Bioequivalence Evaluation of Two Formulations of Celecoxib 200 mg Capsules in Healthy volunteers by using a validated LC/MS/MS method

Research Article

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Abstract

The bioequivalence study to compare a new formulation of celecoxib to its reference formulation was designed as an open-label, randomized, single-dose, two-way crossover, comparative bioavailability study by using a validated LC/MS/MS method. In order to determine the plasma concentrations of celecoxib, a sensitive LC/MS/MS method was developed. The method was validated to possess adequate specificity, linearity, precision, accuracy and stability. The linearity of calibration curve was assessed between the concentration intervals (5–2000 ng/mL) with a correlation coefficient over 0.999. Regarding pharmacokinetic investigation, the mean celecoxib AUC_{0-t} values from the test and reference drug formulations were 7360.44 ± 1714.14 h•ng/mL and 7267.48 ± 2077.68 h•ng/mL, respectively, and the corresponding $AUC_{0-\infty}$ values were 8197.45 ± 2040.31 h•ng/mL and 7905.54 ± 2286.12 h•ng/mL, respectively. The C_{max} of the test and reference drugs was 705.30 ± 290.63 ng/mL and 703.86 ± 329.91 ng/mL, respectively, and the corresponding T_{max} was 3.4 ± 1.6 h and 2.9 ± 1.4 h. Lastly, the $T_{1/2}$ values of the test and reference drugs were 13.9 ± 7.9 h and 12.9 ± 7.7 h, respectively. The 90% confidence intervals for AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were 97.00-108.85, 98.01-112.09, and 93.20-116.13, respectively, satisfying the bioequivalence criteria of 80-125% range. In conclusion, these results demonstrated that the bioequivalence of two formulations of celecoxib was established successfully by utilizing present developed LC/MS/MS method.

Keywords: Bioequivalence; LC/MS/MS; Pharmacokinetics; Celecoxib.

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are not only among the most commonly used therapeutic agents worldwide but also the most commonly prescribed medications for pain relief and arthritis management globally. Despite of tremendous total usage, the side effects correlated with gastrointestinal, hepatic or renal damages and occasional cardiovascular risk leaded to the confinement of NSAIDs - related concomitant therapies [1, 2]. NSAIDs exert anti-inflammatory effects mainly through reduction of prostaglandin biosynthesis by inhibiting the activity of cyclooxygenase (COX) activity, which exists in two predominant isoforms, COX-1 and COX-2 [3-5]. Specifically, COX-1 is constitutively expressed in most tissues to maintain routine functions of organs. COX-2 shows basal expression by tissue-specific distribution and is activated to correspond with inflammatory stimulation [6, 7]. However, recent evidence has revealed that selective inhibition of COX-2 activity-inducing inflammatory events reduce the potential side effects caused by traditional NSAIDs [8, 9].

Celecoxib (Celebrex®) was the first of the COX-2 selective inhibitors (coxibs) approved by Food and Drug Administration (FDA) in 1998 for the following indications: arthritis, acute pain, and familial adenomatous polyposis (FAP) [10, 11]. Although celecoxib has very poor water solubility, orally administered celecoxib shows good absorption and promising gastrointestinal safety. Under fasting condition, the rate and extent of absorption of celecoxib are dose-proportional. Celecoxib is primarily metabolized by cytochrome P4502C9 (CYP2C9) in the liver and cytochrome P4503A4 (CYP3A4) is involved to a small extent. After hepatic metabolism, three metabolites without COX-2 inhibitory activity are derived from celecoxib, and subsequently, some of the parental compound is excreted in urine and feces. Additionally, T_{max} was delayed by ~1 h and AUC increased by 10-20% when celecoxib was administered with a high-fat diet [12].

Based on a recent study, celecoxib accounted for 0.2% to 21.2% of total NSAID sales around the world [13], suggesting a great demand for the drug in the global market. In light of the

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guidance for bioavailability/bioequivalence studies stipulated by Taiwan Food and Drug Administration (TFDA), assessment of bioequivalence to establish therapeutic equivalence via a comparative bioavailability evaluation based on pharmacokinetics of a test (generic) and a reference drug formulation is regarded as the required criterion for marketing approval of generic medicinal products [14]. Certainly, the clinical safety and tolerability of the generic formulation also need to be evaluated.

