EVALUATION OF THE QUALITY ASSURANCE OF FIVE BRANDS AMOXICILLIN TRIHYDRATE CAPSULES SOLD IN THE OPEN DRUG MARKET AT NSUKKA

BY

ONUNZE EMMANUEL CHUKWUDI SUNDAY

PG/M.Sc/09/50668

A PROJECT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARDS OF MASTER OF SCIENCE DEGREE (M.Sc) IN ANALYTICAL CHEMISTRY IN THE DEPARTMENT OF PURE AND INDUSTRIAL CHEMISTRY, FACULTY OF PHYSICAL SCIENCES UNIVERSITY OF NIGERIA NSUKKA.

MAY, 2012.

CERTIFICATION

This is to certify that Onunze Emmanuel Chukwudi Sunday, with Registration Number PG/M.Sc/09/50668 successfully carried out and completed this work in the Department of Pure and Industrial Chemistry, University of Nigeria Nsukka.

Supervisor	Sign/Date
Head of Department	Sign/Date
External Examiner	Sign/Date

DEDICATION

To God Almighty

ACKNOWLEDGEMENT

First and foremost I sincerely wish to appreciate my Lord God almighty for his grace which saw me through this work. To Him be all the Glory and Honour. Amen. He has always been my helper in times of despair and need. Thank You JESUS.

My warm and in ï depth appreciation equally goes to my able and well articulated supervisor Dr. C. O. B Okoye for his thorough scrutiny throughout this work.

My heart felt appreciation equally goes to my H.O.D Prof P.O. Ukoha for his experience and expertise in piloting the affairs of the Department.

More so, Iôm appreciating my lecturersô prof Nwadinigwe, prof U.C. Okoro, Dr. Nwachukwu Ekere, Dr. Ibeto Cynthia, Engr. J. Ani, Engr. Anyanwu, Mr.Obinna Okparaeke and host of other lecturers for their painstaking dedication in lecturing and meticulous striving in ensuring that we become a better person.

Furthermore, my appreciation will also go to my friends Chioma Adiele, Walters, Abimbola Toyin, Igori Wallace, Okenwa Jude, Okoronkwo Kanu, Ugwuowo onyeye bridget, Enoobong Nickolas Sunday, Ikeh Obianuju Adeobi and wonderful Evans Chinyere for their support and encouragement throughout this work.

Without forgetting my dynamic and wonderful in ï law Dr. Uchenna Augustine Akubue and his wife madam Peace Ngozika Akubue (my sister), I truly appreciate them for their support and encouragement throughout this my project work.

I will also like to appreciate in a special way my father Chief Onunze F.A, my mother Lolo Onunze Victoria Ngozi and my brothers Mr. Onunze Tobias Pascal and Mr. Onunze obiora Eugene for their love, care, prayers and financial support I received from them throughout this program.

Finally, I appreciate all those who in one way or the other contributed to the success of this work, more especially my friends pharm. Nnadi Charles (great man), lecturer in Pharmacy UNN and Mr. Agbo who is also a lecturer in Pharmacy UNN and Peter (hard stone) in Federal Water Urban Corporation Enugu, I say thank you all very much and be assured that God in His infinite Mercies will in no short time reward you all more than you have done to me in JESUS Name, Amen.

Thank you all.

TABLE OF CONTENTS

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

ABSTRACT

The active drug content and weight uniformity of five brands (A ï E) of Amoxicillin Trihydrate capsules in Nsukka open drug market were evaluated to ascertain their quality assurance using UV visible spectroscopy and gravimetry. This study has shown that all the brands analyzed showed significant variations with respect to active drug content determination for the three brands B, D and E. Using United State Pharmacopoeia (USP) and British Pharmacopoeia (BP) specifications of 90 ï 110 %, the values obtained were B ï (413.525 mg), D ï (440.325 mg), E ï (414.100 mg). These values were within the general drug acceptance limit of 80 ï 110 % but failed the antibiotic amoxicillin trihydrates USP and BP specifications with acceptable limit of 90 ï 110 % determined at 266 nm. The percentage mean content of all the brands (A ï E) were 77.64, 82.80, 74.24, 88.07 and 82.82. All the brands passed the weight uniformity test with coefficient of variation CV value range of ± 0 ï 5 % for capsules more than 250 mg according to USP and BP specifications. However, with the result obtained, the consequences are that it poses a serious threat to the health of the entire people of West Africa sub regions that depend on these drugs for therapeutical response. As all the drugs assayed were below the acceptance limit requirement of antibiotic amoxicillin trihydrate and thus, can lead to serious health implications such as drug resistance, cardiac failure, etc. Drug regulatory bodies should be at alert and they should conduct strict routine check on all the NAFDAC satisfied companies as all the drugs analyzed compromise their quality because of profit reasons.

CHAPTER ONE

1.0 INTRODUCTION

1.1 ANTIBIOTICS

An antibiotic is a compound or substance that kills or slows down the growth of bacteria [1]. The term is often used synonymously with the term antibacterial; however, with increased knowledge of the causative agents of various infectious diseases, *antibiotic(s)* has come to denote a broader range of antimicrobial compounds, including antifungal and other compounds [2]. It can be loosely defined as the variety of substances derived from bacterial sources (microorganisms) that control the growth of or kill other bacteria. However, synthetic antibiotics, usually chemically related to natural antibiotics, have since been produced that accomplish comparable tasks.

1.2 Modern antibiotics

The term ñantibioticsò was coined by Selman Waksman in 1942 to describe any substance produced by a micro-organism that is antagonistic to the growth of other micro-organism in high dilution [3]. This definition excluded substances that kill bacteria, but are not produced by microorganisms (such as gastric juices and hydrogen peroxide). It also excluded synthetic antibacterial compounds such as the sulphonamides. Many antibiotics are relatively small molecules with a molecular weight less than 2000 atomic mass units [3].With advances in medicinal chemistry, most antibiotics are now semi synthetic modified chemically form of the original compounds found in nature,[4] as is the case with beta-Lactams (which include the penicillins, produced by fungi in the genus penicillium, the cephalosporins, and carbapenems). Some antibiotics are still produced and isolated from living organisms, such as the amino glycosides and others have been created through purely synthetic means, the sulphonamides, the quinolones, and the oxazolidinones[4]. In addition to this origin-based classification into natural, semi synthetic, and synthetic, antibiotics may be divided into two broad groups according to

their effect on micro-organisms. Those that kill bacteria are bactericidal agents, whereas antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity [4]. Most antibiotics target bacterial functions or growth processes. Antibiotics that target the bacterial cell wall (penicillin, cephalosporins), or cell membrane (polymixins), or interfere with essential bacterial enzymes (quinolones, sulphonamides) are usually bactericidal in nature. Those that target protein synthesis, such as the amino glycosides, macrolides and tetracyclines are usually bacteriostatic. Further categorisation is based on their target specificity.

Narrow spectrumò antibiotics target particular types of bacteria, such as Gram-negative or Gram-positive bacteria, where as broad spectrum antibiotics affects a wide range of bacteria. In the last few years, three new classes of antibiotics have been brought into clinical use. This follows a 40-year hiatus in discovering new classes of antibiotics compounds. These new antibiotics are of the following three classes; cyclic lipopeptides (daptomycin), glyclycyclines (tigecyclines), and oxazolidinones (linezolid).Tigecyclines is a broad spectrum antibiotics, where as the other two are used for Gram-positive infections. These developments show promise as a means to counteract the bacterial resistance to existing antibiotics.

1.3 FAKE DRUGS

Fake drugs otherwise called ócounterfeit drugsô can be defined as drug that are unfit for usage and human consumption and therefore constitute hazard to good and sound health. Considering the above definition, the evil effect of fake drugs can be better understood by looking back to what the situation was several years back, before the country got to her present situation. The situation could be said to have gone bad many years back when our hospitals changed from health institutions of excellence to mere consulting clinics occasioned by non-availability of drugs. As a result of this problem, dubious and unpatriotic persons came in with fake drugs which they supplied to the hospitals. Added to these problems are the activities of smugglers who perhaps because of the porous

nature of the Nigerian boarders and the connivance of some law enforcement agents, bring fake drugs into the country [5].

Faking of drugs is a global public health problem, because the effects can be felt from both the country of manufacture to the recipient countries. Hence, national measures for combating fake drugs in a country might be insufficient because of the advanced sophistications of those who manufacture and sell them [5]. Nigeria is not an exception in the problems of fake drugs till date. Some people still prefer to self medicate when they are ill, and often time the drugs are bought from unlicensed drug vendors, whose drug quality is not sure. Through the past two decades in Nigeria, the problem of fake drugs has been a very big issue. In addition, fake drugs proved a major factor in contributing to high death rates. Over 50 children died in 1989 as a result of a formulation error in a drug [5]. Such problems led to the establishment of National Agency for Food Drug Administration and Control (NAFDAC), which would help create a fake-drug-free environment [5]. The intent was to ensure effective registration of good quality drugs that are inexpensive in Nigeria. Since the inception of NAFDAC in April 2001, the commission has worked hard in combating the problems of sale of fake drugs, but yet to no avail [5]. Questions are, ówhy does Nigeria still have in existence open drug markets?

Why do Nigerians in drug business breech the stipulated drug laws and still get away with it and continue with their business, committing mass murder and smiling to their banks? How long do we fight the battle of fake drug even with the threats on our lives who want to preserve the health of the Nation? The consistent raids by NAFDAC on fake drug dealers who contravene the applicable laws and regulations, have helped in clamping down on the illegal drug traders but when things seem as if itôs getting better, these illegal drug sellers begin to emerge from their hideouts [5]. I continue to wonder, why? Could it be that the agency is not doing enough to stop the evil activities, or could the problem be from the drug sellers themselves? The tragic irony is that the problems of fake drug have refused to go away from the shores of Nigeria.

1.4 DRUG REGULATORY AND ENFORCEMENT AGENCY

The role of any drug regulatory agency is the protection and promotion of public health. The enforcement directorate arm of NAFDAC established under the provisions of the counterfeit and fake drugs (miscellaneous provision) act is charged with the responsibility of enforcing the provisions of the counterfeit and fake drug decree, which includes:

- Conducting surveillance on companies and persons suspected to be violating NAFDAC regulations and carrying out investigations on such persons and companies.
- Paying unscheduled visits to all ports of entry and border posts and interrogation of suspects.
- Sampling of NAFDAC regulated products for laboratory analysis and compilation of case files.
- Raiding of drug hawkers and destruction of fake and spurious regulated products.
- Coordination of activities of state task force.
- The establishment of the task force in Nigeria was seen as a welcome development for the fight against fake drugs.

1.5 DRUG PROFESSIONALS

The main professional organization of pharmacist in Nigeria is the Pharmaceutical Society of Nigeria (PSN). The organization was established in 1927, with membership over 7000 pharmacists [6]. Its main functions are to determine the skill and knowledge that is required of anyone who seeks to be registered as a member of the pharmacy profession, preparation and review of the code of conduct, regulate and control the practice of the pharmacy profession. PSN also has a panel that investigates and disciplines erring pharmacists.

According to the PSN president, the main sources of fake drugs in Nigeria are India, China, Pakistan, Egypt and Indonesia [7]. The influx of fake drugs is quite

worrisome to the health experts. It is difficult to get reliable data on mortality or morbidity caused due to the consumption of fake drug in Nigeria. In 1987, an increased number of fake drugs were noticed in some market places, even in some pharmacy outlets [7]. Chemical test showed that they contain smaller amount of the active ingredient of the drugs. Some pharmaceutical companies felt it was due to laxity of inspection that contributes to the successful faking. On 31 October 1987, the Pharmaceutical Society of Nigeria (PSN) discussed the implication of fake drug manufacturing, marketing as well as possible remedies; they identified some major drugs that are often faked viz; antibiotics, antifungal agents, antihypertensive, malaria medicines, bronchodilators and hormonal preparations. They related the problem of drug faking to exchange control situation that causes scarcity and high price for drugs and that government can help reduce the problems through provision of essential drugs at reasonable price to the people which will in turn make fake drugs low priced and less attractive [8].The effect from fake drug consumption usually goes unnoticed, except in cases where it results to mass death. These problems made the PSN as a body to pressure Nigerian government in taking definite step towards the control of fake drug. Hence, the promulgating of the counterfeit and fake drug decree No. 21 of 1988 that prohibits the sale and distribution of fake drugs in open markets and created penalties for anyone who breaches the law [6]. In United States of America, pharmacists are allowed to work with foreign governments, international regulatory bodies as well as law enforcement agencies. This collaboration enables them to detect and combat counterfeiting [9]. In Cuba, almost all pharmaceutical operations are owned and managed by the government who determines how drugs are regulated and the members of their drug professional groups can get involved in drug regulation by joining the advisory committee [10].

1.6 INFORMAL DRUG SELLERS

Informal drug sellers are people that sell drugs in an unregulated manner without professional consultation and with limited knowledge of pharmacy. Their main aim in drug business is the profit they make even when they are aware of fake drug proliferation.

They can store and handle drugs in inappropriate ways that can endanger the drug potency [11].

Informal drug sellers can be found in shops, kiosks, open markets, general stores etc, and can operate as itinerant hawkers. Just like any other business, their existence is maintained in accordance to consumer demands for easy accessibility, convenience and affordable supplies. They can be very friendly, approachable and promising to their customers. Their attraction to those that patronizes them is that they have cheaper products when compared to the formal Outlets and their products can be given on credit because they source their products from cheaper sources. Most of these drug sellers are less knowledgeable about the doses of drug appropriate for a particular illness; their prescription could be higher or lower than the correct dosage because their major aim is more on profit making and meeting up with competition from other sellers [11].

1.7 Dangers associated with purchasing from informal drug sellers

Consumers/buyers are exposed to dangers from hazardous drugs because they are entrapped in the web of fake drugs without respite and any one can be a victim. Many drugs are offered for sale in Nigeria without expiration dates and can be bought and sold over the counter or by hawkers selling alongside newspaper vendors (personal observation). A man who is sick can walk to any drug store and come out with prescriptions loaded with drugs. In some cases, smooth talking drug peddlers in public buses save such a man the walk to a drug outlet. Consumers on the other hand may not know the quality of products they are purchasing [11]. The reasons why consumers prefer to patronize such outlets include geographical accessibility,

Shorter waiting times, longer opening hours, greater confidentiality, more personable social interaction, ease of seeking advice, lower cost, flexible pricing policies and no separate fee charged for advice. However, one of the problems associated with self-medication with drugs from these sellers is that in most cases, neither the drug seller nor the consumer is aware of the correct dosage and duration of treatment [12].

1.8 AIMS AND OBJECTIVES

(a.) To assay some brands of amoxicillin trihydrate in the Nigerian market with a view to ascertain the quality assurance using official standards for quality drug samples.

(b.) To determine the drug claim of active ingredient of the brands of amoxicillin trihydrate with strength 500 mg.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 AMOXICILLIN TRIHYDRATE (AMT)

Amoxicillin trihydrate is a broad spectrum antibiotic [13]. It is one of the extensively prescribed drugs within the list of the antibiotic therapeutic class [14]. Literature survey reveals that for amoxicillin trihydrate, spectrophotometric,[15, 16] few HPLC,[17-18] HPLC with Fluorimetric detection[19], HPLC with photo diode array detection,[20] voltametry[21]. LC-MS [22], electrospray mass spectroscopy[23] and DSC[24] have been reported for determination of amoxicillin trihydrate alone and in *combination with other drugs for pharmaceutical* formulation and biological fluids.

