

THE QUALITY CONTROL OF AMPICILLIN AND
AMPICILLIN/CLOXACILLIN SUSPENSIONS
MARKETTED IN KADUNA AND ZARIA

BY

UDOBI, CHINWEIZU EJKEME
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MICROBIOLOGY, FACULTY OF PHARMACEUTICAL SCIENCES
AHMADU BELLO UNIVERSITY, ZARIA

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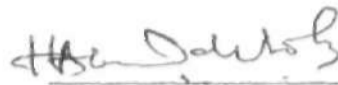
DECLARATION

I hereby declare that the work reported in this Thesis was carried out by me under the supervision of Drs J. A. Onaolapo, P. F. Olurinola and Abdullahi Mustapha of the Departments of Pharmaceutical Microbiology and Pharmaceutical Chemistry, Ahmadu Bello University, Zaria. It has not been presented in any previous application for higher degree. The work of other investigators are acknowledged and referred to accordingly.


UDOBI, CHINWEIZU EJKEME

CERTIFICATION

This Thesis entitled "The Quality Control of Ampicillin and Ampicillin/
Cloxacillin Suspensions Marketed in Kaduna and Zaria" by Udobi,
Chinweizu Ejikeme meets the regulations governing the award of the
Degree of MASTER OF SCIENCE (M.Sc) of Ahmadu Bello University, Zaria
and is approved for its contribution to knowledge and literary presentation.



External Examiner
Prof. H. A. Odelola
Department of Pharmaceutical Microbiology
University of Ibadan,
Ibadan - Nigeria



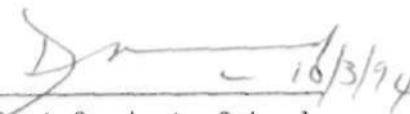
Internal Examiner
Dr J. A. Onaolapo
B.Sc Pharm. (A.B.U), M.Sc (A.B.U)
Ph.D (Aston)
Department of Pharmaceutics and
Pharmaceutical Microbiology
Ahmadu Bello University,
Zaria - Nigeria



Internal Examiner
Dr P. F. Olurinola
B.Sc Pharm. (A.B.U)
Ph.D (Bradford)
Department of Pharmaceutics and
Pharmaceutical Microbiology
Ahmadu Bello University,
Zaria, Nigeria



Internal Examiner
Dr Abdullahi Mustapha
B.Sc Pharm. (A.B.U) Ph.D (London)
Department of Pharmaceutical and
Medical Chemistry
Ahmadu Bello University,
Zaria - Nigeria



Dean, Post-Graduate School
Ahmadu Bello University, Zaria
Prof. Dalhatu Mohammed

DEDICATION

TO GOD ALMIGHTY

FOR HIS STEADFASTNESS

AND TO MAMA

FOR ALL THE ENCOURAGEMENT SHE GAVE

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Ify, who also did the typing, I thank her for being a wonderful sister.

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ABSTRACT

A total of 51 samples of ampicillin and ampicillin/cloxacillin Oral Preparations from 5 different companies labelled A, B, C, D and E commonly used within Kaduna and Zaria township were assayed using a total of 3 assay methods namely, microbiological, chemical and spectroscopic methods. The level of their ampicillin content in relation to the manufacturers claim was ascertained.

Bacillus megatarium NCTC 10342 A76 was found to be suitable for the microbiological assay of ampicillin. Selective determination of ampicillin in its combination with cloxacillin using microbiological assay method was also carried out.

Infra-red spectroscopy confirms the presence of ampicillin in all the samples and the results obtained from the assay demonstrate that the samples had a wide range of amounts of ampicillin. The percentage ampicillin content range from between 57 - 162 using the microbiological, chemical and spectrophotometric assay methods.

The products of Company C assayed had ampicillin content less than 80 and 90%. These are lower than the levels recommended by the BP and USP respectively. Samples from one batch of the products from Company B had ampicillin content greater than 120% which is the upper limit recommended by the BP.

Products of Companies A, D and E had ampicillin contents within acceptable ranges recommended by both the USP and BP.

The non-pharmacopeial methods of Akanni and Ayim (1990) used gave results which correlate with the results obtained from the microbiological assay for the same products.

Results obtained in this work are discussed in the light of the high rate of drug faking, adulteration, manufacture of sub-standard drugs and their implications.

CHAPTER 1

INTRODUCTION

The need to ascertain that the quality of pharmaceutical products are within acceptable limits cannot be over-emphasized. This is even more important these days when the problem of faking, adulteration and production of sub-standard drugs are becoming a serious problem worldwide. Since pharmaceuticals are products that affect human lives, the concept of "a little too much" or that of just a little less cannot be accepted. It is important that pharmaceuticals be prepared right in both their active medicaments and other excipients.

Antibiotics are drug preparations containing some chemical substances produced by micro-organisms and which in low concentrations can wholly or partially inhibit or destroy micro-organisms. Some are also produced by chemical synthesis. All antibiotics whether produced by chemical synthesis or by micro-organisms have the problem of faking, adulteration and standard compromise. Because of these problems, constant checks on the quality of these products have become very important especially when one considers that they are drugs whose presence or absence can determine life or death (Hewitt 1977).

Checks on the quality of antibiotics are basically done by assays. These assay methods help in estimating active Constituents, biological activity and in monitoring the stability of products etc.

For antibiotics, estimation of biological activity (potency) using assay has for a long time now being an acceptable method for determining how good a drug is.

There are many assay methods which are being used in the quality control of pharmaceuticals. These include:

- (i) Biological assay (Bioassay)
- (ii) Chemical assay
- (iii) Spectrophotometric assay

1.1 BIOLOGICAL ASSAY (BIOASSAY)

Biological assay is that assay method which employs the biological properties of medicinal agents in the estimation of their activity.

The method uses the basic principle of comparing a sample of known activity or potency (standard) and one of unknown activity at the same time and under very strict comparable conditions.

The method of comparison of these two methods may be macrobiological as in the assay of insulin where mice is used or microbiological as in the assay of ampicillin where suitable micro-organism like Bacillus megatarium may be used.

Along with biological assay, other assay methods like chemical, physico-chemical and purely physical methods which employ the chemical physico-chemical and physical properties have been and are still in constant use for drug quality analysis. This, though has been possible mostly in cases where the agents consists of a single active substance that can be characterized purely on the basis of these parameters.

It has been argued by Ayim et al (1990) that chemical assay methods take precedence over biological methods since materials to be tested for biological and chemical equivalence must have been shown to be chemically equivalent. The undisputed fact that a substance becomes of recognised therapeutic use (Determined by estimation of biologic activity) before its

chemical composition is sought after goes to show how important biological assay methods have continued to be.

1.1.1 MICROBIOLOGICAL ASSAY TECHNIQUES

For microbiological assays, two principal methods are commonly used:

1. *The Agar diffusion method*
2. *The turbidimetric method*

The two methods are used for comparing the effect of two or more substances which have inhibitory property (in the case of antibiotics) or growth promoting property (in the case of vitamins and amino acids) using a suitable micro-organism as an indicator.

1.1.1.1 THE AGAR DIFFUSION METHOD

The relationship that exists between the dose (Concentration) of an antibiotic and the size of the zone of inhibition that results from it is recognised as a yardstick for comparing substances of unknown potency with those of known potency (standard). The zones of inhibition or clearing can only be observed on solid agar media through which the antibiotic can diffuse. This explains why the method is referred to as the agar diffusion method.

Large assay plates were introduced for use in agar diffusion assays because of its advantages over the conventional assay plates. They are flat bottomed and this has made it possible to obtain agar layer of uniform thickness during assays. It has also minimized the basic problem of variability of response to any one test solution by making it possible to take different readings of inhibition given by the same concentrations if any. This makes room for the use of more reliable average result for the calculation of potency.

1.1.1.2 THE TURBIDIMETRIC METHOD

This method involves the measurement of opacity of a known quantity of a suspension of susceptible organism after an incubation time of about 4 hours. The organism used is one that has a good sensitivity to the antibiotic being assayed so that an appreciable growth inhibition will occur. Like in the plate method, equal concentrations of the standard and sample being assayed and the potency of the sample determined by comparing its inhibition with that of the standard under the same conditions. This is done by opacity measurement in the tubes.

1.1.1.3 FURTHER USES OF BIOLOGICAL ASSAY

Besides potency determination which is almost entirely related to healthcare, other purposes of assay abound just like their methods and designs. The immediate use to which an assay will be put will normally determine its design and the method to be employed e.g. for the control of dosage usage during antibiotic treatment or the studies of the absorption and excretion rate of an antibiotic by animals or humans, a sensitive method which can detect very low levels of antibiotics will have to be employed. How rapid this method chosen should be will depend on how toxic the antibiotic in question is e.g. for Gentamicin speed and a high sensitive method has to be considered in selection because of its toxicity level.

In the manufacture of some antibiotics and some vitamins made by fermentation where the level of active substance is comparatively high, the process control is almost always done by microbiological assay methods. In this case, an assay method which requires a high sensitivity like in the former may not be

necessary or at least as necessary as speed will be in this case.

In research and development, assay of new crude substances (e.g. crude extracts) found to have medicinal effect, supplement chromatographic techniques in giving clues as to the composition of such crude substance and those which have antibacterial activity.

For finished (market) products, an accurate estimate of potency is important. Microbiological assay method stands out as one of the best ways of this determination. In this case, excessive speed is not always as important as accuracy. This is because the result of the assay will say a lot about the manufacturers and could have health and economic implications since it will determine the usability of the product.

1.2 CHEMICAL ASSAY

Chemical assay is the assay method which employs the chemical nature of compounds/agents in their design. It also exploits the ability of the agents in question or its active ingredients to undergo certain reactions. This explains why unlike the biological assay method which is based on the general principle of biologic response, the chemical assay design varies from one agent to the other. For example, the chemical assay of ampicillin trihydrate is based on the ability of ampicillin to react with acetic anhydride - Dioxan solution and Imidazole mercury reagent. Chemical assay methods are used for identification and estimation of the level of active ingredients in pharmaceuticals. These are spelt out in reference books.

1.3 SPECTROPHOTOMETRIC ASSAY

The spectrophotometer is an analytical photoelectric instrument which is used for estimating the concentrations of solutions by measuring either the amount of light passing through (transmittance) or the amount of light absorbed (absorbance). The assay method which involves principally the use of the spectrophotometer is termed spectrophotometric assay. Other very similar instruments which work on the same principle e.g. the colorimeter can also be used in place of the spectrophotometer.

The spectrophotometer works on the principle of the Beer-Lambert's law. Within the range in which the Beer's law is obeyed, it has been effectively used in the assay of pharmaceuticals when made into solutions.

Spectrophotometric assay of pharmaceuticals is highly dependent on the chemical reactions of their active ingredients. The concentration of the ingredients or its characteristic end product can be determined by comparing its light absorption with that of a solution of the same substance of known concentration (standard).

1.4 THE PENICILLIN GROUP OF ANTIBIOTICS

The penicillins are a group of naturally occurring and semi-synthetic antibiotics. They were the first to be used therapeutically and was originally obtained from the mould Penicillium notatum. Better yield however was further obtained from Penicillium chrysogenum.

Penicillins are still the most widely used antibiotics. They are bactericidal in action by inhibiting the synthesis of the bacterial

cell wall. The general structure of the penicillins show that they have a fused ring system of a 5-membered thiazolidine ring and a 4 membered B - lactam ring.

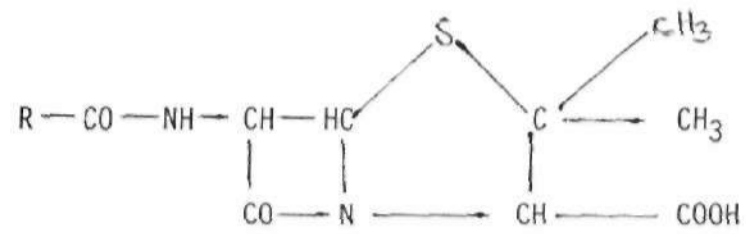


Fig. 1 BASIC STRUCTURE OF PENICILLIN

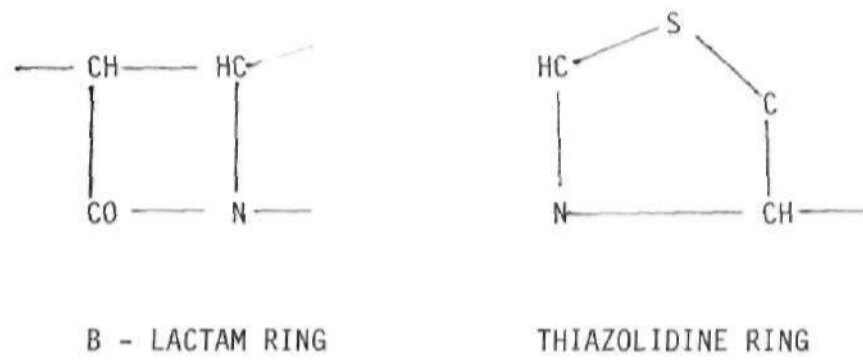


Fig. 2 STRUCTURES OF B-LACTAM AND THIAZOLIDINE RINGS

Penicillins are known to have high activity against gram-positive organisms being bactericidal at high concentrations and bacteriostatic at low concentrations. They have been used to treat infections due to streptococcus pyogenes, anaerobic streptococci, pneumococci, corynebacteria diphtheria etc. They are also active against some gram negative organisms. Though the activity of penicillins against most gram negative bacilli is usually too low to be of any clinical significance, over 80% of the species of salmonella, proteus mirabilis and E.coli are sensitive enough to respond to penicillins (Foye 1976). Penicillins have also been found useful in the treatment of urinary tract infections and biliary tract infections especially those due to gram negative enterobacteriaceae.