The purpose of this study was to investigate the bioequivalence of generic celecoxib (200 mg capsule) in comparison with the reference formulation Celebrex® capsule 200 mg in healthy volunteers by using a validated LC/MS/MS method.

Materials and Methods

Drug information

Capsules containing 200mg celecoxib were obtained from manufacturers. Celecoxib 200mg capsule (Synmosa Biopharma Co., Ltd.) and Celebrex® 200mg capsule (celecoxib 200mg capsule, Pfizer) served as test and reference formulations, respectively.

Chemicals and reagents

HPLC-grade acetonitrile (ACN) was purchased from Mallinckrodt (USA). All other chemicals were analytical grade and obtained from Merck (Darmstadt, Germany).

Preparation of plasma samples for LC/MS/MS analysis

Plasma samples were allowed to thaw in a water bath at room temperature. 200µl of plasma from each sample was mixed with 50µl of ACN/H_2O containing 0.3ng/µl celecoxib-d_4 as an internal standard. The mixture was centrifuged at 3000 rpm for 10 min after addition of 500µl of 100% ACN to each sample. The solvent layer was transferred to which 500µl of H_2O was added for further analysis.

The plasma concentrations of celecoxib were determined by a validated LC/MS/MS method. Chromatographic separation was performed on Agilent ZORBAX XDB-C18, 2.1 × 50 mm internal size, 5µm particle size (Agilent Technologies, USA) with a mobile phase consisting of $ACN/H_2O/formic$ acid (60/40/0.2) via an optimum flow rate of 0.3ml/min. The LC/MS/MS system comprised Waters Alliance 2795 Separations Module and Micromass Quattro Ultima with MassLynx V4.0 SP4.

Validation

Procedures of validation and acceptance criteria were in accordance with "FDA Bio-analytical Method validation guidelines [15]." Validation was performed by evaluating specificity, linearity, precision, accuracy, recovery and stability. The precision was defined by the coefficient of variation (%CV) and accuracy was based on the relative error (%RE) [(mean calculated concentration–nominal concentration) × 100%/ nominal concentration]. The precision should be ≤ 15.0%, except for lower limit of quantification value (LLOQ), where it should be ≤ 20.0%. For accuracy (%RE) acceptance criteria, the % RE of the mean value should be within ± 15.0%, except for LLOQ, where it should be within ± 20.0%.

Ethics and subject enrollment

This study was performed in accordance with the Taiwan Law of Pharmaceutical Affairs, Good Clinical Practices, Good Laboratory Practices, local regulatory requirements, and the principles enunciated in the Declaration of Helsinki. The sample size which would expect to achieve the 80-125% confidence interval limit was based on the result of pilot study. Thereby, a total of 38 healthy Taiwanese volunteers participated in this study. Signed informed consent was obtained from all individual participants enrolled in the study. The enrolled subjects were between the ages of 20 and 36 years and weighed 45.5 to 81.3 kg. All subjects were healthy, as determined by complete physical and clinical examinations before the study. The subjects were instructed to avoid any medication for at least 1 month prior to and during the study.
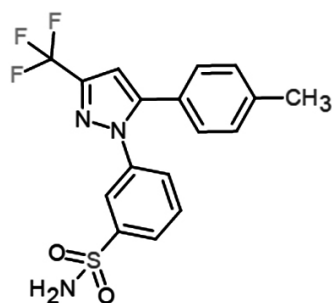
Study design and clinical protocol

This was an open-label, single-dose, randomized, two-period, two-sequence, two-treatment, crossover, comparative bioavailability study under fasting conditions. This trial was conducted to demonstrate therapeutic equivalence through pharmacokinetic means, and hence, the bioavailability of celecoxib from the two study drug formulations was compared. To this end, 38 healthy adult volunteers were screened after obtaining consent for enrollment. Subjects who met the eligibility criteria were randomly assigned in equal numbers to one of the two dosing sequences, T-R or R-T. For example, subjects randomized to the T-R sequence received the test product in Period 1, and then received the reference product in Period 2 after a 7-day washout period.

