QUALITY ASSESSMENT OF DRUGS WITH TLC - A STUDY OF SOME COMMERCIALLY AVAILABLE AMINOPENICILLINS

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ABSTRACT
Thin layer chromatographic (TLC) analytical method was employed in the assay of ampicillin and amoxycillin in commercial capsules. The spot sizes of the reference drug samples at various concentrations were utilized to plot a calibration graph. The slopes of the graphs were used to calculate the drug content in the dosage forms. The results of this assay method were compared with those obtained from the official iodometric method. Both methods yielded similar results with high percentage recoveries. TLC method is suggested as a less cumbersome and faster way of assaying these two drugs in pharmaceutical preparations.

Keywords: Quantitative and qualitative evaluation, aminopenicillins. Thin layer chromatography.

INTRODUCTION

The existence of fake and substandard drugs has become a worrisome problem in the health care delivery system in Nigeria. Ampicillin and amoxycillin, two of the most widely used antibiotics in Nigeria have not escaped this unfortunate development. The official assay methods[1]. For these drugs in pharmaceutical preparations are the iodometric titration and the hydroxylamine spectrophotometric methods. Unfortunately, the iodometric titration method first suggested by Alicino[2] is beset by numerous errors/problems[3] while the hydroxylamine method requires an expensive equipment and some level of expertise.

Recently, Onunkwo and Adikwu[4] utilized the charge transfer complexation with p-chloranil and chloranilic acid in the spectrophotometric analysis of these two drugs. In this paper, we report on the adoption of the method of Gänshirt and Polderma[5] but with a modification as introduced by Touchstone[6] in the quantitative determination of these two drugs in a capsule dosage form. Although this is the first report of this type of quantitation for ampicillin and amoxycillin by TLC, quantitative analysis of drugs by TLC is nothing novel[7,8].

MATERIALS AND METHOD

Reference samples of ampicillin trihydrate and amoxycillin trihydrate certified to have potencies of 980μg and 1020μg respectively were obtained from Doyin Pharmaceuticals Limited, Lagos, Nigeria. Commercial samples of silica gel D and chloranilic acid (Reidel-de Haen®); iodine (Merck®); 1,4-dioxane (BDH®); Reichlin® capsules (Medreich Limited, England); Amoxil® capsules (Beecham Pharmaceuticals, Nigeria) each capsule containing 250mg of antibiotic salt were used as supplied by the manufacturers.

PREPARATION OF STANDARD REFERENCE DRUG SOLUTION

Accurately weighed powder, equivalent to 100mg of ampicillin or amoxycillin respectively was dissolved in 50ml of methanol to give a 2.0mg/ml solution. Solutions containing 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 and 1.8mg/ml ampicillin and amoxycillin base respectively were also prepared.

PREPARATION OF TEST DRUG SOLUTION

Twenty capsules randomly selected were emptied from which quantities of powder equivalent to 100mg of either drug was weighed and dissolved in 100ml of methanol.

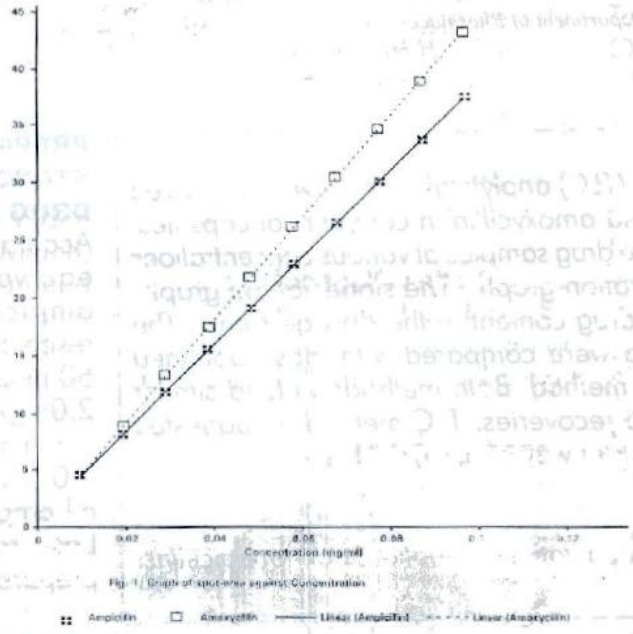
QUALITATIVE ANALYSIS

Standard reference drug solutions of ampicillin or amoxycillin were spotted on 10 x 20cm TLC glass plates with silica gel D, in 0.25mm, developing solvent was chloroform, methanol: acetic acid (5:3:1) and detected with 0.5% chloranilic acid solution in 1,4-dioxane. The chromatographic profile i.e. colour responses, stability of colour, R values and the spot size were noted and recorded.

QUANTITATIVE ANALYSIS

With the aid of a micropipette, 0.05ml corresponding to 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1mg of the prepared standard reference solutions

for each drug were chromatographed as above. A transparent paper was placed on the chromatographic plates and the outline of the spots were inked out. The areas of the spots were obtained by superimposing the inked outlines on a square millimetre graph paper and counting the number of squares occupied by the spot. The mean spot area for each concentration (n = 5 determinations) were used to plot a calibration curve for the two drugs (Fig 1).



A similar procedure was adopted for the test drug solution of either drug prepared above. The mean spot area (n = 5 determinations) were used together with the slope of their respective calibration curves to calculate the drug content in the pharmaceutical capsules. The two drugs were also assayed using the official iodometric titration method[1]. The results from both methods were compared.

RESULTS AND DISCUSSION

Both drugs gave a purple colour with 0.5% chloranilic acid spray reagent. The colour was stable for about 15 minutes before fading. However, the colours reappeared on respraying. The solution containing ampicillin had R_f value of 0.81 $\pm$ 0.04 while those containing amoxycillin showed R_f value of 0.85 $\pm$ 0.02.

The calibration curve showed a linear relationship leveling support to the proportionality between the spot size and the concentration of the material in solution[9]. The two linear equations were found to be:

$A = 358.91C \pm 0.78$ for ampicillin, and

$A = 422.61C \pm 0.23$ for amoxycillin

The small positive intercept from these equations may have originated from minor errors in determining the spot size of the chromatogram (Seher[10] and Dallas[11]). From the analysis of the commercial capsules, ampicillin was found to contain 95.0 $\pm$ 2.22% in and amoxycillin 96.4 $\pm$ 2.34% in from the label claims of 250mg. With iodometric titration method, the results were 95.1 $\pm$ 1.97 and 96.1 $\pm$ 2.06 respectively.

CONCLUSION

The results from the two methods of quantitation are remarkably similar. Whereas the official iodometric titration method is time-consuming beset with many errors/problems, whereas, similarly the spectrophotometric metod is expensive and require a certain level of expertise, this TLC method described in this paper will serve as a faster, cheaper and more easily accessible method for the quantitative evaluation of these drugs in pharmaceutical preparations.

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