The mode of action of the penicillins has been found to be by the inhibition of the mucopeptide component of the cell wall which the organism needs for strength and rigidity. They do this by preventing the incorporation of N-acetyl muramic acid into the mucopeptide layer. Burger (1970).

In some organisms however, especially those that produce the enzyme B-lactamase resistance has been observed against the penicillins. These enzymes are natural antagonists to penicillins and are found in many micro-organisms. When they exist in reasonable amount in the organisms, the B-lactamase leads to the production of an opening in the lactam ring, rendering the penicillin inactive i.e. the conversion of penicillin to an inactive form (Steward 1975).

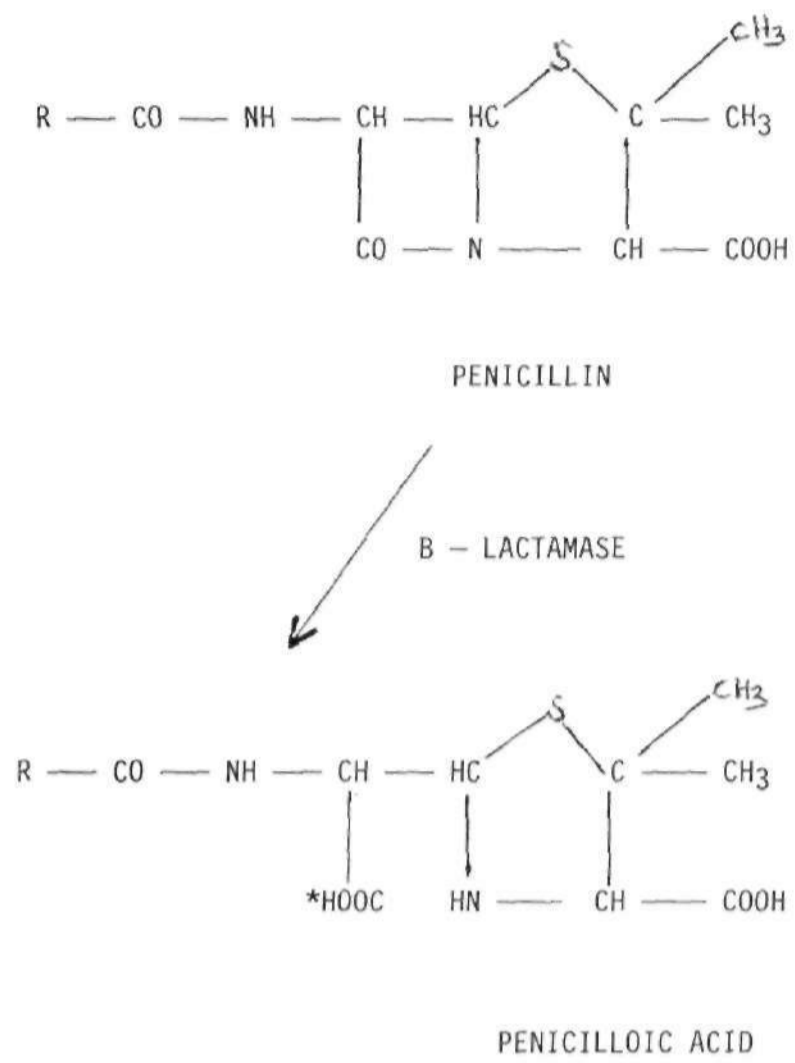
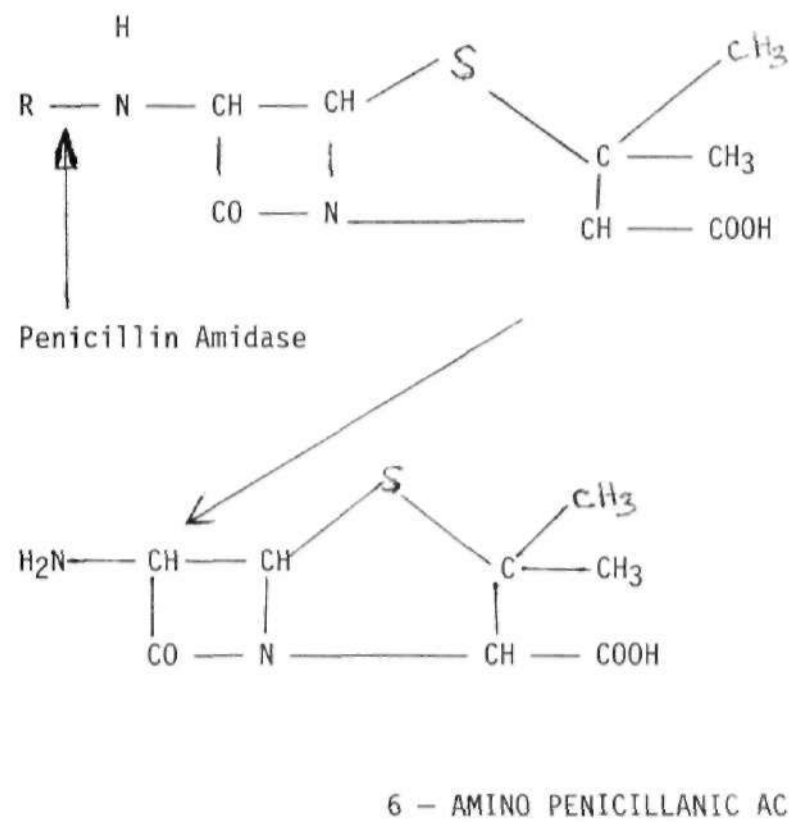


Fig. 3 - INACTIVATION OF PENICILLIN BY B- LACTAMASE

* A nucleophilic attack leading to the opening of the B-lactam ring and the production of penicilloic acid which has no antibacterial activity.

In a few instances also, there is the cleavage of the side chain of the natural penicillin structure by the enzyme penicillin amidase giving rise to 6-amino penicillanic acid (6APA). This is a compound with very little antibacterial activity and very susceptible to attack which leads to the opening of the lactam ring causing a total loss of activity (Jawetz et al 1982).



FORMATION OF 6-AMINO PENICILLANIC ACID (6-APA) BY ENZYMATIC CLEAVAGE

Fig. 4

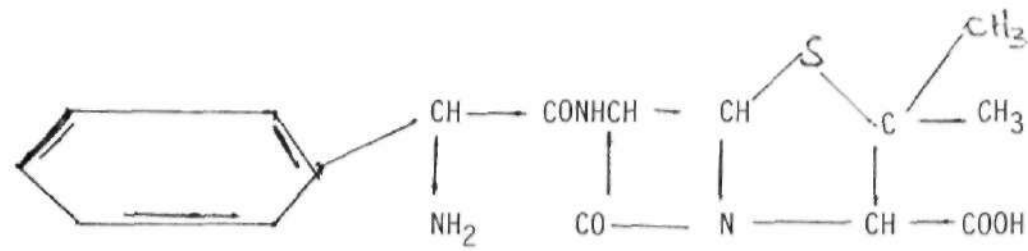
Penicillin resistance can however not be entirely explained by the production of the enzyme penicillinase. It may also result from the lack of the absorption of the antibiotic by the organism or the natural selection of resistant organisms which develops clinically e.g. by the use of subinhibitory concentrations of antibiotics.

The need for compounds (penicillins) which are superior to the original penicillin G in their physical, pharmacological and microbiological properties have led to the synthesis of a wide range of penicillins referred to as semi-synthetic penicillin. They have the basic structure of the penicillins (the thiazolidine and the B-lactam ring) but differ principally in the side group. These semi-synthetic penicillins are comparatively more acid stable, more B-lactamase resistant, have wider spectrum of activity than penicillin G e.g (carbenicillin) is more effective against Gram negative organisms than penicillin G while methicillin and cloxacillin are not readily inactivated by penicillinase. Ampicillin however, is more effective against Gram positive organisms than is penicillin G in addition to other advantages the semi-synthetics have.

1.4.1 AMPICILLIN

Ampicillin is a white crystalline powder, odourless and almost colourless. It is sparingly soluble in water. Solubility is about 1:170. It has been prepared in many dosage forms e.g. capsules, injections (sodium salt of ampicillin), Oral suspension, tablets and paediatric tablets.

Ampicillin is active against the same gram positive organisms that are susceptible to other penicillins and has a broader spectrum of activity than penicillin G. It is also known to have activity against some gram negative organisms and this combined spectrum of activity to both gram positive and gram negative organisms has made it the most commonly used and abused among the penicillin group of antibiotics.



C₁₆H₁₉N₃O₄S

6-(α -D-phenylglycyl amino)
penicillanic acid

Fig. 5 - STRUCTURE OF AMPICILLIN

Ampicillin, though a semi-synthetic penicillin is susceptible to penicillinase. The B-lactam ring can therefore be opened by the penicillinase enzyme rendering it inactive. Ampicillin is an α -amino penicillin because it has an α -amino group side chain attached to the basic penicillin structure. It is believed that the broader spectrum of activity is as a result of this. This explains the ability of the agents to cross cell wall barriers of organisms which other penicillins cannot penetrate.

Ampicillin is well absorbed from the gastrointestinal tract of man to give a peak plasma concentration of about 2-6 mg/ml. Absorption in infants and children however may be reduced with acute shigellosis (Welling and Tse, 1984). Food will always interfere with the absorption of ampicillin so it is taken 30 minutes to one hour before food.

1.4.1.1 USES OF AMPICILLIN

Ampicillin is used for the treatment of respiratory tract infections caused by Haemophilus influenza, Diplococcus, Pneumoniae, enteric and biliary tract infections due to E.coli, Enterococci, Salmonella and Shigella. It is also used in the treatment of meningitis due to Neisseria meningitidis and gonorrhoea due to Neisseria gonorrhoeae.

1.5 WHY THE PROJECT

Ampicillin and Ampicillin/cloxacillin preparations are the agents commonly used for the treatment of most infections especially in children. This is so because of their wide spectrum of activity. They are so widely used that virtually every product in the market labelled ampicillin is used up due to high demand. Ampicillin/cloxacillin is used sometimes in preference to only ampicillin due to the ability of the cloxacillin contained therein to block the the production of penicillinase enzyme (B-lactamase) which makes the organism resistant because it destroys the ampicillin. By this, the ampicillin is save from destruction and can then act on the organism. It has been noted that the combination gives an even broader spectrum of activity than ampicillin alone.

The high rate of use of these ampicillin preparations especially for children has increased the need for making sure that anything labelled ampicillin is not just truly ampicillin but of good quality. This is the primary aim of this work. This appear to be more important especially now that our society is faced with the problem of drug faking quality compromise and adulteration.

It is hoped that this independent effort (unknown to the manufacturers) will go a long way in complementing the efforts of the Federal Ministry of Health and other concerned agencies in identifying those fake and substandard products in our markets so that their sources can be traced.

It is also hoped that this work will confirm or contradict the method of Akanni and Ayim (1991) in determining ampicillin in the presence of cloxacillin by comparing it with the microbiological results of the same products.

CHAPTER 2

2.0 MATERIALS AND METHODS

2.1 MATERIALS

- 2.1.1 Media:- a/Nutrient Agar (NA) Oxoid
b/Nutrient (NB) Oxoid
- 2.1.2 Standard Organism:- Bacillus megatarium NCTC 10342 A76

2.1.3 Instruments and Glassware

1. Centrifuge (Gallenkamp, England)
2. Spectrophotometer Pye-Unican-200
3. Water bath shaker (Gallenkamp, England)
4. Test Tubes
5. Culture Plates
6. Pipettes (Automated)
7. Large assay plates (30 x 30 cm)
8. Cork Borer (Size 4)
9. Sintered Glass Porosity Size 0
10. 100ml Graduated Flask
11. Electric Shaker (Gallenkamp, England)
12. Pipettes (Glass)
13. Stoppered Tubes
14. Ice-cubes

2.1.4 Chemicals

1. Formaldelyde 37% V/v (M x B)
2. Phosphate buffer with 0.5% W/v formaldehyde - (PH 2.5)
3. Pottassium dihydrogen Orthophosphate (M x B)
4. Hydrochloric acid (BDH, England)
5. Sulphuric acid (BDH, England)
6. Sodium Hydroxide (BDH, England)
7. Boric Buffer PH 9.0

8. Acetic Anhydride (Merck)
9. Imidazole (Merck)
10. Mercury II chloride (BDH, England)
11. 1, 4, Dioxan (BDH, England)
12. Ampicillin Trihydrate pure powder (From food and Drug Administration (FDA) Nigeria)
13. Drug samples: A total of 51 samples of ampicillin and ampicillin/cloxacillin preparations taken from 14 different batches and 5 different companies.

