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Pharmacokinetic evaluation of etoricoxib 120mg tablets in healthy human Pakistani volunteers: *In-vivo in-silico* bridging for bioequivalence

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Abstract: **Background**: Etoricoxib is a selective cyclooxygenase-2 inhibitor widely used for the treatment of pain and inflammatory conditions. However, comparative pharmacokinetic and bioequivalence data for locally manufactured etoricoxib formulations in the Pakistani population remain limited. **Objective**: This study aimed to evaluate the pharmacokinetics and bioequivalence of a locally manufactured etoricoxib tablet compared with the innovator product in healthy Pakistani volunteers, with supportive assessment using physiologically based pharmacokinetic (PBPK) modeling. **Methods**: Comparative *in-vitro* dissolution studies were conducted in buffer media of pH 1.2, 4.5 and 6.8 and evaluated using similarity factor (f_2) analysis. A randomized, open-label, two-treatment, two-period crossover bioequivalence study was performed in healthy male volunteers under fasting conditions. Subjects received a single oral dose of 120 mg Etoricoxib (ETO) as either the test product (Etoxib®) or the reference product (Arcoxia®), with a 14-day washout period. Plasma concentrations were quantified using a validated HPLC-UV method and pharmacokinetic parameters were estimated using non-compartmental analysis. PBPK modeling of the test product was conducted using GastroPlus® to assess model predictability. **Results**: The test and reference products exhibited similar dissolution profiles across all media, with f_2 values indicating similarity. The geometric mean ratios (90% confidence intervals) of the test to reference product for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were 0.946 (0.8855–1.0135), 0.923 (0.8705–0.9795) and 0.960 (0.8955–1.0255), respectively, all within the regulatory bioequivalence acceptance range. PBPK model predictions for key pharmacokinetic parameters were within an acceptable fold-error range (≤2) compared with observed data. **Conclusion**: The study demonstrated comparable systemic exposure and confirmed bioequivalence between the locally manufactured and innovator etoricoxib formulations in the Pakistani population. PBPK modeling provided supportive evidence of formulation similarity and model adequacy.

Keywords: Bioequivalence; Etoricoxcib; PBPK; Physiologically based pharmacokinetics

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INTRODUCTION

Etoricoxib (ETO) ($C_{18}H_{15}ClN_2O_2S$), a selective cyclooxygenase-2 (COX-2) inhibitor (Martina *et al*., 2005), is prescribed for the management of inflammatory conditions including osteoarthritis, rheumatoid arthritis and acute pain (Akter *et al*., 2025; Takemoto *et al*., 2008). It offers effective anti-inflammatory and analgesic benefits while minimizing gastrointestinal adverse effects commonly attributed to selective nonsteroidal anti-inflammatory drugs (NSAIDs) (Agrawal *et al*., 2003; Biase *et al*., 2024). It has also been found safe and effective in the management of inflammatory bowel disease (Oliveira *et al*., 2024; Ribaldone *et al*., 2015). The clinical effectiveness greatly depends upon the physiological differences and is apparent by the variability observed in pharmacokinetics of the drugs. Ethnic as well as regional factors may also be pivotal in determining pharmacokinetic variability among populations. Differences in genetic polymorphisms of drug-metabolizing enzymes and extrinsic factors such as diet, nutrition and environmental exposures further influence drug disposition. Such findings emphasize the importance of pharmacokinetic investigations in specific populations since data from one ethnic group may not adequately predict effectiveness in another. In the case of ETO, no prior data were available that addressed the pharmacokinetics with reference to the regional differences present in the population. Bioequivalence studies, on the other hand, facilitate regulatory approval of generic versions by demonstrating comparable pharmacokinetic profiles, thereby ensuring interchangeability with the innovator drug (Chow., 2014). The assessment of bioequivalence involves a cascade of

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processes beginning with pharmaceutical assay and comparative dissolution profiling of the test and reference products, to the drug administration and sample analysis, followed by pharmacokinetic and statistical evaluation. Pharmaceutical quality tests like assay and dissolution in the context of bioequivalence studies ensure the similarity in quality and potency of the reference and test formulations (ICH, 2024). Comparative dissolution profiling evaluates whether the test product exhibits a similar dissolution behavior to the reference product under expected *in-vivo* conditions, which is a prerequisite for establishing bioequivalence (Diaz *et al*., 2016).

Inter-ethnic variability in pharmacokinetics is a recognized determinant of drug exposure, therapeutic response and regulatory decision-making (ICH, 2019). Genetic polymorphisms in drug-metabolizing enzymes, along with population-specific physiological, dietary and environmental factors, can result in clinically meaningful differences in pharmacokinetic behavior among ethnic groups. ETO is primarily metabolized by cytochrome P450 enzymes, including CYP3A4 and CYP2C9, which are known to exhibit inter-ethnic variability in expression and activity (Guengerich, 2008; Zhou *et al*., 2009). The Pakistani population represents a genetically heterogeneous South Asian cohort for which pharmacokinetic data on ETO are limited. Generation of population-specific pharmacokinetic and bioequivalence data is therefore important to support safe clinical use and to meet regulatory expectations for locally relevant evidence.

The present study aims to establish the pharmacokinetics of ETO and bioequivalence of a test formulation to the reference product through a comprehensive approach encompassing pharmaceutical assay, comparative dissolution profiling, drug release kinetics, bioanalytical method development, including validation and bioequivalence assessment. The research was further strengthened by investigating physiologically based pharmacokinetics (PBPK) of the pharmacokinetic data. These findings are expected to provide insights into pharmacokinetics that support its safe and effective use in clinical practice, facilitate regulatory processes and contribute to the export of generic products.

MATERIALS AND METHODS

ETO (99.43% pure) standard was obtained as a gift from Glenmark Pharmaceuticals (Pvt. Ltd). All solvents were of HPLC grade, while the rest of the materials were of analytical grade and procured from Sigma-Aldrich. The test product Etoxib® (120mg) was manufactured by Hiranis Pharmaceuticals (Pvt. Ltd), Karachi while the reference product Arcoxia® (120mg) manufactured by Merck Sharp and Dohme, GmbH was imported from Dubai, UAE.