During each study period, the subjects were hospitalized at a study facility from 9 pm before dosing of day 1 until 12 h after dosing. On Day 1, the subjects' vital signs were checked. Before drug administration, it was confirmed that subjects had fasted by evaluating if their blood sugar levels were in the normal range (60-120mg/dL). Thereafter, the test or reference drug was administered orally with 240 mL of water maintained at room temperature. Water up to 3 L per day was offered on request. Alcohol, coffee, tea, cocoa, or cola were not permitted for 48 h before each dosing until the last sampling in each period. No beverages were allowed 1 h before and until 2 h after drug administration. The subjects were required to fast overnight (for at least 10 h) before dosing and a minimum of 4 h thereafter. Standardized meals were provided on Day 1, including lunch and dinner, at 4 and 10 h after dosing, respectively, during each period. During housing, meal plans were identical for both periods. Information on the standardized meal, and its quantity and time of provision were recorded on the relevant raw data forms. During housing, consumption of tobacco was not allowed.

Blood samples for pharmacokinetic analysis were collected in a pre-labeled vacutainer tubes containing sodium heparin in each period at pre-dose (0), 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 36, and 48 h after administration of each formulation. Approximately 10mL of venous blood sample as blank was collected prior to drug administration. Approximately 10mL of venous blood samples were obtained according to the above sampling schedule. The actual collection time for each blood sample was recorded. The blood samples collected at each time point were centrifuged at 1900 × g for 10 min at 4°C to separate plasma. Then, the separated plasma was stored at - 20°C for further analysis.

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Figure 1. Structure of celecoxib.

Pharmacokinetic and statistical analysis

Pharmacokinetic and statistical evaluations were performed for samples from subjects who completed the study according to the protocol. Any value of plasma concentration below the limit of quantification (LOQ) was considered as zero for computation. The pharmacokinetic parameters of celecoxib from the two formulations, such as AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, K_{el}, T_{max}, $T_{1/2}$ and MRT, were determined and calculated using WinNonlin professional software version 6.3 (Pharsight Corporation, Mountain View, CA). Each parameter was presented as arithmetic mean (Mean) with standard deviation (SD).

The pharmacokinetic parameters of celecoxib from the two formulations were statistically evaluated using analysis of variance (ANOVA) appropriate for the experimental design of this study. The statistical model included factors accounting for the following sources of variations: sequence, subjects within a sequence, period, and treatment. For AUC and C_{max}, the ratio of geometric means for the ln-transformed data was compared. The statistical significance of the ratio was assessed by appropriate analysis of variance (ANOVA) using SAS® version 9.2 (SAS Institute Inc., Cary NC). Statistical inferences including 90% confidence intervals and Schuirmann's two one-sided test procedures were evaluated. To establish bioequivalence, the 90% confidence intervals for the ratio of the geometric means with respect to the test/reference products were to fall within the range of 80-125 % according to ln-transformed data.

Results

Method validation

Method validation was conducted in accordance with the currently accepted "FDA Bio-analytical Method validation guidelines" for industry [15]. In order to confirm the reliability of our method, the accuracy, precision, selectivity, linearity and stability were needed to be validated. First, blank plasma samples from six independent sources were prepared and analyzed without addition of celecoxib and internal standard (IS, celecoxib-d$_4$). As expected, no significant interference peaks were observed in the chromatograms (Figure 2A). The results revealed that minimal endogenous compounds or chemical reagents would affect the retention times of celecoxib or IS due to the specificity of signal. The representative chromatograms demonstrated the retention times of celecoxib and IS were 1.76 and 1.74 minutes, respectively (Figure 2B and C). The linearity was established in terms of eight spiked plasma samples at nominal concentrations range 5-2000ng/ml. The calibration curves were linear at indicated concentration range with a correlation coefficient over 0.999 (Y

= 0.01495 X - 0.00720, r^2 = 1.00). Suitable precision (0.8 to 7.6%) and accuracy were observed which highlighted the reliability of analytical method (Table 1). Additionally, the designated LLOQ (5 ng/mL) was acceptable to determine the plasma concentration of celecoxib in specimens which obtained from endpoint sampling as 48 h post-dose according to present study design.

