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Research Article

Comparative *in-vitro* bioequivalence analysis of some brands of ciprofloxacin HCl tablets marketed in Ethiopia

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ABSTRACT

Peoples who are below poverty prefer to go for inexpensive products to reduce the cost of treatment and there are general psychologies that, less expensive drugs are less effective than the expensive one. There are different brands of ciprofloxacin, most commonly prescribed drug against gram-negative bacterial infections, has a wide price range. Hence, this study was conducted to compare the *in-vitro* bioequivalence profile of the expensive and less expensive brands of ciprofloxacin tablet dosage forms marketed in Ethiopia. Assay, weight uniformity, hardness, friability, disintegration time and dissolution tests were performed and all the four brands tested in this study meets the compendia specifications of hardness, weight uniformity, friability, disintegration time, and dissolution tests. But, hardness and disintegration time of brand A was not correlated. Brand A had disintegrated within 9.16 minutes and crushed in 118.7N force but, the other formulations were crushed at higher pressure and disintegrated in short period relative to brand A. This may be due to the difference in type and amount of additivives like binders and disintegrating agents added during manufacturing. This work revealed that the four formulations included in this study complied with the physicochemical quality parameters claimed in official compendia. But, disintegration time of bran A was statistically significant as compared to with the other brands. Although, there was statistically significant difference ($p<0.05$) between brand A and D in their dissolution profile, all brand included in this were fulfilled the compendia specifications for *in-vitro* dissolution profile. Hence, all brands included under this study are pharmaceutically and chemically equivalent based on the performed *in-vitro* determinations and could be used interchangeably.

Key words: *Bioequivalence; Ciprofloxacin; in-vitro; Dissolution test.*

INTRODUCTION

Ethiopia is a developing country, in which majority of the population are under the poverty line. Hence, they prefer to use less expensive medicines. To reduce the cost of treatment especially for the below poverty peoples of developing countries, it has been recommended to use drug products other than innovator brands and expensive drugs.[1] The substitution of expensive with less expensive drug could be considered when it contains identical amounts of the same active ingredient in the same dose, dosage form and route of administration with the expensive drug as well as it should meet requirements for strength, purity, quantity, and identity specified in official compendia.[2]

To ensure substitution of expensive with less expensive drug for affordability and at the same time achieve therapeutic efficacy, bioequivalence studies, which involves both *in-vivo* and *in-vitro* methods, become necessary.[3] With the introduction of biopharmaceutics classification system (BCS), *in-vivo* bioequivalence studies could be waived for immediate release solid dosage forms for BCS class I (highly soluble and highly permeabile), class III drugs (highly soluble and low permeability)

and highly variable drugs which have wide therapeutic index.[4] Therefore, *in-vitro* comparative analysis could be utilized to determine bioequivalence profile of this type of drugs as this method reduces the cost of *in-vivo* method and determines drug absorption directly. Dissolution test can serve as a tool to determine the drug products are either acceptable or unacceptable.[4,5] It is a surrogate marker for bioequivalence test, which is practical and less costly approach in developing countries, where both technology and resources are limited for *in-vivo* studies. Thus, *in-vitro* dissolution may be important in assessing *in-vivo* drug performances. Ciprofloxacin is a synthetic fluoroquinolone derivative with broad spectrum antibacterial activity which can be used for the treatment of different bacterial infections.[6,7]

Numerous *in-vitro* comparative bioequivalence studies on different brands of ciprofloxacin HCl tablets have been conducted to determine their cost and quality relationship and their interchangeability for therapeutic purpose. One of the brands included in the study was failed to meet USP specification for friability and dissolution test and has been considered as substandard medicine.[8] Similar study have been conducted by Osonwa *et al.* and have been reported that among the five tested brands one brand were failed to satisfy t*he united states pharmacopeia (USP) acceptance criteria and three brands were failed to meet the BP specification for in-vitro dissolution test.*[9] Furthermore, The study conducted by Muhammad and coworkers shown that three local brands had active pharmaceutical ingredient content less than the claimed requirement in USP and one brand has been reported that it failed the disintegration and dissolution tests, thus, the products could not be used interchangeably.[10]

Ethiopia is a developing country in which majority of the population are unable to afford for some brand products because as they are more and more expensive than the locally produced generic products. And also, there is a general psychology low priced products have poor efficacy than the more expensive drugs. Recently many brands of ciprofloxacin are found in Ethiopian market and their price ranges from ETB1.0 to ETB20.0 per tablet in Ethiopian local currency, which means that there is ETB19.0 difference between the less priced ciprofloxacin product and the expensive one. As this drug is prescribed for at least seven and more days, the cost of treatment would raised by at least ETB266. Therefore, this study was aimed to evaluate the *in-vitro* bioequivalence profiles of four selected less priced and expensive ciprofloxacin HCl tablet products of different manufacturers comparatively.

