Pharmacokinetic and bioequivalence studies of immediate release diclofenac potassium tablets (50mg) in healthy volunteers

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Abstract: This study was conducted with the aim to determine the pharmacokinetic and bioequivalence of diclofenac potassium 50 mg test (F4) tablet formulation with reference product (Caflam). Present study was single dose, randomized, two phase cross over design, conducted in 12 healthy Pakistani volunteers and planned in accordance with FDA guidelines. In this study a simple, selective, sensitive and reproducible HPLC procedure was developed and validated for the estimation of diclofenac potassium in plasma. The process was validated in the range of 50 - 0.05 $\mu g.mL^{-1}$ and used in bioequivalence trial of two products. Multiple blood samples were collected at various time points (0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 14 hr after treating volunteers with test (F4) and marketed reference brand. Plasma separation and deproteination were carried out with acetonitrile; samples (20µL) were injected using the validated HPLC method. Various pharmacokinetic parameters (compartmental and noncompartmental) were estimated using Kinetica™ 4.4.1 (Thermo Electron Corp. USA). Bioequivalence among the products was established by calculating the 90% CI with log and non log transformed data for $C_{maxcalc}$, $T_{maxcalc}$, $AUC_{0-\infty}$, AUC_{tot} and AUC_{last} using two way ANOVA and Schirmann's Two one sided *t*- test. No significant difference was found between log and non-log data. The 90% confidence interval values using log transformed data for $AUC_{0-\infty}$ (0.997-1.024), AUC_{tot} (1.004-1.031), AUC_{last} (0.997 - 1.024), $C_{maxcalc}$ (0.994-1.007) and $T_{maxcalc}$ (0.996-1.013) for the trial and reference products were found within the FDA acceptable limits of 0.8-1.25. Results were further verified by the Schirmann's one-sided *t* test. Results showed the bioequivalence of test and reference formulations. Both the products were well tolerated.

Keywords: Pharmacokinetics, Bioequivalence, Diclofenac Potassium, HPLC, validation, Compartmental and Non-Compartmental, Log and Non log transformed,

INTRODUCTION

Diclofenac potassium belongs to the category of non-steroidal anti-inflammatory drug (NSAID), it inhibits iso-forms of cyclo-oxygenase and decreases the synthesis of prostaglandins in the body which is responsible to produces pain and inflammation. It is used for the treatment of mild to severe pain (Mcneely and Goa, 1999), used as an antipyretic agent, particularly useful in the treatment of osteoarthritis and rheumatoid arthritis (Shah *et al*., 2012), migraine (Diener *et al*., 2006) and primary dysmenorrhea (Chang *et al*., 2002).

Process of formulation development become challenging day by day and it is prerequisite to substantiate the formulation change with *in vivo* study to prove the validity of new formulation with respect to pharmacokinetics and its effectiveness. Today there has been significant application of pharmacokinetic investigations to explain various parameters in specific conditions and individualize the dose of the product (Abbas *et al*., 2013). Pharmacokinetic parameters are influenced by various factors particularly gastric residence time, volume and composition of gastrointestinal fluids, critical destructive forces during peristalsis and variations in absorption along the gastrointestinal tract (Kimura and Higaki, 2002; Fadda *et al*., 2010; Coupe *et al*., 1991). In case of any change in the composition of formulation, various pharmacokinetics investigations in healthy human volunteers are involved to develop bioequivalence with the reference formulation. Bioequivalence is the comparative evaluation between reference and test products to certify the relative bioavailability with exact criteria and particular defined objectives (Chen *et al*., 2009). The magnitude of bioequivalence studies is well growing over the couple of years due to the high volume of generic products in local markets and their extensive prescribing utilizations (Vetchy *et al*., 2007). Manufacturers required to conduct the bioequivalence studies to provide assurance for similar drug profiles of the generic version with original drug in bloodstream over time. Metabolic profiles can significantly modifies the pharmacokinetic parameters of the products, so bioequivalence assessment assist in the comparison of the metabolism of different drugs in various populations (Srinivas *et al*., 2009).

