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NOTE

Bioequivalence Study of Aceclofenac Formulations in Animal Model

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A single dose complete crossover study of aceclofenac was carried out in wistar rats. Blood samples were collected after predetermined time intervals. Plasma aceclofenac concentrations were determined using RP-HPLC method. Plasma aceclofenac concentrations as well as other pharmacokinetic parameters obtained were subjected to statistical analysis.

Key Words: Aceclofenac, HPLC, Pharmacokinetics.

Sustained release dosage forms are becoming increasingly important, either to achieve the desired level of therapeutic activity required for a new drug entity or to extend life cycle of an existing drug through improved performance or patient compliance[1]. The drug candidate selected under the present study is aceclofenac, a synthetic NSAID used in treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. It is almost rapidly and completely absorbed from the gastrointestinal tract after oral administration. It is reported to have plasma half life 4 h, time of peak plasma concentration occurs about 1.25-3.00 h after an oral dose. It is reported to have considerable first pass metabolism. Aceclofenac is usually administered as conventional tablet, containing 100 mg, two times daily. These bio- pharmaceutical and physiochemical properties reveal that aceclofenac is an ideal candidate to develop the oral sustained drug delivery system[2]. The short biological half-life (4.0-4.3 h) of the drug and need for chronic therapy make it an ideal candidate for sustained release dosage form.

The aceclofenac sustained release tablets were prepared by wet granulation[4] technique using various ratios of hydroxypropyl methyl cellulose and ethylcellulose polymers with the drug[4]. The drug aceclofenac was passed through sieve No. 40. The release retarding polymer namely hydroxypropyl methyl cellulose, ethyl cellulose and additives namely micro crystalline cellulose and lactose as diluents, propylparaben sodium as preservative, talc and magnesium stearate as glidant and lubricant, respectively were passed through sieve No. 60. Both were mixed well in a mixer. Polyvinylpyrrolidone in isopropyl alcohol was added to the mixture as granulating agent to get coherent mass. The wet granules were dried at room temperature and then the dried granules were passed through sieve No. 14, mixed with talc and magnesium stearate and compressed into tablets on a 16-station rotary cadmach machine using 12/32 punch. The selected matrix tablet (F3) was compared with

marketed sustained release tablet (M1) of aceclofenac to ascertain the bioequivalence of developed product. The experimental protocol has been approved by the institutional animal ethical committee (registration number is 409/01/a/CPCSEA).

The overnight fasted Wistar rats were divided into 3 groups each containing six animals. Group I animals received pure aceclofenac suspended in 0.5 % carboxy methyl cellulose in distilled water given orally at a dose of 10 mg of the drug/kg body weight of animals. Group II animals received formulated granules of aceclofenac sustained release tablet suspended in 0.5 % carboxy methyl cellulose in distilled water given orally at a dose of 10 mg of the drug/kg body weight of animals and group III animals received crushed granules of marketed aceclofenac sustained release tablet suspended in 0.5 % carboxy methyl cellulose in distilled water given orally at a dose of 10 mg of the drug/kg body weight of animals. Blood samples were collected at intervals of 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 12.0 h after postdose into heparinized tubes from the orbital sinus of the animals. The plasma was separated immediately by using cold centrifuge at 3000 rpm for 15 min and the plasma was stored at -20 °C until analysis.

The concentration of aceclofenac in plasma was determined by HPLC method using venlafaxine as internal standard. Chromatographic condition-HPLC system consisted of Shimadzu SPD10ATVP pump and rheodyne injector with 20 µL fixed volume loop and shimadzu SPD10A UV detector controlled by the software kinetica. Separation was carried out at room temperature phenomenax C_{18} (150.0 mm × 4.6 mm with 5 µ particle size) column. The mobile phase was methanol and 0.3 % tri ethyl amine in water (60:40). The detector wavelength was set at 275 nm.

