OBAFEMI AWOLOWO UNIVERSITY

COMPARATIVE ASSESSMENT OF THE PHYSICO-CHEMICAL PROPERTIES AND *IN-VITRO* BIOAVAILABILITY OF CO-TRIMOXAZOLE TABLETS MARKETED IN NIGERIA

BY

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CERTIFICATION

I certify that this thesis work was carried out by BETIKU, Opeyemi in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, under my supervision.

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(Head of Department)

DEDICATION

To God the Father, Son and Holy Spirit

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ABSTRACT

This study investigated the in-vitro bioavailability of some brands of co-trimoxazole tablets in Nigeria and determined the prevalence of fake or substandard co-trimoxazole tablets with a view to providing information on the quality of co-trimoxazole tablets marketed in the country.

Twenty different brands of co-trimoxazole tablets marketed in the country were obtained from retail outlets in Ile-Ife. Basic information such as batch number, NAFDAC registration number, manufacturer's address, production and expiry dates were documented and each brand was tested for weight uniformity. The components of each brand were identified by thin layer chromatography and their percentage content were determined by the British Pharmacopoeia (BP, 2010) method, in which sulphamethoxazole was determined by titrimetry and trimethoprim by UV-spectrophotometry. Further assessment of the active ingredients was undertaken using a High Performance Liquid Chromatographic (HPLC) method for the determination of the contents and dissolution profile of each sample using the United States Pharmacopoeia (USP, 2009) monograph. The results of assay using the BP and HPLC methods were compared using Student's t-test while ANOVA was used to compare the differences in the *in-vitro* release pattern of the tablets.

Only fourteen of the samples conformed to the weight uniformity requirements specified in the BP while the active ingredients, sulphamethoxazole and trimethoprim, were present in only eighteen of the samples. The assay results showed that the samples contained between 54.85 % and 103.37 % of sulphamethoxazole and between 0.24 % and 106.67 % of trimethoprim using the BP method. However, the HPLC method showed that the samples contained between 92.61 % and 99.23 % of sulphamethoxazole and between 94.99 % and 103.26 % of trimethoprim. The

dissolution tests showed that between 3.80 % and 82.25 % of sulphamethoxazole were dissolved in 60 min while 17.04 % and 101.26 % of trimethoprim were dissolved. Only seven of the samples released more than the required amount of trimethoprim after 60 min while only three released more than the required amount of sulphamethoxazole. A non-significant difference ($p>0.05$) was observed between the BP method and the HPLC method while the release pattern of the drug showed that the brands were not equivalent with respect to their *in-vitro* release profile as there was a significant($p<0.05$) difference in the release profile at the two time points (10 min and 60 min).

The study concluded that there is a prevalence of fake and substandard co-trimoxazolein the Nigerian Pharmaceutical market.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

1.1 The Nigerian Pharmaceutical Market and Drug Quality

It has been established that a large market exist for drugs in Nigeria with over 130 existing pharmaceutical manufacturers (Erhun et al., 2001). Despite the enormous numbers of these pharmaceutical industries, only 60 are in active manufacturing. This is against the installed capacity of the industry to produce between 50 and 75% of the nation's drug needs. With the production capacity below 30%, much of the nation's drugs are imported, with a bulk of the import coming from Asia. Drug counterfeiters see Nigeria as a good base for their criminal but lucrative trade. Bate and Boateng (2007) reported that India and China are the market leaders in pharmaceutical manufacturing and the biggest culprits of drug counterfeiting globally because of the porous borders which allow counterfeiters easy access to both legitimate and illegitimate drug markets.

Much of the global outsourcing is contracted to firms in Asia, both for manufacturing and increasingly, for services. A statistics by the European commission described India as the source of 75% of counterfeit drugs (Chika et al., 2011). It is therefore not surprising that most of the counterfeit drugs in Nigeria originate from India (Raufu, 2002). However, this is not to suggest that the problem is limited to Asia. In many cases, the goods are only misbranded in places far from the production site.

The World Health Organization defined a "counterfeit drug as a medicine, which is deliberately and fraudulently mislabelled with respect to identity and/or source''. Counterfeiting is said to

apply to both branded and generic products, may include products with the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient active ingredients or with fake packaging (WHO, 2011).

