Journal of Applied Pharmaceutical Science

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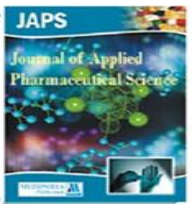

ISSN: 2231-3354
Received on: 07-08-2011
Revised on: 11-08-2011
Accepted on: 13-08-2011

Bioequivalence studies on some selected brands of ciprofloxacin hydrochloride tablets in the Nigerian market with ciproflox® as innovator brand

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ABSTRACT

This work assesses the bioequivalence of four selected brands of ciprofloxacin hydrochloride in the Nigerian market to Ciproflox® as a selected innovator brand. The brands selected were reperesented by samples A, B, C and D all are of Asian origin. The following parameters were employed in the assay- Weight uniformity, tablet hardness, friability, disintegration time and dissolution profile in 0.1N HCl , and minimum inhibitory concentration (MIC) determined by zones of inhibition on clinical isolates of *Staphylococcus aureus.* The mean hardness results were in the order Ciproflox(R) (14.55 ± 3.97) > C (12.51 ± 3.01) > D (9.87 ± 2.38) > B (9.67 ± 1.37) > A (8.98 ± 3.46) and they all fell within the acceptable values of 3 kgs ≤.hardness. The mean friability results were in the order A (0.07) < D (0.12) < Ciproflox (0.17) < B (0.30) < D (0.31). The drug content values were in the order -Ciproflox (500.02) > D (483.54) > Cipromax B > C (458.86) > A (420.29). By the USP standard, only A failed the test; while, by the BP standard only Ciproflox and D passed the test. The disintegration times were in the order- A (1.73±0.27) > C (3.22±0.20)> B (4.15±1.07)> Ciproflox (4.88±0.54)> D (7.60±0.44). The tablet dissolution rates (P_{12min} values) were in the order – A (59.528 ± 8.427) > C (42.468 ± 1.863) > B (35.124 ± 4.408)> Ciproflox (15.403 ± 0.799) > D (8.808 ± 0.856). Ciproflox(R) with the highest drug content had the lowest MIC on *Staphylococcus aureus* (0.064 µg/ml). This shows that Ciproflox is the most effective of the five brands, followed by D, C, B and A. The highest MIC value on *Staphylococcus aureus* was obtained with A 0.255 µg/ml which also had the lowest drug content. Ciproflox (R) with this reseach stands to be the oustanding product among the five brands.

Key words: Ciprofloxacin hydrochloride, tablets, brands, bioequivalence, tablet characteristics, microbiological.

INTRODUCTION

The availability of different brands of the same drug places many prescribers in a difficult position over the choice of an 'ideal' brand (Poli, 2008). The differences in cost between a branded and generic medicine may be as high as 90%. To achieve therapeutic efficacy, therefore, bioequivalence studies are imperative (Government of India, 2005). *In vitro* testing or quality control of drugs helps in obviating this confusion (Poli, 2008). Ciprofloxacin is a quinolone carboxylic acid derivative with an extensive antibacterial spectrum of activity (Campoli-Richard et. al, 1988). It also exhibits a rapid onset of action, and lacks cross-reactivity with penicillin, cephalosporins and the aminoglycosides (Obi, 2009). In the 1990's, there were just a few brands of ciprofloxacin in the Nigerian market but recently many brands have flooded the market

(Obi, 2009). Ciprofloxacin given as an oral tablet is rapidly and well absorbed from the gastrointestinal tract and this can be satisfactorily described as a zero-order process (Campoli-Richard, 1988, Bayer, 2008). The absolute bioavailability is approximately 70% with no substantial loss by first pass metabolism (Ngwuluka et. al, 2009). Maximum (peak) serum concentration are attained in 1 to 2 hours after oral dosing. Serum concentrations increase proportionately with doses up to 100mg (Obi, 2009). The drug has been shown to be active against most strains of the following micro-organisms both *in vitro* and in clinical infections: *Aerobic gram-positive micro-organisms- Enterococcus faecalis, Streptococcus pneumonia, Streptococcus pyrogenes, Staphylococcus saprophyticus, Staphylococcus aureus; Aerobic gram-negative micro-organisms- Enterobacter cloacae, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia, Neisseria gonorrhoeae, Shigella sonnei* (Campoli-Richard, 1988, Bayer, 2008 and U.S.P 2005).

MATERIALS AND METHODS

Materials

The materials used for this research work include the following: ferric chloride (May and Baker, England) (1%w/v), hydrochloric acid (Philip Harris, England), distilled water, Ciprofloxacin (tablets from the companies shown in table I).

Table I: Samples of Ciprofloxacin Tablets.

