

## Comparative Pharmacokinetic Profile of Aceclofenac from Oral and Transdermal Application

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### Abstract

The aim of the present investigation was to compare pharmacokinetic profile (bioavailability) of aceclofenac by transdermal and oral application. Nanoemulsion, nanoemulsion gel and marketed tablet (Aceclofar<sup>®</sup>) were subjected to pharmacokinetic (bioavailability) studies on Wistar male rats. Several pharmacokinetic parameters like  $C_{max}$ ,  $t_{max}$ ,  $AUC_{0 \rightarrow t}$ ,  $AUC_{0 \rightarrow \infty}$ ,  $K_e$ , and  $T_{1/2}$  were determined for each formulation. The absorption of aceclofenac by transdermally applied nanoemulsion and nanoemulsion gel resulted in 2.95 and 2.60 fold increase in bioavailability as compared to oral tablet formulation. Results of these studies indicated that the nanoemulsions can be successfully used as potential vehicle for enhancement of bioavailability of aceclofenac.

**Keywords:** Aceclofenac; Nanoemulsion; Bioavailability

### Introduction

Aceclofenac, a nonsteroidal anti-inflammatory drug (NSAID) has been recommended orally for the treatment of various kinds of pain, inflammation, rheumatoid arthritis and osteoarthritis (Gonzalez et al., 1994; Yamazaki et al., 1997; Yang et al., 2005; Shakeel et al., 2007). Long term oral administration of aceclofenac causes gastrointestinal (GI) ulcers and GI bleeding (Yamazaki et al., 1997). Transdermal route is known to reduce these adverse effects and increased bioavailability of drugs (Shakeel et al., 2007; Shakeel et al. 2008a, b, c). Aceclofenac is well absorbed orally with hepatic first pass metabolism (Gonzalez et al., 1994). It exhibits elimination half life of 4h, volume of distribution 25l, 99 % of protein binding and 60-70 % of bioavailability after oral administration (Gonzalez et al., 1994). Recently lipid based carriers such as microemulsions, nanoemulsions, solid dispersions, solid lipid nanoparticles and liposomes have been used successfully for the enhancement of solubility/bioavailability of drugs (Kommuru et al., 2001; Shakeel et al., 2008b, c).

Nanoemulsions have been reported to make the plasma concentration profiles and bioavailability of drugs more reproducible (Lawrence and Rees, 2000; Kommuru et al., 2001; Shakeel et al., 2008b, c). Nanoemulsions have also been reported as one of the most promising techniques for enhancement of transdermal drug delivery and bioavailability of many drugs (Baboota et al., 2007a, b; Shafiq et al., 2007a, b; Shakeel et al., 2007; Shakeel et al., 2008a, b, c). Nanoemulsions are thermodynamically stable transparent or translucent solutions of oil and water stabilized by an interfacial film of surfactant and cosurfactant molecules having a droplet size 10-100 nm (Shafiq et al., 2007a, b, c). This article evaluates the pharmacokinetic profile of aceclofenac using nanoemulsion technique and its comparison with marketed tablet formulation.

### Materials and Methods

#### Materials

Aceclofenac was a kind gift sample from Ranbaxy Re-

search Laboratory (Haryana, India). Oleolyl macroglycerides-EP (Labrafil) and diethylene glycol monoethyl ether (Transcutol-P) were gift sample from Gattefosse (Cedex, France). Glycerol triacetate (Triacetin) and acetonitrile (HPLC) grade were purchased from E-Merck (Mumbai, India). Tween-80 was purchased from Sigma Aldrich (St. Luis, MO, USA). All other chemicals used in the study were of analytical reagent (AR) grade.

### Preparation of Aceclofenac Nanoemulsion and Nanoemulsion Gel

Nanoemulsion formulation of aceclofenac (F1) was prepared by spontaneous emulsification method (Shakeel et al., 2007). 2 % w/w of aceclofenac was dissolved in 15 % w/w mixture of Labrafil and Triacetin (2:1, oil phase). Then 35.33 % w/w of Tween-80 and 17.66 % w/w of Transcutol-P were added slowly in the oil phase. The final formulation was made 100 % w/w by slow addition of distilled (Shakeel et al., 2008a).

Nanoemulsions gel (NG1) was prepared by dispersing 1 % w/w of Carbopol-940 in sufficient quantity of distilled water. After complete swelling of Carbopol-940 in distilled water. 2 % w/w of aceclofenac was dissolved in 15 % w/w mixture of Labrafil and Triacetin (2:1). Drug solution (aceclofenac) was added slowly to Carbopol-940 dispersion. 0.5 % w/w of triethanolamine (TEA) was added in the above mixture to neutralize Carbopol-940. Then 35.33 % w/w of Tween-80 and 17.66 % w/w of Transcutol-P were added slowly. Then remaining quantity of distilled water was added to get the final formulation 100 % w/w.

