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Post Market In-vitro Bioequivalence Study on Representative Brands of Ciprofloxacin Tablets (500 mg) Prescribed in Typhoid Disease

Muhammad Azeem[1,2,*], Humaira Naureen[1], Madeeha Malik[2]

[1]Department of Pharmaceutics, Riphah Institute of Pharmaceutical Sciences, Islamabad, Pakistan
[2]Department of Pharmacy, Hamdard Institute of Pharmaceutical Sciences, Islamabad, Pakistan
*Corresponding author: azeem.hamdard@gmail.com

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Abstract Seven brands of ciprofloxacin (One multinational and six local) were evaluated in terms of their bioavailability and efficacy. Pharmaceutical Parameters like weight variation, hardness, disintegration, dissolution and content assay were determined. Antibacterial susceptibility assay was performed on standard Strain of *Salmonella typhi* (ATCC 14028). MIC and ZOI were assessed. The results of the study indicated that three local brands had content less than the stated claim of USP and one brand did not pass the disintegration and dissolution tests, so they cannot be used as therapeutic alternatives. Antibacterial assay showed less MIC value of the brands having greater drug content while drug with lesser amount had greater MIC value. Similarly, one local brand showed greater zone of inhibition (ZOI) as compared to multinational brand having more price than the local brand.

Keywords: *minimum Inhibitory concentration (MIC), zone of inhibition (ZOI), United States Pharmacopoeia (USP), World Health Organization (WHO), Gastro-Intestinal Track (GIT), Muller Hinton Agar (MHA), Irrational Drug Use (IRDU)*

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1. Introduction

Post market study involves the monitoring of drugs after being marketed to the local public for its efficacy and standard [1]. Typhoid fever, one of the major bacterial infections worldwide, is caused by the human-adapted *S.Enterica*serovar Typhi [1]. For every ten cases of *S.Typhi* infection, there are one or two cases of paratyphoid fever, caused by the human-adapted *S. Enterica* serovars Paratyphi A, Paratyphi B and Paratyphi C [1]. In developing countries, typhoid is considered as a major health problem. According to WHO, approximately 500,000 deaths are reported each year globally. This disease is prevalent in India, Africa and Central America, where supply of pure water is scarce. Typhoid fever is the sixth most common cause of death in Pakistan. In Pakistan, the prevalence of typhoid is estimated to be 412 cases per 100,000 populations per year [2]. In India the prevalence of typhoid in each year is estimated to be 214.2 per 100,000 [3]. *S. enteric* species are typically orally acquired pathogens that cause one of four major syndromes: enteric fever (typhoid), enterocolitis/diarrhea, bacteremia and chronic asymptomatic carriage [4]. In Pakistan epidemiology of typhoid fever with in the country is heterogeneous both in terms of time and location. Cases of typhoid fever are greater in areas where rainfall is greater like Rawalpindi and Faisalabad as compared to areas where rainfall is less and moderate like Multan and Lahore [5]. Treatment of typhoid disease in past was preferred by oral antibiotics particularly Chloramphenicol, amoxicillin, ciprofloxacin etc. But with the emergence of multidrug resistantance, intravenous cephalosporins are now being used to treat infection [6].

"Rationale use of drugs requires that patients receive medications appropriate to the clinical needs, in doses that meet their own individual requirements for an adequate period of time, at the lowest cost to them and their community". There are number of factors which cause treatment failure of typhoid. Among them late diagnosis of the disease, incompliance to the medications due to affordability issue, lack of knowledge and adherence to the standard treatment guidelines and availability of substandard formulations etc leads to the development of extensively drug resistance typhoid [7].

2. Methodology

All the seven Brands were purchased from Retail pharmacies of Islamabad. Standard ciprofloxacin was gifted by Wilson Pharmaceuticals for conduction of research. Bacterial Strain was purchased from local supplier "The Scientific Laboratories". The reagents used included Hydrochloric acid, ferric chloride, MHA,

nutrient broth agar. The brands were given codes and these codes are used. Cipro-1 is a multinational brand, while cipro-2, cipro-3, cipro-4, cipro-5, cipro-6 and cipro-7 all are local company brands (Table 1).

