

Research Article

A comparative bioavailability study of aceclofenac products in healthy human subjects

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This study was conducted to compare the bioavailability of two branded formulations of aceclofenac and to evaluate their pharmacokinetic behavior. For bioequivalence study of two formulations of aceclofenac; drug A and drug B were administered to 18 healthy human volunteers using a two-treatment, two-way cross over study design in a randomized fashion. For the determination of aceclofenac plasma concentration, validated HPLC method with UV-visible detector, 20 μ l injecting loop and C18 analytical column were used. The lower limit of detection is 0.0195 μ g/ml and quantitation range is 0.039, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5, 5, 10 and 20 μ g/ml. Different pharmacokinetic parameter were determined including T_{max} , $T_{1/2}$, C_{max} , AUC_{0-t} , $AUC_{0-\infty}$, vd , ke for two formulations of aceclofenac in plasma. After log-transformation of plasma data for bioequivalence C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ were tested. The C_{max} values of $7.69 \pm 0.14221 \mu$ g/ml and $6.82 \pm 0.13411 \mu$ g/ml were attained in 3.14 ± 0.0801 h and 2.94 ± 0.1878 h for drug A and Drug B, respectively. AUC_{0-t} was $45332.79 \pm 2096.770 \mu$ g.h/ml and $43842.56 \pm 1046.954 \mu$ g.h/ml, respectively. $AUC_{0-\infty}$ was $45329.97 \pm 2138.871 \mu$ g.h/ml and $43589.97 \pm 1039.78 \mu$ g.h/ml for drug A and Drug B, respectively. The $t_{1/2}$ values were found to be 3.14 ± 0.080 h and 3.01 ± 0.024 h for drug A and Drug B.

Keywords: Bioequivalence, Pharmacokinetics; HPLC, Immediate Release Aceclofenac.

1. INTRODUCTION

Aceclofenac, [2-(2-, 6-dichlorophenylamino) phenyl] acetoxyacetic acid) is a nonsteroidal anti-inflammatory drug (NSAID) of the phenyl acetic acid type that is structurally related to diclofenac as shown in [Figure 1](#). Chemically it is $C_{16}H_{13}Cl_2NO_4$ with a molecular weight of 354.19 (Martindale, 2002). Aceclofenac is indicated for the acute and chronic treatment of the signs and symptoms of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and scapulohumeral periartthritis (Ballesteros et al, 1990; Honorato et al, 1990). It is also indicated for pain of various etiologies such as musculoskeletal pain (e.g. low back pain), dental pain or postsurgical pain (Ballesteros et al, 1990).

Aceclofenac produces its effects by inhibition of an enzyme (cyclooxygenase) production which is essential for the production of inflammatory process. It selectively blocks the lipoxygenase that inhibit the cox-2 dependant production of prostaglandins (Yong et al, 2005; Chandra et al, 2012). Biotransformation of aceclofenac is carried out by cytochrome P-450 2C9 (CYP2C9) mediated hydrolysis and hydroxylation to diclofenac and 4-hydroxy-aceclofenac respectively (Kang et al, 2008).

After absorption of aceclofenac, the drug is progressively hydrolysed to diclofenac in the circulation, which accounts for 51% of the activity. Aceclofenac is metabolized into a large number of compounds; the most important metabolite is 4-hydroxyaceclofenac (Bubani, 1988). It has good analgesic, potent anti-inflammatory and antipyretic (Movilia,

1989; Yong et al., 2005; Naz et al., 2011; Yadave et al., 2009).

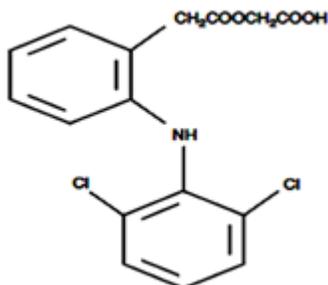


Figure 1: Chemical structure of Aceclofenac

Aceclofenac is absorbed rapidly as unchanged drug when taken orally, and its analgesic effect can begin within 30min of ingestion (Honorato et al., 1990).

It reaches a peak plasma concentration 1–3h after ingestion (Martindale, 2002). C_{max} and AUC increase proportion-ally in the dose range 50–150mg (Honorato et al., 1990). When aceclofenac is administered to fasting and fed healthy volunteers, only the rate but not the extent of aceclofenac absorption is affected by the presence of food in the gastrointestinal tract. The mean plasma elimination half-life is approximately 4h; parent compound and its metabolites are eliminated primarily (66%) in the urine and to a lesser extent in the faeces (Honorato et al., 1990; Martindale, 2002).

