

Quality evaluation and *in vitro* interaction studies between levofloxacin 250mg and diclofenac sodium 50mg tablets

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Abstract: Fluoroquinolones are broad-spectrum antibiotics, work against Gram-positive and Gram-negative bacteria and are a clinically proven option for many resistant infections. Among fluoroquinolones Levofloxacin works best against acute sinusitis, inflammation of the lower airways, acute exacerbation of chronic bronchitis, community acquired pneumonia, complicated urinary tract infection including Pyelonephritis, chronic bacterial prostatitis and skin and soft tissue infection. Levofloxacin is a frequently prescribed antibacterial agent with Diclofenac Sodium for pain management in infectious conditions. The objective of the present work is to evaluate the level of interaction between Levofloxacin and Diclofenac Sodium. In this work market available brands of both drugs were also evaluated for quality. The physicochemical parameters like weight variation, thickness variation, and mechanical strength were determined. Similarly the percentage drug release and content uniformity test were also analyzed; the tested quality attributes were found within the recommended pharmacopeia ranges except brand L₆ that had high drug content 124.629±3.614 while brand L₄ and L₅ were not found similar in pH 1.2. When subjected to model dependent analysis Levofloxacin showed compliance with (first order, Higuchi, Hixson Crowell and Weibull) at pH (1.2, 4.5 and 6.8). However Diclofenac Sodium showed adherence with (first order, Hixson Crowell and Weibull) at pH (1.2, 4.5 and 6.8) but following Higuchi at pH 1.2 and 4.5 only. The interaction studies were also performed spectrophotometrically and simultaneous equation was used to estimate the percentage availability of both the drugs at pH 4.5, 6.8, FaSSGF and FaSSIF. The studies showed that the percent availability of Levofloxacin was increased significantly in FaSSIF i.e. 129.173±0.323 at 45 minutes in the presence of Diclofenac Sodium.

Keywords: *In vitro* interaction, Levofloxacin, Diclofenac sodium.

INTRODUCTION

Chemically Levofloxacin is (S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid (fig. 1). It is the optical S(-) isomer of Ofloxacin. The efficacy of Levofloxacin has been increased to 32-128 folds due to isomerization (Davis and Bryson, 1994; Miyashita *et al.*, 1995; Tanaka *et al.*, 1992, Fujimoto *et al.*, 1988). It inhibits the super coiling activity of bacterial DNA gyrase, halting DNA replication (Furuhama *et al.*, 1992, Sato *et al.*, 1989). Levofloxacin is rapidly and completely absorbed after oral administration. Therapeutically, it is used for the treatment of urinary tract infection, pyelonephritis, sinusitis, chronic bronchitis, and bacterial prostatitis and, other skin and soft tissue infections.

Chemically, Diclofenac Sodium is 2-[2-(2,6-dichloroanilino) phenyl] acetate sodium (fig. 2). It is a potent non-steroidal anti-inflammatory agents specifically indicated for rheumatoid arthritis, degenerative joint disease, ankylosing spondylitis and allied conditions, and in the treatment of pain resulting from minor surgery, trauma and dysmenorrheal (Brogden *et al.*, 1980). Diclofenac Sodium is completely absorbed after oral

administration and achieves peak plasma levels within 2.5 hours.

Antibiotics and analgesics are the most commonly prescribed agents in different clinical conditions and are available in a great number of generic products. The use of these agents has become a challenge with increasing number of available brands. In order to avoid drug resistance and effective bactericidal response with effective analgesia, quality of formulation is prerequisite. Several researchers have suggested evaluating pharmaceutical quality in order to ensure effectiveness (Adegoke *et al.*, 2003). Therefore *in vitro* dissolution testing can be a valuable predictor of *in vivo* bioavailability and bioequivalence of oral solid dosage forms (Prajapati *et al.*, 2005). Some studies have been reported, reflecting interaction of fluoroquinolones with NSAIDs. It has been reported that the concomitant administration of some fluoroquinolones with NSAIDs produces severe convulsions in human beings and animals (Ohtani *et al.*, 2009). Similarly one researcher has also reported a significant decrease in total body clearance of another frequently prescribed fluoroquinolone Ciprofloxacin, with concurrent administration of Diclofenac Sodium (Khan *et al.*, 2009). On the basis of these evidences, the prime objective of current study is to evaluate and compare the physicochemical equivalence

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of different brands of Levofloxacin and Diclofenac Sodium that are available in local market and to determine *in vitro* interaction between two drugs. The study will provide mechanistic basis for proper design of clinical studies using a modeling and simulation approach. The *in vitro* interaction of Levofloxacin from film-coated tablets was analyzed at different pH values i.e. phosphate buffer (pH 4.5 and 6.8), fasted state simulated gastric fluid (FaSSGF) and fasted state simulated intestinal fluid (FaSSIF) simulating certain parts of gastrointestinal tract (Sultana *et al.*, 2010).

MATERIALS AND METHODS

Materials

Levofloxacin and Diclofenac Sodium were kindly gifted by Sanofi-Aventis Pakistan Limited and Abbot Laboratories Pakistan. Different local and multinational brands of Levofloxacin 250 mg tablets and Diclofenac Sodium 50mg tablets were purchased from local market. All the glass ware used were of Pyrex origin, sodium hydroxide, sodium chloride, Triton X, hydrochloric acid, sodium taurocholate, lecithin, tri sodium phosphate anhydrous, potassium dihydrogen phosphate and monobasic sodium phosphate, acetonitrile, methanol and phosphoric acid (HPLC grade) all chemicals were purchased from (Merck, Millipore, Germany).