Table 1. Samples of Ciprofloxacin Tablets

Sr.no	Brands	Company	Batch no	License no	Reg no	Exp Date
1	Cipro-1	Bayer's Pharma	KHO1161	000003	013329	9/2018
2	Cipro-2	Wilson Pharma	6140	00239	016728	07/2016
3	Cipro-3	Warafanapharma	13F0220	000720	072302	6/2015
4	Cipro-4	AmsonPharma	380	000393	017352	01/2017
5	Cipro-5	Pearl Pharma	3110	000479	033359	02/2017
6	Cipro-6	Max Pharma	T015	000671	068588	03/2016
7	Cipro-7	Sami Pharma	010N	000072	011837	12/2016

2.1. Weight Variation Test

Twenty individual tablets of each brand were weighed on analytical weighing balance. The mean, average and percentage deviation was assessed. For tablets weighing more than 500mg, the individual tablet must be in range of ±5% according to USP standards.

2.2. Hardness Test

The resistance of a tablet to physical abrasion or crushing depends upon its hardness. Ten tablets of each brand were taken and their crushing strength was determined with a tablet hardness tester (Monsanto hardness tester). Average hardness was assessed.

2.3. Disintegration test

Six tablets from each brand were randomly selected and were employed for disintegration test using freshly prepared 0.1N HCl solution at 37ºC±1. The tablets should comply with in the specified time limit that is ten minutes. The disintegration time is the time taken where no particles of the tablet remains on the basket assembly of the apparatus [3].

2.4. Dissolution Test

Dissolution test is performed to assess the amount or percentage of drug released from a formulation under simulated conditions of GIT provided during in-vitro analysis. Six tablets of each brand were taken and dissolved in 900mL of 0.01N HCL solution using apparatus II paddle method. After thirty minutes the sample was taken, filtered and assayed using UV-spectroscope at 277nm wavelength to determine the amount of ciprofloxacin released from the tablets.

2.5. Drug Content Assay

A solution of 1% w/v of ferric chloride was freshly prepared. Another solution of 100 mcg/mL of pure ciprofloxacin was prepared. Five tablets from each brand were crushed and 100mg of the powdered samples were weighed, dissolved in 100mL of 0.1N hydrochloric acid and further dilution was made upto 100mcg/mL or each brand. To 5mL of each brand and pure sample, 1mL of ferric chloride was added and made up to 50mL with 0.1N HCl. The absorbance of each sample was taken at 438nm against blank reagent with an ultraviolet spectrophotometer. The percentage content was calculated for each brand [1].

2.6. Anti-bacterial Activity

2.6.1. MIC Determination

Minimum inhibitory concentration is the lowest concentration, amount of the drug at which it shows the highest activity against microorganisms [8,9]. MIC was determined by broth dilution method. All seven herbal and seven allopathic formulations along with the standard ciprofloxacin was assessed and their MIC was determined.

2.6.2. Preparation of Inoculum

Standard stock of bacterial isolate was prepared by suspending a loop full of microbial growth in 4mL of distilled water. It was incubated at 37°C for 12 hours and then its turbidity was compared with 0.5 McFarland's standard giving a bacterial load of about 3.6 × 102 cfu /mL [9].

2.6.3. Preparation of Antimicrobial Solutions

One tablet of 500mg was dissolved in 10mL of sterile water. This gives concentration of 50mg/mL. It was further diluted through two-fold dilution method and a series of serial dilutions were made from 1 to 12µg/mL. Similarly herbal formulations were diluted using sterile water.

2.6.4. Preparation of Media

Muller Hinton Broth media was prepared by using manufacturer's guidelines. It was then autoclaved at 121°C at 15psi pressure for 15 minutes. It was then allowed to cool. This media was poured in sterile test tubes under aseptic conditions using laminar flow Hood.

2.6.5. Inoculation and Incubation

One microliter of the bacterial isolate suspension was taken through micropipette and was inoculated in the test tube containing MH broth media. Then one milliliter of each dilution of the antimicrobial agents of all allopathic was poured in the test tube. These test tubes were incubated at 37°C for 18 – 24 hours.

