MATERIALS AND METHODS

Drug formulations:

Drugs	Manufacturer	Preparation	Lot No
Theo-Dur®	ASTRA Sodertaje, Sweden [Astra, OLIC (Thailand) Limited]	200 mg tablet	B. AB 558
Xanthium®	SMB Technology, Galephar, Belgium [Berlin Pharmaceutical Industry, Bangkok Thailand]	400 mg capsule	98D03
Uni-Dur®	Schering-Plough Products Inc, Puerto Rico, USA [Schering-Plough Ltd, Bangkok, Thailand]	400 mg tablet	91058

The drugs used in this study were donated by the manufacturers.

Subjects:

Since gender was a factor affecting theophylline pharmacokinetics[25], only male volunteers were enrolled in this study. A total of 13 healthy Thai volunteers who ranged in age from 18-30 years (average 22.7 ± 3.5 years) participated. Weight and height of the subjects ranged from 55-68 kg (60.7 ± 4.7) and 160-180 cm (170.3 ± 5.6), respectively. Subjects were judged healthy based on medical history, physical examination, routine blood chemistry and urinalysis. None had a history or evidence of disease especially kidney, liver, and hematological diseases. The laboratory tests included complete blood count with differentials, fasting blood sugar, blood urea nitrogen, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and bilirubin. The demographic characteristics of all subjects are

shown in Table 1. Subjects were refrained from any medication and xanthine-containing foods or beverages for 1 week before, during and 1 week after the study period. Cigarette smokers, alcohol consumer subjects were excluded from the study. Subjects were enrolled to the study after given written informed consent.

Table 1. Demographic characteristics of 13 male subjects in this study.

Characteristics	Subject number													mean ± SD
	1*	2*	3*	4	5	6*	7*	8	9*	10	11*	12*	13*	
Age (yr)	27	26	24	22	25	18	19	23	20	21	30	20	20	22.7 ± 3.5
Height (cm)	165	160	164	171	164	167	180	178	171.5	171.5	173	176	173	170.3 ± 5.6
Weight (kg)	65	55	65	60	55	65	68	58	60	55	67	57	60	60.7 ± 4.7
Hct (40-50%)	47	47	40	44.5	44.2	41	47	41.8	46	46.3	41	42.3	38.3	41.6 ± 8.9
Hb (10-15 g/dl)	16.1	16.8	12.5	14.4	14.9	13	15.7	14	15.6	16.2	14	14.4	13	14.7 ± 1.4
WBC (5000-10,000 /mm^3)	9,295	11,180	15,210	10,600	7,800	8,125	11,115	6,400	9,100	5,800	4,900	6,500	6,200	8,632 ± 2869
Platelets	Adequate													
AST (3-37 u/l)	25	29	30	18	27	27	30	19	26	15	17	24	14	23.1 ± 5.8
ALT (7-42 u/l)	18	31	41	20	71	16	27	16	25	8	12	34	7	25.1 ± 17.1
Alk. phosphatase (21-128 u/l)	178	169	175	67	42	214	252	76	152	78	84	93	78	127.5 ± 65.6
Total bilirubin (0.2-1.1 mg/dl)	0.5	0.6	0.5	1.51	1.18	1.1	0.6	0.97	0.6	2.12	1.02	0.71	1.27	0.98 ± 0.47
Direct bilirubin (0-0.3 mg/dl)	0.1	0.1	0.1	0.55	0.33	0.2	0.1	0.79	0.1	0.75	0.43	0.31	0.52	0.34 ± 0.25
BUN (7-24 mg/dl)	15.2	13.9	14.6	12	12	15.3	17.3	18	14.8	9	14	16	19	14.7 ± 2.7
Creatinine (0.6-1.6 mg/dl)	1	1	0.9	1.2	0.7	1.1	0.9	1.2	1	1.1	1.2	1.4	1	1.05 ± 0.18
FBS (70-110 mg/dl)	86	79	82	85	81	100	88	97	77	95	62	112	87	87.0 ± 1.3

* From Lanna Medical Laboratories Co., Ltd. Normal value for AST 0-37, ALT 0-40, Alk. phosphatase 91-258 u/l.

