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Bioequivalence Study of Diclofenac 150 mg XR: A Single-Dose, Randomized, Open Label, 2-Period Crossover Study in Healthy Adult Volunteers

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Abstract

Objectives: Evaluate the bioequivalence (BE) of two oral tablets formulations of diclofenac 150 mg in healthy male subjects under fasting condition. This was a phase I, randomized, open label, balanced, two period, two sequences, single oral dose, crossover, analyst blind study. **Methods:** Twenty four (24) healthy subjects were randomly assigned to one of two sequences protocol: 150 mg XR of reference formulation (R), diclofenac sodium in the first period or the test formulation (T), diclofenac potassium in the second or vice versa. The plasma concentrations were determined using a validated LC-MS/MS method. Pharmacokinetic (PK) parameters included: maximum plasma concentration (C_{max}), area under the plasma concentration—time curve from time 0 to the last measurable concentration (AUC_{0-t}), and area under the plasma concentration—time from time 0 to infinity ($AUC_{0-\infty}$), were evaluated for BE. **Results:** The results showed that 90% confidence intervals for the test/reference geometric mean ratios (GMR) of C_{max} (90.43 - 107.17), AUC_{0-t} (93.08 - 116.46) and $AUC_{0-\infty}$ (92.52 - 117.39) were within the BE (80% - 125%) acceptance range. **Conclusions:** Two formulations, reference product (R) Voltaren® (diclofenac sodium) of Novartis and test product (T), Diklason Bi (diclofenac potassium) of Laboratorios Leti S.A.V., with a single dose of 150 mg XR, under fasting conditions were bioequivalent. No severe, serious or unexpected adverse events (AEs) were reported in this study.

Keywords

Bioequivalence, Diclofenac, Pharmacokinetics

1. Introduction

Diclofenac is a medication used in the management and treatment of inflamma-

tory conditions and pain. It belongs to the family of phenylacetic acids and acts to decrease inflammation as other class drugs do [1] [2]. Its mode of action consist in inhibiting the activity of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) by supressing the synthesis of prostanoids such as prostaglandin-E2 (PGE2), prostacyclins, and thromboxanes, which are essential components of the inflammatory and nociceptive response It is regarded as one of the most effective inhibitors of the production of PGE2, the primary prostanoids elevated during an inflammatory response [1].

Diclofenac's effect of COX-2 inhibition appears to occur mostly at the site of target tissues such as synovial fluid and joint capsules. The drug concentrates in synovial fluids, where it renders its targeted action as an NSAID for relief musculoskeletal inflammation and ailments [1] [3]. It has both extended-release and immediate-release forms that vary in doses. Oral diclofenac sodium can be administered in delayed-release or immediate-release tablets in 25 to 150 mg tablets to achieve a total daily dose of 100 - 150 mg per day. These doses are for ankylosing spondylitis, osteoarthritis, and rheumatoid arthritis [3] [4].

The growth in NSAID prescriptions has been driven in part by the introduction of new diclofenac drug products [5] [6]. These new products have varied PK properties and dosing regimens and are indicated for the treatment of a range of acute and/or chronic pain conditions. Delayed- and extended-release forms of diclofenac sodium were initially developed with the goal of improving the safety profile of diclofenac and providing convenient, once-daily dosing for the treatment of patients with chronic pain [1] [4]. The development of diclofenac drug products demonstrates how pharmaceutical technology can be used to drive innovation, creating drug products with improved efficacy, safety, and increased clinical utility by improving the pharmacological properties of these agents [7] [6]. These improvements have provided clinical benefits such as reduced dosing frequency and improved adverse event profile. These new formulations of diclofenac require demonstrating their BE with the original forms, therefore it is necessary to demonstrate that they are BE, making comparative studies to evaluate their equivalences [3] [5] [6].

Diclofenac potassium salt is another alternative as it is more water soluble and provide more rapid dissolution and faster absorption than sodium salt, leading to more uniform absorption and rapid onset of pain relief [3] [5] Approximately 60% of the intact diclofenac molecule reaches the systemic circulation due to first-pass metabolism. The main metabolite, 40-hydroxydiclofenac, is known to retain weak anti-inflammatory and analgesic activities. Following second phase metabolism to glucuronides and sulphates metabolites, diclofenac is excreted in the urine (65%) followed by biliary excretion (35%) [3] [8].

The purpose of the present study was assess and compare the PK profile and safety of two formulations, reference product (R) Voltaren® (diclofenac sodium) of Novartis and test product (T), Diklason Bi (diclofenac potassium) of Laboratorios Leti S.A.V., with a single dose of 150 mg XR, under fasting conditions required for BE purposes. This study was conducted in India, by CRO ICBio Clin-

ical Research Pvt, Ltd.