Instrumentation

Rheodyne Syringe (Gastight Hamilton USA), Filtration assembly (Sartorius, Gorringen, Germany), Vacuum Pump (Merck, Millipore Germany), pH meter (Jenway Portable 370 England), Ultrasonic bath (Clifton, Nickel Electro Ltd, Somerset, England), HPLC column (Waters Spherisorb 5 μ m ODSI 4.6x250 mm Analytical column, Ireland), Distillation assembly (Hamilton Laboratories, Kent, England), Dissolution apparatus USP I and II, (Erweka DT, Heusenstamm, Germany) and Spectrophotometer UV 1800 (Shimadzu, Japan) were used for the current studies. Assay was performed using HPLC (LC 10 AT, SPD 10AVP, Shimadzu, Japan).

Method

Six different market brands of Levofloxacin (250 mg tablets) and Diclofenac Sodium (50mg tablets) were selected for quality evaluation.

Physical evaluation of tablets

Tablets were evaluated for various physical parameters including weight variation, hardness, thickness, disintegration and dissolution. Weight variation was carried out by using analytical balance (Mettler Toledo B204-SSwitzerland), hardness was tested with Hardness Tester (OSK Fujiwara, OgawaSeiki Co Ltd, Tokyo, Japan), and thickness was measured using Digital Vernier Calliper (Seikobrand, China). Disintegration time was evaluated by disintegration tester (Erweka ZT2,

Heusenstamm, Germany). Single point dissolution test was carried out in Erweka DT 700, Heusenstamm, Germany dissolution tester by using (USP Apparatus I Rotating Basket, for Levofloxacin and USP Apparatus II paddles for Diclofenac Sodium). Dissolution studies were performed using dissolution media, specified in table 1 as mentioned in USP and BP (BP, 2013, USP37NF32, 2014). Mean and standard deviation were calculated using Microsoft excel.

Content uniformity test for levofloxacin and diclofenac sodium

(a) Levofloxacin

Preparation of mobile phase For Levofloxacin

0.05 M KH₂PO₄: Acetonitrile (82:18) pH 2.6, adjusted with Ortho-phosphoric acid (Pea *et al.*, 2001).

Chromatographic condition for levofloxacin

Injection volume was 20 μ l samples were analyzed at 280 nm, with a flow rate of 1ml/min.

Levofloxacin reference standard and sample preparation

0.025% of test and reference standard solutions of Levofloxacin were prepared in mobile phase, filtered by using 0.45 μ m millex syringe filter (Merck Millipore, Germany) before injecting.

Twenty tablets were weighed individually, crushed into powder, dissolved, diluted and filtered through 0.45 μ m millex syringe filter (Merck Millipore, Germany). Samples were injected and peak area was measured with the same concentration of Levofloxacin reference standard solution.

(b) Diclofenac Sodium

Preparation of mobile phase for Diclofenac Sodium

Equal volumes of phosphoric acid (0.01 M) and monobasic sodium phosphate (0.01M) were mixed, to obtain the desirable pH of 2.5 \pm 0.2 (USP36-NF31, 2013).

Chromatographic condition for Diclofenac Sodium

Injection volume was 10 μ L, detected at 254 nm with a flow rate of 1 mL/min.

Diluent

Methanol and water in the ratio of 7:3 was used as diluent.

Diclofenac sodium reference standard and sample preparation

0.075% of test and reference standard solution of diclofenac sodium was prepared in the diluent, filter through (0.45 μ m) millex syringe filter (Merck, Millipore, Germany) and injected.

Similarly twenty tablets were weighed individually, crushed into powder, and diluted with the mentioned diluent to make the desirable strength. Filtered samples were injected and peak areas were measured.

Tablets should contain not less than 90.0% and not more than 110.0% of the labeled amount of Levofloxacin and Diclofenac Sodium (USP37NF32).

Dissolution profile comparison

Dissolution studies of Levofloxacin 250mg and Diclofenac Sodium 50mg tablet were carried out at 100 and 50 rpm, using 900 ml of hydrochloric acid buffer pH 1.2, phosphate buffer pH 4.5 and pH 6.8 (see table 1) at a temperature of $37 \pm 0.5^\circ\text{C}$. A sample of 10ml was drawn at different time interval i.e., 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180 min and replaced with 10ml of the similar medium maintained at $37 \pm 0.5^\circ\text{C}$. Percentage drug release was determined by UV spectrophotometer UV-Vis 1800 spectrophotometer (Shimadzu Corporation Kyoto, Japan) at a wavelength of 294 nm for Levofloxacin and 276nm for Diclofenac Sodium.

DATA STATISTICAL ANALYSIS

Model dependent approach

The dissolution data was subjected to analysis using different dissolution models like; first order (Eq.1) that is log cumulative percentage drug remaining vs. time, Higuchi model (Eq.2) as cumulative percentage drug release vs. square root of time, Hixson – Crowell cube root law (Eq.3) as cube root percentage drug remaining vs. time and Weibull model (Eq. 4) as log dissolved amount of drug vs. log of time, using DD-solver that is an add-in program to Microsoft excel® for windows (Huo *et al.*, 2010).

