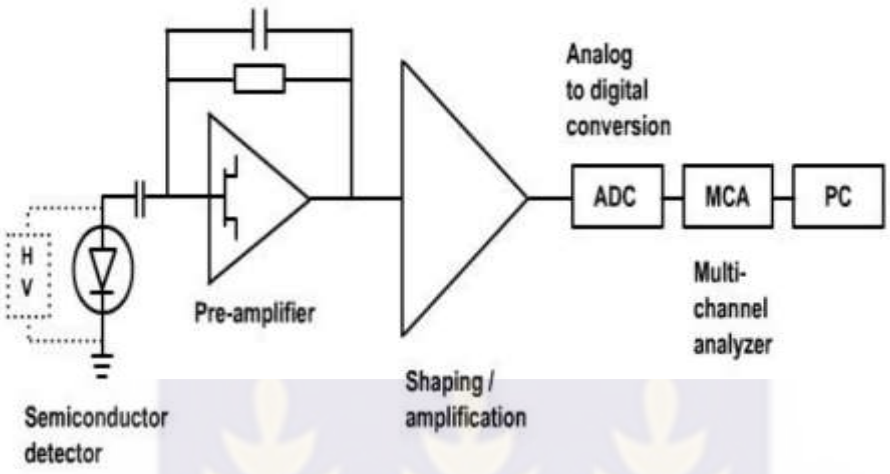

Figure 2. 7 : Detector system

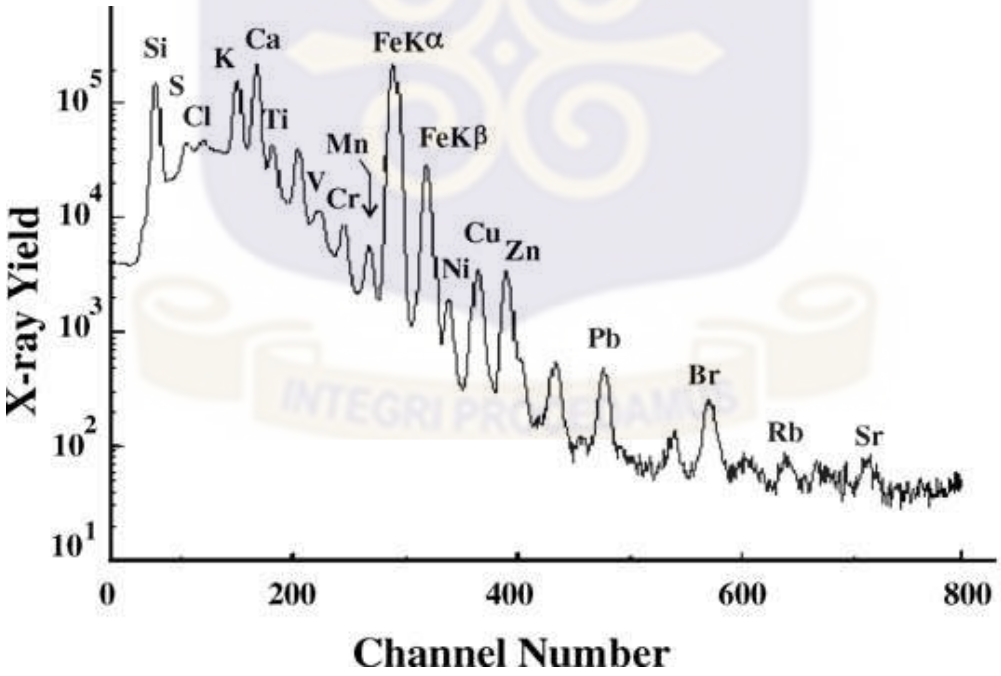

Figure 2. 8 : A typical PIXE spectrum. The names of the elements over the peaks in the spectrum refer to the X-ray lines of those elements''.

2.11 PIXE CALIBRATION

The calibration of PIXE lies in the PIXE technique used to evaluate the concentrations (absolute/ relative) of the elemental components that are selected under inquiry. For complex PIXE spectra analysis, the interface has to be installed in connection to the Gupix set up with windows. These parameters (cross-sections, Coster–Kroning, and fluorescence and probabilities, attenuation coefficients and stopping powers) are all incorporated into the GUPIX database. In any GUPIX software, the possible addition is the energy dependent calibration factor H, but other practical GUPIX possibilities may include analysing spectra in batch, matrix iteration, adding invisible elements to the fit, layer thickness iteration, etc. The H-value method as experimentally determined in the laboratory was used to calibrate the new PIXE setup for quantitative analyses. The following equation was based on the method of the calibration:

A homogeneous thick target sample with the concentration of an element and the x-ray yield is associated with the following equation in theory:

$$Y_i = nC_i wK \in \int_{\epsilon_0}^{0} \sigma_i(E) T_i(E) \left(\frac{-dE}{dx}\right)^{-1} dE \quad \text{(Verma, 2007).} \qquad (5)$$

Where

Y_i……. X-ray yield.

n…….. The number of proton hitting the target.

C_i……. Concentration of element I in the target.

ω…….. The probability of x-rays or the fluorescence field.

K……. The relative line intensity of possible transitions.

ϵ…… The detection efficiency.

$\sigma_i(E)$…. The ionization cross section for proton energy E.

$T_i(E)$…. The transmission of photons from successive depths in the matrix.

$\frac{-dE}{dx}$…… The stopping power of the target for the incoming protons.

E_0…….. The initial proton energy.

In practice, to calculate the x-ray yield, the target sensitivity (S_v) may be determined by (The Gupix approach) which leads to the equation:

$$Y_i = Yl_t C_i \, Q f_q \, \Omega \in \tau \qquad (6)$$

Yl_t…. Theoretical intensity per micro-coulomb of charge per unit concentration per steradian.

C_i…… Concentration of analyte in the sample.

Q…… The measured beam charge.

f_q…... Factor which converts Q to micro-coulombs.

Ω……detector front face solid angle in steradians.

∈…... Intristic detection efficiency.

τ……Transmission factor through any filters or absorbers between target and detector.

Combining $f_q \, \Omega$ in to an instrumental constant H which should in principle be independent of Z and M characterises any PIXE system. H is determined by running standards (preferably having a general similarity to the sample matrix). Hence it is possible to convert measured x-ray yield to concentration for all elements fitted using the equation:

$$C_i = \frac{Y_i}{Yl_t \, H \, Q \in \tau} \qquad \text{..... (7)}$$

2.12 DETECTOR EFFICIENCY

The theoretical predictions or the direct measurements can be used to evaluate the Si(Li) detector energy efficiency. But theoretically, the product of the detector solid angle $\Omega/4\pi$ is the absolute efficiency ε of the detector and its intrinsic efficiencyε_i. If the energy requisite for the detector solid angle is neglected (as a result of different x-ray mean interaction depth in the detector), the efficiency of the detector is given as:

$$\varepsilon = \frac{\Omega}{4\pi} e^{-\sum_{i=1}^{4} \mu_i t_i} f_{\mathrm{E}} (1 - e^{-\mu si^{D}}) \qquad (8)$$

One of the four layers of the exponential term that corresponds to the absorption in front of the detector (ICC layer, window, the contact layer, and ice layer). The Si K X-ray abscond as a result of events loss from the reflection of factor f_{E} as the finite detector dimensions effect was described from the last term in Eq. (8). At X- ray energies higher than 10 keV the term becomes more important at the for 3 mm thick Si(Li) detectors. "For instance, intrinsic efficiency for the 3 mm thick Si(Li) detector with 8 μm Be window, 20 nm of gold contact, 200 nm of ICC layer and no ice on detector surface, is calculated and delivered in Fig. 2.10". Once there is no standard radioisotope source for efficiency, then the direct measurement of the efficiency range is impossible simply because complex portion of the efficiency curve is its low energy part (from 0.5 to 5 keV). The PIXE measurements and the direct use of pure element standards (thin or thick) can be establish if the efficiency measurement of the solution is accepted. However, the only unknown component is the efficiency of the detector as it involves the emission of the characteristic X-ray using the database for the fundamental procedures. The contact layer thickness and the validity of the assumptions for the ICC can be check in this way (IAEA, 2000).

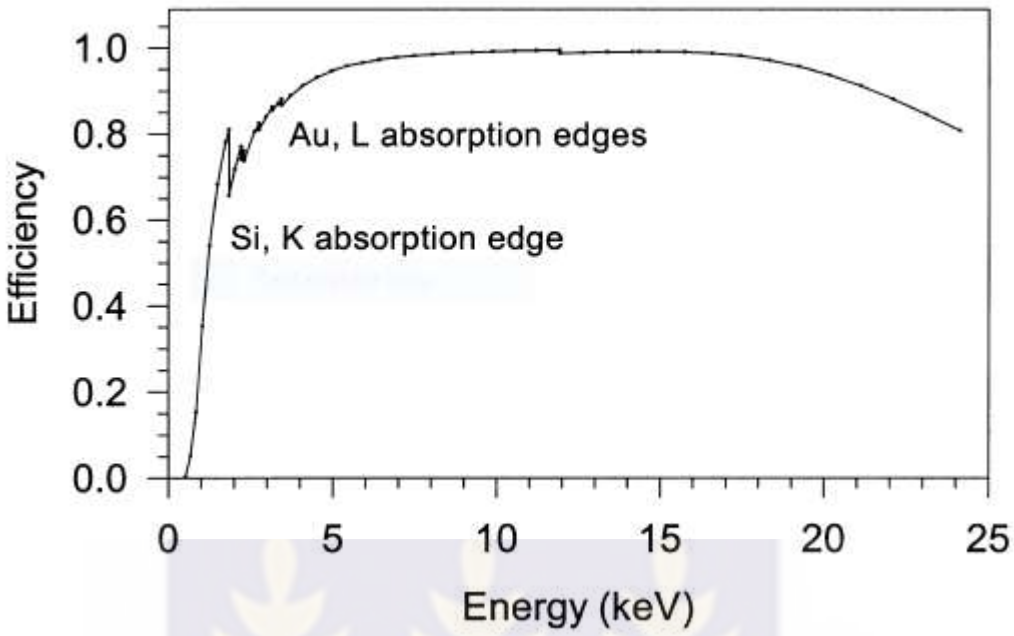

Figure 2. 9 : Intrinsic efficiency curve of a Si(Li) X ray detector calculated for 8 m thick Be window

With very few exceptions the spectroscopy of the characteristic x-rays discharged from a specimen is accomplished using a lithium-drifted silicon detector. The detectors combine the advantage of high efficiency in the x-ray energy region of interest (typically -20 KeV) with a good energy resolution. The resolution, is defined as the full width at half maximum of a spectral peak corresponding to 5.9 KeV energy, is typically 160 eV, which means that one can fully resolve the K_{α} x-ray of neighbouring elements in the transition element region of periodic table. (pixe a novel technique for elemental analysis, Seven A E Johansson ; John L Campbell). At short shaping time the Silicon Drift Detector (SDD) has much lower electronic noise than the planar device. This explains for better resolution means lower noise, at low energies respectively. But for Silicon Drift Detector (SDD) even at large areas the resolution is good.

2.13 DETECTOR AREA AND RESOLUTION

To ascertain the existence of a peak, the fundamental performance parameter is the peak-to-background ratio (P/B) or signal-to-noise ratio. This is somehow related to the "detection limit" This related the number of counts in both the peak (Np) and background (Nb) and can be explained as Np/Nb This improves with acquisition time, t, hence detection limit improves as 1/(t)1/2 But Np and Nb are proportional to detector area (provided there is no overlapping from adjacent peaks) Hence detection limit is proportional to 1/(At)1/2. This is correct for variation of the area of the detector through collimation of a particular detector in an actual change of detector size, increasing area leads to degrading resolution (Adrian, 2005).

2.14 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHODS

Generally, the enormously upgraded configuration (form) of column liquid chromatography (CLC) is the high-performance liquid chromatography (HPLC). Here solvent is supposed to fall/drop into the column through gravity influence but rather was forced to 400 atmospheric pressures through the column, which makes it simple, easier and much faster. Separation of chromatography including HPLC all work with the same simple principle; sample separations into its various constituents as a result of distinctness in the relative aptitude of distinguishable molecules of the mobile phase and stationary phase used in the separation.

Chromatography is a methodology used to identify, quantify and dissociate mixtures of samples into their various components on the foundation of the structural molecule and molecular composition. This is made up of liquid supported on a solid or a solid (stationary phase) and a liquid or gas (mobile phase). The flow of the mobile phase occurs through the stationary phase and carries the elemental components of the

mixture along. Elements that show stronger interactions with the immobile phase will move more sluggishly over the column than elements with the weaker interaction. The rates difference cause the dissociation of components in different forms. A column occupied with a solid adsorbent material relies on a pump which forces the liquid solvent that contained the sample mixture in the HPLC. All the sample's constituents interact with the adsorbent material in different ways, which leads to different flow rates for the dissimilar constituents and hence allow all the constituents to separate as they flow out the column (Vu Ngoc Hanle et al., 2007).

Also, one of the modes of separation frequently used is a reverse phase of the HPLC/UHPLC chromatography. It possesses the hydrophobic with the retention of mixtures provided as both the stationary and mobile phases enable the retention of these mixtures by phase with all the target compound through the interaction of van der Waals type (Tomaz et al., 203).

2.15 HOW HPLC WORKS

The HPLC works under a fundamental principle by taking a small quantity of the mixture (sample) that is to be disassociated and verified as it is sent into a stream of mobile phase transuding or percolating through the column.

The velocities of the compound vary as they move through the column and interact with the sorbent, this is simply referred to as a stationary phase. The chemical nature of each constituent in the compound determine their velocities, the nature of the column and the alignment of the mobile phase. The retention time is simply the time at which every detailed analyte comes out through the column (Calvin, 2002).

The retention identifies the characteristic of every given analyte and also measure it under a specified conditions. Sorbent constituents may be polar or hydrophobic in nature. The organic solvent like acetonitrile and methanol and any combination of miscible water are commonly used as mobile phases. More so, mobile phases without water can also be replaced. Acid like formic could comprise of an aqueous constituent of the mobile phase, salts or phosphoric or trifluoroacetic acid to enable the sample components separation. During the chromatography analysis, the mobile phase composition is either varied or maintained constant throughout the experiment. Also, for the stationary phase, the sample components that are not very dissimilar in their affinity have constant approach separation which makes it effective. The alignment of the mobile phase changes from low to high eluting strength in a varied approach. The analyte retention times have the high eluting strength that produces fast elution as a result of reflection from the eluting strength of the mobile phase (Xiang et al., 2006).

Solvent Reservoir: The glass reservoir is made up of the mobile phase contents. The solvent (mobile phase), in HPLC, is mostly made of a mixture of polar and non-polar liquid components or constituents whose respective concentrations are varied depending on the structure of the sample.

Pump: The solvent stored in the mobile phase is extracted from the pump and forces it through the system's column and detector. Considering the number of factors or what influence the result including the flow rate, column dimensions, the composition of the mobile phase, the particle size of the stationary phase, and the operating pressures of up to 42000 kPa (about 6000 psi) can, however, be manufactured (https://laboratoryinfo.com/hplc/).

Sample Injector: The injector also referred as the introducer is a single injection/an automated injection system. The introducer for an HPLC system should supply the injection of the liquid sample at a range of 0.1-100 mL of volume with high reproducibility and under high pressure (up to 4000 psi) (https://laboratoryinfo.com/hplc/).

Columns: Columns are usually or mostly made up of polished stainless steel, which is ranging between 50 and 300 mm long and has an internal or inner diameter ranging from 2 and 5 mm. They are frequently filled with a stationary or immobile phase with a particle size of 3–10 µm. Micro bore columns are simply known as columns with core diameters of less than 2 mm. In an ideal way, the temperature of the mobile phase and the column should be held constant during an analysis.

Detector: The HPLC detector, is situated at the far end of the column detect as the analytes emerge from the chromatographic column. Some of the commonly used detectors are fluorescence, electrochemical, UV-spectroscopy, and mass-spectrometric detectors.

Data Collection Devices: The electronic integrators also known as chart recorders are used to collect signals from the detector that are varied in their capacity and complexity, and reprocess chromatographic data stored. The data acquisition (computer) integrates the response of the detector to each constituent and places it into a chromatograph that is easy to peruse and describe (https://laboratoryinfo.com/hplc/).

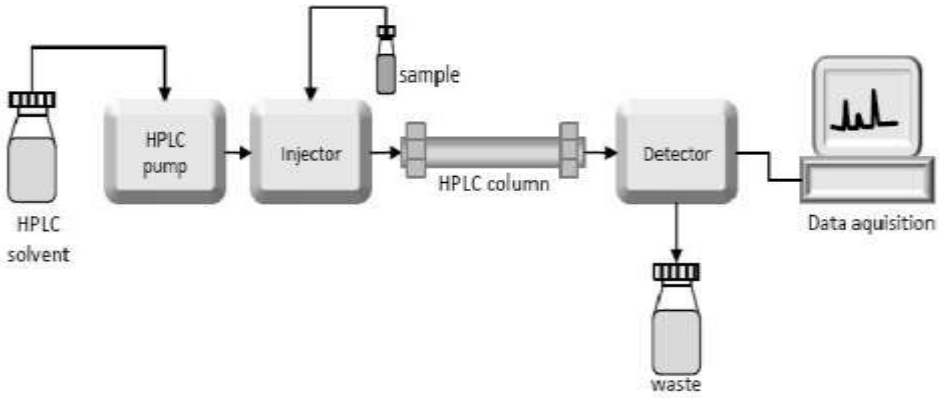

Figure 2. 10 : A typical HPLC setup

CHAPTER THREE

MATERIALS AND METHODS

3.1 SAMPLING

Three categories of amoxicillin drugs were used for this study, standard reference amoxicillin, Brands-name amoxicillin, Generic amoxicillin drugs and Suspected Counterfeit / fake drug.

3.1.1 Standard materials

To verify the analytical procedures of samples it is vital to use certified materials known as a metric (standards) for configuration or as a geometry. An authentic (certified) reference substantial (amoxicillin trihydrate) was procured from a certifiable pharmaceutical company in Accra, Ghana was used to verify the PIXE techniques.

3.1.2 Brand-name drug

A drug that cannot be produced by any other company other than the manufacturing company and are usually emplace on authorization (license) for 12-15years, that is a pharmaceutical product that has a truck or trade name and is safeguard by a patent (can be produced and sold only by the company holding the patent or the manufacturing company). Thirty one (31) amoxicillin capsules manufactured by Bristol and Medriech were purchased in a licensed pharmacy shop in Accra.

3.1.3 Generic drug

A generic drug is a medicinal product that is the same as the brand-name product in quantity (dosage), potency, route of administration, quality, performance, and intended

use. A generic drug must contain the same active constituents as the original brand-name formulation. As soon as the patent protection on brand-name drug is no longer valid or expires, the generic drugs are produced into the market. Nineteen (19) Amoxicillin capsules of the National Health Insurance Scheme (NHIS) produced by Ernest Chemist was obtained from GAEC Hospital, Kwabenya-Accra and anatomized for the goal of this research.

3.1.4 Suspected Counterfeit/Fake drug

Counterfeit or fake/spurious drug is a medicinal or pharmaceutical product which is produced and sold with the intent to deceptively represent its original, authenticity or effectiveness.

Amoxicillin drug suspected to be counterfeit/fake were purchased from an unlicensed drug seller at Okaishie area within the central business district in Accra. Also, another suspected Amoxicillin drug were purchased at Kwame Nkrumah circle (popularly called 'circle') as well from Hawker disputable to be unlicensed and illegal drug seller in Accra. The Amoxicillin drugs will be anatomized (analysed) and assimilate (compare) with the other samples. Thirty (30) amoxicillin capsules were purchased for analysis.

3.2 ANALYSIS OF SAMPLES USIG PIXE METHODOLOGY

3.2.1 Instrumentation

A PIXE instrumentation, as shown in the figure 3.1 below consist of an Ion Source, acceleration tank, steering and focusing section and target chamber.

Figure 3.1: A view of the GAEC pelletron accelerator

Any PIXE analysis requires an ion source, in this study, an alphatross source was used. In an alphatross source, Hydrogen gas is bled into a gas inlet as indicated in the figure 3.2 below.

