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COMPARATIVE *IN VITRO* DISSOLUTION STUDY OF SOME CIPROFLOXACIN GENERIC TABLETS UNDER BIOWAIVER CONDITIONS BY RP-HPLC

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ABSTRACT

This study is aimed to assess the bioequivalence of five generic ciprofloxacin tablets from different manufacturer using in vitro dissolution study under biowaiver conditions by RP-HPLC. Dissolution media were USP buffer solutions at pH 1.2 (hydrochloric acid solution), pH 4.5 (acetate buffer solution), and pH 6.8 (phosphate buffer solution). Other general quality assessment tests of these tablets like weight variation, hardness, friability, disintegration time and assay were also determined according to established methods. All brands complied with the official specification for uniformity of weight, friability and disintegration time. Assay of selected tablets revealed that all samples contained over 99% (w/w) of labeled chemical content. The dissolution profiles showed no significant inter brand and intra brand variability. Dissolution results of all the tablet formulations and the innovator brand were further analyzed with difference factor (f1), similarity factor (f2), dissolution efficiency and dunnet,s test. These results indicated that all generic ciprofloxacin tablets included in this investigation were bioequivalent with the chosen innovator brand and so may be used interchangeably.

INTRODUCTION: The process of dissolution plays a vital role in liberation a drug from its dosage form and making it available for subsequent gastrointestinal absorption.

So, dissolution analysis of pharmaceutical solid dosage forms is a very important test of product quality and it can be used as a sensitive method for differentiating between formulations of the same therapeutic agent [1-2].

Dissolution of a drug from its dosage from is dependent on many factors, which include not only the physicochemical properties of the drug, but also the formulation of the dosage form and the process of manufacturing [3]. So, constant dissolution analysis of marketed drug products is essential to ensure availability of quality medicines.

Ciprofloxacin is a synthetic flouroquinolone derivative with broad spectrum antibacterial activity [4]. 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 [5]. In most of the cases, it would appear that for treatment of above said infections, physicians prescribe ciprofloxacin as a first choice of drug.

Different reports on comparative dissolution study of ciprofloxacin tablets of different countries have been published. Ngwuluka *et al.,* evaluated six brands of ciprofloxacin 500 mg tablets available in Jos, Nigeria and found that only 3 brands (50%) may be used interchangeably with their chosen 'innovator' brand [6]. On the other hand, Amit *et al.,* evaluated six generic ciprofloxacin tablets, manufactured by different

manufacturer in India and reported that all (100%) generic ciprofloxacin tablets were bioequivalent with the chosen innovator brand [7]. Again Soula *et al.,* studied 10 brands of ciprofloxacin tablet available in Lebanese market and found significant variations among some brands in terms of hardness, disintegration and dissolution [8]. No such report is available for ciprofloxacin brands available in Bangladesh. So the present work was undertaken to evaluate the performance of our local products.

Both branded versions and generic products of ciprofloxacin tablets are available in Bangladesh market but people like to use generic products as they are far cheaper than its branded versions. Generic products can only be substituted with the branded version if they are bioequivalent with the innovator band. A product is considered bioequivalent with innovator brand when it contains identical amounts of the same active ingredient in the same dose formulation and there is no difference in the availability at the site of drug action when they are administered at the equal molar dose under similar conditions.

Bioequivalence studies involve both *in-vivo* and *in-vitro* studies. But as bioavailability depends on drug dissolution and the permeability across the gastrointestinal tract *in vitro* dissolution may be vital in assessing bioequivalence. In this study, bio-equivalence of five ciprofloxacin brands were assessed in three different dissolution media by in vitro dissolution study.