Previously several methods were studied and reported for the assessment of diclofenac potassium in plasma by using LC-MS, HPTLC, spectroscopy, HPLC techniques,

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absorption correction method by different investigators (Umarkar *et al*., 2011a,b; Sarfraz *et al*., 2011; Rao *et al*., 2011; Khatal and Kamble, 2010). Because of complicated procedures and difficult preparation methods make them unsuitable for routine analysis.

For this study a sensitive and precise reverse phase High Performance Liquid Chromatography procedure has been utilized for the evaluation of plasma samples of diclofenac potassium with the suitably low values of LOD (limit of detection) and LOQ (limit of quantification). Presented method is the adaptation of the procedure reported in USP 2007 and is validated following ICH guidelines (ICH, 1996; USP, 2007).

In the present study immediate release (IR) diclofenac potassium test (F4) formulation was selected for pharmacokinetic and bioequivalence study which was previously developed and optimized with rotatable central composite design (Huma *et al*., 2013). Various pharmacokinetic parameters of test (F4) formulation were evaluated and then compared with reference (marketed) brand in male healthy Pakistani volunteers.

MATERIAL AND METHODS

Material

Two Formulations were used in pharmacokinetics and bioequivalence Studies. Diclofenac Potassium (Caflam50mg tablet, Novartis Pakistan Ltd.) used as the reference product; Diclofenac potassium (DP) raw material was gifted from Hilton Pharma (Pvt.) Ltd. Test formulations of DP were optimized and developed using CCRD (Huma *et al*., 2013). Trial formulation (F4) was selected as best optimized formulation for pharmacokinetic study.

Chemical and reagents

HPLC grade Methanol and Acetonitrile (Merck, Darmstadt, Germany), Ortho-Phosphoric Acid (Merck, Darmstadt, Germany), monobasic sodium phosphate (Merck, Darmstadt, Germany), Membrane Filters (0.45µ - 47mm & 13mm diameter Millipore, England),

Instrumentation

High performance liquid chromatography; LC 20A, Communication Bus Module; CBM 102 with a Pentium™ IV with software Class; GC 20, Spectrophotometric Detector; SPD-20A (Shimadzu Corp, Japan). Guard column (Merck, Germany), C18 (250 x 4.6 mm x 5 µm), Column (Nucleosil, Germany), Vortex Mixer (Whirl, England), pH meter (3510 Jenway, England), Filtration Assembly (Sartorius, Gottingen, Germany), Centrifuge (Hereues, Osterode, Germany), Ultrasonic Bath (E30H, Elma, Germany), Deionizer (Elga, Highwycombe, England) and Swinney Filtration Assembly (Millipore, England).

Chromatographic conditions

For the mobile phase composition 0.01N methanol - monobasic sodium phosphate was used with equivalent volume 0.01M ortho phosphoric acid in 70:30 ratio. Orthophosphoric acid was used to adjust the pH up to 2.5. Mobile phase was then filtered and degassed before use. Flow rate was adjusted to 1mL min^{-1} with 20µL injection volume. Detection was carried out at 254 nm.

Bioanalytical method validation

Preparation of stock solution and quality control plasma samples

Stock solution (100µg.mL^{-1}) of diclofenac potassium RS was prepared in Acetonitrile. Serial dilutions were prepared in different concentrations i.e. 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.5, 0.1 and 0.05µg.mL^{-1}.Plasma samples were used in the range of 50-0.05µg.mL^{-1}. Blank plasma was spiked with known concentration of working solutions in 1:1 ratio to yield these concentrations. All solutions were stored at -20°C. For the separation of protein, samples should be vortex for 5mins and then centrifugation should be carried out at 3500 rpm for 10 minutes. Finally supernatant was filtered by 0.45µ membrane filter. Injecting volume of sample was 20µl.