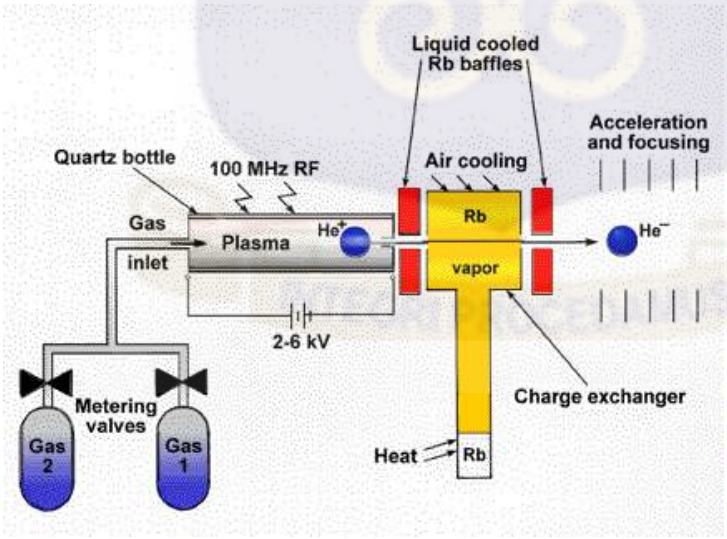

Figure 3. 2 : An Alphatross source

Hydrogen gas passes through this inlet and enters a plasma bottle made of quartz. A 100MHz radio frequency electromagnetic energy is dumped into the quartz bottle containing the hydrogen gas to produce H+ ions. With a potential created across the bottle, the ions produced are accelerated in to a charge exchanger.

At the charge exchanger, the hydrogen ions (H+) pass through a rubidium (Rb) vapor and picks up an extra negative charge through collision, which are further attracted to the positive potential terminal of the accelerator.

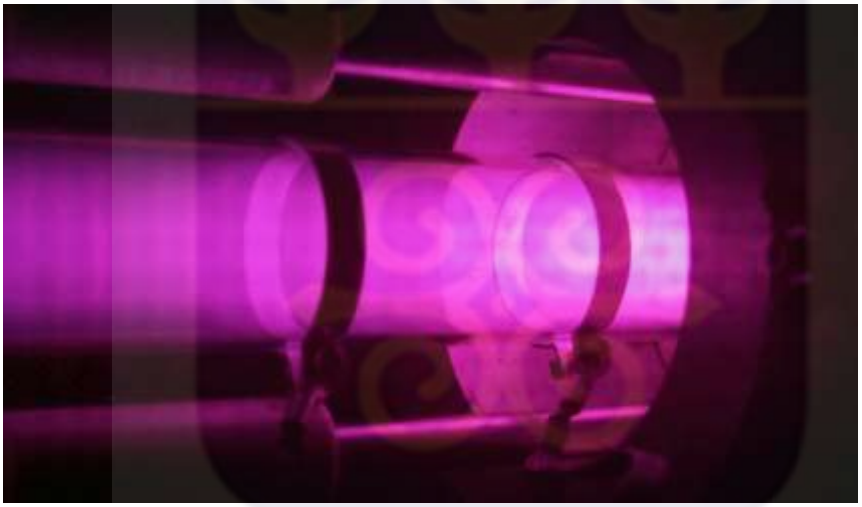

Figure 3. 3: Hydrogen Ion

The Hydrogen ions produced must be given enough energy to cause a nuclear reaction. A tandem accelerator is employed for this task.

Inside the tank, negative ions are accelerated towards the center of the pressure tank by a 1.7MV potential difference. The potential difference is developed by the Pelletron charging system, which consists of metal pellets and insulating connectors. The

terminal is charged by induction and is a very stable and reliable system. The terminal is in the center of the tank.

At the terminal, a nitrogen gas is bled to the terminal to pull off the added electron in a charge exchange collision. The resultant positive particle is accelerated away from the terminal.

The steering section consists of steering magnets and quadrupole magnets. Steering magnets acts as momentum filter which separates charged particle based on their momentum.

The target chamber is where the high energy proton beam interacts with the sample under high vacuum to produce characteristic x-rays. This is where all the experiments are done and the necessary radiations measured.

Inside the chamber, resides a Silicon Drift Detector with energy resolution of 180eV at 5.9KeV placed 45 degrees with respect to the sample holder to detect all characteristic x-rays produced from samples. X-ray spectra acquired are analyzed with GUPIX software.

3.2.2 Sample Preparation

The sample used was amoxicillin (Active Principal Ingredient (API) + Excipient). 2 g of each of the amoxicillin capsule (powdered sample) purchased from the various pharmaceutical stores and hawkers were weighed and pressed with a pelletizer (Carver pelletizer) under a pressure of 20 MPa into pellets of 10 mm diameter and 1 mm thickness.

3.2.3 Transformation of Samples into Pellets

10 grams of the powdered drug is weighed in a plastic petri dish. No sieving was done since the sample was already fine in texture. Pellets of diameter 5mm and thickness of 2mm were done using carver pelletizer.

3.2.4 Conditioning of Sample

Samples are kept in a desiccator for 24 hours prior to analysis to remove any moisture in the sample.

3.2.5 Procedure (Methods)

The pellet was attached to the target holder (sample holder). The target sample with the holder were kept in the vacuum chamber. The target was then placed into the frame holder in the chamber. After placing the target, the chamber was pumped to attain high vacuum 1×10^{-6} Torr.

3.2.6 Irradiation and Detection of X-ray

A process of exposing an object (sample) to a source of radiation is simply referred to as irradiation. The thick target (sample) was exposed to a source of radiation like alpha-particles. That is a positive ion (proton) from the ion source was injected in the pelletron accelerator at an energy of 2.5 Mev by the injector system for the sample to be irradiated.

A 10 nA proton beam current was employed to impinge on the sample at a normal angle in the vacuum chamber at a target collection of 10 μC . A Faraday cup was used to

collect the charge deposited by the incident protons and this was integrated electronically to give the beam current of 10 nA.The characteristic x-rays from the sample are detected with a Silicon drift detector aligned at an angle 45º to the target, the x-ray production cross-section on the proton energy and the atomic number of the sample (target) was taken in to record or written down. The spectrum is made up of discrete x-ray peaks superimposed on a continuous background electromagnetic radiation (bremsstrahlung). The K_α and K_β lines are for the lighter elements (from the filling of the K shell vacancies) and the L lines are for the heavier elements. The peaks matching or corresponding to a given element was integrated to give peak areas. The amounts of that element acquired either from a knowledge of the absolute ionisation cross sections (~1-10^4 barns), fluorescence yields (0.1-0.9), beam current and geometry or by comparison to the results acquired from a thick elemental benchmark (standard). The collected x-ray spectra were processed off-line with the backstop (aid) of GUPIX software package for thick targets. The GUPIX software provided the nonlinear least-squares fitter of the spectrum, together with the subsequent conversion of the fitted X-ray peak intensities to elemental concentration via defined standardisation technique involving fundamental parameters and a user-determined instrumental constant.

3.3 ANALYSIS OF PIXE SPECTRA

3.3.1 Qualitative analysis of the PIXE spectra

Elemental Calibration of PIXE

The Energy Calibration of the spectra acquired from this study was done using DRN-Diorite pelletized reference standard material. This standard contained known elements

with their x-ray energies known. The GUPIX software is utilised in the calibration of the various spectra.

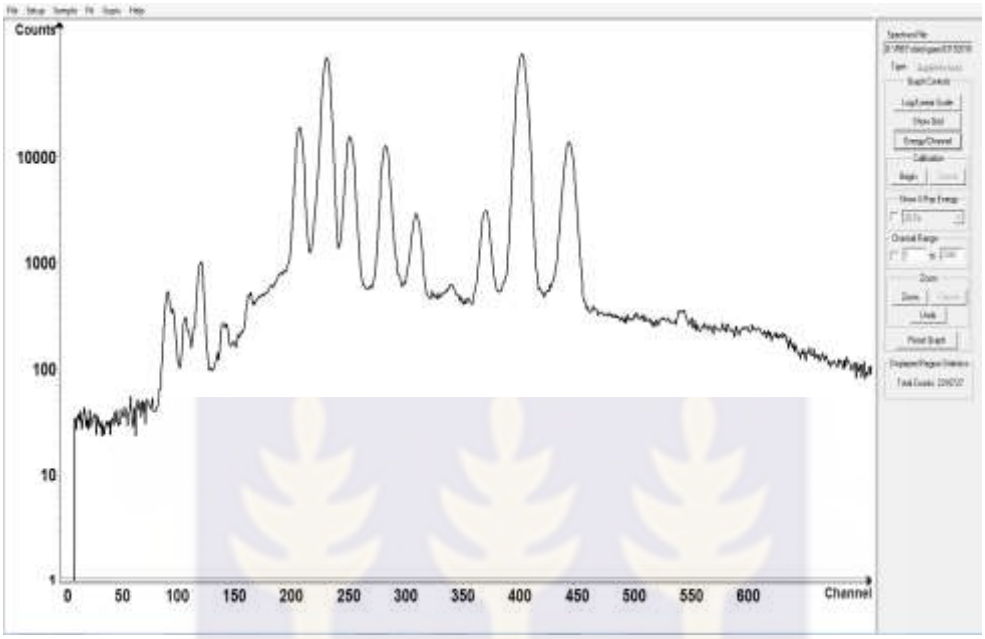

Figure 3. 4: A typical un calibrated spectrum of the DR-N SRM sample in GUPIX

Figure 3.4 illustrates a typical un calibrated spectrum in Gupix, in this spectrum, elemental peaks are not calibrated therefore cannot be related to energies and identified.

This uncalibrated spectrum obtained is then calibrated by ascertaining the two most prominent peaks which are Fe and Ca. On the right side of Figure 3.4, on the Gupix display is the calibration button which when clicked allows the user to enter the x-ray energies corresponding to the two most prominent peaks on the spectrum whose identities are already known. The Figures 3.5 and 3.6 below illustrates this procedure.

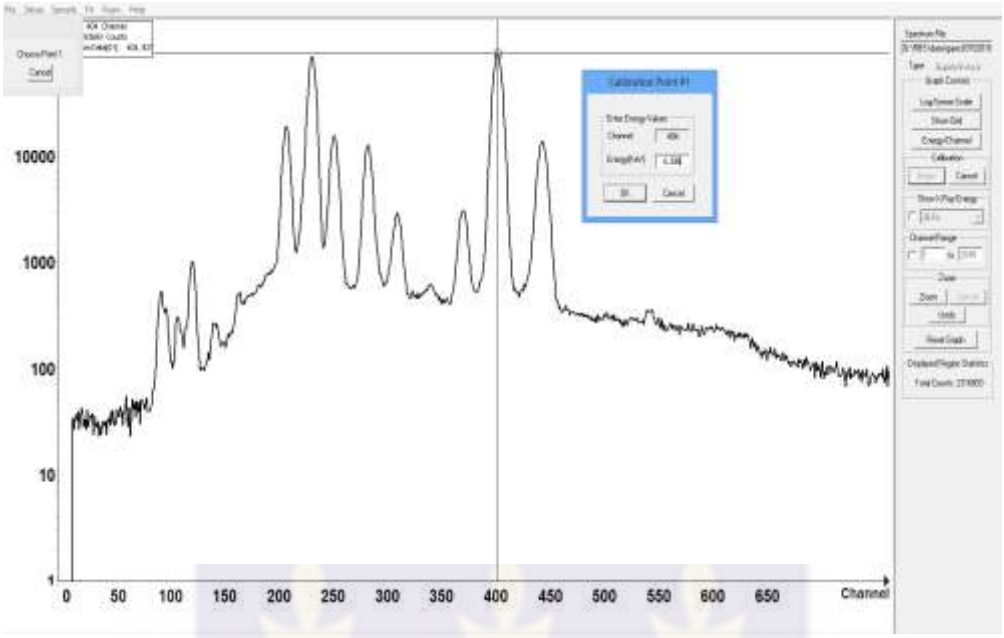

Figure 3. 5 : Energy calibration for Fe peak in Gupix

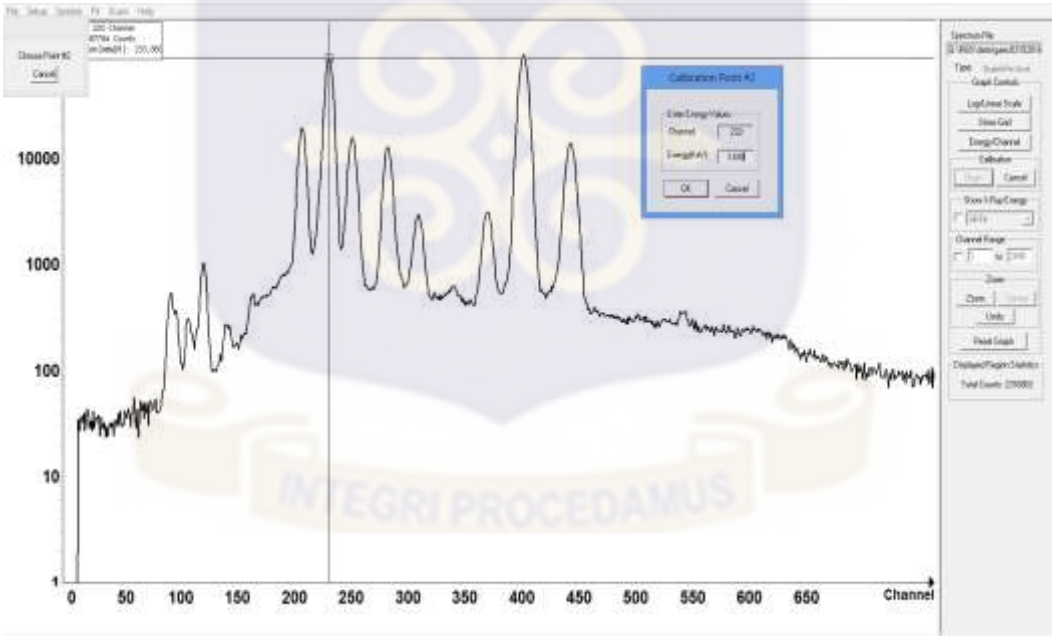

Figure 3.6: Energy calibration for Ca peak in Gupix

After a successful calibration of the spectrum, the fitting parameters are determined; this is shown in Figure 3.7

Two variables A1 and A2 are introduced to define the linear relationship between the Channel number C and X-ray energy E (in keV):

$$C = A1 + A2E \quad (9)$$

Two further variables A3 and A4 similarly define the relationship between the Gaussian peak width (standard deviation in channel units) r and X-ray energy:

$$\sigma^2 = A_1 + A_2E \quad (10)$$

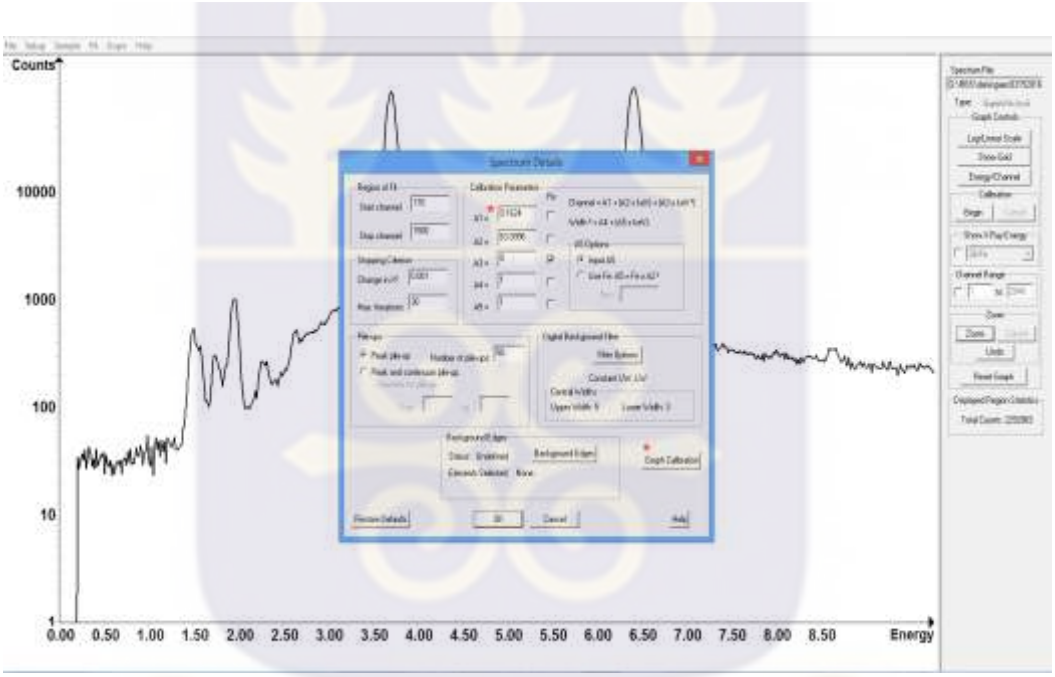

Figure 3. 7 : Fitting parameters for spectrum

A successful completion of all this procedure results in a calibrated spectrum is illustrated in Figure 3.8.

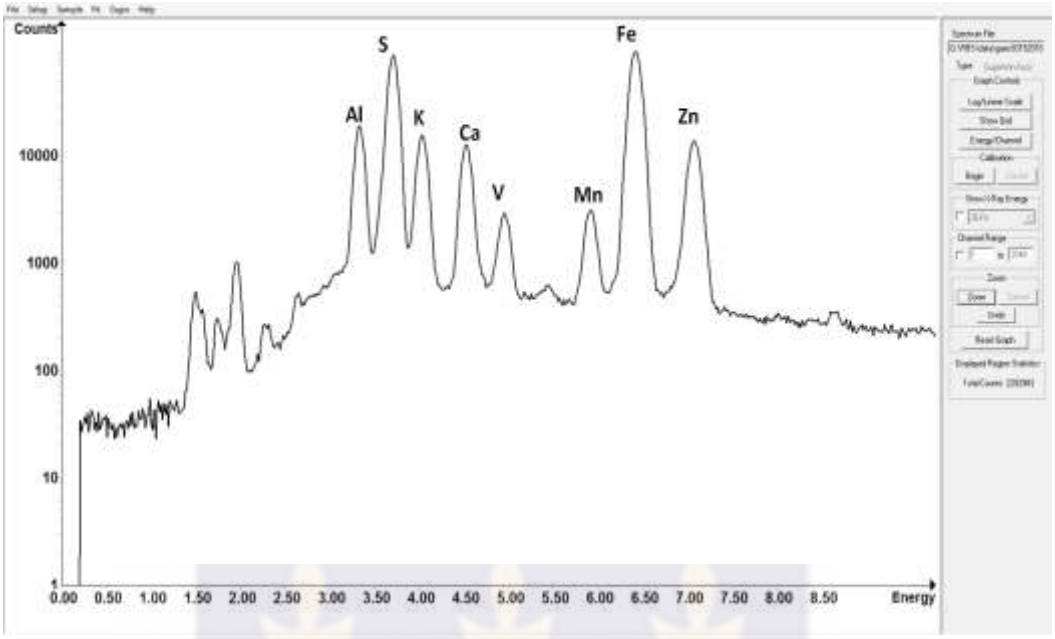

Figure 3. 8: Calibrated spectrum of DR-N SRM sample

3.3.2 Quantitative analysis of PIXE spectra

The GUPIX software was used for the quantitative analysis of all the spectra obtained from this study. Gupix requires a list of basic parameters to assist in its computation. Figure 3.9 shows a typical setup window for GUPIX quantitative analysis.

