QUALITY CONTROL AND STABILITY STUDIES OF ORAL AMOXYCILLIN SUSPENSIONS MARKETED IN NIGERIA

BY

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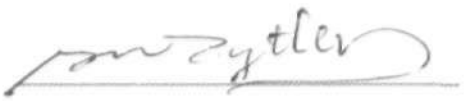
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Department of Pharmaceutics and
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SEPTEMBER, 1995

DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my research work, done under the supervision of Dr. J.A. Onaolapo and Dr. P.G. Bhatia. It has not been presented in any previous application for higher degree. All sources of information are specifically acknowledged by means of references.



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Date: September, 1995

CERTIFICATION

This thesis entitled: "**Quality Control and Stability Studies of Oral Amoxycillin Suspensions Marketed in Nigeria**" by **Tytler**, Babajide Akinyele, meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, Nigeria and is approved for its contribution to Knowledge and literary presentation.

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DEDICATION

To **God Almighty**, who makes all things possible,

My late **Father**, for the push and encouragement,

My **Mother**, for the staying,

Kehinde, Yinka, Damilola, Seyi, Damola and Tolu for their understanding and encouragement, you are all wonderfull.

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TYTLER, B.A.

ABSTRACT

A total of 60 bottles of Amoxycillin Oral suspensions of six different companies obtained from wholesale "drug markets" of Lagos, Onitsha and Kaduna were sampled.

They were qualitatively analysed for the presence of amoxycillin by Infra-red spectroscopy (IR) and thin layer chromatography (TLC) and compared to a reference standard. Quantitative analysis of the samples were done using three methods namely, microbiological assay, pharmacopoeia and non-pharmacopoeia spectrophotometric (as designed by Bungaard and Larsen, 1983) methods.

The suspensions were reconstituted as specified by the manufacturers. The reconstituted samples were stored at 2-8°C (refrigeration temperature), 25°C and 35°C, to represent the three common storage conditions obtainable in homes of consumers in a tropical country like Nigeria. The residual amoxycillin contents of the samples at the various storage temperatures were quantitatively analysed by the three methods earlier mentioned. Inter-brand and inter-batch variations were compared statistically using the Student t- and F-ratio tests.

The three analytical methods were also compared statistically by employing such parameters as F-ratio, Student t- and Pearson correlation matrix tests.

All the products from the six companies sampled were found to contain amoxycillin. The IR spectra for the test and reference samples all showed peaks specified for amoxycillin in the BP and BPC and the Rf values also confirmed the presence of amoxycillin.

In general, all samples contained amoxycillin within officially recommmended ranges of 100 ± 10%, except some batches of brand D. Inter-brand variation was observed. Of the 15 possible paired combinations, for eample in batch I, only 5 showed variations that were not statisically significant. of the six brands, products of company A showed the minimal inter-batch variation in content of amoxycillin whilst samples of brand D exhibited the highest (15%) as judged by their F-ratio and t-test values.

The three analytical methods employed in this study were highly comparable as there were no significant differences when the results they generated were statistically computed and compared.

Reconstituted oral amoxycillin suspensions were found to be most stable at storage temperature of 2-8°C with shelf-life in most cases greater than 7 days while similar suspensions stored at relatively elevated temperature of 35°C have shelf-life of 2-3 days in most cases.

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CHAPTER ONE

1.0 INTRODUCTION

The aim of any drug therapy is the restoration of health to a patient and it is for this reason that drugs should contain medicament(s) at effective concentrations to achieve this purpose. Drugs should also fulfil the specifications presented in the monograph of official compendia on identity, strength, quality and purity. A drug should retain within specified limits, throughout the period of storage and use (i.e. its shelf-life), the same properties and characteristics that it possessed at the time of its manufacture, that is, it should be stable.

Therefore, it is not enough to manufacture and present a drug or medicament that meets the specified requirements at the time of manufacture. It is incumbent on the manufacturer of the drug to ensure that its product as it gets to the consumer and during its use is acceptable in terms of therapeutic efficacy and purity. Of important considerations in the realization of this objective, the distribution network must be known and evaluated and be taken into account during the formulation stage of the drug.

Nigeria is a developing country characterized by a high population and a low number of local pharmaceutical manufacturing companies. Consequentially, the country lacks the necessary manpower and resources to meet the enormous drug requirements of its populace. These factors among others has led to mass and indiscriminate importation of all brands of drugs and medicinal preparations, with its pro

blems. As at today, it is estimated that a large percentage of the drug needs of the country is imported (Adenika, 1991). A high percentage of drugs available in many developing countries have been found to be adulterated, sub-standard and in some cases, even totally devoid of the stated active ingredients (Masland and Marshal, 1990). Moreover, most of these drugs are manufactured in temperate countries where the ambient temperatures are very much lower from that in the tropics. The deleterious effect of higher storage temperatures and humidity on various drug constituents and efficacies, and different formulations are well documented (Wells and Aulton, 1990).

The danger in the use of sub-standard drugs can not be over-emphasized especially in terms of therapeutic failure. The use of sub-standard and adulterated drugs are particularly dangerous with antibiotics where sub-clinic or sub-lethal doses encourage the infecting pathogenic organisms to develop tolerance and subsequent resistance to the drug.

1.1 STABILITY OF DRUGS

Drug stability may be defined as the time from the date of manufacture of a formulation until its chemical or biological activity is not less than a predetermined level of labelled potency and its physical characteristics have not changed appreciably or deleteriously (Lintner, 1973).

Stability can be discussed under five sub-topics:-

i. **Chemical**:- each active ingredient retains its chemical integrity and labelled potency, within the specified limits throughout the shelf-life of the product.

ii. **Physical**:- the original properties, including appearance, taste, uniformity, dissolution profile and suspendibility are retained throughout the shelf-life of the product.

iii. **Microbiological**:- sterility or resistance to microbial growth is retained according to the specified requirements. Antimicrobial agents that are present retain their effectiveness within specified limits.

iv. **Therapeutic**:- the therapeutic effect should not fall below defined limits.

v. **Toxicological**:- no significant increase in toxicity occurs throughout the shelf-life.

Although the above types of stability are recognized, chemical, physical and to some extent, biological (microbiological) stability will give pointers to the other two.

1.1.1 Chemical Degradation

Pharmaceutical preparations are of different chemical composition and as such subject to different forms of chemical degradation. Hydrolysis and oxidation are the major causes of degradation.

1.1.1.1 Hydrolysis

This is the reaction between a compound and water and it may be divided into ionic and molecular hydrolysis. Ionic hydrolysis occurs when the salt of a weak acid and base interact with water to give alkaline and acidic solutions respectively. This is an instantaneous equilibrium process.

Molecular hydrolysis is a much slower irreversible process involving cleavage of the drug molecule. This is the form of hydrolysis observed in the decomposition of pharmaceutical preparations (Enever, 1977). Esters, amides and nitrites are particularly liable to hydrolysis. The reaction is frequently catalyzed by hydrogen (H^+) or hydroxyl (OH^-) ions and is said to be specific acid or specific base catalyzed. In many instances, hydrolysis is specifically acid-base catalyzed and the rate of decomposition is critically pH dependent, esters generally exhibit this type of catalysis. Hydrolysis may also be catalyzed by acidic or basic species other than hydrogen and hydroxyl ions. For example, chloramphenicol undergoes both specific acid-base catalysis and general acid-base catalysis (Higuchi *et al*, 1954).

1.1.1.2 Oxidation

The removal of an electropositive atom, radical or electron or the addition of an electronegative atom or radical from a compound is termed oxidation. It may also be divided into two:-

i. that which involves the reversible loss of electrons without addition of oxygen. This is less frequent; and

ii. that which proceeds slowly under the influence of atmospheric oxygen which is termed auto-oxidation (Ostendorf, 1964).

The rate of oxidation in many reactions is proportional to the concentration of the oxidizable species but independent of the concentration of the oxygen present. The rate may be influenced by temperature, radiation and the

presence of a catalyst. Increase in temperature results in an increase in the rate of oxidation and the process may be at least halved by storage between 0-5°C. Heavy metals like chromic, cupric, ferrous and ferric ions also catalyze oxidation e.g. as little as 0.2 mg of copper ions per liter considerably reduces the stability of penicillin. Hydronium and hydroxyl ions also catalyze oxidative stability for any antibiotic or vitamin preparation which can usually be achieved by using a buffer or adding an acid or alkali.

1.1.1.3 Decarboxylation

This is the elimination of carbon dioxide from a compound. The process is not very common in pharmaceutical products. However, p-aminosalicylic acid undergoes protolytic degradation to m-aminophenol and carbon dioxide. This reaction which occurs only at low pH is catalysed by hydronium ion.

1.1.1.4 Racemization

This is the process of changing from an optically active compound into a racemic compound or optically inactive mixture of corresponding dextro (d-) and levo (l-) forms. Usually the levo form is the most pharmacologically active.

Racemization is influenced by temperature, solvent, catalyst and the presence or absence of light. The process depends on the functional group attached to the asymmetric carbon atom, with aromatic groups tending to speed up the process.

1.1.1.5 Photochemical

If a drug absorbs radiation at a particular wavelength and the energy absorbed exceeds a certain threshold value, a chemical reaction will take place. Ultraviolet radiations, which has a large energy level is the cause of many degradation radiative reactions. If the absorbing molecule take part directly in the reaction, the reaction is said to be photochemical in nature. When the absorbing molecule does not participate directly in the reaction, but passes the energy to another reacting molecule, the absorbing substance is said to be a photo-sensitizer. The intensity and wavelength of the light, the size, shape, composition and colour in which they are packed are all variables that may affect the rate of the reaction.

1.1.1.6 Ultrasonic Energy

Vibrations and waves with frequencies greater than 20,000/sec, promote the formulation of free radicals and alters drug molecules. Ultrasonic radiation has been shown to affect solutions of aromatic esters, l-ascorbic acid and acetylsalicylic acid.

1.1.1.7 Physical Factors Affecting Chemical Degradation

A number of physical factors such as temperature, humidity, light/radiations are known to have potentiating deleterious effects on drug stability.

(a) **Temperature**

It is generally accepted that the rate of chemical reactions increase with increase in temperature. In fact, it is recognized that the rate increases two- to three-fold with every 10°C rise in temperature. This is of special importance for pharmaceutical preparations made in temperate climates meant for use in the tropics or when heat sterilization is employed. According to collision theory, chemical reactions take place when molecules collide, and the thermal energy of colliding molecules is converted into energy necessary to break chemical bonds (activation energy) enabling a reaction to occur. As the temperature of the system is raised, the number of molecules possessing energy of activation increases resulting in greater number of collisions, and therefore, greater reaction rate. This was expressed by Arrhenius as:

$$K = Ae^{-E/RT}$$

where K is the specific reaction constant,
A is a frequency factor (a constant),
E is the energy of activation,
R is the gas constant, and
T is the absolute temperature.

(b) **Moisture**

Absorbed moisture on the surface of solid preparation often increase the rate of decomposition by hydrolysis of susceptible drugs like aspirin, penicillins, streptomycin and tetracyclines. The presence of moisture can

also increase the rate of oxidation of susceptible drugs since oxygen is dissolved in the water layer.

(c) **Light**

On exposure of light-sensitive (photolabile) drugs to sunlight, sufficient energy of activation may be acquired enabling decomposition to take place. Only the radiation absorbed by the drug is effective in producing photochemical reactions.

(d) **pH**

Negative logarithm of hydrogen ion concentration is a measure of acidity/basicity of any chemical system. The rate of decomposition of hydrogen and/or hydroxyl ions catalyzed hydrolysis reactions is critically pH dependent, so also is oxidative decomposition involving reversible oxidation-reduction processes influenced by hydrogen ion concentration. In aqueous solutions, the rate of reaction is proportional to the solution pH.

1.1.2 Biological Degradation

Bacteria, moulds and yeast grow on/in a variety of substrates ranging from distiled water, simple solutions, emulsions, fixed oils, fats, etc, especially when organic matter is present supplying traces of nitrogen and phosphorous. Moulds have been isolated from acriflavin solutions and Syrup of Tolu, the latter developing an odour of toluene indicating the reduction of the side chain (Wills, 1958). Contaminating yeasts and moulds can develop readily in

dextrose and other sugar solutions (Phillips and Miller, 1975).

Hydrolytic, oxidation and reduction reactions may be catalyzed by enzymes produced by the organism e.g. hydrolysis of penicillins by some *Staphylococcus*, *Streptococcus* and *Bacillus* species (Soulal, 1977)

1.1.3 Physical Degradation

Physical stability governs the rate at which the physical properties and structure of a system changes. In some cases, physical changes may be influenced by packaging whilst in others it is totally independent of the container. Some of such changes include evaporation, sedimentation, crystal growth and colour change.

1.1.3.1 Loss Of Moisture

Moisture loss from aqueous solutions will result in change of concentration and therefore change in dose of a substance in a given dosage form, and sometimes, crystallization if the concentration of the original preparation is high and the solubility is near that of a saturated solution. Liquid emulsions suffer a change of strength due to convective circulation. Consistence of creams (semi-solid emulsions) prevent free circulation, and evaporation takes place only at the upper layers since diffusion from below is slow. This may lead to the separation of the non-volatile oily phase on the surface resulting in an unacceptable preparation even with relatively small moisture loss. Some suspensions may form an irreversible coagulation of particles.

1.1.3.2 Absorption of Moisture

Many ingredients of pharmaceutical formulations e.g. calcium chloride and potassium citrate are deliquescent, whilst glycerin and dry extracts are hygroscopic. Absorption of moisture by these substances result in degradation. Some finished products like effervescent powders and tablets may be subject to premature reaction, and capsules also soften on absorption of moisture.

1.1.3.3 Loss Of Volatile Substances

Volatile substances are liable to evaporation at room temperature and their loss from a preparation affects the therapeutic efficacy and potency of the preparation e.g., anaesthetic agents (ether, chloroform, etc), iodine, camphor all evaporate at room temperature. The potency of nitro-glycerine preparations are reduced if improperly stored.

1.1.3.4 Sedimentation

Separation of the diphasic systems will occur under the effect of gravity resulting in caking or creaming, due to density difference in the preparation ingredients.

1.1.3.5 Crystal Growth

Supersaturation of aqueous solutions will result in crystal formulation and the tendency of these crystals to bind together forming a hard cake is undesirable in suspensions of finely divided powders. Higuchi (1958) listed the causes of crystal growth, among others, to include :-

(a) Temperature fluctuation: Temperature rise causing increased solubility whilst a full results in supersaturation;.

(b) Change in crystal structure due to formulation solvent;

(c) Size distribution of particles: small crystals being more soluble;

(d) Polymorphic change: inter-conversion resulting in increased or decreased solubility of particles.

1.1.3.6 Colour Changes

This is usually an indication of underlying chemical or photochemical decomposition. Certain water-soluble dyes added for aesthetics purposes tend to fade rapidly in the presence of reducing substances like lactose and dextrose. The pH of the solution also affects the process.

All the above factors tend to affect the stability of a formulation and sometimes give indication as to the efficacy and therapeutic reliability of pharmaceutical formulations. Antibiotics like other pharmaceutical drugs are susceptible to various kinds of degradation. The penicillin group of antibiotics are particularly susceptible to hydrolysis and to some extent oxidation.

1.2 ANTIBIOTICS

An antibiotic is a substance produced by microorganism, or a similar substance (produced wholly or partly by chemical synthesis), which in low concentrations inhibit the growth of other microorganisms (Russel, 1987).

The quality and stability of antibiotics can be of immense importance, since they are often used in debilitating and sometimes life-threatening conditions. Thus, the use of sub-standard and degraded formulations (sometimes with toxic by-products as in the case of tetracycline), can be dangerous. To determine their purity parameters, a suitable assay procedure must be employed. A number of techniques that have been used include:

i. Biological assay (Bioassay);
ii. Chemical analysis
iii. Spectrophotometric assay;
iv. Others e.g. Chromatographic techniques.

In order to assess the quality of a preparation and the rate of its decomposition, the method of assay must estimate the active substance in the presence of its decomposition products, hence, it is sometimes necessary to check chemical methods against pharmacological or biological assays.

1.2.1 Biological Assay (Bioassay)

This involves the estimation of biological activity of an unknown sample with the confirmed potency of a known (or standard) compound on an acceptable biological indicator. Both samples are tested under strictly comparable conditions and at the same time. The test may be macro-biological as in the case of insulin which uses live rabbit as the biological indicator or it may be microbiological as in the assay of antibiotics using specified or suitable microorganism as test indicator.

1.2.1.1 Microbiological Assay Techniques

Two techniques are generally recognized:

1. The Agar Diffusion Method, and
2. The Turbidimetric Method

Either method may be used to compare the inhibitory properties (as in antibiotics), or growth promoting properties (as in vitamins and amino acids) employing a suitable microorganism as indicator.