Its systematic (IUPAC) name is (2S, 5R, 6R)-6- (2R)-2-amino-2-(4-hydroxyphenyl)-acetyl amino -3,3-dimethyl-7-oxo-4-thia-1-azabicyclo **(**3,2,0)heptane-2-carboxylic acid trihydrate. Itôs molecular formular is $C_{16}H_{19}N_3O_5S.3H_2O$, molecular weight is 419.45[25]. It is of pharmaceutical Grade. Its appearance is white to off-white powder. The pH in aqueous solution is 3.5 - 5.5. It is one of the most common antibiotics prescribed for children, and the liquid forms are helpful where the patient might find it difficult to take tablets or capsules. It has two ionisable groups in the physiological range (the amino group in alpha-position to the amide carbonyl group and the carboxyl group).

2.2 PROPERTIES OF AMOXICILLIN TRIHYDRATE

It is white or almost, crystalline powder, slightly soluble in water and in alcohol, practically insoluble in ether and in fatty oils. It dissolves in dilute acids and dilute solutions of alkali hydroxides.

Amoxicillin is a ß-Lactam antibiotic that interferes with the synthesis of the bacterial cell wall peptidoglycan, after attachment to binding sites on the bacteria, it inhibits the transpeptidation enzyme that cross ï links the peptide chains attached to the backbone of the peptidoglycan. The final bactericidal event is the inactivation of an inhibitor of the

autolytic enzymes in the cell wall; this leads to lysis of the bacterium [26]. Its molecular structure is shown in fig 2.1;-

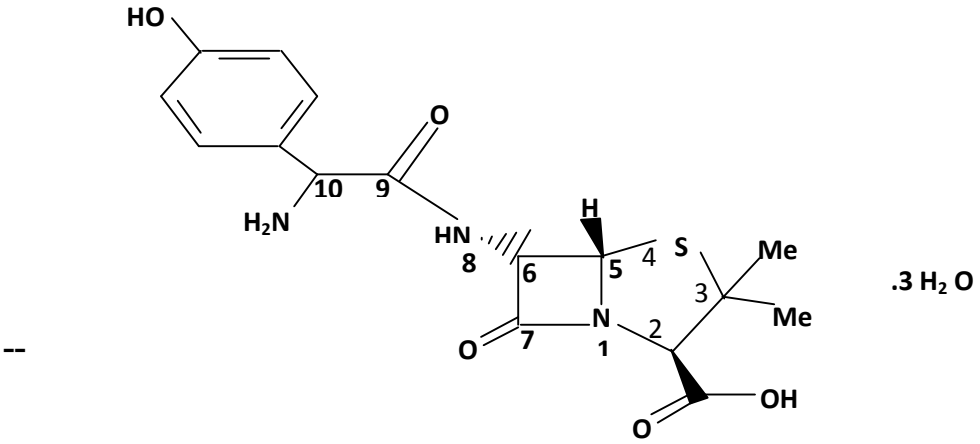


Figure 2.1 Molecular structure of amoxicillin trihydrate. Systematic (IUPAC) name is (2*S*,5*R*,6*R*)- 6-{[(2*R*)-2-amino- 2-(4-hydroxyphenyl)- acetyl]amino}- 3,3-dimethyl- 7-oxo- 4-thia- 1-azabicyclo[3.2.0]heptane- 2-carboxylic acid trihydrate.

Amoxicillin exist in various forms which include the following monohydrate, dihydrate and trihydrate and these forms have been reported, among which the trihydrate is the most stable hydrated form of amoxicillin [13-16]. It is available as capsules, chewable and dispersible tablets plus syrup and pediatric suspension for oral use and as the sodium salt for intravenous administration [27].

2.3 β-LACTAM ANTIBIOTICS

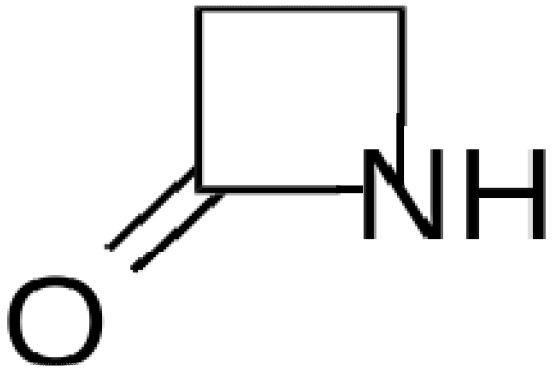


Figure 2.2 A β-Lactam antibiotics. IUPAC name 2-Azetidinone

A **β-lactam** (**beta-lactam**) ring is a four-membered lactam [28]. A lactam is a cyclic amide. It is named as such, because the nitrogen atom is attached to the ɓ-carbon relative to the carbonyl carbon. The simplest ɓ-lactam possible is 2-azetidinone. ɓ-Lactam antibiotics are broad class of antibiotics consisting of all antibiotic agents that contain a ɓ-Lactam nucleus in its molecular structure. These include penicillin derivatives (penams) cephalosporins (cephams), monobactams, and carbanems [29]. Most ɓ-lactam antibiotics work by inhibiting cell wall biosynthesis in the bacterial organism and are the most widely used group of antibiotics. Up until 2003 when measured by sales, more than half of all commercially available antibiotics in use were ɓ-lactam compounds [30]. Bacteria often develop resistance to ɓ-Lactam antibiotics by synthesizing beta lactamase, an enzyme that attacks the ɓ-Lactam ring. However, the resistance can be overcome by use of ɓ-Lactamase inhibitors such as clavulanic acid. Nowadays, antibiotics resistance by plasmide transfer has become a significant clinical problem, because an organism may become resistant to several antibiotics at the same time due to acquisition of a plasmide that encodes resistance to multiple agents [29]. Antibiotics resistance can be developed through the wide and inappropriate use of the drug without following any standard guidelines.

2.4 AMOXICILLIN CAPSULE

Each capsule contains either 250 mg or 500 mg of amoxicillin.
They also contain the following inactive ingredients:

1. **Magnesium Stearate**: also called *octadecanoic acid, magnesium salt*, is a white substance which is solid at room temperature. It has the chemical formula $Mg(C_{18}H_{35}O_2)_2$. It is a salt containing two equivalents of stearate (the anion of stearic acid) and one magnesium cation (Mg^{2+}). Magnesium stearate melts at about 120 °C, is not soluble in water, and is generally considered safe for human consumption at levels below 2500 mg/kg per day [31]. In 1979, the FDA's Subcommittee on GRAS (generally recognized as safe) Substances (SCOGS) reported, "There is no evidence in the available information on ... magnesium stearate ... that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current and in the manner now practiced, or which might reasonably be expected in the future [32].

2. **Microcrystalline Cellulose**: is a term for refined wood pulp and is used as a texturizer, an anti-caking agent, a fat substitute, an emulsifier, an extender, and a bulking agent in food production[32]. The most common form is used in vitamin supplements or tablets. It is also used in plaque assays for counting viruses, as an alternative to carboxymethylcellulose [34].

In many ways, cellulose makes the ideal excipient. A naturally occurring polymer, it is composed of glucose units connected by a 1-4 beta glycosidic bond. These linear cellulose chains are bundled together as microfibril spiralled together in the walls of plant cell. Each microfibril exhibits a high degree of three-dimensional internal bonding resulting in a crystalline structure that is insoluble in water and resistant to reagents. There are, however, relatively weak segments of the microfibril with weaker internal bonding. These are called amorphous regions but are more accurately called dislocations

since microfibril contain single-phase structure. The crystalline region is isolated to produce microcrystalline cellulose.

3. **Gelatine**: It is a translucent, colourless, brittle (when dry), flavourless solid substance, derived from the collagen inside animals' skin and bones. It is commonly used as a gelling agent in food, pharmaceuticals, photography, and cosmetic manufacturing. Substances containing gelatin or functioning in a similar way are said to be *gelatinous*. Gelatin is an irreversibly hydrolysed form of collagen, and is classified as a foodstuff. Gelatine is now classed as a food in its own right and not now subject to the food additives legislation in Europe [35]. It is found in some gummy candies as well as other products such as marshmallows, gelatin dessert, and some low-fat yogurt. Household gelatin comes in the form of sheets, granules, or powder. Instant types can be added to the food as they are; others need to be soaked in water beforehand.

4. **Sodium Lauryl Sulphate**: Sodium dodecyl sulfate (SDS or NaDS), sodium laurilsulfate or sodium lauryl sulfate (SLS) is an organic compound with the formula $CH_3(CH_2)_{11}OSO_3Na$. It is an anionic surfactant used in many cleaning and hygiene products. The salt is an organosulfate consisting of a 12-carbon tail attached to a sulfate group, giving the material the amphiphilic properties required of a detergent. Being derived from inexpensive coconut and palm oils, it is a common component of many domestic cleaning products.Sodium coco-sulfate is essentially the same compound, but made from less purified coconut oil[36][37].

SDS is synthesized by treating lauryl alcohol with sulfur trioxide gas, or oleum, or chlorosulfuric acid to produce hydrogen lauryl sulfate. The industrially practiced method typically uses sulfur trioxide gas. The resulting product is then neutralized through the addition of sodium hydroxide or sodium carbonate. Lauryl alcohol is in turn usually derived from either coconut or palm kernel oil by hydrolysis, which liberates their fatty acids, followed by hydrogenation.

Due to this synthesis, commercial samples of SDS are often a mixture of other alkyl sulfates, dodecyl sulfate being the main component [38].

5. **Colloidal Anhydrous Silicate**: colloidal anhydrous silica is a light blue zone (shellolic acid) accompanied by zones of the same colour but of lower intensity. Other faint grey and violet zones may be visible.

6. **Croscarmellose Sodium**: Croscarmellose sodium is an internally cross-linked sodium carboxymethylcellulose for use as a disintegrant in pharmaceutical formulations [39]. The cross-linking reduces water solubility while still allowing the material to swell (like a sponge) and absorb many times its weight in water. As a result, it provides superior drug dissolution and disintegration characteristics, thus improving formulas' subsequent bioavailability by bringing the active ingredients into better contact with bodily fluids. Croscarmellose sodium also resolves formulators' concerns over long-term functional stability, reduced effectiveness at high tablet hardness levels, and similar problems associated with other products developed to enhance drug dissolution. Croscarmellose sodium is a very commonly used [40] pharmaceutical additive approved by the U.S. Food and Drug Administration. Its purpose in most tablets - including dietary supplements - is to assist the tablet in disintegrating in the intestinal tract at the required location. If a tablet disintegrating agent is not included, the tablet could disintegrate too slowly, in the wrong part of the intestine or not at all, thereby reducing the efficacy and bioavailability of the active ingredients. Croscarmellose sodium is made by first soaking crude cellulose in sodium hydroxide, and then reacting the cellulose with sodium monochloroacetate to form sodium carboxymethylcellulose. Excess sodium monochloroacetate slowly hydrolyzes to glycolic acid and the glycolic acid catalyzes the cross-linkage to form croscarmellose sodium[41]. Chemically, croscarmellose sodium is the sodium salt of a cross-linked, partly O-(carboxymethylated) cellulose.

7. **Sunset Yellow Fcf**: Sunset Yellow FCF (also known as Orange Yellow S, FD&C Yellow 6 or C.I. 15985) is a synthetic yellow azo dye, manufactured from aromatic hydrocarbons from petroleum. When added to foods sold in Europe, it is denoted by E Number E110 [42]. Although there are reports it can induce an allergic reaction, this is not confirmed by scientific research [43].

Sunset Yellow is useful in fermented foods which must be heat treated. It may be found in orange sodas, marzipan, Swiss rolls, apricot jam, citrus marmalade, lemon curd, sweets, beverage mix and packet soups, margarine, custard powders, packaged lemon gelatin desserts, energy drinks such as Lucozade, breadcrumbs, snack chips such as Doritos, packaged instant noodles, cheese sauce mixes and powdered marinades, bottled yellow and green food colouring, ice creams, pharmaceutical pills and prescription medicines, over-the-counter medicines (especially children's medicines) cake decorations and icings, squashes, and other products with artificial yellow, orange or red colours.Sunset Yellow is often used in conjunction with E123, amaranth, to produce a brown colouring in both chocolates and caramel. At high concentrations, Sunset Yellow in solution with water undergoes a phase change from an isotropic liquid to a nematic liquid crystal. This occurs between 0.8 M and 0.9 M at room temperature.

8. **Quinoline Yellow**: Quinoline yellow, Quinoline Yellow WS, C.I. 47005, or Food Yellow 13, is a yellow food dye. Chemically it is a mixture of disulfonates (principally), monosulfonates and trisulfonates of 2-(2-quinolyl) indan-1,3-dione. The color Quinoline Yellow SS (Spirit Soluble), which lacks the sulfonate groups, is a related form that is insoluble in water [44]. Quinoline Yellow is used as a greenish yellow food additive, designated in Europe as the E number E104 [45].

9. **Titanium Dioxide**: also known as titanium (IV) oxide or titania, is the naturally occurring oxide of titanium, chemical formula TiO_2. When used as a pigment, it is called titanium white, Pigment White 6, or CI 77891. Generally it comes in two different forms, rutile and anatase. It has a wide range of applications, from paint to

sunscreen to food colouring [46]. When used as a food colouring, it has E number E171.

Titanium dioxide occurs in nature as well-known minerals rutile, anatase and brookite, and additionally as two high pressure forms, a monoclinic baddeleyite-like form and an orthorhombic Ŭ-PbO_2-like form, both found recently at the Ries crater in Bavaria[47 -48]. The most common form is rutile,[49] which is also the equilibrium phase at all temperatures [50]. The metastable anatase and brookite phases both convert to rutile upon heating.[51][52] Rutile, anatase and brookite all contain six coordinated titanium. Titanium dioxide has eight modifications ï in addition to rutile, anatase and brookite there are three metastable forms produced synthetically (monoclinic, tetragonal and orthorombic), and five high pressure forms (Ŭ-PbO_2-like, baddeleyite-like, cotunnite-like, orthorhombic OI, and cubic phases):

10. **Methyl Hydroxybenzoate**: Methylparaben, is a preservative with the chemical formula $CH_3(C_6H_4(OH)COO)$. It is the methyl ester of *p*-hydroxybenzoic acid.

Methylparaben is found in several fruits, in particular blueberries,[53][54][55] where it acts as an antimicrobial agent. Methylparaben is an anti-fungal agent often used in a variety of cosmetics and personal-care products. It is also used as a food preservative and has the E number E218.Methylparaben is commonly used as a fungicide in *Drosophila* food media. Usage of methylparaben is toxic at higher concentrations, has a estrogenic effect and slow *Drosophila* growth rate in the larval and pupal stages at a lower concentrations [56].

11. **Propyl Hydroxybenzoate**: Propylparaben, the propyl ester of *p*-hydroxybenzoic acid, occurs as a natural substance found in many plants and some insects, although it is manufactured synthetically for use in cosmetics, pharmaceuticals and foods. It is a preservative typically found in many water-based cosmetics, such as creams, lotions, shampoos and bath products. As a food additive, it has the E number E216.

Sodium propyl *p*-hydroxybenzoate, the sodium salt of propylparaben, a compound with formula Na (C_3H_7 (C_6H_4COO) O), is also used similarly as a food additive and as an anti-fungal preservation agent. Its E number is E217.

12. **Shellac**: is a resin secreted by the female lac bug, on trees in the forests of India and Thailand. It is processed and sold as dry flakes, which are dissolved in ethyl alcohol to make liquid shellac, which is used as a brush-on colorant, food glaze and wood finish. Shellac functions as a tough natural primer, sanding sealant, tannin-blocker, odour-blocker, stain, and high-gloss varnish. Shellac was once used in electrical applications as it possesses good insulation qualities and it seals out moisture. Phonograph (gramophone) records were also made of it during the pre-1950s, 78-rpm recording era.

13. **Black Iron Oxide**: Black Iron Oxide (Fe_30_4) Powder**:** Magnetite is a natural iron oxide magnet, hence the name, giving it a very nice distinguishing characteristic. Magnetite is the most magnetic of all the minerals on Earth. Iron oxides and oxide-hydroxides are widespread in nature, play an important role in many geological and biological processes, and are widely utilized by humans, e.g., as iron ores, pigments, catalysts, in thermite. Common rust is a form of iron (III) oxide. Iron oxides are widely used as inexpensive, durable pigments in paints, coatings and colored concretes.