2.2 METHODS

2.2.1 MICROBIOLOGICAL ASSAY

2.2.1.1 INTRODUCTION:-

While the British pharmacopeia (BP) 1988 does not recognise the microbiological method of assay for ampicillin, the USP insists that the microbiological method is the only reliable and acceptable method for the assay of this antibiotic among all other methods. Since most of the products commonly used are purportedly assayed using the USP recommended method, it is only reasonable that the same method be used (among other methods) in testing the manufacturers claims of the potency of their products.

Different methods and designs are used for microbiological assay but no matter the method or design, the underlining principle is basically the same - that of comparing the activities of the known (standard) with the unknown (sample).

2.2.1.2 SELECTION OF INDICATOR ORGANISM

In the absence of Sarcina lutea ATCC 9341 recommended by the USP, efforts were made to find an acceptable standard organism which will be suitable for the microbiological assay of ampicillin. Official books (USP 1980 and BP 1988) recommend that other organisms different from those specified for a particular agent can be used as long as they are susceptible to the agent and show good growth. Since this work also intends to assay the level of ampicillin in combined preparations with cloxacillin, the organism to be selected in addition must be selectively susceptible only to ampicillin and not to cloxacillin.

Different organisms were grown in the shaker bath at 120 throws per minute and monitored by turbidity measurement until they

entered the logarithmic phase. Different concentrations of the standard ampicillin trihydrate powder were tried on the organisms for their susceptibility using the cup plate method. The same was carried out using pure cloxacillin trihydrate powder to confirm the non-susceptibility of the organism to be selected to cloxacillin.

Pure ampicillin powder inhibited the growth of *Bacillus megatarium* NCTC 10342 A76 showing least measurable zone of inhibition from a concentration of 10 $\mu\text{g/ml}$ while the same organism was resistant to pure cloxacillin powder upto a concentration of 100 $\mu\text{g/ml}$.

2.2.1.3 INOCULUM STANDARDIZATION

The inoculum was standardized to ensure that equal number of the selected organism was used during every assay in compliance with the requirement that the conditions of the assay must be strictly comparable.

For the assay, 1 ml of an overnight broth culture of *Bacillus megatarium* NCTC 10342 A76 in nutrient broth containing 1 million cells was used. This was obtained thus. The selected indicator organism *B. megatarium* NCTC 10342 A76 was grown in a sterile nutrient broth overnight at 37°C in a static condition. The organism was washed once with sterile nutrient broth and the optical density (O.D) reading at a wavelength of 470nm (O.D₄₇₀) using a spectrophotometer. The nutrient broth culture in a conical flask was put in the water bath shaker and shaken at 120 throws per minute. The OD₄₇₀ readings of the culture were taken at 30 minutes intervals. The culture was appropriately diluted when necessary before the OD₄₇₀ reading was taken. At each OD₄₇₀ reading, viable counts were taken after appropriate serial dilutions

of the culture and plating out on nutrient agar plates.

The plates were then incubated at 37° for between 18 - 24 hours. A graph of OD_{470nm} against cfu/ml was plotted. The OD₄₇₀ of the culture which contains 1 million cells per ml was thus obtained by extrapolation from the graph (see fig. 10).

2.2.1.4 SAMPLING

Each product tested was bought only after it was confirmed that the samples were found in about 80% of the shops visited. A total of six types of ampicillin and ampicillin cloxacillin from 5 companies were tested. From each product, 3 batches were selected (except products of company E where only two batches were tested). From each batch of products of the companies, 3 bottles were selected randomly (in most cases from different shops).

Total number of batches tested were 15 while the total number of bottles were 51. All the samples were tested when the active ingredients were still within their lifespan.

2.2.1.5 PREPARATION OF STANDARD TEST DOSES

Pure ampicillin trihydrate powder obtained from FDA Nigeria was used as standard. Three concentrations of 10, 20 and 40µg were prepared on the day of the assay using sterile 0.1M phosphate buffer PH 8(USP) as diluent from a stock solution of 100µg/ml bearing in mind that 1.15g of pure ampicillin trihydrate contains 1g of ampicillin e.g for making the stock solution of 100µg/ml, 0.115g of the ampicillin trihydrate powder was dissolved in 100 mls of water. Each stock solution so prepared was used up within one week of its preparation while being stored in the refrigerator.

2.2.1.6 PREPARATION OF SAMPLE TEST DOSES

Samples used were ampicillin and ampicillin/cloxacillin suspensions obtained commonly from pharmacy shops in Zaria and Kaduna. They were reconstituted into suspensions according to the instructions of the manufacturers. Three concentrations of 10, 20 and 40µg/ml of the samples were prepared on the day of the assay using sterile 0.1M phosphate buffer PH 8 (USP) as the diluent.

2.2.1.7 ASSAY PROCEDURE

For the assay, the agar diffusion method was used. The 6 x 6 (3 + 3) dose level latin square design using large assay plates was employed. (Table 1).

Two hundred millilitre of sterile nutrient agar (oxoid) prepared according to manufacturers instructions were poured into a sterile large assay plate and allowed to set. This is the basal layer.

TABLE 1

A REPRESENTATION OF THE 6x6(3+3)
3 DOSE 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	T ₂	T ₃	S ₁	S ₂
T ₁	T ₂	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 stands for Standard, T stands for Sample

S₁ = 10µg/ml

S₂ = 20µg/ml

S₃ = 40µg/ml

T₁ = 10µg/ml

T₂ = 20µg/ml

T₃ = 40µg/ml

Another 200 millilitre (ml) sterile nutrient agar inoculated with 1ml of the test culture of OD₄₇₀ of 0.17 (this contains approximately 1 million cells) i.e. 10^6 cells per ml was poured on top of the basal layer aseptically. This is the seeded layer and it was also allowed to set. Using a sterile No. 4 Cork borer, 36 cups were cut in the agar in a random manner referred to as the latin square design. Twenty microlitre (20 μ l) of each concentration (10,20 and 40 μ g/ml) of the agents were applied into each cup as shown in table 1, the plates were left for two hours to allow for effective diffusion. The plates were then incubated at 37°C for between 16 - 24 hours.

Zones of inhibition produced by both the standard and test concentrations including the holes were measured to the nearest millimetre using a pair of dividers and ruler. The experiment was repeated at least once for the content of each bottle and the average result taken.

2.2.2 SPECTROPHOTOMETRIC ASSAY2.2.2.1 DETERMINATION OF AMPICILLIN IN
COMBINED PREPARATIONS WITH CLOXACILLIN

Ampicillin and cloxacillin are semi-synthetic penicillins which share very close similarity in their structures due to the presence of β -lactam and thiazolidine rings.

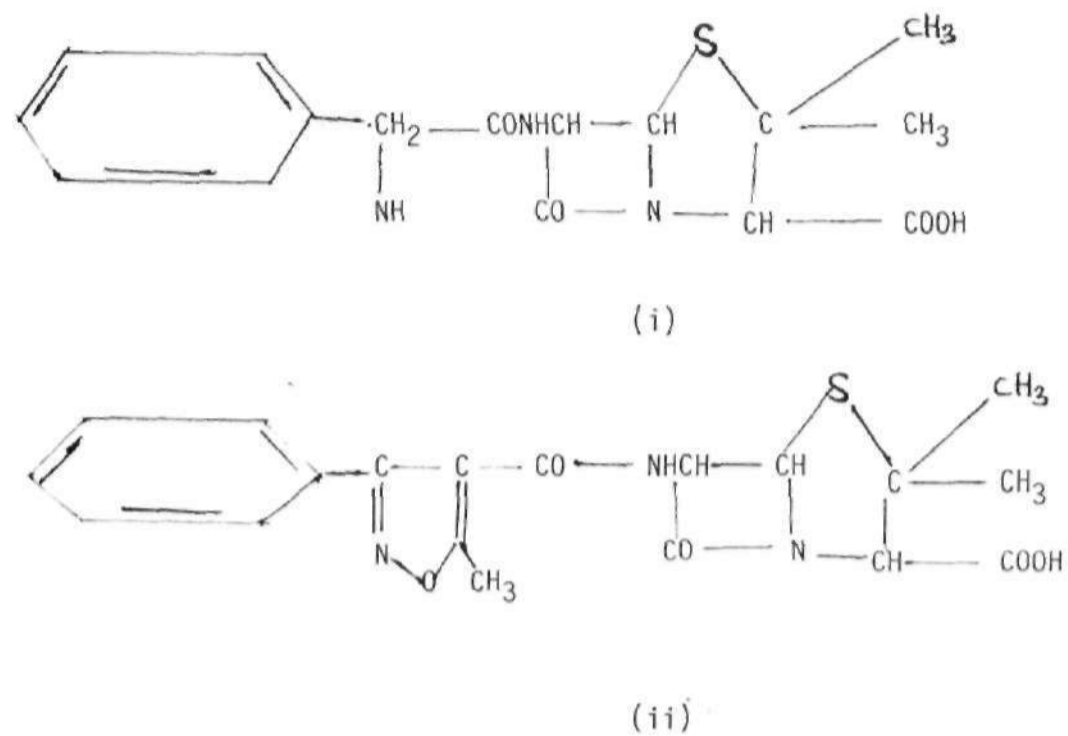


Fig. 6

Structures of (i) Ampicillin and (ii) cloxacillin showing their close structural similarity

This explains why the design of the methods for their analysis in combined preparations must be done with a very high degree of selectivity in mind. Presently, the component penicillins in combinations of ampicillin and cloxacillin are determined only microbiologically after separation by gel electrophoresis (Davidson and Stenlake 1973). Penicillanic and penicilloic acids, the acid and base hydrolysis products respectively of penicillins have been found to react with formaldehyde to give a pyrazinone derivative in acidic medium (Ayim 1973). Akanni and Ayim (1990) concluded that these derivatives so formed could form a basis for the spectrophotometric determination of ampicillin in the presence of cloxacillin.

Davidson and Stenlake (1973) attempted the determination of ampicillin in the presence of cloxacillin by measuring the absorbance of the combined preparations at 268nm. Akanni and Ayim (1990) however reported that the max of 268nm where Davidson and Stenlake (1973) measured their absorbance of penicillin is subject to interference from cloxacillin and other additives in pharmaceutical preparations. They, therefore, suggested a wavelength of 373nm where they confirmed that there will be no interference from cloxacillin and other additives.

The method of Akanni and Ayim (1990) was used for measuring the ampicillin content in ampicillin/cloxacillin combination. This involves:

1. Obtaining a calibration curve for standard ampicillin
2. Determination of ampicillin in ampicillin/cloxacillin oral preparations.

2.2.2.2 CALIBRATION CURVE FOR STANDARD AMPICILLIN

The calibration curve for ampicillin was drawn using results obtained from reactions of ampicillin at concentrations 18 μ g - 91 μ g. This was carried out thus.

A stock solution containing 1 mg/ml of standard ampicillin was made and 2, 4, 6, 8 and 10ml aliquots of the stock were taken into separate flasks and hydrolysed using 2ml of 2M sodium hydroxide. After 20 minutes, 1ml of 2M sulphuric acid was added to each mixture. 50mls of formaldehyde containing phosphate buffer PH 2.5 was added to each flask and heated for 1 hour at 100°C. Each of the flasks so heated was designated solution A.

Equivalent amounts of aliquots (2, 4, 6, 8 and 10mls) of the same 1mg/ml stock solution of standard ampicillin were taken in another set of flasks. Each sample was reacted with 50ml formaldehyde containing phosphate buffer (PH 2.5) i.e. the ampicillin solution here was not hydrolysed. The flasks were designated solution B each. A 1:2 dilution of corresponding solution A and B were made with 2M sulphuric acid and the absorbance of solution A was measured at the wavelength 373nm using solution B as reference.

The absorbance readings so obtained were used with the Corresponding Concentrations to plot a calibration curve for ampicillin.

2.2.2.3 DETERMINATION OF AMPICILLIN IN AMPICILLIN/ CLOXACILLIN ORAL PREPARATIONS

A stock solution of ampicillin/cloxacillin oral preparation containing 1mg/ml of ampicillin was made from reconstituted samples of concentration of 250mg/5ml. Five (5) ml aliquots of the stock solution was then used to prepare reaction mixtures

to make solutions A and B as was done during the calibration.

A 1:2 dilution of corresponding solutions A and B were made using 2M sulphuric acid and the absorbance of solution A determined with solution B as reference. This procedure was strictly followed for all the preparation tested by this method. The absorbance due to each sample is compared with that due to 5mls of the standard ampicillin in Calibration Curve.

2.2.3 CHEMICAL ASSAY

There seems to be a strong argument between chemists and Biologists on which of the methods for assay of antibiotics is better and more reliable (Hugo and Russel 1977), (Akanni and Ayim 1990), (Hewitt 1977). Even standard reference books (USP and BP) take different stands on which of the methods (chemical or biological) takes precedence in the case of some quantities e.g. Ampicillin. This has actually made it necessary to employ the chemical assay method in assaying some products of Companies A, B and C alongside microbiological method. The method used here is strictly that recommended by British pharmacopeia (1988).

2.2.3.1 ASSAY PROCEDURE

0.17g of ampicillin trihydrate standard equivalent to 0.15g of ampicillin was dissolved in 500ml of distilled water. This was shaken for 30 minutes using an electric shaker. The solution was then filtered using a sintered glass number 0 under pressure.

