QUALITY OF AMPICILLIN/CLOXACILLIN PREPARATIONS

IN THE NIGERIAN MARKET

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ABSTRACT

Various brands of the combined preparation of ampicillin/cloxacillin found in the Nigerian market have been analysed for contents. The ampicillin contents varied between 53.7 and 101.7%. Most of the capsules analysed had lower ampicillin content than that specified in either the BP or USP. While the cloxacillin contents were found to be within the compedia specified limits.

INTRODUCTION

The quality of pharmaceutical products is essential to achieve their ultimate goal, therapeutic efficacy. When there are different brands of a drug product, their quality becomes more important since they could be substituted for one another by writing prescriptions with generic names. Although all drug products must of necessity meet the minimum required standards as specified in official pharmacopoiea, these vary from country to country. The problem of generic inequivalence has long been established (1). Hence generics need to be established as being equivalents before they could be substituted for one another. Generic inequivalence is known to arise from three main levels namely chemical, biological and therapeutic (2). Tests to ascertain chemical equivalence takes precedence over the others, since preparations to be tested for biological and therapeutic equivalence must have been shown to be chemical equivalents. Of recent, many brands of the combined preparation of ampicillin/cloxacillin have been found in the market with wide range of prices. Although price index could not be used as a measure of the quality of drug products, it was thought necessary to subject these products to chemical analysis to ascertain the quality of these products in terms of the amount of active ingredients present. This became necessary, following complaints from clinicians and patients about ineffectiveness of some ampicillin/cloxacillin preparations on the market, and the wide price differences of the products on the market.

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EXPERIMENTAL

Reagents:

Standard ampicillin trihydrate and cloxacillin sodium (Beecham Research Laboratories, U.K). Phosphate buffer pH6 was prepared according to BP (1980) (3). Phosphate buffer (pH 2.5) with 0.5% w/v formaldehyde: 31.2g of potassium dihydrogen orthophosphate was dissolved in 900 ml of water; 13.5ml of formaldehyde (3% w/v) was added; the volume was made up to 1 litre with water and the pH adjusted to 2.5 with conc. HCl. All reagents used were of analytical grade. The different brands of the combined preparations of ampicillin/cloxacillin were purchased from pharmacy shops.

Equipment:

Phy Unican SP8-400 UV/visible spectrophotometer was used to obtain the absorption spectra.

Procedure:

The methods used in the determination of the amounts of ampicillin and cloxacillin respectively were adapted from that developed by Akanni and Ayim (4).

Determination of Ampicillin:

100ml of standard solution in water containing lmg/ml ampicillin was prepared with the reference material. Reaction mixtures were prepared as follows: 0.5 to 3.0ml standard solution were pipetted into respective 100ml volumetric flask; into each flask was added 2ml 2M, sodium hydroxide solution; the mixtures were left to stand; after 20 minutes, lml of 2M sulphuric acid and 50ml of phosphate buffer (pH 2.5) with formaldehyde were added into each flask. The mixtures were heated for one hour in a boiling water bath, cooled to room temperature and made up to 100ml with water. A one in two dilution of each reaction mixture was made in 2M sulphuric acid, and the absorbance measured at 373nm using 1M sulphuric acid as blank.

For the dosage forms, the equivalent of 100mg ampicillin of the sample was put into a 100 ml volumetric flask, water was added to the mark, the mixture was shaken for 20 minutes and filtered when necessary. 1.5, 2.0, or 2.5ml of the filtrate was used to prepare the reaction mixtures as described above.

An appropriate dilution of the reaction mixture was made in 2M sulphuric acid and the absorbance determined at 373nm.

Determination of Iodine Absorbing Impurities:

Iodine absorbing impurities were determined according to BP 1980 (3).

Cloxacillin:

The cloxacillin in the combined preparation of ampicillin/cloxacillin was determined by a modified method of Davidson and Stenlake (5).

A stock solution of cloxacillin (1 mg ml -1) was prepared in water. Alfiquots of the stock solution (5 to 24ml) were put into respective 50ml volumetric flasks; 25ml phosphate buffer (pH6) was added to each flask and water was added to 50 ml mark. The absorbance of each solution was measured at 275nm using 1 in 2 dilution of phosphate buffer as blank.