Instruments

The analysis was conducted on a Shimadzu LC-20A system equipped with a UV-VIS detector (SPD-M20A, Shimadzu, Kyoto, Japan), an auto-sampler (SIL-20AC, Shimadzu, Kyoto, Japan), a column oven (CTO-20A, Shimadzu, Kyoto, Japan) and a degasser (DGU-20A5R, Shimadzu LC-20A). Data acquisition was performed using Lab Solution software (version 5.65, Shimadzu, Kyoto, Japan). Sample preparation involved the use of a vortex mixer (Biobase, California, USA), a centrifuge (Mikro, Hettich, Germany) and micropipettes (Eppendorf, Germany). The filtration assembly (Sartorius, Germany) was used to degas and filter mobile phase prior to use.

Chromatographic conditions for assay of ETO products

The mobile phase composition was 0.05M potassium dihydrogen phosphate (KH_2PO_4) and acetonitrile in the ratio 50:50. The flow rate was set at 1.5mL/min, while the detection was performed at 283nm and the column was maintained at 40°C temperature and the injection volume was 20 µl (Patel *et al*., 2007).

Preparation of standard and product samples

For the preparation of the ETO standard, accurately weighed 120mg of ETO was transferred to a 100 mL volumetric flask and dissolved in 50mL of diluent (Water: ACN) (50:50). The volume was made up to 100mL with the same diluent. Subsequent dilutions (60µg/mL) was prepared from the stock solution (Patel *et al*., 2007). Samples of both test and reference products were prepared separately by weighing and crushing 20 tablets and transferring amount equivalent to 120mg of active ingredient to 100mL volumetric flask. It was dissolved in the same diluent as the standard and diluted to nominal concentration of 60µg/mL.

Comparative dissolution testing

The three distinct dissolution media i.e., 0.1 N hydrochloric acid, acetate buffer pH 4.5 and phosphate buffer pH 6.8, were prepared according to United States Pharmacopeia. The dissolution testing was performed on a USP paddle II apparatus (PharmaTest, PT-DT70, Germany) operated at 50rpm paddle speed and 900mL dissolution medium in each vessel (USP, 2023). The absorbance measurements for both standard and sample solutions were conducted using a UV-Vis spectrophotometer (UV-1800 Shimadzu, Kyoto, Japan) at a wavelength of 283 nm, with the dissolution medium serving as the blank. Samples were withdrawn at intervals of 5 min, 10 min, 15 min and 30 min from each vessel. An additional sample was collected at 60 min for pH 4.5 and 90, 120 and 150 min samples were also collected in buffer pH 6.8 owing to reported poor dissolution (Ashokraj *et al.*, 2016). A portion of 10mL was collected and adequately diluted with the same buffer for determination of absorbance while the buffer was immediately replaced in each vessel after sample collection. Similarity factor *(f2)* was applied to compare

the similarity of dissolution profiles (ICH, 2024). Drug release kinetic models, including zero order, first order, and Higuchi models, were fitted to the obtained dissolution data to get insights into the drug release mechanism from film coated immediate release tablets.

Bio-analytical method development and validation

Chromatographic conditions

The separation of analyte from plasma matrix was performed on aWelchrom C-18 column (150 mm×4.6mm, 5µm) equipped with a guard column (LiChrospher® 100, 5 µm, LiChroCART® 4-4). The column oven temperature was maintained at 35 °C. The mobile phase consisted of acidic aqueous medium (0.1% formic acid in water) and acetonitrile with 0.1% formic acid. The two solvents were mixed in the ratio of 78:22 (v/v), delivered at 1 mL/min flow rate (Loh *et al*., 2022). The UV detector was set at a wavelength of 254 nm, while diclofenac (10 µg/mL) was used as the internal standard (Patel *et al*., 2007). The injection volume was 50 µL and the total run time was 14 minutes.

Preparation of calibration standards and quality control

The stock solution of ETO (1 mg/mL) was prepared in methanol and diluted to 10 µg/mLin plasma. Similarly, the stock solution of the internal standard, diclofenac (1mg/mL), was prepared by weighing 10 mg of diclofenac, dissolving it in methanol and diluting the solution to 10 mL with methanol. Furthermore, ETO calibration standards were prepared in plasma at concentrations ranging from 0.175 to 3.5 µg/mL. A system suitability sample containing 2.1 µg/mL ETO and 1 µg/mL diclofenac was prepared for method validation.

Plasma sample pre-treatment

In a 2 mL Eppendorf tube, 400 µL of spiked or blank plasma was added with 50 µL of the I.S (5 µg/mL of diclofenac) and 400 µL of acetonitrile. The mixture was thoroughly mixed on vortex mixer for 30 seconds, then heated at 56 °C for 5 minutes. After heating, the sample was centrifuged at 12,000 rpm for 10 minutes. About 600 µL of the upper layer was carefully collected and filtered into a 0.5 mL micro-centrifuge tube.

Bio-analytical method validation

The proposed bio-analytical method was fully validated according to ICH guidelines on bioanalytical method validation for selectivity, sensitivity, accuracy, precision and stability (ICH; 2019). Selectivity was rigorously evaluated on six different blank plasmas to ensure accurate quantification of ETO in the presence of potential endogenous matrix interferences. The selected chromatographic conditions yielded retention times of 11.0 ± 1 minutes for ETO and 6.6 ± 1 minutes for diclofenac.

The stability of ETO in plasma was investigated under various storage and handling conditions at three concentration levels of quality control samples: low (0.525µg/mL), medium (1.75µg/mL) and high (3.15µg/mL). The stability parameters evaluated include stock solution, auto-sampler, freeze-thaw and long-term stability at -70°C. Triplicate analyses of QC samples were performed by HPLC after a one-week interval.

For freeze-thaw stability, QC samples were prepared and initially analyzed to establish a baseline (zero-hour) concentration. Subsequently, the samples were kept at -20°C and analyzed at 24, 48 and 72h post-initial preparation. The chemical integrity of ETO was assessed by determining the percent recovery at each time point.