10ml of the resulting solution was transferred to a 100ml graduated flask and 10ml of boric acid buffer PH 9.0 added. 1ml of acetic anhydride - Dioxan solution was added, the whole content of the flask was allowed to stand for five minutes and sufficient water added to produce 100ml.

Two 2ml aliquots of this solution were pipetted into 2 separate stoppered tubes A and B. To one tube, 10ml of Imidazole - mercury reagent was added. The tube was then mixed, stoppered and immersed in a water bath at 60°C for twenty-five minutes with occasional swirling.

After twenty-five minutes, the tube was removed from the water bath and cooled rapidly in ice to 20°C and the whole content designated solution A. To the second stoppered tube B, 10ml of water was added and it was designated solution B. Immediately, the absorbance of solution A and B were measured at 325nm using a mixture of 2ml of water and 10ml of Imidazole - mercury reagent as blank for solution A and water for solution B.

For the market samples, 6mls of the reconstituted suspension (125mg/5ml) which contains 0.15g of ampicillin was transferred to 500ml of distilled water, shaken for 30 minutes using an electric shaker and filtered under pressure using the sintered glass filter number 0. The resulting solution was subjected to the whole process the standard solution went through until the absorbance readings of solution A and B were measured at 325nm.

The content of ampicillin was calculated from the difference between the absorbance of solution A and that of solution B and from the difference obtained when 0.17g of the standard was used. The content of ampicillin in the standard powder used was considered to be 100%.

2.2.4 THIN LAYER CHROMATOGRAPHY (TLC)

Thin layer chromatography was carried out using the method described by the BP 1988. It was used as a means of identifying the presence of ampicillin in the market samples assayed.

A silica gel precoated chromatoplate was sprayed with a 0.1% w/v solution of disodium edetate in a 5% w/v solution of dihydrogen orthophosphate. The plate was allowed to dry in air and left in the oven at 105°C for 1 hour.

A quantity of the ampicillin trihydrate which contains 0.125g of ampicillin was weighed out of each sample and diluted with 100 ml of phosphate buffer PH 7.0 (BP 1988) and designated solution 1 each. 0.14% w/v of the standard ampicillin trihydrate (FDA Nigeria) was made in phosphate buffer PH 7.0 (BP 1988) and designated solution 2. 2.1µl of solution 1 from each sample was spotted on the chromatoplate. Same volume of solution was also spotted.

The plate was then introduced into a chroma-tank which had mixture of 50 volumes of butylacetate 30 volumes of glacial acetic acid, 10 volumes of a 0.1% w/v solution of disodium edetate in a 5% solution of sodium dihydrogen orthophosphate and 5 volumes of butan -1- ol as the mobile phase.

The plate was dried in air after removal from the tank. It was then put in the oven at 105°C for 15 minutes and then sprayed with a mixture of 100 volumes of starch mucilage, 6 volumes of glacial acetic acid and 2 volumes of 1% w/v solution of iodine in 4% w/v solution of potassium iodide.

2.2.5 INFRA-RED SPECTROSCOPY

Infra-red Spectroscopy was carried out as a means of ascertaining the presence or absence of ampicillin in the samples. This was achieved by observing the presence or absence of characteristic peaks in the spectrogram.

A little quantity of the sample (in solid form) was finely ground in a small mortar with a few drops of liquid hydrocarbon (Nujol). The mull was then pressed between flat plates of sodium chloride and fed into a perkin Elmer infra-red spectrophotometer. The spectrum for each sample so treated was produced by the instrument within a time of 3 minutes.

CHAPTER 3

3.1

SELECTION OF INDICATOR ORGANISM

Bacillus megatarium NCTC 10342 A76 was selected as the indicator organism on an account of its selective sensitivity to ampicillin and not to cloxacillin as well as showing least measurable zones of inhibition from a concentration of 10µg/ml.

The sensitivity of the test organism to the standard ampicillin is shown in Table 2 below:

TABLE 2
SENSITIVITY OF B. megatarium TO THE
STANDARD AMPICILLIN

Concentration of agent in resevoir µg/ml	Log of Concentration of agent in resevoir	Zone of Inhibition (Hole Inclusive) mm (x)*	Distance from Agar Interphase to Zone Boundary (y)	y ²
10	1.0	12.0	5.0	25.0
20	1.30	15.0	8.0	64.0
30	1.47	17.0	10.0	100.0
40	1.60	18.0	11.0	121.0
50	1.69	22.0	15.0	225.0