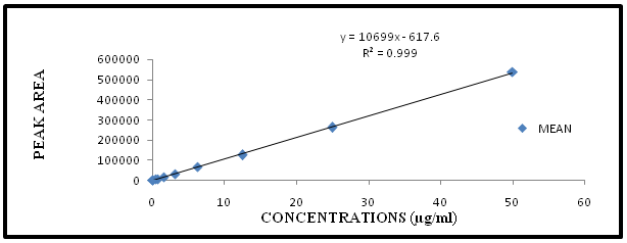

Fig. 1: Calibration curve of diclofenac potassium in plasma

Selectivity/Specificity

For the determination of specificity triplicate placebo, blank plasma and trial samples were injected on HPLC.

Range, linearity, accuracy and precision

For the calculation of regression, SD, CV %, accuracy % and precision %, calibrations curve was plotted for0.05-50µg.mL^{-1}range of plasma concentrations. Similarly, Intra and inter day precision and accuracy were also observed.

Freeze - thaw & long term stability

In this study three freeze and thaw cycles were conducted. Fifteen different plasma samples were prepared of 0.50µg.mL^{-1} and 1.56 µg.mL^{-1} (low &high concentrations), samples were placed at -20°C for 24 hours. For testing only 5 samples were defrosted and rests of samples kept refrozen for next 24 hours. Same method was used for another two cycles i.e., 2 and 3. Fresh samples were compared with freeze- thaw samples and estimated for mean, SD and % CV. Samples were also evaluated at 2nd and 3rd week of storage for long term stability.

Table 1: Intra Day / Interday Accuracy and Precision in Plasma

INTRADAY				
Conc.	50 (µg mL^{1})	25 (µg mL^{-1})	12 (µg mL^{-1})	1.56 (µg mL^{-1})
Mean (N=5)	50.275	24.858	12.498	1.555
SD	0.534	0.185	0.136	0.019
CV (%)	1.062	0.745	1.087	1.205
Accuracy	100.55	99.433	99.981	99.681
INTERDAY				
Mean (N=15)	50.282	24.963	12.493	1.546
SD	0.591	0.096	0.066	0.020
Accuracy	100.564	99.853	99.946	99.145
Precision	1.176	0.387	0.531	1.353

Table 2: Absolute Analytical Recovery

Conc. (µg/mL)	Mean Peak Area (N = 5)		
	MOBILE PHASE	PLASMA SAMPLES	% RECOVERY
50	536024.3	525772.9	98.088
3.125	33584.3	32959.4	98.139
1.56	17672.8	17473.2	98.871
0.5	6068.6	6040.8	99.542

Table 3: Freeze – Thaw (FT) and Long Term Stability of Diclofenac Potassium in Plasma

Freeze-thaw stability of diclofenac potassium					Long-term stability of diclofenac potassium		
High Concentration					1.56 (µg/ml)		
CODE	Fresh sample	FT cycle 1	FT cycle 2	FT cycle 3	Fresh sample	After 2 Weeks at 20°C	After 3 Weeks at 20°C
MEAN (N=5)	1.563	1.562	1.560	1.558	1.564	1.556	1.545
SD	0.010	0.008	0.005	0.006	0.004	0.008	0.014
CV%	0.608	0.491	0.322	0.410	0.278	0.527	0.930
% RECOVERY	100.177	100.149	99.978	99.877	100.233	99.733	99.036
Low Concentration					0.5 (µg/ml)		
MEAN (N=5)	0.509	0.508	0.499	0.497	0.509	0.501	0.490
SD	0.009	0.006	0.009	0.011	0.012	0.009	0.011
CV%	1.679	1.175	1.804	2.291	2.335	1.805	2.300
% RECOVERY	101.866	101.569	99.720	99.478	101.760	100.202	98.039

Table 4: Parameters of System Suitability

Parameters	Mean	RSD%	Limit
	(N=5)		
Tailing Factor	0.83	0.75	□ 2
Retention Time	14.75	0.47	□ 2
Theoretical Plate	11394	1.14	-
Peak Area	525772.0	1.07	-

Absolute and relative analytical recoveries

For relative and analytical recoveries, five samples (replicates) were injected using four different concentrations (0.5, 1.56, 3.125 and 50µg.mL^{-1}). Mean, SD, % recoveries and % CV were estimated.

