Drug Development and Industrial Pharmacy, 32:1219–1225, 2006

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040600608805

informa
healthcare

Evaluation of Bioequivalence of Two Formulations Containing 100 Milligrams of Aceclofenac

K. V. Gowda, D. S. Rajan, U. Mandal, P. S. Selvan, W. D. Sam Solomon, A. Bose, A. K. Sarkar and T. K. Pal
Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India

T. K. Chattaraj
Department of Pharmacology, N. R. S. Medical College & Hospital, Kolkata, India

ABSTRACT The bioequivalence of two oral formulations containing aceclofenac 100 mg was determined in 24 healthy Indian male volunteers. The study was designed as a single dose, fasting, two-period two-sequence crossover study with a washout period of 1 week. The content of aceclofenac in plasma was determined by a validated HPLC method with UV detection. The preparations were compared using the parameters area under the plasma concentration–time curve (AUC_{0-t}), area under the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$), peak plasma concentration (C_{max}), and time to reach peak plasma concentration (t_{max}). No statistically significant difference was observed between the logarithmic transformed $AUC_{0-\infty}$ and C_{max} values of the two preparations. The 90% confidence interval for the ratio of the logarithmic transformed AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were within the bioequivalence limit of 0.80–1.25.

KEYWORDS Bioequivalence, Aceclofenac, Pharmacokinetics, HPLC

INTRODUCTION

Aceclofenac is a phenylacetic acid derivative with anti-inflammatory and analgesic properties similar to those of diclofenac. Aceclofenac is an oral non-steroidal anti-inflammatory (NSAID) that is effective in the treatment of painful inflammatory diseases and has been used to treat more than 75 million patients worldwide. The preclinical studies suggest that the potential of aceclofenac to cause gastrointestinal damage is less than that of diclofenac. The analgesic efficacy of aceclofenac 100 mg is more prolonged than that of paracetamol (acetaminophen) 650 mg (Brogden & Wiseman, 1996). It has proved as effective as diclofenac, naproxen, and piroxicam in patients with osteoarthritis, diclofenac, ketorolac, tenoxicam, and indomethacin in patients with rheumatoid arthritis, and tenoxicam, naproxen, and indomethacin in patients with ankylosing spondylitis. It also provides effective analgesia in other indications, such as dental or gynecological pain, lower back pain, and ear, nose, and throat indications. Aceclofenac appears to be particularly well-tolerated among the NSAIDs, with a lower incidence of gastrointestinal adverse effects. This

Address correspondence to T. K. Pal, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032, India; E-mail: tkpal_12@yahoo.com

good tolerability profile results in a reduced withdrawal rate and hence greater compliance with treatment (Legrand, 2004).

Aceclofenac is metabolized in human hepatocytes and human microsomes to form [2-(2′,6′-dichloro-4′-hydroxyphenylamino)phenyl]acetoxyacetic acid as the major metabolite, which is then further conjugated. Minor metabolites were [2-(2′,6′-dichlorophenylamino)-5-hydroxyphenyl]acetoxyacetic acid and [2-(2′,6′-dichlorophenylamino)phenyl]acetic acid, as well as the hydroxylated derivatives [2-(2′,6′-dichloro-4′-hydroxyphenylamino)phenyl]acetic acid and [2-(2′,6′-dichlorophenylamino)-5-hydroxyphenyl]acetic acid (Bort et al., 1996).

Several HPLC methods have been published for the individual determination of aceclofenac (Zawilla et al., 2002) and diclofenac (Hanses et al., 1995) or for the simultaneous analysis of aceclofenac and diclofenac (Lee et al., 2000) and diclofenac and its monohydroxylated metabolites (Lansdrop et al., 1990), respectively. Hasan et al. (2003) have described about five various methods for the determination of aceclofenac in the presence of its degradation product diclofenac.An HPLC method for the determination of aceclofenac and three of its metabolites in human plasma was also developed (Hinz et al., 2003). Two simple, sensitive, and reproducible spectrophotometric and spectrofluorometric methods were adopted for the analysis of the anti-inflammatory drugs, etodolac and aceclofenac (El Kousy et al., 1999).