First-order kinetic model

According to first order kinetic rate of release is concentration dependent

$$\ln Q = \ln Q_0 - kt/2.303 \quad (\text{Eq.1})$$

The drug release at time t is Q ; initial drug release is Q_0 at time t_0 and the first order rate constant.

Higuchi Square Root Law

$$Q = kt^{1/2} \quad (\text{Eq.2})$$

Where k is the Higuchi release rate constant and t is the time in hours.

Hixson Crowell cube root law

Hixson Crowell in 1931 recognized that the particles regular area is proportional to the cube root of its volume (Hixson and Crowell, 1931).

$$C_0^{1/3} - C_t^{1/3} = K_{HC} t \quad (\text{Eq.3})$$

Where C_0 is the initial concentration of drug in the tablets and C_t is the remaining concentration of drug in the

dosage form at time t . K_{HC} is the Hixson–Crowell constant. (Higuchi, 1963)

Weibull model

An equation described by Weibull was used to explain release procedure (Lin and Cham, 1996). This equation can be used to all types of drug release curves (Romero, Costa *et al.*, 1991, Vudathala and Rogers, 1992).

$$m = 1 - \exp \left[\frac{-(t-T_i)^b}{a} \right] \quad (\text{Eq.4})$$

Where m is the accumulated fraction of the drug in the solution at time t , a defines the time scale of the process, T_i represent the lag time before the onset dissolution release process b characterize the curve as exponent (Costa and Sousa Lobo, 2001).

Model independent approach

FDA has approved following equation (Eq.5) for the pairwise comparison of dissolution profiles of test and reference formulations. The test is said as similarity factor (f_2).

$$f_2 = 50 \times \log \left\{ \left[1 + \left(\frac{1}{n} \right) \sum (R_t - T_t)^2 \right]^{-0.5} \right\} \times 100 \quad (\text{Eq.5})$$

Where R_t is the amount of drug release from the reference (L_1 and D_1 for Levofloxacin and Diclofenac Sodium respectively) at each time point, T_t is the amount of drug release from the test brands of each, and n is the number of dissolution sample time. The profiles would be considered similar when, similarity factor (f_2) is >50 (Kannan *et al.*, 2012).

In vitro interaction study

The interaction studies between Levofloxacin 250mg and Diclofenac Sodium 50mg tablets were carried out spectrophotometrically in different dissolution media i.e., phosphate buffer pH 4.5 and 6.8, fasted state simulated intestinal fluid (FaSSIF) and fasted state simulated gastric fluid (FaSSGF), as specified in table 1 using dissolution test apparatus II.

Quantitation of interacting drug

Levofloxacin and Diclofenac Sodium were observed to follow Beer's law at their respective wave lengths of detections i.e. 294nm and 276nm. The linearity was observed in the range of 0.001-0.0018mM for Levofloxacin and 0.01-0.180mM for Diclofenac Sodium. For the quantitation of both the drugs, molar absorptivities were calculated at their respective wavelength of detection and that of interacting drug i.e. diclofenac sodium (table 7).

In the first phase of the study the percentage availability of both the drugs were determined in the mentioned dissolution media at $37 \pm 0.5^\circ\text{C}$. The samples were drawn at different time intervals. In order to observe interaction

Table 1: Dissolution Media

S. No.	Methodology For Preparation of Buffer			Reference
1	Hydrochloric Acid Buffer pH 1.2			(USP37NF32, 2014)
	Place 50 mL of the potassium chloride solution in a 200-mL volumetric flask, add the 85 ml of 0.2 M hydrochloric acid solution, and then add water to volume.			
2	Potassium Dihydrogen Phosphate Buffer pH 4.5			(BP, 2013)
	Dissolve 6.80 g of potassium dihydrogen phosphate in 1000 ml of water			
4	Potassium Dihydrogen Phosphate Buffer pH 6.8			(USP37NF32, 2014)
	Place 50 mL of the monobasic potassium phosphate solution in a 200-mL volumetric flask, add 22.4 ml of 0.2 M sodium hydroxide solution, and then add water to volume.			
5	Tribasic Sodium Phosphate Buffer pH 6.8			(USP37NF32, 2014)
	Solution A: 76mg/ml tribasic sodium phosphate			
	Solution A and 0.1 N hydrochloric acid (1:3), adjusted with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of 6.8 ± 0.05, if necessary			
Fasted State of Gastric Juice FaSSGF				
6	S. No.	Chemicals	Quantity	(Dressman, 2005)
	1	Sodium Chloride	2 gm	
	2	Hydrochloric Acid	3 gm	
	3	Triton X	1 gm	
	4	Deionized Water q.s	1000 ml	
Blank Fasted State Of Intestinal Fluid (FaSSIF)				
7	1	Sodium Dihydrogen Phosphate	3.438 gm	(Dressman, 2005)
	2	Sodium Chloride	3 gm	
	3	Sodium Hydroxide	0.348 gm	
	4	Deionized water q.s	1000 ml	
Fasted State of Intestinal Fluid (FaSSIF)				
8	1	Sodium Taurocholate	1.65 gm	(Dressman, 2005)
	2	Lecithin	0.591 gm	
	3	Blank FaSSIF q.s	1000 ml	