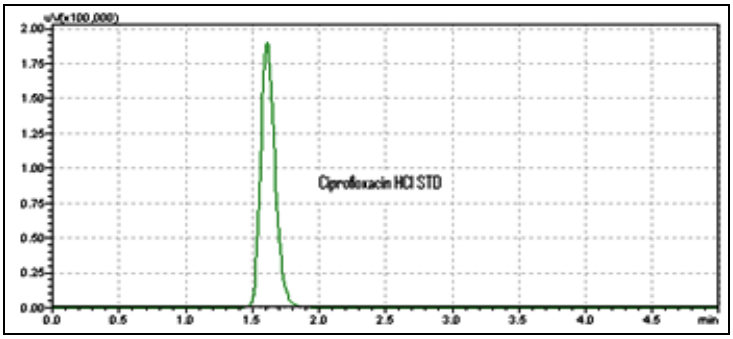
A validated method is essential for the analysis of ciprofloxacin for bio equivalence study. Several methods that are available for ciprofloxacin analysis are not free from limitation [9-12]. So first, we developed and validated an economic, rapid reversed-phase high performance liquid chromatographic method for analysis of ciprofloxacin with lower solvent consumption for the short analytical run time that leads to an environmentally friendly chromatographic procedure and will allow the analysis of a large number of samples in a short period of time.

MATERIALS AND METHODS:

Materials: Ciprofloxacin Hydrochloride was kindly gifted by Incepta Pharmaceuticals Ltd, Bangladesh. Acetonitrile and methanol were of HPLC grade and were purchased from E. Merck, Darmstadt, Germany. Ammonium acetate, acetic acid and other reagents were of analytical-reagent grade and purchased from E. Merck, Darmstadt, Germany. Water was deionised and double distilled. Five brands of ciprofloxacin tablets were purchased from local drug shops in Dhaka city. The samples were properly checked for their manufacturing license numbers, batch numbers, and production and expiry dates. They were randomly coded as A to E and stored properly. The labels of all the products claimed to contain 500 mg of the active ingredient per tablet.

HPLC Analysis of Ciprofloxacin: A simple, selective and rapid reversed phase High Performance Liquid Chromatographic (RP-HPLC) method has been developed and validated for quantification of ciprofloxacin. The chromatographic system consisted of a LC-20 AT pump, SPD-20 A UV/visible detector (Shimadzu, Japan). The Separation was achieved from C18 column (5µ, 4.6 X 150 mm, Waters, USA) at 30^0C temperature with a mobile phase consisting of Buffer (0.025 M orthophoshoric acid): Methanol: Acetronitrile: (ratio:50:15:35) at a flow rate of 1.5 ml/min.

The drug analysis data were acquired and processed using LC solution (Version 1.2, Shimadzu, Japan) software running under Windows XP on a Pentium PC. The method was validated for the parameters like system suitability, selectivity, linearity, accuracy, precision and robustness. The retention time was about 1.605 minutes both for standard solution and sample solution (**Figure 1**). Similar retention time proves the selectivity of the method. The calibration curves were linear over the concentration range of 80% to 120% (R^2 > 0.999). The proposed method is accurate with 100.165% recovery, precise (% RSD < 0.5) and robust.