2.7. Determination of Zone of Inhibition

2.7.1. Preparation of Media

Muller Hinton agar was dissolved is distilled water and simultaneously heated until it gets boiled. Then the media was poured in a conical flask and along with glass petri plates and autoclaved at 121°C and 15psi for 15 minutes.

Then under aseptic conditions these petri plates were filled with MHA and allowed to solidify.

2.7.2. Inoculation and Incubation

After solidification of the medium, sterile cotton swab was dipped in the bacterial inoculum and was spread over the medium on plate. Then using a sterile cork borer of 8mm diameter, wells were made and approximately 100uL of the stock solutions of the standard drug and other formulations of allopathic drugs were incorporated. These plates were divided into four portions and were labeled. After allowing the drug to diffuse these plates were incubated at 37ºC for 24 hours [8]. Zone of inhibition was measured for all allopathic formulations.

3. Results

3.1. Weight Variation Test

Results for weight variations showed that all the brands were within the specified limit that is ±5%. Not a single tablet deviates from this limit. The average weight of cipro-2 was greater than all other brands. The minimum average weight was that of cipro-5. The results in decreasing order are as, cipro-2 > cipro-4 > cipro-6 > cipro-1 > cipro-3 > cipro-7 > cipro-5 (Table 2).

Table 2. Results of Weight Variation Test

Sr. no	Brands	Average weight (mg)	% Deviation
1	Cipro-1	773.3	-0.023
2	Cipro-2	904.85	-0.00525
3	Cipro-3	744.45	0.0027
4	Cipro-4	816.1	-0.0118
5	Cipro-5	670.9	0.00045
6	Cipro-6	773.55	0.0001
7	Cipro-7	726.3	0.0039

3.2. Hardness Test

Results showed that cipro-3 has highest value for hardness that is 12 kg/cm^2, while cipro-5 has the least value that is 7.61 kg/cm^2. Values for hardness in decreasing order are cipro-3 > cipro-1 > cipro-6 > cipro-2 > cipro-4 > cipro-7 > cipro-5 (Table 3).

Table 3. Results for Hardness test

Sr. no	Brands	Average Hardness (kg/cm^2)
1	Cipro-1	9.5
2	Cipro-2	8.2
3	Cipro-3	12
4	Cipro-4	8.02
5	Cipro-5	7.61
6	Cipro-6	9.3
7	Cipro-7	7.8

3.3. Disintegration Test

Six tablets of each brand were taken and their disintegration tests were performed, temperature was maintained at 37°C±1. The maximum time for disintegration was taken by cipro-3 that was 55 minutes. The minimum time was taken by cipro-7 that was 1 minute. Results for disintegration time in decreasing order are as cipro-3 > cipro-2 > cipro-6 > cipro-4 > cipro-1 > cipro-5 > cipro-7 (Table 4).

Table 4. Results for Disintegration Test

Sr. no	Brands	Average Disintegration Time (minutes)
1	Cipro-1	1.28
2	Cipro-2	8.32
3	Cipro-3	55
4	Cipro-4	2.57
5	Cipro-5	1.16
6	Cipro-6	3.7
7	Cipro-7	1

3.4. Dissolution Test

Dissolution test was performed to check the percentage of drug released from the formulation after thirty minutes. The amount of drug released was analyzed by Uv-spectroscopy. The maximum % of drug released was 101.03 % from cipro-4. The minimum amount of drug was released by cipro-3 that was 39.8%. Cipro-7 released 76.7% of the drug after thirty minutes. Cipro-1, cipro-2, cipro-5 and cipro-6 showed 97.3%, 93.7%. 86.82% and 93.4% respectively (Table 5).

