

PHARMACEUTICAL EVALUATION OF DIFFERENT BRANDS OF LEVOFLOXACIN TABLETS (500MG) AVAILABLE IN PAKISTAN

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ABSTRACT

This investigation has been conducted to evaluate the quality of commonly prescribed fluoroquinolones antibiotic, Levofloxacin tablets and examine the possibility of biopharmaceutical classification system biowaiver. As Levofloxacin is important antibiotic for resistant bacteria and price fluctuation of Levofloxacin tablet brands in a Pakistani community were analyzed. The possibility of biowaiver on brand level was also examined. Ten generic brands and the innovator brand were compared on friability, hardness, disintegration and dissolution. In vitro testing shows less variation in case of hardness, disintegration. In vitro dissolution testing was carried out in three different Medias including 0.1 N HCl, PH 4.5 acetate buffer and PH 6.8 phosphate buffer while the samples were taken after every 5 min for up to 30 min. Ten brands of Levofloxacin (500 mg) and 6 dosage units of each brand were randomly selected for study. A total of about 1000 dissolution tests were performed. And all the samples were analyzed by a validated UV spectrophotometer at 293 nm wave length. The dissolution test results obtained were recorded and graphs were prepared for comparison. The dissolution data obtained revealed that though the brands of Levofloxacin tablet manufactured in Pakistan have met the minimum pharmacopoeial requirements but still efforts are certainly further needed to get close to the innovator brand and to meet the requisite criteria for getting the biowaiver status as different brands showed variable results in different dissolution medias. The significance of the observed in vitro differences may be further evaluated and confirmed by in vivo bioequivalence studies.

Keywords: Levofloxacin, BCS, Physicochemical property, Biowaiver Conditions, Dissolution testing.

INTRODUCTION

Since the biopharmaceutics classification system (BCS) was introduced in 1995, it has had an increasing impact on regulatory practice. The BCS presented a new paradigm in bioequivalence, based on scientific principles. According to the tenets of the BCS, certain drug products can be considered for biowaivers, i.e. approving the product based on in vitro dissolution tests rather than requiring bioequivalence studies in human subjects. At first, biowaivers were only applied to Scale-Up and Post approval Changes (SUPAC), but later the biowaivers principle was extended to the approval of new generic drug products. As a result, unnecessary human experiments can be avoided and the costs of developing generic products can be significantly lowered [1]. WHO's Prequalification of Medicines Programme (PQP) has reviewed the existing evidence on the bioavailability and dissolution data of the medicines invited to the PQP evaluation, and has identified the following medicines to be eligible for BCS-based biowaivers applications i.e. Ethambutol, Isoniazid, Levofloxacin, Ofloxacin, Pyrazinamide. Currently biowavier is allowed only for immediate release product of BCS Class 1 drug substances (highly soluble and highly permeable) that exhibit rapid in vitro dissolution. Thus, for such products, demonstration of similar in vitro dissolution profiles using the recommended test methods would provide sufficient assurance of rapid in vivo dissolution, thereby ensuring human in vivo bioequivalence of Levofloxacin falls in same class 1 drug [2]. The objective of this study was to investigate the pharmaceutical quality evaluation of different brands of ciprofloxacin 500 mg tablet and the efficacy and comparison of dissolution profiles of ten brands of Levofloxacin tablets with innovator brand under biowaiver conditions. In the view of importance of conducting BCS based studies and the pharmacology of Levofloxacin, It was thought worthwhile to carry out studies to evaluate bioequivalence status of different brands of Levofloxacin 500 mg tablets used in this country.

MATERIALS AND METHODS

Analysis of Levofloxacin was carried out on UV -Vis Spectrophotometer (T 80 UV/VIS, PG instruments ltd UK), Electronic balance (Mettler Toledo, England), Hardness Tester (Ervaka Germany), pH meter (jenway model no 7530), Friability Apparatus (Pharma test Germany) disintegration apparatus (Pharma test Germany) and Dissolution Apparatus (Instruments UK).

Ten Levofloxacin brands were obtained from local market of Pakistan. Hydrochloric acid (Merck), Potassium Dihydrogen Phosphate, Sodium Hydroxide (sigma Aldrich- Germany) and Distilled water were prepared freshly.

