# Evaluation of Effect of Temperature & Pressure on the Bioavailability of Diclofenac Topical Formulation

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**Abstract—** The study aimed at the assessment of the pharmacokinetic parameters of Diclofenac Sodium Marketed formulations and evaluation of the effect of pressure and temperature by Dermatopharmacokinetic method. This was a single-dose-one arm, open label pharmacokinetic study of marketed formulations of Diclofenac Sodium using healthy Indian male subjects. Skin Stratum Corneum samples were collected in sterile glass test tubes and were analysed for Diclofenac Sodium concentrations. Pharmacokinetic parameters of Diclofenac sodium were calculated as $C_{max}$, $t_{max}$, AUC (0-t) and AUC (0-∞). Diclofenac Sodium was estimated in Stratum Corneum using a validated Spectroscopic method. The bioequivalence values of the test drug A were found to be as: $C_{max}$ of 25.457±2.398 μg/ml $t_{max}$ of 1.23 ±0.261 h, $AUC_{0-t}$ of 100.586±11.15 h. μg/ml $AUC_{0-∞}$ of 178.286± 22.859 h. μg/ml; after applying pressure, $C_{max}$ of 30.651± 2.742 μg/ml $t_{max}$ of 0.83 ±0.342 h $AUC_{0-t}$ of 100.507± 10.455 h. μg/ml $AUC_{0-∞}$ of 166.971± 47.627 h. μg/ml; and after using heat belt $C_{max}$ of 29.344± 2.216 μg/ml $t_{max}$ of 1.23 ±0.261 h $AUC_{0-t}$ of 100.507± 10.455 h. μg/ml, $AUC_{0-∞}$ of 179.887± 21.553 h. μg/ml. The study demonstrated that the bioavailability of the topical formulations increases with the help of pressure and temperature. © 2011 IGJPS. All rights reserved

**Keywords :** Dermatopharmacokonetic, Diclofenac, Bioavailability.

## *INTRODUCTION*

The EMEA (European Agency for the Evaluation of Medicinal Products) defines the term bioavailability as the "rate and extent to which the active substance or active moiety is absorbed from a pharmaceutical form and becomes available at the site of action" [1]. Topical dermatological drug products belong to the class of locally acting drug products [2]. In this case, the site of pharmacological action is the skin. Stratum corneum and skin surface are considered to be the compartments of invasion, whereas the blood system represents the compartment of excretion [3]. Therefore, two different types of bioavailability have to be distinguished for topical application. The topical bioavailability reflects the rate and extent to which the active moiety becomes available at the site of action, i.e. the skin. The systemic bioavailability, instead, may not properly reflect the cutaneous bioavailability for medications intended to treat local skin disorders but becomes important for the toxicological evaluation of the body burden and for transdermal therapeutic systems (TTSs) [4]. Percutaneous absorption is the uptake of a compound into the systemic circulation after topical application and describes the movement through the various layers of the skin with respect to both rate and extent. The Percutaneous absorption process can be divided into the following 3 steps [5]. Penetration is the entry of a substance into a particular layer. Permeation is the passage through one layer into another layer. Absorption is the uptake of a substance into the vascular system (blood and/or lymph vessel), which acts as the central compartment, and reflects the systemic bioavailability. Skin characteristics are an essential consideration for percutaneous absorption. Features of normal skin, barrier changes in the skin, and vascular changes in the skin all play a critical role in absorption. Several approaches have been used to improve entry of drugs into lower skin layer and deeper tissues. Chemical and physical permeation enhancers have been designed to facilitate delivery of high drug concentrations across the skin into systemic circulation or deeper tissues [6]. The classes of enhancers used and the mode of action of these agents vary [7]. Increased drug diffusivity in the skin, stratum corneum lipid fluidization, increase in thermodynamic activity of drug in the skin and vehicle, as well as effect on drug partition coefficient, are the most common modes of action of chemical enhancers. While physical parameters like pH, temperature, pressure, vehicle etc acts as a physical enhancer.