2. Methods

2.1. Ethical Considerations

The study was conducted ethically in accordance with the principles of the ICMR guidelines (2017), New Drugs & Clinical Trials Rules 2019 India, and adhered to the ethical principles of the Declaration of Helsinki, the International Conference on Harmonisation Good Clinical Practice Guidelines [9] [10]. The study protocol was approved by an Independent Ethical Committee (ECR/141/indt/KA/2013/RR-19), and certified by N˚ EC-CT-2018-0029 to ICBio Clinical Research Pvt, Ltd., BA/BE, Study number: ICBio/003/0221 approved on February, 17th 2021. (Start date 23/Nov/2021 to clinic phase, period I and end date 30/Nov/2021 for period II).

2.2. Study Design

The study was an open label, analyst blind, balanced, randomized, two-treatment, two-period, two-sequence, single oral dose, crossover, BE study performed in healthy adult subjects under fasting conditions.

Tablets of Voltaren® (diclofenac sodium) Batch KL8511, expiration date 12/2021, Novartis, Colombia were used as the reference (R) sample and Diklason BI (diclofenac potassium) batch 096, expiration date 11/2022, diclofenac 150 mg XR, as the active pharmaceutical ingredient (API).

As per the randomization schedule, a single dose of the T or R was administered in each period. The clinical phase had a duration of 10 days, (23/11/2021 period I and 30/11/2021 to period II), the subjects who were administered the T product in period I were administered R product in period II and vice versa. There was a washout period of 7 days between the two dosing periods, considering the terminal half-life for diclofenac [3] [5].

Male subjects who fulfilled all the following criteria were included in the study, although the study was open to males and females, only male subjects fulfilled all the following inclusion criteria: aged between 18 and 45 years, body mass index (BMI) within a range of 18 to 30 kg/m^2 with good health based on the results of a complete clinical history and valid for 1 month prior to the start of the study; normal laboratory values as determined by medical history and physical examination at the time of screening; normal vital signs and physical examination, and laboratory, normal chest radiography and negative result in urine drug tests and were eligible to participate. The main exclusion criteria included: severe gastrointestinal bleeding, or renal disease known, history of hypersensitivity to active principle or any excipient or diclofenac, hepatic impairment, haematopoietic disease, positive urine test for drugs of abuse, use of tobacco, alcohol or any medication within the 24 hours (h) prior to study start. All subjects participating in this study received full details of the study before signing the consent forms.

2.3. Drug Administration and Blood Collection

The subjects were confined within the facility one night before study, the subjects were fasted for at least 10 h pre-dose and 4 h post-dose. The subjects received standardized meals at 04.00, 08.00, 12.00 and 24.00 h after dosing in each period. During housing the meal menu was same in both the periods (2200 Kcal) and drinking water was provided *ad libitum*.

Following an overnight fast of at least 10 hr, subjects were scheduled for dosing as per the randomization schedule in each period. The study was conducted with 24 subjects for period I and period II. The randomization list was generated using Statistical Analysis Software, (SAS®). A single oral dose (1 × 150 mg XR, tablets) of either the T or R was administered with 240 mL of water at ambient temperature in each period.

A total of 20 × 6 ml blood samples were collected via cannula from each subject during each period while 24:00 and 48:00 h samples were collected by direct punction. The venous blood samples were withdrawn at pre-dose (00.00 h) and 00.125, 00.25, 00.50, 00.75, 01.00, 01.50, 01.75, 02.00, 02.50, 03.00, 03.50, 04.00, 04.50, 05.00, 06.00, 08.00, 12.00, 24.00 and 48.00 h. Equal allocation of treatments or balanced randomization was ensured. No food was allowed until 4 h after dose administration for each period ("Guidance on the investigation of bioavailability and bioequivalence" CPMP/QWP/EWP/1401/98).