Physicochemical Parameters

Any deviation from the physical parameters can lead to marked differences in the dissolution profiles. Assessment of physicochemical parameters which include uniformity of weight, hardness, friability, disintegration test were done according to United State Pharmacopeia (USP-32) and British Pharmacopeia (BP-2007)

Weight variation: Weight variation test were performed on twenty units of each brands and % deviation was calculated. According to USP for tablets weighing less than 130 mg, between 130-325 mg and above than 325 mg deviation limits should be $\pm 10\%$, $\pm 7.5\%$ and $\pm 5\%$ respectively [3, 4].

Hardness: Crushing strength is an important parameter to evaluate the compression forces. If a tablet requires more force to be broken, dissolution profile will be affected. Ten units of each brand were used. Minimum and maximum force needed to break the tablet was determined (British Pharmacopoea-2007) [5]

Friability test for tablets

The friability test is closely related to tablet hardness and is designed to evaluate the ability of the tablet to withstand abrasion in packaging, handling and shipping.

Ten tablets were weighed and placed in the apparatus where they were exposed to rolling and repeated shocks as they fall 6 inches in each turn within the apparatus. After four minutes of this treatment or 100 revolutions, the tablets were weighed and the weight compared with the initial weight. The loss due to abrasion was a measure of the tablet friability. A maximum weight loss of not more than 1% of the weight of the tablets being tested (BP-2002) [6].

Disintegration Test: 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. The disintegration time was taken to be the time, when no particle remained on the basket. All the tablets of different brands under study were found to be within limit i.e. to disintegrate within 15 minutes.

Dissolution Study

The dissolution profile of Levofloxacin tablets was evaluated in 900ml of buffer pH 1.2, 4.5 and 6.8 using US Pharmacopoeia dissolution apparatus II (USP-32) [7].

Preparation of Reagents

- pH 1.2:** 8.5 ml of HCl and dissolve in 1000 ml of distilled water (USP-23)
- pH 4.5:** 6.8 g of Potassium dihydrogen phosphate and dissolve in 1000 ml of distilled water (B.P 2002)
- pH 6.8:** Potassium Phosphate, Monobasic, (0.2 M). Dissolve 27.22g of Monobasic Potassium Phosphate (KH₂PO₄) in water, and dilute with water to 1000 ml.

Take 250 ml of 0.2 M Monobasic potassium phosphate solution and 112 ml of 0.2 M NaOH solution and make up the volume up to 1000 ml with distilled water. (USP 27) [8]

Dissolution Procedure

The temperature and degree of agitation were set at 37°C ± 0.5 and 50 rpm respectively. Samples (10 ml) were collected at predetermined time intervals 5, 10, 15, 20, 25 and 30 minutes and filtered (Millipore) to remove any insoluble excipients.

10ml of fresh medium already equilibrated to 37 °C was replaced into dissolution medium after each sampling in order to maintain sink condition. Six tablets per brand were used for the study. The filtered samples were analyzed by the Ultra-violet spectrophotometric method (UV) at 293 nm wavelength. The

concentration and the percentage release in each time interval was determined.

Standard preparation:

Accurately 56.26 mg of Levofloxacin hemihydrates was weighed and made up the volume up to 100 ml with the desired media solution. Then took 10 ml of this solution and made up the volume up to 100 ml with the desired media solution and from this solution exactly 2 ml solution was taken and analyzed with a UV spectrophotometer (USP, 2008)

Preparation of sample

Took 5ml of sample from desired dissolution media from the dissolution apparatus after specified time intervals. Added this 5ml sample in 50ml volumetric flask and made up the volume up to the mark. Then 2ml of the solution was taken from volumetric flask and absorbance was recorded with UV spectrophotometer. (USP, 2008)

RESULT

Table 1 show that all the tablets brands have satisfactory hardness, their friability found to be in limit i.e. not more than 1 % (USP 2008) and also disintegrate within 15 minutes, dissolution results (Table 2) shows that only Levofin brand of Levofloxacin rapidly dissolves in three medias and shows more than 85% results in three medias within 15 minutes similar with innovator brand (Tavanic). This clearly satisfies the *in vitro* bioequivalence and Biowaiver status given to Levofloxacin tablets based upon biopharmaceutical classification system. So this brand can be given to the patient with the satisfaction that this brand will disintegrate, dissolve in human GIT and will reach the site of action and will produce the desired therapeutic results.