Bioequivalence of two formulations of the same drug comprises equivalence with respect to the rate and extent of absorption while the area under concentration time curve (AUC) generally serves as the characteristic of the extent of absorption (Hauschke et al., 1990; Schulz and Steinijans, 1992). No single parameter reliably measures the rate of absorption; for instance, the maximal drug concentration (C_{max}) has been widely used, but it depends more on the fraction absorbed than the rate of absorption; the time of maximal concentration (t_{max}) depends on both absorption and elimination rates (Farolfi et al., 1999). However, it is essential that the generic preparations are proven to be bioequivalent to the reference preparation before they can be safely used as a substitute for the latter.

Therefore, in the present study, the bioavailability of a local generic preparation of aceclofenac was evaluated with the reference preparation (Hifenac®, Intas Laboratories, Pvt. Ltd., Ahmedabad, India). An attempt was made to study the pharmacokinetics of aceclofenac in the local population of Indian origin.

MATERIALS AND METHODS

Products Studied

Test product: aceclofenac 100 mg tablet (batch No. AF-004, expiry 04/2006, from Aeon Therapeutics, Pvt. Ltd., Chennai, India).

Reference product: Hifenac® (batch No. D-4282, expiry 12/2005, from Intas Laboratories, Pvt. Ltd., Ahmedabad, India).

Study Design

Twenty-four Indian male volunteers aged between 18 and 45 years (21.2 ± 1.5 years) and with body mass index between 18 and 24 (22.5 ± 1.6) were included in a randomized, single dose, fasting, two-period, two-sequence crossover study with a 1-week washout period. Informed consent was obtained from all the volunteers prior to the start of the study. Various physical, biochemical, and hematological tests were carried out before enrolling the volunteers for the study. Approval from the Drugs Control General of India (DCGI) and Institutional Ethical Committee of Jadavpur University was obtained prior to the start of the study.

Drug Administration and Sample Collection

The study was a single dose, fasting, two period, two-way crossover study with a washout period of 1 week. All the volunteers assembled in the Clinical Pharmacology Unit (CPU) ward at 6 AM on the study day of each session, after overnight fasting of 10 h. Their total pulse rate, blood pressure, was recorded. The subjects received either of the study preparations. According to U.S. Food and Drug Administration (FDA) and European Agency for the Evaluation of Medicinal Products (EMEA) regulations, the sampling schedule should be planned to provide a reliable estimate of the extent of absorption (US FDA, 2000; CPMP, 1991). This is generally achieved if AUC_{0-t} is at least 80% of $AUC_{0-\infty}$. Usually the sampling time should extend to at least three terminal elimination half-lives of the active ingredient. Time periods between sampling should not exceed one terminal half-life (Nation & Sansom, 1994). A total of 15 blood samples were collected at 0 h (before drug administration) and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0,

8.0, 10.0 12.0, 24.0 h (after drug administration) in the test tubes with EDTA at each time point. Breakfast, lunch, and dinner were provided after 3 h, 6 h, and 13 h, respectively, after drug ingestion. Collected blood samples were centrifuged immediately, plasma was separated and stored frozen at –20°C with appropriate labeling of volunteer code number, study date, and collection time, till the date of analysis.

Sample Preparation

A total of 1.0 mL of plasma was taken in a stoppered test tube. To this 0.5 mL of dilute hydrochloric acid was added and mixed for 1 min. Now to this mixture 8 mL of dichloro methane was added and stirred for 10 min with the help of a mechanical stirrer. It is then centrifuged for 5 min at 4000 rpm; 7 mL of organic layer was removed in a separate tube and evaporated in the presence of N_2 atmosphere at low temperature. The residue was reconstructed with a 0.2-mL mobile phase. The same was injected into the HPLC system.

Chromatographic Conditions

Plasma samples were analyzed for aceclofenac by using HPLC. All solvents used were of HPLC grade and were purchased from S.d. Fine Chemicals, Mumbai, India, whereas other chemicals and reagents were of analytical grade. Aceclofenac was obtained from Aeons Therapeutics, Pvt. Ltd., Chennai, India.

The HPLC system was Knauer GmbH, Berlin, Germany, and it consisted of a solvent delivery pump (K-1001), a Rheodyne injector and an UV-visible detector (K-2501). Integration was done using Eurochrom 2000 software. Chromatogram separation was done using a BDS Hypersil C18 (250 × 4.6 mm, 5 µm particle size) column. The mobile phase consisted of acetonitrile and water (pH adjusted to 3.0 with glacial acetic acid) in the ratio of 50:50 v/v and eluted at a flow rate of 1 mL/min. The effluent was monitored using UV detection at 280 nm. The method was as per the following guidelines (Shah et al., 1992).

