BIOEQUIVALENCE STUDY OF DULOXETINE HYDROCHLORIDE 60 MG EC CAPSULES IN FASTING AND FED STATE IN HEALTHY THAI MALE VOLUNTEERS

Isariya Techatanawat[1,*], Pahweenvaj Ratnatilaka Na Bhuket[1], Polsak Teerawonganan[1], Ekawan Yoosakul[1], Vipada Khaowroongrueng[1], Wiriya Paisarnsinsook[1], Bancha Chuasuwan[1], Piengthong Narakorn[1], Archawin Rojanawiwat[2], Achara Eksaengsri[1]

[1] The Government Pharmaceutical Organization, Bangkok 10400, Thailand
[2] Clinical Research Center, Department of Medical Sciences, Ministry of Public Health, Nonthaburi 11000, Thailand

ABSTRACT

Duloxetine is one of serotonin (5-HT) and norepinephrine (NE) reuptake inhibitors (SNRIs). Because it is used extensively in both psychiatric and non-psychiatric conditions, a generic product of duloxetine hydrochloridehas been developed with lower price by the Government Pharmaceutical Organization (GPO) which would be benefit for patients. A randomized, open label, two-treatment, two-period, two-sequence, single dose, crossover, bioequivalence study of generic duloxetine 60 mg EC capsules of GPO, Thailand, and the reference product, Cymbalta®EC capsules, of Lilly S.A., Spain, in healthy human male adult subjects, under fasting and fed conditions was carried out. Washout period was 10 days between treatments. Blood samples were collected at predefined time points up to 72 hours. Plasma concentrations of duloxetine were analyzed using liquid chromatography tandem mass spectrometry. Non-compartmental model was used for pharmacokinetic analysis. The 90% CI for the ratios of mean $AUC_{0\text{-tlast}}$, $AUC_{0\text{-}\infty}$ and C_{max} for the test/reference in fasting condition were 102.3 (95.27-109.95), 102.5 (95.27-110.26) and 99.3 (92.69-106.32), respectively. For fed condition, the 90% CI for the ratios of mean $AUC_{0\text{-tlast}}$, $AUC_{0\text{-}\infty}$ and C_{max} for the test/reference were 98.9 (92.08-106.28), 98.8 (91.80-106.39) and 92.7 (85.18-100.90), respectively. These values were within the acceptable range of 80.00-125.00. Both the formulations were well tolerated. No clinically significant or serious ADRs were observed. Therefore, two formulations of duloxetine, Duloxetine GPO 60 mg EC capsules and Cymbalta®, were bioequivalent and can be used interchangeably.

Keywords: Duloxetine, Pharmacokinetics, Bioequivalence, Liquid chromatography tandem mass spectrometry

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INTRODUCTION

Duloxetine is one of serotonin (5-HT) and norepinephrine (NE) reuptake inhibitors (SNRIs). It is chemically described as (+)-(*S*)-*N*-methyl-γ-(1-napthyloxy)-2-thiophenepropylamine as shown in Figure 1. Because it is acid labile, a formulation that prevents duloxetine from degradation in the

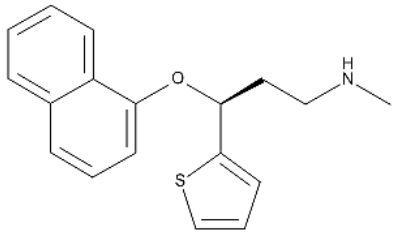

Figure 1 Chemical structure of duloxetine

* *Correspondence to: Isariya Techatanawat*
E-mail: isariya_t@gpo.or.th

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acidic environment of stomach such as enteric-coating is required. Duloxetine is well absorbed at the pH in small intestine. Food affects absorption of duloxetine by delaying time to maximum plasma concentration (t_{max}). However, food does not have significant effect on maximum plasma concentration (C_{max}) of duloxetine. Therefore, patients may take duloxetine with or without food [1].

