

Original article

BIOEQUIVALENCE STUDY OF GENERIC PENTOXIFYLLINE

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Abstract The bioequivalence of two oral formulations of pentoxifylline were evaluated. The two products were administered as a single oral dose in a randomized two-way crossover design to 12 healthy Thai male volunteers. The washout period between each treatment was 1 week. After drug administration, serial blood samples were collected over a period of 30 hours. Plasma pentoxifylline concentrations were measured by HPLC with UV detection. The pharmacokinetic parameters were analyzed by non-compartmental analysis. **RESULTS:** The maximum pentoxifylline concentrations (C_{max}, ng/mL), median time to reach the C_{max} (T_{max}, hr) for the test and the reference were 225.5 (range 374.9–111.1), 1.0 (0.75–2.0) and 218.4 (390.4–133.7), 0.88 (0.5–1.5), respectively. Analysis of variance for bioequivalence was carried out using logarithmically transformed AUC 0–∞ and C_{max}. The mean (90% CI) of the AUC 0–∞ and C_{max} ratios for the Test : Reference were 0.99 (0.81–1.22) and 1.02 (0.91–1.15), respectively. These values were within the bioequivalence range of 0.80–1.25, thus, our study demonstrated the bioequivalence of the test and reference. **Chiang Mai Med Bull 2003;42(1):7-16.**

Keywords : Bioequivalence, pentoxifylline

Pentoxifylline is a synthetic xanthine derivative used for the treatment of chronic occlusive arterial diseases and intermittent claudication.⁽¹⁾ The drug does not act as a vasodilator, however, it improves the flow of blood primarily by improving erythrocyte flexibility and decreasing blood viscosity.⁽²⁾ The inhibiting of platelet aggregation and a decreasing concentration of fibrinogen may also be involved.⁽¹⁻²⁾ An increasing blood flow to affected circulation

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enhances tissue oxygenation and nutrient supply. Moreover, the improvement of circulation results in greater walking distance and the relief of pain, paresthesia and nocturnal leg cramp. The success of treatment usually occurs at 2–6 weeks after the initiation of therapy. Pentoxifylline can be used in the treatment of other vascular disorders, including those associated with diabetes and cerebrovascular insufficiency. Improvements in symptoms such as impaired concentration and memory as well as dizziness and low drive have been reported.⁽²⁾

Pentoxifylline is completely absorbed after oral administration. It undergoes extensive first-pass metabolism and its oral bioavailability is only 20–30%.⁽³⁾ The plasma levels of its major metabolites (Metabolite I, Metabolite V) are 5 to 8 times greater than the parent compound. Peak plasma levels of pentoxifylline and its metabolites are reached within 1 hour.⁽¹⁾ Excretion is mainly via the kidney with an elimination half-life ranging from 0.4–0.8 hours.⁽¹⁾ Oral controlled-release preparations of pentoxifylline have been developed to compensate for its rapid absorption and elimination. These products continuously release an active substance that results in a constant absorption and an improvement in gastrointestinal tolerance. The time to reach the peak plasma levels and the half-life of pentoxifylline are delayed to within 2–4 hours and 3–4 hours, respectively.⁽³⁾ The recommended dosage is 400–mg as a controlled-release tablet taken 2–3 times daily with meals. Food slows the rate, but does not change the extent of

pentoxifylline absorption.⁽⁴⁾ Although pentoxifylline is generally well tolerated, adverse effects such as gastrointestinal symptoms (nausea, vomiting, and dyspepsia) occur in 1%–3% of patients.⁽²⁾ Central nervous system side effects (headache, dizziness) are dose-related and require dosage reduction. Flushing, tachycardia, angina pectoris, hypotension and hypersensitivity reactions are rarely reported. Pentoxifylline is contraindicated in patients with recent cerebral and/or retinal hemorrhage as well as in those previously intolerant to this product or methylxanthine derivatives such as caffeine, theophylline and theobromide.⁽¹⁾ Also the drug should not be administered during pregnancy.

Objective

The objective of the study was to assess the bioequivalence of generic pentoxifylline preparations at 400-mg after a single oral dose had been administered in 12 healthy Thai male volunteers.

