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Research Article

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Predicting the Bioequivalence of Ciprofloxacin HCL Tablet-Brands Using *In Vitro* Dissolution and Rat Intestinal Permeability Tests

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ABSTRACT

Bioavailability of a drug is critical factor affecting its efficacy. This parameter for every new brand or a new batch of the brand can be tested by in vivo bioequivalence studies on human volunteers where the equivalence in bioavailability of the tested brand is compared to that of an innovator reference one. These studies requires availability of huge budget, qualified personnel and specific technical requirements and hence, unfortunately, are difficult if not impossible to be carried out in poor countries such as Yemen. Therefore, the approaches of using alternative methods such as animal models, under ethical approval, may be suitable alternatives to the expensive in vivo methods. The present study was alongside those alternative approaches as it aimed to predict bioequivalence of ciprofloxacin HCl 500 mg tablet-brands marketed in Yemen using in vitro dissolution and animal intestinal permeability tests.

Five test brands and one innovator brand of the drug were investigated in this study. In addition to drug content and in vitro dissolution, the rat intestinal permeability experiment using rat gut sac model, was carried out for each brand. The drug content and dissolution of all brands were within the pharmacopeial limit with exception of one test brand whose cumulative dissolved% was lower than pharmacopeial limit and also not similar to that of the innovator brand. On the other hand, the permeability ratios of only two test brands were within the range of 0.80-1.25. Since the drug has low solubility and permeability, the two brands which showed similar dissolution and permeability to the innovator brand could be predicted to be bioequivalent to it.

Keywords: Ciprofloxacin HCl; Bioequivalence; *In vitro*; Prediction; Intestinal permeability

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INTRODUCTION

Ciprofloxacin HCl (3-Quinoline carboxylic acid, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-,

monohydrochloride, monohydrate) [1] is a broad-spectrum bactericidal anti-infective agent of the fluoroquinolone class approved for treatments of 14 types of infection, especially urinary tract infections such as acute uncomplicated cystitis and chronic bacterial prostatitis, and lower respiratory infections [2,3].

The drug is a biopharmaceutics classification scheme (BCS) class IV drug, which means that the drug has low solubility and intestinal solubility. Therefore, the dissolution and absorption of ciprofloxacin HCl are both the rate-limiting steps in the drug bioavailability [4]. The drug is highly soluble at acidic pH, however, at intestinal pH like 6.8 and 7.5, its solubility is far lower. *In vivo* studies on human volunteers showed that the absolute oral bioavailability of the drug compared to intravenous administration range from 56-77% [4-6]. The permeability of ciprofloxacin HCl was measured in an *in vitro* Caco-2 assay, rat jejunum and In situ rat jejunum and found to be (2.49, 3.2 and 11.1) × 10^6 cm/s, respectively [4,7].

Bioequivalence is defined by FDA as the absence of a significant difference in the rate and extent to which the active ingredient or active moiety becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study. The accepted limit of bioequivalence as reported by FDA is within the range of 0.80-1.25. Bioequivalence studies are mainly *In vivo* studies carried out on human volunteers [8]. However, *in vitro* methods e.g. comparative drug release/dissolution studies under certain conditions may give an indication of drug bioavailability and bioequivalence [9]. Other methods e.g. animal gut sac model, which is an efficient tool for studying in-vitro drug absorption mechanisms, have been reported to provide results which are in agreement with in-vivo findings [10-13].

Although ciprofloxacin HCl rat intestinal permeability was studied by many approaches including rat sac model [4], this study was the first to use one of those approaches to predict the bioequivalence of ciprofloxacin HCl tablet-brands.

EXPERIMENTAL SECTION

Materials

Ciprofloxacin HCl standard was a gift from Global pharma-manufacturer, Yemen. All chemicals used were purchased from the local market and were at least of analytical grade. Six 500 mg tablet brands of the drug were investigated in this study, of which one innovator brand (Cipro®; Batch No. BXGNH61, Bayer Schering Pharma AG, Germany) were purchased from the market.

Instrumentations

UV spectrophotometer (LI-295 Lasany, India) and dissolution apparatus USP II (912, Esico, India) were used in this study.