According to the Food and Drug Administration (FDA) guidelines [8], pharmacokinetic (PK) measurements in blood, plasma and/or urine of topical dermatological drug products are not feasible to document bioavailability since the active ingredient(s) in topical formulation is not intended to be absorbed into the systemic circulation and in addition, concentration in extracutaneous biological tissues would generally not be measurable. This limits determination of bioavailability and assessment of BA of such product to pharmacodynamic measurements, [8]. One method which has received a great deal of attention is TS, also sometimes referred to as a DPK method. It has been widely investigated as a possible method for use in BE and BA studies of topical drug products [8]. TS involve removal of successive layers of the SC using adhesive tape placed on the skin. Each strip removes approximately 0.5 – 1 μm of SC and the procedure is relatively pain free [9]. TS is considered to be a non-invasive sampling technique and although it causes minor distortion of the skin structure, the skin has the ability to rapidly regenerate and restore barrier function [9]. The use of TS is limited by the fact that currently there are no standardized and universally acceptable TS methods to determine the SC concentration of a compound following topical administration. Standardization of such methods are essential for routine use of TS for BA determination and BE assessment of topical dosage forms. Another challenge is that once TS data have been collected it has to be processed in a manner which takes into account the variability of data due to inter-individual differences in the thickness of the SC. Whilst it is essential to quantitatively determine the amount of SC removed by each strip, consensus has not yet been reached on a simple and rapid method to do so [9].

TS have found use in a variety of applications such as:

- assessment of pH of the SC environment [11]

- estimation of the amount of SC removed by TS [10]

- assessment of the BA of topically applied substances [12-17]

- determination of diffusion characteristics at different anatomical sites of the body [18]

- investigation of the effect of certain skin pathologies such as inflammation, neoplastic

   disorders or xerotic conditions [19], amongst other uses.

The technique is particularly useful when considering substances whose intended site of action is the SC such as antifungal, UVA/UVB filters and antiseptics [8]. Thus the use of TS presents a potential technique for use in assessing topical BA and BE of topically applied formulations targeting the SC and underlying tissues of the skin.

Diclofenac is a drug belonging to the class of nonsteroidal anti-inflammatory drugs (NSAIDs). It has analgesic and anti pyretic properties. It is widely used for reducing pain, stiffness and inflammation caused by conditions, including osteoarthritis, rheumatoid arthritis, abdominal cramps associated with menstruation.  The drug is manufactured and supplied as either the sodium or potassium salt. It is also available as a generic drug in a number of formulations. The exact mechanism of action is not entirely known, but it is thought that the primary mechanism responsible for its anti-inflammatory, antipyretic, and analgesic action is inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX). It also appears to exhibit bacteriostatic activity by inhibiting bacterial DNA synthesis.[20] Inhibition of COX also decreases prostaglandins in the epithelium of the stomach, making it more sensitive to corrosion by gastric acid. This is also the main side effect of diclofenac. Diclofenac has a low to moderate preference to block the COX2-isoenzyme (approximately 10-fold) and is said to have therefore a somewhat lower incidence of gastrointestinal complaints than noted with indomethacin and aspirin. The action of one single dose is much longer (6 to 8 hours) than the very short half-life that the drug indicates. This could be partly because it persists for over 11 hours in synovial fluids.[21]

## *SUBJECT & METHOD*

**Study Subjects:**

Sufficient numbers of healthy Indian male human subjects was screened, out of those 09 male subjects were enrolled in the study and 03 male subjects were taken as standby. A total of 12 male subjects were applied with the study medication in the beginning of the study. The screening consent & study consent was taken respectively before drug application. Thereafter, subject's medical records were documented and physical examination was conducted. Inclusion eligibility was also based on successful completion of a clinical health evaluation , which consisted of a personal interview; a complete physical examination (BP, pulse, weight, temperature, and respiratory rate); diagnostic testing that included a 12-lead electrocardiogram and chest radiograph; a laboratory testing that included a complete blood cell count, metabolic and hepatic tests (alanine amino transferase [reference range, 5-55 U/L], aspartate amino transferase [5-34 U/L]), urine analysis, pregnancy test (for female subjects), blood chemistry for glucose (70-109 mg/dL), blood urea nitrogen (7-23 mg/dL), and creatinine (0.-1.3 mg/dL), as well as serologic tests for hepatitis (B and C), and HIV antibodies. Testing was performed by Central Pathology Laboratory, MGM Hospital, N-6 CIDCO, Aurangabad,(MS) INDIA 431005. Subjects were excluded if laboratory values were significantly above or below the reference range and/or if all tests had not been performed. In addition, the laboratory data were reviewed by the investigators of the clinical unit prior to the enrollment of the subjects. Subjects were compensated for participation.

