American Journal of
Bioavailability & Bioequivalence

Research Article

Bioequivalence of two Oral Formulations of Etoricoxib 60 mg Tablets in Healthy Mexican Adults

Araceli G. Medina-Nolasco[1], Karina L. Ortiz-Campos[1], Ericka Lopez-Bojorquez[2], Miguel Angel Arellano-Ibañez[2], Victoria Burke-Fraga[2], and Mario Gonzalez-de la Parra[2]*

[1]*Investigacion Farmacologica y Biofarmaceutica, S.A.P.I. de C.V., Mexico City, Mexico*

[2]*Biokinetics, S. A. de C. V., Mexico City, Mexico*

***Address for Correspondence:** Mario Gonzalez-de la Parra, Privada Jesus del Monte No.77, Col. Cuajimalpa, 05000 Mexico City; Mexico, ORCID: orcid.org/0000-0003-1349-8955, E-mail: mdelaparra@biokinetics.com.mx/ mariobiokinetics@gmail.com

Submitted: 13 April 2018; **Approved:** 15 May 2018; **Published:** 19 May 2018

Citation this article: Medina-Nolasco AG, Ortiz-Campos KL, Lopez-Bojorquez E, Arellano-Ibañez MA, Gonzalez-de la Parra M, et al. Bioequivalence of Two Oral Formulations of Etoricoxib 60 mg Tablets in Healthy Mexican Adults. Am J Bioavailab Bioequiv. 2018;1(1): 010-014.

ABSTRACT

Etoricoxib is a potent selective Cyclo-oxygenase 2 inhibitor with anti-inflammatory and analgesic properties. Here we investigate the bioavailability and the bioequivalence of a test formulation containing 60 mg of etoricoxib with respect to the corresponding reference drug formulation, which was administered as a tablet. A single-dose randomized, open-label, two-sequence, two-period crossover design under fasting conditions with a 12-day washout interval between the two periods was used.

Samples were drawn at baseline and then at 0.25, 0.5, 0.75, 1.00, 1.25, 1.50, 2.00, 3.00, 4.00, 6.00, 8.00, 12.00, 24.00, 36.00, 48.00 and 72.00 hours after administration.

The 90% CIs for etoricoxib C_{max}, and truncated AUC_{0-72} were 99.55% to 119.33%, and 95.97% to 103.06%, respectively. The 90% CIs of the geometric mean ratios of the two parameters fell within the predetermined range of 80% to 125%. Therefore, these results indicate that the bioequivalence criteria were satisfied.

Keywords: Etoricoxib; Bioavailability; Bioequivalence

INTRODUCTION

Etoricoxib is a potent selective Cyclo-oxygenase 2 inhibitor with anti-inflammatory and analgesic properties. The therapeutic indications of etoricoxib in different countries include treatment for the signs and symptoms of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, acute gouty arthritis, chronic low back pain, acute pain, chronic musculoskeletal pain and primary dysmenorrhoea [1-4].

The pharmacokinetics of etoricoxib appears to be linear over a dose range from 5 to 120 mg [5]; its absolute bioavailability has been estimated to be near to 100% [1,6].

The elimination half-life of etoricoxib has been estimated (harmonic mean) to be 24.8 hours [7]. Etoricoxib elimination occurred primarily through metabolism followed by renal excretion [4].

In vitro studies in human liver microsomes have indicated that etoricoxib is metabolized *via* oxidative pathways by cytochromes P450 [1,7].

The single-dose oral pharmacokinetics of etoricoxib are independent of food because the extent of absorption remained intact. Thus, etoricoxib may be taken with or without food [4].

Etoricoxib is available at the strengths of 30, 60, 90 and 120 mg [4]. The sponsor of this study (Laboratorios Liomont, S.A. de C.V., Mexico City. Mexico) wished to obtain the marketing authorization for etoricoxib 60 mg in tablet formulation in Mexico.

Therefore, the aim of this study was to assess the bioavailability and the bioequivalence of a test formulation containing 60 mg of etoricoxib compared with the corresponding reference drug formulation.