Evaluation of system suitability

Peak area, Tailing factor, retention time and theoretical plates of column were determined for system suitability test.

Pharmacokinetic and bioequivalence study

In the present investigation, different pharmacokinetic parameters of trial (F4) and reference product manufactured (Caflam) were determined in 12 healthy male subjects. Bioequivalence study was carried out according to FDA guidelines.

Study design and ethical approval

Present study was designed as open label, single centre, single dose, randomized and conducted in cross over

manner, with one week washout period. Ethical approval was obtained from ethical review body of Ziauddin University, Karachi, Pakistan (Reference no. 0091111HA). The age groups and weight of all subjects were ranged of 20-40 years and 60-70kg respectively. Before the study medical history and other important tests were establish. Written informed consents were taken before the study from all the subjects.

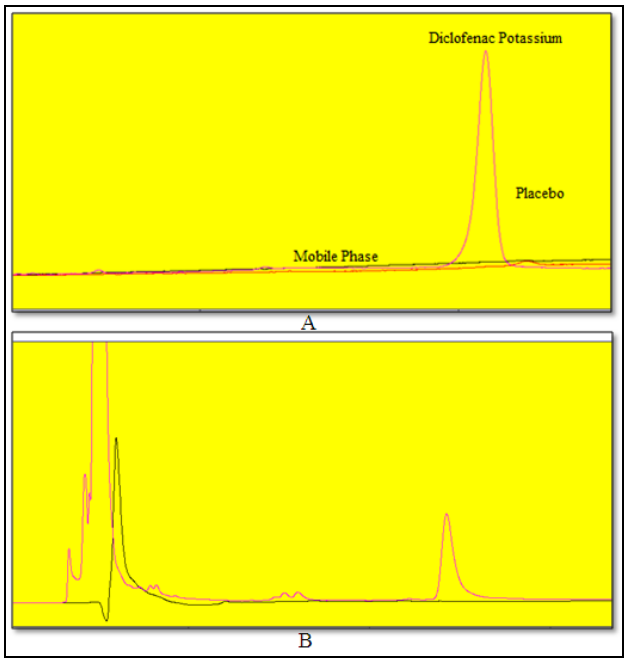

Fig. 2: Specificity at flow rate of 1ml/minute (A): In placebo, mobile phase and active compound (run time: 18 minutes) (B): in blank plasma and plasma sample of drug (DP)

Protocol of sampling

Each volunteer was given single oral dose of DP (F4) 50 mg in phase 1 and after one week washout period, marketed Brand was administered in crossover manner for phase 2. Blood sample of approximately 5mL was collected at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 14 hr after administrating volunteers with F4 (trial) and marketed product. Plasma samples were separated after centrifugation for 10 minutes at 3500 rpm and freezed at -20°C. For the estimation of active compound in plasma, above procedure was adopted and samples were determined by HPLC method.

Pharmacokinetic analysis

Compartmental and noncompartmental analysis was carried out using Kinetica™ (ver.4.4.1, Thermo-Electron Corp, USA). Presented data was fitted into oral two compartmental model. Various compartmental parameters i.e. clearance, K_{el}, K_a, β, α, $AUC_{0-\infty}$, $C_{maxcalc}$, $T_{maxcalc}$, K_{12}, K_{21}, V_c, T_{abs}, $T_{1/2Ka}$,$T_{1/2Kel}$, $T_{1/2\alpha}$, $T_{1/2\beta}$, were measured. While AUMC, MRT,λ_z, AUC_{last}, AUC_{tot} , $AUMC_{tot}$, $AUMC_{last}$, V_z and $T_{1/2\lambda z}$ were calculated as noncompartmental parameters.