14. **Carmoisine (500 Mg Only):** Azorubine, carmoisine, Food Red 3, Azorubin S, Brillantcarmoisin O, Acid Red 14, or C.I. 14720 is a synthetic red food dye from the azo dye group. It usually comes as a disodium salt. It is a red to maroon powder. It is used for the purposes where the food is heat-treated after fermentation. It has E number E122. Some of the foods it can be present in are blancmange, marzipan, Swiss roll, jams, preserves, yoghurts, jellies, breadcrumbs, and cheesecake mixes. It is also present in Oraldene Mouthwash. Chemical names: 2-(4-Sulfo-1-naphthylazo)-1-naphthol-4-sulfonic acid disodium;4-Hydroxy-3-((4-

sulpho-1-naphthalenyl)azo)-1naphthalenesulphonic acid disodium; Disodium 4-hydroxy-3-((4-sulphonatonaphthyl)azo) naphthalenesulphonate

15. **Brilliant blue FCF (500 mg only):** also known under commercial names, is a colorant for foods and other substances to induce a color change. It is denoted by E number E133 and has a color index of 42090. It has the appearance of a reddish-blue powder. It is soluble in water, and the solution has a maximum absorption at about 628 nanometer.

It is a synthetic dye produced using aromatic hydrocarbons from petroleum [57]. It can be combined with tartrazine (E102) to produce various shades of green.

It is usually a disodium salt. Calcium and potassium salts are also permitted. It can also appear as an aluminium lake. The chemical formation is $C_{37}H_{34}N_2Na_2O_9S_3$. The dye is poorly absorbed from the gastro-intestinal tract and 95% of the ingested dye can be found in the feces. It also reacts with certain bile pigments to form green feces. As a blue colour, Brilliant Blue FCF is often found in ice cream, canned processed peas, packet soups, bottled food colourings, icings, ice pops, blue raspberry flavoured products, dairy products, sweets[58] and drinks. It is also used in soaps, shampoos, mouthwash [59] and other hygiene and cosmetics applications. In soil science, Brilliant Blue is applied in tracing studies to visualize infiltration and water distribution in the soil.

2.5 Medical uses of Amoxicillin Trihydrate

Amoxicillin is used in the treatment of a number of infections including: acute otitis media, streptococcal pharyngitis, pneumonia, skin infections, urinary tract infections, *salmonella*, lyme disease, and chlamydia infections[60]. It is used to prevent bacterial endocarditis in high risk people who are having dental work done, to prevent *strep pneumococcus* infections in those without a spleen, and for both the prevention and the treatment of anthrax [60]. It is also a treatment for cystic acne [61].The UK however

does not recommend its use for infectious endocarditis prophylaxis [62]. These recommendations have not appeared to have changed the rates of infection [63].

2.6 SIDE EFFECTS OF AMOXICILLIN TRIHYDRATE

Side effects are as those for other beta-Lactam antibiotics. The side effects include nausea, vomiting, rashes, and antibiotic associated colitis. Loose bowel movements (diarrhea) also may occur rarer, patient-reported, side effects include mental changes, light-headedness, insomnia, confusion, anxiety, sensitivity to light and sounds, and unclear thinking [63]. Immediate medical care is required upon the first signs of these side effects.

2.7 INSTRUMENTATION METHOD OF ANALYSIS

There are many instrumental methods of analysis that can be utilized in the analysis of amoxicillin trihydrate. Among these are chromatographic and spectroscopic methods.

2.7.1 High-performance liquid chromatography

High-performance liquid chromatography (sometimes referred to as high-pressure liquid chromatography, HPLC, is a chromatographic technique used to separate a mixture of compounds in analytical chemistry and biochemistry with the purpose of identifying, quantifying and purifying the individual components of the mixture. Some common examples are the separation and quantitation of performance enhancement drugs (e.g. steroids) in urine samples, or of vitamin D levels in serum [64]. HPLC typically utilizes different types of stationary phases (i.e. sorbents) contained in columns, a pump that moves the mobile phase and sample components through the column, and a detector capable of providing characteristic retention times for the sample components and area counts reflecting the amount of each analyte passing through the detector. The detector may also provide additional information related to the analyte, (i.e. UV/Vis spectroscopic data, if so equipped). Analyte retention time varies depending on the strength of its interactions with the stationary phase, the composition and flow rate of mobile phase

used, and on the column dimensions. HPLC is a form of liquid chromatography that utilizes small size columns (typically 250 mm or shorter and 4.6 mm i.d. or smaller; packed with smaller particles), and higher mobile phase pressures compared to ordinary liquid chromatography. With HPLC, a pump (rather than gravity) provides the higher pressure required to move the mobile phase and sample components through the densely packed column. The increased density arises from the use of smaller sorbent particles. Such particles are capable of providing better separation on columns of shorter length when compared to ordinary column chromatography.

2.7.2 Operation

The sample to be separated and analyzed is introduced, in a discrete small volume, into the stream of mobile2 phase percolating through the column. The components of the sample move through the column at different velocities, which are functions of specific physical or chemical interactions with the stationary phase. The velocity of each component depends on its chemical nature, on the nature of the stationary phase (column) and on the composition of the mobile phase. The time at which a specific analyte elutes (emerges from the column) is called the retention time. The retention time measured under particular conditions is considered an identifying characteristic of a given analyte. The use of smaller particle size packing materials requires the use of higher operational pressure ("backpressure") and typically improves chromatographic resolution (i.e. the degree of separation between consecutive analytes emerging from the column). Common mobile phases used include any miscible combination of water with various organic solvents (the most common are acetonitrile and methanol). Some HPLC techniques use water free mobile phases (see Normal Phase HPLC below). The aqueous component of the mobile phase may contain buffers, acids (such as formic, phosphoric or trifluoroacetic acid) or salts to assist in the separation of the sample components [64]. The composition of the mobile phase may be kept constant ("isocratic elution mode") or varied ("gradient elution mode") during the chromatographic analysis. Isocratic elution is typically

effective in the separation of sample components that are not very dissimilar in their affinity for the stationary phase. In gradient elution the composition of the mobile phase is varied typically from low to high eluting strength. The eluting strength of the mobile phase is reflected by analyte retention times with high eluting strength producing fast elution (short retention times). A typical gradient profile in reversed phase chromatography might start at 5% acetonitrile (in water or aqueous buffer) and progress linearly to 95% acetonitrile over 5ï 25 minutes [64]. Period of constant mobile phase composition may be part of any gradient profile. For example, the mobile phase composition may be kept constant at 5% acetonitrile for 1ï 3 min, followed by a linear change up to 95% acetonitrile. The composition of the mobile phase depends on the intensity of interactions between analytes and stationary phase (e.g. hydrophobic interactions in reversed-phase HPLC). Depending on their affinity for the stationary and mobile phases analytes partition between the two during the separation process taking place in the column. This partitioning process is similar to that which occurs during a liquid-liquid extraction but is continuous, not step-wise. In this example, using a water/acetonitrile gradient, more hydrophobic components will elute (come off the column) late, once the mobile phase gets more concentrated in acetonitrile (i.e. in a mobile phase of higher eluting strength).The choice of mobile phase components, additives (such as salts or acids) and gradient conditions depend on the nature of the column and sample components. Often a series of trial runs are performed with the sample in order to find the HPLC method which gives the best separation.

2.7.3 Partition chromatography

Partition chromatography was the first kind of chromatography that chemists developed. The partition coefficient principle has been applied in paper chromatography, thin layer chromatography, gas phase and liquid-liquid applications. The 1952 Nobel Prize in chemistry was earned by Archer John Porter Martin and Richard Laurence Millington Synge for their development of the technique, which was used for the

separation of amino acids [64]. Partition chromatography uses a retained solvent, on the surface or within the grains or fibers of an "inert" solid supporting matrix as with paper chromatography; or takes advantage of some coulombic and/or hydrogen donor interaction with the solid support [64]. Molecules equilibrate (partition) between a liquid stationary phase and the eluent. Known as Hydrophilic Interaction Chromatography (HILIC) in HPLC, this method separates analytes based on polar differences. HILIC most often uses a bonded polar stationary phase and water miscible, high organic concentration, mobile phases. Partition HPLC has been used historically on unbonded silica or alumina supports. Each works effectively for separating analytes by relative polar differences. HILIC bonded phases have the advantage of separating acidic, basic and neutral solutes in a single chromatogram.[64] The polar analytes diffuse into a stationary water layer associated with the polar stationary phase and are thus retained. Retention strengths increase with increased analyte polarity, and the interaction between the polar analyte and the polar stationary phase (relative to the mobile phase) increases the elution time. The interaction strength depends on the functional groups in the analyte molecule which promote partitioning but can also include coulombic (electrostatic) interaction and hydrogen donor capability. Use of more polar solvents in the mobile phase will decrease the retention time of the analytes, whereas more hydrophobic solvents tend to increase retention times.

2.7.4 Normal-phase chromatography

It was one of the first kinds of HPLC that chemists developed. Also known as normal-phase HPLC (NP-HPLC), or adsorption chromatography, this method separates analytes based on their affinity for a polar stationary surface such as silica, hence it is based on analyte ability to engage in polar interactions (such as hydrogen-bonding or dipole-dipole type of interactions) with the sorbent surface [64]. NP-HPLC uses a non-polar, non-aqueous mobile phase, and works effectively for separating analytes readily soluble in non-polar solvents. The analyte associates with and is retained by the polar

stationary phase. Adsorption strengths increase with increased analyte polarity. The interaction strength depends not only on the functional groups present in the structure of the analyte molecule, but also on steric factors. The effect of steric hindrance on interaction strength allows this method to resolve (separate) structural isomers [64]. The use of more polar solvents in the mobile phase will decrease the retention time of analytes, whereas more hydrophobic solvents tend to induce slower elution (increased retention times). Very polar solvents such as traces of water in the mobile phase tend to adsorb to the solid surface of the stationary phase forming a stationary bound (water) layer which is considered to play an active role in retention. This behaviour is somewhat peculiar to normal phase chromatography because it is governed almost exclusively by an adsorptive mechanism (i.e. analytes interact with a solid surface rather than with the solvated layer of a ligand attached to the sorbent surface; as in reversed-phase HPLC [64]. Adsorption chromatography is still widely used for structural isomer separations in both column and thin-layer chromatography formats on activated (dried) silica or alumina supports. Partition- and NP-HPLC fell out of favour in the 1970s with the development of reversed-phase HPLC because of poor reproducibility of retention times due to the presence of a water or protic organic solvent layer on the surface of the silica or alumina chromatographic media. This layer changes with any changes in the composition of the mobile phase (e.g. moisture level) causing drifting retention times. Recently, partition chromatography has become popular again with the development of HILIC bonded phases which demonstrate improved reproducibility, and due to a better understanding of the range of usefulness of the technique.

2.7.5 Displacement chromatography

The basic principle of displacement chromatography is: A molecule with a high affinity for the chromatography matrix (the displacer) will compete effectively for binding sites, and thus displace all molecules with lesser affinities.[65] There are distinct differences between displacement and elution chromatography. In elution mode,

substances typically emerge from a column in narrow, Gaussian peaks. Wide separation of peaks, preferably to baseline, is desired in order to achieve maximum purification. The speed at which any component of a mixture travels down the column in elution mode depends on many factors. But for two substances to travel at different speeds, and thereby be resolved, there must be substantial differences in some interaction between the biomolecules and the chromatography matrix. Operating parameters are adjusted to maximize the effect of this difference. In many cases, baseline separation of the peaks can be achieved only with gradient elution and low column loadings. Thus, two drawbacks to elution mode chromatography, especially at the preparative scale, are operational complexity, due to gradient solvent pumping, and low throughput, due to low column loadings. Displacement chromatography has advantages over elution chromatography in that components are resolved into consecutive zones of pure substances rather than ñpeaksò. Because the process takes advantage of the nonlinearity of the isotherms, a larger column feed can be separated on a given column with the purified components recovered at significantly higher concentrations.

2.7.6 Reversed-phase chromatography (RPC)

A chromatogram of complex mixture (perfume water) obtained by reversed phase HPLC. Reversed phase HPLC (RP-HPLC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been surface-modified with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With such stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily (early in the analysis). An investigator can increase retention times by adding more water to the mobile phase; thereby making the affinity of the hydrophobic analyte for the hydrophobic stationary phase stronger relative to the now more hydrophilic mobile phase. Similarly, an investigator can decrease retention time by adding more organic solvent to the eluent. RP-HPLC is so commonly used that it is often incorrectly referred to as "HPLC" without

further specification. The pharmaceutical industry regularly employs RP-HPLC to qualify drugs before their release. RP-HPLC operates on the principle of hydrophobic interactions, which originate from the high symmetry in the dipolar water structure and play the most important role in all processes in life science. RP-HPLC allows the measurement of these interactive forces. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand on the stationary phase. This solvophobic effect is dominated by the force of water for "cavity-reduction" around the analyte and the C_{18}-chain versus the complex of both. The energy released in this process is proportional to the surface tension of the eluent (water: 7.3×10^{-6} J/cm², methanol: 2.2×10^{-6} J/cm²) and to the hydrophobic surface of the analyte and the ligand respectively [64]. The retention can be decreased by adding a less polar solvent (methanol, acetonitrile) into the mobile phase to reduce the surface tension of water. Gradient elution uses this effect by automatically reducing the polarity and the surface tension of the aqueous mobile phase during the course of the analysis. Structural properties of the analyte molecule play an important role in its retention characteristics. In general, an analyte with a larger hydrophobic surface area (C-H, C-C, and generally non-polar atomic bonds, such as S-S and others) is retained longer because it is non-interacting with the water structure [64]. On the other hand, analytes with higher polar surface area (conferred by the presence of polar groups, such as -OH, $-NH_2$, COO^- or $-NH_3^+$ in their structure) are less retained as they are better integrated into water. Such interactions are subject to steric effects in that very large molecules may have only restricted access to the pores of the stationary phase, where the interactions with surface ligands (alkyl chains) take place. Such surface hindrance typically results in less retention. Retention time increases with hydrophobic (non-polar) surface area [64]. Branched chain compounds elute more rapidly than their corresponding linear isomers because the overall surface area is decreased [64]. Similarly organic compounds with single C-C-bonds elute later than those with a C=C or C-C-triple bond, as the double or triple bond is shorter than a single C-C-bond. Aside from mobile phase surface tension (organizational strength in eluent structure), other mobile

phase modifiers can affect analyte retention. For example, the addition of inorganic salts causes a moderate linear increase in the surface tension of aqueous solutions (ca. 1.5×10^{-7} J/cm² per Mol for NaCl, 2.5×10^{-7} J/cm² per Mol for $(NH_4)_2SO_4$), and because the entropy of the analyte-solvent interface is controlled by surface tension, the addition of salts tend to increase the retention time [64]. This technique is used for mild separation and recovery of proteins and protection of their biological activity in protein analysis (hydrophobic interaction Chromatography, HIC). Another important factor is the mobile phase pH since it can change the hydrophobic character of the analyte. For this reason most methods use a buffering agent, such as sodium phosphate, to control the pH. Buffers serve multiple purposes: control of pH, neutralize the charge on the silica surface of the stationary phase and act as ion pairing agents to neutralize analyte charge. Ammonium formate is commonly added in mass spectrometry to improve detection of certain analytes by the formation of analyte-ammonium adducts. A volatile organic acid such as acetic acid, or most commonly formic acid, is often added to the mobile phase if mass spectrometry is used to analyze the column effluent. Trifluoroacetic acid is used infrequently in mass spectrometry applications due to its persistence in the detector and solvent delivery system, but can be effective in improving retention of analytes such as carboxylic acids in applications utilizing other detectors, as it is a fairly strong organic acids. The effects of acids and buffers vary by application but generally improve chromatographic resolution. Reversed phase columns are quite difficult to manage compared with normal silica columns; however, many reversed phase columns consist of alkyl derivatized silica particles and should never be used with aqueous bases as these will destroy the underlying silica particle [64]. They can be used with aqueous acid, but the column should not be exposed to the acid for too long, as it can corrode the metal parts of the HPLC equipment. RP-HPLC columns should be flushed with clean solvent after use to remove residual acids or buffers, and stored in an appropriate composition of solvent. The metal content of HPLC columns must be kept low if the best possible ability to separate substances is to be retained. A good test for the metal content of a column is to inject a sample which is a mixture of 2,2'- and 4,4'- bipyridine. Because the 2,2'-bipy can

chelate the metal, the shape of the peak for the 2,2'-bipy will be distorted (tailed) when metal ions are present on the surface of the silica.