A study conducted by the Nigerian National Agency for Food and Drug Administration and Control (NAFDAC) in 2002 (Yankus, 2006) found that nearly 41 percent of pharmaceuticals in the country were counterfeits, and as many as 70 percent were unregistered. At one point, a study estimated that fake anti-malarial drugs in Nigeria comprised 85 percent of the total market. Between 2001 and 2006, Nigerian officials were said to have destroyed counterfeit and substandard products worth USD 109-169 million (Akunyili, 2007). Although combative efforts by NAFDAC led to a significant decrease in the prevalence of substandard drugs since 2002, the level remains high. In 2006, officials of NAFDAC estimated that counterfeits made up 16.7 percent of the total market while 19 percent of the drugs in the market were unregistered. In May 2009, NAFDAC was reported to have seized $675,000 worth of counterfeit medicines at a Lagos airport that included fake antimalarials, diabetes medicines, heart medicines, anti-anxiety drugs and a variety of antibiotics such as amoxicillin, ciprofloxacin, and ampicillin. Also in January 2010, NAFDAC reportedly intercepted a large consignment of fake Lonart-DS antimalarial tablets, valued at $66,530, in a shed near the Lagos airport (Primo-Carpenter and McGinnis, 2009).

Other African countries are not exempted from this ordeal, as reports of drug counterfeiting abound for countries like Niger where about 50,000 people reported received fake meningitis vaccine that led to the death of about 2,500 of the recipients.

Substandard drugs are also prevalent in Ghana, a report suggests up to 50 percent. In 2008, researchers at the Kwame Nkrumah University of Science and Technology (KNUST) tested a selection of artesunate tablets sold in pharmacies in Kumasi, the second-largest city in the country, and found that 82 percent of the drugs did not meet European pharmacopeia guidelines (Ofori-Kawakye et al., 2008). In November 2009, the Chief Executive of Ghana's Food and Drugs Board was reported to have acknowledged these quality issues and ordered the withdrawal of 22 batches of anti-malaria medicines from the Ghanaian drug market due to quality defects (Opuni, 2009).

In 2008, 20 percent of medicines in the capital city of Ouagadougou were estimated to be counterfeit and sold without a prescription or expiration date (Tipke and Diallo, 2008).

Some fake drugs common in regions of Afirica and they includes cancer medications, antimalarial and antiretroviral medications, antibiotics, analgesics, anti-virals, and erectile dysfunction drugs (Jubat and Ongeri, 2009).

Some control measures have been reportedly put in place to regulate the manufacture, sale and distribution of drugs in Nigeria. An example is the deployment of the handheld spectrometers which allows the inspection and authentication of products at the point of sale (Roger and Aparna, 2011). In 2006, the WHO also launched the International Medical Products Anti-Counterfeiting Task Force (IMPACT) to assist countries strengthen their detection and enforcement systems and work with industries to develop secure measures as high-tech pharmaceutical packaging. Moreover, there have been calls for the passage of a new bill, which seeks life jail term and confiscation of assets of counterfeiters upon conviction and compensation of victims, where fake drug is found to be the proximate cause of injury. (Odiegwu,2011).

1.2 Antibiotics

Antibiotics are chemical compounds derived from micro-organisms that kill or suppress the growth of other microorganisms. They are one class of the larger group of antimicrobials which includes anti-viral, anti-fungal, and anti-parasitic drugs. They work as bactericidal when they kill the cell and bacteriostatic by stopping bacteria from multiplying (Rudy and Senkowski, 1973). Examples of bactericidal antibiotics are aminoglycosides, cephalosporins, and fluoroquinolones while bacteriostatic antibiotics include sulphonamide, tetracycline, spectinomycin, trimethoprim, chloramphenicol, macrolide and lincosamide.

Antibiotics also have either a narrow or broad spectrum of activity in which they can inhibit both Gram-positive and Gram-negative bacteria and can be grouped into different classes based on their mode of action against bacterial cells:

(i) Inhibition of protein synthesis e.g. chloramphenicol, tetracycline

(ii) Inhibition of cell wall synthesis e.g. penicillin, cephalosporin

(iii) Inhibition of functions of cell membranes e.g. amphotetericin B, nystatin

(iv) Inhibition of nucleic acid synthesis e.g. sulphonamides

1.3 Sulphonamides

Antibacterial sulphonamides were the first effective chemotherapeutic agents to be used for bacterial infection in humans. The term, sulphonamide, is usually employed as a generic name for derivatives of para-amino-benzene sulphonamides. Sulphonamides inhibit Gram-positive and Gram-negative bacteria, *Nocardia, Chlamydia trachomatis* and some *Protozoa*. Some enteric bacteria such as *Escherichia coli, Kelbsiella*, *Salmonella, Shigella* and *Enterobacter* are also inhibited by sulphonamides. They are used in the treatment of tonsillitis, septicemia, meningococcal meningitis, bacillary dysentery and a number of infections of the urinary tract.

1.3.1 Biosynthesis of dihydrofolic acid and mechanism of action of Sulphonamides

Bacteria are unable to take up folic acid from the environment and therefore must synthesize the vitamin *de novo* from para aminobenzoic acid (PABA), pteridine and glutamate. Mammalian cells, in contrast, use the folate receptors and folate carriers in the plasma membrane to scavenge the intact vitamin (Merck Index, 2000). This fundamental metabolic