METHODOLOGY

2.1 Materials:

Four different brands of ciprofloxacin were purchased from community pharmacies in Ethiopia. Pure ciprofloxacin HCl powder manufactured by Zhejiang Guobang Pharmaceutical Co., Ltd, China, obtained by donation from Addis Pharmaceutical Factory S.C., was used as a standard. The reagent utilized was hydrochloric acid which was manufactured by D.B.H Laboratory supplies, England.

Table 2.1 Ciprofloxacin HCl 500 mg tablet brands evaluated in this study for their quality.

Brand name (symbol)	Country of origin	Strength	Batch No.	Mfg.date	Exp.date
Ciflox (A)	Ethiopia	500mg	B-16113	11/2013	11/2016
Ciprodac (B)	India	500mg	D13025BY38	03/2013	02/2016
Cipropharm (C)	Jordan	500mg	B-14497	10/2014	10/2017
Ciprodenk (D)	Germany	500mg	16036	02/2014	01/2017

2.2 Methods:

2.2.1 Assay

The assay of each brands of ciprofloxacin included in this study was determined according to united States Pharmacopoeia (USP) specifications.[11] 20 tablets of each formulation were weighed and powdered. The powder equivalent to 100mg of ciprofloxacin HCl active ingredient was taken and transferred to 100 ml volumetric flask. Then, the volume was filled up to 100 ml with 0.1 N Hydrochloric acids (HCl). Vigorous shaking was done to dissolve the powdered material. After proper dilution, absorbance values were measured at the maximum wave length (λ_{max}) of 277 nm using UV-VIS spectrophotometer (SHIMAZDU Corporation, JAPAN) against a blank.

2.2.2 Determination of Uniformity of Weight

The determination of weight uniformity of both local and imported products of ciprofloxacin HCl tablets were done according to USP procedures.[11] 20 tablets from each formulation were weighed individually with an analytical balance (Adventurer OHAUS, China). The average weights for each brand as well as the percentage deviation from the mean value were calculated and compared.

2.2.3 Hardness Test

The crushing strength of each ciprofloxacin HCl tablet formulation were determined with a tablet hardness tester. Ten tablets were randomly selected from each brand and then the crushing strength at which each tablet crushed was recorded and discussed.[9]

2.2.4 Friability Test

The friability tests of all formulated products of ciprofloxacin HCl tablets were carried out using the method adopted from the research done by Osonwa and coworkers, by employing 20 tablets from each brand and tested with friability tester rotated 100 revolutions (i.e. 25 revolutions per minute for 4 minutes). The tablets were dedusted, weighed together (W_i) and friabilated. The friabilated tablets were reweighed (W_f) and compared with their initial weights and percentage friability was obtained. Percentage friability was determined by using the following formula:[9]

$$\%\text{Friability} = \frac{(W_i - W_f)}{W_i} \times 100$$

Where, W_i= initial weight and W_f = weight after friability tested

2.2.5 Disintegration Time Test

The disintegration time of local and imported products of ciprofloxacin were determined by employing six tablets from each formulation. All tablets were employed for the disintegration test in water at 37± 0.5°C using disintegration apparatus (Es Eagle Scientific Limited, Nottingham, UK). The disintegration time was taken to be the time when no undispersed particles left on the basket of the system.[12]

2.2.6 Dissolution Test

The dissolution test was carried out using USP apparatus II (paddle method), method described in the British Pharmacopoeia (BP) as it is recommended for tablet dosage forms.[13] The dissolution medium was 900 ml of 0.1N HCl acid which were maintained at 37 ± 0.5°C. Six tablets of each formulation were employed for dissolution test three times repeatedly. In all the experiments, 5 ml of dissolution samples were withdrawn at 5, 10, 15, 30, 45 and 60 minutes, and replaced with equal volume fresh dissolution medium for each samples taken to maintain sink condition. Collected dissolution samples were filtered, diluted and assayed by UV-VIS spectrophotometer at maximum wavelength (λ_{max}) of 277 nm.[14] The concentrations of each sample were determined from a calibration curve that was obtained from pure reference samples of ciprofloxacin powder.