Recovery was determined by comparing the peak area of ETO extracted from plasma samples to that of ETO in mobile phase samples. Recovery assessments were performed over three consecutive days to evaluate reproducibility. Linearity was assessed by injecting a series of standards at seven distinct concentrations spanning the anticipated concentration range. During the validation of the ETO assay, the calibrators demonstrated linearity across the concentration range of 0.175µg/mL to 3.5µg/mL.

Accuracy was defined as the percentage ratio of the calculated mean concentration of spiked samples in comparison to the nominal (theoretical) concentrations. Both intra-day and inter-day accuracy and precision were assessed using the standard curves generated during the linearity assessment. The %CV was calculated for the QC samples across their respective concentration ranges. For sensitivity, ten replicate injections of nominal LLOQ sample were analyzed and the measured concentrations were determined based on the peak area ratio. This assessment provided a robust measure of the method's ability to accurately quantify ETO at low concentrations.

Bioequivalence study design

Subject selection

A controlled clinical investigation was conducted involving twelve (n=12) healthy male adult volunteers (designated as V1–V12). The eligibility of participants was ascertained through comprehensive physical examinations, medical history assessments and clinical laboratory evaluations. Individuals presenting with clinical laboratory values deviating from the standard reference range, those afflicted with acute infections or chronic pathologies and individuals with documented hypersensitivity to NSAIDs were excluded from enrollment. Additionally, subjects with a history of smoking, alcohol consumption, or tobacco use, as well as those who had recently donated blood for therapeutic or research purposes, were precluded from participation.

Study design and blood sampling protocol

This investigation followed an open-label, randomized, single-dose, two-treatment, two-period, two-sequence crossover bioequivalence design.

Table 1: Input values for physiologically based pharmacokinetic (PBPK) model of etoricoxib

Parameters	Value	Reference
Log P	2.794	Jones *et al.*
pKa	4.96	Jones *et al.*
Molecular weight (M/W g/mol)	358.842 g/mol	Jones *et al.*
Aqueous solubility S mg/mL	0.0767 ± 0.0018 mg/mL	Gonzalez *et al.*
Jejunal effective permeability (P_{eff}) (cm/sec x 10-4, fasted state)	4.75 × 10−4 cm/s	Gonzalez *et al.*
Unbound percent in human plasma (%Fup)	8%	Takemoto *et al.*
Human blood to plasma concentration ratio (Rbp)	9.2:0.8 (plasma:blood)	Takemoto *et al.*
Cl IV (L/h)	49 mL/min	Agrawal *et al.*
Cl renal (L/h)	57mL/min	Nayak *et al.*
Cl biliary (mcL/min/106)	14.95	Nayak *et al.*
Vss (L/kg)	119L	Agrawal *et al.*

The reference formulation, Arcoxia® (120 mg film-coated tablet), was designated as treatment A, whereas the test formulation, Etoxib® (120 mg film-coated tablet), was identified as treatment B. Subjects were admitted to the clinical facility 12 hours prior to drug administration to ensure a fast state for at least one-hour post-dosing. Pharmacokinetic (PK) profiling involved the collection of 15 venous blood samples in heparinized tubes (5 mL per sample) at predefined intervals: 0, 0.25, 0.50, 0.75, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 24.0, 48.0 and 72.0 hours post-dosing. A washout period of one week was observed between crossover phases. Immediately following collection, samples underwent centrifugation at 2500 rpm for 5 minutes at ambient temperature to isolate plasma and stored at −20°C until further analysis.

Pharmacokinetic analysis

The pharmacokinetic parameters of both the test and reference formulations were determined through plasma drug concentration assessments utilizing validated bioanalytical methods. Non-compartmental analysis was executed on Kinetica® version 5.1 (Thermo Electron Corp., Waltham, USA) to derive key PK parameters, including the time to reach maximum plasma concentration (T_{max}), maximum plasma concentration (C), area under the plasma concentration-time curve from zero to the last time point (AUC0-t) and area extrapolated to infinity ($AUC_{0-\infty}$).

Statistical evaluation of pharmacokinetic data

In compliance with U.S. FDA regulatory guidelines on statistical approaches to bioequivalence assessment, a Latin square-design two-way ANOVA was employed on log-transformed pharmacokinetic data using a general linear model framework to evaluate the impact of formulation, period, sequence and inter-subject variability within sequences using $\alpha = 0.05$ significance level. A two one-sided *t*-test (TOST) was conducted to compare the mean pharmacokinetic parameter values between the test and reference products. IBM Statistical Package for Social Sciences (SPSS) version 23.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Bioequivalence was confirmed if the 90% confidence intervals for the geometric mean ratios (GMR) of the pharmacokinetic parameters fell within the regulatory acceptance range of 0.80-1.25, signifying comparable systemic exposure between the formulations (FDA; 2021).

Physiologically based pharmacokinetic (PBPK) modeling of pharmacokinetic data

A physiologically based pharmacokinetic (PBPK) model for ETO 120 mg immediate-release tablets was developed using the Advanced Compartmental Absorption and Transit (ACAT) model in GastroPlus® version 9.8.3 (Simulations Plus, Inc., Lancaster, CA). This model aimed to simulate drug absorption in a healthy male population. Physicochemical, physiological and pharmacokinetic properties required for the model were gathered from experimental data and literature sources, as summarized in table 1. The initial step involved validating the model by comparing the simulated intravenous (IV) bolus profile of ETO with corresponding *in-vivo* data from adult studies. Furthermore, the simulated plasma concentration-time profile for the immediate-release tablets was cross-checked against observed *in-vivo* oral data. Before establishing the correlation, the plasma concentration-time profile of the optimized formulation was analyzed using the PKPlus™ module and the resulting compartmental parameters were integrated into the pharmacokinetic section of GastroPlus®. The accuracy of the model was assessed by calculating the fold error (FE) between the predicted and observed pharmacokinetic parameters using a standard formula (Cvijic *et al*., 2018; Jones *et al*., 2013).

RESULTS

Pharmaceutical assay of test and reference products

The assay results for both the reference and test products were found to be within the specified acceptance criteria of 90–110% of the label claim, as per the standards outlined in the USP. Additionally, for bioequivalence studies, it is a regulatory requirement that the test and reference products do not exhibit a difference exceeding 5% (ICH; 2024).