Pharmacokinetic Analysis

The following pharmacokinetic parameters were directly determined or calculated by the standard non-compartmental method. Both maximum plasma concentration (C_{max}) and time to peak plasma concentration (t_{max}) were obtained directly from the data. The elimination half-life ($t_{1/2}$) was calculated as $0.693/K_e$, where K_e is the apparent elimination rate constant. K_e was, in turn, calculated as the slope of the linear regression line of natural log-transformed plasma concentrations. The last seven quantifiable levels were used to determine K_e. The area under the plasma concentration–time curve (AUC_{0-t}) was calculated from the measured levels, from time zero to the time of last quantifiable level, by the linear trapezoidal rule. $AUC_{0-\infty}$ was calculated according to the following formula: $AUC_{0-\infty} = AUC_{0-t} + C_{last}/K_e$, where C_{last} is the last quantifiable plasma level.

Statistical Analysis

An analysis of variance was performed on the pharmacokinetic parameters C_{max}, $AUC_{0-\infty}$ using general linear model (GLM) procedures in which sources of variation were subject, formulation, period. Differences in t_{max} were assessed by the nonparametric Wilcoxon signed rank test at the 5% level of significance. The 90% confidence interval (CI) of the test/reference ratios for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ (log transformed) were determined. Bioequivalence between the two formulations can be concluded when the 90% CIs for the pharmacokinetic parameters of the two products are found within the acceptable range of 80–125%.

RESULTS

In the HPLC method, no interferences were observed in human plasma (Fig. 1). The retention time for aceclofenac was 7 min. The limit of quantification for aceclofenac in plasma was 0.1 µg/mL. The relationship between concentration and peak area was found to be linear within the range of 0.1 to 10 µg/mL. Quality control points at low, medium, and high levels (0.25, 5, and 9 µg/mL) were used to determine absolute recovery and within-day and between-day precision and accuracy. Stability, limit of quantification, and selectivity were also evaluated. The within-day and between-day precision and accuracy data are summarized in Table 1.

Average concentration versus time curves after administration of reference and test products to healthy