Table 1: Physico-chemical Characteristics of ten brands of Levofloxacin (500mg) tablets

Brands	Uniformity of weight (mg) ± SD	Friability	Hardness (kg/cm ²) ± SD	Disintegration (min) ± SD
Tavanic	518.89±8.83	0.08±0.21	8.53±0.78	6±1.02
Levoflox	527.35± 8.72	0.07±0.26	8.63±0.89	7±0.98
Qevo	475.89 ± 1.38	0.19±0.18	7.9 ± 0.56	14.5±0.59
Levosafe	499.03± 4.02	0.28 ±0.21	6.56±1.19	11±0.67
Feelix	492.61 ± 3.99	0.09±0.21	7.91±0.97	13±0.58
Farleo	489.23 ±3.06	0.08±0.26	9.96 ±0.99	14±0.69
Levofin	480.22±2.02	0.59±1.08	7.90±0.01	8.50±0.71
Cravit	510.10±1.02	0.19±3.08	8.02±0.2	9.08±0.5
Lecord	501.01±0.62	0.09±1.07	9.9±0.21	10.5±0.99
Levodin	490.45±2.42	0.49±2.01	8.4±0.02	14.20±0.55

Table 2: Dissolution data of ten brands of Levofloxacin (500mg) tablets

Medium	Time Min	T	L	F	Far	Q	Levo	Lec	C	Levofin	Levodin
		% released	% released	% released	% released	% released	% released	% released	% released	% released	% released
pH 1.2	5	47.79	2.1	29.97	6.64	30.29	7.49	19.23	30.02	16.36	8.72
	10	92.85	3.19	38.07	9.68	67.28	12.74	30.86	47.82	47.76	12.79
	15	94.28	4.18	81.53	12.03	81.72	17.53	51.50	51.52	85.88	27.80
	20	96.38	7.04	107.74	15.96	85.22	25.74	85.15	85.15	92.78	70.46
	25	99.91	7.50	107.80	18.58	107.86	29.94	107.86	107.68	99.41	85.15
	30	100.59	9.27	107.92	20.54	107.86	42.65	107.98	107.92	107.80	99.48
pH 4.5	5	49.28	8.175	26.87	29.73	35.82	26.73	27.03	22.98	22.76	7.23
	10	96.78	14.82	70.37	89.21	88.32	82.43	30.05	26.87	35.49	22.24
	15	99.48	16.02	89.72	88.89	95.83	92.61	35.75	38.74	85.02	30.14
	20	98.30	26.20	95.28	94.78	94.66	96.05	72.36	90.16	93.17	67.18
	25	99.48	35.92	97.21	96.74	97.46	96.65	96.61	93.39	96.66	90.25
	30	100.7	54.94	100.10	99.95	100.36	100.28	100.43	99.26	100.10	96.66
pH 6.8	5	80.97	36.80	54.26	86.58	31.89	7.64	6.15	22.88	31.88	35.13
	10	80.97	37.05	98.77	99.00	38.92	13.19	30.81	61.59	48.87	70.21
	15	102.73	54.75	100.02	101.73	38.87	18.08	61.30	89.95	95.52	92.52
	20	108.53	63.18	111.35	109.85	66.83	26.63	81.43	94.71	88.11	93.75
	25	111.54	72.88	111.52	110.22	96.02	30.93	90.01	95.92	100.17	97.17
	30	111.48	80.67	111.59	109.43	100.15	44.08	94.35	96.19	110.31	103.25

Where, T= Tavanic, L=Levoflox, F=Feelix, Far= Farleo, Q= Qevo, Levo=Levosafe, Lec= Lecord, C= Cravit

Table 2 shows that Levofin brand of Levofloxacin can be used as alternative to Tavanic® tablet i.e. innovator brand while other all brands did not meet the criteria, so the other brands can't be bio waived.