Comparative dissolution test

The comparative profile (Fig. 1) shows that more than 85% of the drug dissolved within 15 minutes in buffer at pH 1.2.

A relatively slower dissolution rate was observed at pH 4.5 for both the products, i.e., around 85% after 30 minutes, whereas the slowest dissolution rate was observed at pH 6.8, with around 85% drug dissolution observed after two hours. The similarity factors (f_2) at each pH were 64, 82 and 85 for pH 1.2, 4.5 and 6.8, respectively. Similarity factors were found above 50 in all cases. The investigations of drug release kinetics revealed the first-order kinetics as the best fit with R2adj= 0.995, 0.895, and 0.900 for pH1.2, 4.5 and 6.8, respectively, which best explained drug release behavior from the immediate release tablets.

Bio-analytical method

The chromatographic conditions employed provided a highly selective assay, as demonstrated by the absence of interfering peaks in the elution regions of both the analyte (ETO) and the internal standard (IS) in representative plasma sample chromatograms (Fig. 2). Critically, no interfering peaks were observed at the retention times corresponding to ETO or the IS, confirming the method's selectivity. The LLOQ was established at 0.175µg/mL. The calibration curves demonstrated linearity across the concentration range of 0.175–3.5 µg/mL (Fig. 3).

In terms of precision and accuracy, the method was deemed satisfactory for both intra-day and inter-day analyses, ensuring high reproducibility and reliability of the method. This was evident from the accuracy and precision values within ±15% of the nominal values (Table 2 and 3). Different stability assessments representing varying environmental conditions indicated that samples remained stable with reference to three freeze thaw cycles (-20 °C), stock solution stability and long-term stability for up to 72 hours and up to four weeks respectively at −70 °C (Table 4). These stability findings were crucial for ensuring proper sample preservation prior to and during analysis, thereby enabling accurate quantification of drug concentrations in biological fluids. Overall, all validation parameters met the predefined acceptance criteria in accordance with the ICH bioanalytical method validation guidelines, confirming the method's reliability and suitability for quantifying ETO in human plasma.

Bioequivalence study design

The demographic analysis of the study population, comprising 12 subjects, revealed an average age of 24 years, with a range spanning from 19 to 27 years. The mean height was observed to be 66 inches (range 61-72 inches), while the average weight was 60.9 kg, (range 50.4-78.4 kg). The BMI distribution indicated a mean of 21.71 kg/m², with a minimum of 18.68 kg/m² and a maximum of 27.14 kg/m². The observed demographic parameters fell within accepted physiological ranges, suggesting a relatively homogenous study population in terms of age, height, weight and BMI. The absence of extreme values enhances the reliability of the dataset for further analysis.

Pharmacokinetic and statistical analysis

Overall, the pharmacokinetic parameters of Arcoxia® (120 mg) and Etoxib® (120 mg) were similar. As can be seen in table 5 The mean C_{max} of the reference product was slightly higher than the test product while minimal inter-individual variability was evident from minor standard deviation values indicating clinical insignificance of the observed results. The T_{max} values for Arcoxia® (0.729 ± 0.198 h) and Etoxib® (0.791 ± 0.208 h) were also found close to each other, both achieving peak plasma concentrations within an hour (Fig. 4). For AUC_{o-t}, slightly higher values were observed for Arcoxia® (3893.91 ± 839.58 ng·h/mL) as compared to Etoxib® (3639.12 ± 1308.0 ng·h/mL), as well as higher inter-individual variability in the latter. The total drug exposure, represented by AUCo–∞, was also similar between the reference (4554.0 ± 886.4 ng·h/mL) and test products (4450.68 ± 1280.68 ng·h/mL), showing minor difference. The elimination half-lives were likewise consistent (Table 5), supporting comparable elimination kinetics. There are other notable studies addressing bioequivalence of ETO, but their results cannot be compared here owing to the higher doses administered to subjects (Meulman *et al.*, 2023; Shohag *et al.,* 2011).

The geometric mean ratio (GMR) for C_{max} was calculated as 0.947, indicating similar peak plasma concentration of the two products (Table 5). The 90% confidence interval (C.I) for C_{max} falls within the accepted bioequivalence range of 80%-125%, as confirmed by the two one-sided t-tests. The T_{max} values for Arcoxia® (0.729 ± 0.198 h) and Etoxib® (0.791 ± 0.208 h) were also similar, demonstrating a comparable absorption rate of two formulations. The geometric mean ratio (GMR) for AUC_{o-t} was calculated as 0.923, indicating that Etoxib® achieved similar systemic exposure as compared to Arcoxia®. The 90% confidence interval (CI) for AUCo-^{t} also fell within the accepted bioequivalence range, supporting the conclusion of bioequivalence. Similarly, the geometric mean ratio (GMR) for AUCo-∞ was determined as 0.961. The elimination half-life ($T_{1/2}$) for Arcoxia® (25.175 ± 6.10 h) and Etoxib® (23.73 ± 6.71 h) exhibited minor variations, suggesting comparable elimination kinetics and duration of systemic drug exposure (Fig. 5).

Overall, the PBPK model demonstrated reasonable predictive performance, with all fold errors within the generally acceptable range. However, the discrepancies in C_{max} and T_{max} suggest the need for further refinement of absorption kinetics to improve the accuracy of the model. The absorption characteristics of ETO tablets manufactured at two different sites has been evaluated previously using comparative dissolution testing and PBPK modeling. Film-coated tablets of three different strengths were assessed, followed by a bioequivalence comparison with reference products. Similar dissolution behavior was observed in acidic media, whereas significant differences were noted at pH 4.5 and 6.8. Despite these

differences, PBPK simulations predicted bioequivalence between products from the two manufacturing sites, which was subsequently confirmed by the bioequivalence study. These findings are in agreement with the results of the present study (Mitra et al., 2015)

DISCUSSION

Pharmaceutical assay of test and reference products

There was no official testing method available for ETO in reputed international Pharmacopoeia. Therefore, an in-house method was developed and validated as per general ICH guidelines (Q2 R1) for quantitative determination of active ingredients in dosage form (ICH, 2005). Researchers have previously reported HPLC based analytical methods for quantification of ETO in tablet dosage forms and bulk material (Gangane *et al*., 2014). They used the λ max wavelength (233nm) for detection of API. Though ETO exhibits two wavelengths of maximum absorbance, the second wavelength 285nm was selected in the current study as reported previously (Cacciari *et al*., 2020). This may enhance specificity of the analytical method. The chromatographic conditions thus applied yielded acceptable results with reference to system suitability parameters. Using this method, the assay was found to be greater than 99% and within ±5% of the reference product.