Table 2: Physical Parameters of market available brands of Levofloxacin 250mg tablets

Brand Code	Levofloxacin					
	Average Weight mg±SD n= 20	Average Hardness kg/cm ² ± SD n=20	Average Thickness mm ± SD n=20	Disintegration Test ± SD n=6	Single Point Dissolution Test n= 6	Assay ± SD n=3
L ₁	319.628±2.190	10.845±1.321	4.16±0.072	4.83±1.29	100.97±1.19	103.461±3.346
L ₂	408.375±2.409	11.115±1.447	3.85±0.034	5.83±1.50	100.22±1.43	104.793±3.534
L ₃	319.204±2.350	8.491±0.616	4.99±0.070	6.25±1.44	100.11±0.77	102.867±3.020
L ₄	322.725±4.084	12.078±1.196	4.07±0.063	5.5±1.18	100.22±0.98	101.930±0.996
L ₅	326.310±3.842	8.132±0.958	4.66±0.041	7.25±1.63	100.20±0.92	106.133±4.320
L ₆	405.391±2.595	8.122±1.294	4.53±0.0311	7.5±1.37	100.02±0.93	124.629±3.614

Limits: ± 5% for tablet weighing and thickness. Hardness is > 5 kg/cm². Disintegration less than 30 min for uncoated and film coated tablets. Single point dissolution not less than 80% (Q) of the labeled amount of levofloxacin is dissolved. Content uniformity tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of levofloxacin (USP37NF32, 2014, www.usp.org/sites/default/.../usp.../USP/NF/pendingStandards/m5751.pdf).

Levofloxacin 250mg tablet and Diclofenac Sodium 50mg tablet were added simultaneously. The samples were analyzed on UV spectrophotometer. As the difference between the absorption maxima of Levofloxacin (294nm) and Diclofenac Sodium (276nm) was observed greater, simultaneous equation was used for the quantitation of drug concentration (Sultana *et al.*, 2010).

$$C_a = \frac{A_{\lambda_{max}(\text{Levofloxacin})} b_2 - A_{\lambda_{max}(\text{interacting drug Diclofenac Sodium})} b_1}{a_1 b_2 - a_2 b_1} \quad (\text{Eq.6})$$

$$C_b = \frac{A_{\lambda_{max}(\text{Levofloxacin})} a_2 - A_{\lambda_{max}(\text{interacting drug Diclofenac Sodium})} a_1}{a_2 b_1 - a_1 b_2} \quad (\text{Eq.7})$$

C_a is the concentration of Levofloxacin and C_b is the concentration of interacting drug Diclofenac Sodium, a₁ and a₂ sequentially were the molar absorptivities of Levofloxacin at λ_{max} (Levofloxacin) i.e.294 and at λ_{max} (interacting drug) i.e.276nm, while b₁ and b₂ were the molar absorptivities of Diclofenac Sodium at λ_{max} (interacting drug

Diclofenac Sodium) i.e.276 nm and at λ_{\max} (levofloxacin) i.e.294, respectively.

RESULTS

To minimize the health related risks and to enhance the drug related safety, it is mandatory to evaluate pharmaceutical quality. In this study, different physicochemical parameters of the selected brands of Levofloxacin 250mg and Diclofenac Sodium 50mg tablets were analyzed and their results were found within acceptable limits (USP37NF32, 2014).

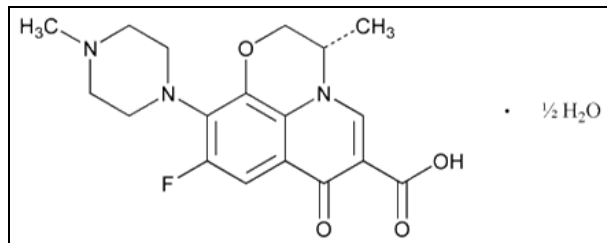


Fig. 1: Levofloxacin

It was observed that the mean weights of the selected brands of Levofloxacin (L₁-L₆) and Diclofenac Sodium

(D₁-D₆) tablets were in the range of 319.628±2.190 mg to 408.375±2.409mg and 145.503±3.158mg to 221.927±4.085mg, respectively. Whereas thickness variation was observed in the range of 3.85±0.034 mm to 4.99±0.07 mm for Levofloxacin and 3.44±0.024 to 4.74±0.024 mm for Diclofenac Sodium tablets. The results are given in table 2 and 3 and showing that they are within the pharmacopeial limits.

Hardness of the selected brands was also evaluated in order to assess tablet resistance against breakage during tablet handling, and was found to be 8.122±1.294 kg/cm² to 12.078±1.196 kg/cm² for Levofloxacin, and 13.30±1.85 to 17.21±2.02 kg/cm² for Diclofenac Sodium tablets. With the good physical strength, tablets of both the brands also showed compliance with the disintegration test limits, i.e. 4.83±1.29 min to 7.5±1.37 min for L₁-L₆. However, in case of enteric coated Diclofenac sodium tablets, no tablet showed disintegration in simulated gastric fluid but disintegrated in simulated intestinal fluid at 20.5±3.83 min to 22.00±3.40 min (tables 2-3).