2.3 Data Analysis

Results were reported as mean plus or minus standard error of mean (M± SEM). Analysis of the data obtained from the experiment was carried out using ORIGIN 7 statistical soft ware packaging and Microsoft office packaging. Analysis of variance (ANOVA) followed by Tukey *post-hoc* analysis was used to compare dissolution profile among the brands.

3. RESULTS AND DISCUSSION

3.1 Assay

Assay of uniformity was performed to determine the percent of active ingredient available in the brand against the claimed amount in the label. The USP states that, the content of ciprofloxacin in a formulation should not deviate from the claimed dose by more than 10%.[11]

As shown in Table 2, the percentage of ciprofloxacin in the formulations was found to be 100.9% (for brand B), 98.9% (for brand D), 97.37% (for brand C) and 96.3% (for brand A). There was no significant difference ($p>0.05$) in the content of ciprofloxacin active ingredients among the four brands. Thus, based on the USP requirements, all formulations included in this study have fulfilled the specifications.

3.2 Weight Uniformity

Uniformity of weight does serve as a pointer to good manufacturing practices as well as serve as a way to predict the uniformity in the amount of the active pharmaceutical ingredient found in the each tablets.[13] As stated in Table 2, each tablet formulations of ciprofloxacin hydrochloride showed acceptable uniformity in their weight based on the USP specification as there was no two tablets deviated in weight from the average by more than 5% and no single tablet deviated by more than 10%.[11]

The significance of the test was to ensure that the tablets are within the appropriate formulation size range. It also showed that there was consistent mixing and die filling during production process.

Table 2: assay, weight variation, hardness, and friability test results of all four generic ciprofloxacin HCl tablets.

Brand	Assay (%), n=20	Weight uniformity (mg), n=20	Harness (N), n=10	Friability (%), n=20
A	96.3 ± 1.33	645 ± 8.86	118.7±6.7	0.16
B	100.9 ± 1.45	749 ± 8.08	138.9 ± 6.79	0.27
C	97.37 ± 0.89	768.6 ± 3.7	≥200	0.19
D	98.9 ± 0.33	733.8 ± 4.38	197.1±3.18	0.05

Where, n is the number of tablets tested from each formulation; N refers to Newton

3.3 Hardness test

Hardness or crushing strength assesses the ability of tablets to withstand handling without fracturing or chipping. It can also influence friability, disintegration and dissolution. The harder a tablet, the less friable and the more time it takes to disintegrate.[9] But, in this study hardness values did not correlate with disintegration time values. As shown in Table 2, brand C had the highest hardness value (≥200N) while brand A had the least crushing strength value (118 ± 6.7N). A force of hardness not less than 50N is accepted as satisfactory for hardness to withstand damage on transportation and handling.[11] But, in this study the disintegration time of brand A was longer than other formulations included under this study. This can be attributed to the difference in properties of excipients employed in the manufacture of the different formulations.

3.4 Friability test

Friability test is used to evaluate the tablets resistance to abrasion. The USP specification is that the amount to friable should not exceed 1% of the total tablets weight.[11] Friability for all the formulations was below 1%, as shown in Table 2, with the most and least friable brand B (losses 0.27%) and brand D (losses 0.05%), respectively. This means that all the formulations were conformed to the pharmacopoeia specification. Although, the formulations were lied in the acceptance limit, there is significant difference ($p<0.05$) in their friability result between formulations B and D.

3.5 *Disintegration time test*

Disintegration could be directly related to dissolution and subsequent bioavailability of a drug. 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. The BP specification is that uncoated tablets should disintegrate within 15mins.[13] The USP specifies that uncoated and film coated tablets should disintegrate within 30 minutes.[11] As shown in Table 3, brand B was disintegrated in 2.47 ± 0.5minutes, a product which was disintegrated quickly, and brand A was disintegrated in 9.16 ± 0.45minutes. Though, there was a statistically significant ($p<0.05$) difference in disintegration time of brand A as compared with the rest formulations, all products included in this study were complied with the compendia specifications for disintegration time.

Table 3: Disintegration time and dissolution profiles of four ciprofloxacin HCl tablet products.