Samples	Dosage form	Country of origin	NAFDAC Reg. No
A	Tablet	India	
B	Tablet	India	
C	Tablet	China	
D	Tablet	India	
Ciproflox (R)	Tablet	Manufactured for Orange drugs Ltd Nigeria by Indonesian Pharm Industries	04-080

NAFDAC – National Agency for food and Drugs Administration and Control
Reg. No - Registration number

The apparatus used for physico-chemical analyses include the following: analytical weighing balance (Mettler), ultraviolet spectrophotometer (Jenway USA 6405 UV-vis spectrophotometer), tablet hardness tester (Shital Scientific, England), friabilator (Roche), disintegration apparatus , dissolution apparatus (USP apparatus – basket method), foil paper , spatula , beakers , masking tape, conical flask, mortar and pestle , measuring cylinders , cardboard paper, soft brush , 1 mm screen (for sieving).

For microbiological assay, the following were used: nutrient medium (Mueller Hilton agar), empty sterile petri-dishes , empty sterile test-tubes , cork-borer (8mm diameter), sterile distilled water in flask, test tube racks , 10 ml and 1ml sterile pipettes graduated tubes containing test on suspensions of *S. aureus* (clinical isolates) standardized to 10^5cfu/ml, drugs for analysis, empty petri-dishes, empty sterile test-tubes , cork borer of sum diameter , sterile distilled water, 10 and 1ml sterile pipettes, test organism suspension (*S. aureus*) standardized population of 10^5cfu /ml.

Determination of Uniformity of Weight

Twenty (20) tablets from each of the brands were weighed individually using an analytical weighing balance. The average weight for each brand as well as percentage deviations were calculated.

Hardness Test

The hardness tests on the tablets were carried out using 6 tablets selected from each brand. The pressure at which each tablet crushed was recorded and the means and standard deviations were determined.

Friability Test

The friability tests were conducted employing 10 tablets from each brand with 100 revolutions (i.e. 25 revolutions per minute for 4 minutes). The tablets were dedusted, weighed together (W_1) and friabilated. The friabilated tablets were reweighed (W_2) and compared with their initial weights and percentage friability was obtained. Percentage friability was calculated as $[(W_1-W_2)/W_1] \times 100\%$.

Drug Content Assay

The method of Ngwuluka et al (2009) was adopted[6]. A solution of 1% w/v ferric chloride was freshly prepared. Five (5) tablets from each brand were crushed and a sample equivalent to 100 mg of the drug was taken from each powdered sample. This was dissolved in 100 ml of 0.1N hydrochloric acid (HCl). To 5 ml of each l drop of ferric chloride was added and made up to 50 ml with 0.1N HCl. The absorbance of each sample was taken at 438 nm against the blank reagent (1ml ferric chloride solution made up to 50ml with 0.1N HCl) with an ultraviolet spectrophotometer. The drug content was calculated for each brand.

Tablet Disintegration Test

Six (6) tablets from each brand were employed for this test in a freshly prepared medium, 0.1N HCl at 37 ^oC using the BP disintegration apparatus. The disintegration time was taken to be the time no particle remained on the basket of the system.

Dissolution Test

The dissolution test was carried out using USP apparatus I (basket method) [5] in 6 replicates for each brand. The dissolution medium was 900ml 0.1N HCL which was maintained at 37± 0.5 ^oC. In all the experiments 5 ml of dissolution sample was withdrawn at 2, 4, 6, 8, 10 and 12 min and replaced with equal volume of dissolution medium to maintain sink condition. The sampling times were selected in due consideration for the short disintegration times. Samples were filtered and assayed by ultraviolet spectrophotometry at 277 nm. The concentration of each sample was determined from a predetermined calibration curve for ciprofloxacin hydrochloride.

MICROBIOLOGICAL TEST

Preparation of the Test Solution

One tablet/caplet was dissolved in test (10ml) of sterile water. This gives a concentration of 50mg/ml (1 tablet = 500 mg) which was appropriately diluted to 50 µg/ml. Using the stock of

50µg/ml, further dilution was made through two-fold serial dilution method to get 25, 12.5, 6.25 and 3.125 µg/ml (Jackson et. al, 2011).

Procedure

The agar medium (Mueller Hilton Agar) was prepared according to manufacturer's recommendations. It was sterilized and kept molten in 20-ml portions. This was followed by the preparation of seeded agar, which was done by transferring 0.1ml of the test organism into the sterile Petri-dish before mixing it with the molten agar sphenoid at 45^{o}C. After thorough mixing, the plate was allowed to set. Using the cork-borer provided, cups were cut into five (5) sectors previously drawn on the back-side of the plate. Into each cup, a drop of each concentration of the antibiotic was added to its corresponding cup. Then a pre-diffusion time of thirty minutes was allowed before incubation at 37 ^{o}C for 24 h (Agboke et. al, 2011).