**Study Design:**

This study was carried out as per the ICH (Step 5), 'Guidance for Good Clinical Practices (GCP)' and the principles of Declaration of Helsinki (Scotland, October 2000).The Independent Ethics Committee shall review the protocol and the informed consent form for this study. This was the open label, three ways, two period, parallel design pharmacokinetic study. Subjects were admitted and housed in the clinical facility at least 2 hour before the application of the dose during each period of the study. Informed consent for the dosing / sampling procedure was obtained from each subject on admission to the clinical facility for the first study period.The marketed fromulation of the diclofenac [Diclofenac Diethylamine BP 30 ml Spray; Duoflam Spray, Lic. No.: AD/248-A, Batch No.: 8001, Mfg. Date: 04/08, Exp. Date: 04/2011, Mfg. By: SVIZERA HEALTH CARE, Mumbai] was applied on the forearm of the study subjects as per the dosing schedule. For the first period of the study drug sample was applied on both the arms. Of which pressure was applied on all the sites of drug application on one arm using pressure belt for the sufficient period for time. While in second period of the study heating belt was used instead of pressure belt on all the sites of drug application.

The dosing procedure was as mentioned below:

- Both the forearms were washed with mild soap and copious amount of water and dried in air.
- Both the forearms were marked for total of 08 application sites of 1 sq.cm area each.
- 5 mm length product (semisolid dosage forms) or sufficient amount of drug sample was applied on all the sites so that the product completely and smoothly covers the site area (Spray dosage forms).
- The stratum corneum samples were collected from the sites on the desired pre decided time.

**Stratum Corneum Sampling:**

Skin Stratum Corneum samples were collected in sterile glass test tubes during each period. The samples were collected pre-dose and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 6.0 hours post-dose application. The stratum corneum samples were analysed for Diclofenac Sodium concentrations only. For each subject the total number of blood draws were 02 (01 for screening and another during post study assessment); the total volume of blood withdrawn (10 ml for the pre-study evaluation and 10 ml for the post study) through the vein puncture were not exceed 20 ml.

**Procedure**

Study samples were collected as follows. The pre-dose samples were collected within one hour prior to drug application. The post-dose samples were collected within 2 minutes of the scheduled time where the end time of collection to the nearest minute would be recorded.

- Before sampling the drug remained on the site was removed by mild force using three cotton swabs to ensure the complete removal of residual drug from the site.
- The pre cut (1 sq. cm) adhesion tape was applied on the site and the mild force was applied to ensure the proper adhesion of the tape on the site area. The tape was removed and discarded.
- Eight adhesion tape pieces were applied on the site area in the same manner and each tape was removed from the site before the next one is applied. The removal was done using the forceps and the removal should be done by one stroke to ensure the complete removal of stratum corneum.
- All 8 samples tapes were collected in a single test tube which were then sealed and stored in the refrigerator at $-20^0$c till analysed.

**Analytical Method:**

A validated UV spectroscopic method was employed by using Chemito-Spectroscan UV 2600, Double Beam UV-Visible Spectrophotometer for the estimation of Diclofenac Sodium in human stratum corneum. This method involves the extraction of the Diclofenac Sodium form sample by using methanol and measuring the absorbance at 285nm. The concentration of Diclofenac Sodium in sample is determined from calibration curve. The standard stock solution of Diclofenac sodium was prepared by weighing 50mg of Diclofenac Sodium powder and shaking it with 60 ml of *methanol* in a 200-ml volumetric flask which was then diluted with *methanol*. From this solution 4ml was diluted up to 100ml with methanol, to get the solution with concentration of 10μg/ml.The test solution was prepared by taking 1, 2, 4, 6, 8, 10 ml from the standard stock solution in six different labelled (1 μg/ml, 2 μg/ml, 4 μg/ml, 6 μg/ml, 8 μg/ml) test tubes and making volume up to 10ml by adding methanol. (Note: no need to add methanol in last μg/ml sample). Methanol was used as blank solution. Calibration Curve was prepared by using various dilutions (1 μg/ml -10μg/ml) as-

transfer required quantity of blank solution in to the cuvette and the absorbance was seen, take the first test tube (1 μg/ml) transfer the required quantity of the test solution into the cuvette then the absorbance at 285 nm was measured and was recorded, the steps 2 and 3 for remaining dilutions was repeated. Finally the graph of concentration versus absorbance (OD) was plotted.