A literature survey using PubMed, MEDLINE and Google Scholar data through March of 2018 yielded no matches for the following terms: etoricoxib, 60 mg, bioequivalence, bioavailability, pharmacokinetics, tablets, Mexico, Mexican and population.

METHODS

Formulations

The test formulation consisted of Doscoxel® tablets containing 60 mg of etoricoxib, manufactured by Laboratorios Liomont, S.A. de C.V. (Mexico). The lot number was 298G0039, and the expiration date was November 30, 2018. The reference formulation consisted of Arcoxia® tablets containing 60 mg of etoricoxib, manufactured by Frosst Iberica; S.A. (Spain) and distributed by Schering-Plough, S. A. de C. V. (Mexico). The lot number was L045340, and the expiration date was May 31, 2017.

Ethical considerations

An independent ethics and research committee (Committee of Ethics in Research and Committee of Investigation of Arete and Projects and Administration) reviewed and approved the study protocol (ETR-03-LIO) and the informed-consent documents on June 29, 2016. This investigation was authorized by the Federal Commission for Protection against Sanitary Risks (Comision Federal para la Protection contra Riesgos Sanitarios (COFEPRIS)) on August 9, 2016.

The study was conducted according to the Declaration of Helsinki (and its amendments) and the International Conference on Harmonization for Good Clinical Practice Guideline. The principal investigator informed the participants of the anticipated risks and potential discomfort associated with the study drug, the procedures and the duration of the study. All of the subjects gave written informed consent prior to the initiation of the study. The clinical stage of the study was conducted in May of 2017.

Subjects

Healthy Mexican adults of both genders with ages between 18 and 55 years were considered to be eligible for this study. The health of each candidate was evaluated. This evaluation included an interview and a physical examination of vital signs, blood pressure, heart rate, temperature, 12-lead electrocardiogram and chest radiography. Furthermore, laboratory tests (hematology and blood chemistry, urinalysis, and tests for alcohol, drug-abuse and a pregnancy test for women) and serological tests (hepatitis B and C, as well as HIV antibodies) were conducted.

Study design and Drug administration

A single-dose randomized, open-label, two-sequence, two-period crossover design under fasting conditions with a 12-day washout interval between the two periods was used. In addition, the design was regarded as truncated (That is, the blood sampling collection period was restricted to 72 hours because of the long elimination half-life of etoricoxib of roughly 24 hours).

The subjects were admitted to a clinical unit (Investigacion Farmacologica y Biofarmaceutica) on the day before the drug administration. They were randomly assigned to one of the two

sequences: the test formulation followed by the reference formulation and *vice versa*.

The subjects were administered a single tablet of the test or the reference formulation with 250 ml of water after fasting for at least 10 hours. Blood samples (7 mL) were drawn from each subject using an indwelling cannula. These samples were placed into heparinazed (sodium heparin) tubes. The samples were obtained at baseline (pre-dose) and then at 0.25, 0.5, 0.75, 1.00, 1.25, 1.50, 2.00, 3.00, 4.00, 6.00, 8.00, 12.00, 24.00, 36.00, 48.00 and 72.00 hours after administration. The subjects then returned to the clinical unit after the washout period to receive the alternative formulation.

The blood samples were centrifuged at 4500 rpm for 5 minutes. The resulting plasma samples were stored at -70°C ± 10°C until being transported to the analytical unit (Biokinetics) where they were stored at -75°C ± 5°C until analysis. The subjects' diet consisted of standardized meals (breakfast, lunch and dinner) which were provided at 4, 8 and 12 hours after drug administration.

Determination of Etoricoxib plasma concentrations

Chemicals: Etoricoxib (batch: 20-SSR-57-1), the reference substance was obtained from the Toronto Research Chemicals Inc (Toronto, Canada) and sildenafil citrate (internal standard, batch: 00139) was obtained from MAPRIMED (Buenos Aires, Argentina). The water was obtained from a Barnstead water purification system (Thermofisher Scientific, OH, USA) and the solvents were HPLC grade (Avantor Performance Materials, LLC, PA, USA and Honeywell International Inc. MI, USA) and all reagents were analytical grade (Sigma-Aldrich, Inc. Missouri, USA).