The composition of nanoemulsion (F1) and nanoemulsion gel (NG1) are given in Table 1.

### Characterization of Nanoemulsion

Prepared nanoemulsion was characterized for surface morphology, droplet size, viscosity and refractive index. Surface morphology was determined using transmission electron microscopy (TEM) (Topcon, Paramus, NJ, USA). Droplet size distribution was determined by photon correlation spectroscopy using a Zetasizer 1000 HS (Malvern Instruments, Worchestershire, UK). Viscosity was determined using Cone and Plate viscometer (Brookfield Engineering Laboratories, Inc, Middleboro, MA). Refractive

index was determined using Abbes type Refractometer (Precision Standard Testing Equipment Corporation, Mumbai, India) (Shakeel et al., 2007).

### Pharmacokinetic Studies

Approval to carry out pharmacokinetic studies was obtained from the local Animal Ethics Committee of Al-Arab Medical University, Benghazi, Libya. These studies were performed on optimized nanoemulsion (F1), nanoemulsion gel (NG1) and marketed tablet (Aceclofar®). Male Wistar rats were stores under standard laboratory conditions (temperature  $25 \pm 2^\circ\text{C}$  and relative humidity of  $55 \pm 5\%$ ). The rats were kept in polypropylene cages (6/cage) with free access to standard laboratory diet. About 15 cm<sup>2</sup> of skin was shaved on the abdominal side of rats in each group except group treated with marketed tablet formulation. They were fasted for the period of 12 h for observations of any unwanted effects. The dose for the rats was calculated according to the surface area ratio method (Shakeel et al., 2008b, c). The rats were divided into 3 groups, each containing 6 rats. Group I received F1 transdermally, group II received NG1 transdermally and group III received marketed tablet orally. The dose for transdermal and oral treatment was similar. The rats were anaesthetized using light ether anesthesia and blood samples (0.5 ml) were withdrawn from the tail vein of rat at 0 (pre-dose), 1, 2, 3, 6, 12 and 24 h in microcentrifuge tubes in which 6 mg of EDTA was added as an anticoagulant. The blood collected was mixed with the EDTA properly and centrifuged at 5000 rpm for 25 min for separation of plasma. The separated plasma was stored at  $-21^\circ\text{C}$  until drug analysis was carried out using high performance liquid chromatographic (HPLC) method.

### Sample Preparations

Plasma samples were prepared by adding 500 µl of plasma, 50 µl standard solution of aceclofenac, 50 µl of internal standard (IS) solution (diclofenac sodium), 1 ml of orthophosphoric acid and 5 ml of chloroform in small glass tubes. The tubes were vortex for 2 min and centrifuged for 25 min at 5000 rpm. Upper layer was discarded and the chloroform layer was transferred to a clean test tube and evaporated to dryness at  $50^\circ\text{C}$  under the stream of nitrogen. The residue was reconstituted with 100 µl of mobile phase for HPLC analysis.

### Analysis of Aceclofenac

Aceclofenac in plasma was determined by the reported validated HPLC method with slight modifications (Hinz et al., 2003). A Shimadzu model HPLC equipped with binary LC-10A VP pumps, variable wavelength programmable UV/VIS detector SPD-10AVP column oven (Shimadzu), SCL 10AVP system controller (Shimadzu), Rheodyne injector fitted with a 20 µl loop software was used for analysis. Analysis was performed on a C<sub>18</sub> RP-HPLC column. The mobile phase consisted of acetonitrile:phosphate buffer (40:60). The mobile phase was delivered at the flow rate of

Ingredients	F1	NG1
Aceclofenac (% w/w)	2.0	2.0
Carbopol-940 (% w/w)	-	1.0
Labrafil (%w/w)	10.0	10.0
Triacetin (%w/w)	5.0	5.0
Tween 80	35.33	35.33
Transcutol-P (% w/w)	17.66	17.66
Distilled water to (% w/w)	100.0	100.0

**Table 1:** Compositions of nanoemulsion (F1) and nanoemulsion gel (NG1).

1.0 ml/ min. Detection was performed at 282 nm. Injection volume was 20 µl. The concentration of unknown plasma samples was calculated from the calibration curve plotted between peak area ratios of aceclofenac to IS against corresponding aceclofenac concentrations.