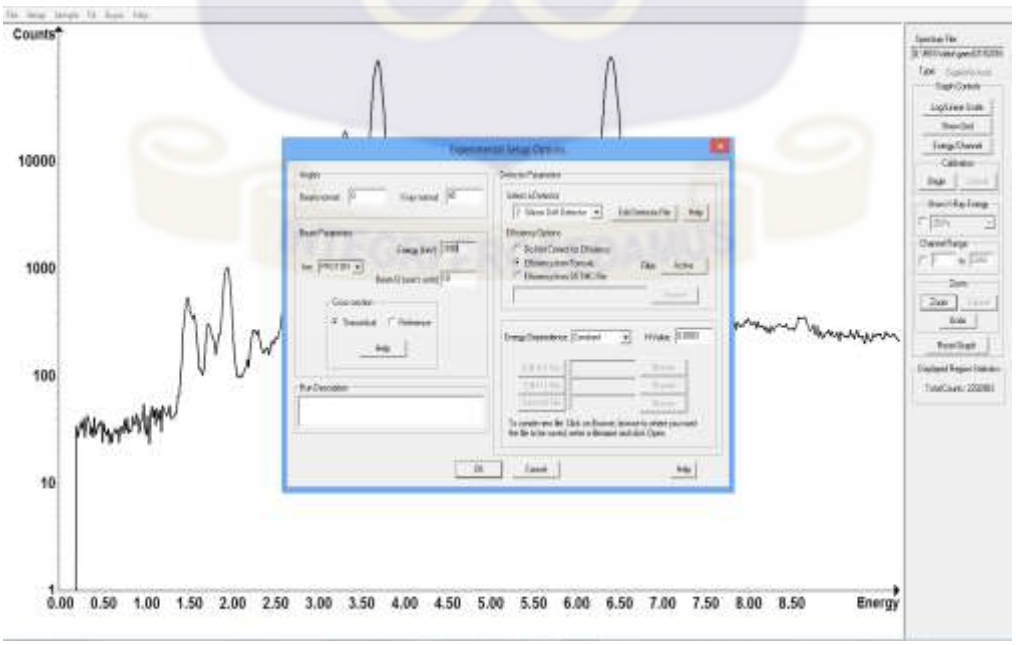

Figure 3. 9: A typical setup window for GUPIX quantitative analysis

In this study, the following parameters were used and entered into the Gupix software incident

- Type of Incident Ion beam: H+
- Incident Ion Energy : 2500 KeV (2.5 MeV)
- Incident Ion Beam normal: 0^0
- Silicon Drift Detector angle to target sample: 45^0
- Incident Ion beam current: 10 µA
- Total Beam Charge collected: 10 µC

The equation below was used for Gupix computation.

$$\mathbf{Y_{(Z,M)} = Y_{1(Z,M)} QC_z T_{(Z)} \varepsilon_z H} \qquad (11)$$

where

$Y_{(Z,M)}$…. Is the measured X-ray yield that is peak area computed by the fitting program,

GUPIX software is utilised to analyse the spectra and provide the Yield, to compute equation (11) for the concentration.

This parameter was obtained by performing a peak area fit of the PIXE spectra using the GUPIX run function. This performed a peak area fit to integrate the area under all the peaks (elements) identified in the spectrum. Fit residuals of values less than 10% were accepted. The area under the given peak is very important on the quantification of the concentration levels in that element.

Figure 3.10 shows the data fit and residues for a standard reference material

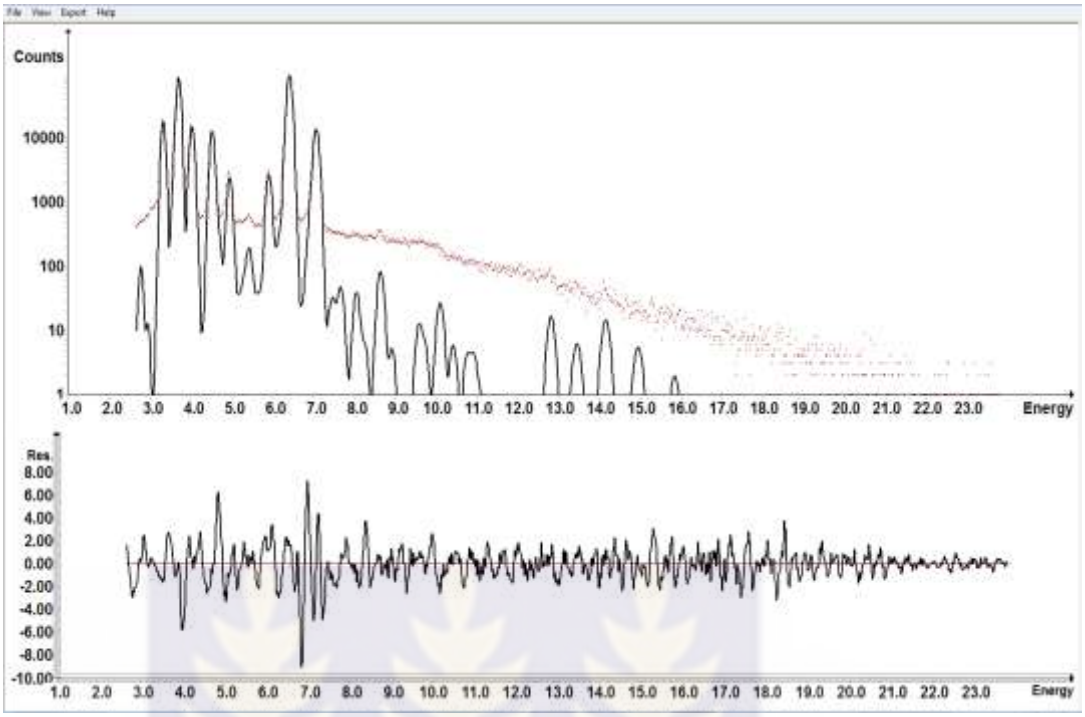

Figure 3. 10: Spectrum fitting and residues of a standard reference material done by GUPIX

From equation (11), $Y_{1(Z,M)}$ which is the theoretical X-ray yield per unit beam charge, per unit solid angle and per unit concentration is computed from the GUPIX database.

Q, which is the measured beam charge is measured by the current integrator system located in the inside the target chamber. This value is experimentally measured.

ε_z , which is the intrinsic or absolute detection efficiency is computed by a Gupix program called DETMC which is based on a Monte Carlo Simulation Tool. The fractional transmission coefficient, $T_{(Z)\ldots\ldots}$ of x-rays travelling through any absorber to the detector is theoretically computed by GUPIX using the stopping power and absorber matrix information in the Gupix database.

The instrumental constant H was determined using one minor or trace elements in a standard reference material. This element was selected to provide characteristic X-ray energies sufficiently high that their attenuation by the Mylar absorber is not large; this ensures that any uncertainty in the absorber thickness or the attenuation coefficient for these X-rays does not translate into a significant uncertainty in the H value. We have chosen to employ the minor element molybdenum in molybdenum-bearing alloys; a molybdenum concentration of a few percent is necessary to obtain adequate spectrum intensities and corresponding statistical counting uncertainty.

C_Z…… Concentration of the element.

H…... H is the product of detector solid angle and correction factor for the charge measurement

3.3.3 Standardisation for elemental analysis

The concentration (C_Z) of the values are known under a direction with reference or standards materials. Once the matrix term starts to run the GUPIX software compute the codes and then iterates the fit of the various peak areas of the various elements to a minimum chi-squared value. In other to compare the code's output concentrations with the known values of H as the instrumental constant is deduced.

3.3.4 Elemental analysis of unknown samples

For an unknown sample to be analysed in the standard of the geometry, the iterated-matrix (IM) has to obtain the GUPIX that is to be used for the H-value, when approaching the Gupix code that is first developed. To optimise the goodness of fit for the first iteration, the sample is treated as infinitely thin because the C_Z values are not

known, thus making the matrix not have any influence. The peak areas come out of the first fit through equation (11), the first estimate of the full C_Z set is obtained with the X-ray yields. But in the second iteration, the matrix terms for all the X-rays to be obtained in each element can be computed with this concentration. The fit is optimised and the C_Z value is acquired through an improved set.

This sequence, which involves iterating to the best fit within each successive matrix iteration, continues until the reduced chi-squared of the fit reaches a minimum and the concentrations are stable. Hence, the final result is acquired from concluding the linear least square fit.

3.4 DISINTEGRATION TEST

Apparatus for disintegration test is fairly understandable. It is made of paddles and baskets, paddles are used for 500 mg amoxicillin and baskets for 250 mg amoxicillin. The dissolution meter contains six different vessels which are filled with 900 mL of water in each vessel. The basket rack holding tubes open at both ends, the bottom is covered with 10 mesh screen. 500 mg Amoxicillin capsules are placed in the vessels directly and the paddles are gently released in the vessels, while the 250 mg capsule is put in the baskets and released in the vessels. All the vessels are contained in a water bath at a temperature of 37 ºC, paddles rotate and baskets move up and down at constant speed until the latter capsule break apart into many small pieces and fall in the vessels through the mesh. The time taken for the whole process is recorded.

3.5 HPLC METHODOLOGY

3.5.1 Sample Preparation

The mobile phase was prepared by weighing 6.8018 g of monobasic potassium phosphate and mixed with a 1000 mL volume of water in a conical flask to form a solution. The solution was sonicated for 10 minutes.The pH of the solution was taken to be 4.92 at 28.2 ºC with the addition of 2 drops of potassium hydroxide as an adjustment at 45 % (w/w) (strong base), the pH rose to 5.1 which is within the standard range (5 ± 0.1). The buffer solution was placed on a hot plate with a magnetic stirrer in it for further mixing which happened for 3 minutes. The whole solution was filtered using membrane filter through the pump. About 12.0 g (1.2 mg/mL) of the USP Amoxicillin reference standard was mixed with the buffer and sonicated to ensure complete dissolution. However, the mobile phase that is acetonitrile and buffer is in the ratio of 1:24 which is supposed to be used within 6 hours of preparation.

Mobile phase B was prepared by measuring 20 volumes of acetonitrile mixed with 80 volumes of pH 5.0 buffer solution. Eight (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 0.5 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 a concentration of [0.5%(w/v)].

3.5.2 HPLC Assay

The sample solutions were filled in each of the valves (vials) and placed in the injector module, the chromatographic condition of the Agilent HPLC 3 of the Food and Drugs Authority (FDA) was already in it automated state and the mobile phase prepared to

start the analysis. The chromatographic analysis starts by introducing 10 μL of the samples into the flow stream of the mobile phase with the support of the injector (ie the automatic sampler). (1.5 mL/min) The heart of the system is the column's stationary phase where dissociations (separations) of sample elements occurs. The column is made of octadecylsilyl silica gel inside it which in separation from samples physical and chemical parameters.

A detector is required to see the divided compound bands as they move out (elute) from the high-pressure column. The information (data) is sent from the detector to a computer which produces the chromatogram. The mobile phase exits the detector and is either sent to a waste or collected, as desired.

3.6 DISSOLUTION METHOD

The dissolution set-up is made up of six (6) different vessels, each vessel was filled with a 900 mL volume of water and heated up to 37 °C this is to match the actual temperature of the human stomach. Paddles are used for 500 mg amoxicillin capsules whiles baskets are used for 250 mg amoxicillin capsules. 500 mg capsules were placed in each of the vessels and the paddles gently lowered in the vessels, the 250 mg was also put in six different baskets and gently lowered in each dissolution bath vessels for the analysis to start. The whole process lasted for 60 minutes (1hr) after which 50 mL of the contents (samples) in each of the vessels was withdrawn with a syringe and poured into six different sizable beakers. 5 mL of the samples in the sizable beakers was pipetted into a 25 mL bottle, the pipetted sample was topped up with the diluents to meet the concentration that is similar to that of the standard [0.0111%(w/v)].

0.1 g of standard amoxicillin trihydrate was matched with 0.1151 g of mass and dissolved in a 100 mL bottle with the diluents used as a solvent. A solution of 2.77 mL was measured (pipetted) into a bottle of 25 mL and then topped up with the diluents to form a concentration of the solution of about [0.0111(%w/v)].

However, isocratic elution was the method used for analysis. 0.5 g of amoxicillin of each sample was measured and diluted with 100 mL of the diluent prepared (mobile phase) to form a solution with a concentration of 0.5 % (w/v).

3.7 VISUAL INSPECTION METHOD

Visual inspection or Visual testing is the most basic method of inspection. It is the process of looking over a piece item using the naked eye to look for flaws. It requires no equipment except the naked eye of a trained inspector. All the medical drugs purchased (amoxicillin capsules) were scrutinised in their various packaging. The packaging of the samples (amoxicillin capsules) did not show any visual discrepancies from standard expectations. In addition to that, the batch numeric, manufacturing dates, expiry dates and the spelling of the products were well tagged on the packs. Samples were also unpacked from their blisters and further examined. Each formulation (capsules) was weighed and measured with their colorations and shapes intact.

3.8 PHYSICAL PARAMETER TEST PROCEDURES

The first step in the assessment of the quality of medical drugs is to carry out prescribed physical parameters' test

In assessing the physical parameters of the samples, the mass of each set of the capsules used was measured. The mass of 10 capsules with and without the powder components were weighed respectively using the Mettler-Toledo analytical balance.

The average weight of 10 capsules of imported amoxicillin was also measured. The detailed and calculated results of the physical parameter test are illustrated in Chapter four.

CHAPTER FOUR

RESULT AND DISCUSSION

4 .1 VISUAL INSPECTION

All the analysed medical drugs (amoxicillin capsules) were scrutinised in their various packaging. The packaging of the samples (amoxicillin capsules) did not show any visual discrepancies from standard expectations. In addition to that, the batch numeric, manufacturing dates, expiry dates and the spelling of the products were well tagged on the packs. Samples were also unpacked from their blisters and further examined. These visual examinations did not reveal any sign of degeneration from any of the capsules. Each formulation (capsules) was weighed and measured with their colorations and shapes intact.

4.2 PHYSICAL PARAMETER TEST

The first step in the assessment of the quality of medical drugs is to carry out prescribed physical parameters' test

In assessing the physical parameters of the samples, the mass of each set of the capsules used was measured. The mass of 10 capsules with and without the powder components were weighed respectively using the Mettler-Toledo analytical balance.

The average weight of 10 capsules of imported amoxicillin was measured to be 6.7272 g, with that of the empty shell was 0.9651 g. This gave the average mass of the constituents in a single capsule to be 0.57621 g. However, the actual range of the mass of the constituents measured individually gave a range of 0.5762 g - 0.7102 g. This complies with the British Pharmacopoeia (B.P 2016), the requirement that the mass of individual capsules should fall within the range of ±7.5% of the average mass of the capsules.

The second imported amoxicillin drug also gave an actual measurement range of 0.5904 g -0.7135 g as compared to the average value 0.59041 g. This also meets the 7.5 % requirement range of the British Pharmacopoeia (B.P, 2016).

Similarly, for NHIS generic amoxicillin on each capsule, the mass of 10 capsules was taken to be 6.9003 g, the weight of empty shells was 0.9347 g, 5.9656 g was the mass of the constituents and 0.59656 g of the average weight of the content was recorded. Hence the B.P ±7.5% weight range was 0.5246 g – 0.6746 g which shows that none of the mass of the capsules was below the range. The actual range was 0.5674 g – 0.6406 g.

Two different 250 mg suspected counterfeit amoxicillin was used, the mass of 10capsules was taken to be 4.0506 g, the weight of empty shells was 0.7753 g, 3.2753 g was the mass of the constituents and 0.32753 g of the average weight of the content was recorded. Hence the B.P ±7.5% weight range was 0.3036 g-0.4536 g and the actual range was 0.3275 g-0.4021 g which indicates that none of the mass of the capsules fell below the range.

For the second suspected counterfeit amoxicillin, the mass of 10 capsules was taken to be 4.8342 g, the weight of empty shells was 0.7814 g, 4.0528 g was the mass of the constituents and 0.40528 g of the average weight of the content was recorded. But the (B.P) of ±7.5% of weight range was taken to be 0.3298 g-0.4798 g with the actual range of the sample been 0.3987 g-0.4113 g indicating that none of the mass capsules was below the standard range.

Table 4. 1 Mass Difference (mg) for Amoxicillin Drugs

IMPORTED GENERIC 1		**IMPORTED GENERIC 11**		**NHIS**		**CIRCLE**		**OKAISHI**	
CAPSULES	*SHELLS*	*CAPSULES*	*SHELLS*	*CAPSULES*	*SHELLS*	*CAPSULES*	*SHELLS*	*CAPSULES*	*SHELLS*
576.2	96.5	590.4	95.3	597.8	94.5	327.5	77.5	405.3	78.1
601.5	96.7	611.6	96.4	601.1	97.6	334.5	75.6	407.4	79.7
670.0	92.1	680.1	96.2	640.6	96.6	376.3	77.3	407.8	78.7
710.2	95.8	682.0	98.5	599.9	95.4	384.7	76.7	402.5	77.7
671.0	94.7	713.5	97.1	589.8	96.3	400.1	75.7	405.7	79.6
695.3	97.4	603.2	96.4	600.6	97.2	402.1	71.6	398.7	87.2
670.0	97.0	670.1	94.5	598.8	97.3	398.5	71.5	401.6	78.4
666.0	93.0	676.1	95.3	601.2	97.5	388.7	76.5	411.3	77.6
694.3	99.6	693.4	97.8	598.7	98.8	399.4	77.2	403.7	78.3
679.0	97.3	689.0	96.5	567.4	97.7	374.1	76.1	404.4	70.4

4.3 CAPSULE DISINTEGRATION TEST

The solubility of a drug is necessary for it to be easily attainable or available to the body for effective curative purposes. The first requirement in solubility is the ready breakdown of the drug into smaller particles or granules. Consequently, disintegration test is another requirement for drug quality assessment.

A critical parameter in the disintegration test is the disintegration time. The solution that was used for the disintegration test was 900 mL of distilled water at a temperature of 37 ℃. these conditions simulate the ambience of the stomach. In addition, the dissolution time during the running of the test must fall within 5 -10 minutes for it to pass this test. All the five different capsules averagely disintegrated within 5 minutes of run-time and this falls within the tolerable time of the British Pharmacopoeia 2016.

4.4 ASSAY USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

The assay was the succeeding test administered on the five arrays of antibiotics drugs samples by applying the HPLC technique tallying with the directives of the third edition of the British Pharmacopoeia 2016 Vol. V.pg V-A357. The essence of this test is to validate the presence and correct concentration of the Active Principal Ingredient (API) of the amoxicillin capsules.

Table 4. 2 Chromatographic System

COLUMN TYPE	agilent hplc 3 c18 stainless steel packed with octadecylsilyl gel for chromatography (5µm)
Column dimension	25 cm × 4.6 mm
Mobile Phase	Acetonitrile
Elution	Isocratic elution
Flow rate	1.5 mL/min
Temperature	28 ℃
detection wavelength	254 nm
Injection size/volume	10 µL
Pressure	58.7 bar- 58.9 bar

4.4.1 HPLC Assay Results for Imported Generic Amoxicillin

There were two (2) different brands of Imported generic amoxicillin used for this work.

Sample 1:

Table 4. 3: Imported Generic Amoxicillin

Mass of sample taken	0.57621 g
Standard (Amoxicillin base)	0.1201 g
Calculated Amoxicillin base	0.6005 g
Percentage purity of standard	99.2 %
The actual amount of Standard taken	0.6053 g

The detailed calculation of the HPLC Assay results for Imported generic amoxicillin sample 1, is illustrated in the appendix A.

Dilution:

0.5 g of amoxicillin trihydrate corresponding to 0.6053 g of the standard was diluted in 100 mL of diluents to obtain a solution of a concentration of [0.5 %(w/v)]. Also, 0.5 g of amoxicillin trihydrate corresponding to 0.5762 g of the sample was diluted in 100 mL of the diluents to obtain a solution of concentration of [0.5% (w/v)]

But from the sequence table (ie the peak summary Appendix D1-D3)

Average area under curve for standard = 13746.933 mAU*s

Average area under curve for (sample 1) that is,

Rep1=14951.75 mAU*s

Rep2= 14994 mAU*s

Rep3= 15029.4 mAU*s

Therefore, the total average area= 14991.82 mAU*s

From theory the percentage of $C_{16}H_{19}N_3O_5S$ in the portion of capsules taken is:

$$Result = (\frac{r_u}{r_s}) \times (\frac{c_s}{c_u}) \times P \times F \times 100$$

r_u=Peak response from the sample solution

r_s=Peak response from the standard solution

c_s=Concentration of USP Amoxicillin RS in the standard solution (1.2 mg/mL)

c_u=nominal concentration of amoxicillin in the sample solution (1.0 mg/mL)

P= Potency of amoxicillin in USP Amoxicillin RS (866 µg/mg)

F= Conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0 % - 120 %

Implies, the percentage content of amoxicillin trihydrate contained in the foreign generic amoxicillin capsule sample 1=

$$\left(\frac{14991.82\ mAU * s}{13746.93\ mAU * s} \times \frac{1.2\ mg/ml}{1.0\ mg/ml} \times 866\ \mu g/mg \times 0.001\ mg/\mu g \times 100\%\right)$$

$$= 113.33\ \%$$

The percentage of sample 1 is accepted once it falls within the range of the acceptance criteria according to the 6th edition of the British Pharmacopoeia.