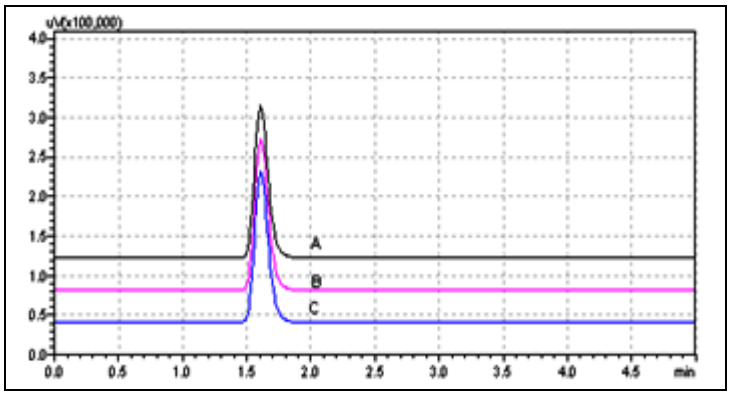


FIG. 1: CHROMATOGRAM OF STANDARD CIPROFLOXACIN HCL AND BRAND A, B, C

Assay: 20 tablets were crushed in a motor pestle. Powdered tablets containing of 2 g of ciprofloxacin was dissolved in 750 ml of the mobile phase, mixed with the aid of ultrasound for 20 minutes and diluted to produce 1000 ml. A portion of the resulting suspension was filtered and the filtrate was diluted with sufficient mobile phase to produce a solution containing the equivalent of 0.05% w/v of ciprofloxacin. 10µl solution was injected in the HPLC and potency was calculated from the calibration curve constructed previously.

Determination of uniformity of weight: 20 tablets from each of the 5 brands were weighed individually with an analytical weighing balance (Model: AY-200, SHIMADZU Corporation, Japan). The average weights for each brand as well as the percentage deviation from the mean value were calculated.

Hardness test: Automatic Tablet Hardness Tester (8M, Dr. Schleuniger, Switzerland) was used to determine the crushing strength. 6 tablets were randomly selected from each brand and the pressure at which each tablet crushed was recorded.

Friability test: 20 tablets of each brand were weighed and subjected to abrasion by employing a Veego friabilator (VFT-2, India) at 25 rev/min for 4 min. The tablets were then weighed and compared with their initial weights and percentage friability was obtained.

Disintegration test: 6 tablets from each brand were employed for the test in distilled water at 37°C using Tablet Disintegration Tester (Model: VDT-2, Veego, India). The time required for disintegrating the tablet and passing completely through the sieve was recorded.

Dissolution test: The dissolution test was undertaken using tablet dissolution tester (TDT-08L, Electrolab, India) in 6 replicates for each brand. Dissolution media were USP buffer solutions at pH 1.2 (hydrochloric acid solution), pH 4.5 (acetate buffer solution), and pH 6.8 (phosphate buffer solution). The medium was maintained at 37±0.5°C.

In all the experiments, 5 ml of dissolution sample was withdrawn at 0, 5, 10, 15, 30, and 45 min and replaced with equal volume to maintain sink condition. Samples were filtered and assayed by validated HPLC method. The concentration of each sample was determined from a calibration curve obtained from pure samples of ciprofloxacin.

Data Analysis: The uniformity of weight was analyzed with simple statistics – percentage deviation while the dissolution profiles were analyzed with difference factor (f1), similarity factor (f2) and some other approaches such as dissolution efficiency and Dunnett's test.

RESULTS AND DISCUSSION: A summary of the results of uniformity of weight, hardness, friability, disintegration and assay are shown in **Table 1**. Uniformity of weight may serve as a pointer to amount of the active pharmaceutical ingredient (API) contained in the formulation.

All the brands complied with the compendial specification for uniformity of weight which states that for tablets weighing more than 324 mg, not more than 2 tablets should differ from the average weight by 5% or more none will deviate 10% of average weight. Highest deviation was found 2.12% in case of brand C.

Hardness is referred to as non-compendial test. The hardness or crushing strength assesses the ability of tablets to withstand handling without fracturing or chipping. It can also influence other parameters such as friability and disintegration.

Brand B required the least pressure (94.67 N). A force of about 40 N is the minimum requirement for a satisfactory tablet [13]. Hence, the tablets of all brands were satisfactory for hardness.

TABLE 1: SUMMARY OF THE QUALITY CONTROL TESTS RESULTS OF CIPROFLOXACIN TABLETS WITH INNOVATOR BRAND

Brand Code	Average Wt	% Deviation	Hardness (N) (Ave ± SD)	Friability (%)	DT (min)	Potency (%)
Innovator Brand (I)	763.00	0.26	240.07 ± 2.54	0.07	3.10	102.30
A	674.42	0.59	185.04 ± 8.28	0.15	2.97	102.78
B	741.70	0.65	153.66 ± 7.23	0.12	9.10	100.68
C	848.10	2.12	161.33 ± 9.02	0.04	10.43	99.35
D	672.12	0.55	94.67 ± 8.08	0.14	9.59	100.45
E	683.54	0.27	149.67 ± 7.51	0.14	9.00	103.51

Friability test is used to evaluate the tablets resistance to abrasion. Friability is now included in the United States Pharmacopeia (USP, 1995) as a compendia test. The compendial specification for friability is 1%. Friability for all the brands was below 1%.