*Reservoir width = 7.0

LOG DOSE AGAINST MEAN ZONE
DIAMETER SHOWN BY PURE
AMPICILLIN

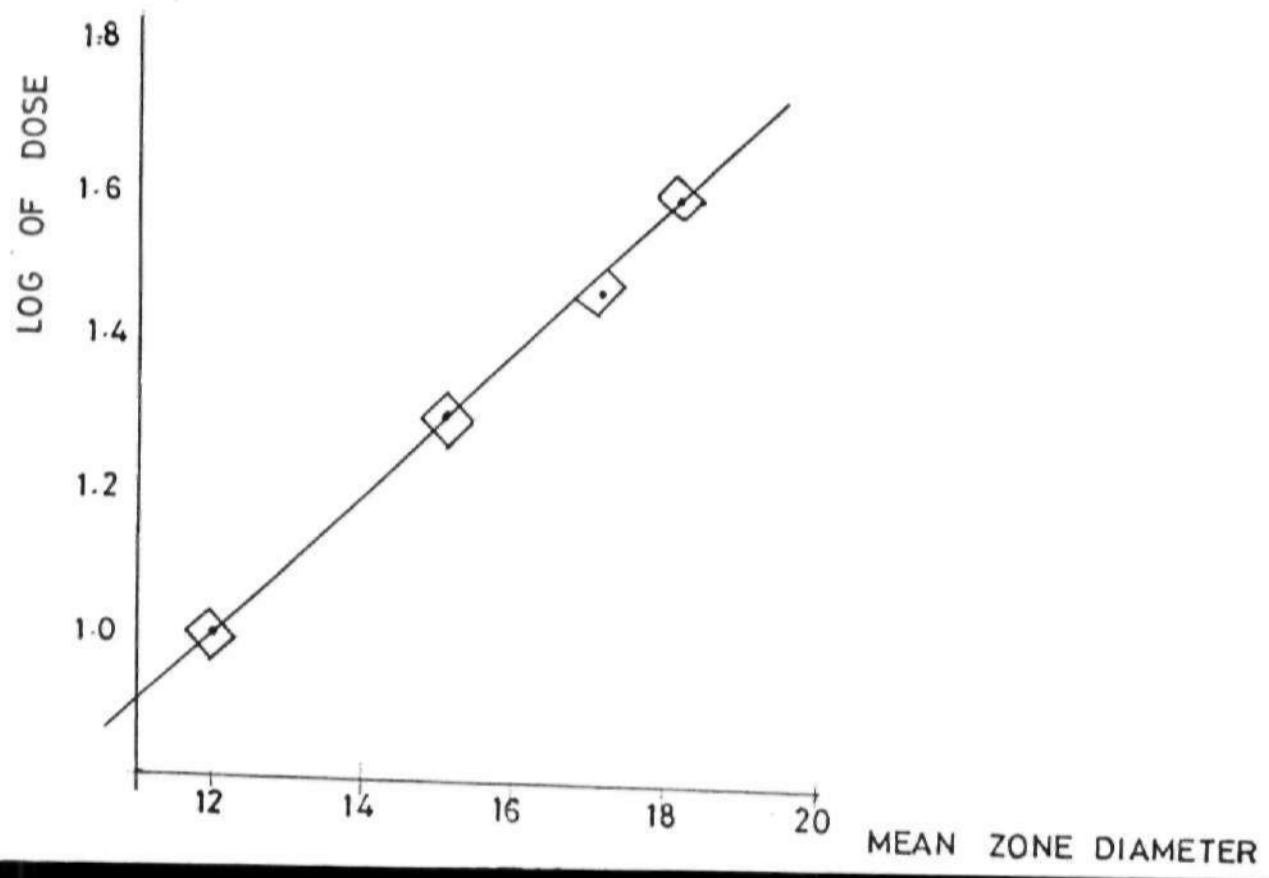


FIG. 8 GROWTH CURVE OF B. MEGATARIUM

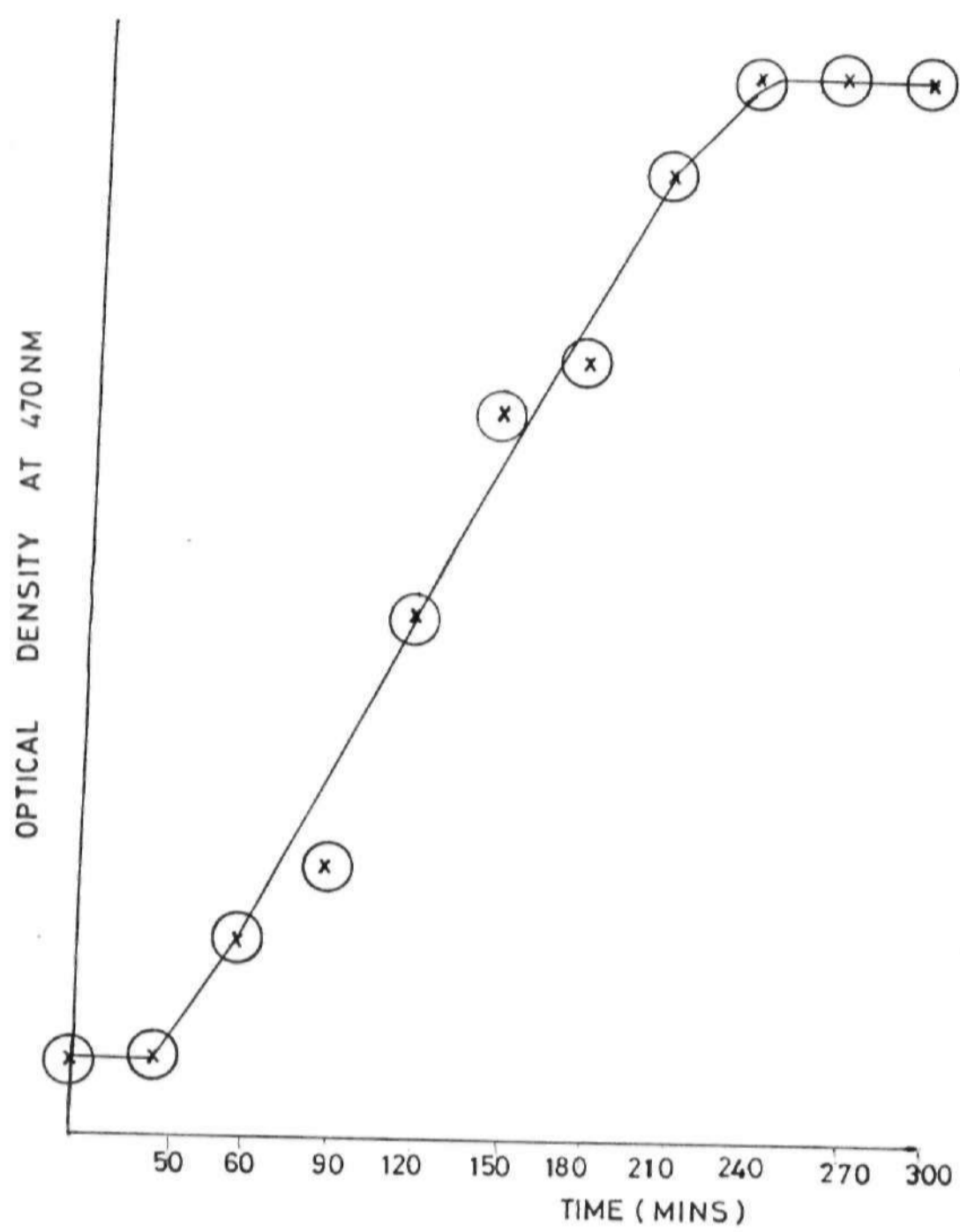
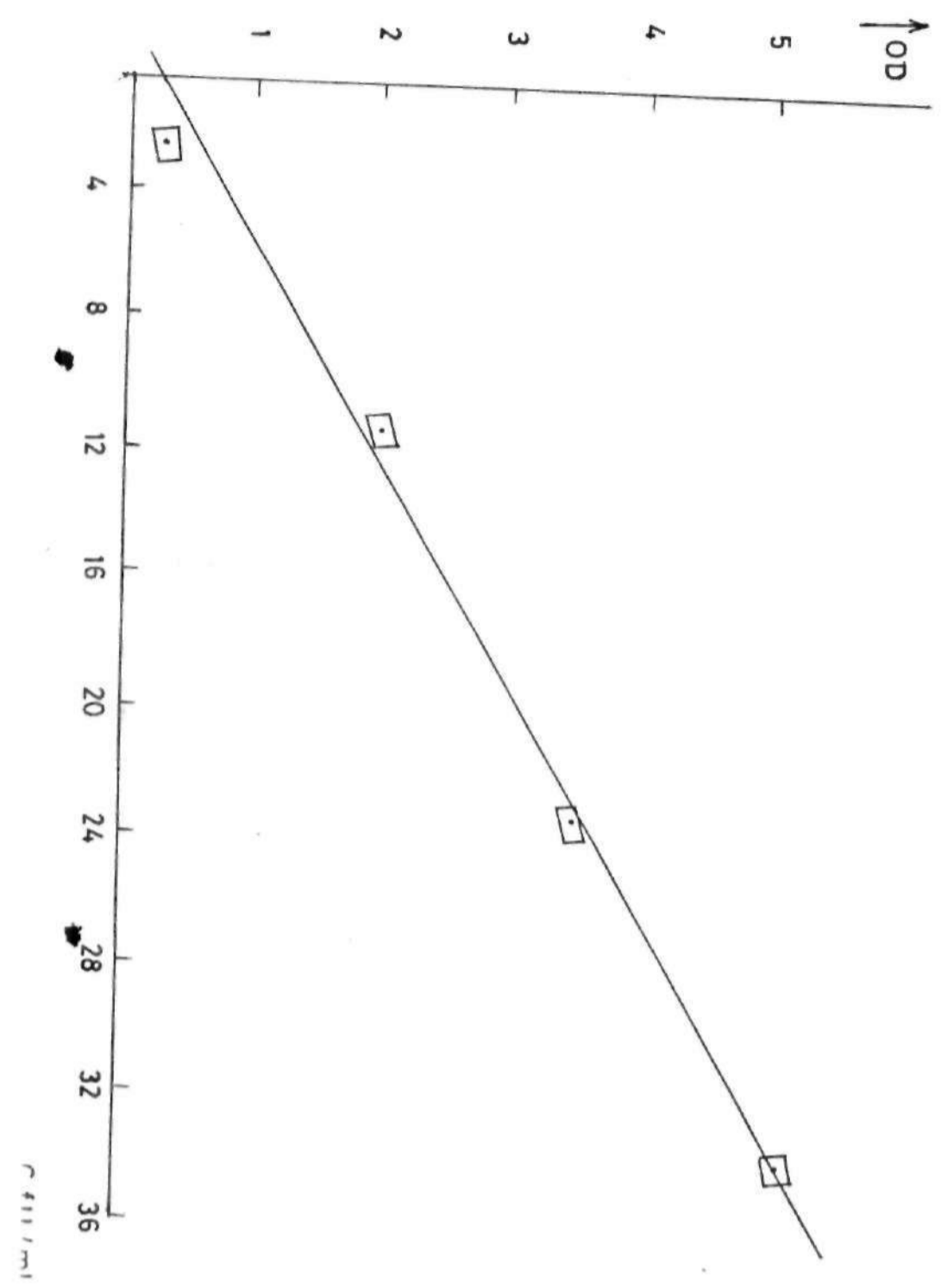
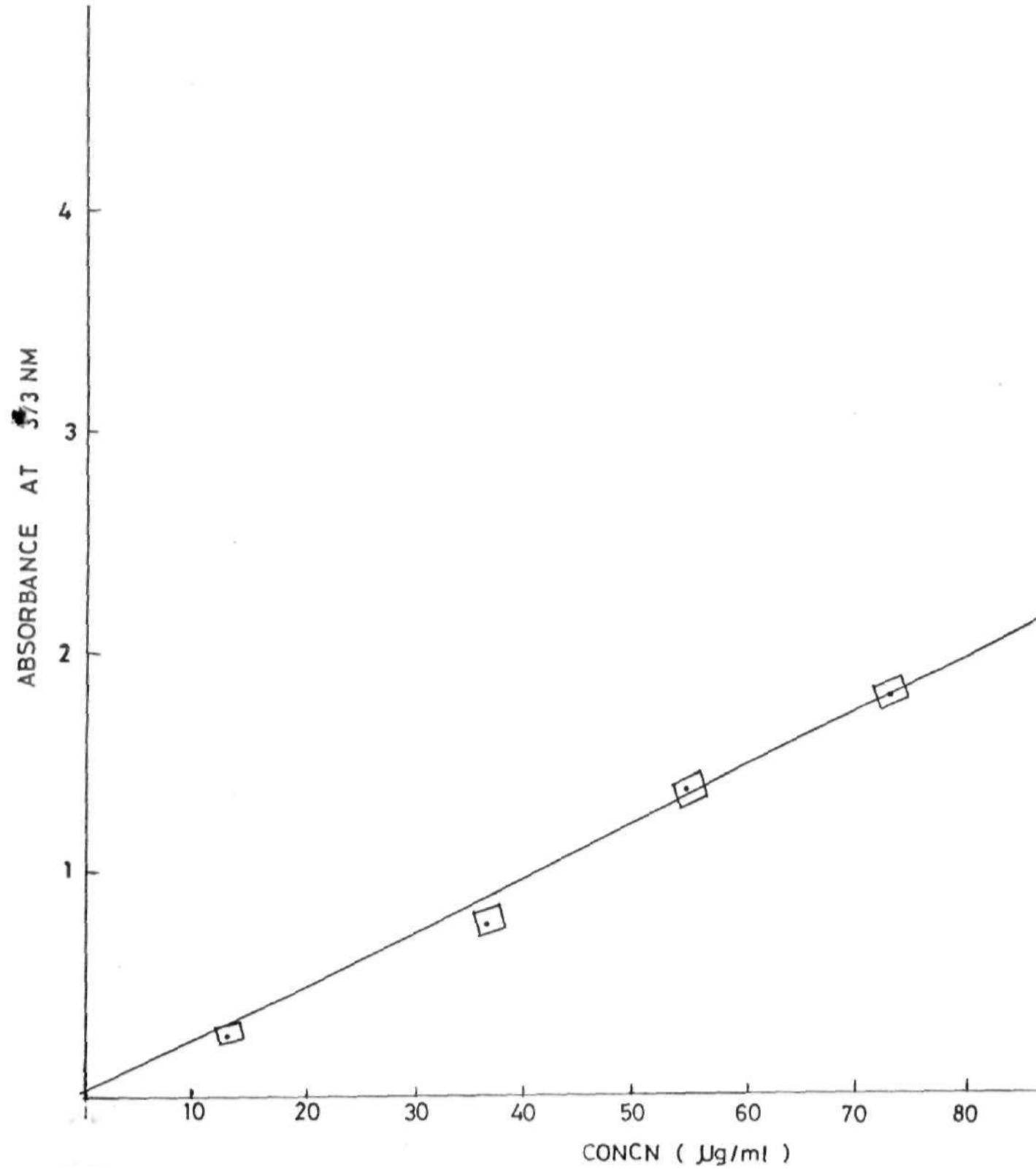


FIG.9 STANDARDIZATION OF B. MEGATARIUM





The table shows the concentration of standard ampicillin used and the corresponding zones of inhibition. The relationship between the log concentration and zones of inhibition is shown in figure 7.

3.2 STANDARDIZATION OF THE ORGANISM AND PREPARATION OF GROWTH CURVE

The Indicator organism B. megatarium NCTC 10342 A76 grows reasonably well. Under the conditions of standardization, it grew up to an O.D of about 8.0 before entering the stationary phase (fig. 8). A plot of OD against cfu/ml shows that a broth culture of OD.17 contains 1 million cells per ml (fig. 9).

3.3 CALIBRATION CURVE FOR STANDARD AMPICILLIN

The result of the absorbance readings of the concentrations of standard ampicillin (18µg/ml - 91µg/ml) used for the calibration curve is shown in Table 3 below:

TABLE 3

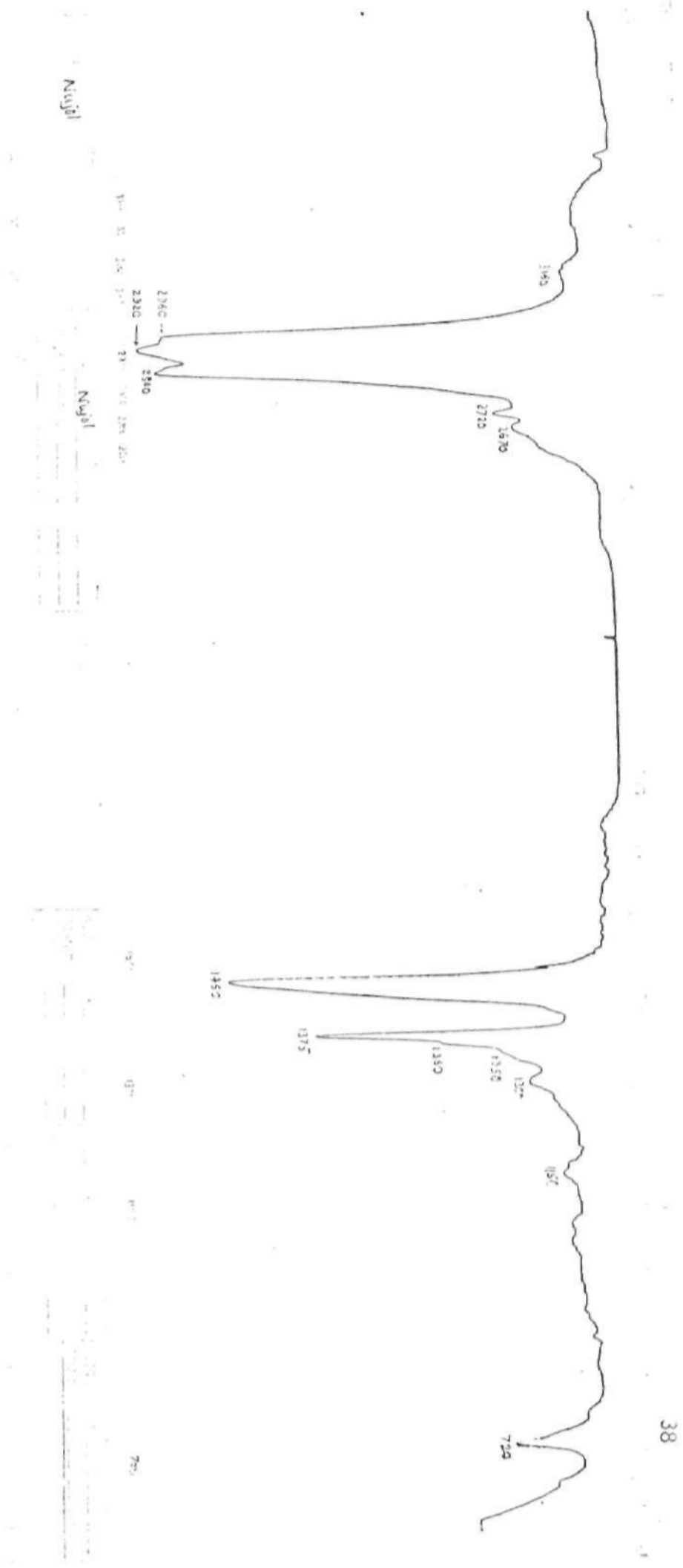
Table showing absorbance of standard ampicillin at different concentrations

Volume of Standard*	Concentration µg/ml	Absorbance 373nm
2	18.2	0.4
4	36.4	0.8
6	54.6	1.4
8	72.8	1.9
10	91.0	2.4

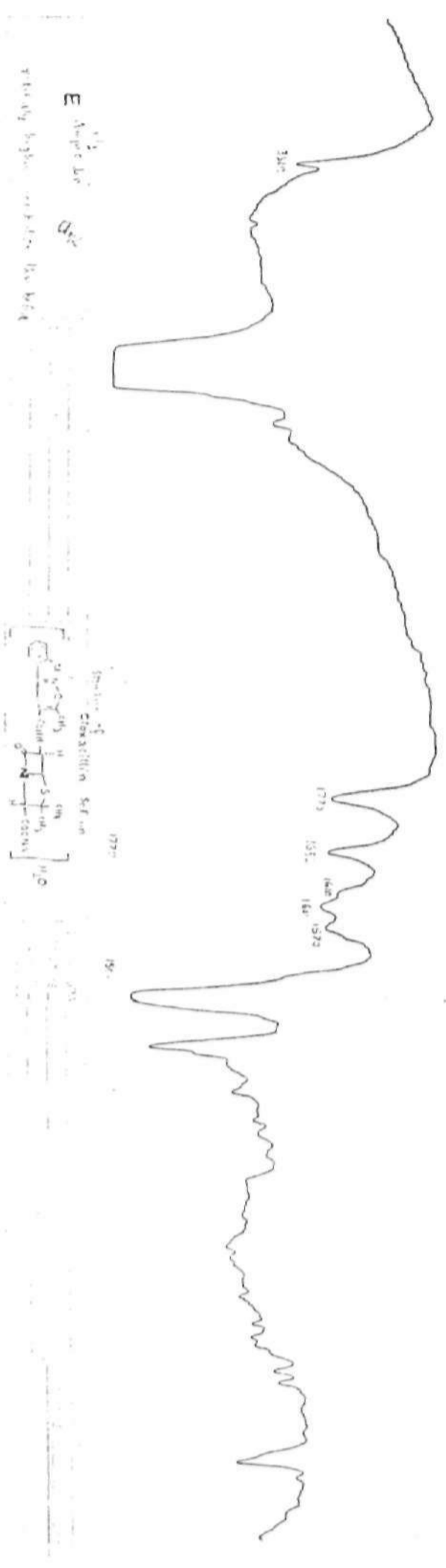
The calibration graph of the standard ampicillin using the concentrations 18 μ g - 91 μ g and the absorbances obtained at 373nm are shown in (figure 10 and Table 3). The calibration gave a straight line graph of reproducible linearity.

