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# COMPARATIVE *IN VITRO* BIOEQUIVALENCE ANALYSIS OF SOME CIPROFLOXACIN HCl GENERIC TABLETS

Amit Kumar Nayak [1] and Dilipkumar Pal *[2]

Seemanta Institute of Pharmaceutical Sciences [1], Jharpokharia, Mayurbhanj, Orissa, India
College of Pharmacy, Institute of Foreign Trade & Management [2], Lodhipur Rajput, Delhi Road, Moradabad (UP), India

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**Correspondence to author:**

**Prof (Dr.) Dilipkumar Pal**
College of Pharmacy, Institute of Foreign Trade & Management, Lodhipur Rajput, Delhi Road, Moradabad (UP), India
Email: drdilip2003@yahoo.co.in

## ABSTRACT

Six generic ciprofloxacin HCl 250 mg tablets from different manufacturer have been evaluated to assess their bioequivalence using *in vitro* tests. Other general quality assessments of these tablets like assay, weight variation, hardness, friability, disintegration time were also determined and all these generic tablets passed compendial specifications. There were no significant differences ($p < 0.05$) in the percentage dissolution of drug from generic tablets at 15 minutes with the same from innovator brand tablet at the same time point. To compare the dissolution profiles of all the tablet formulations and the innovator brand, a model independent approach of difference factor ($f_1$) and similarity factor ($f_2$) was employed with all time points included in the *in vitro* dissolution studies. These results indicated that all generic ciprofloxacin HCl tablets included in this investigation were bioequivalent with the chosen innovator brand and so may be used interchangeably.

**INTRODUCTION:** India is a developing country, majority of population are below the poverty line. Hence, they prefer to go for low priced medicines. To reduce the cost of medicines especially for the below poverty line group of developing countries, the World Health Organization (WHO) has continuously supported the use of generic drug products, aiming to improve the overall health care system[1]. The generic substitution could be considered when a generic copy of a reference drug contains identical amounts of the same active ingredient in the same dose formulation and route of administration as well as meet standards for strength, purity, quality, and identity[2]. Although, the WHO issued guidelines for global standardization and requirements for the registration, assessment, marketing, authorization and quality control of generic drug products[3].

The generic products are usually far cheaper than its branded versions as generic manufacturers do not have the investment costs for the development of a new drug. To assist in substitution of branded with generics for affordability and at the same time achieve therapeutic efficacy, bioequivalence studies become paramount. Bioequivalence has been described as the absence of a significant difference in the rate and extent to which the active moiety in pharmaceutical equivalents or pharmaceutical alternatives become available at the site of drug action (i.e., a significant difference in the bioavailability of two drug products) when they are administered at the equal molar dose under similar conditions in an appropriately designed study[4]. Two pharmaceutical products are considered to be bioequivalent when their bioavailability factors (from the same molar dose) are so similar that they are unlikely to produce clinically relevant differences in therapeutic and/or adverse effects[5]. Bioequivalence studies involve both *in vivo* and *in vitro* studies. With the introduction of biopharmaceutics classification system (BCS), *in vivo* bioequivalence studies could be waived for immediate release solid oral dosage forms for class I (high solubility and high permeability) and class III (high solubility and low permeability)[6-7]. Therefore, only *in vitro* testing may be utilized to determine bioequivalence for highly soluble and highly permeable drugs. Dissolution testing can serve as a tool to distinguish between acceptable and unacceptable drug products[8]. It is a surrogate marker for bioequivalence test is a practical and economic approach in developing countries, where both technology and resources are limited for *in vivo* studies.

The drug release from a drug product (i.e., drug dissolution) under physiological conditions and the permeability across the gastrointestinal tract determines the drug absorption. Thus, *in vitro* dissolution may be vital in assessing *in vivo* performances. Ciprofloxacin is a synthetic fluoroquinolone derivative with broad spectrum antibacterial activity[9]. It is widely used in the treatment of urinary tract infections, lower respiratory tract infections, bacterial diarrhoea, skin and soft tissue infections, bone and joint infections, gonorrhea, and in surgical prophylaxis[9-10]. In most of the cases, it would appear that for treatment of above said infections, physicians prescribe ciprofloxacin as a first choice of drug. This has resulted in higher demand and the need for increasing supply of ciprofloxacin products in generic versions for the use of bellow poverty line group in developing countries. It is a general psychology that the quality of generic products may poor as compared to leading brands available in the market. In the present study, we set out to assess the *in vitro* bioequivalence of some generic ciprofloxacin HCl tablets (250 mg)

and the innovator brand ciprofloxacin HCl tablets (250 mg) to justify the quality of generic substitution of ciprofloxacin brands in the Indian market. Other general quality assessments of the tablets were also determined.