(a) Agar Diffusion Method

This employs a solid growth medium in or on which the indicator organism has been introduced. The principle involves the formation of inhibitory areas (zones) around a reservoir from which antibiotic agent diffuses. Within the zones, no growth of indicator organism can be observed. The method depends on the dose (concentration) response of the selected organism to specific similar strengths of both known (standard) and unknown (test) substances. The zones for both the test and standard samples are compared. Large, flat bottom plates (assay-plate) are often used instead of conventional petri-dishes to minimize variation of response to any one test solution, and also making it possible to take reading of inhibition of the same or varying concentrations. This result give reliable average readings for the calculation of potency.

(b) Turbidimetric Method

Liquid growth media are used. The turbidity or opacity caused by growth of the indicator organism over an incubation period of about four (4) hours is measured. The lowest concentration of both test and standard samples which gives a clear solution (i.e. no growth of indicator organism) is said to be the minimum inhibitory concentration (MIC). This is compared for both the test and standard samples.

Indicator organism selected for both agar diffusion and turbidimetric assay must exhibit good sensitivity to the antibiotic substances in question and be capable of rapid growth.

1.2.2 Chemical Assay

Chemical assays depend on the chemical nature of the substance and its possible reactions with other substances. In this type of assay, either the amount of original unreacted substance (residual) or the degradation product of the reaction is determined using a suitable indicator. This is compared for similar quantities of both the test and standard. For example, the iodometric titration of reaction products of penicillins. In general, chemical assay methods are used for both the identification and estimation of the level of active ingredients in pharmaceutical preparations.

1.2.3 Spectrophotometric Assay

This assay method involves the use of a spectrophotometer, an analytical photoelectric instrument which

estimates the concentration of solution by measuring the amount of light passing through (transmittance) or the amount of light absorbed (absorbance). This method is based on the principles of Beer-Lambert's Law.

Spectrophotometric assays depend on the light absorption characteristics of the substance or its products of degradation. The concentration of test samples as determined by its light characteristics is compared with that of a similar concentration of the standard.

1.2.4 Other Methods of Analysis

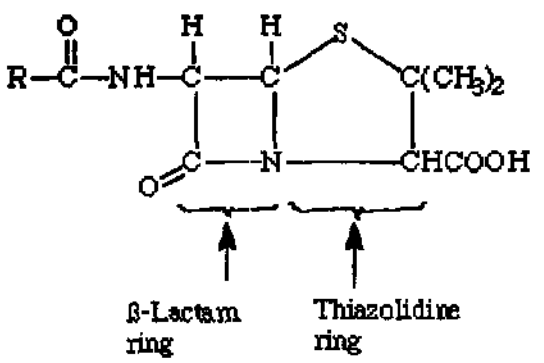
Various other methods have also been employed in the analysis of antibiotics. Abounassif *et al* (1991) showed that liquid chromatography could be used to determine amoxycillin and clavulanic acid in pharmaceutical preparations with comparable results to those obtained by the USP approved method. Kelic *et al* (1994) employed a non-aqueous titrimetric assay method in determining the quality of selected antibiotics including amoxycillin with standard deviation of less than 1% and statistically comparable to other standard methods.

1.3 THE PENICILLIN GROUP OF ANTIBIOTICS

The first antibiotics in clinical practice, the penicillins were first isolated from the mould *Penicillium notatum* and later more efficiently from *Penicillium chrysogenum* (White, 1966). Various strains of both moulds still form the commercial base of production. They may be purely natural or semi-synthetic in origin.

Penicillins are bactericidal, and effect their action by interfering with the development of bacteria cell wall. This effect is achieved by preventing the incorporation of N-acetyl muramic acid into the mucopeptide layer thereby inhibiting the biosynthesis of the dipeptidoglycan that is needed to provide strength and rigidity to the cell wall (Perlman, 1977).

Structurally, the penicillin molecule consist of a fused ring system, made up of a 5-membered thiazolidine ring and the unique 4-membered β-lactam ring.

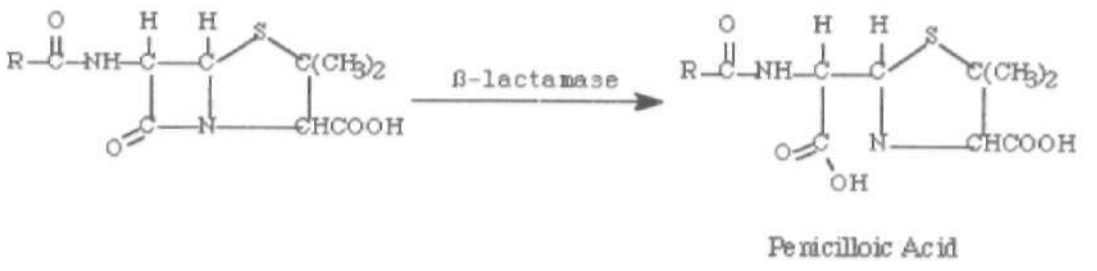


Scheme 1: Basic Structure of Penicillin Molecule

The natural penicillins have been found to be highly active against gram positive micro-organisms being bactericidal even at relatively low concentrations. They are used clinically in gram positive infections due to *Streptococcus pyogenes*, anaerobic *Streptococci*, *Pneumococci*, *Corynebacterium*, *Diphtheria*, etc. They are also active against some gram negative organisms, though the activity of the basic penicillin against most gram negative bacilli is usually too low to be of clinical significance. Over 80% of the species of *Salmonella*, *Proteus mirabilis* and *E. coli* are sensitive

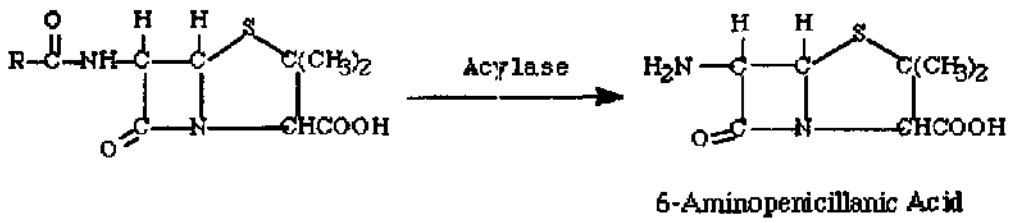
enough to respond to penicillins (Perlman, 1974). Penicillins have also been useful in the treatment of urinary and biliary tract infections especially those due to gram negative Enterobacteriaceae.

Clinically, significant transformation of penicillins is caused by gastric acid and by enzyme generally referred to as penicillinase. The strong acid in the stomach causes hydrolysis of the amide side chain as well as an opening of the ß-lactam ring. The term "penicillinase" refers to two kinds of enzymes, β-lactamase and acylase, that inactivate penicillins. ß-lactamase produces an opening of the ß-lactam ring rendering the penicillin molecule inactive. These ß-lactamase enzymes exist naturally in many organisms and when present in significant quantity, resistance development to the penicillins by micro-organisms results.



Scheme 2: Beta-lactamse Inactivation of Penicillin
(Nucleophillic attack)

Less frequent and less significant is the hydrolysis of the amide side chain by acylase. The enzyme causes the cleavage of acyl group resulting in the production of 6-aminopenicillanic acid (6-APA), a compound of very low antibacterial activity.



Scheme 3: Acylase Attack Resulting in 6-Aminopenicillanic Acid (6-APA)

The above undesirable effects have led to the synthesis of penicillins that are resistant to both acid hydrolysis and penicillinase attack and some of even broader spectrum of activity than the natural penicillins. These are the second generation or semi-synthetic penicillins. Some examples are Phenoxymethylpenicillin and Phenethicillin which are acid stable; Methicillin, Oxacillin and Cloxacillin are penicillinase resistant while Ampicillin and Carbenicillin have broad spectrum of activity, ampicillin being more active against gram negative organisms than it is against gram positive organisms.

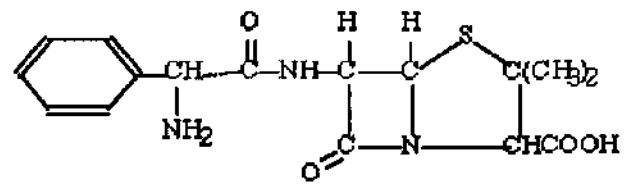
1.3.1 Amoxycillin

Amoxycillin {6-[D-(-)-α-amino-p-hydroxylphenyl-acetamido] penicillanic acid} is a fine white to off white crystalline powder, practically odourless and sparingly soluble in water (1:40). It is available as a trihydrate which is usually formulated as an oral suspension, capsule and chewable tablet as well as an injection.

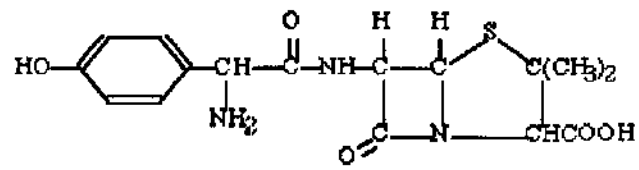
Introduced to medical practice in 1974, amoxycillin belongs to a class of semi-synthetic penicillins which have

an amino group attached to the alpha carbon of the amide side chain, hence an α-amino-penicillin. The α-amino substitution is thought to play a significant role in broadening the activity of the class, possibly by confering an ability to cross cell wall barriers that are impenetrable to other penicillins.

Structurally, amoxycillin differs from ampicillin only in the addition of an hydroxyl group to the para position of the phenyl ring. This substitution is responsible for the better absorption of amoxycillin from the gastrointestinal tract.



Ampicillin



Amoxycillin

Scheme 4: Chemical Structures of Ampicillin, and Amoxycillin

Amoxycillin is susceptible to penicillinase inactivation resulting in β-lactam ring opening. It is generally stable in the presence of acidic gastric secretion, and 74-92% of a single dose of the drug is absorbed from the gastrointestinal tract. Peak serum concentrations are usually reached 1-2 hours after oral administration in fasting and non-fasting adults (i.e. food has little effect or none

at all on absorption). Peak serum ranges between 5.5-11 µg/ml with a single dose of 500 mg (McEvoy, 1990).

1.3.1.1 Uses of Amoxycillin

As with ampicillin, amoxycillin is active against a broad spectrum of micro-organisms including *Streptococcus* species, penicillin sensitive *Staphylococcus* species, *Haemophilus influensae*, *Escherichia coli*, *Proteus mirabilis*, *Salmonella* species and *Neisseria* species. It is used in the treatment of infections of respiratory, urinary, gastro-intestinal, biliary tracts, as well as those of the skin, soft tissue and bone. It is used in the treatment of gonorrhoea (non-penicillinase producing strains), syphilis, septicaemia, endocarditis, peritonitis,and may also be employed in the treatment of dental abscess.

1.3.2 Analysis of Penicillins

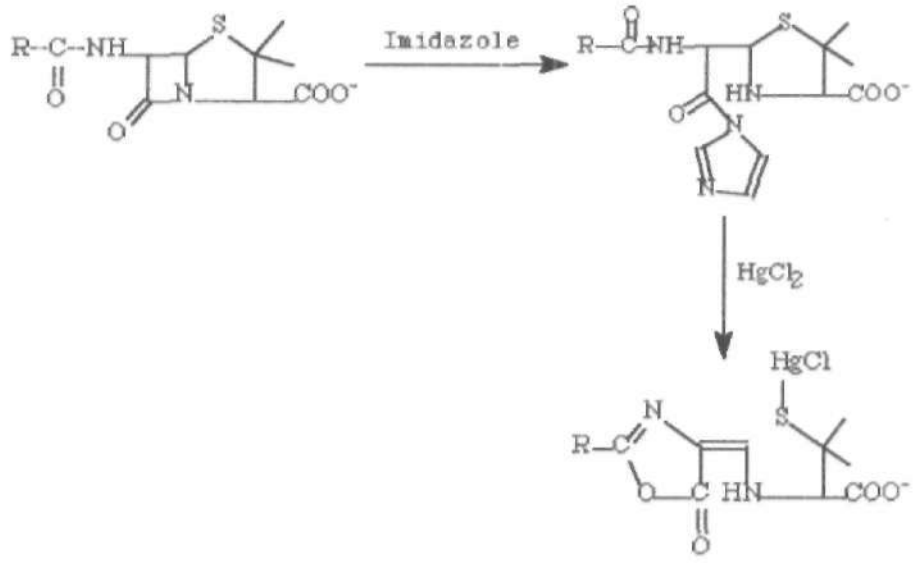
The purity, potency and stability of penicillins have been determined using various techniques of analysis. Various chromatographic techniques- HPLC, TLC etc have been used to determine purity and stability while chemical methods like iodometric titration to determine penicillin content and spectrophotometric methods e.g., pharmacopoeia assay of many penicillins have been employed.

1.3.2.1 Spectrophotometric Analysis of Penicillins

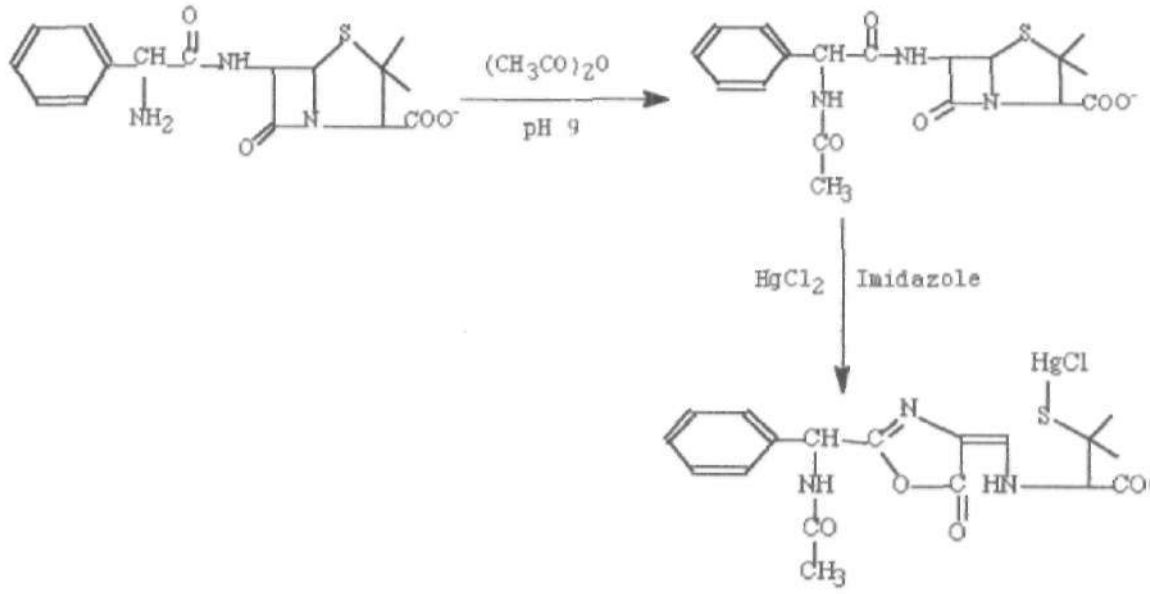
The spectrophotometric method for the qualitative determination of the penicillin group of antibiotics is based on the isomerization of members of the group into their corresponding penicillenic acids. These acids which have a

strong ultraviolet absorption with maximum at 320-360 nm, are formed by degradation of penicillins in acidic aqueous solutions. These are labile but could be stabilized to some extent by copper or mercuric salts. This method have the disadvantage that conversion had to be made in acidic solutions, in which penicillin undergo other degradation reactions (Bundgaard, 1971a). Furthermore, the method involves the addition of a mercuric salt and could not be used for the determination of acid-labile penicillins such as Penicillin G (Tutt and Schwartz, 1971) because the rate of formation of the penicillenic acid in this case is much slower than the rate of its degradation. In neutral aqueous solution, benzylpenicillin and several semi-synthetic penicillins undergo an imidazole-catalyzed rearrangement into the corresponding penicillenic acid which yield the stable mercuric mercaptide in the presence of mercuric (II) chloride as shown in scheme 5 (Bundgaard, 1971b). This is the basis of the spectrophotometric method of quantitative assay of a series of penicillins (Bundgaard and Ilver, 1972).