Disintegration is the process of breaking of tablets in the liquid. Disintegration is a crucial step for immediate release dosage forms because the rate of disintegration affects the dissolution and subsequently the therapeutic efficacy of the medicine. A drug will be released rapidly as the tablet disintegrates. British Pharmacopeia specifies that uncoated tablets should disintegrate within 15 min and film coated tablet disintegrate within in 30 min while USP specification for disintegration is 30 min both for uncoated and film coated tablets. All the brands were coated and complied with the both BP and USP specifications for disintegration as maximum DT was found 10.43 min in case of brand C.

Potency is the average amount of the active ingredient present per tablet. Brand C contains 99.35% ciprofloxacin (lowest potency). On the other hand brand E contains 103.51% ciprofloxacin (highest potency). All the brands complies both BP and USP specification of as USP specification is that the content of ciprofloxacin should not be less than 90% and more than 110% while BP specifies that the content should not be less than 95% and more than 105%.

The results of dissolution studies are graphically represented in **Figure 2 - 4**. All dissolution data are based on the actual drug content of the test tablets as calculated from the assay results. Drug release from innovator brand was found a slight higher in all the dissolution media. All the brands released about 80% drug in acidic media (pH 1.2) within 30 min. Higher amount of drug was released in acetate buffer medium (pH 4.5) from all the brands. But opposite scenario was observed in case of Phosphate buffer (pH 6.8). Only 37.17% ciprofloxacin was released from brand C within 45 min and lowest drug release was 32.17% from brand Band D in this medium within 45 min. This is due to the pH depended solubility of ciprofloxacin.

Ciprofloxacin exhibits a ‘‘U’’ shaped pH-solubility profile, with high solubility at pH values below 5 and above 10, and minimum solubility near the isoelectric point, which is close to neutral[14].

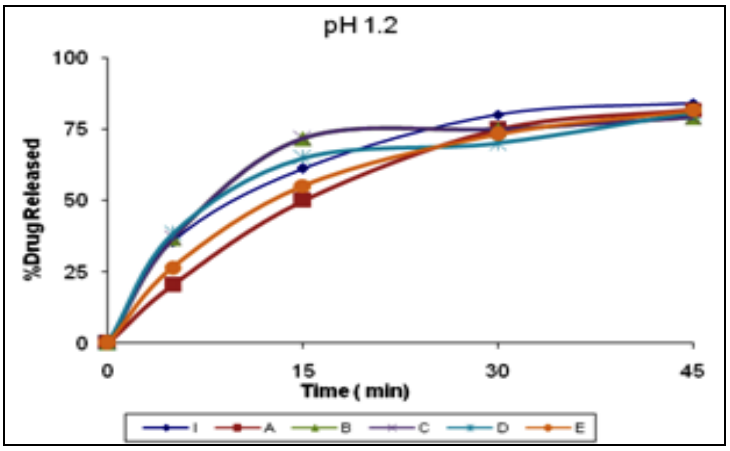


FIG. 2: DISSOLUTION PROFILES OF ALL GENERIC AND THE INNOVATIVE BRAND CIPROFLOXACIN TABLETS IN pH 1.2 (HYDROCHLORIC ACID SOLUTION)