The dosage forms were determined by taking the equivalent of 100mg cloxacillin of the sample to prepare 100ml solution. The solution was filtered when necessary. 5 to 24ml of the filtrates were used to prepare solutions for obsorbance determinations as above.

RESULTS AND DISCUSSION

The calibration graph for ampicillin was linear within the concentration range studied (Fig. 1). It had a correlation coefficient of 0.9996 while the A (1%, 1cm) at 373 was 434 + 5.3 (S.D). The results showing the percentage contents of ampicillin and cloxacillin contained in the various brands are presented in Table 1. The percentage ampicillin contents of the analysed samples varied between 53.7 and 101.7%, with only one of the analysed capsules having its content within the range specified by either the B.P (not less than 95%) or USP (90-120%). The analysed injections had their contents of ampicillin within the specified USP or BP range while one of the syrup had a lower ampicillinn content outside the specified BP/USP range. These results show that there are many capsules having ampicillin content lower than the specified BP/USP range, which is an evidence of the presence of sub-standard drug in the Nigerian market. While some of the preparations had their contents just outside the 90-120% range, one of the capsusles (C) was distinguishable, with a very low content (53.7%). From this result, one reason that may probably be adduced for the low ampicillin content in some of the brands could be the fact that the ampicillin might have been based on the trihydrate rather than the anhydrous form. The BP and USP stipulates 250mg of ampicillin or the equivalent amount of the trihydrate or the sodium salt. When an error is made in adjudging the trihydrate to be equivalent to the anhydrous ampicillin such level of ampicillin (75 - 89%) could be obtained. The low ampicillin content of brand C could not be accounted for by this reason. The percentage of iodine absorbing substances (Table 2) was found to be negligible for all the samples, hence degradation of the products could not have accounted for the low ampicillin contents. Additionally, the problem of low content could not have arisen from the developed method used for the analysis, since the combined preparation made with the authentic samples gave a good recovery value of 100.5%.

The calibration graph for the cloxacillin was linear (Fig. 2) with a correlation coefficient of 0.9992. The combined preparation from the authentic samples gave a good recovery value of 99.5%. The contents of cloxacillin in all the analysed samples were within the range specified in the BP and USP. Thus while the content of ampicillin was variable and do not meet the level stated in compedia in most of the samples, that of cloxacillin was within the limits.

The problem of low ampicillin contents could have considerable effect on the efficacy of these preparations. The

Sample	Batch No.	% Date of Expiry	% Ampiccillin Content	Cloxacillin Content
Capsules				
A	BN 6289	April 1990	101.7	108.7
B	7069	Sept. 1990	76.9	108.8
C	BNOCO 401B	Nov. 1990	53.7	94.0
D	87F24	June 1990	83.4	100.1
E		June 1990	87.3	108.6
Injection				
F	$BN913NO_2$	Oct. 1991	101.4	110.0
G	B1	Jan. 1991	97.2	110.0
Syrup				
H	X2	Oct. 1989	81.5	98.0
I	BNN 224W05	Aug. 1989	96.0	110.0
Standard				
J*			100.5	99.5

* Combined preparation from Standard Samples.

Table 1: Assay Results

Sample	%Iodine Absorbing Substances
A	0.24
B	0.23
C	0.18
D	0.33
E	0.25
F	0.22
G	0.28
H	4.0
I	0.12
Standard Ampicillin	0.14
Standard Cloxacillin	0.21

Table 2: Percentage Contents of Iodine Absorbing Substnces In Each Sample

combined preparation acts synergestically and exhibit bacteriacidal activity against a wide range of Gram-positive and Gram-negative organisms. The cloxacillin inhibits the destruction of ampicillin by beta-lactamase producing organisms. Thus while the ampicillin in the preparation may be secured from being destroyed, and assuming absorption is not a limiting factor, the minimum effective concentration (MEC) level may not be reached due to the low ampicillin content. Hence the patient may not derive the desirable cure from his ailments when given such preparation. This may also lead to the emergence of resistant strains of the microbes being combated. It is, therefore, essential that not only the level of cloxacillin should be ascertained but also that of ampicillin. It is worthy to note that samples from the innovator Company satisfied the BP requirements, for content and iodine absorbing impurities.