Duloxetine has a high mean apparent steady-state volume of distribution (V_{ss}/F) of 1620 to 1800 L [1]. The high V_{ss}/F suggests that duloxetine is extensively distributed throughout the body. It is extensively metabolized to several oxidized and conjugated metabolites and primarily excreted *via* kidney [2].

Duloxetine is effective for major depression, generalized anxiety disorder, diabetic peripheral neuropathy, fibromyalgia, musculoskeletol pain and osteoarthritis [3, 4]. It is the most U.S. FDA-approved indications of any SNRIs [4]. Because it is used extensively in both psychiatric and nonpsychiatric conditions, a generic product of duloxetine hydrochloridehas been developed with lower price by the Government Pharmaceutical Organization (GPO) which would be benefit for patients. Subsequently, the bioequivalence study is conducted to demonstrate the interchangeability between the generic duloxetine hydrochloride and the reference product.

MATERIALS AND METHODS

Drugs

The test product (Duloxetine GPO 60 mg EC capsules) was manufactured from GPO (Batch number S540322, Manufactured on 23 Sep 2011, Expiry date 23 Sep 2013). The reference product (Cymbalta® EC capsules) was manufactured by Lilly S.A., Spain (Lot number A936155, Manufactured on Jun 2011, Expiry date May 2013).

Study design

A randomized, open label, two-treatment, two-period, two-sequence, single dose, crossover, bioequivalence study of generic duloxetine 60 mg EC capsules of the Government Pharmaceutical Organization, Thailand and the reference product, Cymbalta® EC capsules, of Lilly S.A., Spain in healthy human male adult subjects, under fasting and fed conditions was carried out. Washout period was 10 days between treatments. The study protocols of both fasting and fed conditions were approved by Institute for the Development of Human Research Protections (IHRP).

The order of receiving the test and reference products for each subject during both periods of the study were determined according to a randomization schedule which was generated with SAS® software.

Study subjects

Thirty two subjects for fed condition and 30 subjects for fasting condition, were randomly selected from healthy adult Thai male volunteers to participated this study. Number of subjects was determined by considering assumptions as Power ≥ 80%, Significance level = 5%, Bioequivalence limits = 80.00-125.00% with Intra-subject CV (%) ~25% based on literature reviewed. Subject inclusion criteria included age between 18-55 years and Body Mass Index (BMI) between 18-25 kg/m^2. All subjects were determined healthy judged from medical history, physical examination and laboratory examination (complete blood count, hematocrit, hemoglobin, fasting blood sugar, blood urea nitrogen (BUN), serum creatinine, alkaline phosphatase, ALT, AST, total bilirubin, total protein, albumin, hepatitis B test, urine analysis and ECG). The exclusion criteria included history of hypersensitivity to duloxetine or any of excipients, history of illness (like gastrointestinal, hepatic, renal, cardiovascular, diabetes mellitus, and gallstone disease), clinically significant illness within 4 weeks before the start of the study, asthma, urticaria or other allergic type reactions after taking any medication, alcohol abuse, moderate to heavy smoking, consumption of any medication (including over-the-counter products) for 14 days preceding the study, consumption of tea, coffee or xanthine products more than 3 cups/day within 24 hours prior to the first dose of study, participation in any other clinical trial involving drug administration and collection of blood samples or donation blood in the preceding one month prior to the start of the study. Subjects will be evaluated to withdraw from the study when emesis occurs at any time during label dosing interval after dosing of the study drug. The subjects were informed about risks and benefits of the study and signed informed consent before participating into the study.

Blood sampling

Fasting condition: Blood samples were collected into sodium heparin vacutainers by the indwelling catheter for 21 sampling times (0.000, 1.000, 2.000, 3.000, 4.000, 4.500, 5.000, 5.500, 6.000, 6.500, 7.000, 7.500, 8.000, 9.000, 10.000, 12.000, 16.000, 24.000, 36.000, 48.000 and 72.000 hours). The blood samples were centrifuged at 3000 ± 100 rcf for 5 minutes at 10 °C. All plasma samples were

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Table1 The summary of validation results

