Comparison Of Microbiological And Chemical Assay Methods Used In The Quality Assessment Of Ampicillin Suspensions Sold In Zaria-Nigeria

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ABSTRACT

There appears to be a disagreement between chemists and biologists over which of the methods for the assay of antibiotics is better and more reliable. A total of 27 samples of ampicillin suspension from three different companies comprising nine batches and sold within Zaria-Nigeria were therefore assayed to determine their potency using standard chemical and microbiological methods. Results obtained showed that most of the products were of acceptable quality and also confirmed that each of the two methods compared was as good as the other and can therefore be used for the determination of the quality of ampicillin.

Key Words: Chemists, Biologists, Ampicillin, Microbiological, Quality

INTRODUCTION

Since pharmaceuticals are products that affect human lives, it is important that they be prepared right both in their active medicaments and other excipients. The importance of regular checks on the quality of these products meant for the use of the populace cannot be over- emphasised. Quality checks for antibiotics are done using different assay methods. These methods are used to estimate active constituents, biological activity and in monitoring the stability of the products. Generally, in the case of antibiotics like ampicillin, the estimation of potency using either the chemical or biological assay methods is acceptable (Dafale *et al*, 2014). While chemists insist that chemical assay methods take precedence over biological methods (Hewitt, 2003, Li *et al*, 1995). Microbiologists however, are of the view that a substance becomes of recognised therapeutic use (determined by the estimation of its biologic activity) before its chemical composition is sought after.

Biological assays employ the biological properties of medicinal agents in the estimation of their activity. It compares a sample of known potency and one of unknown potency at the same time and under very strict comparable conditions. Biological assays can be macrobiological for example, in the assay of insulin when mice is used or microbiological for example, in the assay of ampicllin where an indicator organism like *Sarcina lutea* or *Bacillus megatharium* are used as indicators. The use to which a biological assay will be employed will determine the method and the design of the assay for example, in some cases, the level of antibiotics to be detected will be very low and a very sensitive method which can detect such low levels will have to be employed. For finished products ready for the market an accurate estimate of potency is necessary and microbiological assay methods stand out as one of the best ways of potency determination.

Chemical assays are used for the identification and estimation of the level of active ingredients in pharmaceuticals. They employ the chemical nature of the drugs to be assayed in their design and take advantage of the ability of the active ingredient in the drug to undergo certain reactions. This is why the design of chemical assays varies from one drug to the other unlike the biological assay which will run on the same principle of growth and inhibition for instance no matter the drug. 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.

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Ampicillin is a beta lactam naturally occurring and semi synthetic antibiotic (Udobi and Otudoh, 2014). It is used for the treatment of respiratory, urinary tract infections among many others. It is a broad spectrum antibiotic showing activity against both Gram positive and Gram negative bacteria (Foye, 1976). Ampicillin acts as an irreversible inhibitor of the enzyme transpeptidase which is needed by bacteria to make their cell walls (AHFS Drug information, 2006). Ampicillin is known to inhibit bacterial cell wall synthesis leading to cell lysis. It does this by preventing the incorporation of the N-acetyl muramic acid into the mucopeptide layer thereby preventing the completion of the cell wall (Dale and Rang, 2007).

Results obtained from this work are intended to be used for comparing the efficiency of the assay methods used and indeed the quality of the products on sale

MATERIALS AND METHODS

Sampling

The products (test samples) used for this work were obtained from pharmacy shops in Samaru-Zaria, Nigeria.

Standard

The standard sample used was pure ampicillin trihydrate powder obtained from the National Agency for Food Drug Administration and control (NAFDAC) Nigeria

Infra-Red Spectroscopy

This was used to confirm the presence of ampicillin in both the standard and test samples. A small quantity of the sample (in solid form) was ground in a mortar with a few drops of 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 was produced and read.

Thin Layer Chromatography

Thin layer chromatography was carried out using the method described by the BP (2009). It was also used as a means of identifying the presence of ampicillin in the market samples assayed.

Microbiological Assay

Preparation of Standard test doses

10, 20 and 40mg/ml of standard ampicillin trihydrate powder were made using sterile 0.1 phosphate buffer (USP) as diluents. (0.17g of ampicillin trihydrate equals 0.15g of ampicillin)

Preparation of sample test doses

Samples of ampicillin suspension to be assayed were first reconstituted according to the manufacturer's instructions. 10, 20 and 40mg/ml of each of the samples were then made using 0.1M phosphate buffer (USP) as diluents.

Microbiological assay proper

The agar diffusion method which employs the 6x6(3x3) dose level latin square design was used. The method was however modified by the use of Bacillus megatharium NCTC10342A$_{76}$ as indicator organism (Udobi *et al*, 2010).

200ml of sterile nutrient agar was inoculated with 1ml of the test culture containing one million cells of the indicator organism. This was then poured aseptically on top of a previously prepared basal layer of nutrient agar. The whole plate was allowed to cool. 36 cups made in a latin square design were cut in the solidified agar using a sterile No 4 cork borer. 20ml each, of the sample and standard test doses were then introduced into each cup as prescribed by the method. The plates were allowed to stand on the table for two hours to allow for diffusion before being incubated at 37^{o}c for 24hours. Zones of inhibition produced were measured after incubation.

Chemical Assay

The method recommended by the British Pharmacopeia (2009) was used.