Materials and methods

Subjects

Twelve healthy Thai male volunteers aged between 20–27 years old, with a body mass index within 18–24, participated in the study. Subjects had abstained from any medication for at least one month prior to the study. Furthermore, subjects were free from medical illnesses judged by physical examination and a routine blood test including complete blood count with differential count and blood chemistry

profiles. The hepatitis B surface antigen, anti-hepatitis-C antibody and anti-HIV were also negative on screening. Cigarette smokers, alcohol consumers as well as subjects currently taking any drug known to induce or inhibit hepatic metabolizing enzymes were excluded from the study. All subjects signed the written informed consent before participating in the study. The Medical Ethic Committee of Chiang Mai University, Thailand approved the study.

Study drugs

The test drug was Pentoxy[®] (400 mg) manufactured by The Biolab Company, Bangkok, Thailand (LOT. PTX-21/coat 11). The reference was Trental[®] 400 manufactured by The HANDOK Pharmaceuticals Co. Ltd, Chungchongbuk, S. Korea (Under license from *Aventis Pharma*, Batch H026B MFG. 20/11/2000 EXP. 19/11/2005).

Study design

1. Method of drug administration :

This was a randomized, two-sequence, two-period crossover study. Each subject was randomly assigned to receive a single dose of pentoxifylline at 400 mg orally, in either the test or reference, during the morning after an overnight starved. Subjects remained fast for at least 2 hours after drug administration. Water and lunch were served at 2-hours and 4-hours consecutively after receiving the study drug. Drinks containing either alcohol or caffeine were not allowed during the study period. Blood samples were collected immediately before and at

0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, 24, and 30 hours after dose administration. Within 30 minutes of taking blood, the samples were centrifuged to separate the plasma. The plasma samples were immediately kept at -20°C until assay. One week after the previous visit, subjects were crossed over to receive another pentoxifylline preparation. The food and water intake were identical in the two study visits.

2. Determination of the plasma pentoxifylline concentrations :

Pentoxifylline and internal standard (IS = chloramphenicol) in the plasma were quantified by high performance liquid chromatography (HPLC) after a liquid-liquid extraction.⁽⁵⁾ The plasma sample was thawed and vortexed for 10 seconds, thereafter, 0.50 mL of the plasma was measured into a Teflon lined screw-capped glass tube. This was followed by adding 50 μL of IS (3.0 $\mu\text{g}/\text{mL}$) and 4 mL of the extraction solvent, dichloromethane. The glass tube was then capped tightly and the mixture was vortexed for 1 minute. After that, the glass tube was centrifuged at 3,500 rpm for 10 minutes. The organic layer was transferred into a 5 mL reactival and evaporated at 35°C under a gentle stream of nitrogen gas until dry. The residue was reconstituted with 100 μL of mobile phase and vortexed for 30 minutes, thereafter, 50 μL was injected into the analytical column (Zorbax SB-C 18, StableBond Analytical, 4.6x250 nm, 5-micron [Agilent Technologies, USA]). The mobile phase comprised 0.02 M phosphoric acid adjusted to pH 4, methanol and tetrahydrofuran (55 : 45 :

1,v/v). An analysis was run at a flow-rate of 1.4 mL/min with a UV detector operated at a wavelength of 273 nm. The retention time for pentoxifylline and IS were 7.1 and 11.6 minutes, respectively. The calibration curve was linear over a concentration range of 12.5–400.0 ng/mL. The linear regression analysis of the peak–height ratio of [pentoxifylline / IS] VS pentoxifylline concentration consistency gave determinant (R^2) coefficients of 0.999 or better. Pentoxifylline concentrations were quantified from the calibration standard lines with the use of linear regression. The method was specific and sensitive, with a detection limit of 12.5 ng/mL. Mean recovery value of the extraction procedure was about 99.9%, while the within-day and between-day coefficient of variation and percent age error values of the assay method were less than 10.0%.

3. Statistical methods and data analysis :

Pharmacokinetic analysis : Maximal plasma concentration (C_{max} , ng/mL) and time to reach the peak concentration (T_{max} , hr) were obtained directly by the visual inspection of each subject's plasma concentration-time profile. The area under the plasma concentration-time curve (AUC), from time 0-infinity ($AUC_{0-\infty}$, ng*hr/mL) and half-life ($T_{1/2}$, hr), were determined by non-compartmental analysis. The slope of the terminal log-linear portion of the concentration-time profile was determined by least-squares regression analysis and used as the elimination rate constant (K_e). The elimination half-life was calculated as