2.7.7 Size-exclusion chromatography

Size-exclusion chromatography (SEC), also known as *gel permeation chromatography* or *gel filtration chromatography* separates particles on the basis of size. It is generally a low resolution chromatography and thus it is often reserved for the final, "polishing" step of purification. It is also useful for determining the tertiary structure and quaternary structure of purified proteins [64]. SEC is used primarily for the analysis of large molecules such as proteins or polymers. SEC works by trapping these smaller molecules in the pores of a particle. The larger molecules simply pass by the pores as they are too large to enter the pores. Larger molecules therefore flow through the column quicker than smaller molecules, that is, the smaller the molecule, the longer the retention time. This technique is widely used for the molecular weight determination of polysaccharides [64]. SEC is the official technique (suggested by European pharmacopeia) for the molecular weight comparison of different commercially available low-molecular weight heparins.

2.7.8 Ion-exchange chromatography

In ion-exchange chromatography (IC), retention is based on the attraction between solute ions and charged sites bound to the stationary phase. Ions of the same charge are excluded. Types of ion exchangers include:

- Polystyrene resins ï These allow cross linkage which increases the stability of the chain. Higher cross linkage reduces swerving, which increases the equilibration time and ultimately improves selectivity.
- Cellulose and dextran ion exchangers (gels) ï These possess larger pore sizes and low charge densities making them suitable for protein separation.
- Controlled-pore glass or porous silica

In general, ion exchangers favour the binding of ions of higher charge and smaller radius. An increase in counter ion (with respect to the functional groups in resins) concentration reduces the retention time. A decrease in pH reduces the retention time in cation exchange while an increase in pH reduces the retention time in anion exchange [64]. By lowering the pH of the solvent in a cation exchange column, for instance, more hydrogen ions are available to compete for positions on the anionic stationary phase, thereby eluting weakly bound cations. This form of chromatography is widely used in the following applications: water purification, preconcentration of trace components, ligand-exchange chromatography, ion-exchange chromatography of proteins, high-pH anion-exchange chromatography of carbohydrates and oligosaccharides, and others.

2.8 Voltammetry

Voltammetry is a category of electroanalytical methods used in analytical chemistry and various industrial processes. In voltammetry, information about an analyte is obtained by measuring the current as the potential is varied [66][67].

Three electrode system

Three-electrode setup: (1) working electrode; (2) auxiliary electrode; (3) reference electrode.

Voltammetry experiments investigate the half cell reactivity of an analyte. Voltammetry is the study of current as a function of applied potential. These curves I = f(E) are called voltammograms. The potential is varied arbitrarily either step by step or continuously, or the actual current value is measured as the dependent variable. The opposite, i.e., amperometry, is also possible but not common. The shape of the curves depends on the speed of potential variation (nature of driving force) and on whether the solution is stirred or quiescent (mass transfer). Most experiments control the potential (volts) of an electrode in contact with the analyte while measuring the resulting current (amperes).[68]

To conduct such an experiment requires at least two electrodes. The working electrode, which makes contact with the analyte, must apply the desired potential in a controlled way and facilitate the transfer of charge to and from the analyte. A second electrode acts as the other half of the cell. This second electrode must have a known potential with which to gauge the potential of the working electrode, furthermore it must balance the charge added or removed by the working electrode. While this is a viable setup, it has a number of shortcomings. Most significantly, it is extremely difficult for an electrode to maintain a constant potential while passing current to counter redox events at the working electrode. To solve this problem, the role of supplying electrons and referencing potential has been divided between two separate electrodes. The reference electrode is a half cell with a known reduction potential. Its only role is to act as reference in measuring and controlling the working electrodes potential and at no point does it pass any current. The auxiliary electrode passes all the current needed to balance the current observed at the working electrode. To achieve this current, the auxiliary will often swing to extreme potentials at the edges of the solvent window, where it oxidizes or reduces the solvent or supporting electrolyte. These electrodes, the working, reference, and auxiliary make up the modern three electrode system. There are many systems which have more electrodes, but their design principles are generally the same as the three electrode system. For example, the rotating ring-disk electrode has two distinct and separate working electrodes, a disk and a ring, which can be used to scan or hold potentials independently of each other. Both of these electrodes are balanced by a single reference and auxiliary combination for an over all four electrode design. More complicated experiments may add working electrodes as required and at times reference or auxiliary electrodes.

In practice it can be very important to have a working electrode with known dimensions and surface characteristics. As a result, it is common to clean and polish working electrodes regularly. The auxiliary electrode can be almost anything as long as it doesn't react with the bulk of the analyte solution and conducts well. The reference is the most

complex of the three electrodes, there are a variety of standards used and its worth investigating elsewhere. For non-aqueous work, IUPAC recommends the use of the ferrocene/ferrocenium couple as an internal standard.[69] In most voltammetry experiments, a bulk electrolyte (also known as a supporting electrolyte) is used to minimize solution resistance. It is possible to run an experiment without a bulk electrolyte, but the added resistance greatly reduces the accuracy of the results. With room temperature ionic liquids, the solvent can act as the electrolyte.

2.8.1 Theory

Data analysis requires the consideration of kinetics in addition to thermodynamics, due to the temporal component of voltammetry. Idealized theoretical electrochemical thermodynamic relationships such as the Nernst equation are modeled without a time component. While these models are insufficient alone to describe the dynamic aspects of voltammetry, models like the Nernst equation and Butler-Volmer equation lay the groundwork for the modified voltammetry relationships that relate theory to observed results[70].

2.9 Liquid chromatography–mass spectrometry

Liquid chromatography–mass spectrometry (**LC-MS**, or alternatively **HPLC-MS**) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry. LC-MS is a powerful technique used for many applications which has very high sensitivity and selectivity. Generally its application is oriented towards the general detection and potential identification of chemicals in the presence of other chemicals (in a complex mixture). The limitations of LC-MS in urine analysis drug screening are that it often fails to distinguish between specific metabolites, in particular with hydrocodone and its metabolites. LC-MS urine analysis testing is used to detect specific categories of

drugs. However, gas chromatography (GC-MS) should be used when detection of a specific drug and its metabolites is required.

2.9.1 Liquid chromatography

A major difference between traditional HPLC and the chromatography used in LC-MS is that in the latter case the scale is usually much smaller, both with respect to the internal diameter of the column and even more so with respect to flow rate since it scales as the square of the diameter. For a long time, 1 mm columns were typical for LC-MS work (as opposed to 4.6 mm for HPLC). More recently 300 μm and even 75 μm capillary columns have become more prevalent. At the low end of these column diameters the flow rates approach 100 nL/min and are generally used with nanospray sources[71].

2.9.2 Flow splitting

When standard bore (4.6 mm) columns are used the flow is often split ~10:1. This can be beneficial by allowing the use of other techniques in tandem such as MS and UV. However splitting the flow to UV will decrease the sensitivity of spectrophotometric detectors. The mass spectrometry on the other hand will give improved sensitivity at flow rates of 200 εL/min or less.

2.10 Electrospray ionization

Electrospray ionization (ESI) is a technique used in mass spectrometry to produce ions. It is especially useful in producing ions from macromolecules because it overcomes the propensity of these molecules to fragment when ionized. The development of electrospray ionization for the analysis of biological macromolecules[72] was rewarded with the attribution of the Nobel Prize in Chemistry to John Bennett Fenn in 2002 [73]. One of the original instruments used by Dr. Fenn is on display at the Chemical Heritage Foundation in Philadelphia, Pennsylvania.Mass spectrometry using ESI is called

electrospray ionization mass spectrometry (ESI-MS) or, less commonly, electrospray mass spectrometry (ES-MS).

2.10.1 Ionization mechanism

The liquid containing the analyte(s) of interest is dispersed by electrospray into a fine aerosol. Because the ion formation involves extensive solvent evaporation, the typical solvents for electrospray ionization are prepared by mixing water with volatile organic compounds (e.g. methanol, acetonitrile). To decrease the initial droplet size, compounds that increase the conductivity (e.g. acetic acid) are customarily added to the solution. Large-flow electrosprays can benefit from additional nebulization by an inert gas such as nitrogen. The aerosol is sampled into the first vacuum stage of a mass spectrometer through a capillary, which can be heated to aid further solvent evaporation from the charged droplets. The solvent evaporates from a charged droplet until it becomes unstable upon reaching its Rayleigh limit. At this point, the droplet deforms and emits charged jets in a process known as Coulomb fission. During the fission, the droplet loses a small percentage of its mass (1.0-2.3%) along with a relatively large percentage of its charge (10-18%) [74][75].

There are two major theories that explain the final production of gas-phase ions:

- The **Ion Evaporation Model** (IEM) [76] suggests that as the droplet reaches a certain radius the field strength at the surface of the droplet becomes large enough to assist the field desorption of solvated ions.
- The **Charge Residue Model** (CRM) [77] suggests that electrospray droplets undergo evaporation and fission cycles, eventually leading progeny droplets that contain on average one analyte ion or less. The gas-phase ions form after the remaining solvent molecules evaporates, leaving the analyte with the charges that the droplet carried.

While there is no definite scientific proof, a large body of indirect evidence suggests that small ions are liberated into the gas phase through the ion evaporation mechanism, while larger ions form by charged residue mechanism.

The ions observed by mass spectrometry may be quasi molecular ions created by the addition of a proton (a hydrogen ion) and denoted $[M + H]^+$, or of another cation such as sodium ion, $[M + Na]^+$, or the removal of a proton, $[M - H]^-$. Multiply charged ions such as $[M + nH]^{n+}$ are often observed. For large macromolecules, there can be many charge states, resulting in a characteristic charge state envelope. All these are even-electron ion species: electrons (alone) are not added or removed, unlike in some other ionization sources. The analytes are sometimes involved in electrochemical processes, leading to shifts of the corresponding peaks in the mass spectrum.

2.10.2 Variants

The electrosprays operated at low flow rates generate much smaller initial droplets, which ensure improved ionization efficiency. In 1994, two research groups coined the name micro-electrospray (microspray) for electrosprays working at low flow rates. Emmett and Caprioli demonstrated improved performance for HPLC-MS analyses when the electrospray was operated at 300-800 nL/min[78]. Wilm and Mann demonstrated that a capillary flow of ~ 25 nL/min can sustain an electrospray at the tip of emitters fabricated by pulling glass capillaries to a few micrometers [79]. The latter was renamed nano-electrospray (nanospray) in 1996[80][81]. Currently the name nanospray is also in use for electrosprays fed by pumps at low flow rates, not only for self-fed electrosprays. There are no well-defined flow rate ranges for electrospray, microspray, and nano-electrospray.

2.10.3 Applications

Electrospray is used to study protein folding [82][83][84].Electrospray ionization is the ion source of choice to couple liquid chromatography with mass spectrometry. The analysis can be performed online, by feeding the liquid eluting from the LC column directly to an

electrospray, or offline, by collecting fractions to be later analyzed in a classical nanoelectrospray-mass spectrometry setup. The effect of various ion-pairing agents such as TFA [85] on Electrospray-LCMS has been studied.

2.10.4 Noncovalent gas phase interactions

Electrospray ionization is also ideal in studying noncovalent gas phase interactions. The electrospray process is capable of transferring liquid-phase noncovalent complexes into the gas phase without disrupting the noncovalent interaction. This means that a cluster of two molecules can be studied in the gas phase by other mass spectrometry techniques. An interesting example of this is studying the interactions between enzymes and drugs which are inhibitors of the enzyme. Because inhibitors generally work by noncovalently binding to its target enzyme with reasonable affinity, the noncovalent complex can be studied in this way. Competition studies between STAT6 and inhibitors [86][86] have been done in this way to screen for potential new drug candidates[86].

2.11 Differential scanning calorimetry

Differential scanning calorimetry or DSC is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined heat capacity over the range of temperatures to be scanned. The technique was developed by E.S. Watson and M.J. O'Neill in 1962[87], and introduced commercially at the 1963 Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. The first adiabatic differential scanning calorimeter that could be used in biochemistry was developed by P.L. Privalov and D.R. Monaselidze in 1964[88]. The term DSC was

coined to describe this instrument which measures energy directly and allows precise measurements of heat capacity [89].

2.11.1 Detection of phase transitions

The basic principle underlying this technique is that when the sample undergoes a physical transformation such as phase transitions, more or less heat will need to flow to it than the reference to maintain both at the same temperature. Whether less or more heat must flow to the sample depends on whether the process is exothermic or endothermic. For example, as a solid sample melts to a liquid it will require more heat flowing to the sample to increase its temperature at the same rate as the reference. This is due to the absorption of heat by the sample as it undergoes the endothermic phase transition from solid to liquid. Likewise, as the sample undergoes exothermic processes (such as crystallization) less heat is required to raise the sample temperature. By observing the difference in heat flow between the sample and reference, differential scanning calorimeters are able to measure the amount of heat absorbed or released during such transitions. DSC may also be used to observe more subtle phase changes, such as glass transitions. It is widely used in industrial settings as a quality control instrument due to its applicability in evaluating sample purity and for studying polymer curing [90][91][92].

2.11.2 DTA

An alternative technique, which shares much in common with DSC, is differential thermal analysis (DTA). In this technique it is the heat flow to the sample and reference that remains the same rather than the temperature. When the sample and reference are heated identically phase changes and other thermal processes cause a difference in temperature between the sample and reference. Both DSC and DTA provide similar information. DSC measures the energy required to keep both the reference and the sample at the same temperature whereas DTA measures the difference in temperature between the sample and the reference when they are both put under the same heat.

2.11.3 DSC curves

The result of a DSC experiment is a curve of heat flux versus temperature or versus time. There are two different conventions: exothermic reactions in the sample shown with a positive or negative peak, depending on the kind of technology used in the experiment. This curve can be used to calculate enthalpies of transitions. This is done by integrating the peak corresponding to a given transition. It can be shown that the enthalpy of transition can be expressed using the following equation: where is the enthalpy of transition, is the calorimetric constant, and is the area under the curve. The calorimetric constant will vary from instrument to instrument, and can be determined by analyzing a well-characterized sample with known enthalpies of transition [93].

2.11.4 Applications

Differential scanning calorimetry can be used to measure a number of characteristic properties of a sample. Using this technique it is possible to observe fusion and crystallization events as well as glass transition temperatures T_g. DSC can also be used to study oxidation, as well as other chemical reactions [91][92][93][94]. Glass transitions may occur as the temperature of an amorphous solid is increased. These transitions appear as a step in the baseline of the recorded DSC signal. This is due to the sample undergoing a change in heat capacity; no formal phase change occurs [91][93]. As the temperature increases, an amorphous solid will become less viscous. At some point the molecules may obtain enough freedom of motion to spontaneously arrange themselves into a crystalline form. This is known as the crystallization temperature (T_c). This transition from amorphous solid to crystalline solid is an exothermic process, and results in a peak in the DSC signal. As the temperature increases the sample eventually reaches its melting temperature (T_m). The melting process results in an endothermic peak in the DSC curve. The ability to determine transition temperatures and enthalpies makes DSC a valuable tool in producing phase diagrams for various chemical systems [91].