Calibration curve: A series of solutions containing 1, 2, 5, 10, 20, 40, 60 and 80 µg/mL of aceclofenac in diluent (methanol and water mixture in the ratio of 70:30) were prepared and used as a working standard. Further internal standard solution containing 500 µg/mL of venlafaxine was prepared in the diluent. Aliquots of 95 µL of untreated rat plasma were pipetted in to a series of 8 µ centrifuge tubes and spiked with 5 µL of respective working standard solution to get spiked plasma sample containing 50, 100, 250, 500 1000, 2000, 3000 and 4000 ng/mL of aceclofenac, respectively. To each of the spiked plasma sample 25 µL of internal standard and 200 µL of acetonitrile were added and mixed for a minute.

To each of the above solutions 675 µL of diluents was added to make up the volume to 1 mL and vortexed for 1 min. The samples were centrifuged at 10000 rpm for 10 min in a cooling centrifuge at 4 °C. After centrifugation, the supernatant was separated and injected in to the HPLC system. Standard curves were obtained by the least square regression analysis of drug/internal standard peak area ratio as a function of theoretical concentration.

Preparation of sample solutions: To 100 µL of plasma, 25 µL of internal standard solution and 200 µL of acetonitrile were added and mixed for 1 min. To this 675 µL of the diluent was added to make up the volume to 1 mL. The resulting solution was vortexed for 1 min and centrifuged at 10000 rpm for 10 min. The supernatant layer was separated and injected in to the HPLC system.

The concentration of the aceclofenac present in the plasma sample was calculated from the area ratio of sample and standard using the calibration curve. The blank plasma sample was also analyzed prior to the analysis of aceclofenac standard preparations. No interference from the blank plasma was observed in the analysis of the drug. The peaks were well resolved and the retention time of aceclofenac and venlafaxin were found to be 10.05 and 17.82 min, respectively.

Aceclofenac was detectable in plasma within 0.5 h after its oral administration in rats. The absorption was rapid with pure aceclofenac as indicated by low T_{max} value (1 h); whereas the sustained release compositions exhibited delayed absorption as demonstrated by high T_{max} (2 h) values. This delayed absorption may be due to the extended release effect of the swellable hydrophilic polymer present in the matrix tablets which might have increased the viscosity and hence reduced the absorption rate. The C_{max} of marketed tablet and prepared tablet were lower as compared with pure aceclofenac. However the C_{max} of marketed tablet and prepared tablet were found to be superimposable. The half-life of pure aceclofenac was found to be less which specifies the rapid removal of drug from plasma and the rapid elimination of pure drug was further supported by high elimination rate constant. On the contrary, sustained release compositions exhibited high half-life and low elimination rate constant values indicating that drug remains in the body for longer period of time and exhibits prolonged effect. The low value of area under the curve (AUC) observed with pure aceclofenac may be due to its rapid absorption and elimination from the body. On the contrary, the sustained release compositions showed high AUC values indicating increased bioavailability of drug. All these parameters clearly reveal that the sustained release formulation prepared by us exhibited prolonged effect of aceclofenac in rats.

TABLE-1
PHARMACOKINETIC PARAMETERS FROM THE PLASMA CONCENTRATION- TIME CURVE IN RATS

Parameters	ACE	F3	M1
C_{max} (µg/mL)	3.48 ± 0.192	2.15 ± 0.474	2.34 ± 0.259
T_{max} (h)	1.00 ± 0.000	2.00 ± 0.000*	2.00 ± 0.000*
$t_{1/2}$ (h)	3.34 ± 0.984	4.33 ± 1.150	4.08 ± 0.718
AUC_{0-t} (µg-h/mL)	13.63 ± 0.755	17.99 ± 0.602*	18.48 ± 0.956*
Ke (h^{-1})	0.207 ± 0.004	0.160 ± 0.001*	0.170 ± 0.004**

All values are expressed as Mean ± SD of six animals each. ACE = pure aceclofenac; M1 = composition of marketed tablet; F3 = composition of F3 tablet; C_{max} = maximum plasma concentration; T_{max}= time for maximum plasma concentration; $t_{1/2}$= biological half life; AUC = area under the curve; K_e= elimination rate constant. *Significant compared to ACE ($p < 0.05$), **significant compared to M1 ($p < 0.05$).

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