$0.693/K_e$. The AUC_{0-t} from time zero to the last quantifiable point (Ct) was calculated using the trapezoidal rule and the extrapolated AUC from Ct to infinity ($AUC_{t-\infty}$) was determined as Ct/K_e . Total AUC was the sum of $AUC_{0-t} + AUC_{t-\infty}$. The TopFit pharmacokinetic data analysis program for PC was used for the calculation.⁽⁶⁾

Statistical analysis⁽⁷⁻⁹⁾

An analysis of variance (ANOVA) was performed to determine the statistical differences of pharmacokinetic parameters ($AUC_{0-\infty}$, C_{max} , and T_{max}). Statistical of AUC and C_{max} was analysis performed on the logarithmically (ln) transformed data. The 90% confidence interval for the ratio of AUC, as well as C_{max} values of the test preparation over those of the reference product, were estimated using the following equation :

$$90 \% \text{ CI } (\mu_T - \mu_R) = (\bar{X}_T - \bar{X}_R) \pm t_{0.1}^v \sqrt{\frac{2S^2}{n}} \quad \text{where } X_T \text{ and } \bar{X}_R \text{ are the}$$

observed means of the (ln) transformed parameters (either C_{max} or AUC) for the test product (T) and references (R); S^2 is the error variance obtained from the ANOVA; n is the number of subjects; $t_{0.1}^v$ is the tabulated two-tail t value for 90% CI; and v is the degree of freedom of the mean square error. The antilogarithm of the 90% CI ($\mu_T - \mu_R$) expresses the bioequivalence as a ratio of the test product and reference [μ_T/μ_R].