Examples

The technique is widely used across a range of applications, both as a routine quality test and as a research tool. The equipment is easy to calibrate, using low melting indium at 156.5985 °C for example, and is a rapid and reliable method of thermal analysis.

2.11.5 Polymers

DSC is used widely for examining polymers to check their composition. Melting points and glass transition temperatures for most polymers are available from standard compilations, and the method can show possible polymer degradation by the lowering of the expected melting point, T_m, for example. T_m depends on the molecular weight of the polymer, so lower grades will have lower melting points than expected. The percentage crystallinity of a polymer can be found from the crystallization peak of the DSC graph since the heat of fusion can be calculated from the area under an absorption peak. DSC can also be used to study thermal degradation of polymers. Impurities in polymers can be determined by examining thermograms for anomalous peaks, and plasticisers can be detected at their characteristic boiling points.

2.11.6 Liquid crystals

DSC is used in the study of liquid crystals. As some forms of matter go from solid to liquid they go through a third state, which displays properties of both phases. This anisotropic liquid is known as a liquid crystalline or mesomorphous state. Using DSC, it is possible to observe the small energy changes that occur as matter transitions from a solid to a liquid crystal and from a liquid crystal to an isotropic liquid [92].

2.11.7 Oxidative stability

Using differential scanning calorimetry to study the stability to oxidation of samples generally requires an airtight sample chamber. Usually, such tests are done isothermally (at constant temperature) by changing the atmosphere of the sample. First, the sample is brought to the desired test temperature under an inert atmosphere, usually nitrogen. Then, oxygen is added to the system. Any oxidation that occurs is observed as a deviation in the baseline. Such analysis can be used to determine the stability and optimum storage conditions for a material or compound [91].

2.11.8 Safety Screening

DSC makes a reasonable initial safety screening tool. In this mode the sample will be housed in a non-reactive crucible (often gold, or gold plated steel), and which will be able to withstand pressure (typically up to 100 bar). The presence of an exothermic event can then be used to assess the stability of a substance to heat. However, due to a combination of relatively poor sensitivity, slower than normal scan rates (typically 2-3 °/min - due to much heavier crucible) and unknown activation energy, it is necessary to deduct about 75-100 °C from the initial start of the observed exotherm to suggest a maximum temperature for the material. A much more accurate data set can be obtained from an adiabatic calorimeter, but such a test may take 2ï 3 days from ambient at a rate of a 3 °C increment per half hour.

2.11.9 DSC in Drug Analysis

DSC is widely used in the pharmaceutical and polymer industries. For the polymer chemist, DSC is a handy tool for studying curing processes, which allows the fine tuning of polymer properties. The cross-linking of polymer molecules that occurs in the curing process is exothermic, resulting in a positive peak in the DSC curve that usually appears soon after the glass transition [91][92][93].

In the pharmaceutical industry it is necessary to have well-characterized drug compounds in order to define processing parameters. For instance, if it is necessary to deliver a drug in the amorphous form, it is desirable to process the drug at temperatures below those at which crystallization can occur [93].

2.11.10 General chemical analysis

Freezing-point depression can be used as a purity analysis tool when analysed by Differential scanning calorimetry. This is possible because the temperature range over which a mixture of compounds melts is dependent on their relative amounts. Consequently, less pure compounds will exhibit a broadened melting peak that begins at lower temperature than a pure compound [92][93].

2.12 REVIEW OF SOME OF THE WORKS ASSAYED ON DRUG ANALYSIS

The quality control assessment of five brands of amoxicillin sodium was carried out by Osadebe and co workers [94] using gravimetry and UV visible spectrophotometer at 227 nm. All the brands analyzed passed the weight uniformity test with percentage deviation value range of 1.51 ï 4.27 %. The results for active drug content shows that 20 % of the brands analyzed passed the USP and BP specifications, 20 % were above the specifications while 60 % were below the manufacturersô claim of 500 mg.

The quality control assay of ampicillin brands of capsules were carried out by Okoro in 2007 [95] using two analytical methods namely iodometric and UV visible spectrophometer showed that 60 % of the different brands analyzed were within the BP specifications while 40 % were below the BP stipulations. This is determined at 322 nm. Two brands of ampicillin capsules were completely out of the range of stipulated values. These two brands therefore, were substandard and adulterated and they pose a serious threat to the health of the entire people of West Africa sub region that depends on these drugs for therapy.

2.13 SPECTROSCOPIC METHOD OF ANALYSIS

The study of either absorption or emission of light is known as spectroscopy and it is the basis of all spectroscopic or optical method of analysis [96]. When light of specific energy is passed through a variety of either solution or suspensions, it is generally observed that the intensity of transmitted light is less than that of the incident light [96]. Measurements based on the light and other forms of electromagnetic radiation are widely used throughout analytical chemistry [97]. We can classify spectroscopic methods according to the region of the electromagnetic spectrum involved in the measurement. The regions of the spectrum that have been used include gamma-ray, x-ray, ultra violet visible, infrared, micro wave and radio frequency [98]. Spectrochemical methods have provided the most widely used tools for the elucidation of molecular structure as well as quantitative and qualitative determination of both inorganic and organic compounds [97].

2.13.1 PHYSICAL METHODS OF ANALYSIS

(1.) **Ultraviolet spectroscopy**;-This involves the spectroscopy of substances in the UV-visible region in the electromagnetic spectrum. Atomic and molecular electronic transitions occur between the wavelength of 10^3-10^4 A^0 which gives rise to visible and UV spectra [98].

(2.) **Infra-red spectroscopy**;-from the electromagnetic spectrum between the wavelength of 10^4-10^6 A^o, the energy of most molecular vibration corresponding to that of the infra red region[98]. The knowledge of infra-red spectroscopy gives information on how the chromophores are joined together in compound [98]. The infrared portion of the electromagnetic spectrum is usually divided into three regions; the near-, mid- and far- infrared, named for their relation to the visible spectrum. The higher-energy near-IR, approximately 14000ï 4000 $cm^{ī 1}$ (0.8ï 2.5 ε m wavelength) can excite overtone or harmonic vibrations. The mid-infrared, approximately 4000ï 400 $cm^{ī 1}$ (2.5ï

25 ε m) may be used to study the fundamental vibrations and associated rotational-vibrational structure. The far-infrared, approximately 400ï 10 $cm^{ī 1}$ (25ï 1000 ε m), lying adjacent to the microwave region, has low energy and may be used for rotational spectroscopy [99-100]. The names and classifications of these subregions are conventions, and are only loosely based on the relative molecular or electromagnetic properties.

(3.) **Proton nuclear magnetic resonance spectroscopy**; - This involves the number and types of protons present [98]. Most commonly known as NMR spectroscopy, is a research technique that exploits the magnetic properties of certain atomic nuclei to determine physical and chemical properties of atoms or the molecules in which they are contained. It relies on the phenomenon of nuclear magnetic resonance and can provide detailed information about the structure, dynamics, reaction state, and chemical environment of molecules [101].

(4.) **Most frequently,** NMR spectroscopy is used by chemists and biochemists to investigate the properties of organic molecules, though it is applicable to any kind of sample that contains nuclei possessing spin. Suitable samples range from small compounds analyzed with 1-dimensional proton or carbon-13 NMR spectroscopy to large proteins or nucleic acids using 3 or 4-dimensional techniques.[101] The impact of NMR spectroscopy on the sciences has been substantial because of the range of information and the diversity of samples, including solutions and solids.

(5.) **Mass spectroscopy**; - This involves the molar mass of a compound and the way the compounds break up during chemical reaction particularly under the influence of energy [98]. (MS) is an analytical technique that measures the mass-to-charge ratio of charged particles [102-104]. It is used for determining masses of particles, for determining the elemental composition of a sample or molecule, and for elucidating the chemical structures of molecules, such as

peptides and other chemical compounds. MS works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios [102-104]. In a typical MS procedure:

1. A sample is loaded onto the MS instrument and undergoes vaporization
2. The components of the sample are ionized by one of a variety of methods (e.g., by impacting them with an electron beam), which results in the formation of charged particles (ions)
3. The ions are separated according to their mass-to-charge ratio in an analyzer by electromagnetic fields
4. The ions are detected, usually by a quantitative method
5. The ion signal is processed into mass spectra

2.13.2 SPECTROPHOTOMETRY

Spectroscopic methods can further be classified into photometry and spectrophotometry. Photometry simply means light measurement and involves the use of a simple instrument known as photometer. The photometer of coloured solutions is termed colorimetry and the instrument is known as colorimeter. However, spectrophotometry is more sophisticated and has wider applications than photometry. It involves the use of a spectrophotometer (a device for measuring light intensity) [98]. It consists of two instruments, a spectrometer and a photometer. The spectrometer is used to split light in bands of wavelength before it is fed to the photometer. To achieve the desired resolution, a spectrometer is equipped with a high resolution wavelength selector known as monochromator [98].

2.13.3 TERMS USED IN UV SPECTROSCOPY

(i.) **Red shift or bath chromic shift**; - This is a shift of the absorption maximum toward the longer wavelength .it could be caused by a change in the medium or by the presence of an auxochrome [98].

(ii.) **Auxochromes**; - Some substituents do not confer colour to an otherwise colourless compound but rather augment the activities of a chromophores such groups are called auxochromes .They intensify the colour of the compounds they include the following NR_2, NH_2, OH,-OCH_3 [98].

(iii.) **Blue Shift**; - This is a shift of the absorption maximum towards shorter wavelength. It could be caused by a change in the medium or disappearance of conjugation [98].

(iv.) **Hypochromic Shift**; - this is an effect that causes a decrease in intensity of absorption maxima [98].

(v.) **Hyperchromic Shift**; - This is an effect that causes an increase in intensity of the absorption maxima [98].

2.13.4 LIGHT AND COLOUR

Light is an electromagnetic radiation resulting from the interaction of mutually perpendicular electric and magnetic vectors. It has both particle and wave nature [96]. As a wave, it is characterized by its wavelength, and frequency, which are related to its energy (E) from equation below

$$E = hv = hc/\lambda \quad \text{.....} \quad (1)$$

Where h = plank´s constant (6.62×10^{27} ergs)

V = Frequency

C = Velocity

λ = Wavelength

The colour of visible light depends on the band of wavelength reaching the eye [96].white colour is observed where the light consists of all the wavelength of the entire visible region. On passing through a medium which selectively absorbs some wavelengths only those reflected or transmitted will reach the eye, which will perceive the colour of the medium in terms of the wavelength band [96]. Table2: below shows the complementary bands of colour and their wavelengths.

Table 2.12: WAVELENGTH AND COLOURS [97]

Wavelength range(nm)	Colour Absorbed	Colour Transmitted
Yellowish-Green	400-435	Violet
Yellow	435-480	Blue
Orange	480-490	Greenish-Blue
Red	490-500	Bluish-Green
Purple	500-560	Green
Violet	560-580	Yellowish-Green
Blue	580-595	Yellow
Greenish-blue	595-610	Orange
Bluish-Green	610-750	Red

Adapted from .....[97]

2.14 ABSORPTION LAWS

There are two empirical laws which govern the intensity of absorption.

Lambert law; - This states that the fraction of the incident light absorbed is independent of the intensity of the source of light [97-98].

$\text{Log } 1_o/1 = k_1L$ ..... (1)

Beer´s Law; **-** This states that the amount of light absorbed is directly proportional to the number of absorbing molecules.

$\text{Log } 1_o/1 = k_2C$ ..... (2)

Replacing $\text{Log } 1_o/1 = A$, $K_1K_2 = E$

And combining equation (1) and (2) will give Beer ï Lambert´s Law.

Beer-Lambert´s law; - This states that the absorption of a solution is directly proportional to the concentration of the absorbing species in the solution and the pathlength.

$A = ELC$ ..... (3)

Where

A = absorbance or optical density

C = concentration

E = molar absorptivity or molar extinction coefficient

L = pathlength of the absorbing solution

1_o, 1 = intensities of the incident and transmitted lights respecting

K_1k_2 = constant

Beer-Lambert´s Law is used in determining the concentration of the absorbing species in a solution. It is necessary to know how quickly the absorbance changes with concentration. This can be determined from a calibration curve .for the beer ï Lambert relation above to hold in a particular case, the light must be sufficiently monochromic that the extinction coefficient used is well defined [97].

It is noteworthy that the beer- Lambert is not obeyed at all concentrations. Only dilute solutions obey it well. Beer Lambert´s Law is useful in spectrophotometry because, absorbance versus concentration yields a straight line. The equation of a Straight line is

$$Y = MX + b$$

Where M = slope

b = intercept

X and Y = variables

If the measurement is made in such a way that b = o and if we substitute absorbance for y, concentration for x and slope for m, we will arrive at the formulation of the beer Lambertôs law.

2.14.1 Instrumentation

In this section, only a brief description of instrumental features will be mentioned. This is important since you may be required to perform some experiments in UV-Vis spectroscopy without enough background.

Two types of instruments are available according to the wavelength selector used.

a. Filter Photometer

This uses filters for the selection of working wavelengths. Photometers are cheap machines that are widely used in most primitive analytical laboratories. The optical system and instrumental components can be represented by Figure 1.

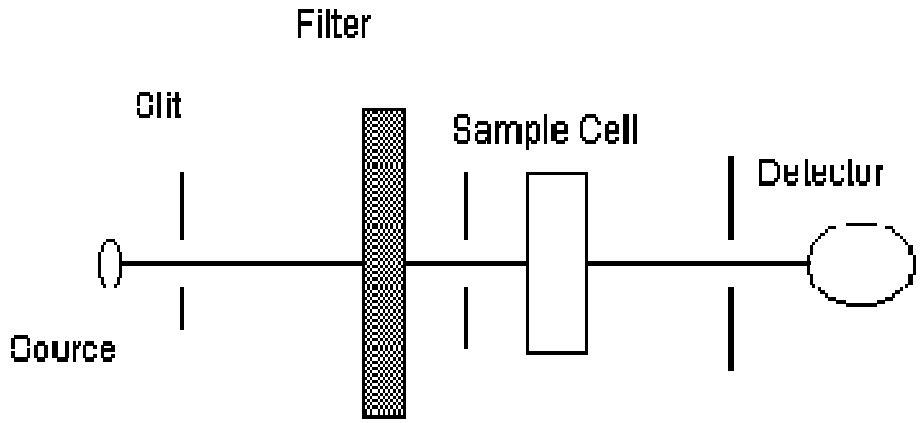


Figure 2.4: Schematic diagram of a photometer

As can be seen from the figure, light is emitted from the source passing through a suitable filter for wavelength selection. Part of the light at the selected wavelength is absorbed by the sample and the transmitted light hits the phototube detector resulting in a signal that is displayed by the instrument as absorbance.

b. Dispersive Spectrophotometers

These use either prisms or gratings for wavelength selection. Prisms and gratings are excellent wavelength selectors where a very narrow band of light at specific wavelength can be chosen especially with good gratings. Dispersive instruments are divided into two types:

1. Single Beam Spectrophotometers

This is similar to the photometer design but the wavelength selector is either a prism or grating instead of the filter. Usually, single beam instruments are of moderate price and require adjustment to zero using a blank before sample measurement. As the

instrument is kept in the operational mode, multiple zero adjustments should be undertaken because there is always some drift in response with time.

2. Double Beam Spectrophotometers

These incorporate places for two cells one for the blank and the other for the sample. The instrument automatically subtracts the absorbance of the blank or reference from that of the sample.

2.14.2 Light Sources

The most commonly used light sources are deuterium lamps in the ultraviolet region and tungsten - halogen lamps in the visible region. Make sure not to look at the deuterium lamp while in the operational mode since UV light is damaging to your eyes.