Statistical analysis of bioequivalence

In the present study different bioequivalence parameters i.e. AUC_{tot}, AUC_{last}, $AUC_{0-\infty}$, C_{max} and T_{max} were selected for F4 (trial) and marketed brand following FDA guidelines (FDA, 2008). Also Schirmann's Two one sided *t*- test was also executed to estimate the difference between two formulations. Two ways ANOVA with Latin Square study design was applied on untransformed and log transformed data following FDA guidelines (FDA, 1992). Wilcoxon Sign Rank test was used for the assessment of untransformed T_{max} using SPSS 20.0 (SPSS Inc).

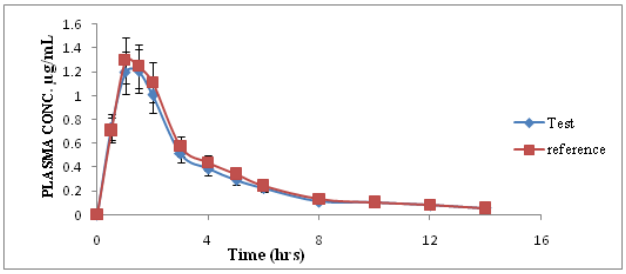

Fig. 3: mean plasma concentration time profile of diclofenac potassium IR (F4) and reference formulation

RESULTS

Method of analysis

In the current study a modified HPLC method was used for the estimation of active compound in plasma. For validation, using 50-0.05µg.mL^{-1} calibration curve was plotted and it was found to be accurate, precise, specific and linear (fig. 1), with coefficient of regression r^2= 0.999 which was described by the equation y=10699x-617.6. No interference was found from the adjuncts as presented in combined chromatogram showing reference, placebo and blank (figs. 2A & B). For the determination of intraday and interday accuracy and precisions different concentrations were used i.e. 50, 25, 12.5 and 1.56 µg.mL^{-1} as shown in table 1. Absolute analytical recovery was also assessed using four concentrations in mobile phase and plasma both as shown in table 2. Present method showed 0.05µg.mL^{-1} for LOD and 0.10µg.mL^{-1} for LOQ. Freeze and thaw stability and long term stability were conducted and presented in table 3.Results showed excellent stability under storage conditions during routine assessment. For system suitability test results were found within limits (table 4).

Pharmacokinetic parameters

Fig. 3 presented average DP concentration in plasma at different time point for reference and test formulations in 12 healthy male volunteers. Mean values of compartmental and noncompartmental parameters of DP test (F4) and marketed brands are presented in table 5 and 6.

Table 5: Mean compartmental parameters of diclofenac potassium after administration of reference and test formulations in 12 male healthy volunteers. S.D. = Standard deviation and C.V. = Coefficient of variation

REFERENCE FORMULATION																
Study Unit	Ka	Beta	Alpha	AUC	T_{max}calc	C_{max} calc	K_{el}	V_c	K_{12}	K_{21}	T_{abs}	$T_{1/2Ka}$	$T_{1/2Kel}$	$T_{1/2\beta}$	$T_{1/2\alpha}$	Cl
Unit	hr^{-1}	hr^{-1}	hr^{-1}	mg/L×hr	Hr	µg/mL	hr^{-1}	L	hr^{-1}	hr^{-1}	hr	hr	Hr	hr	hr	mL/hr
Mean	2.288	0.166	0.831	4.972	1.094	1.303	0.441	23.21	0.244	0.313	1.549	0.310	1.603	4.252	0.882	10.061
SD	0.329	0.023	0.237	0.113	0.010	0.009	0.071	2.572	0.114	0.078	0.264	0.053	0.2055	0.649	0.193	0.2353
%CV	14.39	13.95	28.61	2.290	0.962	0.750	16.11	11.08	46.83	25.03	17.09	17.09	12.818	15.26	21.89	2.338
TEST (F4) FORMULATION																
Mean	2.053	0.180	0.950	4.989	1.099	1.306	0.475	2.133	0.297	0.358	1.746	0.349	1.498	3.889	0.776	10.02
SD	0.368	0.019	0.261	0.119	0.015	0.008	0.085	0.320	0.128	0.071	0.358	0.071	0.237	0.432	0.187	0.249
%CV	17.92	10.69	27.489	2.401	1.379	0.668	17.90	15.04	43.31	19.90	20.54	20.54	15.836	11.12	24.13	2.489

k_a= Absorption rate constant
K_{el} = Elimination rate constant
α = Distribution rate constant
β = Disposition rate constant
CI = Total clearance