Table 5. Results of Dissolution test

Sr. no	Brands	%age of drug released after 30 minutes
1	Cipro-1	97.3
2	Cipro-2	93.7
3	Cipro-3	39.8
4	Cipro-4	101.03
5	Cipro-5	86.82
6	Cipro-6	93.4
7	Cipro-7	76.7

3.5. Drug Content Assay

The United States Pharmacopoeia (2005) states that the content of ciprofloxacin should not differ from the stated dose by more than 10% [6] The results showed that all the brands had amount of active drug >90% except to that of cipro-3 which had 63.45%, cipro-2 had 88.75%,cipro-6 had 88.9% of the total amount of active drug. Cipro-4 had the maximum amount of drug content that was 106.06%. While cipro-1 had 91.28%, cipro-2 had 88.75%, cipro-5 had 91.5%, and cipro-7 had 97.2% of the active drug content respectively (Table 6).

Table 6. Results of Drug Content Assay

Sr.No	Brands	Drug Content (mg)	%age Drug Content	% Drug Content Deviation
1	Cipro-1	456.4	91.28	-8.72
2	Cipro-2	443.75	88.75	-11.25
3	Cipro-3	317.28	63.45	-36.54
4	Cipro-4	530.3	106.06	6.06
5	Cipro-5	457.58	91.5	-8.484
6	Cipro-6	444.5	88.9	-11.1
7	Cipro-7	486.06	97.2	-2.788

3.6. Determination of MIC and Zone of Inhibition

3.6.1. Minimum Inhibitory Concentrations

Minimum inhibitory concentration was determined for all the brands including seven allopathic brands of ciprofloxacin formulations along with that of the standard ciprofloxacin. Results showed that all the allopathic brands showed antibacterial activity. The least MIC value was shown by cipro-4 (1µg/mL) and the maximum value for MIC was shown by cipro-3 (3.2 µg/mL) (Table 7).

Table 7. Results for MIC of Samples

Sr. no	Brands	Minimum inhibitory concentration (µg/mL)
1	Cipro-1	1.8
2	Cipro-2	2
3	Cipro-3	3.2
4	Cipro-4	1
5	Cipro-5	1.8
6	Cipro-6	2
7	Cipro-7	1.4
8	Standard Ciprofloxacin	1.2

3.6.2. Zone of Inhibitions

Zone of Inhibitions was determined after knowing the minimum inhibitory concentrations of all allopathic formulations. As the average MIC value of all the allopathic ciprofloxacin was near to 2µg/mL, zone of inhibitions were determined at 2µg/mL, 4µg/mL, 6µg/mL, 8µg/mL, 10µg/mL and 12µg/mL. Results showed that local brands of ciprofloxacin were more effective and gave bigger zone of inhibition than the multinational brand cipro-1 (Table 8).

Table 8. Results for Zone of Inhibition

Sr.no	Brands	Zone of Inhibition (mm) at 24 hours					
		2 µg/mL	4 µg/mL	6 µg/mL	8 µg/mL	10 µg/mL	12 µg/mL
1	Cipro-1	11	14	15	18	20	22
2	Cipro-2	13	15	18	20	21	23
3	Cipro-3	6	9	10	12	14	15
4	Cipro-4	15	17	18	19	22	24
5	Cipro-5	10	11	11	13	15	17
6	Cipro-6	11	12	14	17	19	22
7	Cipro-7	10	14	16	19	20	21
8	Standard Ciprofloxacin	14	19	20	21	23	25

4. Conclusion

The results revealed that three local (cipro-2, cipro-3, cipro-6) brands were below standard in content assay as per USP standards and cipro-3 has maximum values for disintegration and dissolution time which were out of the standard values according to USP. The results of present study regarding MIC of ciprofloxacin showed that the brand with maximum drug content (cipro-4) had least MIC value and the brand having minimum drug content (cipro-3) had the maximum MIC value against *S.typhi*. Local brands cipro-2 and cipro-4 showed greater zone of inhibitions than the multinational brand cipro-1.

Findings of the current study revealed that lack of treatment guidelines, peer influence of prescribers, influence of pharmaceutical industries might be the contributing factors towards IRDU which resulted in increased treatment cost. Availability of brands having sub-standard levels of the active agent results in resistance to typhoid and ultimately increases the cost of therapy. On the other hand, local brands can be used as therapeutic alternatives to multinational brands which showed greater activity against microorganism and had maximum drug content with least price in market. So Government and Ministry of health should take necessary steps in promoting cost effective treatment of typhoid, in regulating quality and standard of allopathic formulations

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