**Pharmacokinetics and Statistical Analysis**

The plasma concentration of aceclofenac at different time intervals was subjected to pharmacokinetic (PK) analysis to calculate various parameters like maximum plasma concentration ( $C_{max}$ ), time to reach maximum concentration ( $T_{max}$ ), area under the plasma concentration-time curve ( $AUC_{0-t}$  and  $AUC_{0-\infty}$ ), elimination half life ( $T_{1/2}$ ) and elimination rate constant ( $K_e$ ). The values of  $C_{max}$  and  $T_{max}$  were read directly from the arithmetic plot of time and plasma concentration of aceclofenac. The  $AUC_{0-t}$  was calculated by using the linear trapezoidal method. The  $AUC_{0-\infty}$  was calculated by using the equation:

$$AUC_{0-\infty} = AUC_{0-t} + C_t / K_e$$

Where  $C_t$  is the last measurable concentration and  $K_e$  is elimination rate constant.  $K_e$  was determined from the slope of linear portion of graph plotted between logarithm of plasma concentration and time.  $T_{1/2}$  was determined using the equation:

$$T_{1/2} = 0.693 / K_e$$

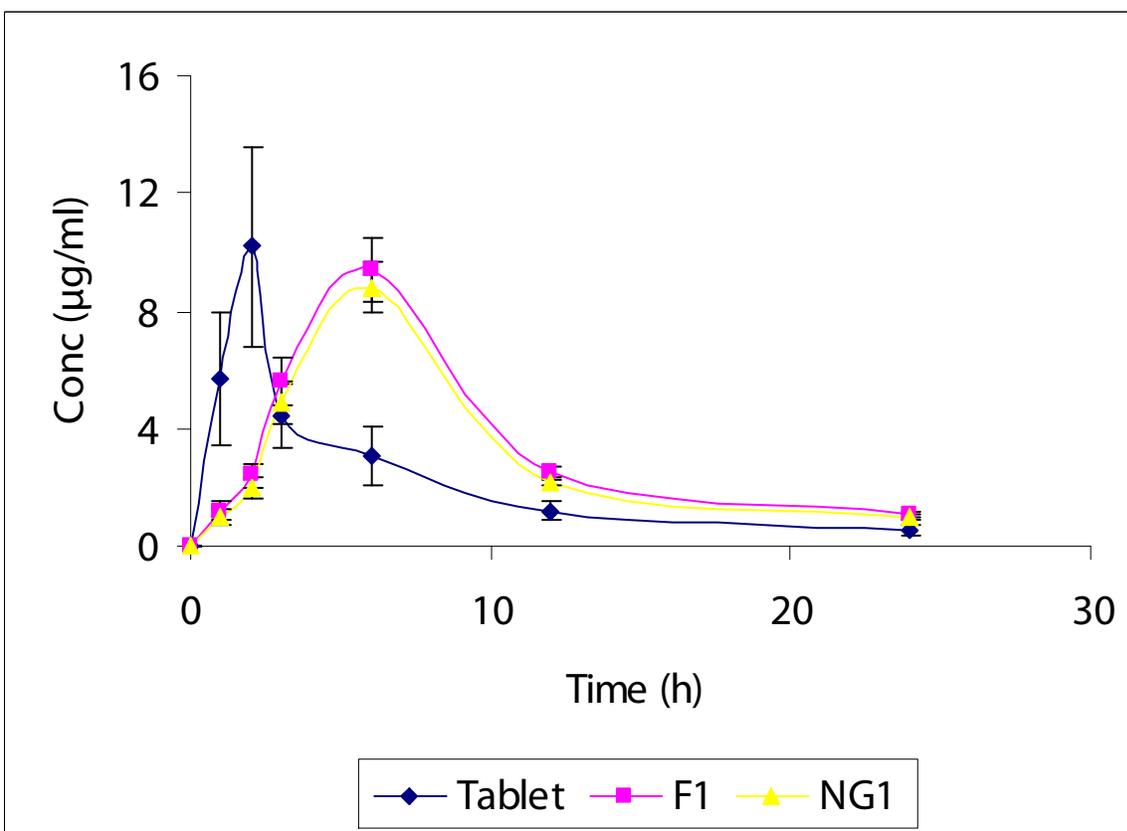
The relative bioavailability of aceclofenac after the transdermal administration versus the oral administration was calculated as follows:

$$F\% = \frac{AUC_{sample}}{AUC_{oral}} \cdot \frac{Dose_{oral}}{Dose_{sample}} \times 100$$

The PK data was compared for statistical significance by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test using GraphPad Instat software (GraphPad Software Inc., CA, USA).