Comparative dissolution test/Drug release kinetics

It was evident from the observed results that the pH-dependent solubility of the drug and the most appropriate model for predicting oral absorption can be explained by drug dissolution at pH 1.2. These observations are in concordance with previously published reports, while a slight difference (approximately 15%) in the results has also been previously reported (Ashokraj *et al*., 2016; Mitra *et al*., 2015). The difference may be attributed to the developed formulation since they tested formulation variability between products developed at an old and new site. The results also signify that due to the high solubility at lower pH, the absorption and bioavailability of the immediate release formulation are expected to be primarily influenced by their initial dissolution in the acidic environment of the stomach (pH < 3). This is further supported by the high absolute bioavailability of ETO (~100%), indicating that solubility does not limit its absorption (Starek *et al*., 2011). The drug release kinetics were best described by first order release at pH 1.2 while Higuchi model best fitted at pH 4.5 and 6.8. The results assure immediate drug release and consequent absorption and therapeutic effect.

Bio-analytical method

Previously reported bioanalytical methods used either LC-MS with stable isotope labeled internal standards (SIL-IS) or solid phase extraction for sample preparation. (Sangoi *et al*., 2008; Ifrah *et al*., 2024; Bräutigam *et al*., 2003). These approaches were very expensive and required sophisticated instrumentation. Therefore, a cost effective HPLC-UV-based analytical method was developed on the basis of a literature survey. Fluorescence detection has previously been employed after converting ETO into a fluorescent chemical derivative (Matthews *et al*., 2001). In addition, a solid-phase extraction approach using a 96-well plate system was employed. However, the derivatization technique is laborious and requires extensive validation, particularly for optimization of the derivatization reaction. In another reported method, a mixture of diethyl ether and dichloromethane (6:4) in a relatively large volume (6 mL) was used for the extraction of ETO (Ashokraj *et al*., 2016).

These solvents are hazardous and using large volumes is not economically viable. Pharmacokinetic studies conducted in rats have therefore employed smaller sample volumes for drug extraction. They used simple protein precipitation and analyzed the samples by HPLC-UV at a detection wavelength of 245nm (Radwan *et al*., 2009). They used gradient elution mode for chromatographic separation and applied the method to pharmacokinetic studies of ETO in rats. These stability findings were crucial for ensuring proper sample preservation prior to and during analysis, thereby enabling accurate quantification of drug concentrations in biological fluids. Overall, all validation parameters satisfied the pre-defined acceptance criteria in accordance with ICH bioanalytical method validation guidelines, confirming the method's reliability and suitability for the quantification of ETO in human plasma.

Pharmacokinetics of reference and test products

The observed variations in C_{max} values across studies, with the present study reporting the highest value, suggest potential differences in the rate or extent of absorption. Comparable C_{max} values have been previously reported, whereas a significant difference has also been observed in another study. Such variability may be attributed to differences in study conditions, population characteristics, or bioavailability (Suyatna *et al.*, 2022; Harikrishnan *et al.*, 2021). The T_{max} values observed in the present study, indicating rapid oral absorption, are also comparable to those previously reported. In contrast, the significant difference in AUC_{0-t} observed between the present study and one previously reported investigation may reflect variations in drug metabolism, study design, or bioanalytical methodology. Furthermore, the close agreement in $AUC_{0-\infty}$ values across all studies, despite variations in T_{max} and AUC_{0-t}, indicates consistency in total systemic exposure. Finally, a moderate variation in elimination half-life has also been reported when compared with previously published data.

Statistical analysis for establishing bioequivalence

The comparative pharmacokinetic evaluation demonstrated similar pharmacokinetic profiles for Arcoxia® and Etoxib®, with minor differences observed in absorption, distribution and elimination parameters.