Method and sample preparation: The etoricoxib plasma concentrations were determined using a HPLC method coupled with ultraviolet spectroscopy; this method was developed and validated by Biokinetics personnel in Mexico City, Mexico.

A sample consisting of 500 µL of plasma and 10 µL of internal standard (sildenafil, 250 µg/ mL) was extracted with 1000 µL of a solvent mixture consisting of ethyl acetate and tert-butyl methyl ether (90:10 v/v). These components were vortexed for one minute and centrifuged at 8000 rpm for 5 minutes at 20°C. The organic phase (800 µL) was separated and placed into a test tube where it was subjected to evaporation to dryness under a nitrogen current, at 50°C for 4 minutes. The residue was reconstituted with a mixture of 100 µL consisting of water and acetonitrile (50:50 v/v) and 20 µL was injected into the chromatographic system (HPLC, Agilent Technologies, model 1200, Palo Alto, CA, California).

Chromatographic conditions: The analytical column was a Zorbax® XDB-C18, (150 × 4.6-mm internal-diameter column of 5-µm particle size (Agilent Technologies)) and the precolumn was a Zorbax® SB-C8 (12.5 × 4.6-mm internal-diameter column of 5-µm particle size (Agilent Technologies)). Etoricoxib and the internal standard were eluted with a mobile phase consisting of a mixture (55:45 v/v) of aqueous ammonium acetate (10 mM, pH = 5.5 ± 0.1) and acetonitrile. The column temperature was 25°C and both analytes were detected by using an ultraviolet detector (Agilent Technologies, model G1314B) at a wavelength of 284 nm. The flow of the mobile phase was 1 mL/ minute. The typical retention times for etoricoxib and the internal standard were 4.9 and 5.9 minutes, respectively. The peak areas were measured to calculate the peak area ratio of etoricoxib with respect to that of the internal standard. We then calculated the concentration.

Method validation: We validated the analytical method in accordance with Mexican and international guidelines [8,9]. Analysis of blank human plasma samples from six different subjects, blank human (hemolyzed and lipemic) plasma samples, as well as anticoagulants (lithium and sodium heparin), xanthines (caffeine and theobromine), and other drug substances commonly used as analgesics (acetylsalicylic acid, diclofenac, paracetamol, ibuprofen and naproxen) were used to test the selectivity of the method. No interferences were observed in the resulting chromatograms**.**

The calibration curve consisted of the following etoricoxib concentrations: 10, 20, 600, 1000, 1800, 2400 and 2800 ng/mL. (i.e., the range of the method was 10-2800 ng/ mL, and the lower limit of quantification (LLOQ) of 10 ng/ mL). The method was linear over this range of concentrations, and the coefficient of determination was 0.99 (average from 4 calibration curves). The intra-assay %CV and accuracy (relative error) of etoricoxib were 1.85% to 3.31% and 1.85% to 9.68%, respectively; and the inter-assay %CV and accuracy were 4.46% to 6.63% and -14.60% to 9.68%, respectively.

Etoricoxib was found to be stable in plasma for at least 24 hours at room temperature (25°C), after three freeze-thaw cycles and after 16 weeks at −75 ± 5°C. We also tested sample dilution to account for etoricoxib concentrations beyond the upper bound of the calibration curve's range. We prepared quality-control samples at three different concentration levels (designated as low (30 ng/ mL), medium (1400 ng/ mL) and high (2100 ng/ mL) of etoricoxib independent of the calibration curve. The acceptance criteria for the approval of the analytical runs and the quality control samples, as well as the criteria for performing sample reanalysis, were consistent with Mexican and international guidelines.

Tolerability: The subjects were interviewed by the principal investigator and/or the study coordinator to determine the occurrence of Adverse Events (AEs) during the study and at the end of the clinical stage of the study. The subjects were asked to spontaneously report any AEs to the principal investigator at any time over the entire duration of the study, including washout period. Adverse events that were life-threatening or led to death, hospitalization, disability, and/ or medical intervention to prevent permanent impairment or damage were considered to be serious.