2.4. Analytical Procedure

The blood samples were collected in pre-labelled K_2EDTA vacutainers and were centrifuged at 4000 rpm for 10 min at 2˚C - 8˚C. Plasma were separated, labelled and stored at −70˚C ± 15˚C prior to analysis. A validated liquid chromatography-mass spectrometry (LC-ESI-MS/MS) analytical technique, plasma samples, calibration curve standards of internal standard (IS) diclofenac D4 (Vivian Life Sciences, Mumbai, India) and quality control samples (QCS) were thawed and vortexed for preparation and analysis. Aliquots of 200 µL plasma were mixed with 200 µL of extraction buffer and vortexed. Solid phase extraction (SPE) on hydrophilic-lipophilic balance (HLB) cartridges was performed. After conditioning (1 mL of methanol), equilibrating (1 mL water) and loading the sample, cartridges were washed (1 mL of water followed by 1 mL of washing solution) and was dried. Cartridges were eluted with 900 µL of methanol and the eluate diluted with 100 µL of methanol. Controls samples were spiked with IS of over the concentration range of 20.641 to 4088.976 ng/mL (linearity range). Samples of plasma, IS and QCS and were transferred to pre-labelled vials arranged into the auto sampler at 5˚C ± 3˚C. Analysis of diclofenac used an LC-ESI-MS/MS instrument (Shimadzu LCMS-8040, Mumbai, India). A BDS Hypersil C_{18} 4.6 × 50 mm id 5 µm HPLC column was used (Thermo Scientific, Mumbai, India) and the mass spectrometer was operated in positive electrospray ionisation mode. Identifications were based on multiple reaction monitoring transitions; *m/z* 296.10 - 214.00 for diclofenac and *m/z* 300.10 - 219.00 for the IS. The inter batch

calibration standard precision was in range 1.55% to 6.10% and accuracy 100.00% to 99.49%.

2.5. Statistical and Pharmacokinetics Analyses

The following PK variables were determined for diclofenac: C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, time to reach C_{max} (T_{max}) and elimination half-life ($T_{1/2}$). PK and statistical analyses were performed using SAS® version 9.3.1 Inc., Cary, North Carolina. USA. The log-transformed pharmacokinetic parameters were analysed using a general linear model (Proc GLM of SAS® Mumbai, India).

The sample size calculation for the study was based on intra-subject coefficient of variation (CV%) for C_{max} of diclofenac of 24% - 28% [5] with the expected coefficient of variation for C_{max} and AUC not exceeding 20% and the ratio falling within 95% to 105%. The study required 20 evaluable subjects to demonstrate bioequivalence with a power of 90% at 5% level of significance. Four additional subjects were included in the study for possible dropouts/withdrawals [3] [5]. The GMR of these primary PK parameters (T/R) and the 90% confidence intervals (CIs) were calculated for the determination of BE. Analysis of variance was applied on the logarithm-transformed PK values. BE between the test and reference formulations of diclofenac was demonstrated if the 90% CI fells within the acceptance range of 80% - 125% for ln-transformed pharmacokinetic parameters C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ [9] [11].

2.6. Safety Assessments

Safety of the subjects was evaluated through the assessment of AEs, vital signs and laboratory test (biochemistry, hematology and urianalysis) throughout the study. Vital signs were measured at baseline screening and at end of the study. Clinical laboratory was carried out at screening and 48 h post study.

3. Results

3.1. Baseline Characteristics

A total of 24 healthy adult male subjects who met the criteria were enrolled and randomized in the study. All completed the study and were valid for the PK analysis and safety evaluation. Demographic data of all evaluable subjects are presented in **Table 1**.

3.2. PK Evaluation

A non-compartmental analysis was applied for the estimation of PK parameters of diclofenac in plasma concentration time data using SAS® (**Table 2**).

Mean C_{max}, AUC_{0-t} and $AUC_{0-\infty}$, were respectively 979.28 ng/mL, 2828.81 ng h/mL, 3454.15 ng/mL for the T formultion and 994.45 ng/mL, 2696.77 ng h/mL, 3302.75 ng h/mL for the R formulation. Median T_{max} was 0.80 h for the T and 0.85 h for the R formulations. Mean diclofenac plasma concentration versus time curve for each formulation a diclofenac for T and R formulations are presented

in Figure 1 and Figure 2.

3.3. Bioequivalence

Analysis of variance for ln-transformed in PK parameters: C_{max} (ng/mL), $AUC_{0\text{-}t}$ (ng * h/mL) and $AUC_{0\text{-}\infty}$ (ng * h/mL) was evaluated, there was no statically significant difference between of two formulations of diclofenac 150 mg XR ($p \geq 0.05$). The test/reference ratio, GMRs for the logarithm transformed of C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ were 98% (100.4% - 112.7%), 104.12% (93.08% - 116.46%), 104.21% (92.52% - 117.39%), respectively (Table 3). These values are within the 90% CI acceptance criteria of 80.00% - 125.00% following EMA-Guidelines [10].

3.4. Safety and Tolerability

All AEs were evaluated throughout the study, using adverse events (AEs) questionnaires in the case report form (CRF) reporting. No AEs serious and no serious were reported for this study.