Sample 2:

Table 4. 4: Imported Generic Amoxicillin

Mass of sample taken	0.59041 g
Standard (Amoxicillin base)	0.1201 g
Calculated Amoxicillin base	0.6005 g
Percentage purity of standard	99.2 %
The actual amount of Standard taken	0.6053 g

The detailed calculation of the HPLC Assay results for Imported generic amoxicillin sample 2, is illustrated in the appendix A.

Dilution:

0.5 g of amoxicillin trihydrate corresponding to 0.6053 g of the standard was diluted in 100 mL of diluents to obtain a solution of a concentration of [0.5% (w/v)]. Also, 0.5 g of amoxicillin trihydrate corresponding to 0.59041 g of the sample was diluted in 100 mL of the diluents to obtain a solution of concentration of [0.5% (w/v)]

But from the sequence table (ie the peak summary)

Average area under curve for standard = 13746.933 mAU*s

Average area under curve for (sample 2) that is,

Rep1=15091.55 mAU*s

Rep2=15172.85 mAU*s

Rep3=15262.8 mAU*s

Therefore, the total average area=15175.73 mAU*s

Hence, the percentage content of amoxicillin trihydrate contained in the foreign generic amoxicillin capsule sample 2=

$$\left(\frac{15175.73\ mAU*s}{13746.93\ mAU*s} \times \frac{1.2\ mg/ml}{1.0\ mg/ml} \times 866\ \mu g/mg \times 0.001\ mg/\mu g \times 100\ \%\right)$$

$$= 114.72\ \%$$

The percentage of sample 2 is accepted because it falls within the range of the acceptance criteria according to the 6th edition of the British Pharmacopoeia.

4.4.2 HPLC Assay Results for NHIS Amoxicillin

Sample:

Table 4. 5: NHIS Amoxicillin

Mass of sample taken	0.5380 g
Standard (Amoxicillin base)	0.1201 g
Calculated Amoxicillin base	0.6005 g
Percentage purity of standard	99.2 %
The actual amount of Standard taken	0.6053 g

The detailed calculation of the HPLC Assay results for NHIS amoxicillin sample, is illustrated in the appendix B.

Dilution:

0.5 g of amoxicillin trihydrate corresponding to 0.6053 g of the standard was diluted in 100 mL of diluents to obtain a solution of a concentration of [0.5% (w/v)]. Also, 0.5 g of amoxicillin trihydrate corresponding to 0.5380 g of the sample was diluted in 100 mL of the diluents to obtain a solution of concentration of [0.5% (w/v)]

But from the sequence table (ie the peak summary)

Average area under curve for standard = 13746.933 mAU*s

Average area under curve for sample is,

Rep1=15197.45 mAU*s

Rep2=15283.55 mAU*s

Rep3=15179.50 mAU*s

Therefore, the total average area=15220.17 mAU*s

Hence, the percentage content of amoxicillin trihydrate contained in the NHIS amoxicillin capsule =

$$\left(\frac{15220.17mAU * s}{13746.93mAU * s} \times \frac{1.2mg/ml}{1.0mg/ml} \times 866\mu g/mg \times 0.001mg/\mu g \times 100\%\right)$$

$$= 115.06\ \%$$

The percentage of the NHIS is accepted because it falls within the range of the acceptance criteria according to the 6th edition of the British Pharmacopoeia.

4.4.3 HPLC Assay Results for OKAISHI Amoxicillin

Sample:

Table 4. 6: Okaishi Amoxicillin

Mass of sample taken	0.3275 g
Standard (Amoxicillin base)	0.1201 g
Calculated Amoxicillin base	0.6005 g
Percentage purity of standard	99.2 %
The actual amount of Standard taken	0.6053 g

The detailed calculation of the HPLC Assay results for Okaishi amoxicillin sample, is illustrated in the appendix C.

Dilution:

0.5 g of amoxicillin trihydrate corresponding to 0.6053 g of the standard was diluted in 100 mL of diluents to obtain a solution of a concentration of [0.5% (w/v)]. Also, 0.5 g of amoxicillin trihydrate corresponding to 0.6550 g of the sample was diluted in 100 mL of the diluents to obtain a solution of concentration of [0.5% (w/v)]

But from the sequence table (ie the peak summary)

Average area under curve for standard = 13746.933 mAU*s

Average area under curve for sample is,

Rep1=15556.25 mAU*s

Rep2=15484.40 mAU*s

Rep3=system error

Therefore, the total average area=15520.33 mAU*s

Hence, the percentage content of amoxicillin trihydrate contained in the OKAISHIE amoxicillin capsule =

$$\left(\frac{15520.33\ mAU * s}{13746.93\ mAU * s} \times \frac{1.2\ mg/ml}{1.0\ mg/ml} \times 866\ \mu g/mg \times 0.001\ mg/\mu g \times 100\ \%\right)$$

$$= 117.33\ \%$$

The percentage of the OKAISHI is accepted because it falls within the range of the acceptance criteria according to the 6th edition of the British Pharmacopoeia.

4.4.4 HPLC Assay Results for Suspected Counterfeit Amoxicillin Sample:

Table 4. 7: Suspected Counterfeit Amoxicillin

Mass of sample taken	0.4053 g
Standard (Amoxicillin base)	0.1201 g
Calculated Amoxicillin base	0.6005 g
Percentage purity of standard	99.2 %
The actual amount of Standard taken	0.6053 g

The detailed calculation of the HPLC Assay results for Suspected Counterfeit amoxicillin sample, is illustrated in the appendix D.

Dilution:

0.5 g of amoxicillin trihydrate corresponding to 0.6053 g of the standard was diluted in 100 mL of diluents to obtain a solution of a concentration of [0.5% (w/v)]. Also, 0.5 g of amoxicillin trihydrate corresponding to 0.8106 g of the sample was diluted in 100 mL of the diluents to obtain a solution of concentration of [0.5% (w/v)]

But from the sequence table (ie the peak summary)

Average area under curve for standard = 13746.933 mAU*s

Average area under curve for sample is,

Rep1=12813.90 mAU*s

Rep2=12880.10 mAU*s

Rep3=13012.90 mAU*s

Therefore, the total average area=12902.30 mAU*s

Hence, the percentage content of amoxicillin trihydrate contained in the suspected fake amoxicillin capsule =

$$\left(\frac{12902.30\ mAU*s}{13746.93\ mAU*s} \times \frac{1.2\ mg/ml}{1.0\ mg/ml} \times 866\ \mu g/mg \times 0.001\ mg/\mu g \times 100\ \%\right)$$

$$= 97.54\ \%$$

The percentage of the suspected fake is accepted because it falls within the range of the acceptance criteria according to the 6th edition of the British Pharmacopoeia.

4.5 CONCENTRATION OF ELEMENTS AND THE HPLC CHROMATOGRAM OF AMOXICILLIN SAMPLES.

The detailed concentration of elements of the various Amoxicillin samples analysed can be obtained the appendices. These includes:

Appendix E: This illustrate the statistical data for standard Amoxicillin Trihydrate.

Appendix F: Shows the statistical data for imported Amoxicillin.

Appendix G: Demonstrate the statistical data for Okaishi Amoxicillin Capsules.

Appendix H1, H2 and H3: Illustrates the peak summary of all the Amoxicillin analysed.

The graphs obtained from the peak summary gives the HPLC Chromatogram of all the samples.

Appendix I: Demonstrate the HPLC chromatogram for standard Amoxicillin Capsules.

Appendix J: Shows the HPLC chromatogram for Imported Amoxicillin Capsules.

Appendix K: Illustrate the HPLC chromatogram for Okaishi Amoxicillin Capsules.

4.6 DISSOLUTION TEST

In order to establish the facts on how the degree and rate at which a substance (drug) is absorbed into a living system is bioavailable, an in vitro dissolution test on the various brands of the amoxicillin capsules was administered.

4.6.1 Dissolution Results for Foreign Generic Amoxicillin (One)

The process prepared by British Pharmacopoeia (B.P 2016) was followed, in which paddles were used for 500 mg amoxicillin capsules. Six capsules of the foreign generic sample each weighing 0.6802 g were each placed in the dissolution paddle containing 900 mL of distilled water. The dissolution template and the results obtained for imported amoxicillin (1) are shown in Tables 4.8 and 4.9.

Table 4. 8: Dissolution Test Template

Apparatus	Paddles
Medium	Water, 900 mL
Blade's speed	75 rpm
Blade's distance from bottom of vessel	25 ± 2 mm
Temperature	37 ± 0.5 ºC
Detection wavelength	272 nm
Time	60 mins

Table 4.9 shows the dissolution percentage of each of the samples put in the paddles.

Table 4. 9: Dissolution Percentage of Imported Generic Amoxicillin (One)

Vessel Number	Average Ordinate Values	Dissolution percentage (%)
1	0.8084	121.1
2	0.8227	123.8
3	0.8134	122.4
4	0.7941	119.5
5	0.7946	119.6
6	0.8209	123.7
STD	0.5758	

$$\% \text{ Dissolution} = \frac{Mean\ Sample\ \ absorbed}{Mean\ Standard\ absorbed} \times \frac{Conc\ of\ Std(\ mg/ml)}{Conc\ of\ Smpl(\ mg/ml)} \times 866\ \mu g/mg \times 0.001 mg/\mu g \times 100\ \%$$

According to the British Pharmacopoeia 2016 edition, the threshold tolerance level is 85 % $\pm$ 5 % below this percentage a medical drug is duly disapproved. The dissolution percentage range from Table 4.8 is 119.5 % - 123.8 % which is above the 85 % tolerance level.

4.6.2 Dissolution Results for Imported (Foreign) Generic Amoxicillin (Two)

Similarly, using the British Pharmacopoeia protocol, results of the test are shown in Table 4.10

Table 4. 10 Dissolution Percentage of Imported Generic Amoxicillin

Vessel Number	Average Ordinate Values	Dissolution percentage (%)
1	0.7476	112.07
2	0.7159	107.33
3	0.7196	107.87
4	0.7691	115.29
5	0.7672	115.01
6	0.7647	114.63
STD	0.5781	

The dissolution percentage range from table 4.9 is 107.33% - 115.29%. This falls above the threshold tolerance level of 85% ± 5% indicating the acceptance of the sample as far as dissolution criteria is concerned.

4.6.3 Dissolution Results for NHIS Amoxicillin

Applying the British Pharmacopoeia protocol, results of the test are shown in Table 4.11

Table 4. 11: Dissolution Percentage of NHIS Amoxicillin

Vessel Number	Average Ordinate Values	Dissolution percentage (%)
1	0.7685	113.50
2	0.7403	109.33
3	0.6199	91.55
4	0.7496	110.70
5	0.7407	109.39
6	0.6589	97.34
STD	0.5868	

According to the British Pharmacopoeia 2016 edition, the threshold tolerance level is 85% ± 5% below this percentage a medical drug is duly disapproved. The dissolution percentage range from Table 4.10 is 91.55% - 113.50% which is above the 85% tolerance level.

4.6.4 Dissolution Results for OKAISHI Amoxicillin

In the case of 250 mg amoxicillin capsules, the dissolution basket approach of the BP protocol was employed. Six capsules of the sample with each containing 0.2857g were used for the test. The dissolution template and results are shown in Tables 4.12 and 4.13

Table 4. 12: Dissolution Test Template

Apparatus	Baskets
Medium	Water, 900 mL
Blade's speed	100 rpm
Blade's distance from bottom of vessel	25 ± 2 mm
Temperature	37 ± 0.5 ºC
detection wavelength	272 nm
Time	60 mins

Table 4. 13: Dissolution Percentage of NHIS Amoxicillin

Vessel Number	Average Ordinate Values	Dissolution percentage (%)
1	0.7681	94.96
2	0.7972	99.38
3	0.7899	98.47
4	0.7925	98.79
5	0.7652	95.39
6	0.7906	98.55
STD	0.6952	-

The dissolution percentage range from Table 4.12 is 94.96% - 99.38% which falls above the threshold tolerance level of 85% ± 5% denoted acceptance in relation to the dissolution test.

4.6.5 Dissolution Results for Suspected Counterfeit Amoxicillin

Similarly, using the dissolution basket approach for 250 mg samples the results of the test are shown in Table 4.14.

Table 4. 14: Dissolution Percentage of Suspected Counterfeit Amoxicillin

Vessel Number	Average Ordinate Values	Dissolution percentage (%)
1	0.7334	84.18
2	0.7401	84.95
3	0.7237	83.06
4	0.7189	82.51
5	0.7310	83.90
6	0.7205	82.70
STD	0.7545	

The dissolution percentage range from Table 4.14 is 82.51% - 84.95% which is below the threshold tolerance tolerance level of the BP requirement, hence indicating failutre in meeting the dissolution test criteria.

Using the approved methodologies of the British Pharmacopoeia, analysis of the amoxicillin samples from Kwame Nkrumah Circle failed the Disintegration and Dissolution tests. This means that even though these sample have Active Principal Ingredients they can be classified as substandard. The failure of the said tests also means that their bioavailability antibiotics in the body will be compromised thus, affecting their curative effectiveness.

4.7 QUALITATIVE ANALYSIS OF PIXE SPECTRA

In the pharmaceutical industries, the Active Pharmaceutical Ingredient (API) is the substantial ingredient which is mostly called the Active substance in medicines, but a substance is formulated/produced alongside the Active Pharmaceutical ingredients of any medications/drugs is the excipients. Excipients are contained in all pharmaceutical formulation, this is because the API is mostly in the small amount of concentration.

More so, the identification of pharmaceutical product is aided by the excipient and that is an inorganic aspect of any drugs that are to be analysed.

PIXE was used in the analysis of standard amoxicillin, imported amoxicillin and two amoxicillin from the market (Okaishi and Kwame Nkrumah Circle), after the bombardment of different brands of antibiotic samples (Amoxicillin Capsules), a detailed PIXE spectrum was acquired using 2.5 MeV proton respectively. After the sample is loaded in the GUPIX software with its corresponding parameter file, a typical uncalibrated spectrum was observed. A click on the calibration button results into

energy calibration for elemental peaks in GUPIX, the spectrum is labelled on top with the corresponding elements.

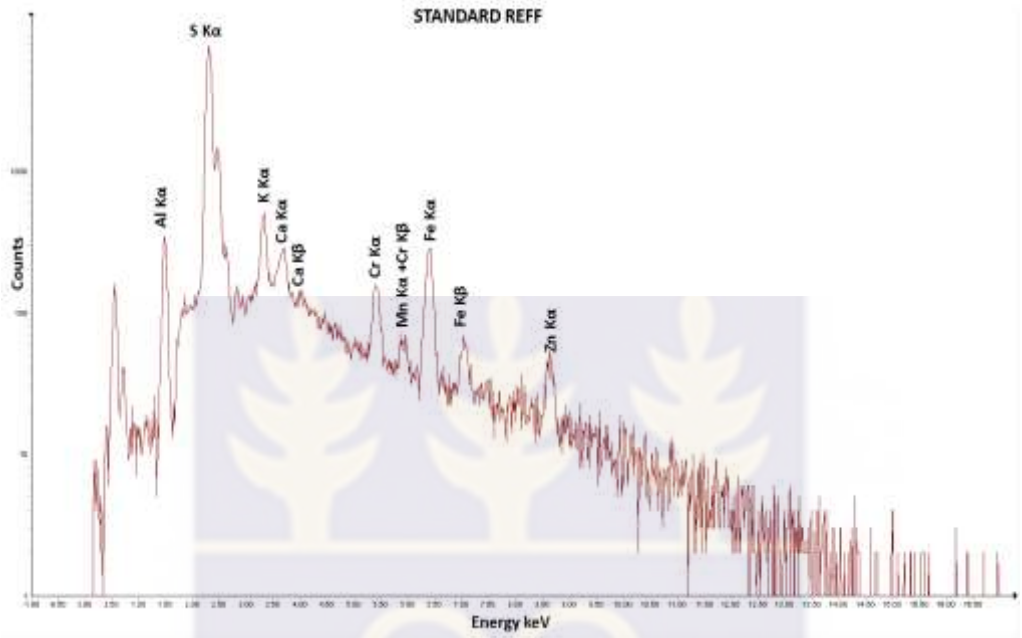

Figure 4. 1: A graph of Pixe spectrum of reference standard

A certified reference standard material of Amoxicillin trihydrate with batch number 14030026070 and purity 98.2% was obtained from Ernest Chemist a pharmaceutical manufacturing company in Ghana. The standard contains mostly the organic elements, the molecular formula of amoxicillin trihydrate is given as $C_{16}H_{19}N_3O_5S$. The standard was however analysed using PIXE to ascertain the inorganic component of the sample.

Fig 4.1. displays the spectrum obtained from the standard sample by PIXE analysis. The elements identified in the spectrum are Al, S, K, Ca, Cr, Mn, Fe and Zn. Other X-ray spectra were generated from the remaining samples, and the elements in the spectra were compared with different brands of amoxicillin obtained from different

pharmaceutical companies. The other samples included the imported amoxicillin, okaishi amoxicillin and suspected counterfeit amoxicillin.

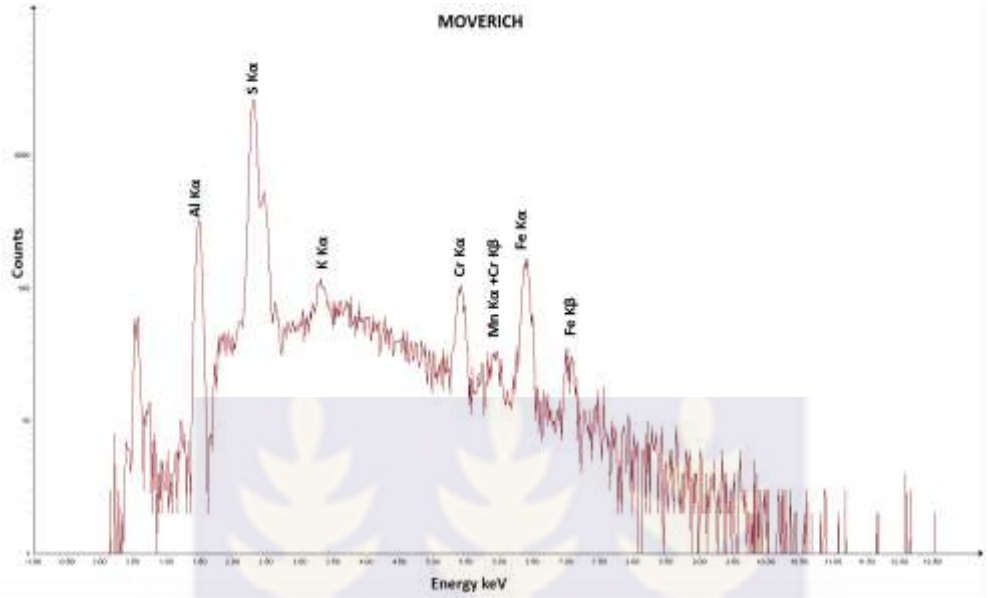

Figure 4.2: Pixe spectrum of Imported amoxicillin

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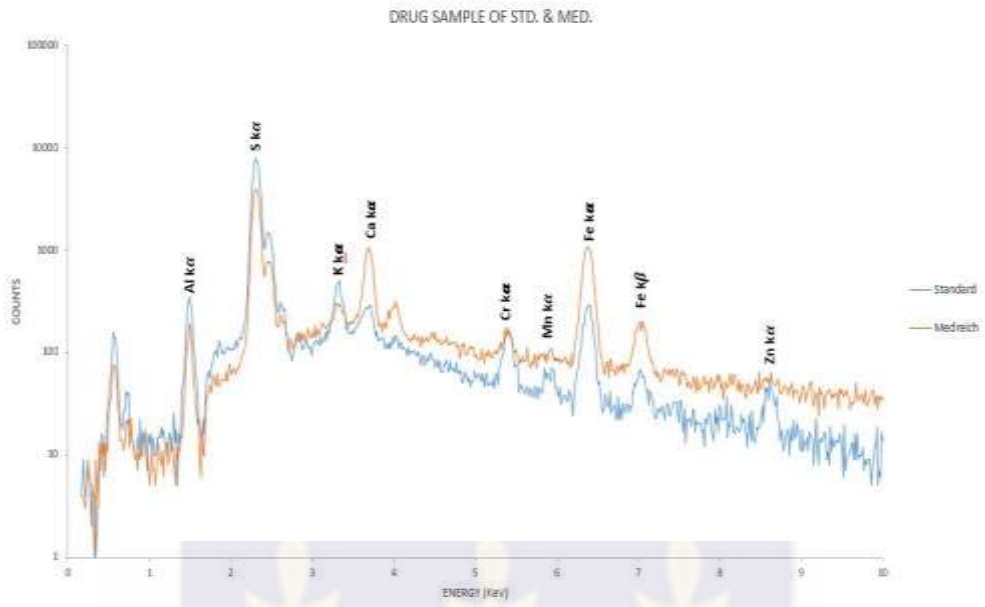

Figure 4. 3 : Pixe spectra of standard and Imported amoxicillin

Fig 4.2, illustrates the spectrum of the imported amoxicillin attained from the analysis. The elements identified are Al, S, K, Cr, Mn and Fe. When the imported standard was superimposed on the reference standard from Fig 4.1, it was observed there were the additional elements of Ca and Fe were present in relatively higher x-ray intensities in the imported sample. It was also noted that sulphur was the prominent peak in both samples.