Pharmacokinetic and statistical analyses: The sample size calculation [10] was based on the within-subject variability of etoricoxib C_{max} with a %CV of 27.67% estimated from data of Shohag et al. [11]. This calculation, performed using the following values: 1 - β = 0.8, α = 0.05, expected ratio (μ_T/μ_R) = 0.95 and an equivalence range of 80% to 125%, yielded a sample size of 34 subjects. Therefore, we planned to recruit 36 subjects to account for potential dropouts.

We directly obtained C_{max} and T_{max} values from the plasma concentration–time curves. Because of the long elimination half-life of etoricoxib, the truncated area under the plasma concentration-time curve from baseline (time 0) to 72 hours (AUC_{0-72}) was used to assess the extent of absorption [12]. From, the terminal log-decay phase, the elimination rate constant (k_e) was estimated using linear regression, and the $t_{½}$ was estimated using the following equation:

$t_{½} = \ln 2/k_e$, where *ln* is the natural logarithm.

To assess the bioequivalence between the test and reference formulations, C_{max}, and the truncated AUC_{0-72} were considered to be primary variables. Using log-transformed data for these parameters, ANOVA for a 2 x 2 crossover design, was carried out at the significance level of 5% (α = 0.05).

The 90% CIs of the geometric mean ratios (test/reference) of the C_{max}, and truncated AUC_{0-72} were calculated. The test and the reference formulations were considered to be bioequivalent if the 90% CIs of these parameters fell within a predetermined range of 80% to 125%. All of the pharmacokinetic and statistical analyses were performed using WinNonlin Version 7.0 (Certara, Princeton, NJ, USA).

RESULTS

Table 1 lists the demographic characteristics of the 36 subjects, who were enrolled in the study. Two subjects were withdrawn from the study because one female subject had a positive result for a pregnancy test and the other subject decided to leave the study at the first period because of personal reasons. Hence, the sample size for the bioequivalence evaluation was 34 subjects.

Pharmacokinetic parameters

Mean plasma concentration-time curves of the two formulations are shown in figure 1. The results suggest that the two formulations have comparable mean plasma concentration-time curves. The pharmacokinetic parameters (C_{max}, T_{max}, $t_{1/2}$, and truncated AUC_{0-72}) for both formulations are listed in table 2.

No significant period or sequence effects were detected based on the ANOVA of C_{max} and truncated AUC_{0-72} (data not provided).

The bioequivalence statistics using the log-transformed data for C_{max} and truncated AUC_{0-72}: geometric means, geometric mean ratios (test/reference), 90% CI, and the intra-subject %CV is listed in table 3.

The 90% CIs for etoricoxib C_{max}, and truncated AUC_{0-72} were 99.55% to 119.33%, and 95.97% to 103.06%, respectively. The 90% CIs of the geometric mean ratios of the two parameters fell within the predetermined range of 80% to 125%. Therefore, these results indicate that the bioequivalence criteria were satisfied.

Tolerability: Two subjects reported a total of two AEs. These events consisted of one headache and one case of diarrhea, which were reported after the administration of the test formulation. None of the AEs was considered to be serious. Rather, both of the AEs were regarded to be mild, and all of the AEs spontaneously resolved under medical surveillance during the clinical stage.

DISCUSSION

All of the 90% CIs of the geometric mean ratios of the pharmacokinetic parameters (C_{max} and AUC_{0-72}) were found to be within the predetermined range of bioequivalence (80%-125%). These results indicate that the bioequivalence criteria were satisfied.

Both formulations were well tolerated, because the two AEs were regarded to be mild and resolved spontaneously during the clinical stage. This study has several limitations. First, it was an open label study. Only healthy adult subjects within a specific age range could participate, and these individuals, were administered a single dose of the formulations. The pharmacokinetic parameters of etoricoxib may differ among patient groups. Therefore, the results of this study might not be generalizable to a target population.