A new, simple, fast and validated HPLC method was developed for the study of bioequivalence of two commercially available brands of Aceclofenac 100mg tablets. The aims and objectives of this study was to develop and standardize an HPLC-UV method for analysis of Aceclofenac in plasma and to determine bioavailability and bioequivalence of two marketed brands of Aceclofenac 100mg tablets in healthy human volunteers.

2. MATERIAL AND METHODS

2.1 Study Design and Selection of Subjects

The study was a single dose, randomized with single treatment and was completed in a period of treatment single dosing. Clinically there was no any major medical history of any volunteer and they were informed not to take any

medication for at least 2 weeks before the study. Awareness about the aim of the study was clear among all volunteers. Before the start of study written informed consent was obtained from each volunteer. Eighteen healthy human volunteers were scheduled to participate in the study. Each volunteer was given single dose of aceclofenac 100 mg immediate release formulation in tablet dosage form orally. Subjects with any disorder or illness were not included in this study.

2.2 Experimental Design for Drug Administration

18 healthy human volunteers were selected for bioequivalence study of two formulations of aceclofenac drug; drug A (Acemed®) and drug B (Airtal®). Volunteers were randomly divided into two groups and each group was contained 9 subjects. One group received drug A and second group received drug B with 250ml of water after an overnight fast (12 hours) by using a two-treatment, two-way cross over study design in a randomized fashion. A one week washout period was given between dosing plan. After the 6 hours of dosing breakfast was given to each subject. Breakfast, lunch and dinner were provided to the subjects according to the schedule. Each subject receives similar type of food.

2.3 Blood Sampling

The blood samples were collected by using intravenous route. A 22 gauge cannula was inserted into vein of forearm and blood is drawn by using disposable syringes at zero time before drug was given. In the same way blood samples were drawn at 0.5,1,2,3,4,5,6,8,10,12 and 24 hours after the administration of 100mg aceclofenac immediate release tablet. 5ml blood samples were collected each time and put the samples in labeled disposable plastic centrifuge tubes containing 100ul of heparin as anticoagulant. Immediately after pouring the blood samples into these tubes, blood samples were centrifuged at 5000 rpm for 10 minutes. The clear upper plasma layer was collected to the labeled sterile glass vials, capped them tightly and stored it immediately at -20C0 until assay. Same procedure was repeated after the 7 days of washout period to complete the crossover design.

2.4 Chromatographic Conditions

Sykam GmbH HPLC system (Germany) equipped with Sykam S2100 solvent delivery system, Sykam 4011 thermo controller, Sykam S3210 UV/VIS detector and Clarity operating software (MS-Windows) was used to control and operate the instrument.

For LC Column and Pump different parameters were used. The mobile phase consisted of 53:24:23 mixture of 0.025M Na₂HPO₄, methanol and acetonitrile, respectively. The pH of mobile phase was adjusted at 7 with 1:1 H₃PO₄ solution at 25°C. The mobile phase was filtered through 0.045µm membrane filter paper and sonicated to degas it. Fresh mobile phase was used and prepared daily (Rhim et al., 2008). Column used was ODS-C18, Flow rate was kept as 1 ml/min. Detection was performed at 278nm. Internal standard used was diclofenac potassium. The blank plasma samples were analyzed before the analysis of test and standard samples. The response factor was measured by the ratio of the peak height of drug to that of the internal standard for both standard and test samples. The peaks obtained were well defined and the retention times for aceclofenac and internal standard (diclofenac) was 5.2 ± 0.4 min and 4.1 ± 0.2 min, respectively.

On the basis of chromatographic conditions prescribed above, the LOD and LOQ values of Aceclofenac were 0.019µg/ml and 0.312µg/ml, respectively. A linear relationship was found between concentration and the peak height ratio in the range of 0.312-20 µg/ml.

2.5 Evaluation of Pharmacokinetic Parameters

Thermo Kinetica" version 4.4.1 was used for pharmacokinetic analysis by using non-compartmental method developed by "Thermo Electron Corporation" and by "Microsoft Excel" of "Microsoft® Corporation". Linear trapezoidal rule was used to calculate the area under time concentration curve (AUC_{0-∞}) by using the same software. Maximum aceclofenac concentration in plasma (C_{max}) and maximum time to these concentrations (T_{max}) were determined by plasma concentration versus time profiles. Elimination half-life (t_{1/2}) was also calculated.