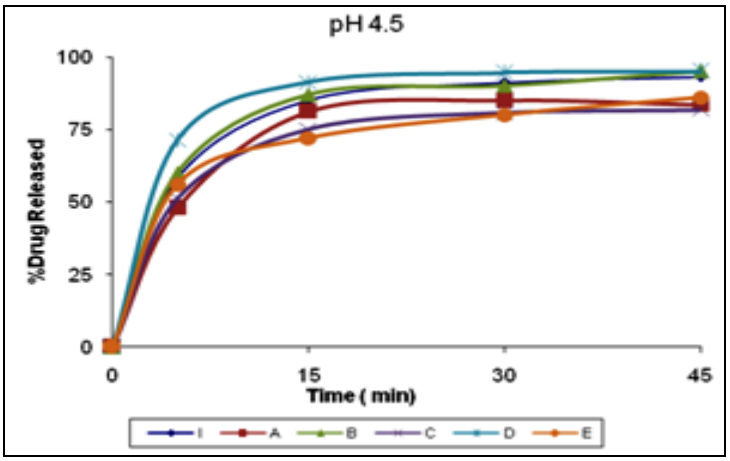


FIG. 3: DISSOLUTION PROFILES OF ALL GENERIC AND THE INNOVATIVE BRAND CIPROFLOXACIN TABLETS IN pH 4.5 (ACETATE BUFFER SOLUTION)

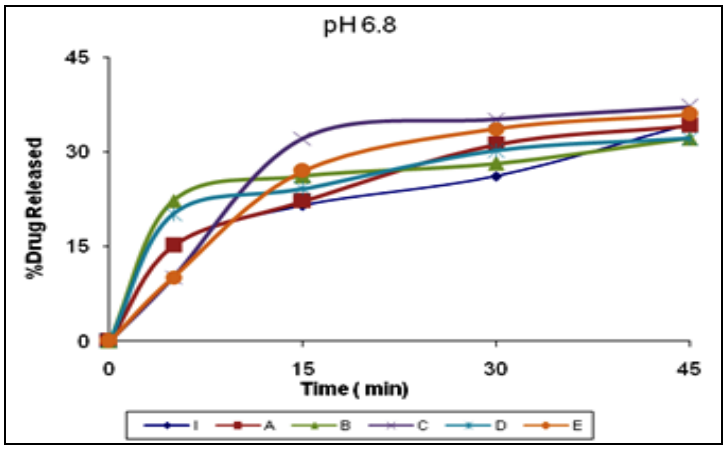


FIG. 4: DISSOLUTION PROFILES OF ALL GENERIC AND THE INNOVATIVE BRAND CIPROFLOXACIN TABLETS IN pH 6.8 (PHOSPHATE BUFFER SOLUTION)

Ciprofloxacin is highly soluble at pH 1.2 and 4.5. So, higher dissolution was obtained in these two media. Ciprofloxacin has limited solubility at pH 6.8 (< .02mg/ml). So, 37.17% dissolution is justified in case of Phosphate buffer medium (pH 6.8).

Analysis of Dissolution data: To compare the dissolution profiles of the brands, a model independent approach of difference factor f1 and similarity factor f2 were employed. Difference factor f1 is the percentage difference between two curves at each point and is a measurement of the relative error between the two curves. The similarity factor (f2) 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 two curves. Difference factor f1 and similarity factor f2 were calculated by using the following formulas:

$$f_1 = \left\{ \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \right\} X100$$

$$f_2 = 50 \log \left\{ \left(1 + \frac{1}{n} \sum_{i=1}^{n} (R_t - T_t)^2 \right)^{-0.5} X100 \right\}$$

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 f2 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 f2 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 (FDA, 1997) and guidance on Waiver of *In-Vivo* Bioavailability and Bioequivalence Studies for Immediate Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System (FDA, 2000) [15-16]. Two dissolution profiles to be considered similar and bioequivalent, f1 should be between 0 and 15 while f2 should be between 50 and 100 (FDA, 1997).