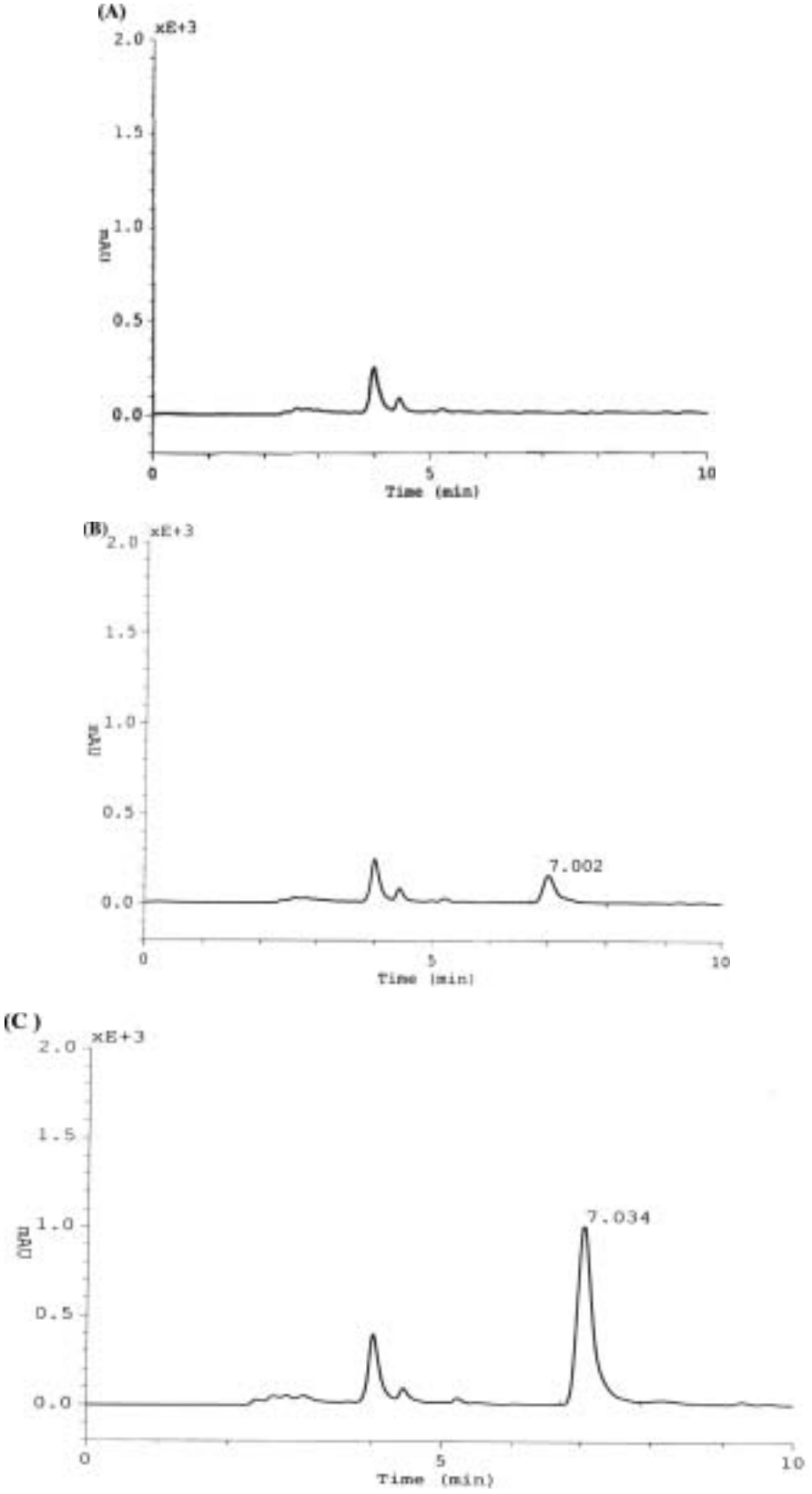

FIGURE 1 **Chromatograms of (A) Blank Plasma, (B) Blank Plasma Spiked With 0.1 μg/mL of Aceclofenac, (C) Volunteer Plasma Containing 1.1 μg/mL of Aceclofenac at 0.5 h After Administration of 100-mg Tablet of Aceclofenac. (A) Peak of Blank Plasma Is Seen at Retention Time of 4.0 Min. (B) Retention Time of Aceclofenac= 7.002 Min; Peak of Blank Plasma Is Seen at Retention Time of 4.0 Min. (C) Retention Time of Aceclofenac= 7.03 Min; Peak of Blank Plasma Is Seen at Retention Time of 4.0 Min.**

TABLE 1 **Within-Day and Between-Day Precision and Accuracy of HPLC Method**

Concentration (μg/mL)	Within day (n= 5)		Between day (n= 5)	
	Accuracy (%)	Precision CV (%)	Accuracy (%)	Precision CV (%)
0.25	99.15	7.58	98.65	9.55
5	96.55	6.86	97.55	7.85
9	97.52	4.91	98.98	10.25

n= 5: Mean value obtained after 5 determinations. CV= Coefficient of variation expressed as %.

volunteers are shown in Fig. 2. The original 24 volunteers concluded the study. Table 2 summarizes the demographic and mean health parameters of all the participants. Average values of pharmacokinetic parameters after administration of reference and test product to healthy volunteers are summarized in Table 3. The limits of the 90% CIs for the ratios of AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} for their log-transformed data fell within 0.80 to 1.25 (Table 3). Nonparametric analysis according to the Wilcoxon signed rank test did not show any statistically significant differences between test and reference formulations ($P > 0.05$). The observed t_{max} values for test formulation were within the acceptable limits (±20% of the mean reference values).

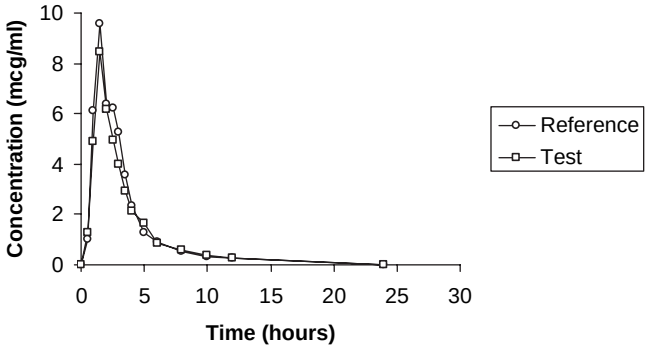

FIGURE 2 **Plasma Concentration–Time Profiles After Administration of Test and Reference Products in Healthy Indian Male Volunteers.**

-○- is Reference Formulation Graph and -□- is Test Formulation Graph Obtained by Plotting Time on X-Axis and Plasma Concentration in Microgram per Milliliter on Y-Axis.