Animal Models

Six adult male Wister rats (286 ± 12 g) were used as models. The animals were incubated in appropriate cage. Prior to test, they were fastened overnight with free excess to water.

Drug Content

The experiment was performed as described by TH Fereja et al. [14] as follows: A stock solution (20 μg/ml) of ciprofloxacin HCl in 0.1 M was prepared. Serial dilution of that stock was performed to prepare 6 diluted standard solution of concentrations (0.5-5 μg/ml). The solutions were filtered and then were analyzed by UV

spectrophotometer at 276 nm. The calibration curve was constructed and the regression equation of the curve was determined and used to analyze the drug in tablets. For the purpose of assaying the drug in the tested tablet brands, 20 tablets of the tested brand were powdered and then a quantity of the powder theatrically equivalent to 50 mg of the drug was accurately weighed. The powder was then dissolved in 75 ml of water, filtered, and then made up to 100 ml with water. 1 ml of the solution was diluted up to 100 ml to provide a theoretical concentration (Ct) of 5 µg/ml. The UV absorbance of that solution was measured at 276 nm. The obtained absorbance was introduced into the calibration regression equation to calculate the practical concentration (Cp). The drug content was calculated as follows:

$$\text{Drug content\%} = 100 \times Cp/Ct$$

***In vitro* Dissolution**

The test was carried out as described in the USP [1] using apparatus II provided with 6 chambers. Each chamber was filled with 900 ml of 0.01 M as dissolution medium. 500 mg of ciprofloxacin HCl standard or one 500-mg tablet of each brand was introduced into each chamber and the system operated at 37 ± 0.5 o C for 30 minutes. 5-ml aliquot was withdrawn from each chamber at 0, 5, 10, 15, 20 and 30 minutes, and 5-ml of fresh dissolution medium was introduced after each withdrawal. The samples were filtered through 0.45 µm filter and the UV absorbance at 276 nm was measured. The dissolved% was then calculated the same as drug content described earlier.

The similarity factor (*f*2) of a test brand dissolution of to that of innovator brand was calculated as follows

$$f2 = 50 * \log \{[1+(1/n)_{\Sigma t=1n} (Rt-Tt)^{2]-0.5} \bullet 100\}$$

where n was the number of time points, Rt was the measured value of the reference original brand at time t, and Tt is the measured value of the test brand at time t**.** The factor for each test brand was calculated once by using pure drug and then by using original brand as references. The test brand with f2 value greater than 50 was considered equivalent to the reference [9].

***In vitro* Rat Intestinal Permeability**

The experiment was carried out as described in the literature [15-18] with appropriate modification. Prior to intestinal permeability test, it was necessary to carry out standard calibration curve of the drug in the incubation medium used in the experiment. Therefore, a stock solution (20 µg/ml) of ciprofloxacin HCl standard in TC 199 solution was prepared. The TC 199 solution was composed of aqueous solutions containing 145 mM NaCl, 4.56 mM KCl, 1.25 mM CaCl2. 2H2O and 5 mM $NaHPO_4$,. Serial dilution of stock solution was made to prepare 6 diluted standard solutions of concentrations (0.5-5 µg/ml). The UV absorbance of those solutions at 276 nm were measured and calibration curve was then constructed, from which the regression equation was determined.

Rats were anesthetized by chloroform. The intestine of the rat was exposed by a midline abdominal incision and 20-25 cm segment of the proximal rat jejunum was excised and was immediately dissected and flushed with TC 199 solution at 10°C for 20 minutes. The excised segment was then gently everted over a glass rod and filled with TC 199 solution (serosal fluid) and tied using surgical suture.