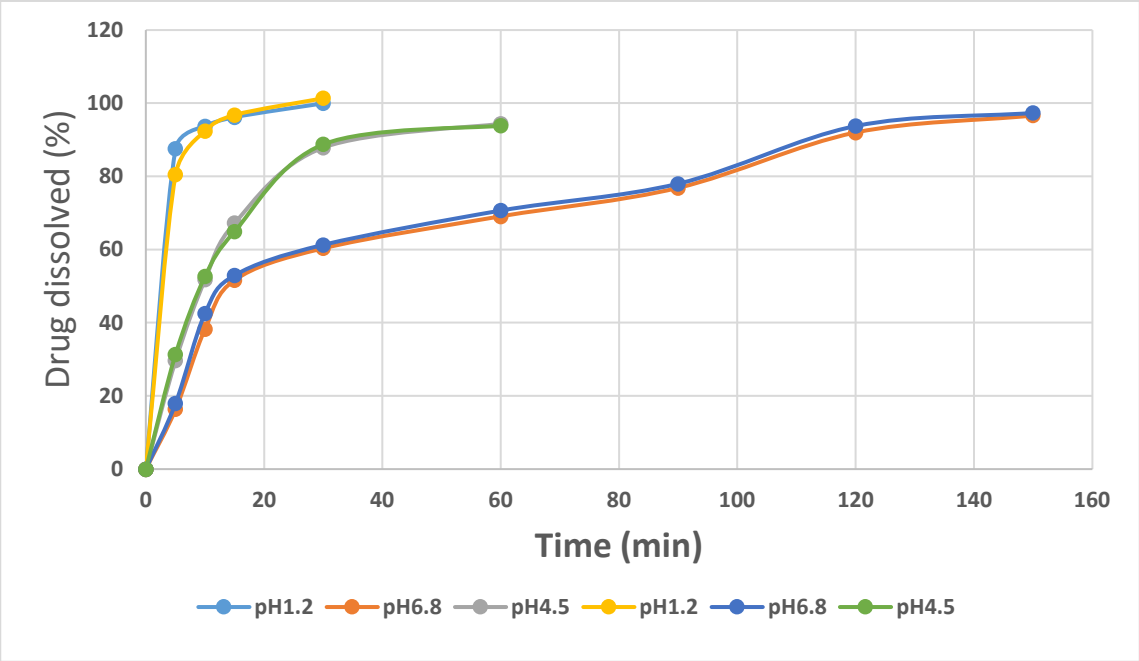

Fig. 1: Comparative dissolution profile of test and reference products

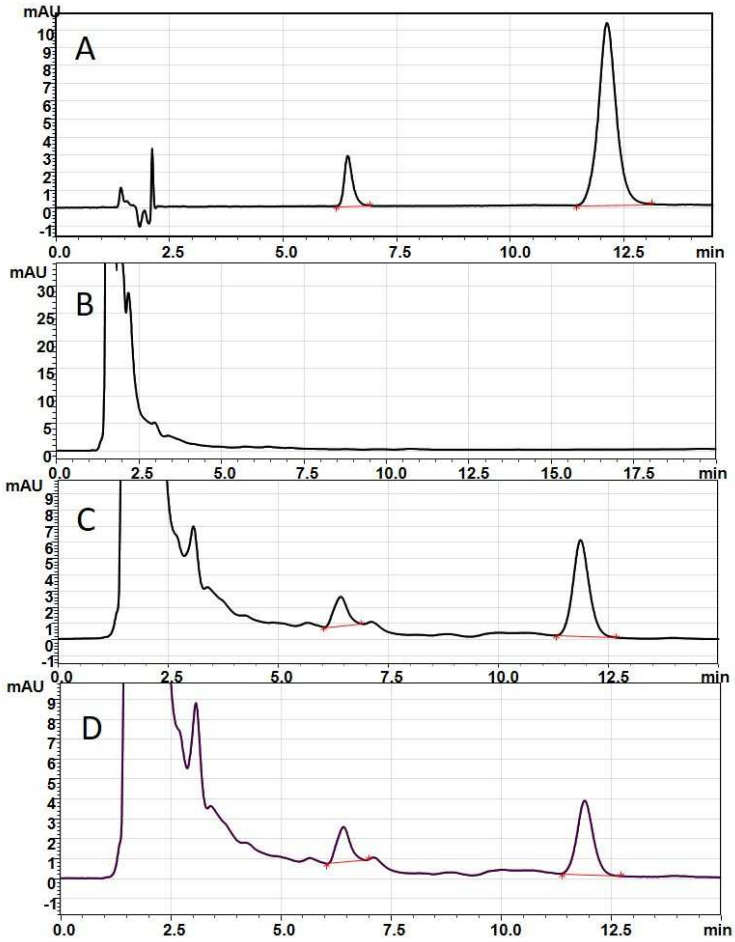

Fig. 2: Chromatograms of (A): Analyte and I.S. in mobile phase. (B): Blank plasma (C): Etoricoxib with I.S. and (D): Plasma sample collected at 2 h.

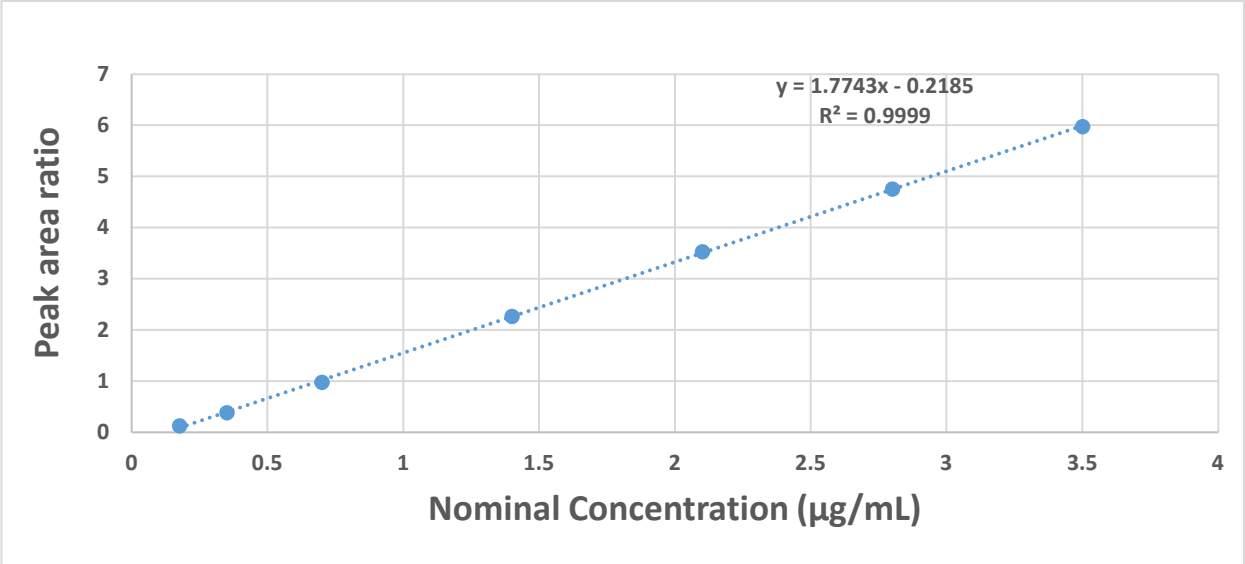

Fig. 3: Linearity curve of etoricoxib in human plasma

Table 2: Accuracy and precision of the bioanalytical method

Concentration level (µg/mL)	Accuracy and Precision Day 1			Accuracy and Precision Day 2			Accuracy and Precision Day 3		
	Mean (µg/mL)	SD	%CV	Mean (µg/mL)	SD	%CV	Mean (µg/mL)	SD	%CV
3.5	3.51	0.05	1.43	3.45	0.16	4.53	3.50	0.13	3.74
2.8	2.84	0.044	1.54	2.75	0.08	2.75	2.75	0.10	3.49
2.1	2.1	0.022	1.06	2.08	0.10	4.64	2.05	0.06	3.05
1.4	1.38	0.029	2.11	1.35	0.04	2.77	1.38	0.05	3.32
0.7	0.71	0.035	4.91	0.71	0.03	4.80	0.71	0.03	3.75
0.35	0.37	0.003	0.8	0.35	0.01	3.82	0.35	0.02	5.16
0.175	0.17	0.003	1.91	0.18	0.01	4.37	0.17	0.01	4.11