With respect to the precision and accuracy corresponding to intra-day and inter-day conditions, the intra-day and inter-day precisions were 2.5-3.1% and 1.7-5.5%, respectively. Meanwhile, the intra-day and inter-day accuracies were 97.4-106.6% and 99.5-102.7%, respectively (Table 2). To evaluate the extraction recovery, the celecoxib chromatograms obtained from plasma and solution were used to determine the ratio based on their mean peak areas. The mean extraction recovery of celecoxib was 87.9% in response to three spiked celecoxib concentrations (15, 100 and 1500ng/mL). Moreover, plasma samples containing celecoxib for two concentrations (15 and 1500ng/mL) were subject to investigate the stability. As results summarized in Table 3, the analyte was found to be stable no matter in short-term, long-term, post-processing and repeated freeze–thaw cycles conditions. Our results demonstrated that present bioanalytical method were reliable in specificity, sensitivity, precision, accuracy and stability over studied range for which it was appropriate to be applied for further bioequivalence study.

Subject demographics

Among the 45 subjects screened for the study, 38 were randomized and completed the study without protocol deviations at Mackay Memorial Hospital Tamshui Branch. Eventually, these 38 subjects were assessed in the pharmacokinetic analysis and bioequivalence evaluation. This subject population included 22 male subjects (57.8%) and 16 female subjects (42.2%) (Table 4).

Pharmacokinetic properties

The mean plasma concentration-time curves of celecoxib after single dose oral administration of the test and reference formulations were presented in Figure 3. Celecoxib plasma concentration-time curves were similar for both formulations. Pharmacokinetic parameters of the test and reference treatments were listed in Table 5. After administration, the time to peak concentration (T_{max}) of the test and reference treatments was 3.4 ± 1.6 h and 2.9 ± 1.4 h, respectively. The maximum plasma level of celecoxib of the test and reference formulations was 705.30 ± 290.63ng/mL and 703.86 ± 329.91ng/mL. AUC_{0-t} was 7360.44 ± 1714.14 h•ng/mL and 7267.48 ± 2077.68 h•ng/mL, and $AUC_{0-\infty}$ was 8197.45 ± 2040.31 h•ng/mL and 7905.54 ± 2286.12 h•ng/mL. The terminal elimination half-life ($T_{1/2}$) was 13.9 ± 7.9 h

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Figure 2. Representative chromatograms of celecoxib and celecoxib-d$_4$ (IS) in human plasma specimens. (A) Blank plasma; (B) plasma sample spiked with celecoxib at LLOQ (5ng/mL); (C) plasma sample spiked with celecoxib-d$_4$ at 1ng/mL.
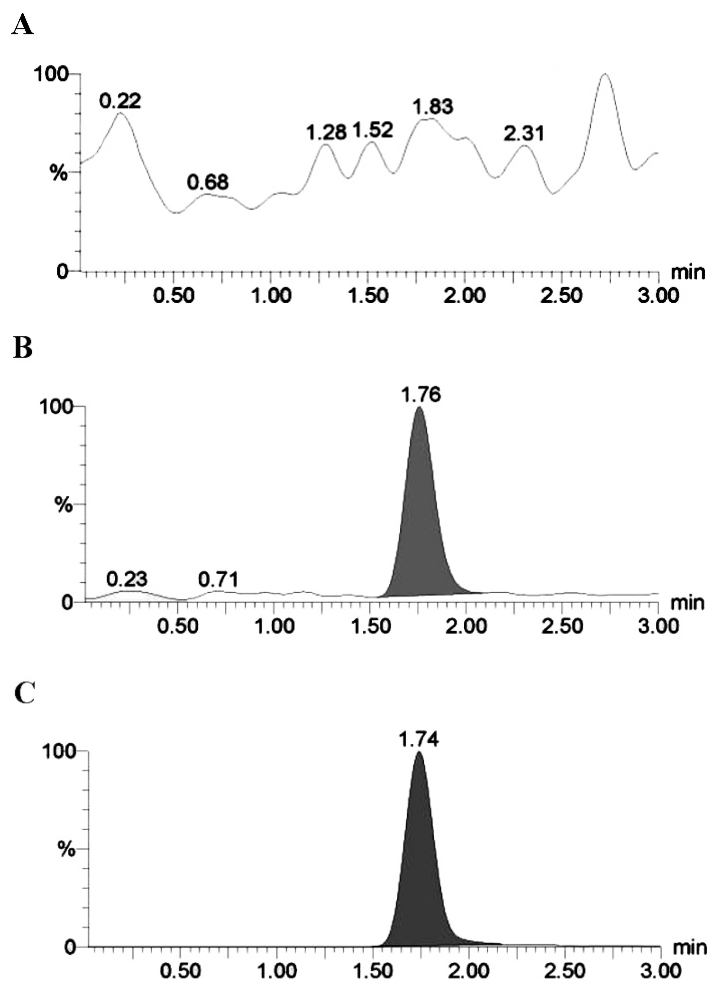
A

B

C

Table 1. Precision and accuracy of celecoxib measurement for linearity.