2.14.3 Cells

Remember that glass absorbs UV light, therefore make sure to use quartz cells when working in the UV region. Glass cells are adequate for measurement of absorbance in the visible region while quartz cells are adequate through the whole UV-Vis range.

2.14.4 Routine Methodology in Spectrophotometric Analysis

The first step of an analytical procedure in UV-Vis is to find the wavelength that yields maximum absorbance. This is done by scanning through the UV or Vis spectroscopy range, depending on the characteristics of the absorbing species. The spectrum is plotted with absorbance on the Y-axis and the wavelength on the X-axis. Then the wavelength that yields maximum absorbance is chosen for further work. This also gives maximum molar absorptivity.

When the problem involves the determination of an unknown analyte concentration, standard analyte is used to construct a calibration curve at the preselected wavelength and the unknown absorbance is measured which can be correlated with concentration from the curve.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

The following drugs were procured; pure amoxicillin trihydrate powder (Juhel Pharmaceutical, Enugu) and five different brands of amoxicillin capsules coded A ï E, shown in Table 4.7, which were purchased randomly at the open drug market, Nsukka in December 2011. The brands were procured based on availability in the market. Other materials include hydrochloric acid (BDH, England), freshly distilled water prepared by Science and Training Centre, University of Nigeria Nsukka. The equipments used are: electronic weighing balance (Thomas Wiley, USA) and UV-visible Spectrophotometer (Model UNICO 2100).

3.2 METHODS

3.2.2 Active drug content Determination

One hundred milligram (100 mg) of pure amoxicillin trihydrates powder was accurately weighed and dissolved in 100 mL of 0.1M HCl to obtain 1000 ppm of pure amoxicillin trihydrate solution.

Then 10, 20, 30, 40 and 50 ppm solutions were prepared by diluting the appropriate volume of the 100 ppm solution with 0.1M HCl in 50 mL standard flask. Exactly 5 mL of the 30 ppm solution was put in the curvet of a UV-visible spectrophotometer and scanned to establish the wavelength of maximum absorption $\lambda_{max.}$ Prominent peaks appeared at 266 nm and 275 nm as shown in Fig 4.3. Then 5 mL of the different dilutions were run separately and their absorbances recorded at wavelength values of 266 nm and 275 nm. Calibration graphs were plotted at the wavelengths to determine the more accurate wavelength for determination of amoxicillin. The mean content of active ingredient of each of the five commercial brands of amoxicillin trihydrates was extrapolated from the calibration graphs. The mean amount of drug content of the different brands was estimated by multiplying the dilution factor of the drug sample with the drug

concentration in ppm that is being extrapolated from the graph. This is then multiplied with hundred and divided by one thousand which would convert the drug from ppm to mg/mL as shown in Table 4.8 and 4.9. The dilution factor is obtained from the drug sample by preparing 20 ppm from the stock solution of 5000 ppm using 250ml standard flask.

3.2.1 Weight uniformity test

Twenty capsules of each brand of amoxicillin trihydrate were randomly selected and weighed singly using an analytical balance. The capsules were opened singly without loss of shell-material and the content removed. The shell was weighed again and the differences in weights representing the weight of the content were noted. Mean weight, standard deviation and Coefficient of Variation (CV) for the weight of each brand were calculated; also analysis of variance was done to compare the five brands.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

The features of the five brands of amoxicillin trihydrate used in this study are shown in table 4.7. Three of the brands were manufactured in Nigeria while two were imported from India. They are all registered by the National Agency for Food Drug Administration and Control (NAFDAC). Manufacturing dates were 2010 and 2011 while expiry dates range from 2013 to 2015.

The weight uniformity studies are shown in Tables 4.1 ï 4.6. The results reveal that all the five brands sampled conformed to the United State Pharmacopoeia (USP) and British Pharmacopoeia (BP) specifications of maximum coefficient of variation (CV) of ±5 %.

The regression equation for the Beer- Lambert´s plot of pure amoxicillin trihydrate at 266 nm is shown in Fig 4.1. The equation was found to be Y = 0.0035X and the correlation coefficient R^2 = 0.9642 while at 275 nm in Fig 4.2 the equation was Y = 0.0031X and the R^2 = 0.9432. Absorptions seem to be good at both wavelengths and the drug may equally be analysed at both wavelength.

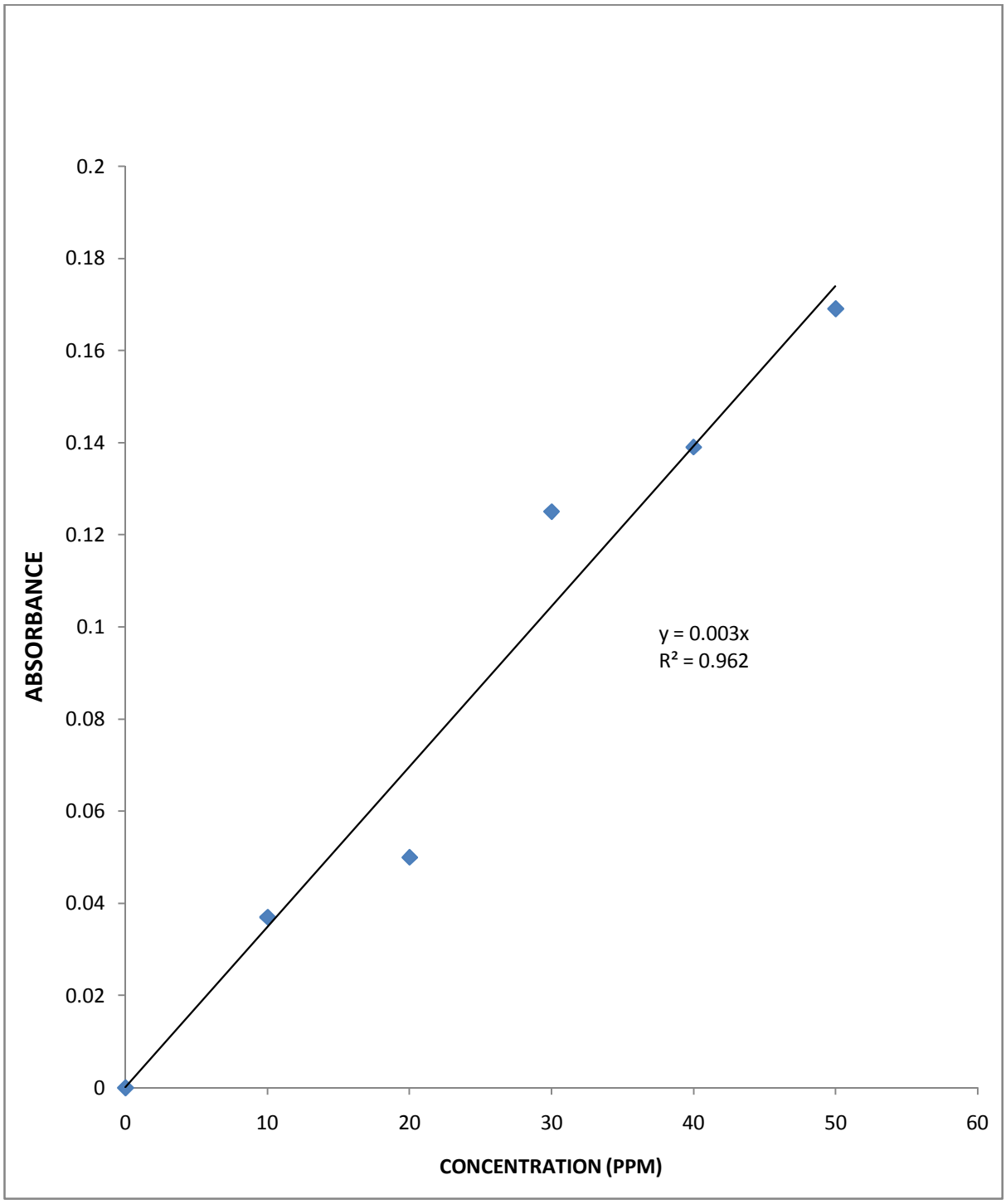


Fig 4.1: BEER' S PLOT OF AMOXICILLIN TRIHYDRATES AT 266 nm

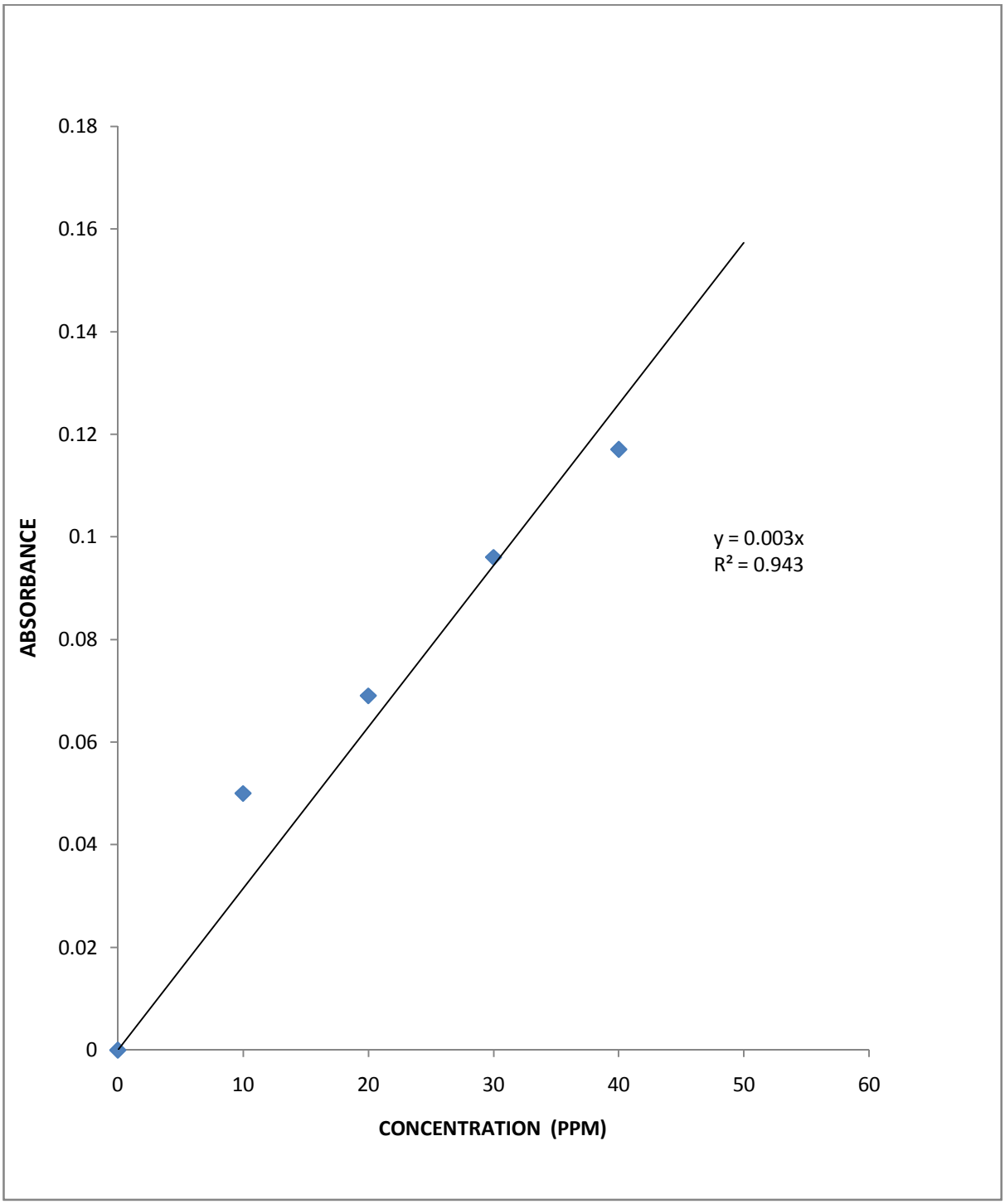
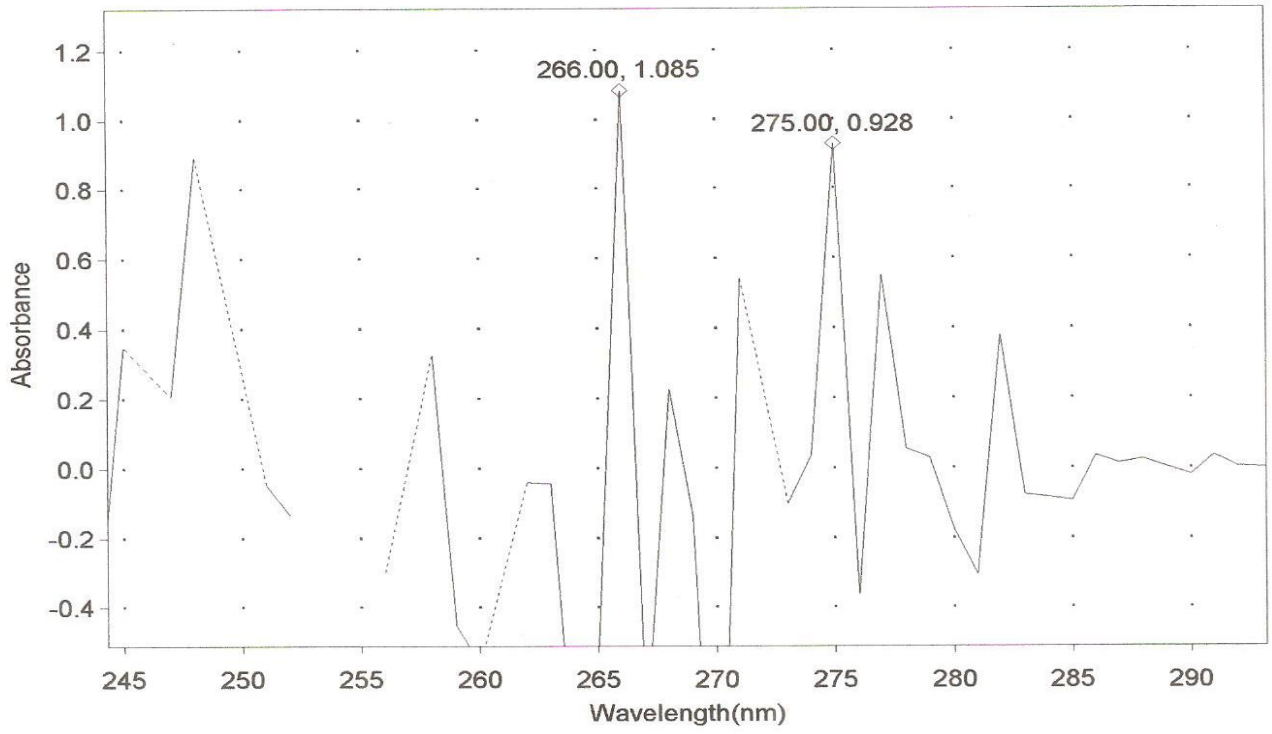


Fig 4.2: BEER' S PLOT OF AMOXICILLIN TRIHYDRATES AT 275 nm

THERMO ELECTRON ~ VISIONpro SOFTWARE V4.10

Operator Name	(None Entered)	Date of Report	25/01/2012
Department	(None Entered)	Time of Report	18:06:34PM
Organization	(None Entered)		
Information	(None Entered)		

Scan Graph



Results Table - Amoxil 1ppm005.sre,1PPM AMOXIL,Cycle01

nm	A	**Peak Pick Method**
266.00	1.085	Find 8 Peaks Above -3.0000 A
275.00	0.928	Start Wavelength 200.00 nm
		Stop Wavelength 300.00 nm
		Sort By Wavelength
Sensitivity	Auto	

Fig 4.3: SPECTRUM OF PURE AMOXICILLIN TRIHYDRATE SHOWING TWO PROMINENT PEAKS

Table 4.8: Active drug content of the sample brands of Amoxicillin trihydrates Capsules at 266 nm