## MATERIALS AND METHODS:

**Materials:** Ciprofloxacin HCl was gifted from Dr. Reddy's Laboratories (Hyderabad, India). Six generic ciprofloxacin HCl tablets, manufactured by different manufacturer and the innovator brand product with labeled contents of 250 mg each, were obtained from local market. All tablets were of same manufacturing year. All other reagents were of analytical grade.

**Assay:** Weighed and powdered 20 tablets of each generic product and the innovator brand product. The powder equivalent to 100 mg of ciprofloxacin was taken and transferred to 100 ml of volumetric flask. Then, the volume made up to 100 ml with 0.1 N HCl. Vigorous shaking was done to dissolve the powdered material. After proper dilution, absorbance values were measured at the maximum wavelength ($\lambda_{max}$) of these concentrations was measured using a UV-VIS spectrophotometer (U. V. 2440 Double beam spectrophotometer, SHIMADZU Corporation, JAPAN) against a blank. Maximum wavelength ($\lambda_{max}$) was obtained by scanning all samples from 200 to 400 nm and this was 276 nm.

**Weight Variation Determination:** 20 tablets from each generic and innovator brand products were weighted individually using a weighing balance (Mettler 1180). The average weights of the tablet as well as their percentage deviation were calculated.

**Hardness Testing:** Hardness was determined using a tablet hardness tester (Monsanto).

**Friability Testing:** Friability test was conducted by employing a Friability tester USP 23 (Electro lab, Mumbai, India) at 25 rev/ min for 4 minutes. Percent friability was determined by using the following formula:

**% Friability = 1 – F X 100 / I …… (1)**

Where, I = Initial weight and, F = Weight after friability

**Disintegration Testing:** 6 tablets from each generic and innovator brand products were employed for the disintegration test in water at 37 ± 0.5 °C using a disintegration apparatus (E.D-2L, USP). The disintegration time was taken to be the time, when no particle remained on the basket.

***In-vitro* Dissolution Studies:** *In-vitro* dissolution studies were carried out using a dissolution apparatus IP/USP/BP (basket type). The dissolution medium was 900 ml of 0.1 N HCl, pH 1.2, which was maintained at 37 ± 0.5 °C. In all dissolution experiments, 5 ml of dissolution samples were withdrawn and replaced with equal volume fresh dissolution medium at regular intervals. Collected dissolution samples were used for determination of released ciprofloxacin concentrations by using a UV-VIS spectrophotometer (U. V. 2440 Double beam spectrophotometer, SHIMADZU Corporation, JAPAN) against a blank. Maximum wavelength ($\lambda_{max}$) obtained by scanning all samples from 200 to 400 nm and this was 276 nm.

**Statistical Analysis:** The uniformity of weight was analyzed with simple statistics, while dissolution profiles at 15 minutes were analyzed for significant differences by one- way analysis of variance (ANOVA) using a Student - Newman-Keuls test for all pair wise comparisons in this study. The statistical analysis was conducted using MedCalc software version 9. 6. 4. 0.

**RESULTS AND DISCUSSION:** All the generic and innovator brand ciprofloxacin HCl 250 mg tablets used in this investigation were within their shelf life. All tablets obtained from local market were subjected to a number of tests in order to assess their *in vitro* bioequivalence along with other quality parameters like assay, weight variation, friability, hardness, and disintegration time. All the tablets, both generics and the innovator brand contained ciprofloxacin within 100 ±10 % of the labeled claim. The USP[11] and IP[12] specifications for assay are that the ciprofloxacin content should be less than 90 % and not more than 110 % (Table 1). Therefore, the assay results ascertain the presence and compendia quality of ciprofloxacin in all the products. Weight variation does serve as a pointer to good manufacturing practices (GMP) maintained by the manufacturers as well as amount of active pharmaceutical ingredient (API) contained in the formulation. The weight variation for all the tablets used in this study showed compliance within the official specifications (USP, 2000; BP, 1998)[11, 13], as none of the products deviated by up to 5 % from their average weight (Table 1).