Observation

Inhibition zones formed cups due to antibiotic activity of the drug against the test organism were measured diametrically and the values were recorded in mm. The minimum inhibitory concentration was determined from a graph of IZD^2 *vs* log of drug concentration. The MIC of the different samples was compared to that of the INOVATOR (Ciproflox$^{(R)}$).

RESULTS AND DISCUSSION

Weight Uniformity

Uniformity of weight does serve as a pointer to good manufacturing practices (GMP) as well as amount of the active pharmaceutical ingredient (API) (B.P 2004), ciprofloxacin hydrochloride contained in the formulation. The USP (2005) states that, 'for tablets weighing more than 324 mg, weights of more than two tablets should not differ from the average weight by more than 5% (U.S.P 2005). The mean tablet weights obtained were in the order- Ciproflox > D > B > C > A (see table II).

Table II: Percentage Weight Deviations from Mean Weights.

S/N	(A) Mean = 0.733 g	(B) mean = 0.779g	C mean = 0.766g	D mean = 0.788g	CIPROFLOX $^{(R)}$ mean = 0.812g
1	1.910	-1.540	3.394	-0.635	0.246
2	-2.319	-0.385	-1.305	-0.508	0.123
3	-5.866	-2.311	-1.044	0.761	0.123
4	1.501	0.642	1.175	-1.269	-1.601
5	3.001	4.750	-0.914	-0.127	-0.369
6	-4.366	5.135	1.697	3.046	-0.369
7	-1.091	-2.054	-0.522	-1.269	0.123
8	4.366	0.000	0.392	0.127	0.123
9	3.138	1.027	-0.522	1.523	-1.601
10	0.819	0.257	0.131	0.000	0.985
11	0.546	0.128	-1.044	0.254	-0.123
12	0.273	-0.513	0.522	-2.792	0.493
13	-0.273	-1.669	-0.261	-0.508	0.246
14	-2.319	1.926	-0.522	0.761	-0.616
15	3.138	-1.926	-0.392	-0.761	0.123
16	-2.729	-0.513	2.089	1.650	2.094
17	-0.273	-1.540	-0.131	-0.254	0.123
18	-1.091	-4.878	-0.131	0.761	0.369
19	1.910	4.108	-1.436	-1.269	1.108
20	0.409	-1.284	-1.436	0.127	-2.217

All the brands complied with the USP (2005) specification for uniformity of weight as no two tablets deviated in weight from the average by more than 5 % and no single tablet deviated by more than 10% (U.S.P 2005). Thus, all the batches complied with the USP specification and thus the drug contents would be uniform.

Table III: Results of Physico-technical Assay on the Tablets.

Samples	Mean weight (mg)	Mean hardness (kgf)	Mean Friability (%)
A	736.2	8.98 ± 3.46	0.07
B	779.6	9.67 ± 1.37	0.30
C	774.0	12.51 ± 3.01	0.12
D	786.5	9.87 ± 2.38	0.31
Ciproflox $^{(R)}$	813.3	14.55 ± 3.97	0.17

Table IV: Results of Bioequivalence Assay.

Samples	Mean Drug Content (mg)	% Drug Content Deviation	Mean tablet disintegration time	P_{12min}	MIC (µg/ml)
A	420.29	-15.942	1.73±0.27	59.528 ± 8.427	0.255
B	462.18	-7.564	4.15±1.07	35.124 ± 4.408	0.193
C	458.86	-8.228	3.22±0.20	42.468 ± 1.863	0.147
D	483.54	-3.292	7.60±0.44	8.808 ± 0.856	0.095
Ciproflox$^{(R)}$	500.02	0.004	4.88±0.54	15.403 ± 0.799	0.064

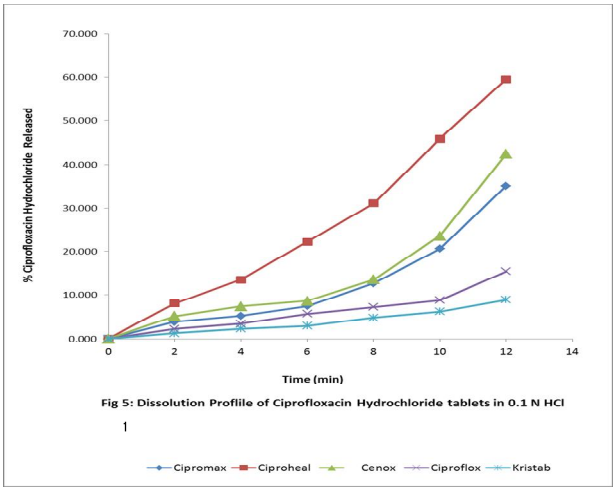

Fig 5: Dissolution Profiile of Ciprofloxacin Hydrochloride tablets in 0.1 N HCl

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The mean hardness results (table III) were in the order: Ciproflox > C > D > B > A. Differences in hardness are usually encountered when different compression pressures are employed (B.P 2004).