$AUC_{0-\infty}$ = Area under plasma concentration time curve
$C_{maxcalc}$ = Maximum plasma concentration
$T_{maxcalc}$ = Time to achieve C_{max}
V_c = Volume of the central compartment
K_{12} = Rate constant from central to peripheral compartment
K_{21}= Rate constant from peripheral to central compartment

$T_{1/2ka}$= Absorption Half Life
T_{abs} =Duration of absorption
$T_{1/2\alpha}$= Distribution Half Life
$T_{1/2\beta}$= Disposition Half Life
$T_{1/2kel}$= Elimination Half Life

Table 6: Mean non± compartmental parameters of Diclofenac Potassium after administration of Reference and Test formulations in 12 male healthy volunteers

REFERENCE						
Study Unit	AUC_{tot}	$AUMC_{tot}$	λ_z	MRT	$t_{1/2\lambda z}$	AUMC
	mg/L*h	mg/L*(h)²	$hr^{\pm 1}$	h	h	mg/L*(h)²
MEAN	5.016	22.844	0.200	4.552	4.110	22.257
SD	0.089	1.686	0.036	0.278	0.388	1.748
%CV	1.772	7.379	17.791	6.097	9.447	7.854
TEST (F4)						
MEAN	5.105	23.980	0.176	4.696	3.889	21.661
SD	0.087	1.540	0.031	0.258	0.433	1.521
%CV	1.698	6.424	17.418	5.485	11.127	7.021

Table 7: Statistical assessment for establishing bioequivalence of DP with log and Non ± Log transformed data

Parameters	*Log±Transformed Data*				Non ± log Transformed Data			
	Geo mean Ratio (Test/ Reference)	Geometric Mean Values		*90 % Confidence Interval*	*Geo-mean Ratio (Test/ Reference)*	Geometric Mean Values		*90 % Confidence Interval*
		Reference	Test			Reference	Test	
$C_{maxcalc}$	1.0005	1.303±1.007	1.305±1.006	0.994±1.007	1.0005	1.303±1.007	1.305±1.006	0.994±1.006
$T_{maxcalc}$	1.0047	1.093±1.009	1.098±1.013	0.996±1.013	1.0048	1.093±1.009	1.098±1.013	0.996±1.013
AUC_{last}	1.0110	4.727±1.014	4.779±1.016	0.997±1.024	1.0110	4.727±1.014	4.779±1.016	0.997±1.024
AUC_{tot}	1.0177	5.015±1.017	5.104±1.017	1.004±1.031	1.0173	5.015±1.017	5.104±1.017	1.004±1.031
$AUC_{0\pm\infty}$	1.0033	4.971±1.023	4.987±1.024	0.986±1.020	1.0033	4.971±1.023	4.987±1.024	0.986±1.020

Table 8: Schirmann's two one-sided *t* test for establishing bioequivalence

Parameters	TWO ONE±SIDED T±TESTS		
	Lower: t (10df)	Upper: t (10df)	t (0.05±10df)
$C_{maxcalc}$	65.152	66.136	1.8125
$T_{maxcalc}$	47.762	49.854	
AUC_{last}	28.963	31.949	
AUC_{tot}	28.193	33.017	
$AUC_{0\pm\infty}$	23.136	23.834	

Table 9: Analysis of variance for the evaluation of different effects in test and reference formulations, using logarithmic transformed data