Ampicillin (an aminopenicillin) however, differs from other penicillins in that it reacts with imidazole in the presence of mercuric (II) chloride to form an unstable product with an absorption maximum at a different wavelength (311 nm rather than 325 nm). Pre-acetylation of the side chain amino group however, allows ampicillin to be assayed by the same method as in scheme 6 (Bundgaard, 1974)



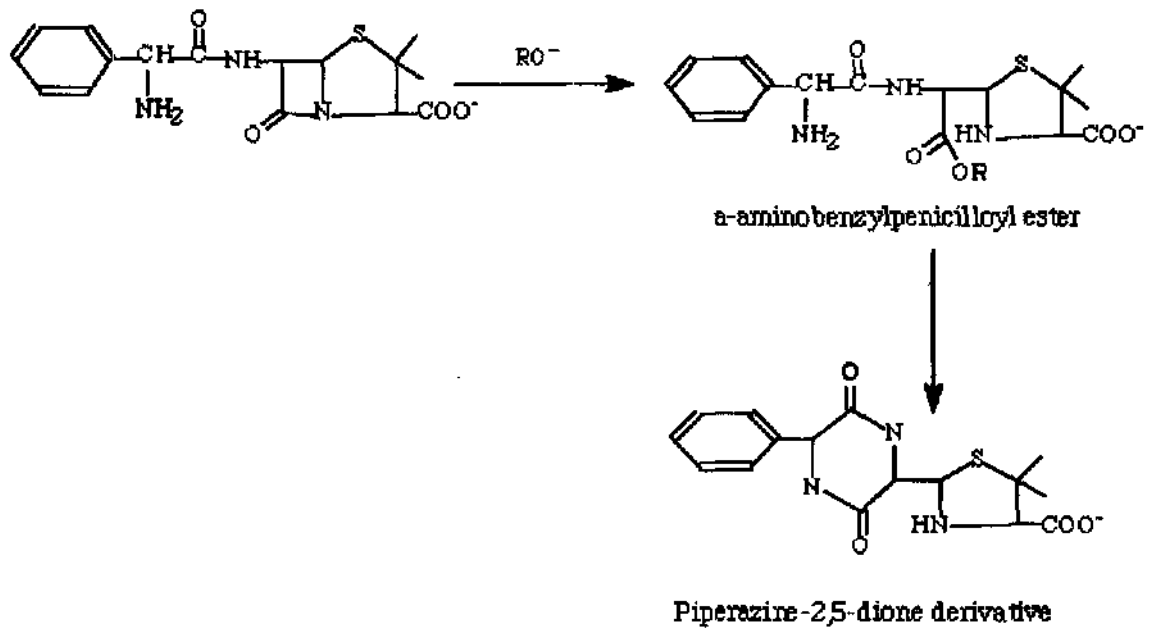
Scheme 5: Degradation of Penicillin in Imidazole



Scheme 6: Degradation of Pre-acetylated Ampicillin in Imidazole

A major limitation of the above method is the interference from di- and polymerization products, which are strongly antigenic and are considered to play an important part in penicillin allergy (Dewdney *et al*, 1971; Smith *et al*, 1971).

Ampicillin has been shown to react with various carbohydrates and polyhydric alcohols (e.g. sorbitol) with the formation of corresponding α-aminobenzyl ester which subsequently undergoes a fast intramolecular aminolysis to the piperazine-2,5-dione derivative (scheme 7).



Scheme 7: Degradation of Ampicillin in Sorbitol

The piperazine-2,5-dione derivative was observed to react rapidly with 0.5-2 M sodium hydroxide to give a product with product absorption maximum at 322 nm (Bundgaard and Hansen, 1981). This forms the basis of a rapid and

convenient method for the selective determination of ampicillin and other related aminopenicillins like amoxycillin (Bundgaard and Larsen, 1983). It is free from interference from degradation and polymerization products since an intact β-lactam moiety and free amino group side chain of the penicillin molecule are required for piperazine dione formation.

1.4 AIMS AND OBJECTIVE OF THE RESEARCH

Studies in the treatment of typhoid fever has shown that there is an increasing number of resistant strains of *Salmonella typhi* to chloramphenicol (Baine *et al*, 1977; Dupont *et al*, 1980). Amoxycillin has been shown to be very effective especially in the prevention of relapse associated with typhoid fever (Robertson *et al*, 1968, Snyder *et al*, 1976). The toxic effects associated with chloramphenicol in infants (Evans and Kleinman, 1986) has led to increasing use of amoxycillin in the treatment of typhoid fever and other conditions. The broad spectrum cephalosporins [cefuroxime (Zinacef^{R}), Ceftriaxone (Rocephin^{R})] are given parenterally, and are therefore not very convenient in typhoid therapy in children. A more recent antibiotic known for its exceptional efficacy in typhoid fever- Ciprofloxacin (Ciproxin^{R}) is contraindicated in children below the age of 10 years (Schluter, 1987).

The ever increasing incidences of typhoid fever in the populace has subsequently increased the demand for and use of amoxycillin oral suspensions as an alternative to chloramphenicol in children for reasons earlier enumerated, beside the added advantage that it is relatively cheap.

Different brands of amoxycillin oral suspensions are available in the market, either manufactured locally or imported. These various brands will be analyzed qualitatively and quantitatively for their active constituents. The effect of storage conditions on reconstituted amoxycillin suspensions as obtained in the consumers homes (i.e., at temperatures of 2-8°C (refrigeration), 25°C (room temperature) and 35°C) will also be investigated.

Various methods are routinely available for the pharmaceutical analysis of amoxycillin in different types of formulations. Three methods of analysis namely: microbiological and chemical (pharmacopoeial and non-pharmacopoeial) methods will be used in this work and their results statistically compared.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Growth Media

a. Nutrient Agar (Oxoid Limited, Basingstoke, England)

b. Nutrient Broth (Oxoid Limited, Basingstoke, England)

2.1.2 Test Organism

Bacillus megaterium (clinical isolate obtained from Department of Microbiology, A,B.U., Zaria)

2.1.3 Instruments and Glassware

1. Spectronic 20 Spectrophotometer (Bausch & Lomb)
2. Petri-dishes
3. Cork borer (size 4)
4. Test tubes
5. Assay Plates (30 x 30 cm)
6. Shaker Water-bath (Gallenkamp, England)
7. Centrifuge (Gallenkamp, England)
8. Automated Pipettes
9. Glass Pipettes
10. Graduated Volumetric flasks
11. Electric Flask Shaker (Gallenkamp, England)
12. Ultraviolet Spectrophotometer, Pye-Unicam 200

13. Infra-red Spectrophotometer, Philips PU 9706
14. pH Meter Model 7060 (Electric Instruments Ltd)
15. Stoppered tubes
16. Thermometer

2.1.4 Chemicals

1. Acetic Anhydride (Merck, Darmstadt, Germany)
2. Imidazole (Merck, Darmstadt, Germany)
3. Mercury (II) Chloride (M & B, England)
4. 1,4-Dioxan (BDH, England)
5. Hydrochloric Acid (M & B, England)
6. Potassium Iodide (M & B, England)
7. Acetone (M & B, England)
8. Glacial Acetic Acid (M & B, England)
9. Zinc Sulphate (BDH, England)
10. Sorbitol (BDH, England)
11. Sodium Hydroxide (BDH, England)
12. Phosphate Buffer pH 6.0; 7.0
13. Boric Buffer pH 9.0
14. Starch Mucilage
15. Iodine
16. Potassium Bromide
17. Amoxycillin Trihydrate (Pure powder, used as standard).
18. Amoxycillin Test Samples:

    Amoxil^{R},

    Amoxycillin (APOTEX^{R}),

    Cliffmoxin^{R},

Amox^{R},

Moxy^{R}

Moxycilin^{R}.

2.2 METHODS

2.2.1 Sampling

Samples were obtained from wholesale markets of Lagos, Onitsha and Kaduna, which are considered as major drug markets in Nigeria. The samples were brands available in these markets at the time of sampling and have been observed to be the most widely available brands for a number of years in Nigeria. The brands sampled for use in this work were:

Amoxil^{R}, designated "A"

Amoxycillin (APOTEX^{R}), designated "B"

Cliffmoxin^{R}, designated "C"

Amox^{R}, designated "D"

Moxy^{R} designated 'E", and

Moxycilin^{R} designated "F"

A total of 60 bottles, comprising 15 batches (three from each brand of A, D and E and two from each of brand B, C and F) brands were sampled. The analytical tests were carried out within the stated shelf-life of the products.

2.2.1.1 Treatment of Samples

Samples were reconstituted in accordance with manufacturers specifications. Three bottles of each brand and

batch were stored under refrigeration (mean temperature of 4°C), at room temperature (25°C) and at 35°C.

2.2.2 **Identification Of Samples**

2.2.2.1 Infra-red Spectroscopy

This is the principal method of identification officially stated in the British Pharmacopoeia 1988.

A small quantity of the reference standard was finely ground and triturated with potassium bromide to form a homogeneous powder. A sample of this was then placed in a special die and pressed in a hydraulic press giving a thin translucent disc.

The disc so formed was mounted in the Spectrophotometer (Philips PU 9706) and the spectrum run for three minutes. The process was repeated for samples of each brand and the spectra later compared with that of the standard.

2.2.2.2 Thin Layer Chromatography (TLC)

The procedure was carried out as specified for amoxycillin in the BP 1988.

A chromatoplate pre-coated with silica gel was activated by heating in an oven at 150°C for five minutes. Solutions containing 0.25% w/v amoxycillin trihydrate in potassium buffer (pH 7.0) were prepared from each of the test samples and standard. One microlitre (1 µl) each of these solutions was applied separately on to the chromato-plate.

The plate was transferred into a chromatotank containing a mixture of 4 volume phosphate buffer (pH 6.0) and 1 volume acetone as mobile phase.

After removal, the chromatoplate was allowed to dry in air. It was then sprayed with 0.125M sodium hydroxide and again allowed to dry. The plate was then sprayed with a mixture containing 100 volumes of starch mucilage, 6 volumes of glacial acetic acid, and 2 volumes of a 1% w/v solution of iodine in 4% w/v of potassium iodide (BP 1988).

The plate was then observed. The principal spots for the reference standard and test samples were then compared in terms of their Rf values.

2.2.3 Assay Methods

The samples were analyzed using both microbiological and spectrophotometric (Pharmacopoeia and Non-pharmacopoeia) methods. This is informed by the fact that there has not been any unanimous agreement between biologists and chemists as to which assay method is best and most reliable for analysis of antibiotics (Hugo and Russel, 1977; Hewitt, 1977). The British Pharmacopoeia relies on chemical/spectrophotometric assay whilst the United States Pharmacopoeia makes no mention of chemical analysis but rather advocates bioassay procedure.

The percentage potency of the reconstituted samples at various storage temperatures was determined at storage periods of 1, 8, 15 and 22 days. These storage periods covers the 7 days shelf life of reconstituted samples as

recommended by the British Pharmacopoeia and most product leaflet inserts, as well as satisfying the 14 days shelf life as recommended by the American Society of Hospital Pharmacist (AHFS Drug Information 90, 1990).

2.2.3.1 Microbiological Assay

This is the assay method recognized by the United States Pharmacopoeia as being the most reliable. The fundamental principle of this method of assay irrespective of design is basically to compare the antibacterial activity of the test sample against that of an established standard.

(a) **Selection of Indicator Organism**

There is no specific micro-organism selected for use in this method. Instead, the official monographs (USP 1980 and BP 1988) recommend that any organism susceptible to the agent and showing good growth characteristics may be used.

Three organisms: *Bacillus megatarium, Ps. aeruginosa* and *Staph. aureus* (clinical isolates obtained from the Department of Microbiology, A.B.U., Zaria), were tested against the standard amoxycillin trihydrate using the cup plate method. Of these organisms, *Bacillus megatarium* was found to be the most sensitive. It showed good growth pattern (Fig. 1) and was susceptible to the standard amoxycillin trihydrate

producing measurable zones of inhibition at concentration of 10 µg|ml.

Concentrations of 10, 20, 30, 40 and 50 µg/ml of the standard amoxycillin sample tested against the chosen organism gave results enhancing its claim of suitability (Table 3).

(b) **Inoculum Standardization**

In order that the conditions of experiment be strictly comparable, the number of organisms in an inoculum must as much as possible, be equal.

For standardization, an overnight culture of the indicator organism was spun down in a centrifuge at 80 rpm and washed with warm sterile nutrient broth. It was then resuspended in fresh warm sterile broth and the process repeated. 1 ml of the subsequent resuspended indicator organism was then introduced into 29 ml of sterile nutrient broth in a 100 ml conical flask. The optical density (OD) of this culture suspension was taken at a wavelength of 470 nm (OD_{470}). This culture was then placed into a thermostatically controlled shaker Water-bath at 37°C and at 80 throws per minute. The optical density reading (OD_{470}) was then taken at 30 minutes interval with appropriate dilution when necessary. At each OD_{470} reading, the appropriately diluted culture was plated out on nutrient agar. The inoculated plated were incubated at 37°C for 18 hours and the number of

colonies that developed counted. A graph of optical density (OD_{470}) against viable count (cfu/ml) was plotted. The OD_{470} of culture containing one million cells per millilitre was obtained by extrapolation from the graph.

Having standardized the culture, each experiment was carried out by concentrating the overnight culture, so that addition of 1 ml culture into 199 ml of molten Nutrient agar will produce a count of 10^6 cfu/ml of the agar.

(c) Preparation of Standard and Test Sample Doses of Amoxycillin

i. Standard Sample Doses

Doses of standard sample were prepared on the day of experiment by weighing 0.115 g of the standard amoxycillin trihydrate (equivalent to 0.1 g amoxycillin), dissolved in some water and made up to 1000 ml with distiled water. This gave a solution of 100 µg/ml. From this stock solution, three concentrations: 40, 20 and 10 µg/ml were made and used.

ii. Test Sample Doses

From the reconstituted samples, 40, 20 and 10 µg/ml solutions were prepared as with the standard.

(d) **Assay Procedure**

The agar diffusion method was employed, using the 6 x 6 (3 + 3) dose level latin square design on large assay plate (Table 1) (USP 1980).

Two hundred millilitres (200 ml) of sterile agar, prepared in accordance with manufacturers' instruction was poured into a sterile plate and allowed to set, forming a basal layer. 1 ml of molten agar was removed from a second 200 ml of sterile agar and replaced with 1 ml inoculum of the test organism (OD_{470} of 0.09). The seeded layer was poured on top of the basal layer aseptically and was allowed to set.

Thirty six (36) cups were bored in the agar in a randomized manner referred to as the latin square design. Twenty five microlitres (250 µl) of each concentrations (10, 20 and 40 µg/ml) of both the standard and test samples were introduced separately into the cups as shown in Table 1. The plates were left undisturbed for two hours at room temperature to allow for effective diffusion of the antibiotic. The plates were then incubated at 37°C for 18 hours.

Zones of inhibition produced by both the standard and test concentrations, holes inclusive, were measured to the nearest millilitre using a pair of calipers and ruler. The procedure was carried out for samples stored at 4°C (refrigerated), 25°C (room temperature) and 35°C.

Table 1: Representation of the 6 x 6 (3 + 3) Three Doses Level Latin Square Design

S_1	S_2	S_3	T_1	T_2	T_3
S_2	S_3	T_1	T_2	T_3	S 1
S_3	T_1	T2	T_3	S_1	S_2
T_1	T2	T_3	S_1	S_2	S_3
T_2	T_3	S_1	S_2	S_3	T_1
T_3	S_1	S_2	S_3	T_1	T_2

KEY: S represent reference standard

T represent test sample

T_1 and S_1 = 10 µg/ml

T_2 and S_2 = 20 µg/ml

T_3 and S_3 = 40 µg/ml

The test was repeated on day 8, 15 and 22 for the same set of samples. The test was repeated twice for all the brands to ascertain reproducibility.

2.2.3.2 Spectrophotometric Assay Procedure

Two methods of analysis, one official and the other unofficial but proven (Bungaard and Larsen, 1983), were used in the analysis of the samples.

The procedures were carried out on the same days of sampling as for microbiological method.

(a) **Pharmacopoeia Method (BP, 1988)**

0.17 g of standard reference amoxycillin trihydrate equivalent to 0.15 g of amoxycillin base was dissolved in about 200 ml of distiled, sufficient water was then added to make 500 ml. This solution was shaken for 30 minutes using an electric flask shaker and filtered through a sintered glass funnel number 0 under pressure. 10 ml of the resulting solution was transferred to a 100 ml volumetric flask and 10 ml of boric buffer pH 9.0 was added. 1 ml of acetic anhydride-dioxan solution was also added and the whole content was allowed to stand for 5 minutes. Sufficient water was added to make 100 ml.

Two millilitres (2 ml) aliquots of this solution were pipetted into two separate tubes A and B. To tube A, 10 ml of imidazole-mercury reagent was added, thoroughly mixed, stoppered and immersed in a water-bath at 60°C for 20 minutes with occasional swirling. The tube was then removed from the water-bath and cooled rapidly in ice to 20°C. 10 ml of water was added to tube B and mixed. The absorbance of both solutions were measured without delay at 325 nm, using a mixture of 10 ml imidazole-mercury reagent and 2 ml water as blank for solution A and water for solution B.

All the marketed samples had a label strength of 125 mg/5 ml on reconstitution except batch II of brand B comes in a strength of 250 mg/5 ml. Six millilitres

(6 ml) of the reconstituted test samples (3 ml in case of batch 2 of brand B) containing 0.15 g of amoxycillin was transferred to a 500 ml volumetric flask and sufficient water added. Each was shaken for 30 minutes and filtered in the same way as the reference standard. The resultant solutions were treated like the reference standard.