3.4 IDENTIFICATION OF AMPICILLINKEY TO I.R. SPECTRA

C	=	COMPANY	B(1)	PRODUCT
AM 8923	=	"	C	"
B	=	"	E	"
A	=	"	B(2)	"
E	=	"	A	"
AM 8924	=	"	C	"
AM 8922	=	"	C	"
F AND G	=	REFERENCE STANDARD (FDA NIGERIA)		







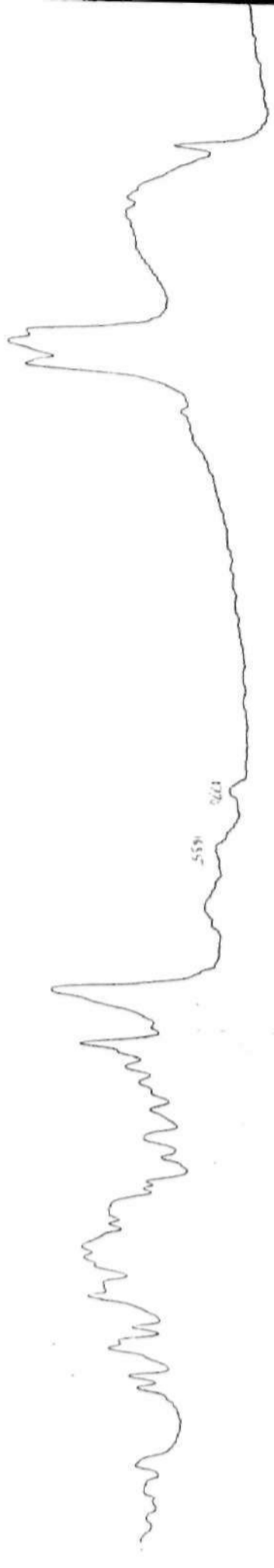
118024

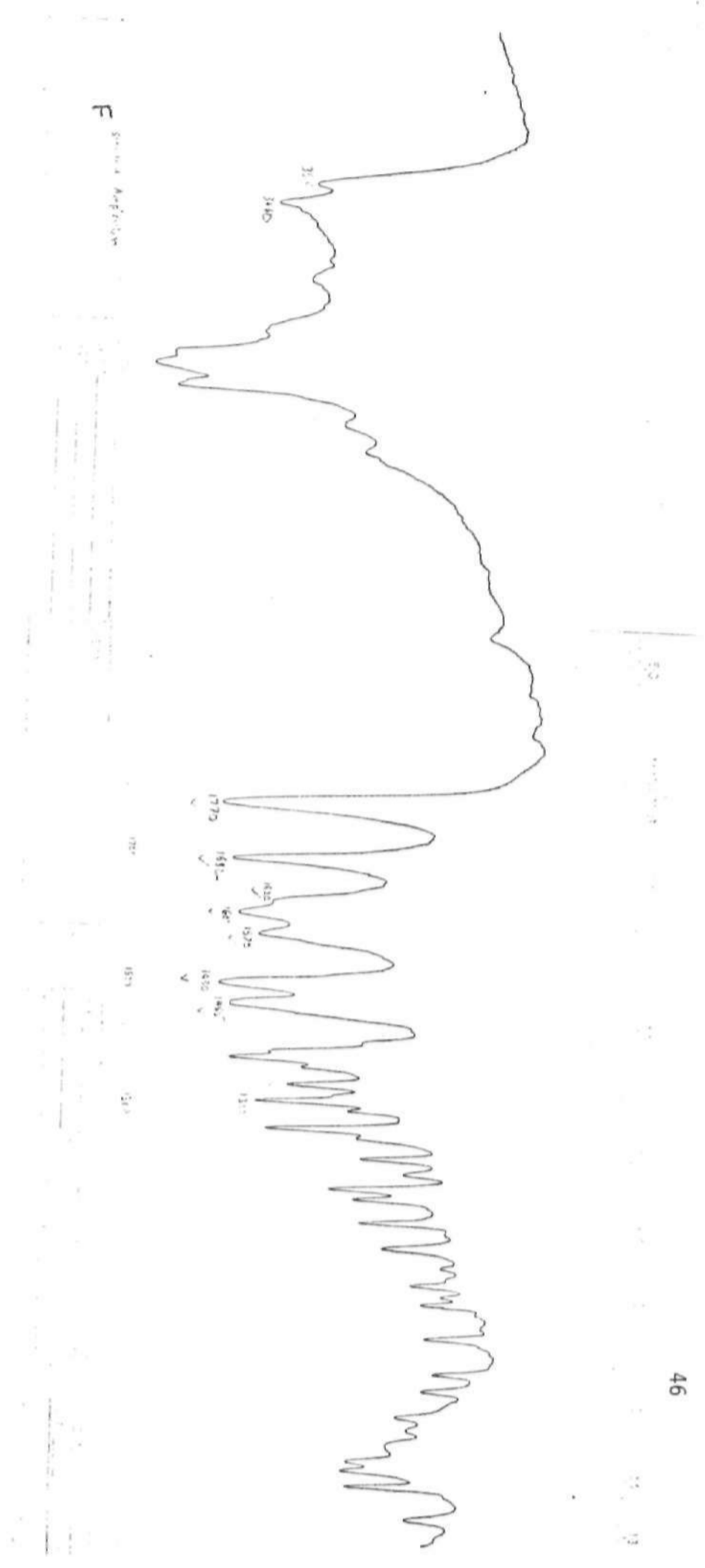
Nigel

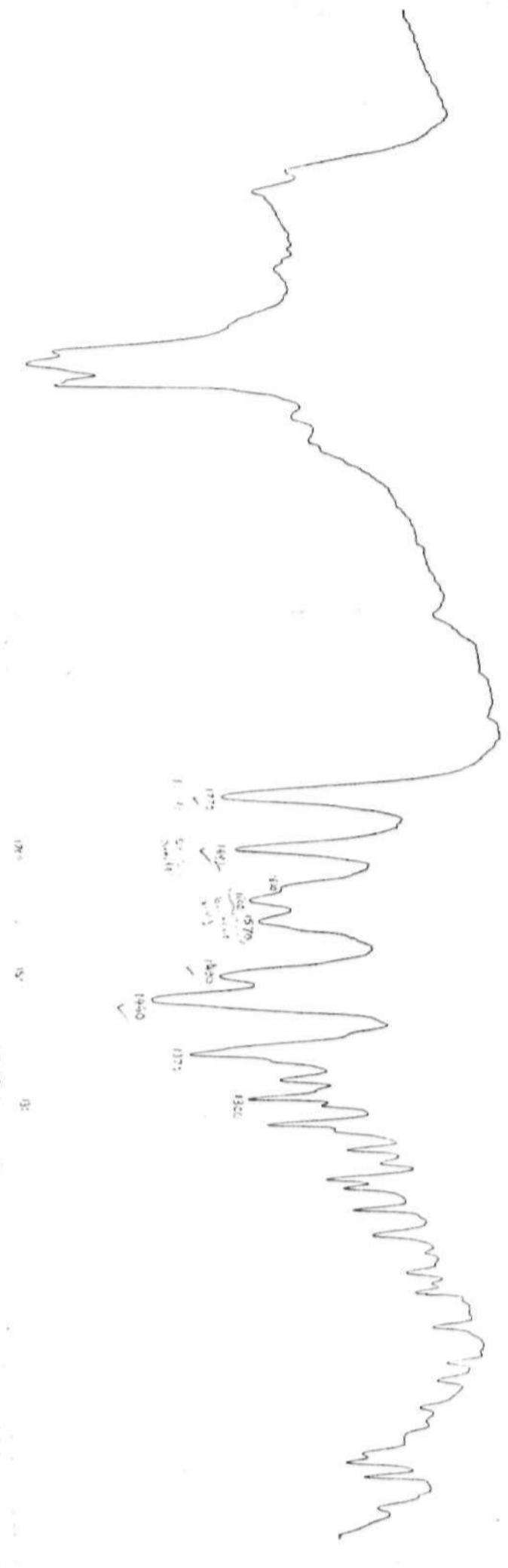
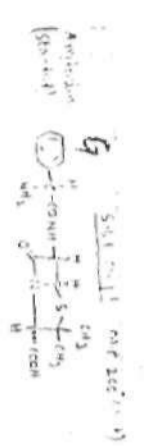
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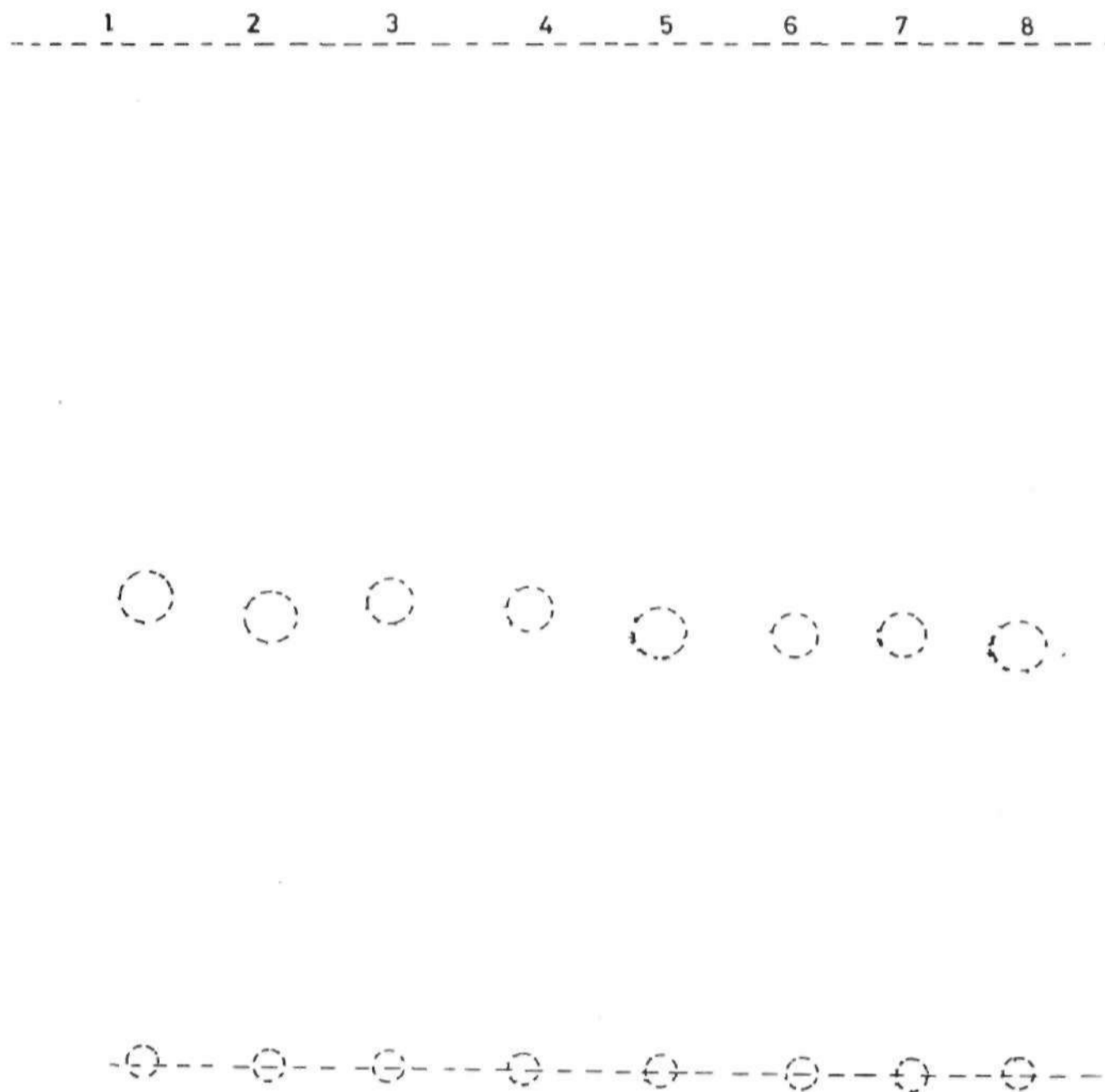


KEY TO TLC CHROMATOGRAM

1 = C = COMPANY B PRODUCT
2 = AM 8923 = " C "
3 = B = " E "
4 = A = " B "
5 = E = " A "
6 = AM 8924 = " C "
7 = AM 8922 = " C "
8 = Reference Standard (FDA Nigeria)

FIGURE

A REPRESENTATION OF THE CHROMATOGRAM OBTAINED FROM THE THIN LAYER CHROMATOGRAPHY IS AS SHOWN



COMMENTS

The need to select an indicator organism different from that recommended by the USP (1980) arose due to the non-availability of the recommended standard organism. The organism so selected *B. megatarium* NCTC 10342 A76 showed reasonable sensitivity to the agent being assayed (Table 2). This is in conformity with the BP 1988 on the criteria for selecting alternative indicator organisms for assay in the absence of that recommended by reference books. It also showed very good growth under the conditions at which it was grown. This was shown by its ability to grow up to an O.D reading of about 8.0 before entering the stationary phase.