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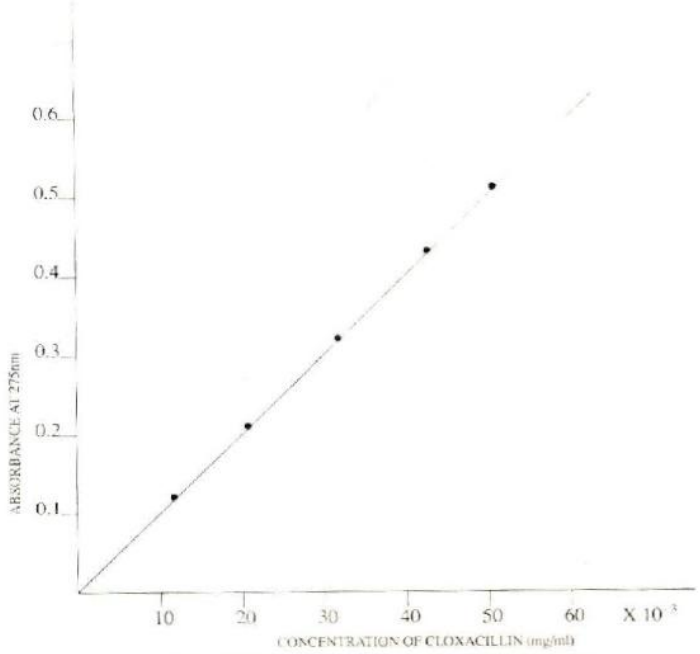

FIG. 2. CALIBRATION GRAPH FOR CLOXACILLIN SHOWING ABSORBANCE AGAINST CONCENTRATION

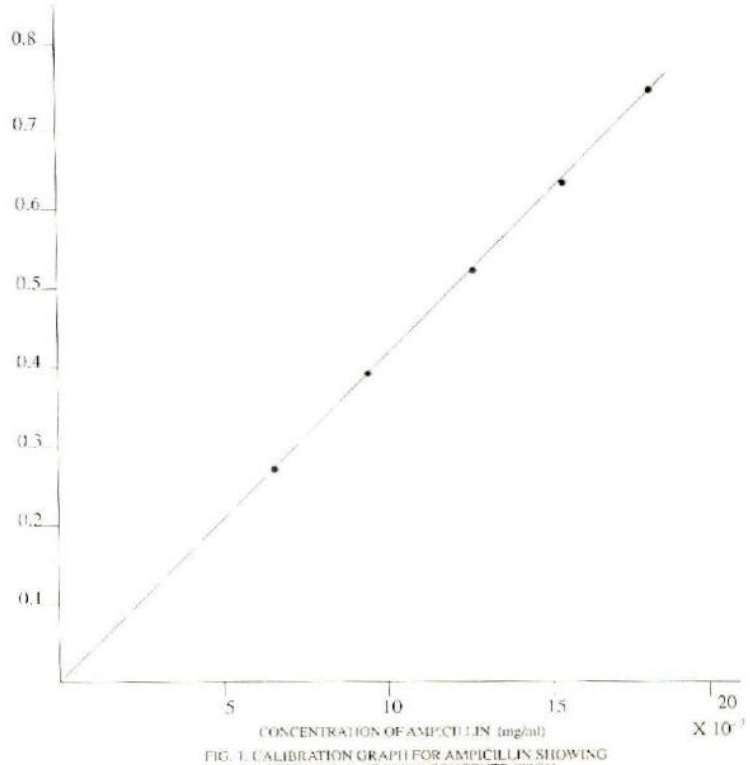

FIG. 1. CALIBRATION GRAPH FOR AMPICILLIN SHOWING ABSORBANCE AGAINST CONCENTRATION.