Brands	Strength (mg)	Conc (ppm) 1st	Conc (ppm) 2nd	Conc (ppm) 3rd	Amt found (mg) 1st	Amt found (mg) 2nd	Amt found (mg) 3rd	Mean Amt found (mg)	% Content 1st	% Content 2nd	% Content 3rd	% Mean Content	Std	CV
A	500	18.357	13.803	14.424	458.93	345.075	360.60	388.20	91.79	69.015	72.12	77.64	2.470	3.2
B	500	19.204	17.101	13.317	480.10	427.53	332.93	413.53	96.02	85.505	66.585	82.71	2.983	3.6
C	500	16.352	13.422	14.766	408.80	335.55	369.15	371.18	81.76	67.11	73.83	74.24	1.467	2.0
D	500	18.440	19.506	14.894	461.00	487.65	372.35	440.33	92.20	97.53	74.47	88.065	2.415	2.7
E	500	17.484	15.347	16.862	437.10	383.675	421.55	414.10	87.42	76.735	84.31	82.82	1.099	1.3

Table 4.9: Active drug content of the sample brands of Amoxicillin trihydrates Capsules at 275 nm

Brands	Strength (mg)	Conc (ppm) 1st	Conc (ppm) 2nd	Conc (ppm) 3rd	Amt found (mg) 1st	Amt found (mg) 2nd	Amt found (mg) 3rd	Mean Amt found (mg)	% Content 1st	% Content 2nd	% Content 3rd	% Mean Content	Std	CV
A	500	17.324	16.904	14.186	433.10	422.60	354.65	403.45	86.62	84.52	70.93	80.69	1.705	2.1
B	500	19.912	18.200	17.765	497.80	455.00	444.13	465.65	99.56	91.00	88.825	93.13	1.135	1.2
C	500	14.940	18.155	16.808	373.50	453.88	420.20	415.850	74.70	90.78	84.04	83.170	1.615	1.9
D	500	13.770	18.792	12.690	344.25	469.80	317.25	377.100	68.85	93.96	63.45	75.42	3.256	4.3
E	500	18.599	14.502	17.120	464.98	362.55	428.00	418.500	93.00	72.51	85.60	83.70	2.075	2.5

Table 4.1: UNIFORMITY OF WEIGHTS OF BRAND A OF AMOXICILLIN CAPSULES (Beecham)

S/N	CAPSULE + CONTENT (g)	SHELL ONLY (g)	CONTENT ONLY (g)
1	0.682	0.100	0.582
2	0.686	0.103	0.583
3	0.681	0.103	0.578
4	0.685	0.103	0.582
5	0.701	0.102	0.599
6	0.663	0.100	0.563
7	0.677	0.102	0.575
8	0.684	0.101	0.583
9	0.692	0.103	0.589
10	0.688	0.101	0.587
11	0.697	0.101	0.596
12	0.690	0.105	0.585
13	0.697	0.101	0.596
14	0.694	0.099	0.595
15	0.697	0.102	0.595
16	0.679	0.101	0.578
17	0.683	0.100	0.583
18	0.695	0.099	0.596
19	0.702	0.103	0.599
20	0.687	0.101	0.586

Table 4.2: UNIFORMITY OF WEIGHTS OF BRAND B OF AMOXICILLIN CAPSULES (HEALMOXY)

S/N	CAPSULE+CONTENT (g)	SHELL ONLY (g)	CONTENT ONLY (g)
1	0.702	0.091	0.611
2	0.680	0.093	0.587
3	0.682	0.094	0.588
4	0.658	0.092	0.566
5	0.657	0.087	0.570
6	0.692	0.092	0.600
7	0.693	0.092	0.601
8	0.668	0.085	0.583
9	0.662	0.087	0.575
10	0.655	0.092	0.563
11	0.674	0.093	0.581
12	0.681	0.091	0.590
13	0.668	0.093	0.575
14	0.676	0.093	0.583
15	0.688	0.096	0.592
16	0.714	0.093	0.621
17	0.718	0.094	0.624
18	0.676	0.093	0.583
19	0.693	0.087	0.606
20	0.685	0.093	0.592

Table 4.3: UNIFORMITY OF WEIGHTS OF BRAND C OF AMOXICILLIN CAPSULES (LAMOX)

S/N	CAPSULE + CONTENT (g)	SHELL ONLY (g)	CONTENT ONLY (g)
1	0.681	0.100	0.581
2	0.645	0.088	0.557
3	0.677	0.101	0.576
4	0.675	0.097	0.578
5	0.651	0.090	0.561
6	0.666	0.093	0.573
7	0.650	0.094	0.556
8	0.666	0.087	0.579
9	0.645	0.093	0.552
10	0.662	0.097	0.565
11	0.690	0.096	0.594
12	0.655	0.094	0.561
13	0.646	0.095	0.551
14	0.643	0.095	0.548
15	0.665	0.090	0.575
16	0.667	0.094	0.573
17	0.667	0.089	0.578
18	0.669	0.096	0.573
19	0.660	0.081	0.579
20	0.658	0.098	0.560

Table 4.4: UNIFORMITY OF WEIGHTS OF BRAND D OF AMOXICILLIN CAPSULES (MOXITIN)

S/N	CAPSULE+CONTENT (g)	SHELL ONLY (g)	CONTENT ONLY (g)
1	0.674	0.103	0.571
2	0.685	0.105	0.580
3	0.698	0.110	0.588
4	0.698	0.105	0.593
5	0.695	0.070	0.625
6	0.699	0.110	0.589
7	0.703	0.111	0.592
8	0.700	0.106	0.594
9	0.698	0.110	0.588
10	0.702	0.102	0.600
11	0.699	0.112	0.587
12	0.693	0.110	0.583
13	0.697	0.108	0.589
14	0.704	0.116	0.588
15	0.701	0.112	0.589
16	0.690	0.109	0.581
17	0.695	0.108	0.587
18	0.686	0.104	0.582
19	0.703	0.113	0.590
20	0.700	0.110	0.590

Table 4.5: UNIFORMITY OF WEIGHTS OF BRAND E OF AMOXICILLIN CAPSULES (CIKAMOX)

S/N	CAPSULE + CONTENT (g)	SHELL ONLY (g)	CONTENT ONLY (g)
1	0.670	0.097	0.573
2	0.669	0.097	0.572
3	0.685	0.097	0.588
4	0.664	0.090	0.574
5	0.681	0.097	0.584
6	0.662	0.093	0.569
7	0.640	0.098	0.542
8	0.681	0.100	0.581
9	0.659	0.093	0.566
10	0.664	0.096	0.568
11	0.665	0.093	0.572
12	0.650	0.086	0.564
13	0.661	0.096	0.565
14	0.683	0.097	0.586
15	0.657	0.096	0.561
16	0.662	0.092	0.570
17	0.686	0.098	0.588
18	0.672	0.091	0.581
19	0.685	0.095	0.590
20	0.681	0.093	0.588

Table 4.6: The Mean Drug Content, Standard deviation and Coefficient of Variation (CV) of the five brands of Amoxicillin Trihydrate for weight uniformity

Brand	Mean Drug Content (g)	Standard Deviation	CV
A	**0.587**	**±0.009**	**1.533**
B	**0.590**	**±0.018**	**3.051**
C	**0.569**	**±0.013**	**2.285**
D	**0.589**	**±0.010**	**1.698**
E	**0.574**	**±0.010**	**1.742**

Table 4.7: List of Brands of Amoxicillin Trihydrate Capsules used in this project.

Brands	Country	Name Of Manufacturer	Batch Number	NAFDAC Number	Manufacturing Date	Expiring Date
A	India	Medreich sterilab Ltd	100380	04-2481	04/2010	04/2015
B	India	Maxheal pharm LTD	H021020	04-4310	06/2011	11/2013
C	Nigeria	Me cure LTD	Lam-417	04-4359	10/2011	09/2013
D	Nigeria	Clarion Medicals LTD	110706	04-4800	07/2011	07/2014
E	Nigeria	Michelle LTD	CM -31	A4-3776	10/2011	07/2014

4.2 DISCUSSION

Having worked with two wavelengths (266 nm and 275 nm), the result obtained at 266 nm for active drug content revealed that out of the five brands assayed it was only three brands; B, D and E (Table 4.8) that met the general drug specifications of 80 ï 110 % of the claim of 500 mg: B - 413.525 mg, D - 440.325, E - 414.100 mg. These however, failed the antibiotic amoxicillin trihydrates USP and BP specifications of 90 ï 110 %. The result obtained at 275 nm revealed that out of the five brands assayed four brands; A, B, C and E (Table 4.9) met the USP and BP general drug specifications of 80 ï 110 % of the claim of 500 mg: A - 403.45 mg, B - 465.65 mg, C - 415.85 mg, and E - 418.50 mg. while only brand B met the antibiotic amoxicillin trihydrates specifications of 90 ï 110%, having 465.65 mg. However, at 266 nm, the ranges of coefficient of variation were 1.3 ï 3.6 while at 275 nm the range were 1.2 ï 4.3.Therefore, it is better to work at 266 nm.

The weight uniformity studies result of the brands in Table 4.1 - 4.6 showed that the five brands passed the maximum Coefficient of Variation (CV) of ± 5 % for capsules more than 250 mg according to USP and BP specifications. Also the analyses of variance in Appendix 1- 3 shows that the weight uniformity of the drug content for all the brands compared to the specification of 90 ï 110 % were significant. (P ’ 0.05)

CHAPTER FIVE

5.0 CONCLUSION

Five brands of amoxicillin trihydrate capsules purchased from Nsukka, open drug market were analyzed for active drug content and weight uniformity to ascertain their quality assurance using UV visible spectroscopy and gravimetry. This study has shown that all the brands evaluated showed significant variations ($P < 0.05$) in active drug content using USP and BP specifications. The drug contents analyzed were below the claim of the manufacturer 500 mg.

However, with the result obtained, the consequences are that it poses a serious threat to the health of the entire people of West Africa sub regions that depend on these drugs for therapeutical response. As all the drugs analyzed were below the acceptance limit requirement of antibiotic amoxicillin trihydrate and thus, can lead to serious health implications such as drug resistance, cardiac failure, etc. Drug regulatory bodies should be at alert and they should conduct strict routine check on all the NAFDAC satisfied companies as all the drugs analyzed compromise their quality because of profit reasons.

REFERENCES

1. Dorlands Medical Dictionary:antibacterial. (2008), Archived from the original on http://www.mercksource.com/pp/us/cns/cns_hl_dorlands_split.jsp?pg=/ppdocs/us/common/dorlands/dorland/one/000005889.htm. Retrieved 2010-10-29
2. Waksman, S.A. (1947), What Is Antibiotic or an Antibiotic Substance. Pp. 565ï 569.
3. Von Nussbaum, F. (2006), Medicinal Chemistry of Antibacterial Natural Products ï Exodus or Revival?". *Angew. Chem. Int. Ed.* **45** (31): 5072ï 5129
4. Calderon,C.B., Sabundayo, B.P .(2007), Antimicrobial Classifications: Drugs for Bugs.. ISBN 978-0-8247-4100-6
5. Zadik, Y., Findler, M., Livne, S, (2008), "Dentists' knowledge and implementation of the 2007 American Heart Association guidelines for prevention of infective endocarditis". *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **106** (6): e16-9.
6. Pankey, G.A., Sabath, L.D. (2004), "Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections.ò *Clin Infect Dis.* **38** (6): 864ï 870.
7. Bates. (2008), Fake drugs kill people all over the world. Available at www.koreatimes.co.kr/www/news/opinion/2008/07/160_17728.html accessed 22/7/08.
8. NAFDAC Consumer safety. (2003), bulletin volume 2. No1.
9. Erhun, W.O., Erhun, M.O., Babalola, O.O. (2001), Drug Regulation and control in Nigeria: The challenge of counterfeit drugs. Journal of health and population in developing countries, 4 (2):
10. Raufu, A. (2002), Influx of fake drugs to Nigeria worries health experts. BMJ. 324 (7339):698.

11. Anonymous. (1989), Drug Distribution and Fake Drugs in Nigeria. International Workshop in Lagos, Nigeria.

12. Spies, A.R., Dusen, V.V. (2003), Counterfeit drugs, a menace keeps growing, Available at www.uspharmacists.com/index.asp?show=article&page=8_1014.htm

13. Ratanawijitrasin, S., Wondemagegnehu, E. (2002), Effective drug regulation- A multicountry study. A book published by World Health Organization. Pp. 45-48

14. Goodman, C., Brieger, W., Unwin, A., Mills, A., Meek, S., Greer, G. (2007), Medicine sellers and malaria treatment in sub-Saharan Africa: What do they do and how can their practice be improved? American Journal of tropical medicine and Hygiene .Pp. 203-218

15. Agege, C.O. (1988), Products and the consumers: An analysis of food and drug legislation in Nigeria. Hein online Food and drug law Journal, 43:201

16. Okeke, T.A, Uzochukwu, B, Okafor, H. (2006), An in-depth study of patent medicine sellerôs perspectives on malaria in rural Nigeria community. Malaria Journal in Pubmed central vol. 5:

17. United States Pharmacopoeia. (2007), 30 National Formulary 25. Rockville MD: USP Convention Inc, 1402.

18. Rang, H.P., Dale M.M., Ritter, J.M., and Moore, P.k. (2003), Pharmacolgy, 5th Edition, Churchill, Living Stone Edinburgh. Pp. 639-643.

19. Rahman, S., Ahuja, A., Ali, J., Khar, K. (2004), Simultaneous spectrophotometric determination of amoxycillin trihydrat and metronidazole in dental films. Indian J Pharm Sci; 66:135-36.

20. Bobrowska, G.E. (2001), Determination of amoxicillin and clavulanic acid in some pharmaceutical preparations by derivative spectrophotometry. Mikrochim Acta; 136(1-2): 31-34.

21. Daharwal, S.J., Saraf, S. (2007), Spectrophotometric Determination for the Simultaneous Estimation of Amoxicillin and Tinidazole in Tablet Dosage Form. Indian J Pharm Sci; 41(1): 35-41.

22. Mascher, J.H., Kikuta, C. (1998), Determination of Amoxicillin in Human Serum and Plasma by High Performance Liquid Chromatography and Online Post Column Derivatisation. JChromagr A; 812: 221-26.

23. Abreu, L.R., Ortiz, R.A. (2003), HPLC determination of amoxycillin comparative bioavailability in healthy volunteers after a single dose administration. J Pharm Sci; 6(2):223-230.

24. Yuan, Z., Russlie, H.Q., Canafax, D.M. (1997), High Performance Liquid Chromatographic Analysis of Amoxycillin in Human and Chinchilla Plasma, Middle Ear Fluid and Whole Blood. Chromatogr B; 692:361-66.

25. Zarapkar, S.S., Rane, S.H. (2000), Reverse phase high performance liquid chromatographic determination of amoxicillin and ambroxol hydrochloride in tablets. Indian drugs; 37(5): 246-250

26. Menotuou, A., soxmogy, A.A., Barclay, M.L., Bochmer, F. (1999), simultaneous quantification of amoxicillin and metronidazole in plasma using high performance liquid chromatography with photodiodes array detection.J chromatogr. B; 731(2); 261 266.

27. Qurestn, S.Z., Qayoom, T., helalet, M.I. (1999), Simultaneous spectrophotometric and voltametric determinations of amoxicillin,ampicillin and cloxacillin in drug formulations reactions mechanisms in the base catalyzed hydrolysis followed by oxidation with iodate in dilute acid solution.J Pharm .Biomed anal,21; 473-483.