Dissolution testing is a well-established technique widely used to evaluate percentage drug release from solid

Table 3: Physical Parameters of market available brands of Diclofenac Sodium (enteric coated) 50mg tablets

Brand Code	Diclofenac Sodium						
	Average Weight mg ± SD n= 20	Average Hardness kg/cm ² ± SD n=20	Average Thickness mm ± SD n=20	Disintegration Test ± SD n = 6 in simulated gastric fluid	Disintegration Test ± SD n = 6 in simulated intestinal fluid	Single Point Dissolution Test n = 6	Assay ± SD n=3
D ₁	221.927±4.085	17.21±2.02	3.63±0.031	No Disintegration after 60 mints	21.41±2.04	100.36±1.003	98.461±1.308
D ₂	184.980±4.212	13.31±2.23	4.74±0.024	No Disintegration after 60 mints	20.50±3.83	100.14±0.197	99.319±1.767
D ₃	156.944±3.112	11.92±2.15	3.44±0.024	No Disintegration after 60 mints	21.16±3.81	100.07±1.066	98.013±2.057
D ₄	145.503±3.158	13.77±1.99	3.44±0.032	No Disintegration after 60 mints	22.00±3.40	100.09±0.666	99.378±1.239
D ₅	205.163±2.671	13.30±1.85	4.04±0.033	No Disintegration after 60 mints	21.66±4.16	99.95±0.897	98.160±1.497
D ₆	204.413±2.43	13.23±2.48	3.6185±0.05	No Disintegration after 60 mints	20.66±4.71	99.85±0.911	99.268±3.404

Limits: ±7.5% for weight variation and ±5% for thickness variation. Hardness is > 5 kg/cm². Disintegration no evidence of softening or cracking after 60 minutes, Single point dissolution test not less than 75% (Q) of the labeled amount of diclofenac sodium is dissolved. Content uniformity tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of diclofenac sodium (USP37NF32, 2014)

Table 4: Similarity factors (*f*₂) at different pH with L₁ and D₁ as reference brand

Brand Codes	Levofloxacin			Brand Codes	Diclofenac Sodium		
	pH				pH		
	1.2	4.5	6.8		1.2	4.5	6.8
L ₂	57.220	62.462	56.945	D ₂	86.324	85.912	64.408
L ₃	81.256	51.921	68.552	D ₃	86.037	65.883	58.471
L ₄	35.190	59.610	51.325	D ₄	79.190	62.606	58.356
L ₅	37.215	62.322	56.235	D ₅	76.796	55.060	53.414
L ₆	70.503	64.204	57.494	D ₆	81.625	56.192	54.906

dosage forms. All the selected brands of Levofloxacin (L₁-L₆) showed 100.02 ± 0.93% to 100.97±1.19% drug releases at 30 minutes while Diclofenac Sodium tablet exhibited percentage drug release in the range of 99.85± 0.911% to 100.36±1.003 % at 45min.

The percentage drug content was estimated to evaluate the label claim of drug strength. For Levofloxacin brands the content assay results were, 101.930±0.996% to 124.629 ±3.614% while that of Diclofenac Sodium tablet brands were 98.013±2.057% to 99.378±1.239%. The results of content uniformity revealed that the percentage drug content were within the USP limits (tables 2-3).

The similarity factor (*f*₂) values for Levofloxacin and Diclofenac Sodium tablet brands are shown in table 4, and found to be highest 81.256% at pH 1.2 for Levofloxacin (L₃) and 86.324% at pH 1.2 for Diclofenac Sodium (D₂). The graphical presentations of drug release are presented by figs. 3 and 4, using Origin Pro 9.0. First order kinetic model has extensively been used for studying drug release profile. The highest value of coefficient of correlation (*r*²) was observed to be 0.9971 for L₁ at pH 1.2 and 0.9985 for D₁ at pH 1.2. Higuchi developed numerous models to explain the release of water soluble compounds from solid and /or semisolid matrixes (Higuchi, 1963). In present work the Higuchi model showed highest value of coefficient of correlation of 0.9973 for L₁ at pH 4.5 and 0.9944 at pH 1.2 for D₂. Hixson-Crowell model elaborates that the rate of release is restricted by the release of the particle and is independent of diffusion (Costa and Sousa Lobo, 2001). The coefficient of correlation (*r*²) for Hixson-Crowell was observed to be highest for L₁ i.e. 0.9998 (pH 1.2), 0.9909 (pH 4.5) and 0.9785 (pH 6.8). Weibull demonstrates S-shaped release of drug. The parameter of shape (*β*) was found to be < 1 for L₂, L₄, and L₅ at pH 6.8 and D₂, D₄ and D₅ at pH 1.2 and 4.5 indicating parabolic curve (tables 5-6).

In present work simultaneous equation was used to estimate the percentage drug release of these drugs from tablets and their availability was calculated in the presence of each other at phosphate buffer pH 4.5, 6.8, fasted state simulated gastric fluid (FaSSGF) and fasted state simulated intestinal fluid (FaSSIF) for 180 minutes. The results are presented by figs. 3 and 4, generated by Origin Pro 9.0. It was observed that percentage release of Levofloxacin increased to 105.625±0.213% at pH 4.5, in the presence of Diclofenac Sodium and the release amount of Diclofenac Sodium was also increase 95.420±2.764 (see fig. 5-6). At pH 6.8 it was found that the release of Levofloxacin in the presence of Diclofenac Sodium, got earlier i.e. 29.25±1.891% at 5 min to 90.307±2.895% and similar pattern was observed for Diclofenac Sodium i.e. 42.79±2.419% at 5 min to 47.661±2.295% (fig. 5-6). A tremendous rise in the availability of Levofloxacin was observed in the presence

of Diclofenac Sodium i.e. 129.173±5.80% at 45 minutes, and decrease in the availability of Diclofenac Sodium was observed i.e., 94.018±1.741 to 80.703±2.092 at 180 minutes in fasted state simulated intestinal fluid (FaSSIF).