In addition, we did not evaluate the effect of food on the bioavailability of etoricoxib because it has been reported that the single-dose oral pharmacokinetics of etoricoxib are independent of food intake due to the fact that the extent of absorption of etoricoxib remains intact [13].

Additional future studies are necessary to compare the test

Table 1: Demographic characteristics of subjects.

Characteristic	Values
Total No. of recruited subjects (female/male)	36 (20/16)
Age, mean (SD), range, years	34 (9),18-54
Weight, mean (SD), range, kg	62.7 (10.1), 41.0-89.0
Height, mean (SD), range, m	1.62 (0.09), 1.48-1.85
BMI , mean (SD), range, kg/m^2	23.71 (2.63), 18.59-26.93

BMI = Body Mass Index
SD = Standard Deviation

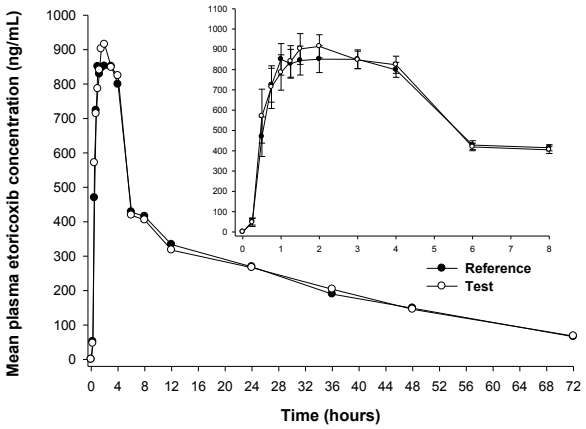

Figure 1: Mean plasma concentration-time curves after a single-dose administration of a test (trademark: Doscoxel®, Laboratorios Liomont, S. A. de C. V., Mexico) and a reference (trademark: Arcoxia®, Schering-Plough, S. A. de C. V; Mexico) oral tablet, containing etoricoxib 60 mg in healthy Mexican adult subjects (n = 34). Inset: mean (± SE) concentrations over the first 8 hours after administration

Table 2: Pharmacokinetic parameters of a reference and a test formulation of etoricoxib after a single-dose administration of etoricoxib 60 mg in healthy Mexican adult subjects (n = 34). Values are mean (SD).

Parameter	Reference†	Test*
C_{max}, ng/ mL	1250.38 (347.53)	1396.32 (522.99)
AUC_{0-72}, ng•h/ mL	17538.86 (4395.91)	17462.07 (4427.36)
T_{max}, h	2.04 (1.35)	1.93 (1.24)
$t_{1/2}$, h	24.45 (9.45)	24.85 (7.48)

C_{max} = Maximum plasma drug concentration
AUC_{0-72} = truncated AUC from time 0 (baseline) to 72 hours
*Trademark: Doscoxel® (Laboratorios Liomont, S. A. de C. V., Mexico City, Mexico)
†Trademark: Arcoxia (Schering-Plough, Mexico, S. A. de C. V., Mexico City, Mexico)

Table 3: Geometric means, geometric mean ratios, 90% CIs and the intra-subject %CV of the pharmacokinetic parameters determined for etoricoxib after a single-dose administration of etoricoxib 60 mg in healthy Mexican adult subjects.

Parameter	Geometric Means Test/Reference	Geometric Mean Ratio (%)	90% CI	Intra-subject %CV
C_{max}, ng/ mL	1311.39/1203.24	108.99	99.55, 119.33	22.29
AUC_{0-72},ng•h/mL	16938.98/17031.82	99.45	95.97, 103.06	8.67

C_{max} = Maximum plasma drug concentration
AUC_{0-72} = truncated AUC from baseline to 72 hours.

formulation with the reference formulation in Mexican patient groups. We expect that the findings of this study may serve as a reference for future controlled studies of etoricoxib in a Hispanic population.

CONCLUSION

This study, which included healthy, fasting Mexican adult subjects, showed that the test formulation of etoricoxib 60 mg met the Mexican regulatory requirements to assume bioequivalence based on the rate and extent of absorption. Both formulations were well tolerated.