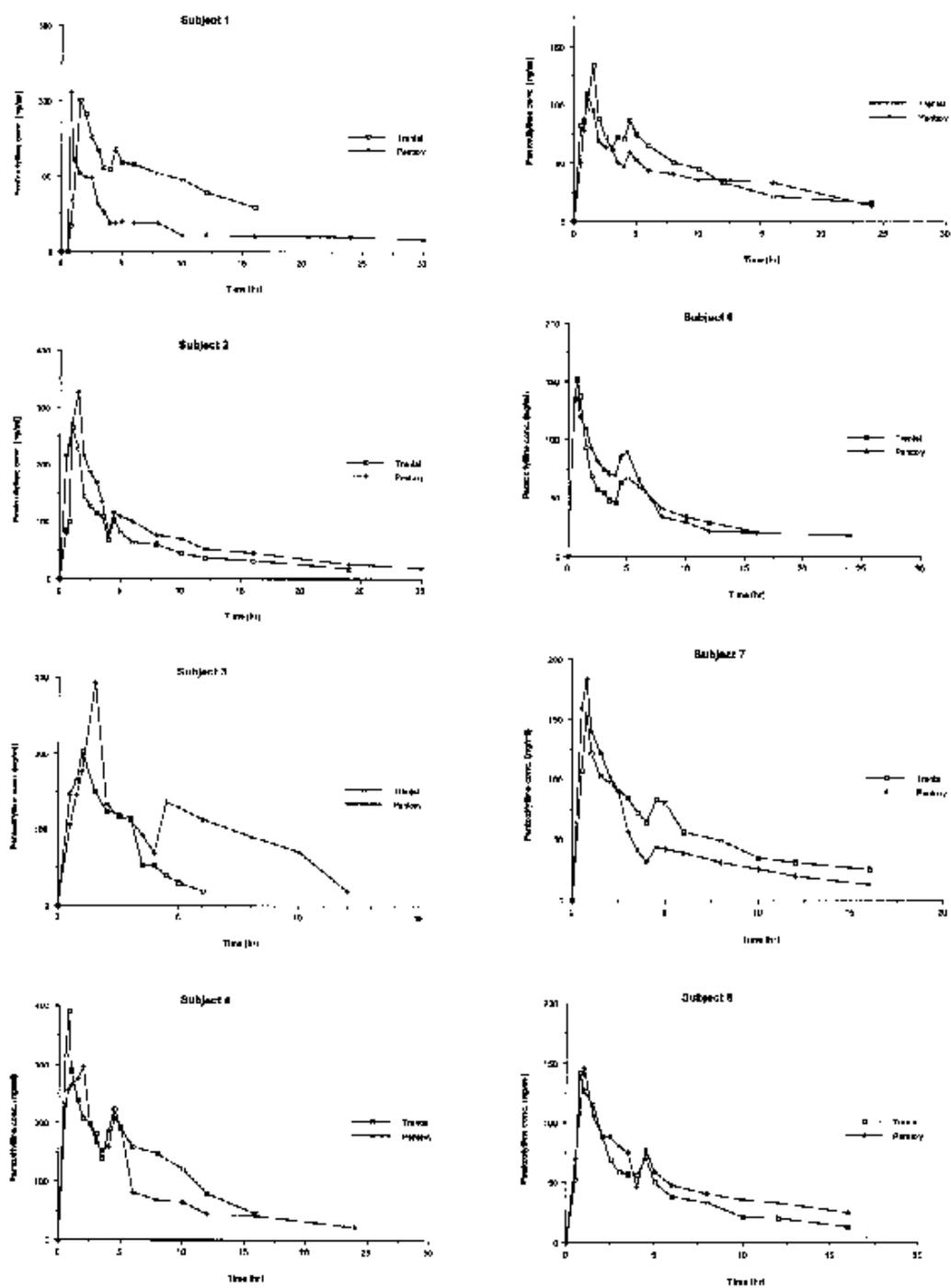


Figure 1. Pairwise intraindividual comparison of plasma concentration-time profiles after single oral administration of the reference - - and the test -◆-.

Bioequivalence acceptance criteria

The bioequivalence intervals of 0.8–1.25 for the ratio test : reference of the average $AUC_{0-\infty}$ and C_{max} were accepted by the Thai FDA. Regarding analysis of T_{max} , the limit for the bioequivalence range was expressed as untransformed data, and the bioequivalence range of T_{max} difference [Test-Reference] was $\pm 20\%$ of the T_{max} of the reference formulation.

Result and discussion

All subjects completed the study without any adverse events. Pair wise presentation of individual plasma concentration-time profiles, as well as those of mean plasma, of the reference drug and the test product are depicted in Fig. 1 and

Fig. 2, respectively. The mean plasma concentration-time curves of the reference drug and test product were comparable, although the peak pentoxifylline concentration of the test was slightly higher than that of the reference drug. Similarly, the pair wise intraindividual concentration-time profiles of the test and reference were relatively similar, except in subject No. 1, where plasma concentration-time curves of the reference were higher than those of the test, and vice versa for subject No 3. The relative bioavailability (Frel) of AUC (T/R) was 0.50 and 2.34 for subject No. 1 and 3, respectively. Table 1 compares individual calculated pharmacokinetic parameters (C_{max} , T_{max} , $AUC_{0-\infty}$ and $T_{1/2}$) of the reference and test product. Following