DISCUSSION

The main objective of current study was to evaluate the pharmaceutical quality of Levofloxacin (250mg) and Diclofenac Sodium (50 mg) tablets, and to assess the degree of *in vitro* interaction between them. Many regulatory guidelines do not address specific study designs for *in vitro* and *in vivo* drug-drug interaction studies. There is a common desire by regulatory authorities and by industry sponsors to harmonize approaches, in order to allow better assessment of the significance of findings across different studies and drugs (Callaghan *et al.*, 2003). This study will provide a mechanistic basis to design clinical methodology using a modeling and simulation approach.

There are many researchers who had evaluated pharmaceutical quality of different brands of Levofloxacin and Diclofenac Sodium tablets. Bano *et al.*, 2010 also observed the similar results of weight and thickness variation when evaluated the quality of different Levofloxacin brands (Gauhar *et al.*, 2010). Similarly many researchers have evaluated pharmaceutical quality of different enteric coated brands of Diclofenac Sodium (Badwaik and Hosny, 1996).

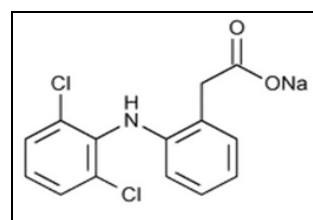


Fig. 2: Diclofenac Sodium

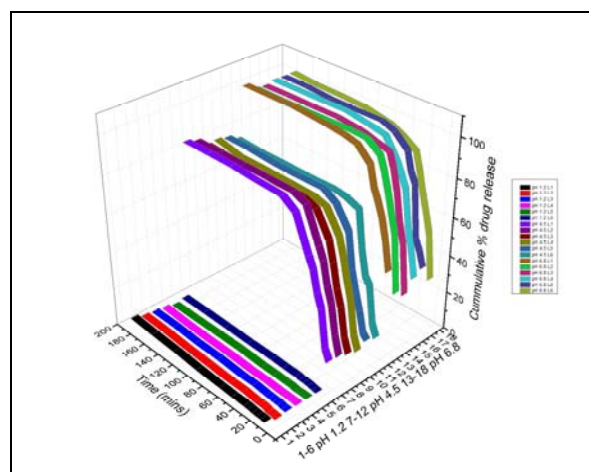


Fig. 3: % Drug release of different brands of Diclofenac sodium in pH 1.2, 4.5 and 6.8 (n=12)

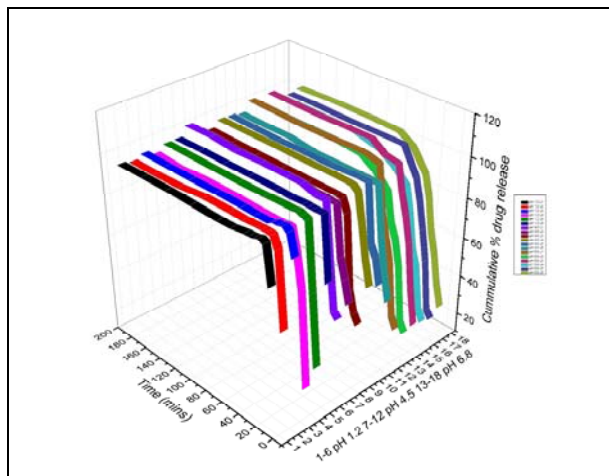


Fig. 4: % Drug release of different brands of Levofloxacin in pH 1.2, 4.5 and 6.8 (n=12)

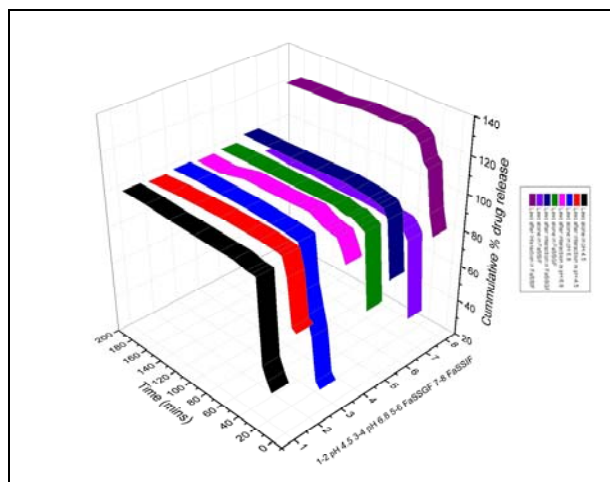


Fig. 5: % Drug release of Levofloxacin before and after interaction with diclofenac sodium at pH 4.5, 6.8, FaSSGF and FaSSIF (n=6)

approaches (similarity factor f_2) and different models like, first-order, Higuchi, Hixson–Crowell and Weibull, to the drug dissolution profiles to understand the similarity and drug release mechanisms (Nainar, Rajiah *et al.*, 2012, Siepmann and Peppas, 2001).