Table 2 shows the f1, f2 values of different brands in respect of chosen innovator brand. In f2 calculation only one measurement is generally considered after the comparator product has reached 85 % dissolution. F1 and f2 values are calculated for dissolution data obtained from two medium (pH 1.2 and 4.5). f1 and f2 are not calculated for phosphate buffer medium as maximum drug release was only 37.17%. All the values for f2 are more than 50 and all the f1 values are less than 15. So, we can say that all the brands are equivalent with the innovator band.

TABLE 2: CALCULATED DIFFERENCE FACTOR (*F1*) AND SIMILARITY FACTOR (*F2*) OF ALL GENERIC CIPROFLOXACIN TABLETS

Brand	pH 1.2		pH 4.5	
	f2	f1	f2	f1
A	51.12	13.27	55.58	9.13
B	56.77	10.16	77.97	3.07
C	56.77	10.16	50.84	11.80
D	57.50	9.29	55.87	7.94
E	57.38	10.18	51.89	10.16

Difference factor f1 and similarity factor f2 are not applicable for dissolution data obtained from phosphate buffer (pH 6.8) due to lower drug release. So, we compare the mean dissolution at 45min by dunnett's test. Pair wise comparisons of brands A, B, C, D, and E against innovator brand (I) were performed by multiple comparisons using Dunnett's test and the outcome at 0.05 level is as shown in **Table 3**. Values above the critical value (3.61) indicate that the mean % dissolved difference is significant while values below the critical value indicate that the difference is not significant. It can be seen that all the brands are not significantly different from innovator brand (I) at 45 min point.

TABLE 3: DUNNETT'S TEST ON THE BRANDS AT 0.05 LEVEL (TWO-TAILED) WITH CRITICAL VALUE 3.61

Time (min)	Pair comparison	Mean difference (% dissolved)	Significance
45	A vs I	-0.33	0.999
	B vs I	-2.33	0.3
	C vs I	2.67	0.194
	D vs I	-2.33	0.3
	E vs I	1.50	0.692

Critical Value is obtained from a table of Dunnett's test; Mean difference is obtained by subtracting mean % dissolved of brand I reference) from mean % dissolved of other brands (five products)

Again dissolution efficiency (DE) was also employed to compare the drug release from various brands. Dissolution efficiency is the area under the dissolution curve within a time range (t1 - t2) expressed as a percentage of the dissolution curve at maximum dissolution, over the same time frame [17]. This was calculated from the equation:

$$AUC = \sum_{i=1}^{i=n} \frac{(t_i - t_{i-1})\,(y_{i-1} + y_i)}{2}$$

Where *y* is the percentage dissolved at time *t.*

Table 4 shows the dissolution efficiency of different brand along with the difference with innovator brand. The reference and the test product can be said to be equivalent if the difference between their dissolution efficiencies is within appropriate limits (± 10%, which is often used) [17]. DE of all the brands did not differ by 10 with the innovator brand. So, we can say that all the brands are equivalent with the innovator brand.

TABLE 4: DISSOLUTION EFFICIENCIES (D.E) OF THE FIVE BRANDS OF CIPROFLOXACIN TABLET WITH INNOVATOR BRAND

Brand	pH 1.2		pH 4.5		pH 6.8	
	DE	Difference with reference	DE	Difference with reference	DE	Difference with reference
I	69.45	0.00	85.54	0.00	25.02	0.00
A	61.64	7.81	78.91	6.63	26.92	1.90
B	69.94	-0.49	86.42	-0.88	27.56	2.54
C	69.94	-0.49	75.54	10.00	31.48	6.46
D	66.57	2.88	90.85	-5.31	27.42	2.40
E	63.36	6.09	75.79	9.75	29.08	4.06

CONCLUSION: Our results indicated that all generic ciprofloxacin tablets included in this study seem to have very good bioavailability. All of them comply with BP and USP specifications. They can be considered bioequivalent with the chosen innovator brand. However in vivo test may be required for final comments regarding the quality of marketed brands of ciprofloxacin.

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