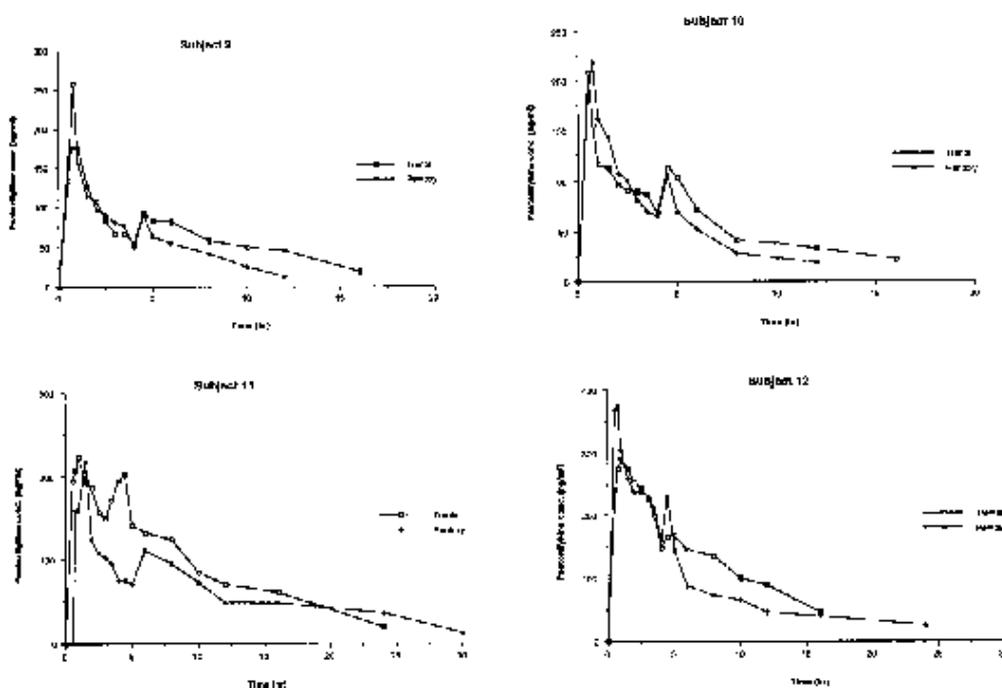


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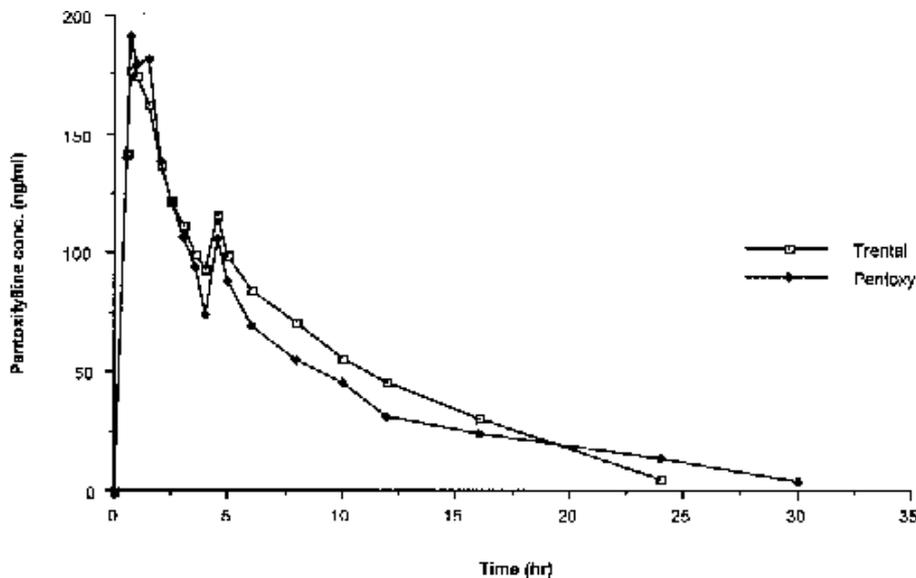


Figure 2. Mean plasma concentration-time profiles after single oral administration of the reference - - and the test -◆-.

a single oral dose, the median time to reach the T_{max} for the test (1.0 hr, range 0.75–2.0 hr) was slower than that of the reference (0.88 hr, range 0.5–1.5 hr). The 90% CI for the T_{max} difference ($\mu_T - \mu_R$) ranged from -0.11 to 0.44 hour, outside the stipulated bioequivalence range of ± 0.19 hour. Therefore, the equivalence with respect to the T_{max} could not be concluded. In spite of this, the average \pm SD of the C_{max} and $AUC_{0-\infty}$ for the test were not significantly different from those of the reference drug (225.53 ± 80.61 v.s. 218.42 ± 74.32 ng/mL, and 1481.13 ± 644.1 v.s. 1528.85 ± 721.8 ngh/mL, respectively). Moreover, the C_{max} of pentoxifylline obtained from this study

was comparable to the values reported in the literature [average C_{max} 266.35 ± 36.0 and 326.38 ± 39.8 ng/mL].⁽¹⁰⁾ The average, relative bioavailability (F_{rel}) calculated for the C_{max} and $AUC_{0-\infty}$ of test : reference was 104% and 107%, respectively. Bioequivalence analysis (Table 2) showed that the mean (90 % CI) of the C_{max} and $AUC_{0-\infty}$ ratios for test : reference were 1.02 (0.91–1.15) and 0.99 (0.81–1.22), respectively. Since these values were well within the bioequivalence range of 0.80–1.25, our study demonstrated the bioequivalence of the generic pentoxifylline.

Table 1. Comparison of pentoxifylline pharmacokinetic parameters after oral administration of the test (T) and the reference (R).