28. Khuroo, A.H., Monif, Verma, P.R.P., Gurule, S. (2008), *Journal of Chromatographic Science.* Vol.46. 10: 854-861.

29. Abdil Ozdemir and Aysenur Korkmaz. (2007), *Journal of Food and Drug Analysis*. Vol.15, 2: 118-125.

30. Alireza Ghassempour., Hasan Rafati., LalehAdlnasab., Yosef Bashour.,Homeira Ebrahimazadeh and Mohammad Erfan. (2007), *Journal of AAPS Pharmsci. Tech*. 8(4). Article

31. Blaser, M.J.J. (1990), Infect Dis.; 161; 626-633

32. Rang, H.P., Dale, M.M., Ritter, J.M and Moore, P.k. (2003), Pharmacology, 5th Edition, Churchill, Living Stone Edinburgh. Pp. 639-643.

33. Goncalves, L.R.B, Sousa, R, Fernandez-lafuente, R. (2002), Enzymatic synthesis of amoxicillin; avoiding limitations of the mechanistic approach for reaction kinetics,Biotechnol bioeng. 80; 622-631.

34. Holten, K.B., Onusko, E.M. (2000), "Appropriate prescribing of oral beta-lactam antibiotics". *American Family Physician* **62** (3): 611ï 20.

35. Dolezalova, M., kunteova, B., Jobanet, R. (2004), Dermination of the purity of ampicillin by miceller electrokinetic chromatography and reversed phase liquid chromatography on a monolithic silica column.J sep Sci; 27; 560-568

36. Kolar, p., Shen, W., Tsuboi, A., Ishikawa, T. (2002), Solvent selection for pharmaceuticals. Fluid phase equilib; 194-197; 771-782.

37. Wei, D.Z, Yang, L. (2003), Effects of the ethylene glycol on the synthesis of ampicillin using immobilized penicillin. Biotechol; 78; 431-436.

38. FDA's SCOGS Database; (1979), Report No. 60; ID Code: 557-04-0;

http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5066975

39. Matrosovich., Mikhail., Tatyana Matrosovich., Wolfgang Garten., and Hans-Dieter Klenk. (2006), "New low-viscosity overlay medium for viral plaque assays". *Virology Journal* (BioMed Central) **3**: 63.

40. Ward, A.G., Courts, A. (1977), *the Science and Technology of Gelatin*. New York: Academic Press. ISBN 0127350500.

41. "Sodium Coco-Sulfate". *TightlyCurly.com*. Retrieved 26 March 2012.

42. LaFlesh., Teri. (2010), *Curly Like Me: How to Grow Your Hair Healthy, Long, and Strong*. John Wiley & Sons. Pp. 189.

43. European Pharmacopoeia: Sodium laurilsulfate

44. Sworbrick., James., James, C., Boylan. (1990), *Encyclopedia of pharmaceutical technology*. Pp. 2623ï 2624.

45. http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm

46. Wood., Roger., M. (2004), *Analytical methods for food additives*. Boca Raton: CRC Press. ISBN 1-85573-722-1.

47. Middleton, Elliott; N. Franklin Adkinson; John Yunginger; William Busse; Bruce Bochner; Stephen Holgate. (2003), *Middleton's allergy principles & practice*. St. Louis: Mosby. ISBN 0-323-01425-9.

48. Entry at chemicalland 21.com

49. "Current EU approved additives and their E Numbers", Food Standards Agency website, retrieved 15 Dec 2011.

50. Zumdahl., Steven, S. (2009), *Chemical Principles 6th Ed*. Houghton Mifflin Company. p. A23. ISBN 0-618-94690-X.

51. El, Goresy., Chen, M., Dubrovinsky, L., Gillet, P., Graup, G. (2001), "An ultradense polymorph of rutile with seven-coordinated titanium from the Ries crater.ò *Science* **293** (5534): 1467ï 1470.

52. Goresy, E.l., Ahmed., Chen., Ming., Gillet., Philippe., Dubrovinsky., Leonid., Graup., GüNther., Ahuja, Rajeev. (2001), "A natural shock-induced dense polymorph of rutile with Ŭ-PbO2 structure in the suevite from the Ries crater in Germany". *Earth and Planetary Science Letters* **192** (4): 485.

53. Greenwood., Norman, N., Earnshaw, A. (1984), *Chemistry of the Elements*. Oxford: Pergamon. Pp. 1117ï 1119.

54. Jamieson, J., Olinger, B. (1969), "Pressure Temperature Studies of Anatase, Brookite, Rutile and TiO2 II: A discussion". *Mineralogical Notes* **54**: 1477.

55. Hanaor, D., Sorrell, C. (2011), "Review of the anatase to rutile phase transformation". *Journal of Materials Science* **46** (4): 855ï 874.

56. Al-Shamma, A., Drake, S., Flynn, D.L., Mitscher, L.A., Park, Y.H., Rao, G.S.R., Simpson,A., Swayze, J.K., Veysoglu, T., Wu, S.T.S. (1981), "Antimicrobial Agents from Higher Plants. Antimicrobial Agents from Peganum harmala Seeds". *J Nat Prod* **44** (6): 745ï 747.

57. Bais, H.P., Vepachedu, R., Vivanco, J.M. (2003), "Root specific elicitation and exudation of fluorescent beta-carbolines in transformed root cultures of Oxalis tuberosa". *Plant Physiology and Biochemistry* **41** (4): 345ï 353.

58. Ward, E. H. (2001), In Vitro Cellular and Developmental Biology". *Plant* **37** (6): 730ï 741.

59. Gu, T., Wei. (2009), oxicity and Estrogen Effects of Methyl Paraben on Drosophila melanodaster. Food Science. Pp. 252-254.

60. El Ali., Bassam, M., Bassam., Mohammad Farahat. (2005), *Handbook of industrial chemistry: organic chemicals*. New York: McGraw-Hill. ISBN 0-07-141037-6.

61. Nestle Aero packet ingredients listing barcode: 7613031579334

62. "LISTERINE Antiseptic Mouthwash, SMART RINSE, WHITENING, ADVANCED, Fluoride Rinse, and Tartar Protection Products". Listerine.com. Retrieved 2009-07-31.

63. Clin, J. (1979), Immunol Allergy.; VOL 64 ISS P32-37, (REF 25)

64. Amoxicillin. *The American Society of Health-System Pharmacists*. http://www.drugs.com/monograph/amoxicillin.html. Retrieved 3 April 2011.

65. Douglas, D.J. (1998), Size exclusion chromatography.Pp.119 -129.

66. Faulkner. (2011), Importance and uses of voltammetry. Pp.56-63.

67. Thornhill, M.H., Dayer, M.J., Forde, J.M., Corey, G.R., Chu, V.H., Couper, D.J., Lockhart, P.B. (2011), Amperometry and voltammograms .Pp.342- 2392.

68. Lindsay, S., Kealey, D. (1987), *High performance liquid chromatography*. Wiley. http://www.osti.gov/energycitations/product.biblio.jsp?osti_id=7013902.

69. Hung, L. B., Parcher, J. F., Shores, J. C., Ward, E. H. (1988), "Theoretical and experimental foundation for surface-coverage. Pp.56 -59.

70. Ray, A.K. (1994), Programming in gas-solid chromatography with an adsorbable carrier gas". *J. Am. Chem. Soc.* 110 (11): 1090.

71. Sacheminc. (2011). Displacement Chromatography. Pp.71-78.

72. Kissinger, Peter., William, R., Heineman. (1996), *Laboratory Techniques in Electroanalytical Chemistry, Second Edition, Revised and Expanded* (2nd ed). CRC. ISBN 0824794451.

73. Zoski., Cynthia, G. (2007), *Handbook of Electrochemistry*. Elsevier Science. ISBN 0444519580.

74. Bard., Allen, J., Larry, R., Faulkner. (2000), *Electrochemical Methods: Fundamentals and Applications* (2nd ed). Wiley. ISBN 0471043729.

75. Gritzner, G. J., Kuta. (1984), "Recommendations on reporting electrode potentials in nonaqueous solvents". Pure Appl. Chem. **56** (4): 461ï 466.

76. Nicholson, R. S., Irving Shain. (1964), "Theory of Stationary Electrode Polarography. Single Scan and Cyclic Methods Applied to Reversible, Irreversible, and Kinetic Systems". *Analytical Chemistry* **36** (4): 706ï 723.

77. Kenneth, B., Tomer, M., Arthur Moseley., Leesa, J., Deterding, Carol., E. Parker. (1994), Mass Spectrometry Reviews, Vol. 13. Pp. 431-457.

78. Zeleny, J. (1914), "The Electrical Discharge from Liquid Points, and a Hydrostatic Method of Measuring the Electric Intensity at Their Surfaces". *Physical Review* **3** (2): 69.

79. Dole, M., Mack, L. L., Hines, R. L., Mobley, R. C., Ferguson, L. D., Alice, M. B. (1968), "Molecular Beams of Macroions". *Journal of Chemical Physics* **49** (5): 2240ï 2249.

80. Iribarne, J. V., Thomson, B. A. (1976), "On the evaporation of small ions from charged droplets". *Journal of Chemical Physics* **64** (6): 2287ï 2294.

81. Emmett, M.R., Caprioli, R.M. (1994), "Micro-electrospray mass spectrometry: ultra-high-sensitivity analysis of peptides and proteins". *J. Am. Soc. Mass Spectrom.* **5** (7): 605ï 613.

82. Wilm, M.S., Mann. M. (1994), "Electrospray and Taylor-Cone theory, Dole's beam of macromolecules at last?". *Int. J. Mass Spectrom. Ion Proc.* **136** (2-3): 167ï 180.

83. Li, K.Y., Tu, H.H., Ray, A.K. (2005), "Charge Limits on Droplets during Evaporation". *Langmuir* **21** (9): 3786ï 3794.

84. Wilm, Mann, M. (1996), "Analytical properties of the nanoelectrospray ion source". *Anal. Chem.* **68** (1): 1ï 8.

85. Gibson. (2009), *Mass Spectrometry Reviews* **28** (6): 918ï 936, doi:10.1002/mas.20248.

86. Konermann, L., Douglas, D.J. (1998), "Equilibrium unfolding of proteins monitored by electrospray ionization mass spectrometry: Distinguishing two-state from multi-state transitions", *Rapid Communications in Mass Spectrometry* **12** (8): 435ï 442,

87. Nemes. (2008), "Conformational and Noncovalent Complexation Changes in Proteins during Electrospray Ionization", *Analytical Chemistry* **80** (2): 387ï 395.

88. Sobott; Robinson. (2004), "Characterising electrosprayed biomolecules using tandem-MS the noncovalent Groel chaperonin assembly", *International Journal of Mass Spectrometry* **236** (1-3): 25ï 32.

89. Ferguson, L. D., Alice, M. B. (2005), Garcia Journal of Chromatography B 825: 111-123.

90. Touboul. (2009), Distinguishing two-state from multi-state transitions, JASMS 20: 303-311.

91. Czuczy. (2009), Differential scanning calorimeters JASMS 20:227-237.

92. Rosu., Biochimie. (2008), Differential thermal analysis 1074-1087.

93. U.S. Patent 3,263,484.

94. Osadebe,P.O., Ezealisiji,K.M., Agbo,C.I., (2010), Evaluation of some quality control assessment of five brands of amoxicillin sodium capsules; International journal of current research and review; vol 03.

95. Okoro, A.I.,Ejike,N.E.,(2007), Quality control assay of ampicillin brands of capsules in Onitsha market, southeastern Nigeria; Research journal of pharmacology 1 (1); 9 ï 11.

96. Okoye, C .O .B. (2005), Undergraduate Analytical Chemistry Nigeria. Pp.98-103.

97. Skoog, West, Holler, Crouch. (2009), fundamentals of Analytical Chemistry (8^{th} ed) India. Pp.710.

98. Akpanisi, L .E .S. (2004), Organic spectroscopy, Louis Chumez printing Enterprises Pp.3-18.

99. Paula., Peter Atkins., Julio de. (2009), *Elements of physical chemistry* (5^{th} ed). Oxford: Oxford U.P. Pp. 459.

100. Laurence, M., Harwood., Christopher, J., Moody. (1989), *Experimental organic chemistry: Principles and Practice* (Illustrated ed.). Wiley-Blackwell. Pp. 292.

101. James Keeler. (2007), "Chapter 2: NMR and energy levels" (reprinted at University of Cambridge). *Understanding NMR Spectroscopy*. University of California, Irvine Pp.34 - 38.

102. Sparkman, O., David, (2000), *Mass spectrometry desk reference*. Pittsburgh: Global View Pub. ISBN 0-9660813-2-3.

103. Merriam Webster. Accessed. (2008), "Definition of spectrograph."

104. Downard, K.M. (2007), "William Aston ï the man behind the mass spectrograph". *European Journal of Mass Spectrometry* **13** (3): 177ï 190.

Appendix 1: Oneway

Descriptives

CONTENT ONLY	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
AMOXIL CAPSULE (Beecham)	20	.586500	.0092991	.02222232 *020793	.582148	.590852	.5630	.5990
HEALMOXY CAPSULES	20	.589550	.0169130	.0037819	.581634	.597466	.5630	.6240
LAMOX CAPSULES	20	.568500	.0122496	.0027391	.562767	.574233	.5480	.5940
MOXITIN CAPSULES	20	.589300	.0103420	.0023126	.584460	.594140	.5710	.6250
CIKAMOX CAPSULES	20	.574100	.0119424	.0026704	.568511	.579689	.5420	.5900
Total	100	.581590	.0149606	.0014961	.578621	.584559	.5420	.6250

Appendix 2: ANOVA

CONTENT ONLY

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.007	4	.002	12.121	.000
Within Groups	.015	95	.000		
Total	.022	99			

Appendix 3: Post Hoc Tests

Multiple Comparisons

Dependent Variable: CONTENT ONLY

LSD

(I) Drugs	(J) Drugs	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
AMOXIL CAPSULE (Beecham)	HEALMOXY CAPSULES	-.0030500	.0039298	.440	-.010852	.004752
	LAMOX CAPSULES	.0180000*	.0039298	.000	.010198	.025802
	MOXITIN CAPSULES	-.0028000	.0039298	.478	-.010602	.005002
	CIKAMOX CAPSULES	.0124000*	.0039298	.002	.004598	.020202
HEALMOXY CAPSULES	AMOXIL CAPSULE (Beecham)	.0030500	.0039298	.440	-.004752	.010852
	LAMOX CAPSULES	.0210500*	.0039298	.000	.013248	.028852
	MOXITIN CAPSULES	.0002500	.0039298	.949	-.007552	.008052
	CIKAMOX CAPSULES	.0154500*	.0039298	.000	.007648	.023252

LAMOX CAPSULES	AMOXIL CAPSULE (Beecham)	-.0180000*	.0039298	.000	-.025802	-.010198
	HEALMOX Y CAPSULES	-.0210500*	.0039298	.000	-.028852	-.013248
	MOXITIN CAPSULES	-.0208000*	.0039298	.000	-.028602	-.012998
	CIKAMOX CAPSULES	-.0056000	.0039298	.157	-.013402	.002202
MOXITIN CAPSULES	AMOXIL CAPSULE (Beecham)	.0028000	.0039298	.478	-.005002	.010602
	HEALMOX Y CAPSULES	-.0002500	.0039298	.949	-.008052	.007552
	LAMOX CAPSULES	.0208000*	.0039298	.000	.012998	.028602
	CIKAMOX CAPSULES	.0152000*	.0039298	.000	.007398	.023002
CIKAMOX CAPSULES	AMOXIL CAPSULE (Beecham)	-.0124000*	.0039298	.002	-.020202	-.004598
	HEALMOX Y CAPSULES	-.0154500*	.0039298	.000	-.023252	-.007648
	LAMOX CAPSULES	.0056000	.0039298	.157	-.002202	.013402
	MOXITIN CAPSULES	-.0152000*	.0039298	.000	-.023002	-.007398

*.The mean difference is significant at 0.05 level.