The amoxycillin content was calculated from the difference between the absorbance of solution A and that of B for both the standard and the test samples, the potency of reference standard being taken as 100%.

(b) **Non-Pharmacopoeia Method**

0.459 g of the reference standard amoxycillin trihydrate equivalent to 0.4 g amoxycillin base was dissolved in abut 300 ml of distiled water,and sufficient water later added to make 500 ml giving a strength of 0.8 mg/ml. This was shaken electrically for 30 minutes and filtered through number 0 sintered glass funnel under pressure. To 500 µl aliquots of the solution were pipetted into separate tubes A and B.

To tube A, was added 5 ml of sorbitol reagent, stoppered and immersed in a water-bath at 60°C for 10 minutes. 500 µl of the solution was pipetted into a curvette containing 2 ml of 1M sodium hydroxide. The absorbance was monitored at 322 nm using a recorder in series, until maximum absorbance was reached within 1-

3 minutes. A mixture of 3 ml water and 12 ml 1M sodium hydroxide was used as reference solution.

Content of tube B was mixed with 5 ml of water and treated with sodium hydroxide as with content of tube A. 3.2 ml of the reconstituted suspension of all the brands, were introduced into 100 ml graduated flasks except batch II of brand B in which the same quantity was measured but introduced into a 200 ml flask. Sufficient water was added to each flask to the calculated mark to give a strength of 0.8 mg/ml. Each flask was shaken electrically for 30 minutes and filtered. The resultant solutions were treated as for the reference standard. The amoxycillin content was similarly determined as described for the Pharmacopoeia method.

CHAPTER THREE

3.0 RESULTS

3.1 IDENTIFICATION

3.1.1 Infra-red (IR) Spectra

The Infra-red spectra of the test samples and standard are shown in Figs. 1 and 2.

3.1.2 Thin Layer Chromatography (TLC)

The Thin Layer Chromatogram of the various test and reference samples are depicted in Fig. 3.

The Rf values of the chromatogram which were calculated using the formula below and are as shown in Table 2 indicates that both the test samples and the reference standard had virtually the same values.

$$Rf = \frac{\text{Distance moved by Sample}}{\text{Distance moved by solvent}}$$

3.2 INDICATOR ORGANISM

Bacillus megaterium was found to be suitable. It grew well, reaching an optical density of 8.3 before going into the stationary phase (Fig. 4). It gave a good dose-response to the standard amoxycillin (Table 3) and a straight line graph when log dose vs mean zone diameter was plotted (Fig. 5).

Table 2: Rf Values of Various Test and Reference Samples of Amoxycillin Oral Suspensions obtained from TLC Analysis

Sample	Distance Moved (cm)		Rf Value
	Sample	Solvent	
A	8.80	16.50	0.533
B	8.90	16.50	0.539
C	8.60	16.20	0.531
D	8.70	16.20	0.537
E	8.60	16.20	0.531
F	8.70	16.20	0.537
S	8.60	16.20	0.531

S represents the reference standard.

Extrapolation of the plot of optical density (OD) against cell count (cfu/ml) showed that a broth culture of optical density of 0.09 contained 10^6 cfu/ml (Fig. 6). This was used as standard inoculum size.

Table 3: Response of *B. megatarium* to Reference Amoxycillin

Concentration of Agent in Reservoir (µg/ml)	Log Concentration of Agent in Reservoir	Zone of Inhibition (Cup Inclusive (mm) (X)	Distance from Agar Interface to Boundary (Y)	y^2
10	1.00	20.0	10.0	100
20	1.30	22.0	12.0	144
30	1.47	23.0	13.0	169
40	1.67	24.0	14.0	196
50	1.69	25.0	15.0	225

Reservoir diameter = 10.0 mm

Plot of log concentration against zone of inhibition response as shown in Fig. 5 gave a straight line which indicates that the response of the organism to amoxycillin is dose-dependent.

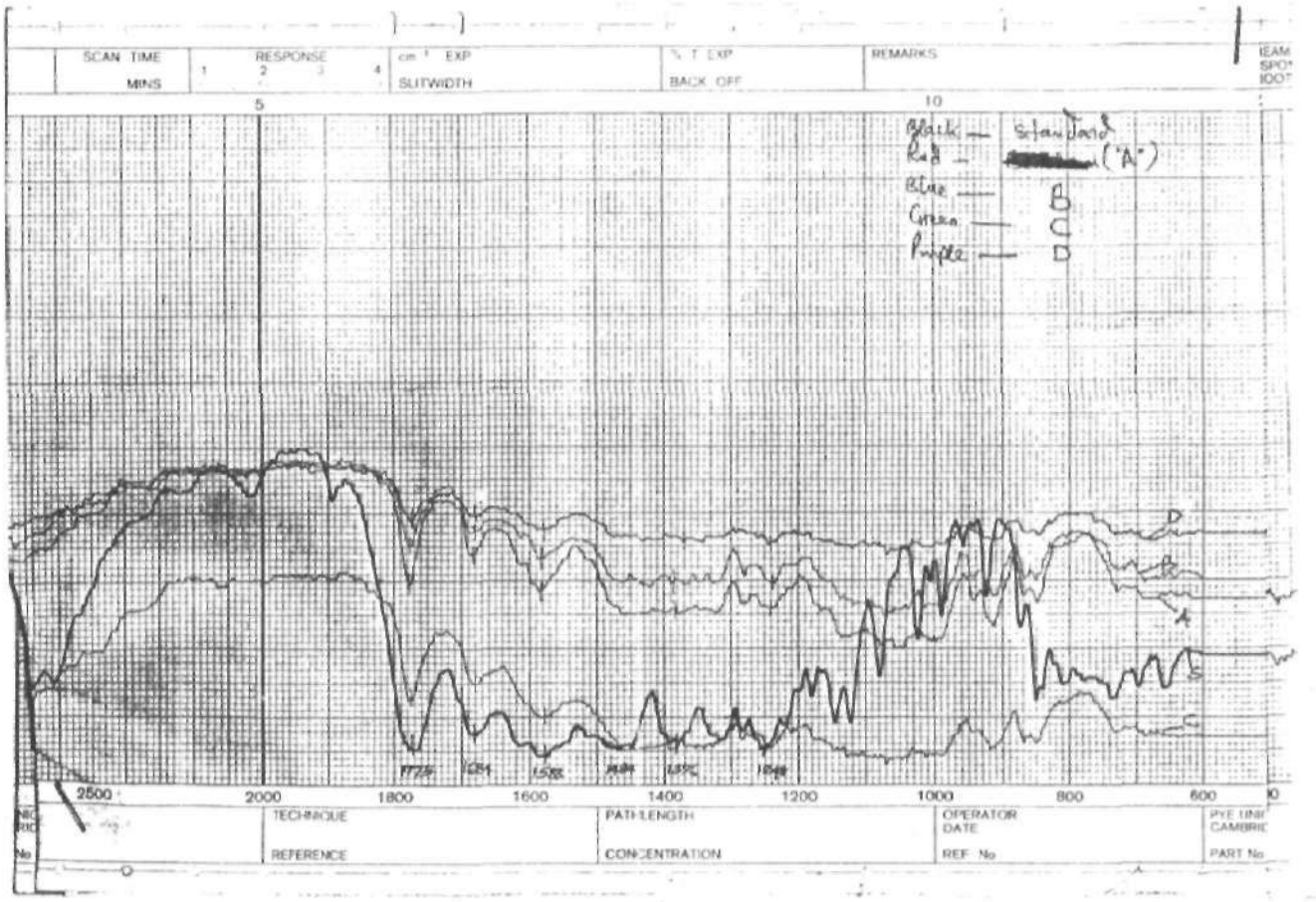


Fig. 1: Infra-red Spectra of Reference Amoxycillin and some Test Samples of Oral Amoxycillin Suspensions (A-D)

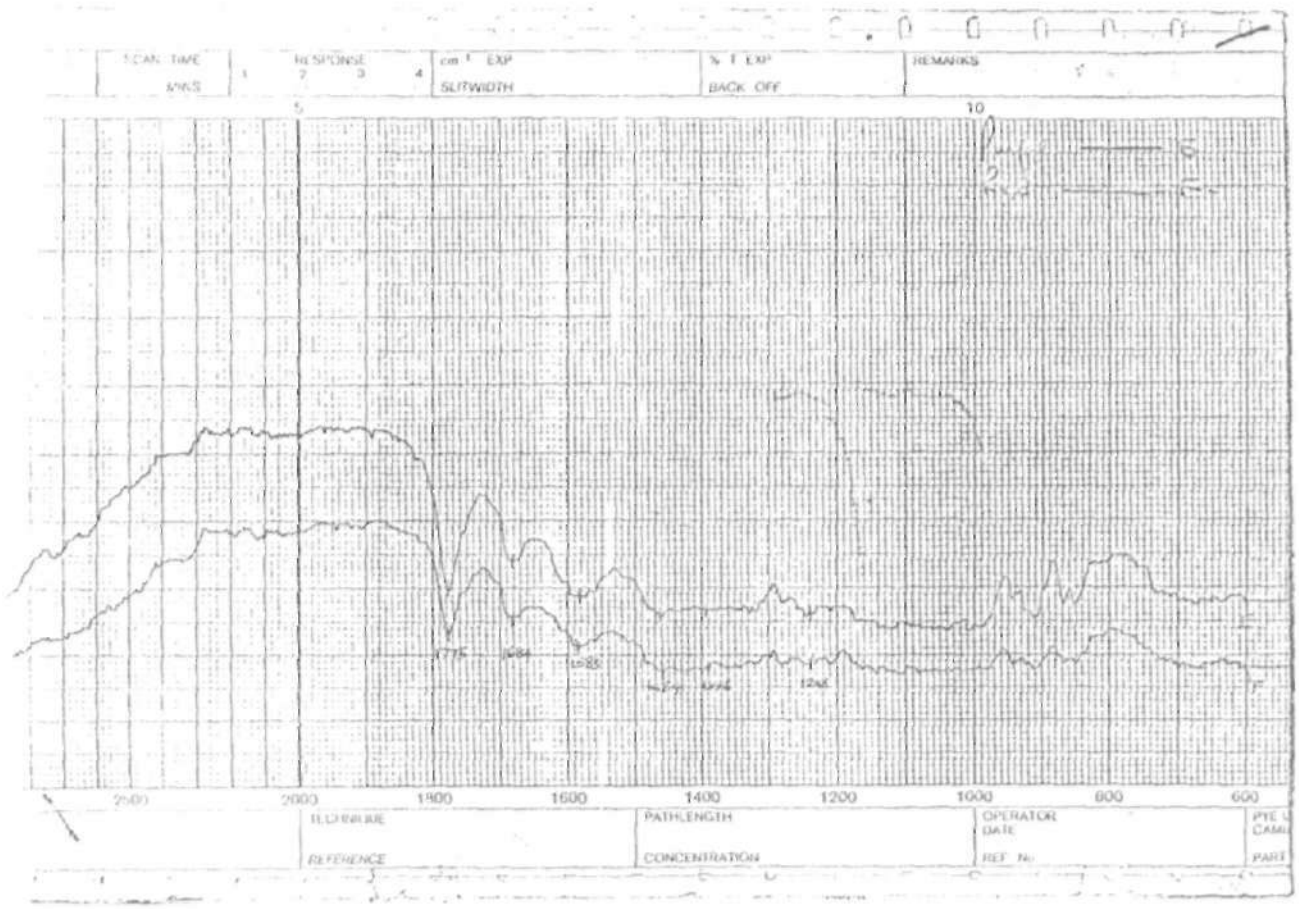


Fig. 2: Infra-red Spectra of some Test Samples of Oral Amoxycillin Suspensions (E-F)

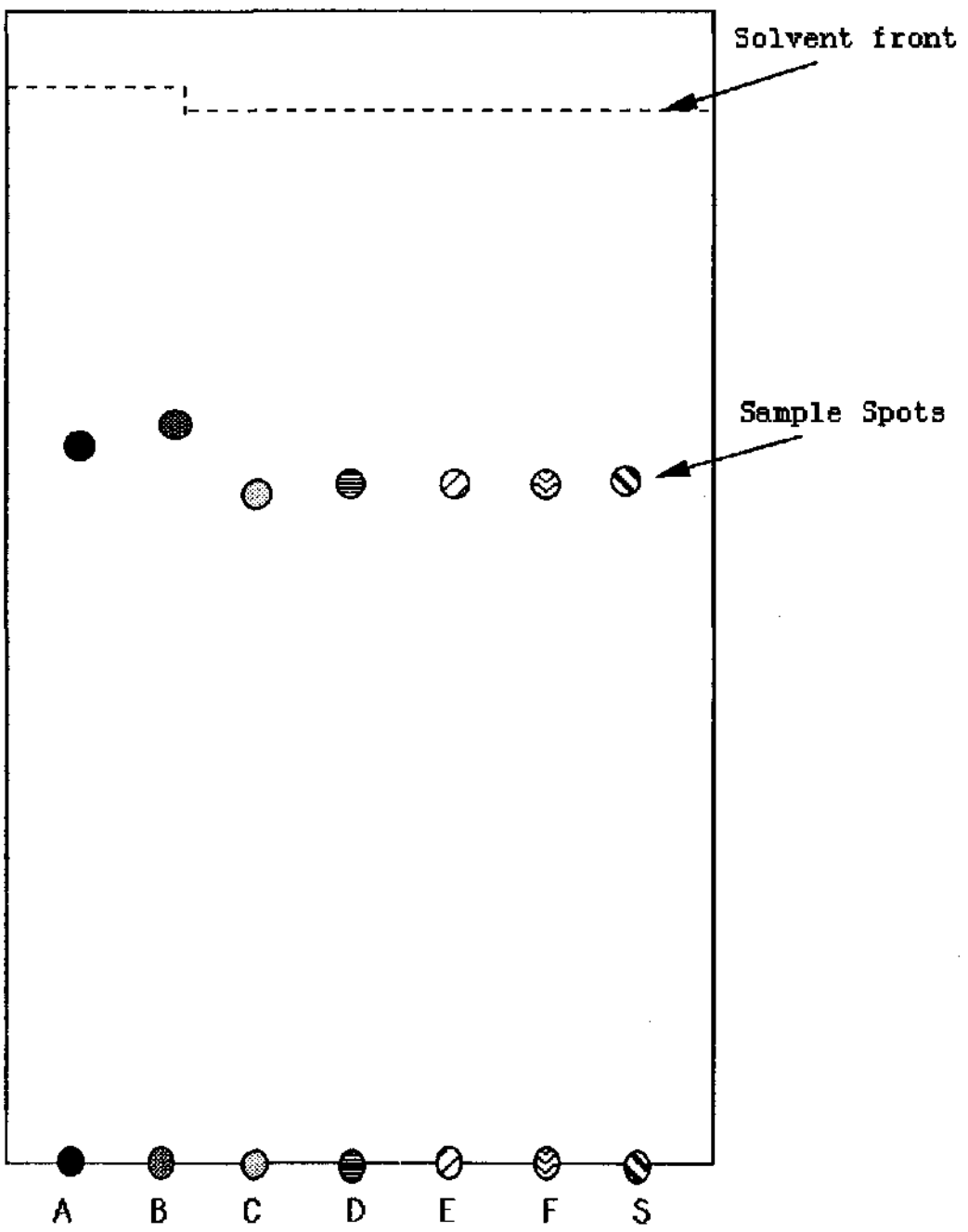


Fig. 3: Representation of Thin Layer Chromatgram of Test and Reference Samples

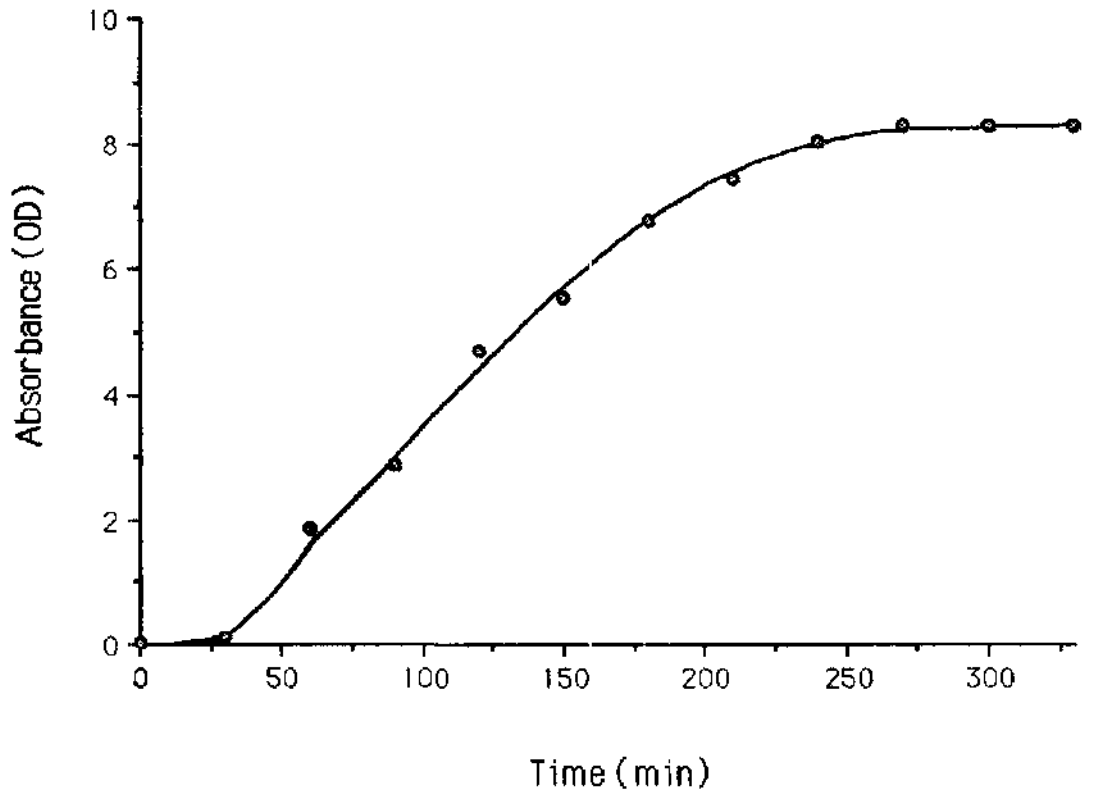


Fig. 4: Growth Curve of *Bacillus megaterium* in Nutrient Broth based on Optical density Measurement