An increase in the concentration of an agent gives a corresponding increase in the diameter of the zone of inhibition due to it. A logdose - response graph should therefore give a straight line. Most potency calculation procedures (including the one used for this work) is based on this. A graph of this relationship considering the agent and the organism used for this work is a perfect straight line (figure 7).

Up to a concentration of 91µg/ml, the corresponding OD_{470nm} readings obtained using the spectrophotometric method showed a linear relationship with concentration (figure 10). This means that up to this concentration and using the method of Akanni and Ayim (1990) ampicillin level can still be determined by absorbance readings. The result agrees with that of Akanni and Ayim (1990) and goes further to show that even at higher concentrations (up to 91µg) a linear relationship between absorbance and concentration could still be obtained.

The I. R spectrum of the standard ampicillin (FDA Nigeria) used as a reference standard superimposed that of another working standard obtained from an independent source. The both showed peaks which are very characteristic of ampicillin e.g. peaks were observed at the following wavelenghts:

1770 cm^{-1} , 1682 cm^{-1} , 1600 cm^{-1} , 1570 cm^{-1}
1370 cm^{-1} , 1308 cm^{-1} (BPC 1973).

The fact that the two reference standards obtained from two different sources gave the same I.R spectra characteristic of ampicillin confirm that the working standard used is indeed ampicillin.

The 1570 cm^{-1} and 1600 cm^{-1} peaks are shown by most six membered aromatic ring systems like benzenes (William and Fleming 1980). These two peaks constitute a valuable identification of the benzene ring in the spectrum of the (reference) ampicillin. The 1770 cm^{-1} peak represents the Imide group while the secondary amide group is represented by the 1682 cm^{-1} peak and the characteristic gem-dimethyl group is shown by the peak on 1370 cm^{-1} (William and Fleming 1980).

Representative samples of all the company products assayed shown characteristic peaks which indicate the presence of the Imide, the secondary amide groups and the benzene ring which all go to confirm the presence of ampicillin in the samples.

The characteristic peaks which is supposed to confirm the presence of ampicillin B appear very small. Some factors may affect the length of these peaks and this include the quantity of the sample which was used for running the spectrum. However, the presence of the peaks is very important to confirm the presence of ampicillin in the sample however small. Like in all the other samples however,

the length of the peak cannot be used to quantify the ampicillin because it was actually intended to be used for ampicillin presence identification only.

The TLC result also confirms the presence of ampicillin in all the samples. This is shown by the presence of a principal spot due to each of the samples on the chromatogram which corresponds to that due to the standard (figure 21) (BP 1988) and the R_f values which are almost equal to the standard.

CALCULATION OF POTENCY

The following method recommended by the BP 1985 was used for the computation of the potency of the sample of suspension in relation to the manufacturers claim considering the zones of inhibition observed.

E = Mean difference due to the highest and lowest doses

$$= 1/4 (T_3 - T_1) + (S_3 - S_1)$$

F = Mean difference between standard and test

$$= 1/3 (T_1 + T_2 + T_3) - (S_1 + S_2 + S_3)$$

I = Log ratio of doses (used)

$$= \text{Log } 2 = .3010$$

b = Slope = $E/I = E/0.3010$

M = Log of potency ratio (test/standard) = F/b

Percent potency of sample = antilog $M \times 100$

where S_1 = Mean of inhibition zone diameter in mm due to S_1

S_2 = " " " " " " " " " " S_2

S_3 = " " " " " " " " " " S_3

T_1 = " " " " " " " " " " T_1

T_2 = " " " " " " " " " " T_2

T_3 = " " " " " " " " " " T_3

For this work the following doses were used:

Standard (S) $S_1 = 10\mu\text{g}$, $S_2 = 20\mu\text{g}$, $S_3 = 40\mu\text{g}$

Sample (T) $T_1 = 10\mu\text{g}$, $T_2 = 20\mu\text{g}$, $T_3 = 40\mu\text{g}$

Using the method recommended by BP 1988,

a typical result is shown below in Table 4

(This is the result of bottle 1, Batch 1, Company A)

TABLE 4

ZONES OF INHIBITION PRODUCED BY STANDARD
AND SAMPLE TEST DOSES OF AMPICILLIN

STANDARD (mm)			SAMPLE (mm)			
S_1	S_2	S_3	T_1	T_2	T_3	
11	15	19	11	15	18	
11	14	20	11	15	18	
11	14	19	11	15	18	
11	15	19	11	15	18	
11	14	20	11	15	18	
11	15	20	11	15	18	
MEAN:-	11.0	14.5	18.0	11.0	15.0	18.0

CALCULATIONS:-

$E =$ Mean difference due to the highest and lowest doses

$$= \frac{1}{4}(18 - 11) + (18 - 11.5)$$

$$= 7.0 + 6.5 = \frac{13.5}{4} = 3.37$$

$$\begin{aligned}
 F &= \text{Mean difference between Standard and Test} \\
 &= 1/3(T_1 + T_2 + T_3) - (S_1 + S_2 + S_3) \\
 &= 1/3(11 + 15 + 18) - (11 + 14.5 + 18) \\
 &= 1/3(44 - 43.5) = \frac{.5}{3} = 0.16
 \end{aligned}$$

$$\begin{aligned}
 I &= \text{Log ratio of doses} \\
 &= \text{Log } 2 = 0.3010
 \end{aligned}$$

$$b = E/I = \frac{3.37}{0.3010} = 11.19$$

$$\begin{aligned}
 M &= \text{Log of potency ratio } T/S = F/b \\
 &= \frac{0.16}{11.19} = 0.014
 \end{aligned}$$

$$\begin{aligned}
 \text{Percentage potency of Sample} &= \text{Antilog } M \\
 &= 103.3\%
 \end{aligned}$$

Showing that the ampicillin content of the Sample assayed is 103.3% of the manufacturers claim i.e. the Sample contains 103.3% of the quantity (125mg/5ml) claimed by the manufacturers. This conforms with the BP 1988 standard which recommends percentages between 80 - 120 and USP which recommends 90 - 110.

These results are relative to the standard ampicillin potency which is taken to be 100%.

All results shown are average computations from at least two assay readings.

Note:- The potency of the market Sample of ampicillin Suspension Samples assayed is claimed to be 125mg/5ml by the manufacturers. This was found on all the bottle labels.

TABLE 5 The percentage of ampicillin syrup from Companies A, B and C obtained by microbiological assay methods is show in Table 5 below

PERCENTAGE POTENCY OF AMPICILLIN
SYRUP FROM COMPANIES A, B AND C.

	COMPANY A		COMPANY B		COMPANY C	
	Calculated % Potency	Equivalent Concentration $\mu\text{g}/5\text{ml}$	Calculated % Potency	Equivalent Concentration $\mu\text{g}/5\text{ml}$	Calculated % Potency	Equivalent Concentration $\mu\text{g}/5\text{ml}$
BATCH <u>1</u>						
1	103.3	129.12	156.30	195.0	71.60	89.50
2	97.7	122.37	161.00	201.25	59.80	74.75
3	101.8	127.25	162.18	202.72	57.0	71.25
BATCH <u>2</u>						
1	95.0	118.75	94.40	118.0	67.80	84.75
2	99.03	123.78	97.05	121.31	68.50	85.62
3	97.20	121.50	97.20	121.50	61.90	77.37
BATCH <u>3</u>						
1	100.80	126.00	105.40	131.75	63.80	79.75
2	101.10	126.37	103.10	128.87	61.80	77.25
3	104.00	130.00	101.80	127.25	60.82	76.02

All results are relative to the Standard ampicillin potency which is taken to be 100%.

Results are average computations from at least two assay readings.

Note:- The potency of the market Samples of Combinations of ampicillin and cloxacillin suspensions is claimed to be 250mg/5ml. This was found on all the bottle labels i.e. 125mg/5ml ampicillin and 125mg/5ml cloxacillin.

TABLE 6. The level of ampicillin in ampicillin/cloxacillin (expressed in %age) from Companies B₂, D and E determined by the microbiological assay method is shown in Table 6

QUALITY (PERCENTAGE POTENCY) OF AMPICILLIN IN AMPICILLIN/CLOXACILLIN COMBINATION

	COMPANY B ₂		COMPANY D		COMPANY E	
	Calculated % Potency	Equivalent Concentration µg/5ml	Calculated % Potency	Equivalent Concentration µg/5ml	Calculated % Potency	Equivalent Concentration µg/5ml
BATCH <u>1</u>						
1	104.80	131.00	89.20	111.50	91.80	114.75
2	97.80	122.25	91.16	113.95	92.50	115.62
3	102.40	128.00	90.15	112.68	90.20	112.75
BATCH <u>2</u>						
1	90.80	113.50	89.50	111.80	98.80	123.50
2	89.10	111.37	90.20	112.75	107.50	134.37
3	92.90	116.12	89.12	111.40	109.20	136.50
BATCH <u>3</u>						
1	112.20	140.25	92.60	115.75		
2	109.80	137.25	95.71	119.63		
3	108.00	135.00	103.10	128.75		

COMMENTS

The results gave information of reasonable interest.

Twelve (12) out of the 24 bottles of ampicillin suspension

tested from the three companies failed the microbiological test. Samples from batch 1 of products from Company B has higher contents of ampicillin than officially recommended (Table 5). The most probable reason for this is the lack of in-process control during the manufacture of this product. Carelessness on the part of the production staff and wrong weighing during the filling of the dry powder in the final bottle may account for this. The point stands however that quality control was compromised during the production of this batch of product. If not, the wrong concentration should have been detected and taken care of before sending the products into the market. The high content of active medicament is potentially dangerous, may be even more dangerous than when the concentration is low because this is likely to lead to a toxicity problem. Considering the immunity level of the children who use the product, this high concentration will most certainly lead to a serious complication that may cause death.

The two other batches, 2 and 3 from the same Company (B) considered, gave percentage potencies between 94 and 105, these fall within the standard recommended ranges of 80 - 120% by the BP and 90 - 110% by the USP. Interestingly, this Company is an indigeneous one whose product was found in almost all the pharmac shops visited during the sampling.

The products of Company A tested showed percentage potency within acceptable limits of both the BP and USP (Table 5).

The products of the 3rd Company (C) had so many things in common with that of B. They were made in India for a Nigerian Company which markets the products, and are commonly found in shops within Kaduna and Zaria. These same products have been reported to be widely available in many other Cities around e.g. Kano (personal communications). These products were sold for half the price of the other ampicillin suspensions found in the same shops. On inquiry, they were said to be selling faster than the other products (especially in patent medicine shops) definitely because of their low price. Their assay results showed that all nine bottles from the 3 batches fell below the USP and BP recommended level. One is tempted to believe that this product was knowingly and intentionally produced to these specifications because the concentration of ampicillin was just about half of the manufacturers claimed potency of 125mg/5ml. As much as lack of proper or even complete absence of in-process control can be adduced as a reason for this result, the cost of the product is a very strong reason to believe that they were intentionally manufactured to the specifications.

A publication of the authoritative American monthly Newsweek (November 1990) mentioned this same country where these products (that failed) came from as one of the countries where most fake and adulterated pharmaceutical products originate. The publication reported that from researches carried out, finished (fake and/or adulterated) pharmaceutical preparations including antibiotics are shipped through a series of middlemen to anywhere in the world

especially the third world countries where nobody checks what is sold. These products especially those from Company C must have come from these fakes.

The implications of giving this kind of substandard drugs to our children may be fatal. At times, when death does not result, directly, resistance to the drug (and many more) has always developed making it very difficult to cure most common infections amongst children.

The microbiological assay results of Companies B, D and E (Table 6) shows that the ampicillin content in the combination products were within the BP and USP acceptable levels of 80 - 120% and 90 - 110%. Interestingly, all the three Companies are indigeneous companies with manufacturing plants in Nigeria and the final products were all prepared locally.

TABLE 7

SPECTROPHOTOMETRIC ASSAY RESULTS OF
AMPICILLIN IN AMPICILLIN/CLOXACILLIN
COMBINATIONS FROM COMPANIES B₂, D AND E

COMPANY	B ₂			D			E		
	ABSORBANCE	CALCULATED % POTENCY	CORRESPONDING CONCENTRATION	ABSORBANCE	CALCULATED % POTENCY	CORRESPONDING CONCENTRATION	ABSORBANCE	CALCULATED % POTENCY	CORRESPONDING CONCENTRATION
BATCH 1									
1	1.20	104.3	130.37	1.10	95.60	119.50	1.03	89.50	111.87
2	1.18	102.6	128.25	1.20	104.30	130.37	1.04	90.49	113.11
3	1.20	104.3	130.37	1.10	95.60	119.50	1.08	93.91	117.38
BATCH 2									
1	1.18	102.6	128.25	1.06	92.17	115.21	1.28	113.0	141.25
2	1.14	99.0	123.75	1.10	95.60	119.50	1.30	111.10	138.87
3	1.14	99.0	123.75	1.08	93.90	117.37	1.30	113.0	141.27
BATCH 3									
1	1.28	113.3	139.12	1.16	100.80	126.00			
2	1.28	113.3	139.12	1.14	99.13	123.91			
3	1.28	111.3	139.12	1.19	103.40	129.25			

All results are average of at least three assay readings.