In order to exclude the influence of drug dissolution on the permeability result, a solution of each tested substance (pure drug or a brand powder) was prepared as follows: A powder of the tested substance was prepared by appropriate milling and then sieved through a mesh No. 60. 5 mg of the sieved powder of pure drug, or an amount of

the brand powder equivalent to 5 mg of the drug, was dissolved in 15 ml of TC 199 solution, filtered and the volume was made up to 20 ml with the same solvent to provide a simulated mucosal fluid containing a theoretical initial concentration of the drug of 0.2 mg/ml. The solution was transferred to an Erlenmeyer flask and was oxygenated (O_2:CO_2=95:5) throughout the experiment time. The everted sac was placed in the flask containing. The flask was then stoppered and kept at 37°C on a oscillating water bath at (80 cycle/minute) for 2 hours. At the end of the experiment, the everted sac was emptied and the volume of the fluid was measured and filtered. The UV absorbance of that fluid was measured by UV spectrophotometer at 276 nm. The test was performed in triplicates. Similarly, a sample of mucosal fluid with a volume equal to that of serosal fluid was taken and filtered and its UV absorbance was also measured at 276 nm. The average UV absorbance was determined and introduced into the calibration regression to calculate practical concentration (Cp µg/ml) of the drug in the serosal or mucosal sample. These concentrations were used to calculate the amount of drug (Q) (as mg) present in each fluid after experiment, as follows:

$$Q=Cp.\ V/1000$$

Where V was the volume of serosal or mucosal fluid tested.

The apparent intestinal permeability coefficient (of the pure drug or a brand) was calculated as follows:

$$P=(dQ/dt).\ (1/AC_0)$$

where P_{app} (cm/s) was the apparent permeability coefficient, dQ/dt (mg/s) was the amount of drug transported across the gut sac membrane per unit time, i.e. the result of subtracting the amount of drug in serosal from that in mucosal fluids; A (cm^2) is the surface area, of gut sac, and C_0 (mg/mL) represented the initial concentration of the drug in mucosal fluid.

In order to make sure that the innovator brand could be valid to compare test brands with it, it was necessary to determine the reference permeability ratio (R_r). This parameter was calculated as follows:

$$R_r=P_{in}/P_p$$

Where P_{in} and P_p were the intestinal permeability coefficients of the innovator brand and pure drug, respectively.

On the other hand, the test permeability ratio of the test brand was calculated as follows:

$$T_r=P_t/P_{in}$$

Where P_t and P_{in} were the intestinal permeability coefficients of test brand and innovator brand, respectively.

Because ciprofloxacin is BCS class IV, only the test brand which showed similar dissolution behavior and permeability ratio of 0.8-1.25, could be predicted to be bioequivalent to the innovator brand.

RESULTS AND DISCUSSION

Standard Calibration Curves

Figure 1A and 1B, respectively, show the UV standard calibration curve, at 276 nm, of ciprofloxacin HCl in 0.1 M (for drug content and dissolution experiments) and in TC 199 solution (for intestinal permeability experiment). The two curves had optimum linearity of 0.995 and 0.994, respectively, and their regression equation that was used to calculate practical concentration of the drug in the sample were (y=0.1009x-0.0058) and, (y=0.1131x-0.0082), respectively.

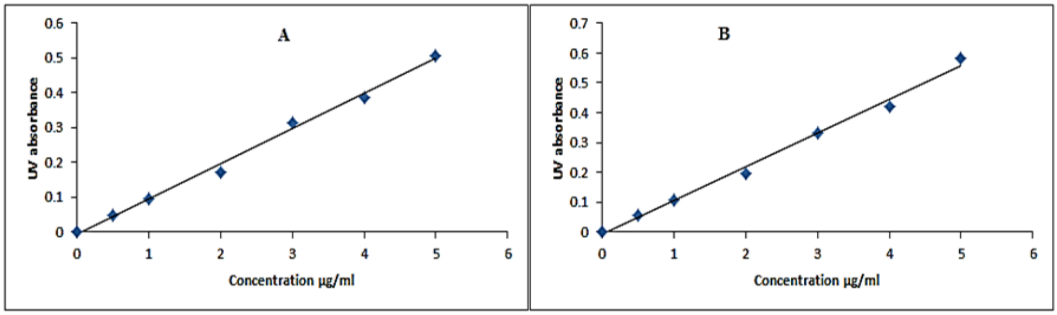

Figure 1. UV standard calibration curves at 276 nm of ciprofloxacin HCl in 0.1 M (A) and TC 199 solution (B)

Drug Content

The drug content% of ciprofloxacin HCl among the tested tablet-brands was found to be within the USP limit (90-110%) and ranged from 99.5 (±3.032) to 102.2 (±0.97).