Nominal conc. (ng/mL)	Observed value (ng/mL) (Mean ± SD) (n = 5)	Precision (%)	Accuracy (%)
5	4.87 ± 0.37	7.6	97.4
10	10.3 ± 0.6	5.8	103
20	19.9 ± 1.0	5	99.5
50	49.7 ± 1.2	2.4	99.4
200	199 ± 4	2	99.5
500	512 ± 12	2.3	102.4
1000	988 ± 18	1.8	98.8
2000	2001 ± 17	0.8	100.1

Table 2. Inter and Intra-day precision and accuracy of celecoxib in human plasma.

Nominal value (ng/mL)	Intra-day (ng/mL) (n = 6)			Inter-day (ng/mL) (n = 5)		
	Mean ± SD	Prec. (%)	Acc. (%)	Mean ± SD	Prec.	Acc.
5	5.33 ± 0.15	2.8	106.6	5.10 ± 0.28	5.5	102
15	15.7 ± 0.4	2.5	104.7	15.4 ± 0.5	3.2	102.7
100	97.4 ± 2.7	2.8	97.4	99.7 ± 3.2	3.7	99.7
1500	1496 ± 46	3.1	99.7	1493 ± 2.7	1.7	99.5

SD, standard deviation; Prec., Precision; Acc., accuracy

Table 3. Stability of celecoxib in human plasma.

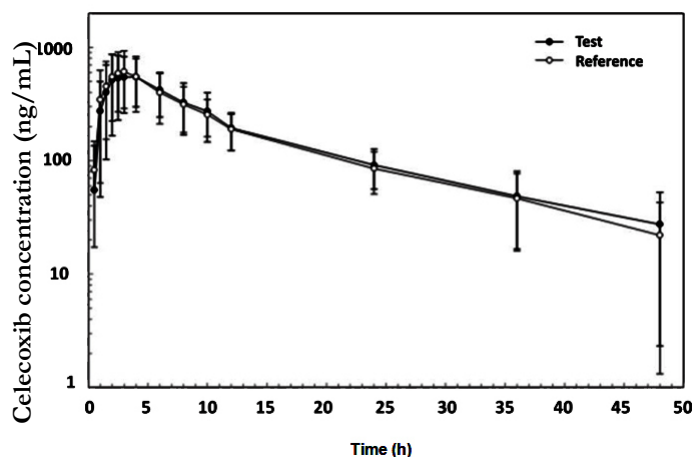| Parameter	Celecoxib		
Stability	Nominal conc. (ng/mL)	Mean conc. ± SD (ng/mL)	Accuracy (%)
Short-term[a]	15	14.2 ± 0.6	94.7
	1500	1512 ± 43.9	100.8
Long-term[b]	15	14.6 ± 0.2	99.3
	1500	1573 ± 45	103.1
Long-term[c]	15	15.0 ± 0.8	102
	1500	1548 ± 25	101.4
Freeze-thaw[d]	15	14.8 ± 0.2	98.7
	1500	1496 ± 63.0	99.7
Freeze-thaw[e]	15	14.6 ± 0.4	97.3
	1500	1570 ± 21.1	104.7
Process[f]	15	14.6 ± 0.4	97.3
	1500	1510 ± 75.2	100.7
Process[g]	15	13.8 ± 1.2	92
	1500	1481 ± 80.9	98.7

[a] At room temperature (25°C) for 26 h, [b] at -20°C for 36 days, [c] at -80°C for 36 days, [d] after four cycle at -20°C, [e] after four cycle at -80°C, [f] autosampler at room temperature for 48 h, [g] sample processing at room temperature.

Table 4. Demographic characteristics.