Study Design

Prescreening for Theophylline clearance

All volunteers were screened for theophylline clearance rate. After an overnight fast they received 400 mg Franol® (Sanofi, Zuellig Thailand), the rapid release theophylline formulation with 240 ml of water. Serial blood samples were collected at predose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hr after drug administration. Pharmacokinetic parameters were obtained with the aid of TopFit 2.0, the pharmacokinetic data analysis program. The theophylline clearance was calculated as dose (=400 mg) /$AUC_{0-\infty}$. Ten subjects with average theophylline clearance of 0.30–0.80 ml/min/kg were selected for the subsequent multiple dose study.

Multiple dose study

Ten subjects participated in this multiple dose study. The study was an open, randomized crossover design with one-week washout period. The assigned treatments were; A: Uni-Dur® (400 mg tablet), B: Theo-Dur® (2 × 200 mg tablets) and C: Xanthium® (400 mg capsule). The randomized schedule of drug administration is shown in Table 2. Each subject received once-daily dosing of one of the SRT product at 07.00 AM after an overnight fast and 2 hours prior to breakfast for 7 consecutive days (Day1 – Day7). Thereafter, they were free from study medication for a week. After a week each subject was cross over to receive the different SRT product in the same manner.

Venous blood samples (3 ml) were collected prior to the morning doses on Day4 and Day5 to ascertain steady state. On the study Day6 and Day7 after an overnight fast at 07.00 AM subjects were admitted at the Clinical Pharmacology Unit

of the Faculty of Medicine, Chiang Mai University. Subjects were given a single dosing of 400 mg SRT (either Uni-Dur®, Theo-Dur® or Xanthium®) with 240 ml of water. After dosing, subjects were fasted for 2 hours. Venous blood samples (3 ml) were collected before the morning doses and at 2, 4, 6, 7, 8, 9, 10, 11, 12, 13, 15 and 24 hours after drug administration. Meal and fluid intake were identical for all study visit. Alcohol and xanthine-containing foods or beverages (e.g., chocolate, cola, coffee and tea) were prohibited for 48 hours before the SRT administration, during (throughout 3 visits and washout period) and 24 hours thereafter.

Table 2. The randomized schedule of drug administration

Subject no.	Visit 1	Visit 2	Visit 3
2	A	B	C
3	C	B	A
4	C	B	A
6	A	B	C
7	C	B	A
9	A	B	C
10	A	B	C
11	C	B	A
12	A	B	C
13	A	B	C

Determination of Serum Theophylline Concentrations

Blood samples were allowed to clot at room temperature and centrifuged for 10 minutes at 2,500 rpm to separate the serum which will be immediately kept at –20 $^{\circ}$C until assay. Serum theophylline samples were analyzed for theophylline concentrations by fluorescence polarization immunoassay (FPIA) technique using the Abbott TDx clinical analyzer (Abbott Laboratory, North Chicago, IL., U.S.A.). The FPIA procedure (as described below) is an automated method for drug level monitoring routinely performed at the Clinical Pharmacology Unit, Department of Pharmacology, Faculty of Medicine, Chiang Mai University. The assay was conducted according to the manufacturer's protocol without modification, and 3 controls (low 7 μg/ml, medium 12 μg/ml and high 26 μg/ml) were run with each carousel of serum samples. The coefficient of variation between measurement was less than 5 % (n=80) for the theophylline levels of 6.3-28.6 μg/ml and the results were shown below:

Standard concentration (μg/ml)	Reading	Coefficient of Variation (%)
6.30 - 7.70	7.03 ± 0.16*	2.23
10.80 - 13.20	11.98 ± 0.17	1.44
23.40 - 28.60	25.12 ± 0.36	1.42

* data represents as mean ± SD.

Principle of Fluorescence Polarization Immunoassay

The Abbott TDx System uses a competitive binding immunoassay methodology[43], to allow tracer-labeled antigen and patient antigen to compete for binding sites on the antibody molecules. The components in this binding reaction are the antibody, the patient antigen, and the antigen labeled with fluorescein (tracer-antigen complex). When competitive binding occurs, the more antigen-antibody complex then becomes a part of very large antibody molecule, and consequently the less tracer-antigen complex that remains in solution.

While the tungsten halogen lamp in the TDx System emits light of different wavelengths or colors in a random spatial orientation. An interface filter, located in front of the light source, allows blue light (481-489 nm) to pass through. The light is then passed through a liquid-crystal polarizer to produce a beam of plane polarized blue light. The plane polarized blue light excites the tracer, or fluorophore, and raises it to an excited state. After excitation, the fluorophore returns to steady state and green light (525-550 nm) is emitted from the fluorophore.