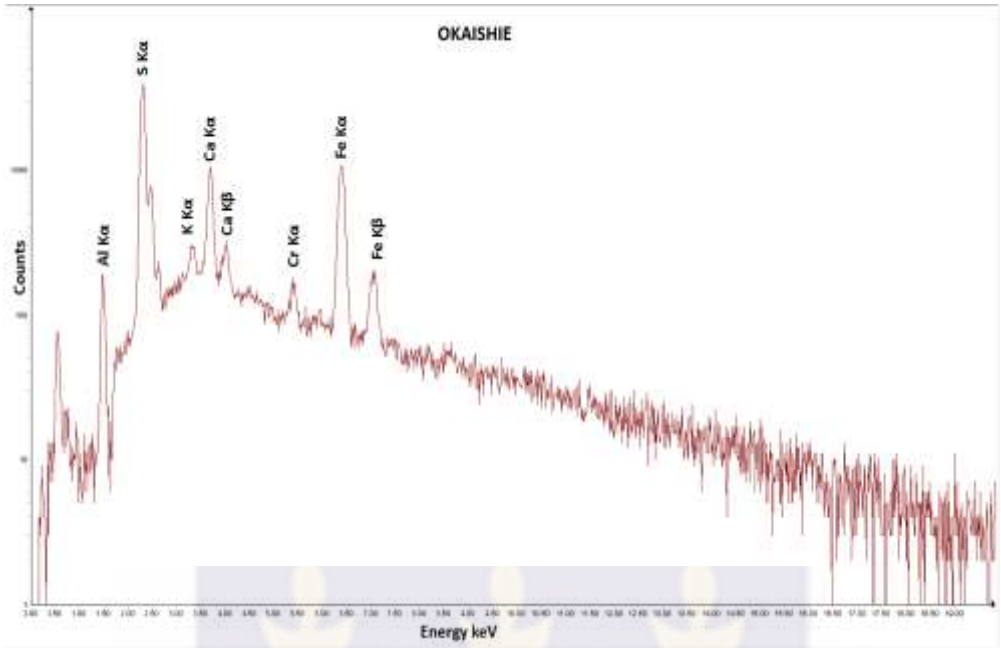

Figure 4. 4:Pixe spectrum of Okaishi amoxicillin

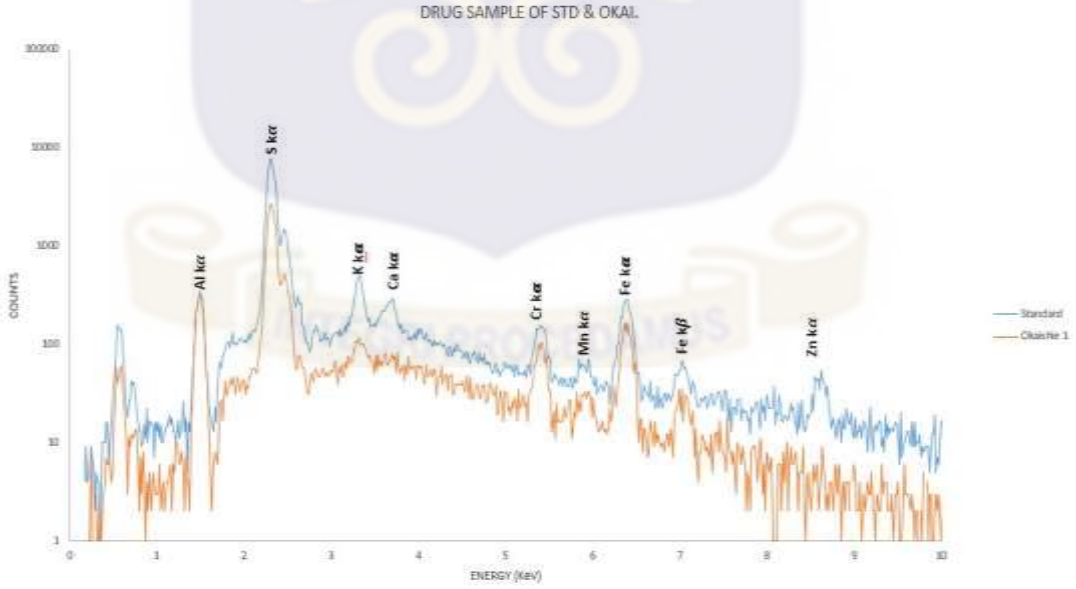

Figure 4. 5 : Pixe spectra of standard and Okaishi

Figure 4.4, shows the spectra of Okaishi amoxicillin acquired from the PIXE analysis. Elements that were singled out include Al, S, K, Ca, Cr and Fe. By overlapping the spectra of Okaishi amoxicillin with the reference standard (Figure 4.1), Fig 4.5 was obtained. It was also realised that Mn and Zn were not present. Here again, the prominent peak in both samples is sulphur.

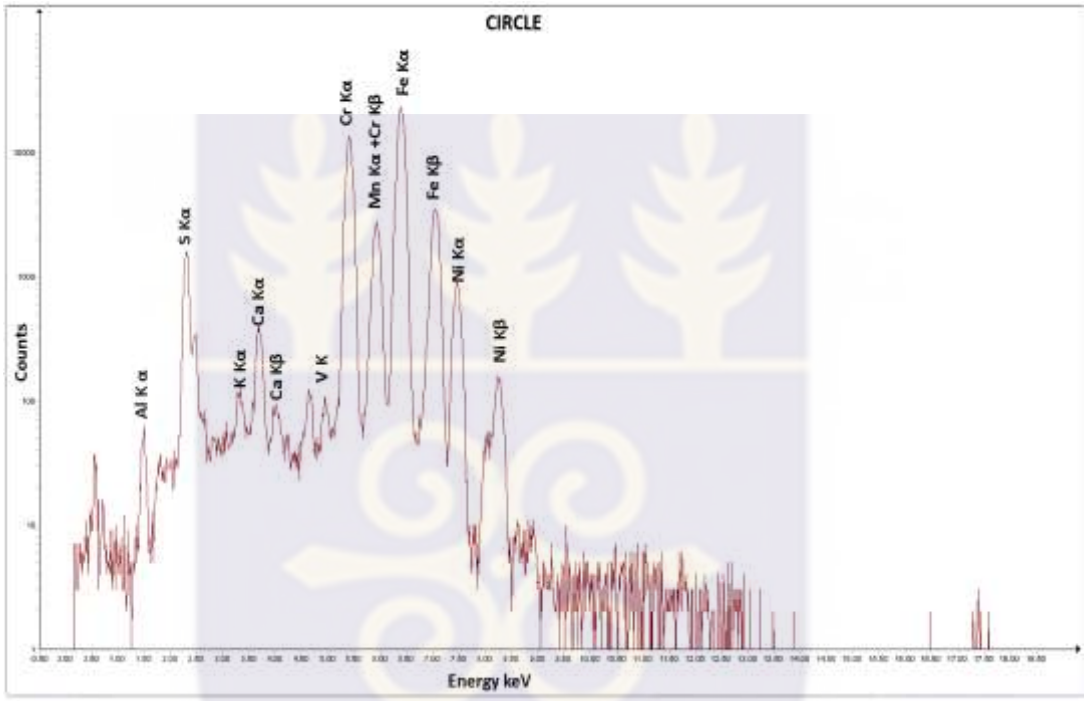

Figure 4. 6:Pixe spectrum of suspected counterfeit amoxicillin

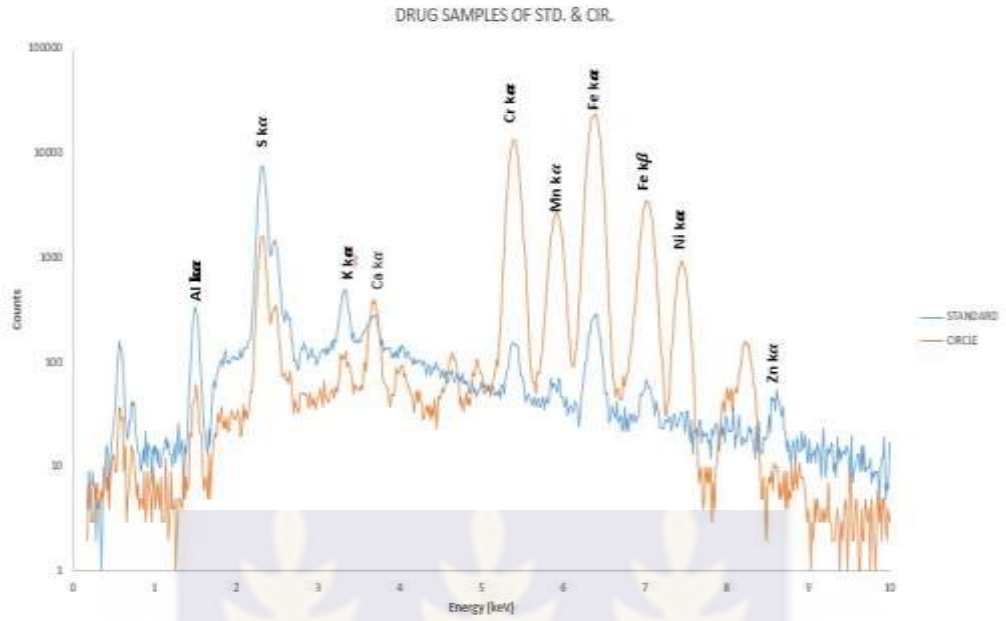

Figure 4. 7:Pixe spectra standard and suspected counterfeit amoxicillin

Figure 4.6, shows the spectrum of suspected counterfeit amoxicillin. The analysis revealed that Al, S, K, Ca, V, Cr, Mn, Fe and Ni were present. Upon comparing the suspected counterfeit amoxicillin with the reference standard as illustrated above (Figure 4.1), fig 4.7 was realised, additional elements like V and Ni were observed. Besides, the elements Cr, Mn, and Fe were present with very high intensities. Sulphur (S) , however, was noted to have a much lower intensity compared to the standard amoxicillin.

All the samples analysed contained the elements Al, S, K, Ca, Cr, and Fe but in varying X-ray intensities. V, Mn, Zn and Ni were the additional elements found in some the amoxicillin samples.

4.8 QUANTITATIVE ANALYSIS OF PIXE SPECTRA

Quantitative analysis is carried out to determine the concentration of the elements identified in the qualitative analysis. This requires the extraction of the X-ray intensities of the elements, sensitivity calibration and the evaluation of the quantities of elements present in the sample. The fitting parameter file was stored and used for the energy calibration of all the spectra obtained from this work. The quantitative analysis of all the spectra attained from this work was through the use GUPIX software.

However, the GUPIX run was performed to attain the peak area fit of the PIXE spectra. The peak area fits integrate the area under all the peaks (elements) identified in the spectrum. Moreso, the accepted fit residuals is less than 10%, this is to check the error margin during the GUPIX run. The area under every given peak enbles the quantification of the concentration levels of that element. Figure 4.8 below shows the data fit and residues for the standard reference material. Aluminium (Al) used as a trace element was heavily attenuated by the mylar filter to provide characteristic x-ray energies. The spectrum intensities and corresponding energies were obtained from the statistical counting data.

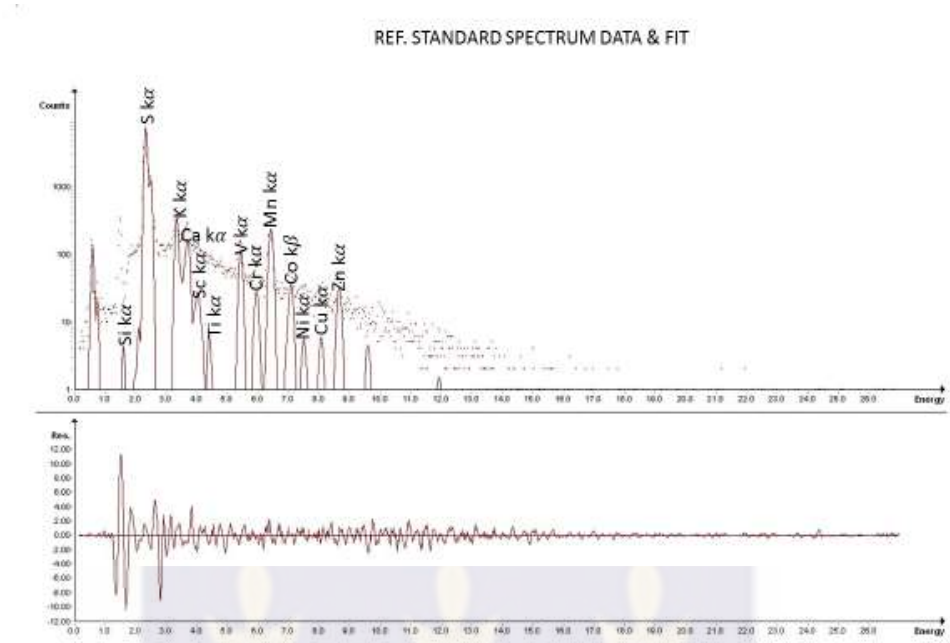

Figure 4. 8: Spectrum fitting and residues of a standard reference material done by GUPIX.

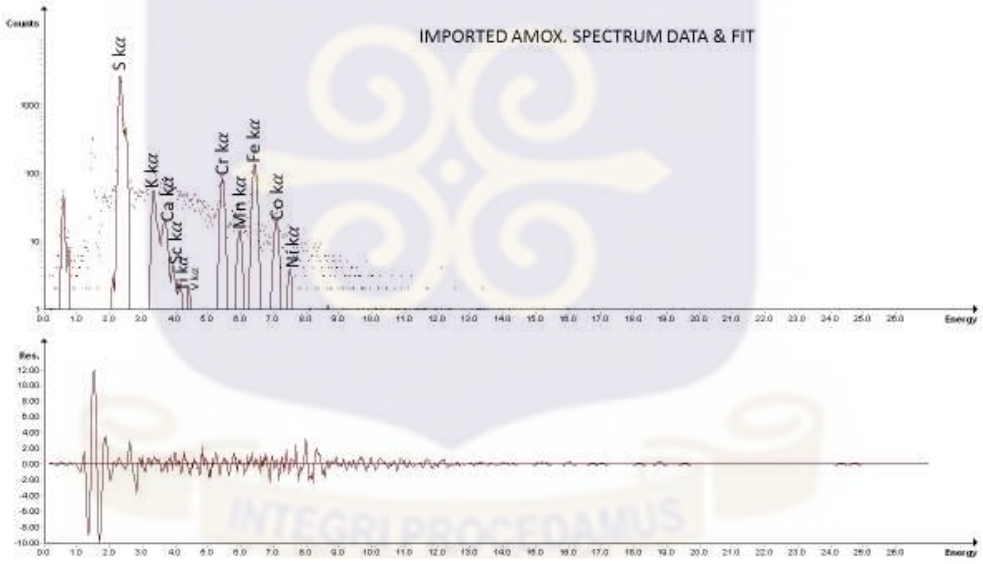

Figure 4. 9: Spectrum fitting and residues of imported amoxicillin done by GUPIX

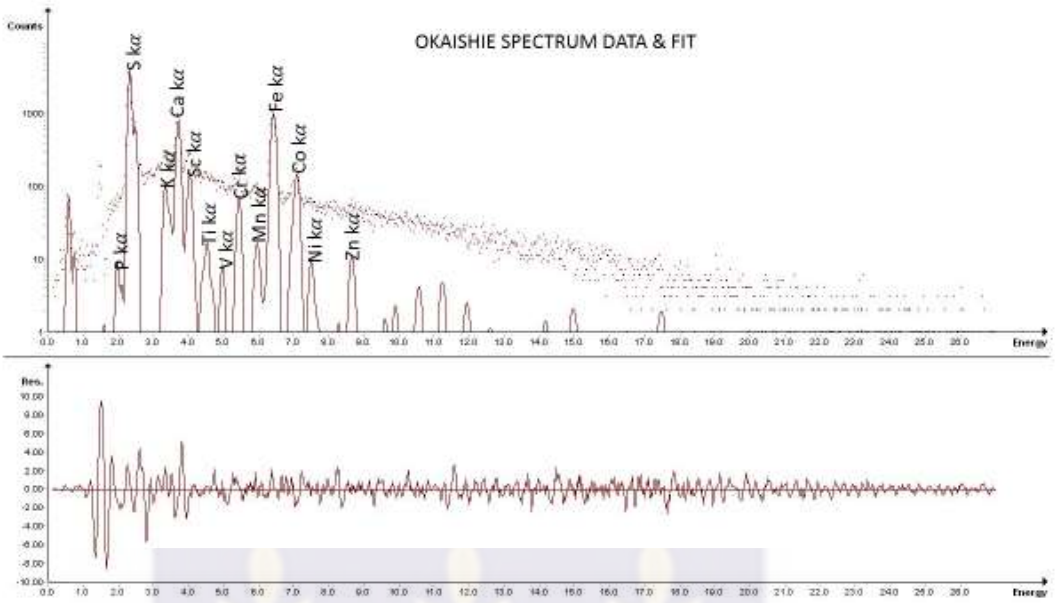

Figure 4. 10: Spectrum fitting and residues of Okaishi amoxicillin done by GUPIX

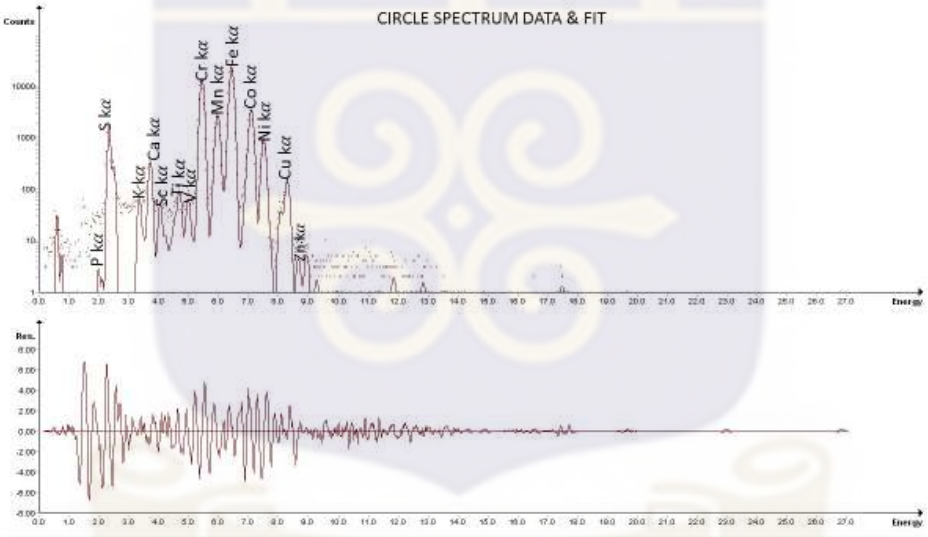

Figure 4. 11: Spectrum fitting and residues of Kwame Nkrumah Circle amoxicillin done by GUPIX

Validation of the quantitative method was implemented using standard reference materials, results of which are as shown in Tables 4.14 – 4.16