Subject No	Tmax* (h)		Cmax (ng/mL)		AUC (ng.h/mL)		F _{rel} (%)	T _{1/2} (hr)	
	T	R	T	R	T	R		T	R
1	0.75	10.60	211.19	200.34	1149.52	2,318.78	0.50	10.60	8.85
2	1.50	9.75	327.03	264.79	2300.35	1,648.96	1.40	9.75	11.40
3	1.50	4.43	292.39	203.74	1347.71	575.73	2.34	4.43	1.56
4	2.00	12.20	296.20	390.39	2398.03	2,527.34	0.95	12.20	4.20
5	1.00	11.20	111.12	133.74	1148.12	1,203.78	0.95	11.20	7.83
6	0.75	8.86	150.75	151.77	1191.66	964.79	1.24	8.86	6.54
7	0.75	6.29	183.51	154.27	804.24	1,118.95	0.72	6.29	6.49
8	1.00	8.19	145.51	141.47	1108.86	811.93	1.37	8.19	8.06
9	1.00	3.10	178.59	257.80	812.97	1,124.99	0.72	3.10	5.02
10	0.75	6.81	219.15	209.04	952.92	1,125.87	0.85	6.81	4.90
11	1.50	10.70	216.05	223.31	2,022.76	2,385.64	0.85	10.70	6.31
12	0.75	11.30	374.91	290.41	2536.46	2,539.49	1.00	11.30	5.20
Mean	1.00	9.31	225.53	218.42	1481.13	1,528.85	1.07	8.62	6.36
SD	0.42	2.91	80.61	74.32	644.10	721.78	0.48	2.91	2.52
% CV	37.96	33.76	35.74	34.03	43.49	47.21	44.82	33.76	39.55
Min	2.00	12.20	374.91	390.39	2536.46	2,539.49	0.50	12.20	11.40
Max	0.75	3.10	111.12	133.74	804.24	575.73	2.34	3.10	1.56

T_{max} presented as median values

Table 2. Parametric 90% CI of the ratio test : reference of the pharmacokinetic parameters (AUC_{0-∞}, C_{max} and T_{max}).

PK parameters	Mean	90% CI	Acceptable range
AUC _{0-∞} (test : reference)	0.99	0.81-1.22	0.80-1.25
C _{max} ((test : reference)	1.02	0.91-1.15	0.80-1.25
T _{max} (test : reference)	0.17	(-0.11) – 0.44	± 0.19

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การศึกษาชีวสมมูลของยาสามัญเพนทอกซิฟลีน

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บทคัดย่อ การศึกษาชีวสมมูลของยาเพนทอกซิฟลีน ขนาด 400 มก. ในอาสาสมัครชายไทยสุขภาพดี โดยอาสาสมัครแต่ละคนจะได้รับการสุ่มไขว้เพื่อรับประทานยา ทั้งยาต้นแบบและยาทดสอบ โดยรับประทานยาหนึ่งครั้งหลังจากงดน้ำและอาหาร ระยะเวลาการศึกษาห่างกัน 1 สัปดาห์ ตัวอย่างเลือดจะเก็บตามเวลาที่กำหนดในช่วงเวลา 30 ชม. หลังจากรับประทานยา และนำไปตรวจวัดหาความเข้มข้นของยาเพนทอกซิฟลีน โดยวิธีโครมาโตกราฟี และประเมินค่าทางเภสัชจลนศาสตร์โดยวิเคราะห์แบบ non compartment ผลการศึกษาพบว่า ระดับยาในเลือดสูงสุดและเวลาที่ระดับยาในเลือดสูงสุดของยาทดสอบและยาต้นแบบมีค่าเท่ากับ 225.5 และ 218.4 นาโนกรัมต่อมิลลิลิตร เวลา 1.0 และ 0.88 ชั่วโมงหลังรับประทานยาตามลำดับ การวิเคราะห์ทางสถิติโดยใช้เอชไอเอ (ANOVA) พบว่าค่าเฉลี่ย (ช่วงความเชื่อมั่นร้อยละ 90) ของอัตราส่วน [ยาทดสอบ / ยาต้นแบบ] ของพื้นที่ใต้กราฟที่เวลา 0 ถึงสองไขว้ และความเข้มข้นสูงสุดของยาในเลือดมีค่าเท่ากับ 0.99 (0.81–1.22) และ 1.02 (0.91–1.15) ตามลำดับ ซึ่งอยู่ในช่วงของชีวสมมูลที่ยอมรับคือ 0.80-1.25 จากการศึกษาครั้งนี้สรุปได้ว่ายาทดสอบและยาต้นแบบมีชีวสมมูลเท่าเทียมกัน *เชียงใหม่เวชสาร 2546;42(1):7-16.*

คำสำคัญ : ชีวสมมูล เพนทอกซิฟลีน