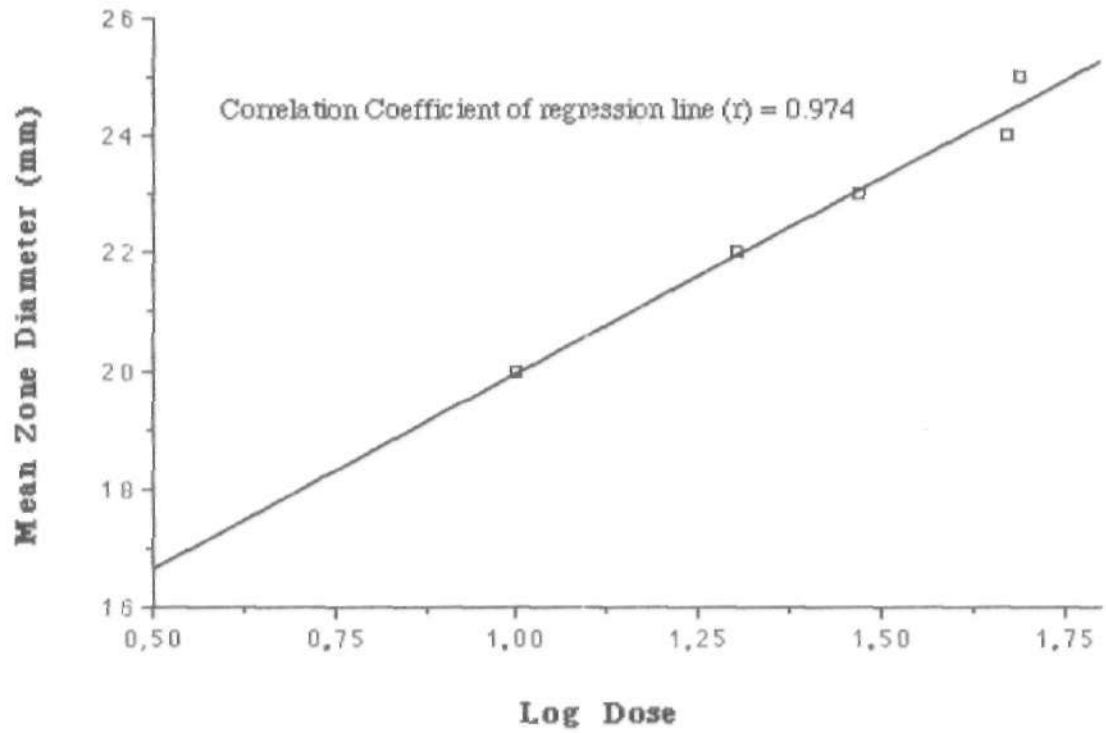


Fig. 5: Log of Concentration of Amoxycillin against Diameter of Zone of Inhibition elicited against B. Megatarium

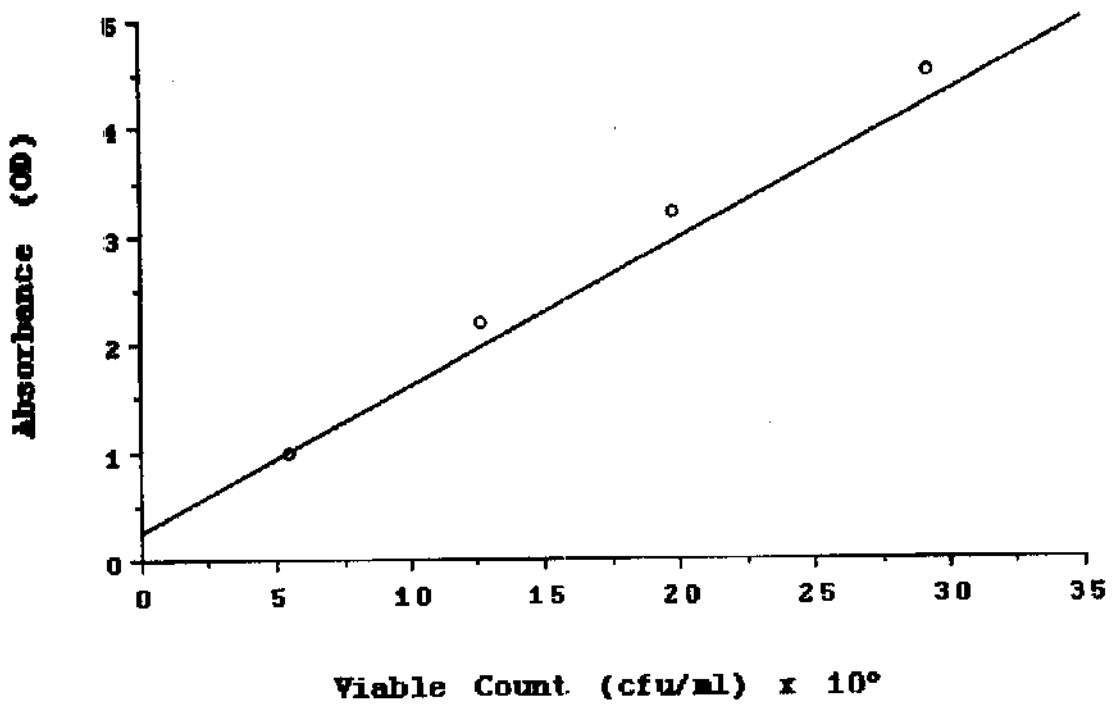


Fig. 6: Optical density of Broth Culture of Bacillus Megatarium against Cell count

3.3 CALCULATION OF POTENCY

3.3.1 Microbiological Assay

The method recommended by the British Pharmacopoeia was used to compute the potency of samples from the observed zones of inhibition as shown below.

Percentage Potency = antilog M x 100

where M = log potency ratio $(\frac{\text{test}}{\text{standard}}) = \frac{F}{b}$, and

F = mean difference between standard and test doses

$= \frac{1}{3}[(T_1 + T_2 + T_3) - (S_1 + S_2 + S_3)]$, and

b = slope = $\frac{E}{I}$

where:

E = mean difference due to the highest and lowest doses

$= \frac{1}{4}[(T_3 - T_1) + (S_3 - S_1)]$, and

I = log ratio of doses

= log 2

Reference Standard (S)

S_1 = 10 µg/ml; S_2 = 20 µg/ml; S_3 = 40 µg/ml

Test Sample (T)

T_1 = 10 µg/ml; T_2 = 20 µg/ml; T_3 = 40 µg/ml

Employing the formula above, the percentage potencies of the various test samples were calculated as shown below in which the mean values of the zones of

inhibition elicited by the various concentrations of a test sample (Sample A, batch I) and reference amoxycillin, depicted in Table 4, were used as an example.

Table 4: Mean Zone Inhibition (mm) of *B. megaterium* Obtained at Various Concentrations of a Test and Standard Amoxycillin Samples

MEAN ZONE OF INHIBITION (mm)					
T_1	T_2	T_3	S_1	S_2	S_3
19.33 ± 0.07	22.00 ± 0.05	24.17 ± 0.06	19.33 ± 0.04	21.53 ± 0.03	23.88 ± 0.04

Calculation:

$$E = \frac{1}{4}[(T_3 - T_1) + (S_3 - S_1)]$$

$$= \frac{1}{4}[(24.17 - 19.33) + (23.88 - 19.33)]$$

$$= \frac{1}{4}[4.84 + 4.55] = \frac{1}{4}[9.39]$$

$$= 2.35$$

$$F = \frac{1}{3}[(T_1 + T_2 + T_3) - (S_1 + S_2 + S_3)]$$

$$= \frac{1}{3}[(19.33 + 22.00 + 24.17) - (19.33 + 21.53 + 23.88)]$$

$$= \frac{1}{3}[65.50 - 64.79] = \frac{1}{3}[0.71]$$

$$= 0.024$$

$I = \log 2 = 0.3010$

$b = \frac{E}{I} = \frac{2.35}{0.3010}$

$= 7.81$

$M = \log \text{ of potency ratio } (\frac{T}{S}) = \frac{P}{b}$

$= \frac{0.024}{7.81}$

$= 0.03$

Percentage potency of sample = antilog 0.03 x 100

= 107.33%

This means that the test sample contains 107.33% (i.e, 134.16 mg/5 ml) of the active constituent (amoxycillin) instead of the manufacturer's stated concentration of 125 mg/5 ml. This percentage potency is based on the fact that the reference standard which is pure, is assigned a percentage content of 100%. This falls within the specification of official monographs such as the British Pharmacopoeia (1988) and the United States Pharmacopoeia (1980) recommendation of 90-110%.

3.3.2 Spectrophotometric Analysis

The method of computation is as recommended by the BP 1988. This formula was employed both for the Pharmacopoeia and Non-Pharmacopoeia methods of analysis, as shown using readings taken for samples of batch I of Companies A and B (Tables 5a and 5b).

Table 5: Absorbance Readings of Some Amoxycillin Samples Analyzed by Pharmacopoeia and Non-Pharmacopoeia Methods

(a) Pharmacopoeia Method

SAMPLE	ABSORBANCE	
	Solution 'A'	Solution 'B'
A	0.127	0.011
B	0.114	0.006
S	0.110	0.004

(b) Non-Pharmacopoeia Method

SAMPLE	ABSORBANCE	
	Solution 'A'	Solution 'B'
A	1.250	0.031
B	1.200	0.060
S	1.127	0.018

S = standard amoxycillin

3.3.2.1 Pharmacopoeia Method

Difference in absorbance between solution 'a' and 'b' for standard S = 0.110 − 0.004

= 0.106

Difference in absorbance between solution 'a' and 'b' for brand A = 0.127 − 0.011

= 0.116

Percentage potency content $= \frac{0.116}{0.106} \times 100$

= 109.43%

Difference in absorbance between solution 'a' and 'b' for brand B = 0.114 − 0.006

= 0.108

Percentage potency content $= \frac{0.108}{0.106} \times 100$

= 101.89%

3.3.2.2 Non-Pharmacopoeia Method

Difference in absorbance between solution 'a' and 'b' for standard S = 1.127.0.018

= 1.109

Difference in absorbance between solution 'a' and 'b' for standard S = 1.200 − 0.031

= 1.219

Percentage potency content $= \frac{1.219}{1.109} \times 100$

= 109.92%

Difference in absorbance between solution 'a' and 'b' for Sample B = 1.200 - 0.060

= 1.140

Percentage amoxycillin content $= \frac{1.14}{1.09} \times 100$

= 102.79%

Using the above formula, the percentage potency of the various test samples were obtained and is shown in Tables 6a, 6b and 6c.

Table 6: Percentage Amoxycillin Content of Marketed Amoxycillin Oral Suspensions as Computed for the Three Analytical Methods.

(a) Batch I

Brand	Pharmacopoeial Spectrophotometric Method	Non-Pharmacopoeial Spectrophotometric Method	Microbiological Assay
A	109.43 ± 1.10	109.92 ± 0.98	107.33 ± 1.43
B	101.89 ± 1.20	102.79 ± 1.24	102.69 ± 1.51
C	115.09 ± 1.30	113.98 ± 1.18	113.68 ± 1.54
D	109.43 ± 1.30	108.93 ± 1.65	109.39 ± 1.29
E	100.94 ± 1.35	100.99 ± 1.58	100.53 ± 1.60
F	97.17 ± 1.15	96.93 ± 1.13	95.24 ± 1.34

(b) Batch II

Brand	Pharmacopoeial Spectrophoto-metric Method	Non-Pharma-copoeial Spectrophoto-metric Method	Microbio-logical Assay
A	107.23 ± 1.30	108.21 ± 1.38	110.63 ± 1.24
B	107.23 ± 1.38	109.40 ± 1.21	108.31 ± 1.28
C	108.78 ± 1.23	110.40 ± 1.21	110.27 ± 1.33
D	124.82 ± 1.50	121.92 ± 1.55	121.56 ± 1.56
E	106.57 ± 1.41	107.99 ± 1.53	110.27 ± 1.61
F	105.11 ± 1.20	104.98 ± 1.46	104.25 ± 1.31

(b) Batch III

Brand	Pharmacopoeial Spectrophoto-metric Method	Non-Pharma-copoeial Spectrophoto-metric Method	Microbio-logical Assay
A	110.48 ± 1.13	111.18 ± 1.34	109.90 ± 0.93
B	109.52 ± 1.22	106.94 ± 1.28	109.90 ± 1.12
D	113.33 ± 1.25	111.99 ± 1.55	111.91 ± 1.58

3.4 Stability Of Test Samples of Amoxycillin Oral Suspensions

The results of mean percentage amoxycillin content in the test samples per week of each batch at the stated storage temperatures on day 1, 8, 15 and 22 is as shown graphically in Figs. 7a, 7b and 7c using the stated methods of analysis. The mean percentage potency loss per week or rate of degradation per week is also illustrated in Tables 7a, 7b and 7c (see Appendix).

3.5 RESULT OF THE STATISTICAL ANALYSIS

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3.5.1 Inter-Brand Variation

To compare the differences between samples from different companies, student paired t-test statistical analysis of mean content of amoxycillin in the samples (Table 9) was done. The result showed that out of a possible 15 paired combinations, there were no statistical difference in 5 paired cases only, namely Samples A vs B, A vs D, A vs E, B vs E and C vs D. All paired combinations involving sample F exhibited significant differences in their mean contents. The same was true for combinations involving samples of C except in paired combination with D.

3.5.2 Inter-Batch Variation

Examination of the summary of the date obtained for the difference batches of the various samples showed

some marked differences in their contents of amoxycillin. Only Sample A showed minimal variation in content among the three batches tested. The statistical F-ratio and t-tests as depicted in Table 8, also indicated that differences in batches of sample A was not significant at p' = 0.05 and df = 5 compared with other brands that showed varying degrees of differences. However, in some cases (Sample C, batch I and II), while the t-test indicated significant difference, the F-ratio showed no significance.

It is interesting to note that the product from Company A is generally not only of high quality, but also exhibited the minimum content variation. This is not very surprising because Company A is a highly reputable foreign-based company and with an affiliate marketing outlet in Nigeria. Companies B and C are also foreign based companies with no marketing outlet in Nigeria. Similarly, products from Companies E and F which are locally based pharmaceutical manufacturers with Asian connection, conform to official specifications, while Company D with similar background to E and F, exhibited pronounced variation in mean amoxycillin content among its batches as measured by student t-test and F-ratio analysis.