**TABLE 1: ASSAY AND WEIGHT VARIATION RESULTS OF ALL SIX GENERIC CIPROFLOXACIN HCL TABLETS WITH ITS INNOVATOR BRAND**

| TABLETS* | ASSAY (%) | WEIGHT VARIATION (mg) |
|---|---|---|
| G 1 | 98.35 ± 0.29 | 390.54 ± 0.84 |
| G 2 | 98.08 ± 1.68 | 387.58 ± 0.79 |
| G 3 | 97.73 ± 1.89 | 397.25 ± 0.46 |
| G 4 | 96.94 ± 2.46 | 392.17 ± 0.88 |
| G 5 | 98.34 ± 1.53 | 364.82 ± 1.02 |
| G 6 | 97.03 ± 0.59 | 390.12 ± 0.93 |
| Innovator brand | 101.12 ± 1.71 | 392.63 ± 0.40 |

*G 1-G 6: denotes all generic ciprofloxacin HCl tablets

The hardness test all tablets (both generics and the innovator brand) were done to assess the ability of tablets to withstand handling without fracturing or chipping. A force of about 4 kg/cm$^2$ is the minimum requirement for a satisfactory hardness of tablets[14]. The results of the hardness testing showed that hardness of all generic tablets were within the range between 5.42 ± 0.25 to 7.04 ± 0.26 kg/cm$^2$, whereas in case of the innovator brand, it was 7.28 ± 0.33 kg/cm$^2$ (Table 2).

Hence, the results of the hardness testing were satisfactory. Friability test is used to evaluate the tablet resistance to abrasion. The compendial specifications of friability for tablets are less than 1 % w/w (USP, 2000; BP, 1998)[11, 13]. The friability (%) of all generic tablets was within the range of 0.11 to 0.22, while the friability (%) of innovator brand was 0.11 (Table 2).

**TABLE 2: HARDNESS AND FRIABILITY DETERMINATION OF ALL SIX GENERIC CIPROFLOXACIN HCL TABLETS WITH ITS INNOVATOR BRAND**

| TABLETS* | HARDNESS (kg/cm$^2$) | FRIABILITY (%) |
|---|---|---|
| G 1 | 5.42 ± 0.25 | 0.11 |
| G 2 | 6.76 ± 0.44 | 0.17 |
| G 3 | 5.82 ± 0.11 | 0.16 |
| G 4 | 6.52 ± 0.19 | 0.22 |
| G 5 | 6.04 ± 0.51 | 0.22 |
| G 6 | 7.04 ± 0.26 | 0.20 |
| Innovator Brand | 7.28 ± 0.33 | 0.11 |

*G 1-G 6: denotes all generic ciprofloxacin HCl tablets

All the tablets, both generics and the innovator brand compiled with the compendia specifications for disintegration (Table 3). The BP specification is that the uncoated tablets should

disintegrate within 15 minutes [13], while USP specifies that uncoated tablets should disintegrate within 30 minutes [11]. The drug incorporated in a tablet is released rapidly as the tablet disintegrates. Therefore, disintegration is a vital quality parameter of tablet as this is directly related with drug dissolution and subsequent bioavailability of drug.

**TABLE 3: DISINTEGRATION PROFILES OF ALL SIX GENERIC CIPROFLOXACIN HCL TABLETS WITH ITS INNOVATOR BRAND**

| TABLETS* | DISINTEGRATION TIME (MIN) |
|---|---|
| G 1 | 3.50 ± 0.55 |
| G 2 | 3.33 ± 0.52 |
| G 3 | 3.50 ± 0.54 |
| G 4 | 3.83 ± 0.75 |
| G 5 | 3.50 ± 0.84 |
| G 6 | 3.33 ± 0.52 |
| Innovator Brand | 3.67 ± 0.82 |

*G 1-G 6: denotes all generic ciprofloxacin HCl tablets

According to the FDA guidance for industry, for the dissolution testing of immediate release solid oral dosage form, the BCS suggests that for class I and few class III drugs 85 % w/w dissolution of the labeled content in 0.1 N HCl within 15 minutes ensure that the bioavailability of the drug is not limited by dissolution [15]. Ciprofloxacin is a class III drug candidate.

The amount released by all generic and the innovative brand ciprofloxacin HCl tablets were over 85 % (Table 4 and Figure 1) within 15 minutes. There were no significant differences ($p < 0.05$) in the percentage dissolution of drug from generic tablets at 15 minutes with the same from innovator brand tablet at the same time point.