Table 3: Label information of ten different brands of Levofloxacin tablets (500mg)

S. No.	Product name	Batch No.	Mfg Date	Exp. Date	Price/10 units
1	Tavanic	HC001	Nov-10	Oct-13	821
2	Levoflox	109F09	Jan-10	Dec-13	185
3	Qevo	Q127	Aug-10	Aug-13	230
4	Levosafe	149	Nov-10	Nov-13	250
5	Feelix	28	Nov-10	Nov-13	300
6	Farleo	T145	Jul-10	Jul-13	416
7	Levofin	G33	Jan-10	Jan-13	410
8	Cravit	91180	Mar-10	Feb-13	795
9	Lecord	090010	Jul-10	Jun-13	416
10	Levodin	032	May-10	Apr-13	425

DISCUSSION

Bioequivalence testing is considered as a surrogate for clinical evaluation of the therapeutic equivalence of drug products based on the fundamental bioequivalence assumption that when two drug products (for example, a brand-name drug and its generic copy) are equivalent in bioavailability, they will reach the same therapeutic. Bioavailability for in vivo bioequivalence studies is usually assessed through the measures of the rate and extent to which the drug product is absorbed into the bloodstream of human subjects, but in few cases in vitro bioequivalence studies are accepted based upon biopharmaceutical Classification system.

In the present study Levofloxacin (500 mg) was selected on this same principle. In Pakistan more than 50 brands of Levofloxacin tablets are available mostly manufactured by local pharmaceutical companies. Most of these companies have not done bioequivalence studies on these brands which shows concern that whether these brands are equivalent to innovator brand and by doing this we assess the bio waiver status of Levofloxacin. We reached to a conclusion that 9 brands of Levofloxacin 500mg tablets are not showing dissolution not less than 85% within 15 minutes in all 3 medias used which were our strict criteria for getting Bio waiver status i.e. the tablet should dissolve not less than 85% within 15 minutes in 0.1N HCL, 4.5 acetate buffer and in 6.8 phosphate buffer media. Only one brand showed this result and fulfils the criteria (Tab. 2). It is also suggested that there should be strong correlation between in vivo and in vitro bioavailability studies and once that correlation is established that we can rely more on in vitro studies. But care should be taken that all conditions should be same after maintaining the correlation between in vivo and in vitro bioavailability studies because any change will alter the results in future. It's possible to add statistical formula as given in different guidelines to get bio waiver for brands dissolves not less 85% in all 3 Medias in 30 minutes but not in 15 minutes but we in this research intentionally avoided this statistical formula so that we can better control our results and conclusions.

Now the issue arises whether all these brand studied are interchangeable or not on the bases of our dissolution results in different medias. When we stick to our strict criteria i.e. to dissolves not less than 85% in all medias within 15 minutes then only 2 brands meet this criteria one is Tavanic tablet itself and the other is Levofin tablet so it can be said that only Levofin tablet is found to be interchangeable with Tavanic® tablet.

Price of Levofloxacin 500mg tablets (a pack of 10 tablets) ranges from Rs 185 to 821 (Tab. 3) as we can clearly see that range is very wide. There is need of standardizing the price and people are paying the high price for non equivalent brands. And some brands are even not meeting the minimum criteria. During study it was observed that such studies should be conducted for every batch manufactured by the company itself so that quality can be build into the product because in our local Pharma industry its very common practice to use excipients from different sources in different batches of the same product and this practice leads to differ in results of dissolution and finally

bioavailability also suffers. According to the SUPAC-SS [9] guidance (scale-up and post approval changes: FDA May 1997) "An in vitro release rate can reflect the combined effect of several physical and chemical parameters, including solubility and particle size of the active ingredient and rheological properties of the dosage form. In most cases, in vitro release rate is a useful test to assess product sameness between pre change and post-change products." [10]. The national authority should be mindful that some excipients can influence motility and/or permeability in the gastrointestinal tract. Therefore, the excipients used in the multisource product formulation should be scrutinized. In this regard, the national authority can draw on the experience of formulations which have been approved on the basis of human bioequivalence studies in their own or in other jurisdictions (WHO guidelines)

Our local industry is growing day by day and many Pharma companies are also exporting the products. But export of medicinal products is not as easy as companies have to follow the strict rules and one of the strict rule is to provide data about the bioavailability and bioequivalence studies of that particular product. Bioavailability is expensive process and very few companies can afford it but on the other hand Bio waiver status of medicinal drugs as given by WHO on the bases of biopharmaceutical classification system gave our Pharma companies an opportunity to do such studies and get their share in global economy.

CONCLUSION

From the present investigation it has been concluded that, brands of Levofloxacin tablet manufactured in Pakistan have met the minimum pharmacopoeial requirements but still efforts are certainly further required to get close to the innovator brand and to meet the requisite criteria for getting the bio waiver status as different brands showed variable results in different dissolution medias. The significance of the observed in vitro differences may be further evaluated and confirmed by in vivo bioequivalence studies.

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