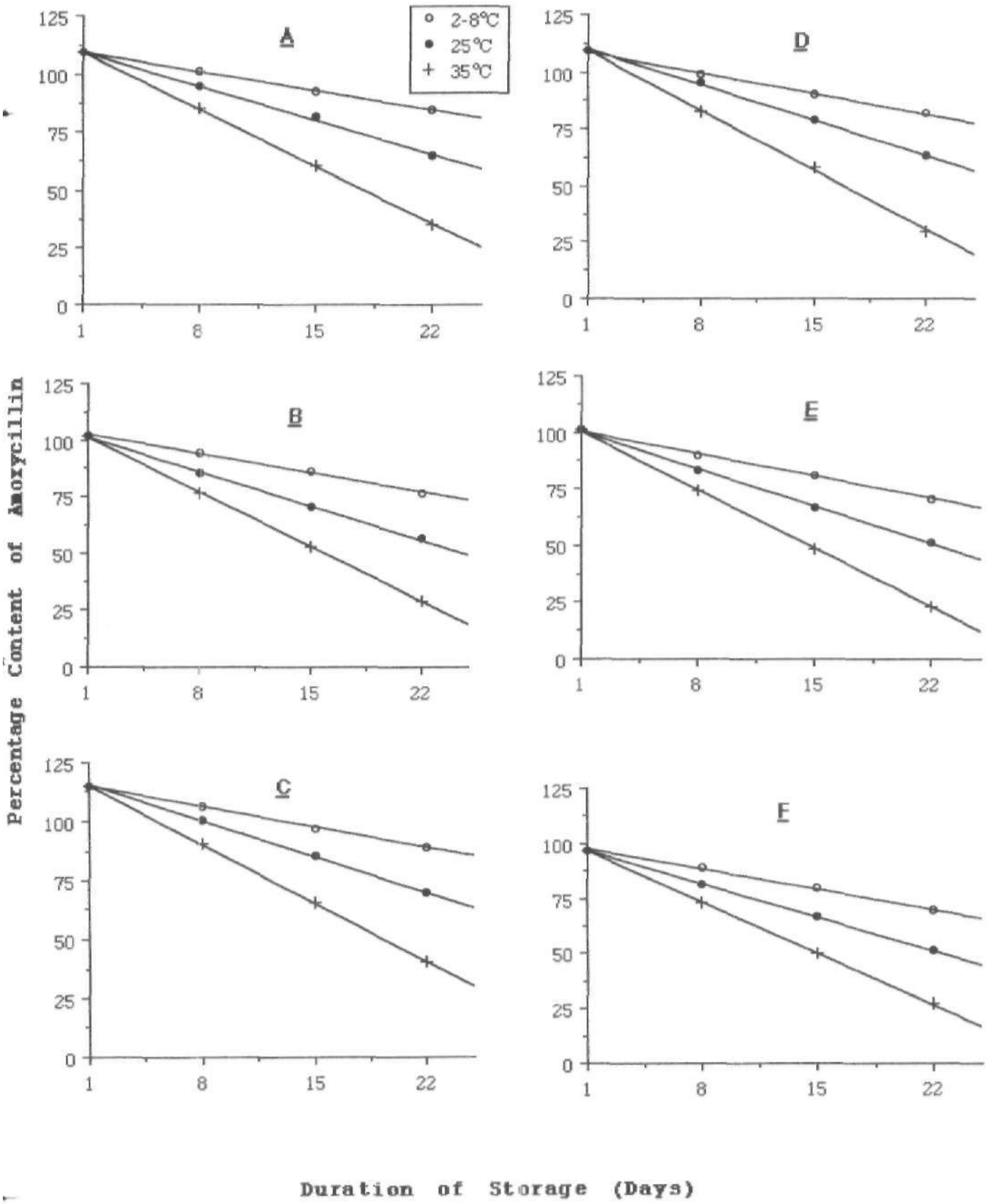


Fig. 7a: Percentage Content of Amoxycillin in reconstituted Batch I Test Suspensions at Different Storage Periods and Temperatures Analyzed by Pharmacopoiea Spectrophotometric Method

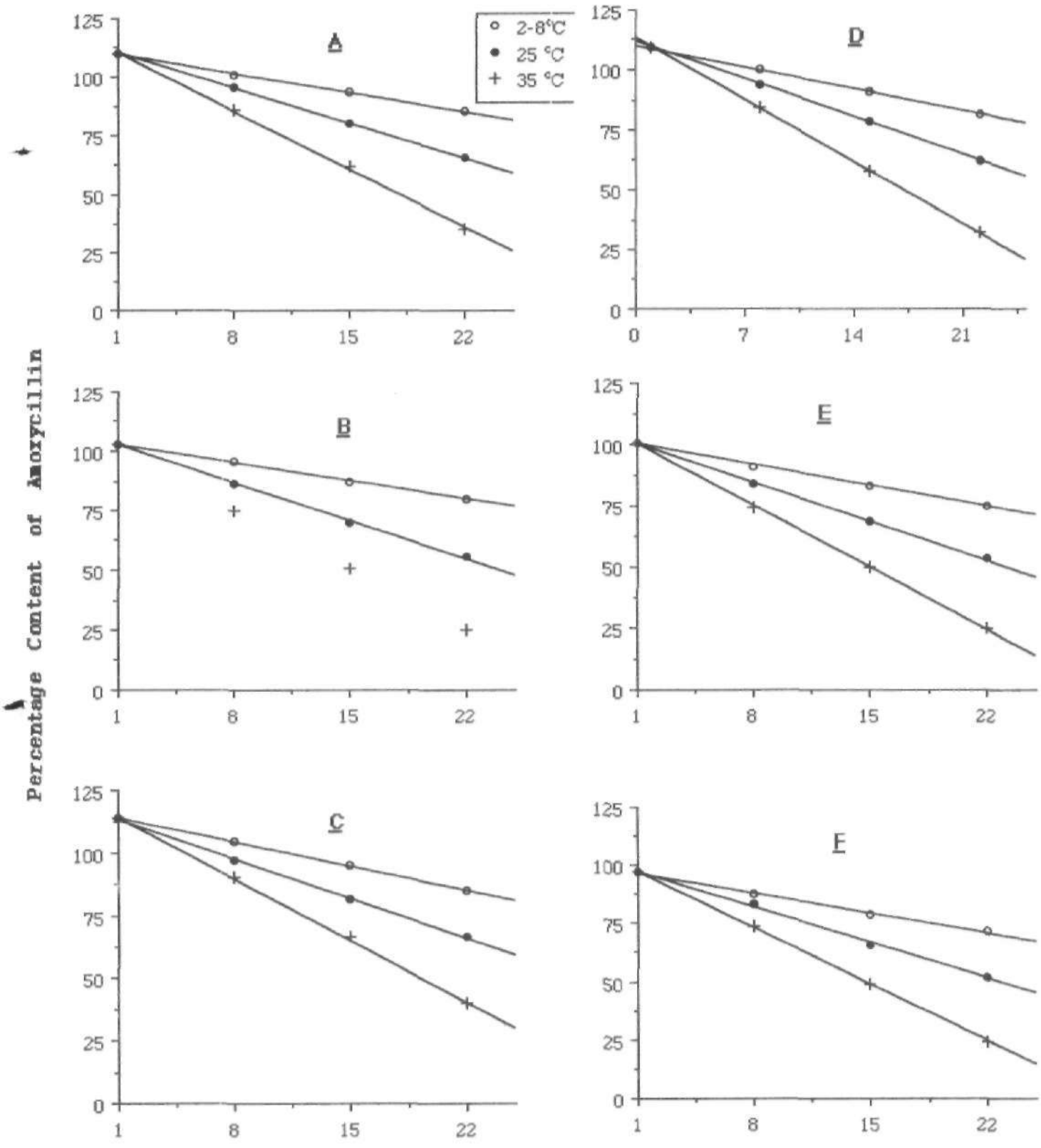


Fig. 7: Percentage Content of Amoxycillin in reconstituted Batch I Test Suspensions at Different Storage Periods and Temperatures Analyzed by Non-Pharmacopoiea Spectrophotometric Method

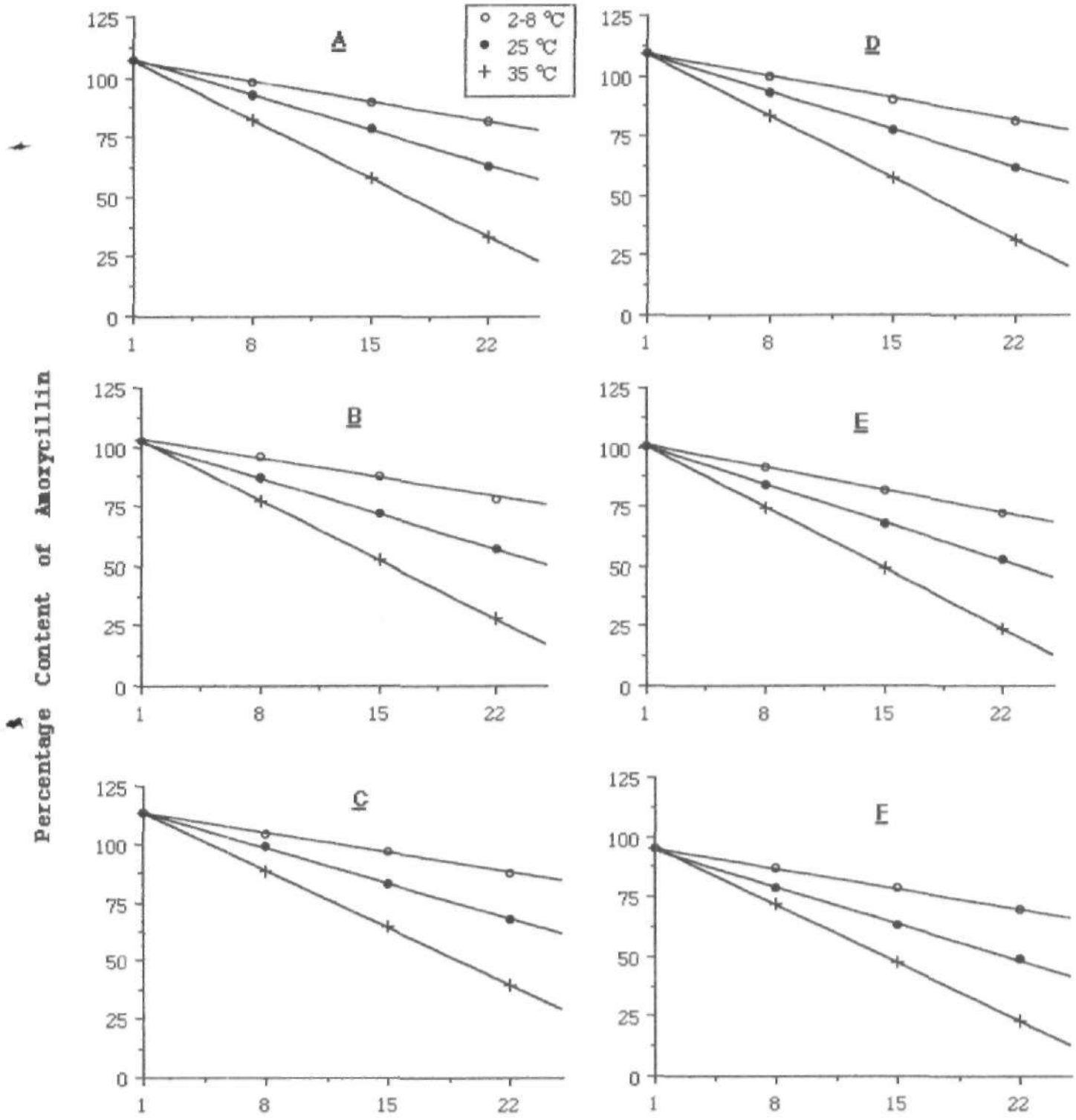


Fig. 7c: Percentage Content of Amoxycillin in reconstituted Batch I Test Suspensions at Different Storage Periods and Temperatures Analyzed by Microbiological Method

Table 7: Mean Percebtage Loss of Amoxycillin Per Week in the Various Test Samples at Different Storage Temperatures

(a) Pharmacopoeial (Spectrophotometric) Method

BATCHES/ Storage Temper-atures	Mean Percentage Loss Per Week					
	A	B	C	D	E	F
BATCH I						
2–8°C	8.32 ± 0.84	8.40 ± 1.10	8.59 ± 0.78	9.35 ±1.109	10.02 ±0.48	9.09 ± 1.24
25°C	14.79 ±1.77	15.19 ± 0.98	15.06 ± 0.66	15.44 ±1.04	16.52 ± 0.78	15.24 ± 0.70
35°C	24.83 ±1.06	24.25 ± 0.69	24.77 ± 0.14	26.45 ±1.81	25.88 ±1.01	23.60 ±1.13
BATCH II						
2–8°C	7.91 ± 2.20	7.59 ± 0.84	8.43 ± 0.56	9.54 ± 0.44	9.63 ± 0.93	8.17 ± 1.29
25°C	14.71 ±1.43	15.05 ± 0.65	15.87 ± 0.53	15.71 ±1.47	15.29 ±1.28	14.87 ± 0.39
35°C	24.09 ± 0.38	25.06 ± 0.39	24.61 ±1.69	25.43 ±1.60	25.49 ± 2.27	23.71 ± 0.76
BATCH III						
2–8°	7.77 ± 0.86	7.76 ± 0.16	-	9.04 ± 1.31	-	-
25°C	14.81 ± 0.42	14.81 ± 0.42	-	15.76 ±0.63	-	-
35°C	23.37 ± 1.67	24.89 ± 0.45	-	25.24 ± 1.48	-	-

(b) Non-Pharmacopoeial (Spectrophotometric) Method

BATCHES/ Storage Temper-atures	Mean Percentage Loss Per Week					
	A	B	C	D	E	F
BATCH I						
2–8°C	8.18 ± 1.16	7.63 ± 0.74	9.64 ± 0.33	9.12 ± 0.10	8.73 ± 1.20	8.54 ± 0.79
25°C	14.74 ± 0.36	15.81 ± 0.80	15.85 ± 0.83	15.58 ± 0.89	15.89 ± 0.67	14.96 ± 1.65
35°C	24.61 ± 1.09	25.93 ± 1.66	24.65 ± 1.44	25.64 ± 1.03	25.33 ± 1.01	24.16 ± 0.96
BATCH II						
2–8°C	8.51 ± 0.99	8.00 ± 0.35	9.16 ± 0.55	9.60 ± 0.32	9.33 ± 1.01	8.06 ± 0.82
25°C	15.28 ± 1.30	16.24 ± 1.39	16.11 ± 1.25	16.83 ± 1.45	16.50 ± 0.93	15.13 ± 0.36
35°C	23.70 ± 0.25	24.76 ± 1.02	24.22 ± 0.62	24.52 ± 0.82	24.87 ± 1.81	24.47 ± 0.47
BATCH III						
2–8°	8.24 ± 0.80	8.68 ± 0.53	-	9.17 ± 0.24	-	-
25°C	14.44 ± 0.61	15.77 ± 0.15	-	16.72 ± 1.60	-	-
35°C	24.26 ± 0.52	25.34 ± 0.38	-	26.13 ± 0.57	-	-

(c) Microbiological Assay Method

BATCHES/ Storage Temper-atures	Mean Percentage Loss Per Week					
	A	B	C	D	E	F
BATCH I						
2–8°C	8.51 ± 0.64	8.15 ± 1.24	8.64 ± 0.47	9.36 ± 0.38	9.50 ± 0.72	8.51 ± 0.57
25°C	14.64 ± 0.49	15.11 ± 0.77	15.19 ± 0.46	15.85 ± 0.35	15.91 ± 0.92	15.41 ± 0.90
35°C	24.56 ± 0.10	24.93 ± 0.55	24.80 ± 1.30	25.99 ± 0.21	25.59 ± 0.45	24.18 ± 1.03
BATCH II						
2–8°C	8.16 ± 1.58	7.96 ± 0.93	8.58 ± 0.58	9.60 ± 0.49	9.22 ± 0.60	7.89 ± 0.62
25°C	15.31 ± 1.04	16.20 ± 1.55	16.26 ± 1.57	16.48 ± 0.73	17.09 ± 1.56	14.92 ± 0.63
35°C	24.31 ± 1.29	24.50 ± 0.93	24.21 ± 1.57	25.91 ± 0.86	25.79 ± 0.68	24.26 ± 0.89
BATCH III						
2–8°	7.95 ± 0.90	8.30 ± 0.96	-	8.53 ± 0.60	-	-
25°C	15.13 ± 0.78	15.55 ± 0.46	-	16.80 ± 0.66	-	-
35°C	23.89 ± 0.30	24.75 ± 0.35	-	25.30 ± 0.64	-	-

Table 8: Statistical Analysis of Various Batches of Marketed Brands of Amoxycillin Oral Suspensions

Statistical Test/ Brand	Batch I vs Batch II	Batch I vs Batch III	Batch II vs Batch III
T-Test			
A	0.116	2.421	1.427
B	15.203*	5.772*	0.332
C	4.269*	–	–
D	13.991*	7.871*	18.093*
E	6.172*	–	–
F	24.523*	–	–
F-Ratio			
A	1.618	4.602	7.452
B	4.844	10.675	2.204
C	1.987	–	–
D	41.493*	259.234*	6.248
E	13.737	–	–
F	5.144	–	–

critical $t_{(p = 0.05, df = 4)} = 2.776$

critical F-ratio$_{(p = 0.05, df = 2)} = 19.00$

* difference is significant.