Table 4. 15: Validation of PIXE analysis setup for metal samples using NIST SRM 1157

3 MeV Helium

Beam current 10 nA

Charge collected 10 µC

Elements	Concentration (%), SRM 1157		Absolute Deviation (%)
	Measured Value	Certified Value	
V	1.88	1.82	3.29
Cr	4.52	4.30	5.11
Mn	0.31	0.34	8.82
Fe	81.14	81.00	0.17
Co	ND	0.03	-
Ni	0.21	0.23	17.39
Cu	0.08	0.09	15.56
Mo	4.73	4.86	2.67
W	6.06	6.28	3.50

Table 4. 16: Validation of PIXE analysis setup for glass samples using NIST SRM 611

2.5 MeV Proton

Beam current 10 nA

Charge collected 10 µC

Elements	SRM 6115 (%)	Measured (%)	Dev (%)
CaO	12	11.09	7.58
SiO2	72	65	9.72
Na2O	14	ND	ND
Al2O3	2	ND	ND
Sb	415.3	ND	ND
As	340	337	0.88
Ba	453	ND	ND
Cd	244	387	58.60
Cr	415	428	3.13
Fe	458	467	1.97
Mn	457	464	1.53
Ni	458.7	459	0.07
Rb	425.7	395	7.21
Se	115.2	144	25.00
Sr	515.5	526	2.04
Cu	444	449	1.13
Th	457.2	ND	ND
Co	390	397	1.79
K	461	413	10.41
Ti	437	1020	133.41
Zn	433	380	12.24

Table 4. 17: Validation of PIXE analysis setup for powdered pelletized rock samples using

SRM Diorite sample

2.5 MeV Proton

Beam current 10 nA

Charge collected 10 µC

Elements	SRM DR-N (Diorite) (%)	Measured (%)	Dev (%)
SiO2	52.85	60.0	13.53
Al2O3	17.52	ND	ND
Fe2O3	97	97.65	0.67
MnO	0.22	0.21	4.55
MgO	4.4	ND	ND
Na2O	2.99	ND	ND
CaO	7.05	6.84	2.98
K2O	1.7	2.00	17.64
TiO2	1.09	1.13	3.67
S	0.0350	0.0338	3.43
Zn	0.0145	0.0143	1.38
SiO2	52.85	60.0	13.53
Al2O3	17.52	ND	ND

Table 4. 18: The concentrations of the elements identified in the amoxicillin drug samples

Element Z	Sym	#	Area counts	value (-6)	/uC/ ppm	Eff. (-3)	Trans. (-5)	Conc. ppm	%Stat. Error	%Fit Error	LOD ppm	
16	S K	1	32477.2	309	7785	749	873	396805.6	0.48	2.17	1174.9	Y
19	K K	1	1678.1	309	2808	906	19661	2084.2	3.32	4.31	98.7	Y
20	CaK	1	643.0	309	2857	931	30829	487.3	8.32	8.38	73.2	Y
22	TiK	1	0	309	2414	962	52592	0	0	0	53.8	N
23	V K	1	0	309	2176	971	61627	0	0	0	41.8	N
24	CrK	1	654.2	309	1997	977	69150	301.4	5.25	5.44	23.3	Y
25	MnK	1	80.8	309	1715	982	75268	39.6	34.25	31.75	26.6	?
26	FeK	1	1460.7	309	1483	987	80177	774.3	2.72	2.90	21.5	Y
27	CoK	1	18.1	309	1208	989	84062	11.2	139.05	120.44	29.1	N
28	NiK	1	36.3	309	1027	990	87147	25.5	57.47	53.66	27.3	?
29	CuK	1	38.9	309	793.5	991	89592	34.3	50.91	48.00	29.3	?
30	ZnK	1	216.9	309	636.0	988	91530	234.3	9.49	9.88	31.3	Y
31	GaK	1	0	309	486.3	981	93072	0	0	0	67.8	N
32	GeK	1	0	309	373.6	965	94303	0	0	0	75.0	N
33	AsK	1	0	309	288.9	941	95290	0	0	0	86.9	N
34	SeK	1	0	309	218.4	908	96085	0	0	0	109.6	N
35	BrK	1	10.2	309	172.6	866	96729	44.0	89.94	100.76	74.8	?
37	RbK	1	0	309	102.2	763	97681	0	0	0	171.2	N
38	SrK	1	0	309	79.41	704	98032	0	0	0	166.0	N
39	Y K	1	3.5	309	62.27	646	98322	55.8	137.26	169.14	154.3	N
40	ZrK	1	0	309	48.47	591	98562	0	0	0	187.4	N
41	NbK	1	0	309	39.76	538	98762	0	0	0	201.0	N
42	MoK	1	4.8	309	31.04	484	98929	200.4	78.50	128.34	310.1	?
47	AgK	1	0	309	9.59	288	99444	0	0	0	1262.2	N
48	CdK	1	0	309	7.48	259	99506	0	0	0	1273.2	*N
50	SnK	*	0	309	4.70	211	99604	0	0	0	5020.9	N
51	SbK	1	0	309	3.74	190	99644	0	0	0	6914.1	N
50	SnLA	1	314.5	309	1059	916	23472	858.8	24.62	26.60	326.5	?
80	HgLA	1	6.8	309	183.2	962	94474	25.5	313.69	274.15	152.4	N
82	PbLA	1	8.5	309	151.5	941	95301	39.0	220.82	273.17	150.9	N

Figures 4.12, 4.13 below shows the percentage concentration of Sulfur in all the samples analysed and the comparisons between the percentages of all other elements in the samples.

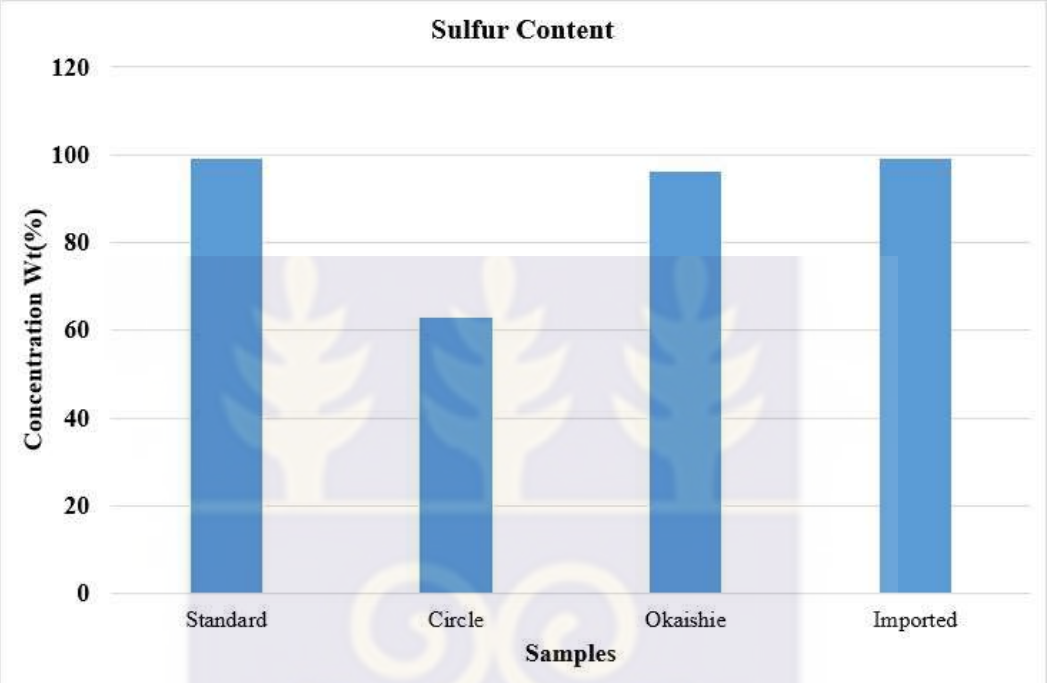

Figure 4. 12: Sulphur concentrations of Samples

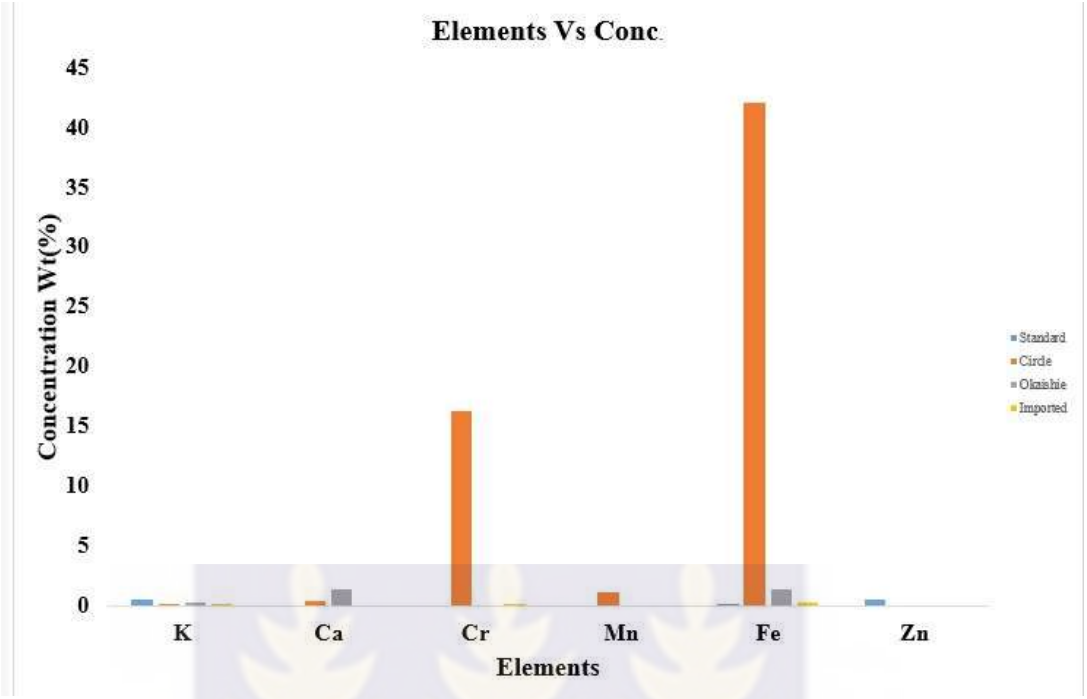

Figure 4. 13: Concentration of other elements

4.9 ELEMENTAL SIGNATURES

The structural formula for the API amoxicillin, is presented in Fig 4.14.

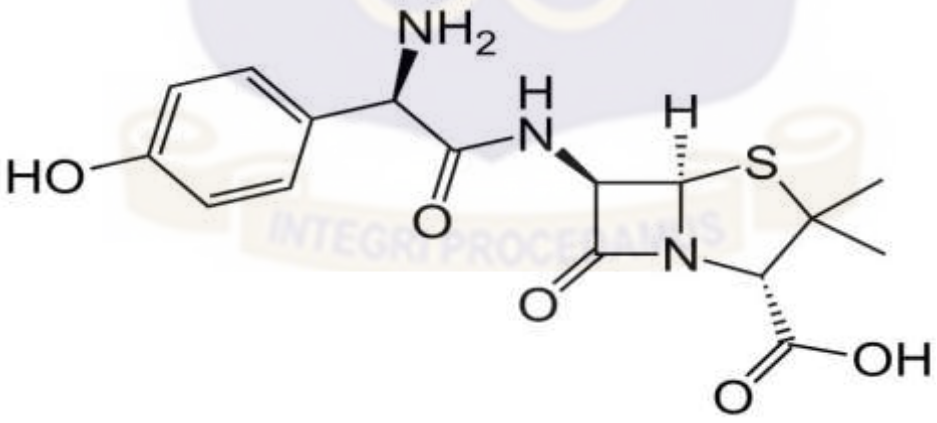

Figure 4. 14: Amoxicillin structural formular

From the amoxicillin structure, sulphur is a dominant inorganic element. Furthermore, the statistical counting data also indicates how prominent sulphur appeared in all the sample analysed. Consequently, Factor Analysis was carried out to verify correlations

with the other elements present in order to ascertain elements that lend themselves as elemental signatures for amoxicillin antibiotic drug.

From the Factor Analysis, the elements Cr, Mn, and Fe correlates with sulphur (S) and these suggest the use of these elements as inorganic signature of amoxicillin. The correlation of Sulphur against all the other elements are illustrated in graphs below;

4.9.1 Strength of Correlations

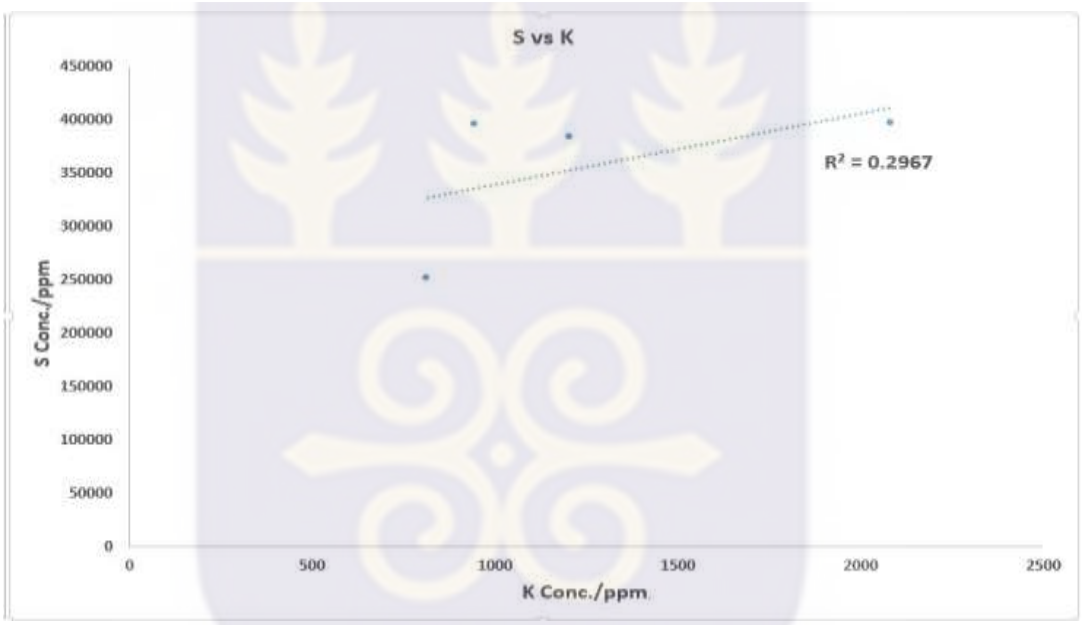

Figure 4. 15: A graph of sulphur versus potassium

From the graph above sulphur against potassium showed a weak positive correlation.

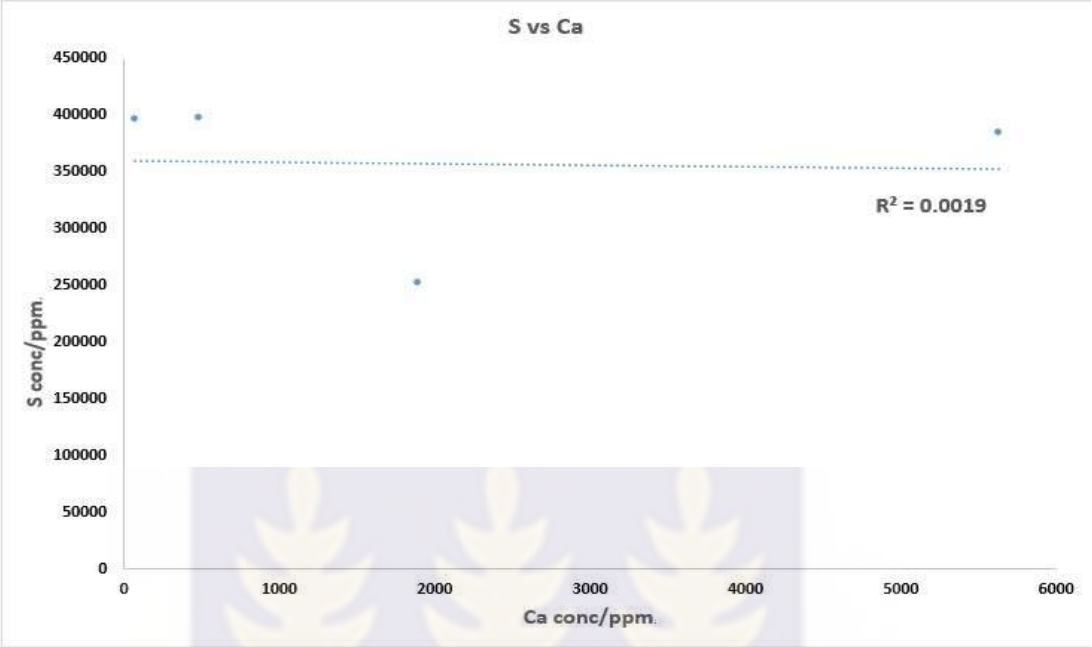

Figure 4. 16: A graph of sulphur against calcium

The graph above shows negligible or no relationship between the two elements.

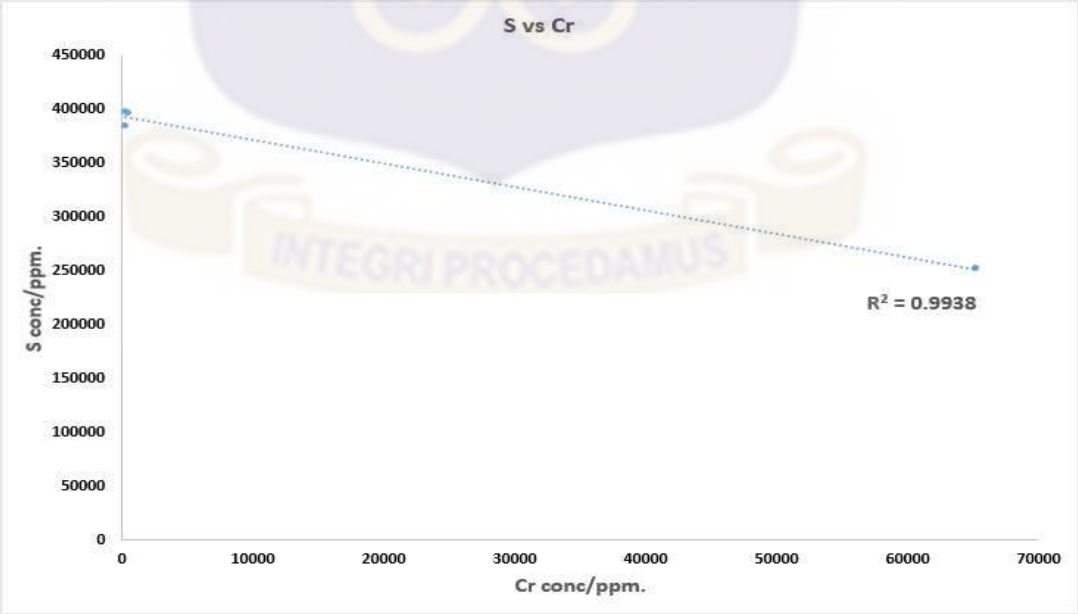

Figure 4. 17: A graph of sulphur versus chromium

The elements above indicates a very strong positive correlation or relationship, though is a negative correlation. However, two relationships with the same value have the same strength in terms of correlation whether positive or negative. Thus the variables move in an opposite or inverse direction, implies as Sulphur increases, the Chromium decreases and as Chromium increases, Sulphur also decreases.