Table 3: Inter-day accuracy and precision for 3 days

Concentration level (µg/mL)	Calculated concentration (ug/mL)			Mean (µg/mL)	SD	%CV
	Day1	Day2	Day3			
3.5	3.53	3.33	3.41	3.43	0.10	2.88
2.8	2.8	2.69	2.73	2.74	0.06	2.06
2.1	2.07	2.00	2.06	2.04	0.04	1.75
1.4	1.35	1.29	1.34	1.33	0.03	2.36
0.7	0.73	0.70	0.69	0.71	0.02	3.01

Table 4: Results of stability studies of etoricoxib in plasma

Stability parameter	% Recovery		
	QCL	QCM	QCH
Freeze-thaw (24 h)	98.89	100.59	96.30
Freeze-thaw (48 h)	98.42	93.43	97.70
Freeze-thaw (72 h)	95.55	96.16	98.56
Long term (week 1)	96.67	102.20	97.15
Long term (week 2)	95.97	100.00	98.08
Long term (week 3)	96.99	99.55	96.46
Long term (week 4)	102.19	98.69	96.29
Stability week	Week 1	Week 2	Week 3
Stock solution (%) ETO	98.96	97.46	96.83
Stock solution (%) I.S	98.57	96.86	96.30

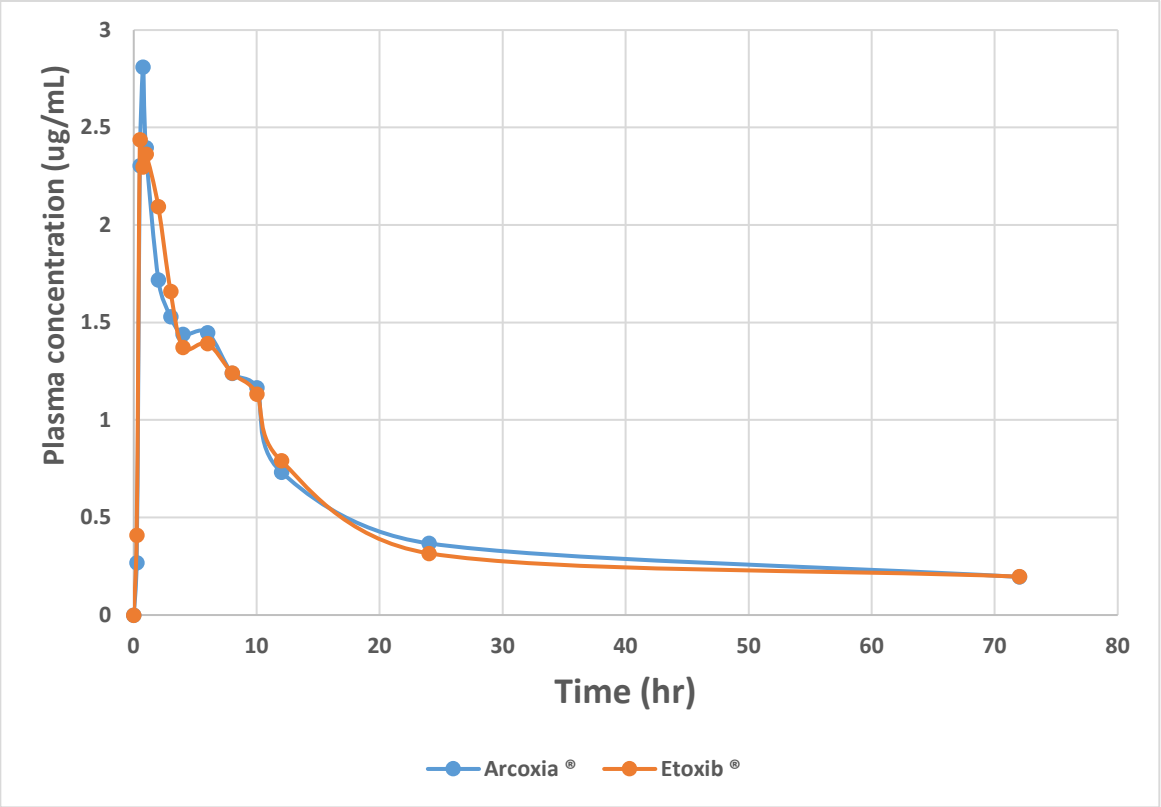

Fig. 4: Concentration *vs.* Time profile of reference vs test products

Table 5: Pharmacokinetic parameters of etoricoxib (120 mg) reference and test products

Pharmacokinetic parameter	Arcoxia® (120mg)	Etoxib® (120 mg)	Geometric mean ratio (B/A)	90% C.I
C_{max}(µg/mL)	3.12 ±0.496	2.91 ±0.350	0.946	(0.8855, 1.0135)
T_{max}	0.729 ±0.198	0.791 ±0.208	-------	---------
AUC $_{0\text{-}t}$	3893.91 ±839.58	3639.12 ±1308.0	0.923	(0.8705, 0.9795)
AUC$_{0\text{-}inf}$	4554.0 ±886.4	4450.68± 1280.68	0.960	(0.8955, 1.0255)
$T_{1/2}$	25.175±6.10	23.73±6.71	-------	---------