Table 9: Student Paired t-test Statistical Analysis of Different Brands of Amoxycillin Oral Suspensions

Paired Samples		t-value
Company One	Company Two	
A	B	2.290
A	C	3.183*
A	D	2.247
A	E	2.441
A	F	4.097*
B	C	2.927*
B	D	5.705*
B	E	1.390
B	F	6.529*
C	D	0.964
C	E	2.837*
C	F	4.030*
D	E	7.009*
D	F	12.225*
E	F	5.931*

critical $t_{(p=0.05, df=4)} = 2.776$

* differences are significant

Table 10: Student Paired t-test Analysis of Effect of Storage Temperature on Amoxycillin Content of Reconstituted Oral Suspensions of Batch I Samples

Storage Period	Paired Variables	t-value
7 Days	2-8°C vs 25°C	9.702
	2-8°C vs 35°C	28.211
	25°C vs 25°C	12.837
14 Days	2-8°C vs 25°C	17.335
	2-8°C vs 35°C	38.119
	25°C vs 25°C	18.943

critical $t_{(p = 0.05, df = 5)} = 2.614$

Differences between the various storage temperatures were significant

Table 11: Statistical Analysis of the Three Methods Used in the Analysis of Amoxycillin in Various Test Samples.

Test Parameter	P vs N	P vs M	N vs M
<u>T-Test</u>			
Batch I	0.234	1.807	1.684
II	0.764	0.858	0.514
III	1.128	1.038	0.423
<u>F-Ratio</u>			
Batch I	1.106	1.025	1.079
II	1.567	1.645	1.050
III	1.873	2.915	5.461*

* signifies that the difference is significant.

For batches I and II, df = 10, and 4 for batches III

critical $t_{(p=0.05,\,df=10)} = 2.226$

$t_{(p=0.05,\,df=4)} = 2.776$

$F\text{-ratio}_{(p=0.05,\,df=5,5)} = 5.05$

P = Pharmacopoeia Spectrophotometric Method

N = Non-Pharmacopoeia Spectrophotometric Method

M = Microbiological Assay Method

Table 12: Correlation Coefficients of Data Obtained by the Three Methods Used in the Analysis of Amoxycillin in Various Test Samples.

Test Parameter	P vs N	P vs M	N vs M
Correlation Coefficient Obtained from Regression Plot			
Batch I	0.991	0.971	0.971
II	0.968	0.901	0.928
III	0.631	0.941	0.389*
Pearson Correlation Matrix Analysis			
Batch I	r = 0.984 df=4; t=11.04 ∴ p < 0.01	r = 0.949 df = 4; t = 6.04 ∴ p < 0.01	r = 0.963 df = 4; t = 7.15 ∴ p < 0.01
II	r = 0.995 df = 4; t = 19.92 ∴ p < 0.01	r = 0.970 df = 4; t = 11.42 ∴ p < 0.01	r = 0.986 df = 4; t = 11.83 ∴ p < 0.01
III	r = 0.794 df = 4; t = 8.59 ∴ p < 0.01	r = 0.970 df = 4; t =7.98 ∴ p < 0.01	r = 0.624 df = 4; t = 1.60 ∴ p > 0.10, therefore significant

Table 13: Rate Of Hydrolysis of Reconstituted Amoxycillin Test Samples

COMPANY	RATE OF HYDROLYSIS (%/DAY)		
	2-8°C (Refrigeration)	25°C (Room Temperature)	35°C
A	1.2010	2.0930	3.5661
B	1.1920	2.1643	3.4510
C	1.2396	2.1583	3.5653
D	1.3240	2.2151	3.7370
E	1.4266	2.3536	3.5146
F	1.3070	2.1656	3.3273

All figures above were computed from results of the Pharmacopoeia spectrophotometric analysis. Both Non-Pharmacopoeia spectrophotometric and Microbiological qave similar fiqures.

Table 14: Estimated Shelf-Life (T_{90}) of Reconstituted Amoxycillin Test Samples (Batch I) Analyzed Using Pharmacopoeia Method

COMPANY	TIME (DAYS)		
	2–8°C (Refrigeration)	25°C (Room Temperature)	35°C
A	16.178	8.83	5.45
B	9.97	5.49	3.45
C	20.24	11.59	7.04
D	14.68	8.77	5.20
E	7.57	4.65	3.11
F	5.49	3.31	2.15

Table 15: Regression Equations and Correlation Coefficients of Batch I Test Samples Stored at Room Temperature and Analyzed by Pharmacopoeia Method

COMPANY	REGRESSION EQUATION	CORRELATION COEFFICIENT
A	Y = 109.80 - 2.0930x	0.998
B	Y = 101.38 - 2.1643x	0.998
C	Y = 115.51 - 2.1583x	0.999
D	Y = 109.96 - 2.2151x	0.999
E	Y = 100.43 - 2.3536x	0.999
F	Y = 97.146 - 2.1656x	1.000

Y = residual amoxycillin content at each defined storage periods, and

x represents the storage periods in days at which the reconstituted Oral amoxycillin suspensions are stored.

CHAPTER FOUR

4.0 DISCUSSION AND CONCLUSION

4.1 DISCUSSION

The British Pharmaceutical Codex (BPC) 1980 and British Pharmacopoeia (BP) 1988 specified that the infra-red (IR) spectra of amoxycillin trihydrate should exhibit characteristic peaks at wavenumbers 1248 cm^{-1}, 1396 cm^{-1}, 1483 cm^{-1}, 1583 cm^{-1}, 1684 cm^{-1} and 1775 cm^{-1}.

Peaks at 1500 cm^{-1} and 1600 cm^{-1} are indicative of a six-membered aromatic ring system (e.g., benzene ring) and these peaks shift to 1483 cm^{-1} and 1584 cm^{-1} respectively following para-substitution of the benzene ring. The peak at 1775 cm^{-1} indicates the presence of imide group while that at 1684 $cm^{-1}1$ shows the presence of a secondary amide group. The characteristic gem-dimethyl group is shown at 1396 cm^{-1} (William and Fleming, 1980).

The IR spectra of the reference amoxycillin trihydrate used as standard in this work gave characteristic peaks at wavenumber characteristic of amoxycillin. This confirmed that the reference standard sample used in the IR spectroscopy contain amoxycillin.

The IR spectra of the various test samples (A-F), (Fig. 4 & 5), also showed characteristic peaks indicative of the presence of imide, secondary amide and

benzene corresponding to specifications of the BPC (1980). One can thus infer that the sampled marketed products contained amoxycillin. Undoubtedly, the magnitude or prominence of the peaks (which can often be quantitatively correlated with amount of available active ingredient), were different. This was due to the fact that equipotent amounts of samples were not used for the IR analysis, since the desire was solely to determine the presence or absence of amoxycillin in the samples.

The result obtained by thin layer chromatography (TLC) confirmed the deduction made from the IR spectroscopy study i.e., the test samples contained amoxycillin. The R_f values of the principal spots shown in the TLC chromatogram of the reference standard and test samples were very similar (0.531-0.539) and not statistically different from Rf of 0.530 specified for amoxycillin in the USP 1980. The additional spots in the TLC chromatogram of the test samples were probably due to other excipients of the various formulations.

Quantitative analysis of amoxycillin content of the various test samples as analyzed by pharmacopoeial spectrophotometric method for the different batches indicated that sample F had the lowest percentage amoxycillin content while sample C gave the highest percentage content.

Based on the USP and BP specifications on acceptable active ingredient concentrations (100 ± 10%),

only samples of brands A, B, E and F satisfied the requirements.

As the student paired t-test result showed, there were significant differences between most of the brands sampled with regards to their amoxycillin content. Variation between brands of the same drug are not unexpected and similar findings have been reported (Udobi *et al*, 1993). Such significant differences are mostly due to marked variation in formulation composition, processing operations, quality control analytical methods, equipment and desired objective. Often, some manufacturers attempt to mask the deficiencies in their formulation by increasing the percentage of the active component of their products.

It is clear from both the t-test and F-ratio that essentially, only brand A samples exhibit consistency in its percentage content of amoxycillin among the various batches. This is particularly obvious in samples of brand D which showed on average, the highest F-ratio and t-test values, followed by brand F that gave the highest single t-test value. The high degree of variation in contents in some brands (D > F > E) and to a lesser extent in brands B and C, among the batches points to questionable in-process quality control measures in the manufacturing process of these companies. It is ethically unacceptable for drugs from one company to vary in its content by as much as 15%. While variation between batches is normal, it is not expected that such variation be statistically significant unless there have

been recent changes in the formulation composition and or methodology or different processing equipment is being used.

The apparent lack of correlation between the t-test and F-ratio results with respect to significance and non-significance in the differences between the different batches in some brands is principally due to the type of statistical inferences such test is set to highlight. While the t-test analyses the closeness of the means of sets of data, the F-ratio tests the scatter or variability of the sets of data around the mean. This implies that two sets of data may have similar scatter of observations but exhibit relatively large differences in their mean. Generally, t-test ranks higher than F-ratio in data comparison because it is more robust and realistic for non-parametric analysis. In essence, t-test can be said to measure the accuracy of data while F-ratio test measures the precision of the data.

As depicted in Fig. 7, the rate of hydrolysis of all the samples at elevated storage temperature (35°C) was much higher than at refrigeration temperature and is is in most cases almost three times higher (Table 13). The differences in the loss of amoxycillin among the various samples at the various shelf storage temperature and time were highly significant (Table 10), for example, the minimum t-value was 9.702 as against 2.16 at $p = 0.05$ and $df = 5$.

In general, increase in reaction temperature usually leads to greater free energy as can be deduced

from Arrhenius equation, which will subsequently enhance chemical reaction, thus the rate of degradation was higher at room temperature and 35°C than at refrigeration temperature. Typically, a 10°C increase in reaction temperature produces a 2-3 fold increase in the rate of reaction. In this study, however, a 1.5 fold increase in reaction rate was observed when the rate of hydrolysis at 35°C is compared with those at 25°C.

Amoxycillin suspension, like other penicillins have been found to degrade by a first order kinetics, as a result of a nucleophilic attack of the C=O bond at position 7 by the hydroxyl ion of water (Wells, 1988). It is however, clear from the plots of concentration against time /Fig. 7) that the rate of hydrolysis is not a first order but a zero order kinetic, since a straight line was obtained at the various temperatures, with high correlation coefficients. It thus followed that the hydrolysis of amoxycillin in the reconstituted suspension obeyed the zero order equation given below:

$$Ct - Co = -Kt$$

where Ct = concentation at time t

C = initial concentration, and

K = reaction constant.

This is in conformity with the equations of the regression lines obtained for the various samples as stated in Table 15.

It is known that zero order reaction predominated where water is present in excess and the concentration of the drug is fixed, as is the case in a suspension. A zero order (which occurs by chance), is independent of the concentration and is due to processes other than simple collision. Similar zero order reaction has been observed with aspirin suspension (Wells, 1988), and this type of kinetic order reaction is characteristic of most break-down processes.

Commercial pharmaceutical products are supposed to contain at least 90% of its labelled strength throughout its shelf life. The t_{90} values of all the samples as calculated from the reaction rate (Table 14) showed that reconstituted amoxycillin suspensions stored at 2-8°C must be use within one week as officially recommended by the manufacturers, except in the case of sample F which should not exceed 5 days after reconstituttion. These manufacturers' 7 days recommendation is however, in contrast to 14 shelf life recommended by the American Society of Hospital Pharmacists as stated in the AHFS 90 (1990). In fact, the assertion by this body that refrigeration of the reconstituted amoxycillin suspension is not necessary and that it it is stable at room temperature for 14 days is contradicted by this result. It must be noted however, that the ambient room temperature in USA is definitely not same for a tropical country like Nigeria. The shelf life of 7 days is also reasonable for storage at room temperature for samples of companies A, C and D, which had initial amoxycillin

content significantly greater than 100%, whereas samples B and E that had about 100% mean content should be used within 5 days of reconstitution. The deleterious effect of storing reconstituted suspensions of antibiotics at tropical ambient temperature that are more often greater than 30°C is amply illustrated in this study (Table 14). The high hydrolytic degradation of amoxycillin suspension at elevated temperature of 35°C implies that reconstituted amoxycillin suspension should as much as possible be used within 3 days and the residual discarded.

In his treatise on drug stability, Wells (1988) pointed out that because of the slower rate of degradation, refrigeration can be used to prolong the shelf life of products by as much as 90%. In this study, the shelf life of the various reconstituted suspensions was extended by 67-83% when stored at refrigeration temperatures, as compared with storage at room temperature.

The t-values for the three methods of analysis for the different batches were all below the critical value ($t_{0.05}$ = 2.228, df = 10 for batches I and II; and $t_{0.05}$ = 2.776, df = 4 for batch III). This indicates that the differences between the result from the various methods are not statistically significant, hence the methods are favorably comparable. This is virtually confirmed by the results of the F-ratio test except for batch III in which the F-value of Non-Pharmacopoeia vs Microbiological methods was slightly significant (Table 11).

Results of the correlation coefficient obtained from regression plots confirmed the deductions from those of the student t-test except in batch III, where the coefficient value for Non-Pharmacopoeia spectrophotometric method vs Microbiological method gave a relatively low value of correlation (Table 12). This is probably due to the very low number of products sample in this batch.

The results of the Pearson correlation coefficients is in agreement with those obtained from regression plots and that of the F-ratio test. While the correlation coefficient of Non-Pharmacopoeia method vs Microbiological methods was apparently high, analysis of the significance of the correlation gave t-value of 1.60 which is considerably less than the critical t-value of 4.604 at df = 4, giving $p > 0.10$ which further buttresses the earlier observation of low correlation between Non-Pharmacopoeia method vs Microbiological assay due to the low number of samples used in batch III.

Although many methods are available for comparing result from different methods, student t-test, correlation and regression analysis as well as F-test are more commonly used. Abounassif *et al* (1991) used the statistical t-test and F-test to compare two methods of analysis of amoxycillin in formulation while Belsey *et al* (1978) used correlatien coefficient to compare a table top analyzer with those of standard laboratory technique. Bland and Altman (1986) advocated a modification of the use of correlation coefficient in

assessing agreement between two methods of measurement. This is principally responsible for additionally comparing the different methods using the Pearson Correlation Matrix and calculating the significance of the correlates.

In general, the four statistical analytical test are very much in agreement and indicate that there are no statistically significant differences between the three methods of analysis. One can further infer that when the procedural specifications are strictly and precisely followed, the three methods have the same degree of accuracy and precision.

4.2 CONCLUSION

The Amoxycillin Oral Suspension brands marketed within Nigeria can be said to contain at least the minimum acceptable amounts of the active ingredients although some brands contain over and above official recommended acceptable limits in some batches.

The reconstituted suspensions were also shown to be stable in terms of residual amoxycillin content for about 7 days when refrigerated, 5 days when left at ambient room temperature and only about 2-3 days at elevated temperature. The latter storage temperature has serious implication in some parts of the country where temperatures can sometimes exceed 40°C during the day.

The results of this study also indicate that the methods are highly comparable in terms of accuracy and precision, and can be substituted with each other. The

microbiological method though reliable can sometimes be cumbersome and time-consuming especially when rapid determination is required. The BP spectrophotometric method compares very well with the Non-pharmacopoeia method designed by Bundgaard and Larsen (1980) but does not distinguish between free active ingredient from its degradation and polymerization products. The Non-pharmacopoeia spectrophotometric analytical process has the added advantage of being less involving and faster in terms of speed of result generation.