**Pharmacokinetic Analysis:**

AUC from time 0 (baseline) to 6 hours (AUC0–6) was calculated using the trapezoidal rule (Chow and Liu, 2000; Chow and Liu, 2007). From the terminal log-decay phase, elimination rate constant (ke) was estimated using linear regression, and t½ was estimated using the following equation: t½ = ln2/ke where ln was defined as the natural logarithm. Extrapolation of AUC from baseline to infinity (AUC0–□) was calculated as follows: AUC0–□□= AUC0–6 + (C6/ke) where C6 was defined as concentration at 6 hours.

To compare the bioavailability of the study drug for the parameters like pressure and temperature, Cmax, AUC from baseline to time t (AUC0–t), and AUC0–□□was carried out. Ratios of Cmax, AUC0–t, and AUC0–□□for all formulations were calculated, and 90% CIs were obtained. The 90% CIs for the corresponding ratios of Cmax, $t_{max,}$ $AUC_0$–t, and AUC0–□ should be within the 80% to 125% range.

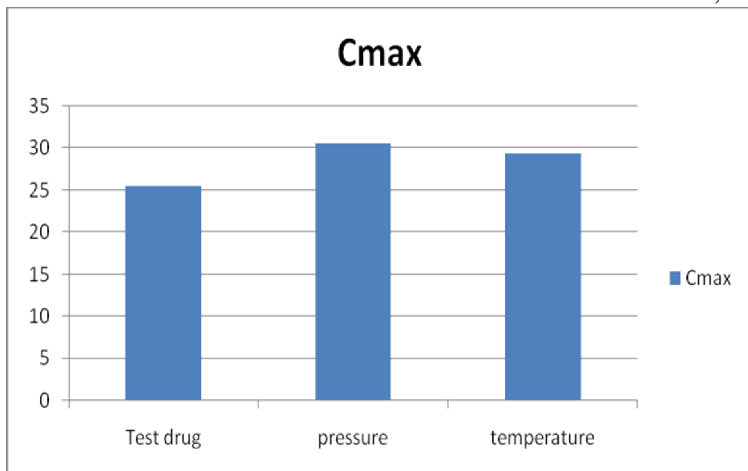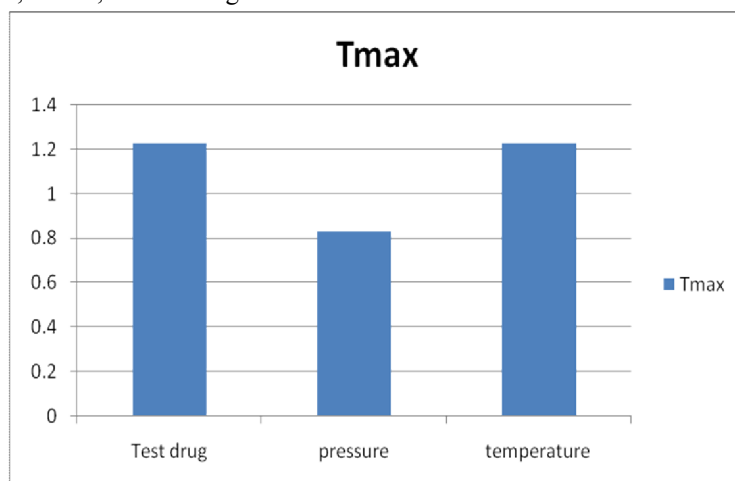| Parameters | Test drug | Pressure | Temperature |
|---|---|---|---|
| Cmax | 25.457±2.398 μg/Ml | 30.651± 2.742 μg/mL | 29.344± 2.216 μg/mL |
| Tmax | 1.23 ±0.261 h | 0.83 ±0.342 h | 1.23 ±0.261 h |
| AUC0-t | 100.586±11.15 h. μg/mL | 100.507± 10.455 h. μg/mL | 100.507± 10.455 h. μg/mL |
| AUC0-∞ | 178.286± 22.859 h. μg/mL; | 166.971± 47.627 h. μg/mL; | 179.887± 21.553 h. μg/mL |