Note:- 5mls of the standard ampicillin gave an absorbance of 1.15

3.7 CALCULATION OF THE AMPICILLIN CONTENT
OF SAMPLES FROM COMPANIES A, B AND C
USING THE CHEMICAL METHOD

The calculation of the ampicillin content is based on the formula suggested by B.P. 1988.

STANDARD AMPICILLIN:

TUBE A	(Absorbance)	TUBE B	(Absorbance)
BLANK	= 0	BLANK	= 0
SOLUTION A	= .15	SOLUTION B	= .02

$$\begin{aligned} \text{Difference in absorbance of solution B from A} \\ = 0.15 - 0.02 = .13 \end{aligned}$$

COMPANY A

BATCH 1

TUBE A	Absorbance	TUBE B	Absorbance
BLANK	= 0	BLANK	= 0
SOLUTION A	= .145	SOLUTION B	= .02

$$\begin{aligned} \text{Difference in absorbance of solution B from A} \\ = .145 - .02 = .125 \end{aligned}$$

$$\begin{aligned} \text{Content of ampicillin in sample (\%)} &= \frac{.125}{.13} \times 100 \\ &= 96.15\% \end{aligned}$$

BATCH 2

TUBE A	Absorbance	TUBE B	Absorbance
BLANK	= 0	BLANK	= 0
SOLUTION A	= .15	SOLUTION B	= .02

$$\text{Difference in absorbance of solution B from A} = .15 - .02 = .12$$

$$\begin{aligned} \text{Content of ampicillin in sample (\%)} &= \frac{.13}{.13} \times 100 \\ &= 100\% \end{aligned}$$

BATCH 3

TUBE A	Absorbance	TUBE B	Absorbance
BLANK	= 0	BLANK	= 0
SOLUTION A	= .15	SOLUTION B	= .02

$$\begin{aligned} \text{Difference in absorbance of solution B from A} \\ = .15 - .02 = .13 \end{aligned}$$

$$\begin{aligned} \text{Content of ampicillin in sample (\%)} &= \frac{.13}{.13} \times 100 \\ &= 100\% \end{aligned}$$

COMPANY B₁

BATCH 1

TUBE A	Absorbance	TUBE B	Absorbance
BLANK	= 0	BLANK	= 0
SOLUTION A	= .22	SOLUTION B	= .02

$$\begin{aligned} \text{Difference in absorbance of solution B from A} \\ = .22 - .02 = .20 \end{aligned}$$

$$\begin{aligned} \text{Content of ampicillin in sample} &= \frac{.20}{.13} \times 100 \\ &= 153.84\% \end{aligned}$$

BATCH 2

TUBE A	Absorbance	TUBE B	Absorbance
BALNK	= 0	BLANK	= 0
SOLUTION A	= .14	SOLUTION B	= .02

$$\text{Difference in absorbance of solution B from A} = .14 - .02 = .12$$

$$\begin{aligned} \text{Content of ampicillin in sample} &= \frac{.12}{.13} \times 100 \\ &= 92.30\% \end{aligned}$$

BATCH 3

TUBE A	Absorbance	TUBE B	Absorbance
BLANK	= 0	BLANK	= 0
SOLUTION A	= .16	SOLUTION B	= .02

$$\begin{aligned} \text{Difference in absorbance of solution B from A} \\ = .16 - .02 = .14 \end{aligned}$$

$$\text{Content of ampicillin in Sample} = 107.69\%$$

COMPANY C

BATCH 1

TUBE A	Absorbance	TUBE B	Absorbance
BLANK	= 0	BLANK	= 0
SOLUTION A	= .11	SOLUTION B	= .01

$$\begin{aligned} \text{Difference in absorbance of solution B from A} \\ = .11 - .01 = .10 \end{aligned}$$

$$\begin{aligned} \text{Content of ampicillin in sample} &= \frac{.10}{.13} \times 100 \\ &= 76.9\% \end{aligned}$$

BATCH 2

TUBE A	Absorbance	TUBE B	Absorbance
BLANK	= 0	BLANK	= 0
SOLUTION A	= .10	SOLUTION B	= .01

$$\begin{aligned} \text{Difference in absorbance of solution B from A} \\ = .10 - .01 = .09 \end{aligned}$$

$$\begin{aligned} \text{Content of ampicillin in Sample} &= \frac{.09}{.13} \times 100 \\ &= 69.2\% \end{aligned}$$

BATCH 3

TUBE A	Absorbance	TUBE B	Absorbance
BLANK	= 0	BLANK	= 0
SOLUTION A	= .195	SOLUTION B	= .01

Difference in absorbance of solution B from A
= .195 - .01 = .085

Content of ampicillin in Sample = 65.38%

TABLE 8 The summary of the chemical assay (calculated) result of the products of Companies A, B and C is shown in Table 8.

AVERAGE PERCENTAGE POTENCY OF AMPICILLIN
SAMPLES OF COMPANIES A, B AND C ASSAYED BY THE
CHEMICAL METHOD

	COMPANY A	COMPANY B ₁	COMPANY C
BATCH 1	96.15%	153.84%	76.90%
BATCH 2	100.00%	92.30%	69.20%
BATCH 3	100.00%	107.69%	65.38%

COMMENTS

The results of the 24 samples of ampicillin/cloxacillin suspensions from total of 8 batches and three Companies tested by the spectrophotometric method show that they all contain the acceptable level of ampicillin in the combination by USP and BP standards. The same samples have been tested by the microbiological method (chapter 2) and the results presented in Table 3.

The highest difference in the calculated percentage potency of different bottles of the same batch is that obtained from the bottle of the first batch of the products of Company D which is around 9%. All other batches show differences within 0 - 5%. If these errors are either from the weighing, mixing or other manufacturing process, they are within reasonably accepted ranges.

The combination ampicillin and cloxacillin is believed to have a synergistic effect (Martindale 29th ed.). Its mode of action has also been suggested to be by a combination effect especially when penicillinase producing organisms are involved.

Here, cloxacillin is believed to block the production of penicillinase while the ampicillin goes to destroy the organism.

Whatever the method, the fact remains that the combination as a dosage form will not have any desired effect when either of the combinants is below the standard recommended level. The synergy between the two penicillins makes the determination of the level of one in the other much difficult using microbiological methods. The products have had to be subjected to the process of gel electrophoresis before the determination.

The method of Akanni and Ayim (1990) is however spectacular for this combination because it can be used to determine the levels of ampicillin and cloxacillin separately with one not affecting the activity of the other. This method is more reliable for the determination of ampicillin level in its combination with cloxacillin due to its specificity. The method is less tedious and takes less time when compared with the microbiological method. One likely problem that may be encountered with this method is its high sensitivity. As a result of this, a little change in absorbance reading which can result from any mistake in timing or weighing will make an appreciable difference in the corresponding percentage potency. It requires a very high degree of accuracy in all the steps and very skilled personnel if reproducible results are to be obtained.

The cloxacillin content of the samples was not determined but from the results of the ampicillin content, it is believed that they will be good products. It is noted that all these companies B, D and E are registered to manufacture pharmaceuticals in Nigeria.

The results of the samples tested by the chemical method were very similar to those obtained by the microbiological assay of same samples. The method is a standard method very specific for ampicillin. The similarity of the results of the method confirm that the microbiological assay method is as good as the chemical assay. The products of Company A were within the acceptable ranges of both the BP and USP. Two bottles of Company B product were also within acceptable ranges while that of one batch had ampicillin content greater than the 120% acceptable higher limit.

Company C products result like in the microbiological assay, were lower than the lower limit (80 or 90%) recommended by official books. Though the percentages vary very slightly (from those of the microbiological assay). The closeness in the results goes a long way in confirming the sub-quality of the products in question.

CHAPTER 4

Quality control started as far back as the thirteenth century when man's success as a craftsman depended to a large extent on the quality of his products (Ottaviano and Disalvo, (1977)). Methods used at that time were most likely limited to usual inspection as work progressed. Today however, the increased worldwide awareness on the importance of quality especially for pharmaceuticals and the increased problem of drug quality compromise reported in many places has made quality control more necessary and the purpose more defined.

Pharmaceutical quality control is an important aspect of its manufacture which no manufacturer is supposed to compromise for obvious reasons. For these same reasons, no consumer will knowingly accept a compromise on the quality of drugs he or she takes.

In Nigeria, constant complaints from doctors and patients about the ineffectiveness of some drug preparations in the 'market' (including ampicillin suspensions) has made constant independent quality checks (like this work) very necessary, Ayim et al (1990). That fake, adulterated and substandard drugs are being used by Nigerians have been suggested. The source of these drugs are believed to be the mushroom and unhygienic backwood rural factories which are not registered to manufacture and/or sell these drugs. Adenika (1991).

This work like a few before it, confirms that substandard drugs e.g. ampicillin suspension preparations are being sold and used by our people. Out of the 51 samples from five companies assayed, both the chemical and microbiological assays confirm that 9

samples from 3 batches had ampicillin content below the standard recommended level. This work also shows that much as it can be true that local mushroom and unregistered companies produce some of the fake and substandard pharmaceuticals, a lot of other factors and sources which are equally responsible have probably not been taken into serious considerations. All the samples that had low level of ampicillin (products of Company C) were made in India, finely packaged and sold at half the cost of the other products. So many other products with similar problems are likely to abound in our markets.

One of the basic purposes of quality control is to measure, calculate, predict and importantly, to control the variations inherent in any manufacturing process. This involves the concept of in-process control which today include techniques ranging from physical observation to biological, chemical, physico-chemical tests etc. Today, these techniques have reached a high degree of refinement, automation and procedural complexities to advancement in technology and complexity of the products made. There is every indication however that quality control as a manufacturing process has been given far less attention than it should have been in Nigeria. Before this work started, every effort made to get a recommended standard organism from Companies and even from the Food and Drug Administration (Drug monitoring Unit) failed. One wonders therefore, what microbiological quality (potency) checks are carried out in the microbiology laboratories, yet ampicillin products are daily being passed as fit for use from them.

Most of the pharmaceutical products that come into our country from other countries have little or no checks done on them before they are allowed in.

This could either be due to the trust reposed on the manufacturers for the fact that they are abroad or simply because the importers exploit the unpatriotic nature of the relevant authorities at the entry posts. The results of this work show that these products from 'Abroad' are even more responsible for the substandard products of ampicillin available in the pharmaceutical premises. This persisting problem has not been made any better by the proliferation of generics from many countries and the indiscriminate way they are marketed here. A great number of the generics used in this country currently, come from Asian countries particularly India which incidentally, is a country that does not recognise patent right Marsland and Marshal (1990).

These drugs are well packaged and sold at prices far less cheaper than the locally made products.

The methods used for the tests showed acceptable comparable results e.g. the ampicillin samples of companies A, B and C tested showed average batch potency values between 96.21 - 103.43 for microbiological assay and 92.3 - 107.69 for chemical assay. The simplicity and fastness of the method of Akanni and Ayim (1991) can be exploited by setting up the experiment at entry points for on the spot test of imported suspensions. This will help reduce the entry of substandard products which are allowed in temporarily because importers do not want their wares left at the entry points so that they will not be made to pay for it. Most times, before the results come from the laboratories the products would have found their way into many shops and homes (personal communication with FDA Nigeria Inspectors).

The problem of drug quality compromise is a serious one which can only be tackled from the source. It has become necessary to suggest therefore, that among all the steps being taken to see that fake and substandard drugs are not found in the shelves of our pharmacy shops, extra care about products from India be taken. Those made specifically for and marketed by Nigerian companies should be given even more serious considerations.

The fact that products of companies B, D and E which are local companies gave good results that indicate good product quality points to the local manufacture as another means of reducing low quality pharmaceutical products. Strict quality monitoring of these locally made products, regular factory inspection along with strict enforcement of existing regulations on the manufacturing and sell of fake and substandard drugs will help further in this effort.

In conclusion, quality surveillance of products commonly prescribed and found around especially those from countries and factories we can no longer trust should be encouraged in different laboratories by the Government. If this is done without the consent of the manufacturers or authorised marketters, the sources of most faked and substandard drugs can easily be pinned down. If the manufacturers know that this is done, they will certainly become conscious of the quality of products they put in the market. The bottomline however is that local manufacture should be encouraged as against importation since it will be much easier to monitor the quality of local product and punish the producers accordingly.

The findings of this work can be summarized thus:-

1. The Organism Bacillus megatarium NCTC 10342A76 can be effectively used for the microbiological assay of ampicillins in the absence of the standard recommended Organism.
2. Substandard oral ampicillin suspensions and possibly similar pharmaceutical products are being used within Kaduna and Zaria towns
3. The method of Akanni and Ayim (1991) for the determination of ampicillin in the presence of cloxacillin compares very favourably with microbiological assay method for the same determination. Prior separation of the two components by electrophoresis will however provide better microbiological results than when they were not separated. The simplicity, straight forward nature and the short time this method takes can be exploited to set it up at entry posts for on the spot tests.
4. Pharmaceutical companies outside Nigeria are mostly responsible for the presence of sub-standard products e.g. ampicillin suspension in the areas sampled.
5. The encouragement of local manufacture of pharmaceutical products will help reduce the incidence of substandard drugs in our 'markets' since it will reduce imported products which account for most of the substandards.

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