TABLE 2 **Demographic and Health Parameters of Healthy Volunteers Considered in the Bioequivalence Study**

	Age (yr)	Weight (kg)	Height (cm)	BMI (kg/m^2)
Mean	23.5	59.8	161	21.85
SD	4.20	5.66	0.08	3.52

SD= Standard deviation; BMI= body mass index.

DISCUSSION

The described analytical method used for measurement of aceclofenac was shown to be accurate and sensitive. An internal standard was not used in the study. The linearity achieved for this assay (0.1 to 10 μg/mL) effectively covers the therapeutic range, and the linearity was assessed by the graph of peak height versus concentration of aceclofenac. The run time was 10 min and the retention time of aceclofenac was 7.0 min (Fig. 1). The peak of aceclofenac was well resolved (Fig. 1). Table 1 shows the data of between-day and within-day precision and accuracy. The high recovery of extraction (95.8%) with the proposed method allows the nonuse of internal standard without compromising precision and accuracy.

Throughout the stability tests, aceclofenac proved stable in biological samples for at least two freeze and thaw cycles with a final mean recovery of 95.18% and a coefficient of variation (CV) of 6.8%. Aceclofenac in plasma was stable at room temperature for at least 6 h. The limit of quantification was 0.1 μg/mL with CV of 4.2%.

It can be observed from Table 2 that the volunteers formed a homogeneous population in terms of age, weight, height, and body mass index. Aceclofenac was well tolerated by all the volunteers. No adverse events were reported and there were no dropouts. Gastrointestinal disorders, the most common adverse effect associated with the use of NSAIDs were not reported.

TABLE 3 **Pharmacokinetic Parameters of Both Brands of Aceclofenac Tablets**

Parameter	Test	Reference	90% CI
AUC_{0-24} (μg/mL.h)	27.523 ± 4.0	28.459 ± 2.10	89.73–102.69
$AUC_{0-\infty}$ (μg/mL.h)	28.642 ± 3.705	29.627 ± 2.074	90.35–102.28
C_{max} (μg/mL)	9.019 ± 0.616	9.455 ± 0.474	89.99–100.90
t_{max} (h)	1.708 ± 0.257	1.750 ± 0.544	
$t_{1/2}$ (h)	2.826 ± 0.762	3.056 ± 0.673	

AUC_{0-t}, $AUC_{0-\infty}$ C_{max}, t_{max}, and $t_{1/2}$ are the area under the plasma concentration–time curve up to 24 h, area under the plasma concentration–time curve up to infinity, maximum plasma concentration, time to reach maximum plasma concentration, and half-life of the drug, respectively.

The elimination half-life of aceclofenac was in the range 2–3 h. Thus, the washout period of 1 week was sufficient due to the fact that no sample prior to administration in phase 2 showed any aceclofenac levels. Time to reach maximum plasma concentration (t_{max}) was observed at 1.70 h after drug administration, and the last samples were sufficient for calculating at least 80% of $AUC_{0-\infty}$. All calculated pharmacokinetic parameters summarized in Table 3 agree with the previously reported values (Najib et al., 2004). C_{max} levels were observed after 1.70 ± 0.257 h (test) and 1.75 ± 0.544 h (reference). The C_{max} and $AUC_{0-\infty}$ of the test and reference were 9.019 ± 0.616 versus 9.455 ± 0.474 μg/mL and 28.642 ± 3.705 versus 29.627 ± 2.074 μg/mL.h. The mean $t_{1/2}$ values for the test and reference were 2.826 ± 0.762 h and 3.056 ± 0.673 h, respectively. The relative bioavailability between test and reference was 0.95.

As can be seen from Table 3, 90% CIs for all the compared pharmacokinetic parameters (ratios of AUC_{0-t}, $AUC_{0-\infty}$, C_{max}) were obtained within the range of 80–125%. Moreover the analysis of variance for all the analysed parameters showed no significant differences.

The aim of the bioequivalence trials is to assure interchangeability between an innovator and a generic formula in terms of efficacy and safety. When a pharmacological effect is difficult to measure, the plasma levels of a drug may be used as an indirect indicator of clinical activity. Therefore, the aceclofenac plasma levels obtained in this study suggest an equal clinical efficacy of the two brands tested and provide pharmacokinetic data from an Indian population.