ACKNOWLEDGEMENT

This research was supported by Laboratorios Liomont, S.A. de C.V., Mexico City, Mexico. The authors have indicated that they have no conflicts of interest regarding the content of this article. We acknowledge Dr. Luis J. Garcia-Aguirre for his valuable help with the preparation of the study protocol and his comments that improved the quality of the manuscript.

REFERENCES

1. Takemoto JK, Reynolds JK, Remsberg CM, Vega-Villa KR, Davies NM. Clinical pharmacokinetic and pharmacodynamic profile of etoricoxib. Clin Pharmacokinet. 2008; 47: 703-720. https://goo.gl/p3w8vm
2. Dallob A, Hawkey CJ, Greenberg H, Wight N, De Schepper P, Waldman S, et al. Characterization of etoricoxib, a novel, selective COX-2 inhibitor. J Clin Pharmacol. 2003; 43: 573-585. https://goo.gl/7b1SAV
3. Baraf HS. Efficacy of the newest COX-2 selective inhibitors in rheumatic disease. Curr Pharm Des. 2007; 13: 2228-2236. https://goo.gl/tj7nAr
4. Merck Sharp, Dohme Corp. Product information Arcoxia® tablets (etoricoxib, MSD). 2011; 1-28. https://goo.gl/M3XLFW
5. Agrawal NG, Porras AG, Matthews CZ, Woolf EJ, Miller JL, Mukhopadhyay S, et al. Dose proportionality of oral etoricoxib, a highly selective cyclooxygenase-2 inhibitor, in healthy volunteers. J Clin Pharmacol. 2001; 41: 1106-1110. https://goo.gl/6JTHpn
6. Agrawal NG, Porras AG, Matthews CZ, Rose MJ, Woolf EJ, Musser BJ, et al. Single- and multiple-dose pharmaco-kinetics of etoricoxib, a selective inhibitor of cyclooxygenase-2, in man. J Clin Pharmacol. 2003; 43: 268-276. https://goo.gl/S6KZTy
7. Rodrigues AD, Halpin RA, Geer LA, Cui D, Woolf EJ, Matthews CZ, et al. Absorption, metabolism, and excretion of etoricoxib, a potent and selective cyclooxygenase-2 inhibitor, in healthy male volunteers. Drug Metab Dispos. 2003; 31: 224-232. https://goo.gl/J1mxsX
8. COFEPRIS. Federal commission for the protection against sanitary risks official Mexican Standard NOM 177-SSA1-2013: Tests and procedures to prove that a medication is interchangeable [in Spanish]: General Directorate of Standards. Mexico. 2013.
9. Center for Drug Evaluation and Research (CDER). Guidance for Industry. Bioanalytical method validation. 2001. https://goo.gl/T5zKTF
10. Chow SC, Liu JP. Design and analysis of bioavailability and bioequivalence studies. 2nd ed. New York, NY: Marcel Dekker. 2000. https://goo.gl/4Z1SbV
11. Shohag MH, Islam MS, Ahmed MU, Joti JJ, Islam MS, Hasanuzzaman M, et al. Pharmacokinetic and bioequivalence study of etoricoxib tablet in healthy Bangladeshi volunteers. Arzneimittelforschung. 2011; 61: 617-621. https://goo.gl/Y8U8bi
12. Francis Micheal, Saranya S, Aparna N, Sridevi, Chithra R, Judith MP. Concepts of bioequivalence and it's impact on Truncated Area Under Curve (AUC) of drugs with long half-life in point estimate and intra-subject variability. J Pharm Sci & Res. 2012; 4: 1890-1896. https://goo.gl/NE2h3W
13. Agrawal NG, Porras AG, Matthews CZ, Rose MJ, Woolf EJ, Musser BJ, et al. Single- and multiple-dose pharmacokinetics of etoricoxib, a selective inhibitor of cyclooxygenase-2, in man. J Clin Pharmacol. 2003; 43: 268-276. https://goo.gl/qzndae