**Table 1**

Figure 1



Figure 2

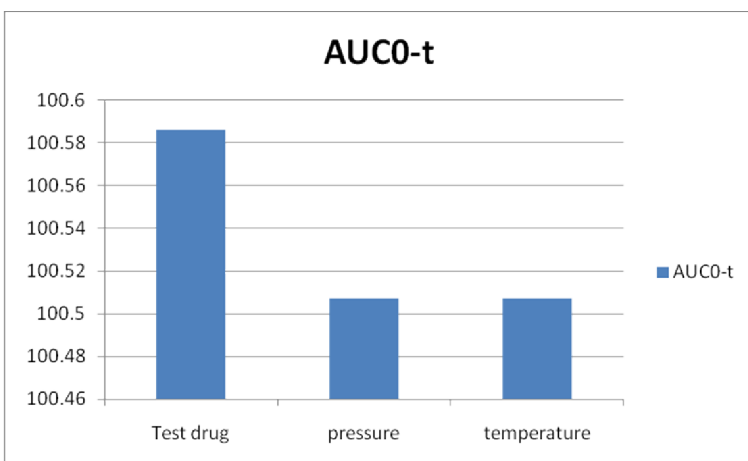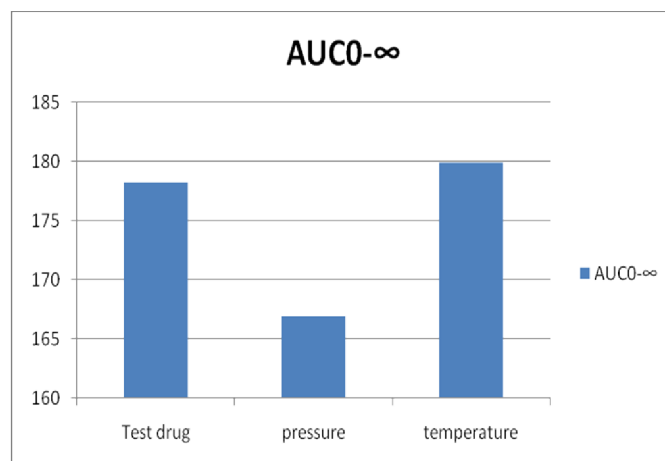

Figure 3



Figure 4

## RESULTS & DISCUSSION

Twelve subjects were enrolled in the comparison between three formulations of Diclofenac (mean age, 25.16 years).The bioequivalence values of the test drug A were $C_{max}$ of 25.457±2.398 μg/Ml $t_{max}$ of 1.23 ±0.261 h, $AUC_{0-t}$ of 100.586±11.15 h. μg/mL $AUC_{0-\infty}$ of 178.286± 22.859 h. μg/mL; after applying pressure, $C_{max}$ of 30.651± 2.742 μg/mL $t_{max}$ of 0.83 ±0.342 h $AUC_{0-t}$ of 100.507± 10.455 h. μg/mL $AUC_{0-\infty}$ of 166.971± 47.627 h. μg/mL; and after using heat belt $C_{max}$ of 29.344± 2.216 μg/mL $t_{max}$ of 1.23 ±0.261 h $AUC_{0-t}$ of 100.507± 10.455 h. μg/mL, $AUC_{0-\infty}$ of 179.887± 21.553 h. μg/mL.

Mean and SD values of tmax, Cmax, AUC0–T, and AUC0∞for each formulation are shown in Table 1 and depicted in Figure 1 to 4. The results revealed that when we apply pressure on all the site of drug application the time required to achieve the Cmax get recued as well as Cmax achieved was greater. On the other hand when we use heat belt the Cmax was increases but the time required to this remains constant.

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## *CONCLUSION*

The present study concludes that the pressure and temperature plays important role in the bioavailability of the topical formulation of Diclofenac.

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