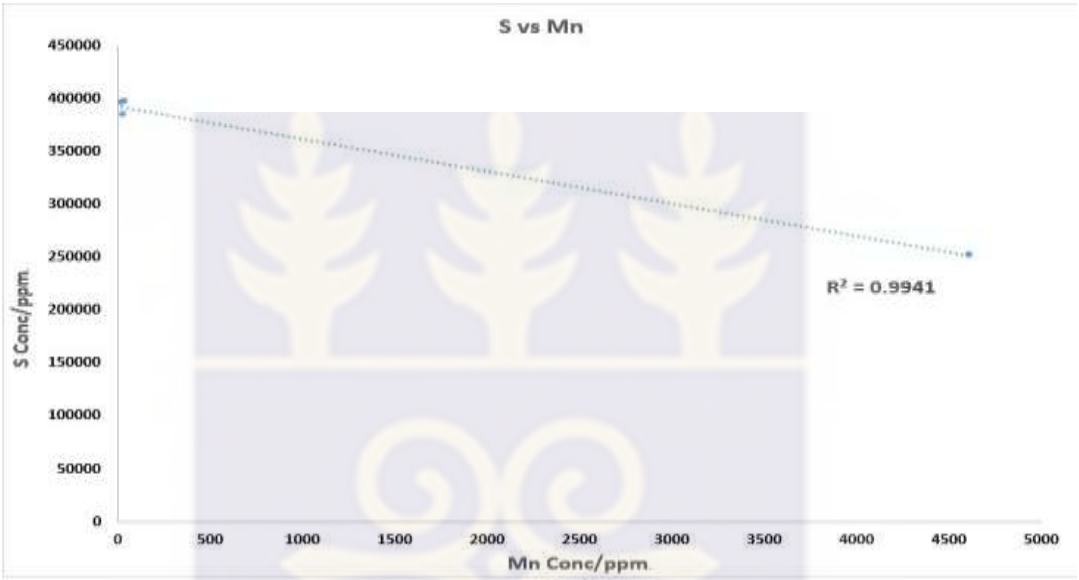

Figure 4. 18: A graph of sulphur against manganese

The elements in the graph above indicate a very strong positive correlation or relationship, though is a negative correlation. More so, two correlation or relationship with the same value have the same strength in terms of correlation whether positive or negative. Thus the variables move in an opposite or inverse direction, this means as Sulphur increases, the Manganese decreases and also as Manganese increases, Sulphur decreases.

A graph of Sulfur Versus Iron

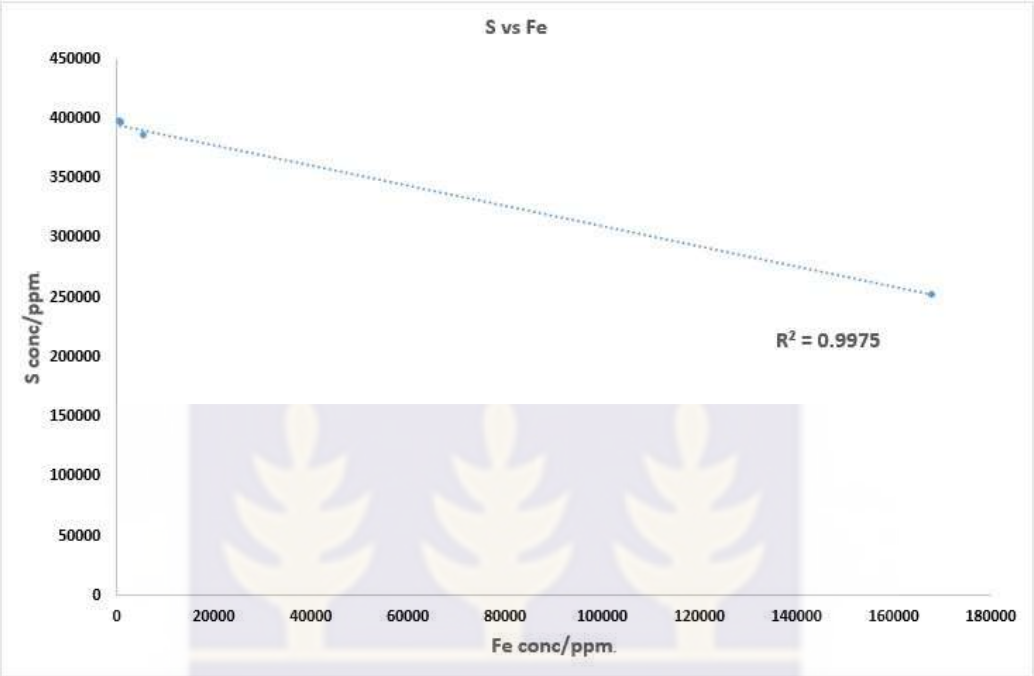

Figure 4. 19: A graph sulphur versus Iron

The elements above indicates a very strong positive correlation or relationship, though negative correlation. However, two relationships with the same value have the same strength in terms of correlation whether positive or negative. Thus the variables move in an opposite or inverse direction, implies as Sulphur increases, the Iron decreases and also Iron increases as Sulphur decreases.

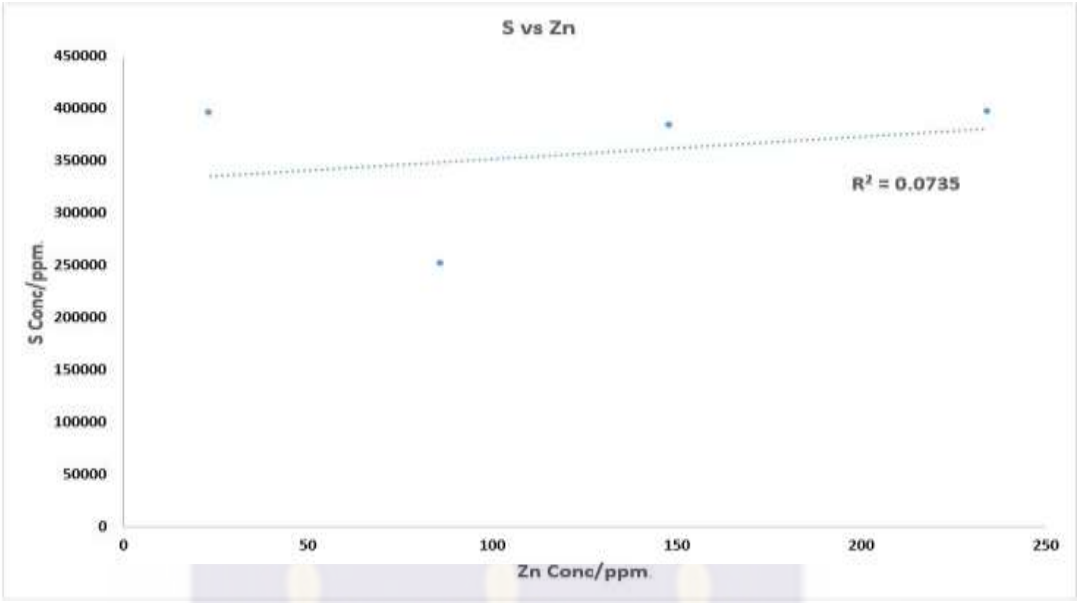

Figure 4. 20: A graph sulphur against zinc

The graph above indicates negligible or no relationship between the two elements.

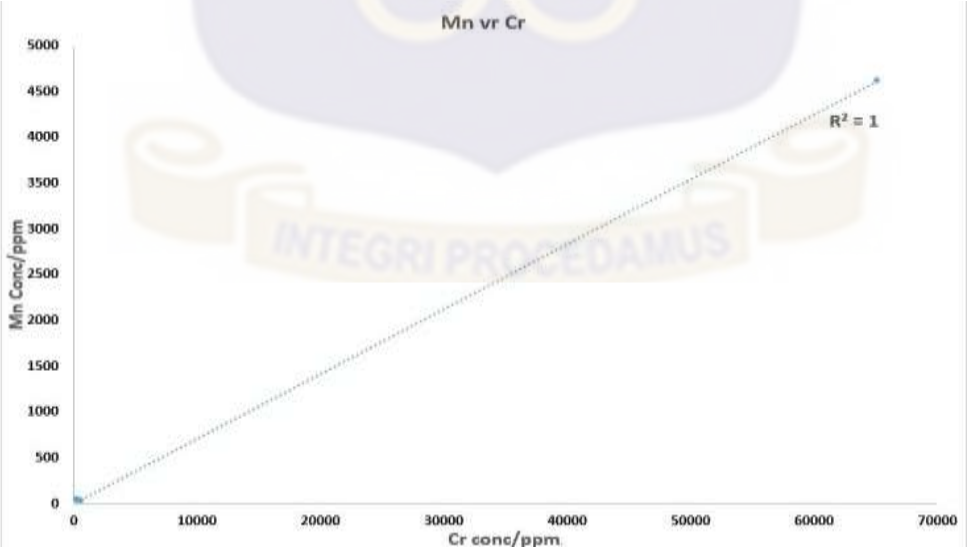

Figure 4. 21: A graph of magnesium versus chromium

The elements above indicates a perfect positive relation or correlation, which means the two elements move in tandem. Here Manganese increases as chromium increases or Manganese decreases while chromium decreases. However, the two element have 100% relationship.

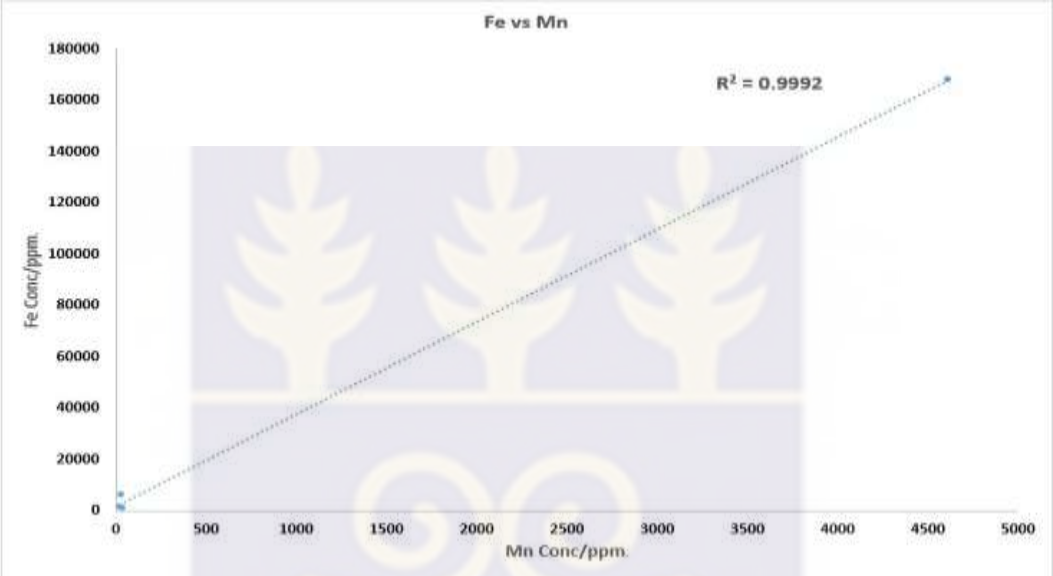

Figure 4. 22: A graph of Iron against Manganese

Figure 4.22 a very strong positive correlation or relationship from the above graph.

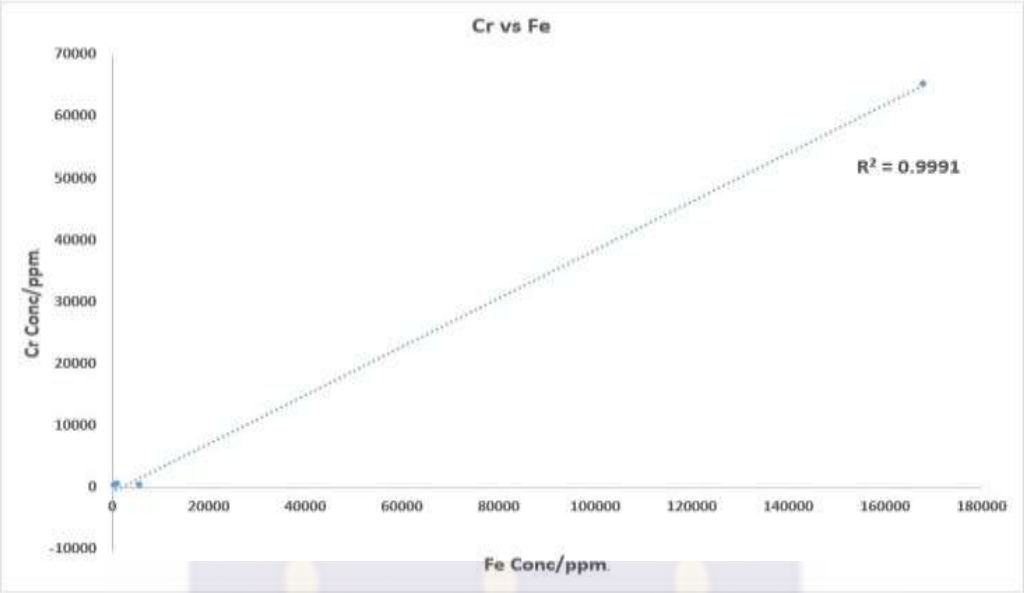

Figure 4. 23: A graph of chromium versus Iron

Fig 4.23 has a very strong positive correlation or relationship from the above graph

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

PIXE and HPLC were the two analytical methods employed in the analysis of amoxicillin antibiotic drugs purchased from pharmacy shops and the local markets in Accra, Ghana. The samples selected were imported and locally manufactured drugs (amoxicillin), as well as amoxicillin drugs of dubious source purchased from illegal sellers on the local market. PIXE was used to determine the elemental composition and concentrations in each brand of the amoxicillin sample. HPLC, being a prescribed method for drug quality control, was used for careful examination of the quality of the drugs from the point of view of their active principal ingredient (API). The HPLC and the physical parameter tests carried out on the samples helped by throwing more light that aids the interpretation of the inorganic element analysis.

The PIXE analysis identified the following elements in the samples analysed: Al, S, K, Ca, V, Cr, Mn, Fe, Ni, and Zn from the constituents in the antibiotic capsules. Whereas Al, S, K, Ca, Cr, and Fe were identified in all the samples; Mn, Ni, and Zn were the additional elements found in some of the samples The major element identified was Sulphur (S) having concentrations ranging from 25% - 40% with the lowest value being recorded for the amoxicillin drugs purchased from the market at "Kwame Nkrumah Circle". Additionally, the elements Cr, Mn, and Fe were found to be relatively higher in the sample from "Kwame Nkrumah Circle" than all the other samples. Hence the S, Cr, Mn, and Fe concentrations of samples from "Kwame Nkrumah Circle" raises eyebrows in regard to its quality. This query on the quality of this sample has been validated by the "Kwame Nkrumah Circle" sample failing the Disintegration and Dissolution tests. The combined analysis of PIXE and HPLC thus confirm the

amoxicillin antibiotic capsules bought from the local market at Kwame Nkrumah Circle as substandard or fake. On the other hand, the standard sample, the imported sample, and the sample from Okaishi passed the required tests according to the protocols of the British Pharmacopoeia (BP). Additionally, the Sulphur concentrations in these three brands of the amoxicillin antibiotics were similar and fell within the range of 38% - 40%. It should be noted that the elements that appeared in all the samples with concentration values higher than their lower limits of determination were Al, S, K, Ca, Cr, Mn, Fe, Ni, and Zn. These elements, therefore, provide the profile of major and minor elements for the amoxicillin antibiotic drug.

Since Sulphur is a constituent of the active principal ingredient (API), correlations with the other identified elements in the samples were investigated. Strong correlations with correlation coefficients within the range 0.9936 – 0.9978 were found with the elements Cr, Mn, and Fe. Hence the elements S, Cr, Mn, and Fe lend themselves as element signature for amoxicillin medical drug.

Drug quality assessment is usually carried out using the organic profile of the API with methods such as HPLC, X-ray Diffraction (XRD) analysis, and proton nuclear magnetic resonance (H-NMR) among others. PIXE, therefore, can also provide an additional analytical technique that can be employed for routine quality assessment of antibiotic drugs. Considering the simplicity in sample preparation, the non-distractive nature of the analysis, and also fast turn out in producing results.

PIXE offers a considerable advantage when analysing antibiotic drugs on a large scale.

5.2 RECOMMENDATIONS

a) Further studies should be conducted using larger quantities of amoxicillin drugs sampled from different brands, and cities in Ghana. The analysis should also cover the elemental composition of the empty capsules.

b) Further research should be carried using other technique of the pelletron accelerator like PIGE, RBS to mention a few.

c) The Food and Drugs Authority and additional supervisory organisations in Ghana ought to be vigilant and steady in checking of medications and antibiotics specifically on the marketplace.

d) Additional research should be carried out on other brands of antibiotics.

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APPENDICES

Appendix A: HPLC Assay Results for Imported Generic Amoxicillin

There were two (2) different brands of Imported generic amoxicillin used for this work.

Sample 1:

$$0.5\ g\ of Amoxicillin = 0.57621\ g$$

$$Mass\ of\ the\ sample\ taken = 0.57621\ g$$

Standard:

$$0.1\ g\ of\ Amoxicllin\ base = 0.1201g$$

$$0.5\ g\ of\ Amoxicillin\ base = \left(\frac{(0.5g \times 0.1201g)}{(0.1g)}\right) = 0.6005\ g$$

But the percentage purity of the standard = 99.2%

$$\text{Hence, the amount of standard to be used for the analysis} = \left(\frac{(100\% \times 0.6005g)}{(99.2\%)}\right) =$$

$$0.6053\ g$$

Therefore, the actual amount of standard taken = $0.6053\ g$

Sample 2:

$$0.5\ g\ of Amoxicillin = 0.59041\ g$$

$$Mass\ of\ the\ sample\ taken = 0.59041\ g$$

Standard:

$$0.1\ g\ of\ Amoxicllin\ base = 0.1201\ g$$

$$0.5\ g\ of\ Amoxicillin\ base = \left(\frac{(0.5\ g \times 0.1201\ g)}{(0.1\ g)}\right) = 0.6005\ g$$

But the percentage purity of the standard = 99.2%

Hence, the amount of standard to be used for the analysis = $\left(\frac{(100\ \% \times 0.6005\ g)}{(99.2\ \%)}\right) =$ $0.6053\ g$

Therefore, the actual amount of standard taken = $0.6053\ g$

Appendix B: HPLC Assay Results for NHIS Amoxicillin

Sample:

$$0.5\ g\ of Amoxicillin = 0.5380\ g$$

$$Mass\ of\ the\ sample\ taken = 0.5380\ g$$

Standard:

$$0.1\ g\ of\ Amoxicllin\ base = 0.1201\ g$$

$$0.5\ g\ of\ Amoxicillin\ base = \left(\frac{(0.5\ g \times 0.1201\ g)}{(0.1\ g)}\right) = 0.6005\ g$$

But the percentage purity of the standard = 99.2 %

Hence, the amount of standard to be used for the analysis = $\left(\frac{(100\ \% \times 0.6005\ g)}{(99.2\ \%)}\right) =$ $0.6053\ g$

Therefore, the actual amount of standard taken = $0.6053\ g$

Appendix C: HPLC Assay Results for OKAISHI Amoxicillin

Sample:

$$0.25\ g\ of Amoxicillin = 0.3275\ g$$

$$0.5\ g\ of\ Amoxicillin = 0.6550\ g$$

$$Mass\ of\ the\ sample\ taken = 0.6550\ g$$

Standard:

$$0.1\ g\ of\ Amoxicllin\ base = 0.1201\ g$$

$$0.5\ g\ of\ Amoxicillin\ base = \left(\frac{(0.5\ g \times 0.1201\ g)}{(0.1\ g)}\right) = 0.6005\ g$$

But the percentage purity of the standard = 99.2 %

Hence, the amount of standard to be used for the analysis = $\left(\frac{(100\ \% \times 0.6005\ g)}{(99.2\ \%)}\right) =$ $0.6053\ g$

Therefore, the actual amount of standard taken = $0.6053\ g$

Appendix D: HPLC Assay Results for Suspected Counterfeit Amoxicillin Sample:

$0.25\ g\ of Amoxicillin = 0.4053\ g$

$0.5\ g\ of\ Amoxicillin = 0.8106\ g$

$Mass\ of\ the\ sample\ taken = 0.8106\ g$

Standard:

$0.1\ g\ of\ Amoxicllin\ base = 0.1201\ g$

$0.5\ g\ of\ Amoxicillin\ base = \left(\frac{(0.5\ g \times 0.1201\ g)}{(0.1\ g)}\right) = 0.6005\ g$

But the percentage purity of the standard = 99.2 %

Hence, the amount of standard to be used for the analysis = $\left(\frac{(100\ \% \times 0.6005\ g)}{(99.2\ \%)}\right) =$ $0.6053\ g$