2.6 Statistical Methods

Standard error of mean (SEM), standard deviation (SD) and Mean were determined by using descriptive statistical formulas either by Kinetica 4.4.1 or MS Excel. Pharmacokinetic parameters such as AUC_{0-t}, AUC_{0-∞}, T_{max}, C_{max}, t_{1/2}, V_d, K_e, Cl and V_{ss} were calculated by using kinetic for the purpose of bioequivalence analysis.

3. RESULTS AND DISCUSSION

The study was a randomized, two treatments, two way crossover design, where Aceclofenac in a dose of 100 mg of Product A was administered to Group A and Aceclofenac 100mg of Product B was administered to Group B. After a wash out period of 1 week, Group A received Product B and Group B received Product A. The Mean ± SEM (n=18) bioavailability and pharmacokinetic parameters of Aceclofenac Product A and B are shown in [Table 1](#).

For the development of HPLC-UV method for the analysis of Aceclofenac, different mobile phases with different compositions, pH, flow rates and different stationary phases (i.e. columns) were used to develop and optimize a new, simple and fast method.

The objectives of the study were to develop, standardize a new HPLC-UV analytical method to study in vivo pharmacokinetic parameters and bioequivalence of two commercially available tablet dosage forms containing Aceclofenac as active pharmaceutical ingredient. The validation of analytical method was carried out according to FDA guidelines for bio-analytical method validation. Aceclofenac is safest drug among NSAID, as there was no any harmful effects were observed in all volunteers till the end of study. Both products are readily absorbed from the gastrointestinal tract and the concentration time profile of both products shows that both products are bioequivalent. After the administration of product A and product B, the peak concentration obtained were 7.69 ± 0.36 µg/ml and 6.82 ± 0.13 µg/ml for aceclofenac at 3.0 ± 0.14 h and 2.94 ± 0.19 h respectively. Clearance is one of the parameter that determines the maintenance dose rate required to achieve a target plasma concentration and therefore, effect at steady state. The total body clearance (Cl_T) (Mean ±

SEM) values of drug A (Acemed® and drug B (Airtal®) were 2.27 ± 0.10 L/h and 2.18 ± 0.06 L/h. The value of total body clearance has not been reported in literature. elimination rate constant (Ke) (Mean \pm SEM) of drug A (Acemed®) and drug B (Airtal®) was found as 0.22 ± 0.004 h⁻¹ and 0.23 ± 0.002 h⁻¹, respectively. As F- value for Relative Bioavailability or Comparative bioavailability lies between 0.8 and 1.25 i.e. 1.04, hence both

products of Aceclofenac are bioequivalent (Bourne, 2002).

After log-transformation of the data, there was no significant difference observed statistically between the two products of aceclofenac. 90% confidence intervals shows that the ratios of AUC_{0-∞}, AUC_{0-t}, (C_{max}) of two aceclofenac products lie in the range of 80%-125% that is acceptable according to the FDA guideline

Table 1: Comparison of Mean \pm SEM (n=18) bioavailability and pharmacokinetic parameters of Aceclofenac Product A and B administered in a dose of 100 mg

S. No.	Bio-parameters	Product A	Product B	P-Value
1	AUC _{0-∞} µg. h ² /ml	45329.97 \pm 2138.87	43589.97 \pm 0.1842	P = 0.250
2	AUC _{0-t} µg. h/ml	45332.79 \pm 2096.77	43842.56 \pm 1048.95	P = 0.285
3	C _{max} µg/ml	7.69 \pm 0.36	6.82 \pm 0.13	P = 0.08
4	T _{max} (hr.)	3.0 \pm 0.14	2.94 \pm 0.19	P = 0.369
5	MRT (hr.)	5.75 \pm 0.08	5.76 \pm 0.06	P = 0.489
6	Vd L/Kg	10.10 \pm 0.52	9.97 \pm 0.22	P = 0.413
7	t _{1/2} (h)	3.14 \pm 0.08	3.01 \pm 0.024	P = 0.065
8	Ke 1/h	0.22 \pm 0.005	0.23 \pm 0.002	P = 0.045
9	Cl mL/min/Kg	2.27 \pm 0.10	2.18 \pm 0.06	P = 0.190
10	Ka 1/h	9.29 \pm 0.04	9.28 \pm 0.02	P = 0.374

4. CONCLUSION

All Pharmacokinetic parameters have similar values for Aceclofenac 100mg tablet Acemed and Airtal. Therefore, on the basis of above study it is

concluded that both Aceclofenac 100mg drug A (Acemed®) and drug B (Airtal®) is bioequivalent. Both products have the same efficacy and can be used alternative to each other.

Conflict of Interests

Authors declared no competitive interests for the presented work.

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