Table 1. Demographic data of subjects completing the bioequivalence study.

Baseline Characteristics	Total (N = 24)
Sex (Men)	100%
Age (years)	34.08 ± 5.5
Weight (kg)	67.83 ± 10.0
Height (m)	1.71 ± 0.06
Body Mass Index (kg/m^2)	23.12 ± 2.9
ABP S/D mmHg	118.9/75.42
BP (beats/min)	72.83

Results are displayed as n (%) or mean ± standard deviation (SD).

Table 2. Pharmacokinetics parameters after a single 150 mg oral dose of the T and R formulations.

ANALYTE: Diclofenac (*n* = 24)		
PK Parameters	Test (T)	Reference (R)
C_{max} (ng/mL)	979.287 ± 353.118	994.653 ± 365.338
$AUC_{0\text{-}t}$ (ng * h/mL)	2828.814 ± 1309.465	2696.771 ± 1229.322
$AUC_{0\text{-}\infty}$ (ng * h/mL)	3454.156 ± 1866.272	3302.754 ± 1749.327
T_{max} (h)	0.802 ± 1.049	0.859 ± 1.088
Kel (h^{-1})	0.231 ± 0.232	0.212 ± 0.221
$T_{1/2}$ (h)	4.754 ± 2.927	4.983 ± 2.720

Data presented as mean ± SE. C_{max}: maximum concentration, $AUC_{0\text{-}t}$: area under the plasma concentration—time curve from time 0 to the last measurable concentration; $AUC_{0\text{-}\infty}$: area under the plasma concentration—time curve from time 0 to infinity, T_{max}: time to reach C_{max}, Kel: elimination rate constant, $T_{1/2}$ time required for plasma.

Table 3. Pharmacokinetics parameters ln-transformed, 90% CI for the T/R T and R ratio in two diclofenac 150 XR, oral formulations. N = 24.

PK Parameters	GMR (T/R)%	GMR		90% confidence interval	
		Test	Reference	Lower	Upper
C_{max} (ng/mL)	98.44	915.06	929.55	90.43	107.17
AUC_{0-t} (ng * h/mL)	104.12	2530.74	2430.65	93.08	116.46
$AUC_{0-\infty}$ (ng * h/mL)	104.21	3045.47	2922.29	92.52	117.39

Data presented as a % mean ln transformed. C_{max}: maximum concentration, AUC_{0-t}: area under the plasma concentration—time curve from time 0 to the last measurable concentration; $AUC_{0-\infty}$: area under the plasma concentration—time curve from time 0 to infinity. GMR: Geometric mean ratios n = 24, PK: Pharmacokinetics, CI: Confidence interval, ln: natural logarithm.

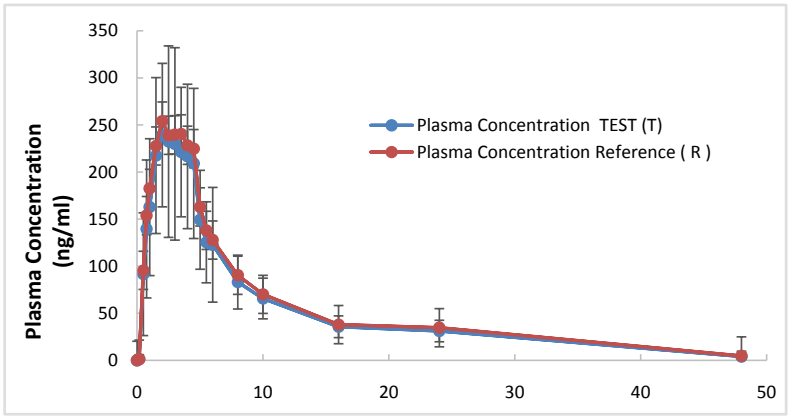

Figure 1. Mean diclofenac 150 mg XR plasma concentration versus time (h) profile for each formulation are presented in a linear scale, following a single oral dose. Blue line indicate diclofenac potassium (Test product of Laboratorios Leti S.A.V. República Bolivariana de Venezuela), and red line indicate diclofenac sodium (Reference product of Novartis).