Brand	Disintegration time (min), n=6	% Dissolution at 30 min, n=6
A	9.16 ± 0.45*[a]	91.56 ± 0.68*[b]
B	2.47 ± 0.5	94.46 ± 0.66
C	5.96 ± 0.57	92.92 ± 1.20
D	4.38 ± 0.32	95.16 ± 2.05

*Where, n is the number of tablets tested from each formulation; * refers result was statistically significant ($p<0.05$); [a] refers to as compared with brand B, C and D; [b] refers to as compared with brand D*

3.6 Dissolution test

Dissolution test is performed to check the percentage of drug released from the brand After 30 minutes.[10] Formulations of different manufacturers, with different inactive ingredients, and different brand Design may have different dissolution profiles or release characteristics and therefore may have different bioavailability.

The amount of drug released to the medium was analyzed by Ultroviolet-Visible spectroscopy at wavelength (λ_{max}) of 277nm. All the formulations of ciprofloxacin tablets studied released more than 80% within 30 minutes as this is an acceptance criterion. As detailed in Table 3 and Figure 1, the maximum percent of drug released was 95.16±2.05% from brand D with the minimum percent of drug release (91.56±0.66%) observed from brand A. Thus, the studied ciprofloxacin formulations meet the compendia specifications. There was statistically significant ($p<0.05$) difference with *in-vitro* dissolution profile of brand A and brand D. how ever there was no stastical significance on the dissolution profile of brands B, C and D.

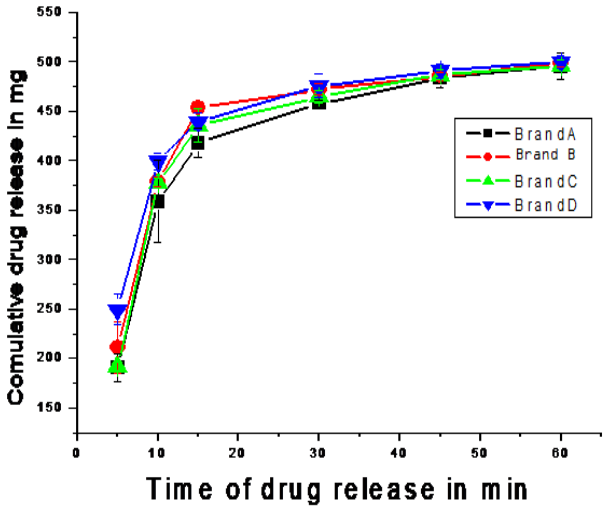

Figure 1: Dissolution profile of four formulations of
ciprofloxacin. Where, n=6; n is the number of tablets
tested in each formulation

Several post marketing surveillances of *in-vitro* bioequivalence analysis have been done in many developing countries to compare and evaluate the price and quality of different brands of ciprofloxacin tablets against the innovator tablet formulation or the reference standard to ensure product quality and eliminating poor quality products from distribution, eventually to have better therapeutic outcome.[15]

Different brands of ciprofloxacin hydrochloride tablets have been evaluated for their assay test, weight uniformity, friability, hardness, disintegration time and dissolution tests by different researchers and have been reported that all brands included in their studies had meet the compendia requirement. They have been also reported as there were no significant difference among i*n-vitro* bioequivalence profile of the brands.[12,16-18] All this four studies were suggested all the brands of the ciprofloxacin hydrochloride tablets evaluated could be regarded as being pharmaceutically and chemically equivalent.

Similarly, this study were also revealed that, all the four brands of ciprofloxacin HCl tablets complied with USP and BP specifications claimed for assay test, weight variation, hardness, friability and disintegration time tests. And the dissolution profile of the less priced and expensive brand products included in this study were shown to conform with the official compendia requirements and had similar dissolution profile even though there was statistically significant difference *in-vitro* drug release between brand A and brand D. In this study hardness values obtained did not correlate with friability values and disintegration time values. This might be attributed to the difference in properties and quantities of excipients, particularly binders and disintegrating agents, employed in the manufacture of the different brand.[9]

To conclude, all the four brands of ciprofloxacin tablets included in this investigation were shown an acceptable *in-vitro* dissolution profile and could be used interchangeably as they had physicochemical equivalence among themselves. Additionally, this investigation will help to change the attitude of peoples on the efficacy of cheapest medicines.

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