There are several methods reported by researchers to quantitate Levofloxacin and Diclofenac Sodium using spectrophotometer (Savaşer, Özkan *et al.*, 2005, Thakkar, Shah *et al.*, 2009).

When Levofloxacin tablet release profile was taken in Fasted state simulated gastric fluid (FaSSGF) in the presence of Diclofenac Sodium tablet, no significant change was observed in percentage availability of Levofloxacin and Diclofenac sodium. Whereas when availability of Levofloxacin was determined in fasted state simulated intestinal fluid (FaSSIF) in the presence of Diclofenac Sodium, a tremendous rise in availability was observed however, the availability of Diclofenac Sodium was observed low in the presence of Levofloxacin. The changed availability of Levofloxacin in the presence of Diclofenac Sodium at FaSSIF may be associated with the formation of charge-transfer complex, due to rearrangement of electrons (Sultana *et al.*, 2010).

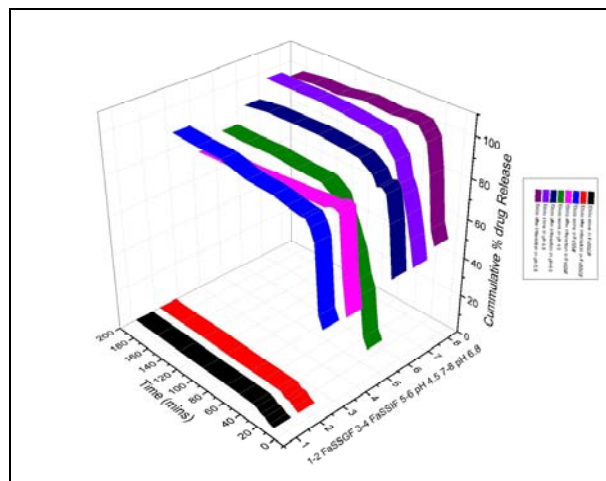


Fig. 6: % Drug release of Diclofenac sodium before and after interaction with Levofloxacin at pH 4.5, 6.8, FaSSGF and FaSSIF (n=6)

Hosnyet *et al.*, worked on enteric coated beads of Diclofenac Sodium and evaluated for their particle size distribution, drug loading efficiency, *in vitro* drug release at pH 1.2 and pH 6.8, in comparison with commercially available enteric-coated tablets (El-Mahrouk *et al.*, 1998).

The *in vitro* drug release characteristics are best quantitated by dissolution profile studies, which not only remain helpful in evaluating quality of product but also in formulation development and optimization, as well as for regulatory surveillance. Ylenia and Giacomo studied *in vitro* release behavior of Diclofenac Sodium from matrices based on chitosan (Zambito and Di Colo, 2003). Yeole *et al.*, reported release kinetics of Diclofenac Sodium through model dependent approach, from matrix tablets (Yeole, Galgatte *et al.*, 2006). Similarly, efforts have been put by Thakkar *et al.*, to study the release mechanisms and kinetics of Levofloxacin. Many researchers have applied both, model independent

CONCLUSION

The current study reveals that physiochemical parameters evaluation of drug products is pre-requisite to obtain efficient drug product. It was also observed that availability of Levofloxacin increased in the presence of Diclofenac Sodium in FaSSIF however availability of Diclofenac observed to be decreased in the same medium in the presence of Levofloxacin. The study will be helpful for *in vivo* pharmacokinetic interaction studies between Levofloxacin and Diclofenac Sodium.

Table 5: Release Kinetics of different brands of Levofloxacin in different pH

Brand Code	First Order						Higuchi								
	pH 1.2		pH 4.5		pH 6.8		pH 1.2		pH 4.5		pH 6.8				
	$K_1 (h^{-1})$	r^2	$K_1 (h^{-1})$	r^2	$K_1 (h^{-1})$	r^2	$K_H (h^{-1/2})$	r^2	$K_H (h^{-1/2})$	r^2	$K_H (h^{-1/2})$	r^2			
	pH 1.2		pH 4.5		pH 6.8		pH 1.2		pH 4.5		pH 6.8				
L ₁	0.351	0.9971	0.108	0.9494	0.061	0.9780	19.436	0.9080	21.0.92	0.9973	15.733	0.9429			
L ₂	0.207	0.9637	0.116	0.9588	0.049	0.9864	18.868	0.8188	12.321	0.8028	8.461	0.7884			
L ₃	0.561	0.8828	0.078	0.9394	0.065	0.9933	7.246	0.6354	11.331	0.7438	13.254	0.8743			
L ₄	0.108	0.9560	0.135	0.9762	0.045	0.9878	5.659	0.4529	5.373	0.5607	8.625	0.8231			
L ₅	0.122	0.9590	0.121	0.9433	0.048	0.9876	23.952	0.9466	18.326	0.9461	13.101	0.9135			
L ₆	0.263	0.9629	0.050	0.9912	0.05	0.9913	13.345	0.6797	6.137	0.5836	9.655	0.8368			
Brand Code	Hixon Crowell						Weibull Model								
	pH 1.2		pH 4.5		pH 6.8		pH 1.2		pH 4.5		pH 6.8				
	$K_{HC} (h^{-1/3})$	r^2	$K_{HC} (h^{-1/3})$	r^2	r^2	α	β	r^2	α	β	r^2	α	β	r^2	
	pH 1.2		pH 4.5		pH 6.8		pH 1.2		pH 4.5		pH 6.8				
L ₁	0.080	0.9998	0.023	0.9909	0.019	0.9785	12.290	1.886	0.9987	79.918	1.543	0.9850	14.002	1.160	0.9486
L ₂	0.053	0.9873	0.006	0.8049	0.008	0.9340	66.779	1.851	0.9944	63.994	1.386	0.9934	6.372	0.877	0.9502
L ₃	0.028	0.9132	0.007	0.8126	0.008	0.8982	35.44	1.274	0.9953	55.479	1.454	0.9959	22.587	1.207	0.9472
L ₄	0.741	0.9996	0.006	0.7818	0.008	0.9521	66.729	1.851	0.9944	64.394	1.348	0.9938	6.209	0.925	0.9776
L ₅	0.046	0.9902	0.006	0.7748	0.008	0.9253	71.855	1.938	0.9921	134.372	1.625	0.9957	6.721	0.916	0.9193
L ₆	0.039	0.9260	0.007	0.7941	0.010	0.9673	52.015	2.552	0.9999	51.227	1.312	0.9951	13.390	1.112	0.9730