Information requested		Data
Linearity (Range)		0.505 to 101.933 ng/mL
Selectivity		No interference at the retention time and transition of drug and internal standard.
Selectivity in presence of co-administered drugs		No interference at the retention time and transition of drug and internal standard.
Verification of interfering potential by co-administered drugs		No interference with acceptable precision and accuracy.
Coefficient of determination (r^2)		Greater than 0.98
Lower limit of quantification		0.505 ng/mL
Limit of detection		0.051 ng/mL
Precision	Within-batch (Intra-day precision)	1.1% to 7.6%
	Between-batch (Inter-day precision)	4.8% to 7.8%
Accuracy	Within-batch (Intra-day accuracy)	86.3% to 100.9%
	Between-batch (Inter-day accuracy)	94.5% to 98.3%
Robustness and Ruggedness		Method is rugged and robust (up to 150 injections)
Recovery of drug (%) (HQC, MQC, LQC)		105.2%, 107.3%, 101.9%
Recovery of internal standard (%)		107.5%
Dilution integrity		152.899 ng/mL diluted 2 and 10 fold
Partial volume verification		77.042 ng/mL diluted 2 and 4 fold
Matrix effect		No ion suppression or enhancement
Reinjection reproducibility		Up to 3rd reinjection
Auto sampler/ Wet extract stability		92.0 hours (within 2 to 8°C)
Dry extract stability		90.0 hours (at -22±5°C)
Freeze and thaw stability		3 cycles
Bench top stability		6.0 hours (at room temperature)
Wet extract bench top stability		2.0 hours (at room temperature)
Dry extract bench top stability		2.0 hours (at room temperature)

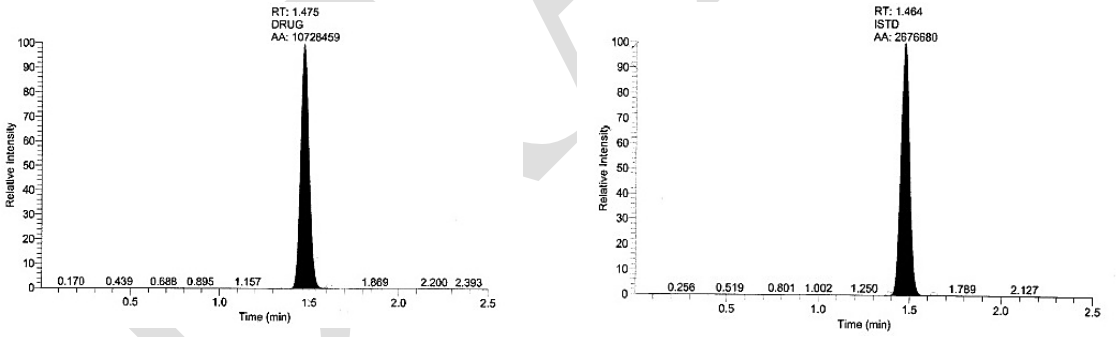

Figure 2 Representative chromatograms of duloxetine and internal standard

transferred to pre-labeled polypropylene tubes and stored frozen at -65 ± 10 °C until analysis.

Fed condition: Blood samples were collected into sodium heparin vacutainers by the indwelling catheter for 21 sampling times (0.000, 1.000, 2.000, 3.000, 4.000, 5.000, 6.000, 7.000, 8.000, 9.000, 9.500, 10.000, 10.500, 11.000, 12.000, 14.000, 16.000, 24.000, 36.000, 48.000, and 72.000 hours). Sample preparation, handling and storage were processed as same as in fasting condition.