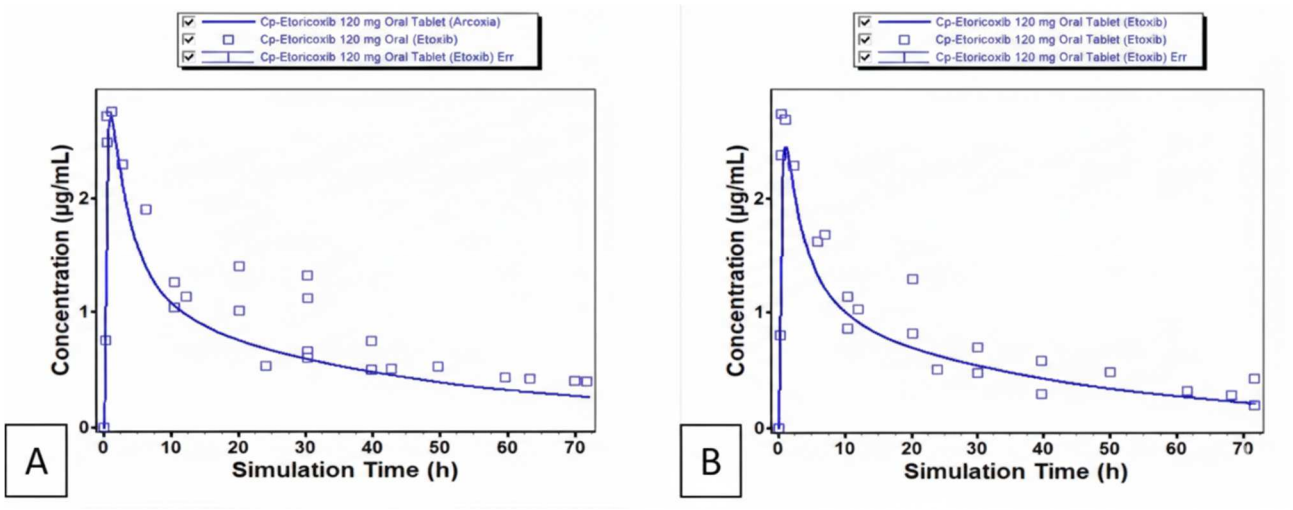

Fig. 5: Observed and predicted plasma concentration vs time curve for: (A). ETO reference product and (B). Test product

Table 6: Predicted pharmacokinetic parameters of etoricoxib reference and test products

Etoxib®				Arcoxia®			
Result	Observed	Simulated	Fold error	Result	Observed	Simulated	Fold error
C_{max} (µg/mL):	2.44	1.792	1.361	C_{max} (µg/mL):	2.81	1.792	1.568
T_{max} (h):	0.6	1.1	1.833	T_{max} (h):	0.75	1.1	1.466
AUC $_{0\text{-}inf}$ (µg-h/mL):	37.744	39.988	1.059	AUC $_{0\text{-}inf}$ (µg-h/mL):	38.256	40.168	1.049
AUC $_{0\text{-}t}$ (µg-h/mL):	33.618	33.318	1.009	AUC $_{0\text{-}t}$ (µg-h/mL):	34.588	35.246	1.019

The slightly higher C_{max} values for Arcoxia® suggest marginally higher peak plasma levels, though the difference is not substantial, indicating comparable bioavailability. The GMR and 90% CI for C_{max} and AUC values confirmed the bioequivalence of the two formulations, as supported by the two one-sided t-tests. Similar T_{max} values indicate a comparable rate of absorption. The slight differences in AUCo-^{t} and AUCo-∞ values suggest minor variations in the extent of drug exposure, but these variations are within the accepted bioequivalence range. The comparable elimination half-life ($T_{½}$) values indicate similar elimination kinetics and durations of systemic drug exposure. These minor variations in C_{max} and AUC values may be attributed to formulation differences or inter-individual variability in drug absorption. However, the consistent T_{max} and $T_{½}$ values support the comparable pharmacokinetic behavior of both formulations.

Physiologically based pharmacokinetic (PBPK) modeling of pharmacokinetic data

The predictive performance of the PBPK model for Etoxib® and Arcoxia® was evaluated by comparing observed and simulated pharmacokinetic parameters, including C_{max}, T_{max}, AUCo–∞ and AUC_{o-t} (Table 6). In the present study, the PBPK model demonstrated moderate under prediction of C_{max} and over-estimation of T_{max}, suggesting limitations in the description of early phase absorption processes. These deviations may reflect incomplete characterization of formulation dependent dissolution or intestinal transit kinetics. In contrast, the close agreement between predicted and observed AUCo–t and AUCo–∞ indicates that the model reliably captured the extent of absorption. It suggests that, despite minor inaccuracies in rate related parameters, the model is suitable for evaluating formulation equivalence and systemic exposure consistency.

These observations are consistent with the findings of Mitra *et al*., (2015) who demonstrated that variability in the dissolution of ETO at higher pH values influenced absorption kinetics in PBPK simulations, while overall systemic exposure remained comparable among products manufactured at different sites. Their PBPK based bioequivalence predictions were subsequently confirmed by *in-vivo* studies, aligning well with the outcomes of the present investigation. Collectively, these results support the utility of PBPK modeling as a mechanistic tool for understanding formulation-dependent absorption behavior and for complementing *in-vivo* bioequivalence assessments.

CONCLUSION

The study established the pharmacokinetics of ETO in local population and bioequivalence of the generic ETO tablet, meeting the regulatory acceptance criteria. PBPK modeling yielded acceptable fold errors, supporting the consistency of the findings and thus establishing the test product as therapeutically equivalent to the reference formulation. This also demonstrates the safe and effective use of the generic product by clinicians and facilitates the manufacturer's contribution to the export of the generic product.

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Authors' contribution

Syed Hakim Masood (SHM), Iyad Naeem Muhammad (INM), Fahad Siddiqui (FS), Muhammad Talha Saleem (MTS), Muhammad Liaquat Raza (MLR). Study concept and design: SHM and INM, Methodology: SHM and FS, Acquisition of data: SHM and FS, Analysis and interpretation of data: SHM, FS and MTS, Administrative, technical and material support: INM and SHM, Study Supervision: INM, Validation: SHM, FS, MTS and MLR, Visualization: SHM, FS, MTS, MLR and INM, Drafting of the manuscript: SHM, FS, MTS and MLR, Critical revision of the manuscript for important intellectual content: SHM, FS, INM and MLR, Statistical analysis: All authors read and approved the final manuscript.

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Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval

The ethical approval was obtained from the ethics committee of the University of Karachi (IBC-KU-358/2023).

Conflict of interest

The authors declare no conflict of interest

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