**TABLE 4: THE AMOUNT RELEASED BY ALL GENERIC AND THE INNOVATOR BRAND CIPROFLOXACIN HCL TABLETS**

| TABLETS* | % DISSOLUTION AT 15 MINUTES¥ |
|---|---|
| G 1 | 86.28 ± 0.44 |
| G 2 | 86.30 ± 1.36 |
| G 3 | 86.08 ± 0.98 |
| G 4 | 89.03 ± 1.92 |
| G 5 | 89.01 ± 1.20 |
| G 6 | 90.99 ± 1.63 |
| Innovator Brand | 91.13 ± 0.44 |

*G 1-G 6: denotes all generic ciprofloxacin HCl tablets;
¥Statistically significance ($p<0.05$) compared with all formulations determined by one way ANOVA followed by using Student- Newman- Keuls test for all pair wise comparisons

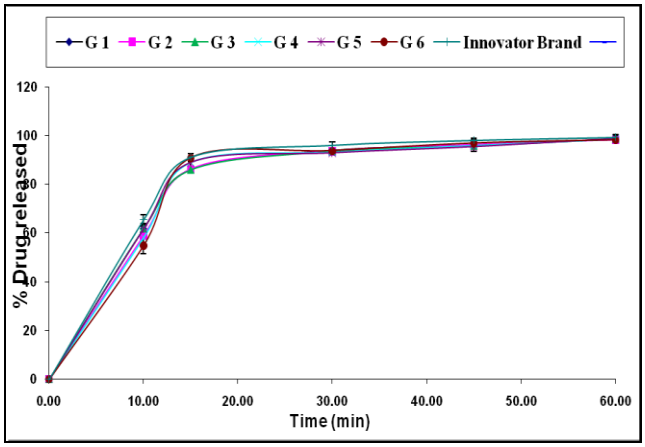


**FIG. 1: DISSOLUTION PROFILES OF ALL GENERIC AND THE INNOVATIVE BRAND CIPROFLOXACIN HCL TABLETS**

To compare the dissolution profiles of all the generic tablets and the innovator brand, a model independent approach of difference factor ($f_1$) and similarity factor ($f_2$) was employed with all time points included in the *in vitro* dissolution studies [15]. Difference factor ($f_1$) is the percentage difference between two curves at each time point and is a measurement of the relative error between the two curves:

$$f_1 = \{[\sum_{t=1}^{n}|R_t - T_t|]/[\sum_{t=1}^{n}R_t]\} \bullet 100 \ldots (2)$$

Where, n is the number of time points, $R_t$ is the dissolution value of reference product at time t and $T_t$ is the dissolution value for the test product at time t.

Similarity factor ($f_2$) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution between these two curves:

$$f_2 = 50 \bullet \log \{[1 + (1/n) \sum_{t=1}^{n}(R_t - T_t)^2]^{-0.5} \bullet 100\} \ldots (3)$$

Similarity factor ($f_2$) has been adopted by FDA and the European Agency for the Evaluation of Medicinal Products (EMEA) by the Committee for Proprietary Medicinal Products (CPMP) as a criterion to compare the similarity of two or more dissolution profiles. Similarity factor ($f_2$) is included by the Centre for Drug Evaluation and Research (CDER) in their guidelines such as guidance on dissolution testing of immediate release solid oral dosage forms [15] and guidance on Waiver of *in vivo* Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System [6].

For two dissolution profiles to be considered similar and bioequivalent, $f_1$ should be in between 0 and 15, while $f_2$ should be in between 50 and 100 [15]. The calculated $f_1$ and $f_2$ values are shown in Table 5. These values were within the acceptable range as per above specifications. Thus, all generic ciprofloxacin HCl tablets were bioequivalent with the innovator brand and so may be used interchangeably.

**TABLE 5: CALCULATED DIFFERENCE FACTOR ($F_1$) AND SIMILARITY FACTOR ($F_2$) OF ALL SIX GENERIC CIPROFLOXACIN HCl TABLETS WITH ITS INNOVATOR BRAND**

| TABLETS* | DIFFERENCE FACTOR ($f_1$) | SIMILARITY FACTOR ($f_2$) |
|---|---|---|
| G 1 | 2.87 | 75.09 |
| G 2 | 3.62 | 70.01 |
| G 3 | 3.02 | 74.61 |
| G 4 | 3.33 | 70.45 |
| G 5 | 2.67 | 77.33 |
| G 6 | 3.29 | 65.35 |

*G 1-G 6: denotes all generic ciprofloxacin HCl tablets

**CONCLUSION:** In conclusion, our results indicated that all generic ciprofloxacin HCl tablets included in this study seem to have good overall quality with high dissolution rate and hence very good bioavailability. All of them can be considered bioequivalent with the chosen innovator brand. It is a general psychology that the quality generic medicines may poor as compared to leading brands available in the market. But, this investigation will help to change the view of people towards generic medicines.

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