**Results and Discussions**

Nanoemulsion formulation of aceclofenac was successfully developed and characterized in terms of surface morphology, droplet size, viscosity and refractive index (Shakeel et al., 2007). Plasma concentration of aceclofenac from formulations F1, NG1 and marketed tablet at different time intervals was determined by reported HPLC method (Hinz et al., 2003). The graph between plasma aceclofenac concentration and time was plotted for each formulation (Figure 1). It was seen from Figure 1 that the plasma concentration profile of aceclofenac for F1 and NG1 showed greater improvement of drug absorption than the oral tablet formulation. Peak (maximum) plasma concentration ( $C_{max}$ ) of aceclofenac in F1, NG1 and tablet was  $9.4 \pm 1.1$ ,  $8.8 \pm 0.89$  and  $10.2 \pm 3.4$  µg/ml respectively whereas  $t_{max}$  was  $6 \pm 0.31$ ,



**Figure 1:** Plasma concentration (Mean ±SD) time profile curve of aceclofenac from F1, NG1 and tablet (n = 6).

6 ± 0.34 and 2 ± 0.27 h respectively (Table 2 & Figure 1). AUC<sub>0→t</sub> and AUC<sub>0→∞</sub> in formulations F1, NG1 and tablet were 61.4 ± 2.98, 54.2 ± 2.58 and 20.8 ± 3.5 µg.h/ml respectively and 77.5 ± 3.1, 69.4 ± 2.85 and 29.1 ± 4.2 µg.h/ml respectively (Table 2). These pharmacokinetic parameters (C<sub>max</sub>, t<sub>max</sub>, AUC<sub>0→t</sub> and AUC<sub>0→∞</sub>) obtained with formulations F1 and NG1 were significantly different from those obtained with oral tablet formulation (P<0.05). This indicated that transdermal application can significantly modify pharmacokinetic profile of aceclofenac. The significant AUC values observed with F1 and NG1 also indicated increased bioavailability of the aceclofenac from F1 and NG1 in com-

parison with oral tablet formulation (P<0.05). The K<sub>e</sub> and T<sub>1/2</sub> for F1, NG1 and tablet were found 0.154, 0.152 and 0.159 h<sup>-1</sup> respectively & 4.50, 4.55 and 4.35 h respectively (Table 2). There was no significant variation in K<sub>e</sub> and T<sub>1/2</sub> for F1, NG1 when compared with tablet formulation (P≥0.05). This indicated that transdermal application could not change intrinsic pharmacokinetic parameters such as K<sub>e</sub> and T<sub>1/2</sub>. The formulations F1 and NG1 were found to enhance the bioavailability of aceclofenac by 2.95 and 2.60 folds (percent relative bioavailability 295 and 260) with reference to the oral tablet formulation (Table 2). This increased bioavailability from transdermal formulations (F1

Formulation	t <sub>max</sub> ± SD (h)	C <sub>max</sub> ± SD (µg/ml)	AUC <sub>0→t</sub> ± SD (µg.h/ml)	AUC <sub>0→∞</sub> ± SD (µg.h/ml)	K <sub>e</sub> (h <sup>-1</sup> )	T <sub>1/2</sub> (h)	F %
F1	6* ± 0.31	9.4* ± 1.1	61.4* ± 2.98	77.5* ± 3.1	0.154	4.50	295
NG1	6* ± 0.34	8.8* ± 0.89	54.2* ± 2.58	69.4* ± 2.85	0.152	4.55	260
Tablet	2 ± 0.27	10.2 ± 3.4	20.8 ± 3.5	29.1 ± 4.2	0.159	4.35	---

\* p<0.05 when compared with tablet formulation using one way ANOVA followed by Tukey Kramer multiple comparison test t<sub>max</sub> is time of peak concentration, C<sub>max</sub> is peak of maximum concentration, AUC<sub>0→t</sub> is area under the concentration time profile curve until last observation, AUC<sub>0→∞</sub> is area under the concentration time profile curve from time 0 to infinity, K<sub>e</sub> is elimination rate constant, T<sub>1/2</sub> is elimination half life and F is relative bioavailability

**Table 2:** Relative bioavailability and mean pharmacokinetic parameters (± SD, n=6) of aceclofenac from F1, NG1 and marketed tablet.

and NG1) may be due to the enhanced skin permeation and avoidance of hepatic first pass metabolism of aceclofenac in the form of transdermal formulations.

## Conclusion

These studies revealed significantly greater extent of absorption of aceclofenac than the oral tablet formulation (P<0.05). The absorption of aceclofenac from F1 and NG1 resulted in 2.95 and 2.60 fold increases in bioavailability as compared to the oral tablet formulation. Results of these studies indicate that nanoemulsions can be successfully used for enhancement of bioavailability of aceclofenac.

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