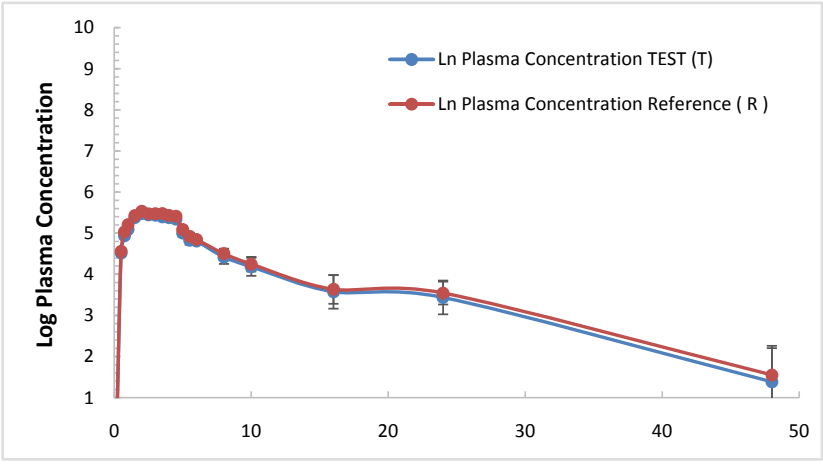

Figure 2. Mean diclofenac 150 mg XR plasma concentration versus time (h) profile for each formulation are presented in a logarithmic scale, following a single oral dose. Blue line indicate diclofenac potassium (Test product of Laboratorios Leti S.A.V. República Bolivariana de Venezuela), and red line indicate diclofenac sodium (Reference product of Novartis).

4. Discussion

For regulatory reasons and to comply with all the requirements established for the commercialization of generic products [10] [11]. It is necessary to carry out bioequivalence studies following international standards, (EMA-Guidelines on the investigation of Bioequivalence 01/08/2010) to demonstrate that the R and the T are interchangeable [9] [10].

This study was designed to assess the BE of a single 150 mg XR dose oral tablet formulation of diclofenac in healthy indian volunteers under fasting condition. The bioequivalence of both formulations with respect to the rate and extent of absortion was demonstrated.

A validate LC-MS/MS method for determination of diclofenac in human plasma was used. Liquid-liquid extraction procedure for sample preparation was accomplished by using ethyl acetate. The method was linear between 20.641 - 4088.976 ng/ml to diclofenac D4 as IS.

BE was assessed by measuring of the PK parameters; C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$. This is evidenced by the results showing that the 90% CI for ln-transformed ratios of C_{max} and $AUC_{0\text{-}t}$ fell within the BE acceptance range (80% - 125%). To others parameters as T_{max} h (0.802 ± 1.049) to T and (0.859 ± 1.088) to R products and Kel·h^{-1} was (0.231 ± 0.232) to T and (0.212 ± 0.221) to R and V1/2 h was (4.754 ± 2.927) to T and (4.983 ± 2.720) to R. All data on T_{max} and T1/2 and Kel were aligned with previous PK studies with these formulations [3] [5].

This study demonstrated that diclofenac 150 mg was well tolerated among healthy subjects and no adverse events, serious or no serious, were reported. An additional advantages of using sustained release formulations of diclofenac is also avoid multiples peaks in plasma concentrations and thereby decrease concentration dependent adverse drug reactions [12].

5. Limitations

We could not assess pharmacokinetics parameters of female volunteers, although the study was open to males and female, only male patients were included. Based on the summary of the product, no pharmacokinetic differences have been reported between male and female subjects [3].

6. Conclusion

This single dose study found that the two drugs were bioequivalent, according to the EMA guidelines, the primary pharmacokinetics parameters were within of acceptable range (80.0 - 125.0 percent). Our study demonstrated that test product Diklason Bi 150 mg (diclofenac potassium) XR tablet should be considered bioequivalent to reference product Voltaren® 150 mg (diclofenac sodium) XR tablet, evaluated in healthy male subjects under fasting condition.

Acknowledgements

This study was conducted at the third party ICBio Clinical Research Pvt. Ltd,

located in Vidyaranyapura, Bangalore, India.

Authors Contributions

EP, AI and XSM performed the statistical analysis, interpretation, writing and revision of the manuscript.

Declaration of Patient Consent

The study was conducted ethically in accordance with the principles of the ICMR guidelines (2017), and adhered to the ethical principles of the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice Guidelines. The study protocol was approved by an Independent Ethical Committee (ECR/141/indt/KA/2013/RR-19), and certified by CDSCO/DGHS to ICBio Clinical Research Pvt, Ltd., BA/BE/2020/053. Study number: ICBio/003/0221. The authors certify that they have obtained patient consent.

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Conflicts of Interest

All authors are Industrias Biocontrolled C.A., (Leti Group Company), employees and may hold share and/or stock options in the company. The authors have no other potential conflicts of interest relevant to this study.

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