If the fluorophore is bound to a very large antibody molecule and does not rotate freely, the emitted green light will be in the same plane as the blue excitation light and polarization is retained. Conversely, if the fluorophore is free to rotate because the small free tracer molecule is not bound, the emitted green light will be in a different plane than the blue excitation light and polarization is lost. Because of the rotational properties of molecules in solution, the degree of polarization is directly proportional to the size of the molecule. That is, polarization increases as molecular size increases.

Therefore, if a patient sample contains a low concentration of antigen, after a competitive binding reaction reaches steady-state, there will be a high concentration of bound tracer in the reaction mixture and polarization will be high. Conversely, if

there is a high concentration of antigen in the sample being tested, after the competitive binding reaction reaches the steady state, there will be a low concentration of bound tracer in the reaction mixture and polarization will be low. The precise relationship between polarization and concentration of the unlabeled drug in the sample is established by measuring the polarization values of calibrators with known concentrations of the drug.

Data Analysis

Pharmacokinetic Parameters Measurements

The attainment of steady state was verified using the observed theophylline serum concentrations at 24 hours before the morning doses of Day 4 and Day 5. From the 24-hour serum concentration-time profiles on study Day 6 and Day 7, the following pharmacokinetic parameters were determined

1. Time to reach the maximal concentration at steady state (Tss_{max}, hr)
2. Maximal serum concentration at steady state ($Css_{max,}$ μg/ml)
3. Minimum serum concentration at steady state ($Css_{min,}$ μg/ml)
4. Fluctuation index (FI, %)
5. Area under the curve at steady-state, 0-24 hrs ($AUCss_{0-24}$, μg.hr/ml)
6. The Wagner-Nelson absorption profile

$Tss_{max,}$ $Css_{max,}$ and Css_{min} were obtained directly by visual inspection of each subject's serum concentration-time profile. Fluctuation index were calculated as $[(C_{max} - C_{min})/ C_{min}] \times 100$. The $AUCss_{0-24}$ and Wagner-Nelson absorption profile were determined with use of TopFit 2.0, the pharmacokinetic and pharmacodynamic data analysis program.

Assessment of the Bioequivalence Based on Drug Bioavailability

Pair comparison of Css_{max}, Css_{min} and $AUCss_{0-24}$ of the three preparations; Uni-Dur® versus Xanthium®, Uni-Dur® versus Theo-Dur® and Xanthium® versus Theo-Dur® were determined for bioequivalence concerning the rate and extent of theophylline absorption. These pharmacokinetic data were logarithmically transformed and an analysis of variance (ANOVA) was performed to determine the differences between the preparations, subjects, and sequence of drug administrations. Thereafter, the 90% confidence interval (90%CI) for the mean difference between each treatment was calculated by using the variance estimate (S^2) obtained from the ANOVA according to the formulation[44] :

$$90\%CI\ (\mu_T - \mu_R) = (\ \bar{X}_T - \bar{X}_R) \pm t^{v}_{0.1} \sqrt{\frac{2S^2}{n}}$$

while

$\bar{X}_T$ and $\bar{X}_R$	the observed means of the transformed parameters (either C_{max}, C_{min} or AUC) for the test drug (T) and the reference drug (R)
S^2	the error variance of the residual mean square
n	the number of subjects
$t^{v}_{0.1}$	the tabulated two-tail t value for a 90% confidence interval
v	the number of degree of freedom of the residual mean square from ANOVA
μ_T and μ_R	the lower and upper limit of the confidence interval

The antilogarithm of the confidence interval would express the bioequivalence as a ratio of the test and reference products (μ_T/μ_R). Bioequivalence acceptance criteria required that the 90%CI for the Css_{max}, Css_{min} and $AUCss_{0-24}$ ratio μ_T/μ_R fell within the bioequivalence interval of 0.7-1.43 (Css_{max}, Css_{min}) and 0.8-1.25 ($AUCss_{0-24}$), respectively. An analysis of Tss_{max} difference [Test-Reference] was determined in the same manner but using untransformed data. Bioequivalence range of Tss_{max} difference was $\pm$ 20 % of the value of Tss_{max} of the reference formulation.