Pharmacokinetic Parameter	ANOVA (*p* - value) Variation Source				90% CI
	Formulation	Period	Subject	Sequence	
$C_{maxcalc}$	0.6336	0.3202	0.9528	0.2269	0.994 - 1.007
AUC_{last}	0.1662	0.3362	0.883	0.2593	0.997 - 1.024
$AUC_{0-\infty}$	0.734	0.1003	0.6716	0.1652	0.986 - 1.020

Table 10: Wilcoxon sign rank test for T_{max}

		Ranks			
		N	Sum of Ranks	Sum of Ranks	p = 0.05
Test - standard	Ranks (Negative)	6[a]	35.00	5.83	Significant if smaller rank sum ≤ 13
	Ranks (Positive)	6[b]	43.00	7.17	
	Ties	0[c]			
	Sum	12			

a. Test < standard
b. Test > standard
c. Test = standard

Bioequivalence assessment

Various bioequivalence parameters were compared for test (F4) and reference formulations i.e. AUC_{tot}, AUC_{last}, $AUC_{0-\infty}$, C_{max} and T_{max} using two way ANOVA and aby Schirmann's two one sided *t* test. 90% confidence interval (CI) value was used for trial and comparator. If AUC_{tot}, AUC_{last}, $AUC_{0-\infty}$, C_{max} and T_{max} fell between 0.8-1.25 for natural log (*ln*) transformed, and for non-log transformed data values lies between 0.8-1.20, then products are considered bioequivalent. In present study the mean log-transformed and non-transformed values of above parameters with 90% Cl were found within the acceptable range as shown in table 7. Bioequivalence assessment was also inveterate with Schirmann's two one-sided *t* test as shown in table 8**.** Analysis of variance was used for the evaluation of formulation, period, subject and sequence effects for the ratio of $C_{maxcalc,}$ AUC_{last} and $AUC_{0-\infty}$ values of trial and reference formulations, using logarithmic transformed data with 90% CI are shown in table 9.

DISCUSSION

In the present study a simple and reproducible reverse phase HPLC procedure has been utilized for the assessment of active compound in human plasma. For specificity study no interfering peaks were detected and method was found selective linear and sensitive in 50-0.05µg.mL^{-1} range. For system suitability test % RSD for retention time were (0.47), tailing factor (0.75), peak area (1.02) and theoretical plates (1.14). The intraday and interday accuracy of 50, 25, 12.5 and 1.56µg.mL^{-1} were from99.14%-100.55%. Several other techniques were also reported in literature for the estimation of diclofenac potassium in plasma by different investigator (Francis *et al*., 2011). But due to the complex solvent systems, high cost of equipments, complicated extraction procedures of sample preparation make them unsuitable for routine practice. This modified procedure was applied successfully for the estimation of active compound in plasma, which was further used for calculation of various pharmacokinetic parameters.

In this study both compartmental and noncompartmental pharmacokinetic parameters were estimated using Kinetica 4.4.1 (table 5 and 6). No significant difference was found in the mean$AUC_{0-\infty}$for the reference and test formulations. In present study using compartmental analysis average AUC values of reference brand and test (F4) were found to be 4.972±0.113 and 4.989±0.119 mg/L×h (table 5). In non compartmental method, for reference and F4 formulation AUC_{last} (4.728±0.069 mg/L×h; 4.780±0.078mg/L×h) and AUC_{total} (5.015±0.088 mg/L×h; 5.10±0.086mg/L×h). Average values for reference of calculated C_{max} (1.303±0.098µg/mL) and T_{max} (1.094±0.011 hr) and for F4 product of calculated C_{max} (1.306±0.087µg/mL) and T_{max} (1.099±0.015 hr) were in accordance of previously reported literature (Kowalski *et al*., 2010; Bele and Derle, 2012).