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APPENDIX

Table A1: Residual Percentage Content of Amoxycillin Per Week in Batch I of Test Samples at Different Storage Temperatures analyzed by Pharmacopoeial Spectrophotometric Method

Storage Temperatures/ Duration of Storage	Mean Percentage Content Per Week					
	A	B	C	D	E	F
2–8°C :						
Day 1	109.43 ± 1.10	101.89 ± 1.20	115.09 ± 1.30	109.43 ± 1.30	100.94 ± 1.35	97.17 ± 1.15
8	101.92 ± 1.13	94.23 ± 1.24	106.73 ± 1.32	99.04 ± 1.43	90.38 ± 1.42	89.50 ± 1.20
15	92.73 ± 1.15	86.36 ± 1.25	97.27 ± 1.35	90.00 ± 1.45	80.73 ± 1.45	79.82 ± 1.23
22	84.47 ± 1.33	76.70 ± 1.28	89.32 ± 1.40	81.55 ± 1.55	70.87 ± 1.52	69.90 ± 1.26
25°C :						
Day 1	109.43 ± 1.10	101.89 ± 1.20	115.09 ± 1.30	109.43 ± 1.30	100.94 ± 1.35	97.17 ± 1.15
8	95.19 ± 1.22	85.58 ± 1.46	100.96 ± 1.56	95.19 ± 1.86	83.31 ± 1.75	81.73 ± 1.16
15	81.82 ± 1.10	70.82 ± 1.66	85.45 ± 1.48	79.09 ± 1.63	67.27 ± 1.94	67.27 ± 1.36
22	65.05 ± 1.32	56.31 ± 1.32	69.90 ± 1.64	63.11 ± 1.86	51.37 ± 1.77	51.46 ± 1.26
35°C						
Day 1	109.43 ± 1.10	101.89 ± 1.20	115.09 ± 1.30	109.43 ± 1.30	100.94 ± 1.35	97.17 ± 1.15
8	85.56 ± 1.32	76.92 ± 1.34	90.38 ± 1.38	82.69 ± 1.58	74.04 ± 1.65	73.08 ± 1.43
15	60.91 ± 1.56	52.73 ± 1.55	65.45 ± 1.59	58.18 ± 1.82	48.18 ± 1.85	50.00 ± 1.68
22	34.95 ± 1.76	29.13 ± 1.82	40.78 ± 1.37	30.09 ± 1.99	23.30 ± 1.97	27.18 ± 1.84

Table A2: Residual Percentage Content of Amoxycillin Per Week in Batch II of Test Samples at Different Storage Temperatures analyzed by Pharmacopoeial Spectrophotometric Method

Storage Temperat-ures/ Duration of Storage	Mean Percentage Content Per Week					
	A	B	C	D	E	F
2–8°C :						
Day 1	107.23 ± 1.30	107.23 ± 1.88	108.78 ± 1.23	124.82 ± 1.50	106.57 ± 1.41	105.11 ± 1.20
8	101.85 ± 1.35	99.07 ± 1.60	100.93 ± 1.53	114.81 ± 1.68	96.30 ± 1.75	96.30 ± 1.45
15	92.45 ± 1.48	92.45 ± 1.64	92.45 ± 1.62	105.66 ± 1.69	87.74 ± 1.68	89.62 ± 1.42
22	83.50 ± 1.67	84.47 ± 1.83	83.49 ± 1.67	96.20 ± 1.56	77.67 ± 1.84	80.58 ± 1.58
25°C :						
Day 1	107.23 ± 1.30	107.23 ± 1.88	108.78 ± 1.23	124.82 ± 1.50	106.57 ± 1.41	105.11 ± 1.20
8	92.59 ± 1.45	91.67 ± 1.54	93.52 ± 1.67	96.20 ± 1.56	77.67 ± 1.84	80.58 ± 1.58
15	76.42 ± 1.63	77.36 ± 1.67	77.36 ± 1.59	92.45 ± 1.85	74.53 ± 1.82	75.47 ± 1.54
22	63.11 ± 1.68	62.14 ± 1.78	61.17 ± 1.88	77.68 ± 1.98	60.71 ± 1.99	60.50 ± 1.66
35°C						
Day 1	107.23 ± 1.30	107.23 ± 1.88	108.78 ± 1.23	124.82 ± 1.50	106.57 ± 1.41	105.11 ± 1.20
8	83.33 ± 1.78	82.41 ± 1.77	86.11 ± 1.79	98.15 ± 1.89	80.56 ± 1.74	81.48 ± 1.46
15	59.43 ± 1.86	57.55 ± 1.89	60.35 ± 1.78	74.53 ± 1.96	57.55 ± 1.86	58.49 ± 1.59
22	34.95 ± 1.84	32.04 ± 1.94	34.95 ± 1.97	48.54 ± 2.05	30.10 ± 2.00	33.98 ± 1.88

Table A3: Residual Percentage Content of Amoxycillin Per Week in Batch III of Test Samples at Different Storage Temperatures analyzed by Pharmacopoeia Spectrophotometric Method

Storage Temperatures/ Duration of Storage	Mean Percentage Content Per Week		
	A	B	D
2–8°C :			
Day 1	110.48 ± 1.13	109.52 ± 1.22	113.33 ± 1.25
8	102.88 ± 1.33	101.92 ± 1.42	103.85 ± 1.48
15	94.17 ± 1.40	94.17 ± 1.48	95.14 ± 1.49
22	87.16 ± 1.64	86.24 ± 1.52	87.16 ± 1.66
25°C :			
Day 1	110.48 ± 1.13	109.52 ± 1.22	113.33 ± 1.25
8	95.19 ± 1.64	95.19 ± 1.73	98.08 ± 1.82
15	80.58 ± 1.67	81.00 ± 1.78	82.52 ± 1.82
22	66.06 ± 1.82	65.14 ± 1.82	66.06 ± 1.88
35°C			
Day 1	110.48 ± 1.13	109.52 ± 1.22	113.33 ± 1.25
8	86.54 ± 1.83	84.62 ± 1.90	87.50 ± 1.93
15	65.05 ± 1.84	84.62 ± 1.90	61.17 ± 1.94
22	40.37 ± 1.86	34.86 ± 1.86	37.61 ± 1.98

Table B1: Residual Percentage Content of Amoxycillin Per Week in Batch I of Test Samples at Different Storage Temperatures analyzed by Non-Pharmacopoeia Spectrophotometric Method

Storage Temperatures/ Duration of Storage	Mean Percentage Content Per Week					
	A	B	C	D	E	F
2–8°C :						
Day 1	109.92 ± 0.98	102.79 ± 1.24	113.98 ± 1.18	108.93 ± 1.65	100.99 ± 1.58	96.93 ± 1.13
8	100.56 ± 1.32	95.77 ± 1.35	104.78 ± 1.28	99.89 ± 1.54	91.10 ± 1.67	87.54 ± 1.31
15	93.49 ± 1.39	87.32 ± 1.58	94.93 ± 1.51	90.80 ± 1.83	83.61 ± 1.69	79.12 ± 1.46
22	85.41 ± 1.54	79.90 ± 1.48	85.06 + 1.54	81.56 ± 1.83	74.80 ± 1.79	71.30 ± 1.56
25°C :						
Day 1	109.92 ± 0.98	102.79 ± 1.24	113.98 ± 1.18	108.93 ± 1.65	100.99 ± 1.58	96.93 ± 1.13
8	95.58 ± 1.37	86.56 ± 1.71	97.22 ± 1.33	93.77 ± 1.56	84.32 ± 1.63	82.98 ± 1.53
15	80.70 ± 1.65	70.26 ± 1.78	81.59 ± 1.65	78.79 ± 1.69	68.79 ± 1.78	66.11 ± 1.59
22	65.69 ± 1.88	55.36 ± 1.81	66.44 ± 1.67	62.18 ± 1.71	53.31 ± 1.77	52.04 ± 1.83
35°C						
Day 1	109.92 ± 0.98	102.79 ± 1.24	113.98 ± 1.18	108.93 ± 1.65	100.99 ± 1.58	96.93 ± 1.13
8	85.98 ± 1.64	75.03 ± 1.61	90.10 ± 1.49	84.43 ± 1.65	74.64 ± 1.68	73.86 ± 1.77
15	61.95 ± 1.79	50.51 ± 1.67	66.33 ± 1.81	57.91 ± 1.81	50.31 ± 1.87	48.97 ± 1.80
22	35.09 ± 1.90	24.99 ± 1.87	40.02 ± 1.92	32.02 ± 2.03	24.99 ± 1.99	24.46 ± 1.89

Table B2: Residual Percentage Content of Amoxycillin Per Week in Batch II of Test Samples at Different Storage Temperatures analyzed by Non-Pharmacopoeia Spectrophotometric Method

Storage Temperatures/ Duration of Storage	Mean Percentage Content Per Week					
	A	B	C	D	E	F
2-8°C :						
Day 1	108.21 ± 1.38	109.40 ± 1.21	110.80 ± 1.15	121.92 ± 1.55	107.99 ± 1.53	104.98 ± 1.46
8	100.76 ± 1.42	101.74 ± 1.43	101.20 ± 1.28	112.01 ± 1.73	97.93 ± 1.65	96.98 ± 1.49
15	91.36 ± 1.65	93.38 ± 1.47	92.66 ± 1.45	102.39 ± 1.62	89.20 ± 1.64	88.07 ± 1.63
22	82.69 ± 1.73	85.40 ± 1.66	83.32 ± 1.67	93.11 ± 1.82	80.80 ± 1.59	80.81 ± 1.71
25°C :						
Day 1	108.21 ± 1.38	109.40 ± 1.21	110.80 ± 1.15	121.92 ± 1.55	107.99 ± 1.53	104.98 ± 1.46
8	94.00 ± 1.60	92.15 ± 1.55	95.20 ± 1.41	105.00 ± 1.65	90.42 ± 1.73	90.19 ± 1.41
15	79.09 ± 1.61	77.50 ± 1.68	80.00 ± 1.43	89.66 ± 1.81	74.55 ± 1.79	75.11 ± 1.65
22	62.36 ± 1.79	60.69 ± 1.73	62.46 ± 1.59	71.43 ± 1.83	58.50 ± 1.91	59.60 ± 1.83
35°C						
Day 1	108.21 ± 1.38	109.40 ± 1.21	110.80 ± 1.15	121.92 ± 1.55	107.99 ± 1.53	104.98 ± 1.46
8	84.41 ± 1.62	85.61 ± 1.51	85.93 ± 1.71	97.15 ± 1.73	81.03 ± 1.65	80.26 ± 1.83
15	60.00 ± 1.72	60.95 ± 1.71	61.02 ± 1.83	72.75 ± 1.83	57.27 ± 1.90	55.50 ± 1.85
22	36.16 ± 1.81	35.12 ± 1.79	37.33 ± 2.00	47.36 ± 1.95	31.37 ± 1.96	31.58 ± 1.95

Table B3: Residual Percentage Content of Amoxycillin Per Week in Batch III of Test Samples at Different Storage Temperatures analyzed by Non-Pharmacopoeia Spectrophotometric Method

Storage Temperatures/ Duration of Storage	Mean Percentage Content Per Week		
	A	B	D
2-8°C :			
Day 1	111.18 ± 1.34	106.94 ± 1.28	111.99 ± 1.55
8	103.45 ± 1.63	98.78 ± 1.22	102.56 ± 1.76
15	95.62 ± 1.68	90.12 ± 1.46	93.60 ± 1.76
22	86.46 ± 1.65	80.91 ± 1.53	84.47 ± 1.83
25°C :			
Day 1	111.18 ± 1.34	106.94 ± 1.28	111.99 ± 1.55
8	97.44 ± 1.55	91.00 ± 1.43	95.33 ± 1.38
15	82.60 ± 1.53	75.31 ± 1.73	76.99 ± 1.54
22	67.86 ± 1.77	59.63 ± 1.78	61.84 ± 1.61
35°C			
Day 1	111.18 ± 1.34	106.94 ± 1.28	111.99 ± 1.55
8	86.76 ± 1.51	81.87 ± 1.56	85.32 ± 1.57
15	63.08 ± 1.71	56.70 ± 1.71	59.15 ± 1.81
22	38.39 ± 1.86	30.92 ± 1.86	33.61 ± 1.94

Table C1 Residual Percentage Content of Amoxycillin Per Week in Batch I of Test Samples at Different Storage Temperatures analyzed by Microbiological Assay Method

Storage Temperatures/ Duration of Storage	Mean Percentage Content Per Week					
	A	B	C	D	E	F
2-8°C :						
Day 1	107.33 ± 1.43	102.69 ± 1.51	113.68 ± 1.54	109.39 ± 1.29	100.53 ± 1.60	95.24 ± 1.34
8	98.11 ± 1.48	95.66 ± 1.61	104.61 ± 1.63	100.00 ± 1.38	91.84 ± 1.63	87.38 ± 1.43
15	90.12 ± 1.53	87.73 ± 1.67	96.47 ± 1.75	90.27 ± 1.46	81.79 ± 1.65	78.64 ± 1.54
22	81.19 ± 1.59	78.24 ± 1.71	87.76 ± 1.56	81.30 ± 1.53	72.03 ± 1.82	69.71 ± 1.71
25°C :						
Day 1	107.33 ± 1.43	102.69 ± 1.51	113.68 ± 1.54	109.39 ± 1.29	100.53 ± 1.60	95.24 ± 1.34
8	93.19 ± 1.65	86.69 ± 1.65	98.76 ± 1.48	93.18 ± 1.51	84.07 ± 1.67	79.10 ± 1.46
15	78.52 ± 1.57	72.05 ± 1.71	83.03 ± 1.59	77.36 ± 1.63	67.64 ± 1.71	63.40 ± 1.54
22	63.40 ± 1.73	57.37 ± 1.85	69.11 ± 1.83	61.84 ± 1.49	52.79 ± 1.74	49.00 ± 1.58
35°C						
Day 1	107.33 ± 1.43	102.69 ± 1.51	113.68 ± 1.54	109.39 ± 1.29	100.53 ± 1.60	95.24 ± 1.34
8	82.70 ± 1.48	77.13 ± 1.56	88.24 ± 1.62	83.40 ± 1.43	74.57 ± 1.58	72.21 ± 1.67
15	58.05 ± 1.72	52.59 ± 1.67	64.94 ± 1.71	57.20 ± 1.59	48.85 ± 1.62	47.73 ± 1.70
22	33.64 ± 1.83	27.91 ± 1.83	39.29 ± 1.79	31.42 ± 1.83	23.76 ± 1.94	22.70 ± 1.84

Table C2: Residual Percentage Content of Amoxycillin Per Week in Batch II of Test Samples at Different Storage Temperatures analyzed by Microbiological Assay Method

Storage Temperatures/ Duration of Storage	Mean Percentage Content Per Week					
	A	B	C	D	E	F
2-8°C :						
Day 1	110.63 ± 1.24	108.31 ± 1.28	110.27 ± 1.27	121.56 ± 1.56	110.27 ± 1.61	104.25 ± 1.31
8	104.28 ± 1.43	100.44 ± 1.39	102.34 ± 1.45	111.50 ± 1.58	100.44 ± 1.63	96.85 ± 1.42
15	95.00 ± 1.71	91.51 ± 1.75	93.31 ± 1.61	102.42 ± 1.73	91.24 ± 1.67	88.26 ± 1.26
22	86.14 ± 1.71	84.44 ± 1.75	84.53 ± 1.69	92.75 ± 1.84	82.61 ± 1.86	80.59 ± 1.56
25°C :						
Day 1	110.63 ± 1.24	108.31 ± 1.28	110.27 ± 1.27	121.56 ± 1.56	110.27 ± 1.61	104.25 ± 1.31
8	96.28 ± 1.48	93.33 ± 1.54	95.13 ± 1.51	104.56 ± 1.64	91.56 ± 1.59	90.00 ± 1.49
15	81.12 ± 1.62	77.60 ± 1.67	79.55 ± 1.61	88.92 ± 1.71	74.59 ± 1.63	74.51 ± 1.53
22	64.71 ± 1.68	59.67 ± 1.69	61.50 ± 1.71	72.12 ± 1.83	59.00 ± 1.85	59.49 ± 1.63
35°C						
Day 1	110.63 ± 1.24	108.31 ± 1.28	110.27 ± 1.27	121.56 ± 1.56	110.27 ± 1.61	104.25 ± 1.31
8	87.81 ± 1.55	83.93 ± 1.52	86.10 ± 1.53	94.75 ± 1.61	83.83 ± 1.71	80.93 ± 1.49
15	62.71 ± 1.59	60.29 ± 1.52	61.49 ± 1.69	70.57 ± 1.74	58.00 ± 1.74	56.56 ± 1.83
22	37.69 ± 1.78	34.80 ± 1.76	37.65 ± 1.78	44.68 ± 1.98	32.91 ± 1.90	31.46 ± 1.85

Table C3: Residual Percentage Content of Amoxycillin Per Week in Batch III of Test Samples at Different Storage Temperatures analyzed by Microbiological Assay Method

Storage Temperatures/ Duration of Storage	Mean Percentage Content Per Week		
	A	B	D
2–8°C :			
Day 1	109.90 ± 0.93	109.90 ± 1.12	111.91 ± 1.58
8	102.50 ± 1.05	102.61 ± 1.41	103.48 ± 1.66
15	93.52 ± 1.29	93.40 ± 1.59	94.31 ± 1.78
22	86.06 ± 1.48	85.00 ± 1.60	86.33 ± 1.84
25°C :			
Day 1	109.90 ± 0.93	109.90 ± 1.12	111.91 ± 1.58
8	94.53 ± 1.46	94.53 ± 1.54	94.35 ± 1.54
15	80.28 ± 1.65	78.46 ± 1.71	77.92 ± 1.63
22	64.52 ± 1.71	63.26 ± 1.83	61.60 ± 1.81
35°C			
Day 1	109.90 ± 0.93	109.90 ± 1.12	111.91 ± 1.58
8	85.66 ± 1.40	85.37 ± 1.63	86.84 ± 1.69
15	61.95 ± 1.68	60.81 ± 1.77	62.04 ± 1.85
22	38.28 ± 1.75	35.66 ± 1.94	36.02 ± 2.06

On exposure of light-sensitive (photolabile) drugs to sunlight, sufficient energy of activation may be acquired enabling decomposition to take place. Only the radiation absorbed by the drug is effective in producing photochemical reactions.

(d) **pH**

Negative logarithm of hydrogen ion concentration is a measure of acidity/basicity of any chemical system. The