Table 6: Release Kinetics of different brands of Diclofenac Sodium in different pH

Brand Code	First Order						Higuchi								
	pH 1.2		pH 4.5		pH 6.8		pH 1.2		pH 4.5		pH 6.8				
	$K_1 (h^{-1})$	r^2	$K_1 (h^{-1})$	r^2	$K_1 (h^{-1})$	r^2	$K_H (h^{-1/2})$	r^2	$K_H (h^{-1/2})$	r^2	$K_H (h^{-1/2})$	r^2			
	pH 1.2		pH 4.5		pH 6.8		pH 1.2		pH 4.5		pH 6.8				
D ₂	0.000	0.9985	0.031	0.9801	0.081	0.9822	0.564	0.9926	7.670	0.8537	7.158	0.7837			
D ₃	0.000	0.9779	0.031	0.9748	0.086	0.9968	0.486	0.9944	7.447	0.8314	6.932	0.6735			
D ₄	0.000	0.9689	0.024	0.9467	0.078	0.9959	0.443	0.9903	7.157	0.8292	7.292	0.7023			
D ₅	0.000	0.9890	0.000	0.6422	0.061	0.9961	0.343	0.9888	7.072	0.8250	7.596	0.7711			
D ₆	0.000	0.9319	0.021	0.9432	0.059	0.9786	0.292	0.9675	7.307	0.8602	7.633	0.7566			
D ₆	0.000	0.9133	0.021	0.8958	0.059	0.9966	0.098	0.6554	6.795	0.7848	7.588	0.7822			
Brand Code	Hixon Crowell						Weibull Model								
	pH 1.2		pH 4.5		pH 6.8		pH 1.2		pH 4.5		pH 6.8				
	$K_{HC} (h^{-1/3})$	r^2	$K_{HC} (h^{-1/3})$	r^2	r^2	α	β	r^2	α	β	r^2	α	β	r^2	
	pH 1.2		pH 4.5		pH 6.8		pH 1.2		pH 4.5		pH 6.8				
D ₂	0.000	0.9985	0.007	0.9609	0.007	0.9456	71.157	0.337	0.9667	19.645	0.840	0.9713	7.003	0.794	0.9922
D ₃	0.000	0.9775	0.007	0.9510	0.007	0.8719	208.230	0.508	0.9944	15.784	0.777	0.9618	15.007	1.071	0.9965
D ₄	0.000	0.9685	0.006	0.9337	0.007	0.8871	144.915	0.419	0.9924	13.368	0.686	0.9403	20.935	1.141	0.9962
D ₅	0.000	0.9889	0.006	0.9232	0.007	0.9364	204.488	0.437	0.9826	12.628	0.663	0.9350	15.265	0.989	0.9958
D ₆	0.000	0.9315	0.006	0.9391	0.007	0.9223	168.374	0.366	0.9812	18.114	0.741	0.9396	18.483	1.042	0.9789
D ₆	0.000	0.9129	0.004	0.8266	0.007	0.9369	117.747	0.337	0.9790	10.951	0.618	0.9057	14.701	0.958	0.9966

Table 7: Molar absorptivities of levofloxacin and diclofenac sodium

S. No.	Dissolution medium	←Levofloxacin→		←Diclofenac Sodium→	
		λ	(ϵ moles ⁻¹ Lcm ⁻¹)	λ	(ϵ moles ⁻¹ Lcm ⁻¹)
1	Fasted state of gastric juice (FaSSGF)	294 nm*	20400	294 nm	5500
		280 nm	16300	280 nm*	9077
2	Fasted state of Intestinal fluid (FaSSIF)	288 nm*	28549	288 nm	5781
		276 nm	9999	276 nm*	9899
3	Hydrochloric acid buffer pH 1.2	294 nm*	31500	294 nm	-
		276 nm	15200	276 nm*	-
4	Phosphate buffer pH 4.5	294 nm*	62700	294 nm	7721
		276 nm	30229	276 nm*	23584
5	Phosphate buffer pH 6.8	288 nm*	26500	288 nm	6833
		276 nm	16055	276 nm*	9933

*= λ max of drug

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