Analytical procedure

The plasma concentrations of duloxetine in study samples were determined by a validated LC-MS/MS method using duloxetine-d7 as an internal standard. The US FDA guidance for industry, bioanalytical method validation and the European Medicines Agency guideline on bioanalytical method validation were followed. The summary of validation results and representative chromatograms of analyte and internal standard are shown in Table 1 and Figure 2, respectively. The analyte and internal standard were extracted from plasma using liquid-liquid extraction techniqueand monitored in the positive ion mode using ESI probeat MRM transitions of m/z 298.100→154.100 and m/z 305.150→154.100 for analyte and internal standard, respectively. The chromatographic

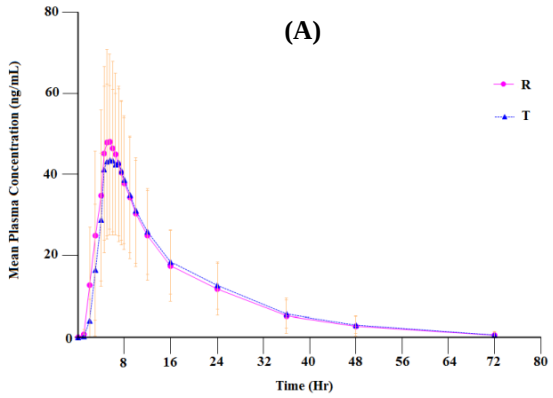

Figure 3a Linear plot of mean (±SD) plasma concentration of duloxetine versus time curves after oral administration of test product-T and reference product-R in healthy Thai male volunteers under fasting conditions (N = 24)

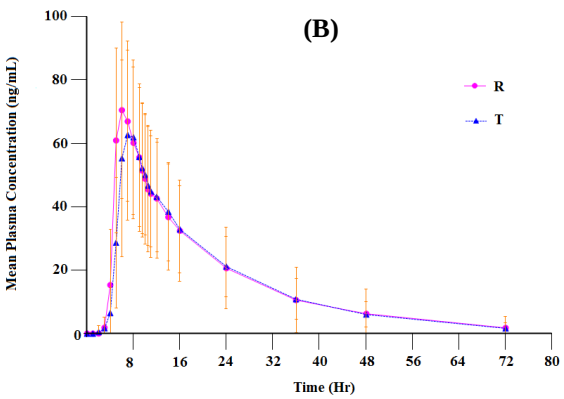

Figure 3b Linear plot of mean (±SD) plasma concentration of duloxetine versus time curves after oral administration of test product-T and reference product-R in healthy Thai male volunteers under fed conditions (N = 30)

Table 2 The pharmacokinetic parameters of the test and reference products for both fasting and fed conditions

Condition	Product	$AUC_{0\text{-}tlast}$ (ng.hr/mL)	$AUC_{0\text{-}\infty}$ (ng.hr/mL)	C_{max} (ng/mL)	t_{max} (hr)	λ_z (1/hr)	$t_{1/2}$ (hr)
Fasting	Test	734.285 ±312.7489	757.394 ± 318.9508	50.398 ± 20.1198	5.000 (4.500-7.500)	0.065 ± 0.0139	11.027 ± 2.2583
	Reference	733.480 ± 367.9644	757.065 ± 381.1285	52.017 ± 23.3665	5.000 (4.500-7.500)	0.066 ± 0.0131	11.067 ± 2.8447
Fed	Test	1139.002 ± 499.3392	1177.155 ± 528.4688	71.048 ± 27.7275	7.000 (5.000-10.000)	0.059 ± 0.0122	12.282 ± 2.5462
	Reference	1183.959 ± 706.5836	1234.446 ± 804.7746	75.578 ± 26.5869	6.000 (5.000-9.000)	0.060 ± 0.0126	12.010 ± 2.8838

system consisted of ACE 3 C18 100 x 4.6 mm column. The mobile phase was a mixture of 2 mM ammonium formate (pH 3.00) and acetonitrile (10:90%v/v) with a flow rate of 0.9 mL/min at 40°C.

Pharmacokinetic analysis

The pharmacokinetic parameters ($AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$, C_{max}, t_{max}, λ_z and $t_{1/2}$) were determined by non-compartmental model using Phoenix WinNonlin Software Version 6.3. Values below lower limit of quantification (0.505ng/mL) were set as zero for calculation purposes.