The Cl (mean clearance) and V_z (volume of distribution) values of reference (10.061±0.235mL/h/kg; 61.555±8.283 L) and test (10.28±0.249mL/h/kg and 59.733±3.530L) formulations were comparable. Rate constants k_a and K_{el} found to be (reference, 2.288±0.329h^{-1}and 0.441±0.071h^{-1} respectively; test, 2.053±0.368h^{-1}and 0.475±0.085h^{-1}respectively). Similarly absorption and elimination half lives were $T_{1/2ka}$ (reference, 0.310±0.053hr; test, 0.349± 0.071hr), $T_{1/2Kel}$ (reference, 1.603±0.205h; test, 1.498± 0.2372h). While other compartmental parameters including T_{abs}, α, β, $T_{1/2abs}$, distribution ($T_{1/2\alpha}$), disposition ($T_{1/2\beta}$), $K_{12}$and K21 were also estimated (table 5). Similarly values of $t_{1/2}$ (1.0-2.5 hrs) and K_{el} (0.2-0.9 hrs^{-1}) for diclofenac potassium were also reported in various literature (Marzo *et al*., 2000). Scientists determined the terminal half lives of diclofenac potassium (12.5mg) was found to be (0.8±0.2 hr) and 25mg (0.9±0.3 hr) (Burkhard *et al*., 2005). In another investigation absorption rate constant (K_a) and K_{el} of diclofenac potassium were found to be 0.362±2.332, 0.394±4.307, h^{-1} (Mahmood *et al*., 2010). Also other noncompartmental parameters were also analyzed as shown in (table 6). Also two-way ANOVA test was used for the assessment of F4 and reference products. Different parameters i.e. subject, formulation, sequence and the period effects were considered using Latin Square ANOVA for bioequivalence evaluation (table 9). Multivariate investigation revealed the absence of formulation, period, subject and sequence effects for $C_{maxcalc}$, $T_{maxcalc}$, $AUC_{0-\infty}$, AUC_{last} and AUC_{tot}.

In this investigation, the geometric mean values of $C_{maxcalc}$ for reference (1.294±1.008) and test (1.295±1.0068) formulation, $T_{maxcalc}$ for reference (1.093±1.0097) and test (1.098±1.013) formulation, $AUC_{0-\infty}$ for reference (4.972 ±0.1134) and test (4.989±0.119) formulation, AUC_{last} for reference (4.727±1.014) and test (4.779±1.016) formulation, AUC_{tot} for reference (5.015±1.017) and test (5.104±1.017) formulation for log transformed data was shown in table 7. Also the geometric mean ratio of (Test/Reference) for $C_{maxcalc}$ (1.0059), $T_{maxcalc}$ (1.00479), $AUC_{0-\infty}$ (1.011), AUC_{last} (1.011) and AUC_{tot} (1.017). Similarly, the 90% CI values were in order of $C_{maxcalc}$ (0.994-1.007), $T_{maxcalc}$ (0.996-1.013), $AUC_{0-\infty}$ (0.986-1.020), AUC_{last} (0.997 - 1.024) and AUC_{tot} (1.004-1.031). The Schirmann'stwoone sided *t* test also inveterate the results of BE as shown in table in 8.

Similarly Basmenj *et al*., 2011 carried out the bioequivalence study of two brands of diclofenac sodium. Results showed 90 % CI for the log transformed values for C_{max}, $AUC_{0-t}$and $AUC_{0-\infty}$ following FDA and EMEA guidelines. On non log transformed data analysis of variance was also executed with the 90% confidence interval (CI) limit and P>0.05 for non significance which

were then evaluated by using geometric means with the range from 0.8-1.2.Results of log and non log transformed data were presented in table 7. For the evaluation of T_{max}, Wilcoxon Sign Rank test was also carried out using SPSS 20.0 (SPSS Inc). Table 10 showed no carry over effect and the results of rank sum were found to be <13.The relative bioavailability of F4 was found to be 101.27%.

It was concluded that both formulations were found to be equivalent and comparable related to their rate and extent of absorption, having comparable plasma concentration time profile. Hence no prejudice in therapeutic activity can be warranted.

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