***In Vitro* Dissolution**

As demonstrated in Figure 2 and Table 1, the cumulative dissolved% of ciprofloxacin HCl, up to 30 minutes, for the pure drug and from the innovator and all test 500-mg tablet brands, except test brand (T5) were all in agreement with the USP limit of the drug dissolution (not less than 80%). In addition, the dissolution similarity factor (*f*2) of all test brands except (T5) was found to be >50 which revealed similar dissolution behavior of those brands.

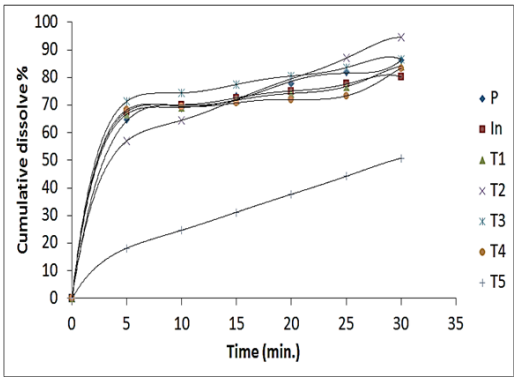

Figure 2. Dissolution profile of ciprofloxacin HCl from pure drug (P), innovator brand (In), and test 500-mg tablet brands (T1, T2,T3,T4,T5)

Table 1. *In vitro* dissolution and rat intestinal permeability of ciprofloxacin HCl for pure drug, innovator and test 500-mg tablet brands

Test	Cumulative dissolved% 0-30 min.	Dissolution similarity factor (f2)	Apparent Intestinal permeability coefficient (x 10^6 cm/s)	Test Permeability Ratio (Tr)
P	86.2$^{\square}$ ± 3.22	-	2.98 ± 0.071	-
In	80.1$^{\square}$ ± 1.03	-	2.502 ± 0.105	-
T1	84.1$^{\square}$ ± 2.13	94.4*	3.867 ± 0.064	1.79$^{\blacksquare}$
T2	94.5$^{\square}$ ± 7.37	59.2*	2.403 ± 0.083	1.11$^{\#}$
T3	86.6 $^{\square}$ ± 4.86	66.9*	2.613 ± 0.057	1.21$^{\#}$
T4	83.4$^{\square}$ ± 4.05	80.1*	1.613 ± 0.031	0.75$^{\blacksquare}$
T5	50.7$^{\clubsuit}$ ± 1.73	20.2**	1.486 ± 0.088	0.69$^{\blacksquare}$

P: pure drug, In: innovator brand, T1,T3, T4, T5: test brands, $^{\square}$: within USP limit (not less than 80%); $^{\clubsuit}$: lower than USP limit; *: similar dissolution profile to that of innovator brand (*f*2>50); **: dissolution profile is not similar

($f2$<50); ♯: equivalent to innovator brand (0.8-1.25); ▪: not equivalent to innovator brand

***In vitro* Rat Intestinal Permeability**

The apparent intestinal permeability (mucosal to serosal) of pure ciprofloxacin HCl, as shown in Table 1, was 2.98 × 10^6 cm/s which was close to that reported in the literature (3.2 × 10^6 cm/s) [18].

Since the FDA bioequivalence limit is 0.8-1.25, this study applied the same limit to describe the degree of equivalence in intestinal permeability. Concerning the innovator brand, it was found that this brand showed an equivalent reference permeability ratio of 0.84 compared to pure drug. With respect to the permeability ratios of test brands (compared to the innovator brand), results revealed that 2 brands (T2, T3) showed ratios within the accepted equivalence. In the contrary, 3 brands showed ratios outside the accepted range with one brand (T1) showed ratios greater than 1.25 and 2 brands (T4 and T5) showed ratios lower than 0.8.

CONCLUSION

Based on the results obtained from this study, only two brands of the investigated ciprofloxacin 500-mg tablet brands could be predicted to be bioequivalent to the innovator brand as they showed similar dissolution behavior and intestinal permeability.

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