Statistical analysis

The statistical analysis was conducted using SAS® Version 9.3. The primary pharmacokinetic parameters ($AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$ and C_{max}) were transformed to natural logarithm scale (ln) before statistical analysis. Bioequivalence of Test Product-T vs. Reference Product-R was concluded, if the 90% confidence interval of ratio of geometric least square mean fell within the acceptance range of 80.00-125.00% for ln-transformed pharmacokinetic parameters $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$ and C_{max} of duloxetine.

RESULTS AND DISCUSSION

For fasting state, 30 enrolled subjects had a mean age of 27.47±6.53 years and an average BMI of 21.92±1.95 kg/m^2. Only 24 subjects were used for pharmacokinetic and statistical calculation. Five of enrolled subjects were withdrawn from the study due to vomiting after drug administration (four for test product and one for reference product). One subject was withdrawn due to severe headache. However, safety evaluation was shown to be safe for the rest subjects without any serious ADR throughout the study period. In fed state, 32 subjects with an average age of 29.69±7.94 year and a mean BMI of 21.64±1.93 kg/m^2 were recruited. Two subjects were dropped out from the study due to personal reason. Both the test and the reference products were well tolerated. No clinically significant or serious ADRs were observed.

The mean plasma concentrations versus time profiles of duloxetine are depicted in Figure 3a and 3b for fasting and fed states, respectively. The pharmacokinetic parameters including $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$, C_{max}, t_{max}, λ_z and $t_{1/2}$ of the test and reference products for both conditions are shown in Table 2.

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Table 3a 90% Confident intervals of the ln-transformed primary pharmacokinetic parameters for fasting state

Parameters	Ratio	90% CI
$AUC_{0\text{-}tlast}$	102.3	95.27-109.95
$AUC_{0\text{-}\infty}$	102.5	95.27-110.26
C_{max}	99.3	92.69-106.32

Table 3b 90% Confident intervals of the ln-transformed primary pharmacokinetic parameters for fed state

Parameters	Ratio	90% CI
$AUC_{0\text{-}tlast}$	98.9	92.08-106.28
$AUC_{0\text{-}\infty}$	98.8	91.80-106.39
C_{max}	92.7	85.18-100.90

In a randomized, open-label, single- and multiple-dose bioequivalence study in 30 healthy Chinese volunteers under fasting conditions, revealed that C_{max}, $AUC_{0\text{-}\infty}$ and t_{max} of single dose of 60 mg of duloxetine administration were 44.40±17.18 ng/mL, 733.82±343.40 ng.hr/ml and 6.10±1.29 hr, respectively[5]. In pharmacokinetics study of single oral dose of duloxetine 60 mg and Caucasian subjects shown that C_{max}(90%CI), AUC(90%CI) and $t_{1/2}$(90%CI) in Japanese subjects were 45.6(39.6, 52.5) ng/mL, 714(595, 857) ng.hr/ml and 10.9(10.2, 11.8) hr, respectively, and in Caucasian subjects were 38.7(33.6, 44.6) ng/mL, 584(486, 702) ng.hr/ml and 10.1(9.41, 10.9) hr, respectively [6].

The 90% confidence intervals for $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$ and C_{max} in ln-transformed unit were within the bioequivalence range of 80.00 – 125.00% as presented in Table 3a for fasting condition and 3b for fed condition. These results indicated that the test product was bioequivalent to the reference product.

The power of all primary pharmacokinetic parameters including as $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$ and C_{max} for fasting state were 99.0, 99.0 and 100.0, respectively. For fed state, the power for $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$ and C_{max} were 99.9, 99.9 and 99.5, respectively. Because all reported powers of primary pharmacokinetic parameters were greater than 80%, it suggested that the number of subjects was enough to confirm the bioequivalency between the two formulations.

CONCLUSION

The test product, Duloxetine GPO 60 mg EC capsules, when compared with the reference product, Cymbalta®60 mg EC capsules, met the bioequivalence criteria of 80.00-125.00% with respect to the rate and extent of absorption in both fasting and fed conditions. Both products were well tolerated. Therefore, Duloxetine GPO 60 mg EC capsules can be used interchangeably to Cymbalta®60 mg EC capsules because of their bioequivalency.

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