Hardness or crushing strength assesses the ability of tablets to withstand handling without fracturing or chipping (Ofoefule, 2002). It can also influence friability, disintegration and dissolution. The harder a tablet, the less friable and the more time it takes it disintegrate (Merchant et. al, 2006). From table III, it can be seen that Ciproflox had the highest hardness value (with average of 14.55 kgs) while Ciproheal had the lowest value (8.98 kgs). A force of not less than 4 kgs is accepted as satisfactory for hardness (B.P 2004). Friability test is used to evaluate the tablets resistance to abrasion. The USP (2005) specification is that the value should not exceed 1% (U.S.P, 2005). Friability for all the brands was below 1% can be seen from table III. This means that

all the brands complied with the compendial specification. The mean friability results were in the order: A < C < Ciproflox < B < D. Thus, the brand most likely to lose particles during handling is A while the least likely to lose particles is Ciproflox. Hardness values did not correlate with friability values. This is can be attributed to the difference in properties of excipients employed in the manufacture of the different brands (Merchant et. al, 2006).

The drug content results (table IV) shows the values to be in the order -Ciproflox > D > B > C > A. The United States Pharmacopoeia (2005) states that the content of ciprofloxacin should not differ from the stated dose by more than 10%, while deviation should not be more than 5 % according to the British Pharmacopoeia (2004) . Thus, by the USP standard, only A failed the test; while, by the BP standard only Ciproflox and D passed the test.

Disintegration could be directly related to dissolution and subsequent bioavailability of a drug Merchant et. al, 2006). A drug incorporated in a tablet is released rapidly as the tablet disintegrates; a crucial step for immediate release dosage forms because the rate of disintegration affects the dissolution and subsequently the therapeutic efficacy of the medicine (Ofoefule 2002). All the brands complied with the compendial specifications for disintegration. The BP (2004) specification is that uncoated tablets should disintegrate within 15mins and film coated tablets in 30 mins. The USP (2005) specifies that uncoated and film coated tablets should disintegrate within 30 mins. The disintegration times were in the order- A < C < B < Ciproflox < D.

The tablet dissolution rates were in the order – A > C > B > Ciproflox > D, (table IV; figure 5). Usually, the higher the tablet hardness the slower the drug dissolution rate (U.S.P. 2005). This obtains when the same processing conditions apply. Therefore, it would have been expected that Ciproflox would have the lowest dissolution rate and sample A the fastest. This discrepancy must be as a result of the different excipients used in each formulation (Ofoefule 2002). The MIC determinations show the efficacy of each formulation against the bacterial isolates which gives a good correlation with activity *in vivo* (Chambers, 2006**).** The antimicrobial activity directly correlates with the drug concentration, and hence drug content, when there excipients used in the formulation do not interfere with drug release (B.P. 2004). Thus, Ciproflox with the highest drug content had the lowest MIC on *Staphylococcus aureus*. This shows it is the most active of the five brands (Esimone et. al 2006, Agboke and Esimone 2011). The highest MIC value on *Staphylococcus aureus* was obtained with sample A which also had the lowest drug content. The drug contents corresponded with the MIC values on *Staphylococcus aureu*s (Vincent, 2005). Thus, in heavy *staphylococcal* infections, Ciproflox would be a drug of choice above the other four brands A, B, C and D.

CONCLUSION

The results show that of the four selected brands of ciprofloxacin currently in the Nigerian market– Sample A, Sample B, Sample C and Sample D - none can be used to replace Ciproflox$^{(R)}$ in *Staphyloccocal* infections.

It is therefore pertinent for the drug regulatory bodies, especially, the National Agency for Food and Drug Administration and Control (NAFDAC) to monitor more closely the products being shipped into Nigeria. It is noteworthy that all the four brands assayed against Ciproflox$^{(R)}$ showed lesser quality. Ciproflox is from Asian country showing that good drugs are also being imported from Asian cuntries into Nigeria, but there should be proper drug assay and monitring by the regulatory body NAFDAC to ensure that people have access to quality and potent drugs.

APPRECIATION

The authors wish to appreciate the following people for assisting in some practical procedures in this work- Mrs E.D. Umoh of the Faculty of Pharmacy, University of Uyo, Nigeria, E. O. Omeje and Nnadi, both of the Faculty of Pharmacy, University of Nigeria, Nsukka. Mr. Ogboso Kalu of the Department of Pharmaceutics, University of Nigeria Nsukka carried out the microbiological assay.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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