Therefore, the actual amount of standard taken = $0.6053\ g$

Appedix E: Statistical data for Standard Amoxicillin Trihydrate

Element		Layer	Area	H value	Yield /uC/	Det. Eff.	Filter Trans.	Conc.	%Stat.	%Fit	LOD	
Z	Sym	#	counts	(-6)	ppm	(-3)	(-5)	ppm	Error	Error	ppm	-
16	S K	1	32477.2	309	7785	749	873	396805.6	0.48	2.17	1174.9	Y
19	K K	1	1678.1	309	2808	906	19661	2084.2	3.32	4.31	98.7	Y
20	CaK	1	643.0	309	2857	931	30829	487.3	8.32	8.38	73.2	Y
22	TiK	1	0	309	2414	962	52592	0	0	0	53.8	N
23	V K	1	0	309	2176	971	61627	0	0	0	41.8	N
24	CrK	1	654.2	309	1997	977	69150	301.4	5.25	5.44	23.3	Y
25	MnK	1	80.8	309	1715	982	75268	39.6	34.25	31.75	26.6	?
26	FeK	1	1460.7	309	1483	987	80177	774.3	2.72	2.90	21.5	Y
27	CoK	1	18.1	309	1208	989	84062	11.2	139.05	120.44	29.1	N
28	NiK	1	36.3	309	1027	990	87147	25.5	57.47	53.66	27.3	?
29	CuK	1	38.9	309	793.5	991	89592	34.3	50.91	48.00	29.3	?
30	ZnK	1	216.9	309	636.0	988	91530	234.3	9.49	9.88	31.3	Y
31	GaK	1	0	309	486.3	981	93072	0	0	0	67.8	N
32	GeK	1	0	309	373.6	965	94303	0	0	0	75.0	N
33	AsK	1	0	309	288.9	941	95290	0	0	0	86.9	N
34	SeK	1	0	309	218.4	908	96085	0	0	0	109.6	N
35	BrK	1	10.2	309	172.6	866	96729	44.0	89.94	100.76	74.8	?
37	RbK	1	0	309	102.2	763	97681	0	0	0	171.2	N
38	SrK	1	0	309	79.41	704	98032	0	0	0	166.0	N
39	Y K	1	3.5	309	62.27	646	98322	55.8	137.26	169.14	154.3	N
40	ZrK	1	0	309	48.47	591	98562	0	0	0	187.4	N
41	NbK	1	0	309	39.76	538	98762	0	0	0	201.0	N
42	MoK	1	4.8	309	31.04	484	98929	200.4	78.50	128.34	310.1	?
47	AgK	1	0	309	9.59	288	99444	0	0	0	1262.2	N
48	CdK	1	0	309	7.48	259	99506	0	0	0	1273.2	*N
50	SnK	*	0	309	4.70	211	99604	0	0	0	5020.9	N
51	SbK	1	0	309	3.74	190	99644	0	0	0	6914.1	N
50	SnLA	1	314.5	309	1059	916	23472	858.8	24.62	26.60	326.5	?
80	HgLA	1	6.8	309	183.2	962	94474	25.5	313.69	274.15	152.4	N
82	PbLA	1	8.5	309	151.5	941	95301	39.0	220.82	273.17	150.9	N

(A "*" by the LOD value indicates the minimum background was used to calc LOD)
(A "*" in the Layer# column indicates that elmt NOT used in matrix iteration)
In the matrix iteration section it was found that a correction needed to be applied to the H or uC value in order for the concentrations to sum to unity.
The concentrations listed in the table above include this correction value.
With the user supplied H & uC values alone the conc would be the above values multiplied by 0.521 (See the documentation for further discussion.)

Appedix F: Statistical data for Imported Amoxicillin

Element Z	Sym	Layer #	Area counts	H value (-6)	Yield /uC/ ppm	Det. Eff. (-3)	Filter Trans. (-5)	Conc. ppm	%Stat. Error	%Fit Error	LOD ppm	
16	S K	1	11647.3	309	7734	749	873	395753.6	0.79	2.55	1203.4	Y
19	K K	1	275.2	309	2809	906	19661	944.2	11.69	12.73	182.6	Y
20	CaK	1	34.7	309	2861	931	30829	72.6	86.81	81.82	119.8	?
22	TiK	1	4.1	309	2406	962	52592	5.8	566.61	511.07	62.8	N
23	V K	1	0	309	2168	971	61627	0	0	0	80.8	N
24	CrK	1	468.7	309	1991	977	69150	598.3	5.40	5.70	37.6	Y
25	MnK	1	17.3	309	1710	982	75268	23.5	110.97	103.46	53.9	N
26	FeK	1	839.6	309	1478	987	80177	1232.9	3.38	3.59	22.8	Y
27	CoK	1	3.0	309	1206	989	84062	5.1	538.21	470.83	52.5	N
28	NiK	1	25.2	309	1024	990	87147	49.0	49.24	43.79	38.3	?
29	CuK	1	0	309	792.2	991	89592	0	0	0	61.6	N
30	ZnK	1	7.9	309	635.2	988	91530	23.5	105.87	104.43	46.1	?
31	GaK	1	2.7	309	485.9	981	93072	10.5	266.28	236.56	50.9	N
32	GeK	1	5.0	309	373.5	965	94303	25.4	119.18	113.43	55.6	?
33	AsK	1	0	309	288.9	941	95290	0	0	0	91.6	N
34	SeK	1	3.9	309	218.4	908	96085	35.1	101.95	116.29	62.4	?
35	BrK	1	0	309	172.7	866	96729	0	0	0	93.6	N
37	RbK	1	4.8	309	102.3	763	97681	108.9	75.13	101.56	150.9	?
38	SrK	1	0	309	79.48	704	98032	0	0	0	123.4	N
39	Y K	1	0	309	62.34	646	98322	0	0	0	170.9	N
40	ZrK	1	0	309	48.53	591	98562	0	0	0	239.5	*N
41	NbK	1	0	309	39.82	538	98762	0	0	0	320.1	N
42	MoK	1	0	309	31.10	484	98929	0	0	0	454.3	*N
47	AgK	1	0	309	9.65	288	99444	0	0	0	2448.8	*N
48	CdK	1	0	309	7.50	259	99506	0	0	0	3508.6	*N
50	SnK	*	0	309	4.71	211	99604	0	0	0	6836.5	*N
51	SbK	1	0	309	3.75	190	99644	0	0	0	9540.5	*N
50	SnLA	1	104.4	309	1058	916	23472	788.6	40.37	45.98	558.6	?
80	HgLA	1	3.0	309	183.1	962	94474	31.0	288.45	266.75	161.1	N
82	PbLA	1	0	309	151.5	941	95301	0	0	0	167.8	N

(A "*" by the LOD value indicates the minimum background was used to calc LOD)
(A "*" in the Layer# column indicates that elmt NOT used in matrix iteration)
In the matrix iteration section it was found that a correction needed to be applied to the H or uC value in order for the concentrations to sum to unity.
The concentrations listed in the table above include this correction value.
With the user supplied H & uC values alone the conc would be the above values multiplied by 0.591 (See the documentation for further discussion.)

Appedix G: Statistical data for Okaishi Amoxicillin Capsules

Element		Layer	Area	H value	Yield /uC/	Det. Eff.	Filter Trans.	Conc.	%Stat.	%Fit	LOD	
Z	Sym	#	counts	(-6)	ppm	(-3)	(-5)	ppm	Error	Error	ppm	-
16	S K	1	16660.7	309	7519	749	873	384710.7	0.68	2.45	902.9	Y
19	K K	1	535.7	309	2827	906	19661	1206.3	10.11	12.01	213.5	Y
20	CaK	1	4041.9	309	2836	931	30829	5632.7	1.89	2.28	128.6	Y
22	TiK	1	90.0	309	2343	962	52592	86.1	49.17	45.58	78.5	?
23	V K	1	30.6	309	2120	971	61627	27.3	128.91	118.83	66.4	N
24	CrK	1	408.5	309	1955	977	69150	350.8	10.07	9.94	60.8	Y
25	MnK	1	39.3	309	1684	982	75268	35.8	97.16	89.10	67.5	?
26	FeK	1	5989.4	309	1461	987	80177	5882.5	1.22	1.35	57.8	Y
27	CoK	1	58.0	309	1195	989	84062	66.2	77.01	63.24	95.2	?
28	NiK	1	60.4	309	1010	990	87147	78.6	53.32	47.07	76.0	?
29	CuK	1	0	309	783.9	991	89592	0	0	0	147.3	N
30	ZnK	1	74.4	309	630.3	988	91530	148.0	40.07	38.08	109.4	?
31	GaK	1	0	309	483.3	981	93072	0	0	0	201.5	N
32	GeK	1	16.8	309	372.2	965	94303	56.3	155.84	151.69	165.4	N
33	AsK	1	16.9	309	288.4	941	95290	74.1	148.93	175.94	211.4	N
34	SeK	1	37.7	309	218.4	908	96085	224.9	59.16	61.23	246.6	?
35	BrK	1	19.9	309	172.9	866	96729	155.8	104.04	112.55	280.0	?
37	RbK	1	0	309	102.7	763	97681	0	0	0	808.3	N
38	SrK	1	12.8	309	79.92	704	98032	262.6	135.60	150.27	656.3	N
39	Y K	1	19.6	309	62.82	646	98322	556.0	84.94	91.85	876.7	?
40	ZrK	1	0	309	48.89	591	98562	0	0	0	1717.8	N
41	NbK	1	7.3	309	40.19	538	98762	385.7	171.48	207.45	1351.0	N
42	MoK	1	20.3	309	31.45	484	98929	1531.7	59.00	73.80	1465.2	?
47	AgK	1	0	309	9.72	288	99444	0	0	0	7237.9	N
48	CdK	1	14.8	309	7.53	259	99506	8645.8	50.89	74.66	5830.5	?
50	SnK	*	0	309	4.73	211	99604	0	0	0	14522.8	N
51	SbK	1	0	309	3.77	190	99644	0	0	0	14492.4	N
50	SnLA	1	268.4	309	1064	916	23472	1332.0	27.33	35.22	618.9	?
80	HgLA	1	0	309	182.4	962	94474	0	0	0	543.5	N
82	PbLA	1	21.6	309	151.1	941	95301	181.3	159.80	273.08	565.1	N

(A "*" in the Layer# column indicates that elmt NOT used in matrix iteration)
In the matrix iteration section it was found that a correction needed to be applied to the H or uC value in order for the concentrations to sum to unity.
The concentrations listed in the table above include this correction value.
With the user supplied H & uC values alone the conc would be the above values multiplied by 0.285 (See the documentation for further discussion.)

Appedix H 1: Peak Summary of Amoxicillin Analysis

Sample Name	Sample Amt [ng/ul]	Multip.* Dilution	FileName .D	Sig	RetTime [min]	Area [mAU *s]	Height [mAU]
Sys Suit	0.00000	1.0000	AMOXCAP000	1	1.079	15.97851	3.24501
				1	2.370	1.37450e4	1900.33374
Sys Suit	0.00000	1.0000	AMOXCAP000	1	1.076	15.99205	3.24940
				1	2.370	1.37462e4	1902.61023
Sys Suit	0.00000	1.0000	AMOXCAP000	1	1.075	16.03090	3.24052
				1	2.364	1.37617e4	1883.34558
Sys Suit	0.00000	1.0000	AMOXCAP000	1	1.078	16.00284	3.23929
				1	2.371	1.37535e4	1901.73999
Sys Suit	0.00000	1.0000	AMOXCAP000	1	1.077	16.03036	3.23817
				1	2.371	1.37457e4	1893.09814
Std Run	0.00000	1.0000	AMOXCAP000	1	1.076	16.07283	3.25487
				1	2.366	1.37384e4	1892.17822
Std Run	0.00000	1.0000	AMOXCAP000	1	1.074	16.09727	3.25528
				1	2.360	1.37524e4	1894.31628
Std Run	0.00000	1.0000	AMOXCAP000	1	1.078	16.04929	3.23582
				1	2.368	1.37500e4	1891.21155
Rep 1 (Bristol)	0.00000	1.0000	AMOXCAP000	1	1.594	24.11550	3.34223
				1	2.004	18.60277	2.96767
				1	2.365	1.49436e4	2058.05322
Rep 1 (Bristol)	0.00000	1.0000	AMOXCAP000	1	1.593	23.62327	3.38663
				1	1.973	19.25351	3.18196
				1	2.127	1.49599e4	2086.20508
Rep 2 (Bristol)	0.00000	1.0000	AMOXCAP000	1	1.594	24.45695	3.40582
				1	1.998	19.47466	3.12341
				1	2.358	1.49836e4	2066.38647
Rep 2 (Bristol)	0.00000	1.0000	AMOXCAP000	1	1.593	24.42337	3.42281
				1	2.000	17.84019	3.03926
				1	2.360	1.50044e4	2071.14990
Rep 3 (Bristol)	0.00000	1.0000	AMOXCAP000	1	1.071	9.33001	1.81940
				1	1.594	24.54157	3.44446
				1	2.002	18.04255	3.07684
				1	2.361	1.50344e4	2072.30713
Rep 3 (Bristol)	0.00000	1.0000	AMOXCAP000	1	1.074	9.45348	1.88489
				1	1.593	24.80175	3.47715
				1	2.000	18.33196	3.13882
				1	2.358	1.50250e4	2073.39648

Appedix H 2: Peak Summary of Amoxicillin Analysis

Sample Name	Sample Amt [ng/ul]	Multip.* Dilution	FileName .D	Sig	RetTime [min]	Area [mAU *s]	Height [mAU]
Std Chk	0.00000	1.0000	AMOXCAP000	1	1.077	16.89825	3.32230
				1	1.995	9.47651	1.71925
				1	2.350	1.37714e4	1907.47314
Rep 1 (Medreich)	0.00000	1.0000	AMOXCAP000	1	1.160	26.61060	3.57011
				1	1.589	19.71373	3.13925
				1	1.990	13.05371	2.27205
				1	2.343	1.50863e4	2090.01025
Rep 1 (Medreich)	0.00000	1.0000	AMOXCAP000	1	1.159	29.05267	3.61807
				1	1.584	19.25832	3.13759
				1	1.988	13.41484	2.31240
				1	2.343	1.50968e4	2081.08301
Rep 2 (Medreich)	0.00000	1.0000	AMOXCAP000	1	1.162	28.53776	3.61336
				1	1.591	19.52911	3.14920
				1	1.992	15.06691	2.45675
				1	2.344	1.51585e4	2107.18774
Rep 2 (Medreich)	0.00000	1.0000	AMOXCAP000	1	1.162	28.12061	3.58623
				1	1.587	19.20585	3.16239
				1	1.984	15.16588	2.50798
				1	2.334	1.51872e4	2116.65259
Rep 3 (Medreich)	0.00000	1.0000	AMOXCAP000	1	1.156	28.47711	3.51431
				1	1.576	18.69278	3.14674
				1	1.972	17.05990	2.65098
				1	2.319	1.52608e4	2127.87109
Rep 3 (Medreich)	0.00000	1.0000	AMOXCAP000	1	1.147	28.90201	3.50908
				1	1.574	18.58362	2.98493
				1	1.944	23.96470	3.22323
				1	2.276	1.52648e4	2192.65210
Std Chk	0.00000	1.0000	AMOXCAP000	1	1.076	18.19413	3.36696
				1	1.860	51.47341	4.14309
				1	2.179	1.38029e4	1997.64160
Rep 1 (ECL)	0.00000	1.0000	AMOXCAP000	1	1.548	22.20234	3.36593
				1	1.924	24.77654	4.12054
				1	2.257	1.51881e4	2160.28296
Rep 1 (ECL)	0.00000	1.0000	AMOXCAP000	1	1.464	22.60710	3.60841
				1	1.836	26.85838	4.02817
				1	2.175	1.52068e4	2097.65991
Rep 2 (ECL)	0.00000	1.0000	AMOXCAP000	1	1.377	24.07540	6.60435
				1	1.498	32.01761	5.76981
				1	1.808	1.53710e4	1549.32556
Rep 2 (ECL)	0.00000	1.0000	AMOXCAP000	1	1.556	22.22491	3.42110
				1	1.937	25.15786	4.24696
				1	2.271	1.51961e4	2161.01514
Rep 3 (ECL)	0.00000	1.0000	AMOXCAP000	1	1.559	22.15112	3.42374
				1	1.941	25.66094	4.25643
				1	2.277	1.51783e4	2153.53394
Rep 3 (ECL)	0.00000	1.0000	AMOXCAP000	1	1.563	21.00763	3.41010
				1	1.947	25.34801	4.26213
				1	2.288	1.51807e4	2146.65015
Std Chk	0.00000	1.0000	AMOXCAP000	1	1.076	15.20664	3.21106
				1	1.939	13.55983	2.42349
				1	2.273	1.38287e4	1971.62061
Rep 1 (Letap)	0.00000	1.0000	AMOXCAP000	1	1.559	23.97779	2.88485
				1	1.940	20.10302	3.36994

Appedix H 3: Peak Summary of Amoxicillin Analysis

Sample Name	Sample Amt [ng/ul]	Multip.* Dilution	FileName .D	Sig	RetTime [min]	Area [mAU *s]	Height [mAU]
Rep 1 (Letap)	0.00000	1.0000	AMOXCAP000	1	2.278	1.55514e4	2197.91602
Rep 1 (Letap)	0.00000	1.0000	AMOXCAP000	1	1.563	24.38481	2.91468
				1	1.944	19.91251	3.38134
				1	2.282	1.55611e4	2204.33105
Rep 2 (Letap)	0.00000	1.0000	AMOXCAP000	1	1.721	20.50076	2.78549
				1	2.234	26.71267	3.55007
				1	2.676	1.54716e4	1964.01440
Rep 2 (Letap)	0.00000	1.0000	AMOXCAP000	1	1.760	17.70540	2.68927
				1	2.251	26.48945	3.98005
				1	2.640	1.54972e4	2022.87000
Rep 3 (Letap)	0.00000	1.0000	AMOXCAP000	1	2.050	13.61017	1.74472
Rep 3 (Letap)	0.00000	1.0000	AMOXCAP000	1	2.307	16.10892	1.98517
Std Chk	0.00000	1.0000	AMOXCAP000	1	1.088	25.89333	3.62007
Rep 1 (M&G)	0.00000	1.0000	AMOXCAP000	1	1.078	84.16039	19.29773
				1	1.298	16.46239	2.32280
				1	2.079	38.36012	4.79891
				1	2.854	45.38602	5.81918
Rep 1 (M&G)	0.00000	1.0000	AMOXCAP000	1	0.615	59.49224	4.45724
				1	0.918	25.10208	2.39654
				1	1.079	92.74596	19.98320
				1	1.299	19.21613	2.28875
				1	2.144	39.17583	4.92878
				1	2.956	53.90215	6.55250
				1	3.602	1.28139e4	1279.48486
Rep 2 (M&G)	0.00000	1.0000	AMOXCAP000	1	1.082	88.72596	19.61583
				1	1.328	20.94273	2.31431
				1	2.207	35.21353	5.11107
				1	2.930	55.33982	6.85325
				1	3.556	1.28801e4	1362.21606
Rep 2 (M&G)	0.00000	1.0000	AMOXCAP000	1	1.081	93.45974	19.76393
				1	1.323	37.16728	3.74965
				1	1.626	92.63549	4.90922
				1	2.078	50.16261	4.79351
				1	3.050	55.58832	4.93603
				1	3.915	4696.89453	601.89319
Rep 3 (M&G)	0.00000	1.0000	AMOXCAP000	1	0.921	61.10939	4.21811
				1	1.069	136.68536	22.62006
				1	1.231	25.08800	2.60546
				1	1.922	55.19194	7.15105
				1	2.476	47.81979	5.63297
				1	2.963	1.29515e4	1559.50244
				1	3.517	48.46085	3.89849
Rep 3 (M&G)	0.00000	1.0000	AMOXCAP000	1	1.074	84.69932	19.38822
				1	1.246	17.59900	2.54859
				1	1.884	44.23545	5.49464
				1	2.537	46.68328	5.34857
				1	3.064	1.30743e4	1474.88293
				1	3.652	35.67870	3.27659
Std Chk	0.00000	1.0000	AMOXCAP000	1	1.089	14.64959	3.31684
				1	1.932	188.94312	5.00336
				1	2.650	37.32370	3.45787
				1	3.306	1.38173e4	1366.69519

Appendix I : HPLC Chromatogram for Standard Amoxicillin

Appendix J: HPLC Chromatogram for Imported Amoxicillin

Appendix K: HPLC Chromatogram for Okaishi Amoxicillin