Characteristic	Total (n = 38)	Male (n = 22)	Female (n = 16)
Age, y	23.3 ± 4.4	24.2 ± 4.9	22.1 ± 3.2
BMI, kg/m²	21.8 ± 2.4	173.0 ± 6.0	159.1 ± 6.1
Weight, kg	61.4 ± 11.0	67.8 ± 8.5	52.6 ± 7.5
Height, cm	167.1 ± 9.1	22.6 ± 2.3	20.7 ± 2.1

*Data were shown as mean ± SD

Figure 3. The mean concentration-time profile of plasma samples on a semi-logarithmic scale.

and 12.9 ± 7.7 h. In addition, a total of four adverse events were reported by three subjects. Neither serious adverse event nor obvious abnormality of vital signs was observed throughout the study. Thereby, these results indicated that two formulations were well tolerated.

Statistical bioequivalence

In order to evaluate the bioequivalence between the two formulations, statistical analysis was performed for AUC_{0-t}, $AUC_{0-\infty}$, and C_{max}. The results are presented in Table 5. The 90% confidence intervals (CI) for the ratios (test versus reference) obtained using ln-transformed values of AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were 97.00%–108.85%, 98.01%–112.09%, and 93.20%–116.13%, respectively. In summary, the above-mentioned parameters showed no significant differences ($p > 0.05$) between the two formulations in the ANOVA assessment, and the 90% CI were within the acceptable range of 80–125% for bioequivalence evaluation.

Discussion

While avoiding the traditional NSAID-induced adverse effects, celecoxib showed clinical efficacy and tolerability equal to those

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Table 5. A summary of the pharmacokinetic parameters and confidence interval of two formulations of celecoxib (reference, R and test, T) after single oral administration in 38 healthy adult volunteers under fasting condition.

Parameter*	Test (T)	Reference (R)	Confidence interval (%)
AUC_{0-t} (hr·ng/mL)	7360.44 ± 1714.14	7267.48 ± 2077.68	97.00-108.85
$AUC_{0-\infty}$ (hr·ng/mL)	8197.45 ± 2040.31	7905.54 ± 2286.12	98.01-112.09
C_{max} (ng/mL)	705.30 ± 290.63	703.86 ± 329.91	93.20-116.13
MRT (hr)	18.7 ± 9.4	17.2 ± 10.3	-
T_{max} (hr)	3.4 ± 1.6	2.9 ± 1.4	-
$T_{1/2}$ (hr)	13.9 ± 7.9	12.9 ± 7.7	-
k_{el} (1/hr)	0.07 ± 0.03	0.07 ± 0.03	-

*Data were shown as mean ± SD

of the first COX-2 selective inhibitors. In contrast, many other COX-2 selective inhibitors identified after celecoxib have been withdrawn from the U.S. market due to the cardiovascular risks associated with long-term usage of these drugs. Thus, it is important to develop an interchangeable generic product of celecoxib in response to medical requirements. In the present study, 38 healthy volunteers completed the clinical trial for establishment of bioequivalence and evaluation of safety. We found that the mean C_{max} of celecoxib was 705.3 ± 290.6ng/mL and 703.9 ± 329.9ng/mL in the test and reference products, and these findings were consistent with previous reports that assessed the results obtained following oral administration of 200mg celecoxib [12, 16, 17]. Meanwhile, the T_{max} values of the two formulations were comparable to those obtained in earlier studies [18]. Above observations demonstrated that those formulations of celecoxib had similar absorption rates after oral administration. However, highly variable AUC values ranging from 5157 to 26630 h•ng/mL were observed in these findings, which may be related to hepatic metabolism differences associated with CYP2C9 polymorphism, which is predominantly responsible for celecoxib metabolism [19, 20]. Intriguingly, we found that C_{max} values obtained from younger women (age range, 20-32) in the present study showed 20% higher than those in men, which was in agreement with the data for elder women as stated in the medical label of celecoxib. This increase is believed to be attributed to the lower body weight of women. However, the present findings are different from those obtained in earlier reports, which indicated a 13% lower C_{max} and longer half-life in women [21].

Conclusion

A sensitive LC/MS/MS method for quantifying celecoxib in human plasma was validated effectively for the purpose of bioequivalence assessment of two celecoxib formulations. The results showed that the generic formulation was not only well tolerated, but also within the acceptable range of 80%–125% for bioequivalence validation. Therefore, on the basis of the data obtained from the present study, the test formulation exhibits therapeutic equivalence to the reference formulation. Thus, both formulations can be prescribed interchangeably.

Acknowledgements

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