Preparation of Standards

0.17g of ampicillin trihydrate equivalent to 0.15g of ampicillin was dissolved in 500ml of distilled water and shaken for 30 minutes using an electric shaker. The solution was filtered using a sintered glass number 0 under pressure. 10 ml of the resulting solution was transferred into a 100ml graduated flask. To the flask was further added 10ml of boric acid buffer pH 9.0 and 1ml of acetic anhydride-Dioxan solution. The flask was allowed to stand for five minutes after which water was added to produce 100ml.

2 ml aliquots of this solution were transferred into two separate stoppered tubes A and B. To one tube (Solution A), 10mls of Imidazole mercury reagent was added. The tube was shaken (to mix the content), stoppered and immersed in water bath at 60^{o}C for twenty five minutes with occasional swirling. After, the tube was removed from the bath and rapidly cooled in ice to 20^{o}C. To the second

stoppered tube, 10ml of water was added and designated solution B.

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.

Preparation of Test samples

60mls of each reconstituted suspension (125mg/5ml) containing 0.15g of ampicillin was transferred to 500ml of distilled water, shaken for 30 minutes using an electric shaker and filtered under pressure using sintered glass filter number 0. The resulting solution of each sample was treated like the standard solution and the absorbance of solution A and B were measured at 325nm.

The content of ampicillin in each sample was calculated from the difference between the absorbance of solution A and B and from the difference obtained when 0.17g of the standard ampicillin trihydrate powder (equivalent to 0.15g of ampicillin was used.

RESULTS

Infra- Red Spectroscopy

Representative samples of all the company products assayed showed characteristic peaks which indicated the presence of the Imide group and the benzene ring confirming the presence of ampicillin in the samples.

Thin Layer Chromatography

Results of TLC obtained confirmed the presence of ampicillin in all the samples. This was 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.

Microbiological Assay

Results of the microbiological assay of ampicillin suspension from companies A, B and C are shown in Table 1. They show the calculated percentage potency of each of the bottles and their equivalent concentration in µg/5ml and by implication, the quality of the product.

Chemical Assay

Results of the chemical assay of ampicillin suspension from companies A, B and C are presented in Table 2.

Table 1: Percentage potency of ampicillin suspension from companies A, B and C obtained from microbiological assay.

COMPANY A		COMPANY B		COMPANY C	
Calculated %Potency	Equivalent Concentration µg/5ml	Calculated %Potency	Equivalent Concentration µg/5ml	Calculated %Potency	Equivalent Concentration µg/5ml
103.3	129.12	156.30	195.0	71.60	89.50
97.7	122.37	161.00	201.25	59.80	74.25
101.8	127.25	162.18	202.72	57.0	71.25
95.0	118.75	94.40	118.0	67.80	84.75
99.03	123.78	97.05	121.31	68.50	85.62
97.20	121.50	97.20	121.50	61.90	77.37
100.80	126.00	105.40	131.75	63.80	79.75
101.10	126.37	103.10	128.87	61.80	77.25
104.00	130.00	101.80	127.25	60.82	76.02

Table 2: Average percentage potency of ampicillin suspension from companies A, B and C obtained using the chemical assay method

SAMPLE	COMPANY A	COMPANY B	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

They show the average percentage potency of each batch and their equivalent concentration in ug/5ml which points to the quality of the product.

DISCUSSION

Some of the basic purposes of quality control are; to measure, calculate, predict and importantly, control the variations inherent in any manufacturing process. Choosing a method which will help the researcher to achieve this goal is a serious and fundamental decision. Today, the techniques employed by scientists to achieve these have reached a high degree of refinement, automation and procedural complexities due to advancement in technology. While the results of this work determined the quality of the products assayed and can help us to make statements about them, it has also helped us compare the efficiency

of the two assay methods used to achieve this purpose.

Even though the potency of antibiotics is known be determined by both chemical and biological assay methods, biologists, related scientists and even official books argue that biological methods of assay(bioassays) are more convenient for the determination of antibiotics (Cazedey and Salgado, 2015). For so many other advanced reasons, they are adjudged to be better and more reliable. For instance, it is known that bioassays do not require specialized equipments or toxic solvents like chemical assays will do. (Pinto *et al*, 2007*)* When compared with chemical methods, biological assays are known to measure the true response of antibiotics in a biological system. They are also used to do a more realistic and precise measurement of potency among other things. (Nishant *et al*, 2014). Since bioassays measure antimicrobial activity, they are able to detect small changes in antibiotic molecule which chemical methods may not be able to detect. (USP, 2009)

The results of the samples tested using the chemical assays were similar to those obtained by the microbiological assay (Tables 1 and 2). The similarity of the results obtained using the two methods confirm that one is as good as the other. The products of company A were all within the acceptable potency ranges of both the BP and the USP. Two bottles of company B product were within acceptable ranges while that of one batch had ampicillin content greater than 120% acceptable higher limit.

Company C products were shown by both of the assays to be lower than the acceptable lower limit of 80-90% recommended by the official books. Though the percentages obtained by both methods vary slightly, the results tell the same story about the products.

While the reasons for the observed slight quality variations may not be our purpose for this work, it is important to observe that the pattern of result points to processing errors on the part of the production staff more than any other reason. Most of the times, this is due to either an under weighing or over weighing during production or due to contamination with impurities or some other unwanted substances. It is most probable that any slight differences observed may not be from any of the methods but from the production process. It may therefore, not be out of place to suggest the use of either of the two methods for the effective quality check of ampicillin products.

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