CONCLUSION

The 90% CI of $AUC_{0-\infty}$ and C_{max} ratios of aceclofenac of these two preparations were in the acceptable range. Both formulations were equal in terms of rate and extent of absorption. Consequently, bioequivalence between two formulations can be concluded.

ACKNOWLEDGEMENTS

The authors are thankful to Aeon Therapeutics, Pvt. Ltd., Chennai, India, for sponsoring the present study and DST (PRDSF) for providing us the necessary instrumental facilities.

REFERENCES

Bort, R., Ponsoda, X., Carrasco, E., Gomez-Lechon, M. J., & Castell, J. V. (1996). Metabolism of aceclofenac in humans. *Drug Metab. Dispos., 24*, 834–841.

Brogden, R. N., & Wiseman L. R. (1996). Aceclofenac: a review of its pharmacodynamic properties and therapeutic potential in the treatment of rheumatic disorders and in pain management. *Drugs, 52*, 113–124.

Committee for Proprietary Medicinal Products (CPMP). (1991). *Note for Guidance*: *Investigation of Bioavailability and Bioequivalence*. London: Working Party on the Efficacy of the Medicinal Products.

El Kousy, N. M. (1999). Spectrophotometric and spectrofluorimetric determination of etodolac and aceclofenac. *J. Pharm. Biomed. Anal., 20*, 185–194.

Farolfi, M., Power, J. D., & Rescigno, A. (1999). On the determination of bioequivalence. *Pharmacol. Res., 39*, 1–4.

Hanses, A., Saphn-Langguth, H., & Mutschler, E. (1995). A new rapid and sensitive high performance liquid chromatographic assay for diclofenac in human plasma. *Archiv der Pharmazie, 328*, 257–260.

Hasan, N. Y., Abdel-Elkawy, M., Elzeany, B. E., & Wagieh, N. E. (2003). Stability indicating methods for the determination of aceclofenac. *Farmaco, 58*, 91–99.

Hauschke, D., Steinijans, V. W., & Diletti, E. (1990). A distribution free procedure for the statistical analysis of bioequivalence studies. *Int. J. Clin. Pharmacol. Ther. Toxicol., 28*, 72–78.

Hinz, B., Auge, D., Rau, T., Rietbrock, S., Brune, K., & Werner, U. (2003). Simultaneous determination of aceclofenac and three of its metabolites in human plasma by high-performance liquid chromatography. *Biomed. Chromatogr., 17*, 268–275.

Lansdrop, D., Janssen, T. J., Guelen, P. J. M., & Vree, T. B. (1990). High performance liquid chromatographic method for the determination of diclofenac and its hydroxy metabolites in human plasma and urine. *J. Chromatogr., 528*, 487–494.

Lee, H. S., Jeong, C. K., Choi, S. J., Kim, S. B., Lee, M. H., Ko, G. I., & Sohn, D. H. (2000). Simultaneous determination of aceclofenac and diclofenac in human plasma by narrowbore HPLC using column-switching. *J. Pharm. Biomed. Anal., 23*, 775–781.

Legrand E. (2004). Aceclofenac in the management of inflammatory pain. *Expert Opin. Pharmacother., 5*, 1347–1357.

Najib, N., Idkaidek, N., Beshtawi, M., Bader, M., Admour, I., Alam, S., Zaman, Q., & Dham, R. (2004). Bioequivalence evaluation of two brands of aceclofenac 100 mg tablets (Aceclofar and Bristaflam) in healthy human volunteers. *Biopharm. Drug. Dispos., 25*, 103–108.

Nation, R. L, & Sansom, L. N. (1994). Bioequivalence requirements for generic products. *Pharmacol. Ther., 62*, 41–55.

Schulz, H. U., & Steinijans, V. W. (1992). Striving for standards in bioequivalence assessment: a review. *Int. J. Clin. Pharmacol. Ther. Toxicol., 30*, S1–S6.

Shah, V. P., Midha, K. K., & Sighe, S. (1992). Analytical method validation: bioavailability, bioequivalence and pharmacokinetic studies. *Eur. J. Drug Metab. Pharmacokin., 16*, 249–255.

U.S. Food and Drug Administration. (2000). *Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products—General Considerations*. Rockville, MD: Center for Drug Evaluation and Research.

Zawilla, N. H., Mohammad, M. A., El Kousy, N. M., & El-Moghazy Aly, S. M. (2002). Determination of aceclofenac in bulk and pharmaceutical formulations. *J. Pharm. Biomed. Anal., 27*, 243–251.