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การศึกษาชีวสมมูลและเภสัชจลนศาสตร์ของยา sildenafil ในอาสาสมัครชายไทยที่มีสุขภาพดี

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ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

Bioequivalence and Pharmacokinetic Study of Sildenafil in Healthy Thai Male Volunteers

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หลักการและเหตุผล: Sildenafil เป็นยารักษาโรคหย่อนสมรรถภาพทางเพศ ที่นิยมใช้กันอย่างแพร่หลาย ซึ่งปัจจุบันยานี้ที่มีจำหน่ายในท้องตลาดมาจากผู้ผลิตหลายบริษัทดังนั้นเพื่อประกันถึงประสิทธิผลและความปลอดภัยของยาสามัญที่จำหน่ายในประเทศไทย จึงจำเป็นที่จะต้องศึกษาเปรียบเทียบชีวสมมูลของยาระหว่างตำรับทดสอบและตำรับอ้างอิง

วัตถุประสงค์การวิจัย: เพื่อศึกษาว่ายาเตรียม sildenafil 2 ตำรับ คือ Erec® (บริษัท ยูนิสัน จำกัด, ประเทศไทย) และ Viagra® (บริษัท ไฟเซอร์ จำกัด, ประเทศออสเตรเลีย) มีชีวสมมูลกันหรือไม่

รูปแบบการวิจัย: เป็นการศึกษาแบบสุ่มไขว้สลับ ภายใต้สภาวะที่ต้องอดอาหาร โดยการให้ยาแบบรับประทานครั้งเดียว ในขนาด 100 มก.

ประชากรที่ศึกษา: อาสาสมัครชายไทยที่มีสุขภาพดีจำนวน 15 ราย

สถานที่ศึกษาวิจัย: ภาควิชาเภสัชวิทยาและ โรงพยาบาลศรีนครินทร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

วิธีการวิจัย: อาสาสมัครได้รับยาในขนาด 100 มก. และทำการเก็บเลือดผ่าน catheter ที่เวลาต่างๆ ภายหลังการได้รับยาจนกระทั่ง 12 ชั่วโมง เพื่อนำไปวิเคราะห์หาปริมาณยา sildenafil โดยวิธี HPLC ค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ที่ใช้ในการตัดสินความเท่าเทียมกันทางชีวสมมูลได้แก่ ค่าระดับยาสูงสุดในเลือด (C_{max}) และพื้นที่ใต้เส้นโค้งความสัมพันธ์ระหว่างระดับยาในเลือดกับเวลาที่เวลา 0 ถึง เวลาที่จุดสุดท้ายที่สามารถวัดความเข้มข้นของยาได้ ($AUC_{0\text{-}t\ last}$)

Background: Sildenafil is a popular drug used for improving penile erectile function that has been commercially available through several manufacturers and distributors in Thailand. Therefore, it is necessary to study bioequivalence of the drugs obtained from the original manufacture and from a local manufacturer to ascertain that they can be medicated interchangeably.

Objective: To determine whether two sildenafil preparations: Test (Erec®, Unison Laboratories, Co., Ltd., Thailand) and reference, (Viagra®, Pfizer Pty Limited., Australia) are bioequivalent.

Design: Single oral dose and double-blind randomized two-way crossover.

Population and samples: Fifteen healthy Thai male volunteers.

Setting: Department of pharmacology, and Srinagarind Hospital, Faculty of Medicine, Khon Kaen University.

Methods: The subjects received either 100 mg of the reference or test formulation. Blood samples were collected from catheter at several time points after sildenafil administration up to 12 hours. The bioequivalence between the two formulations was assessed by comparison of the peak plasma concentrations (C_{max}) and area under the curve of time, from 0 to the last measurable concentration ($AUC_{0\text{-}t\ last}$).

ผลการวิจัย: หลังได้รับยา sildenafil อาสาสมัครทนต่อยาทั้ง 2 ตำรับได้ดี โดยไม่มีอาการไม่พึงประสงค์ที่ร้ายแรงจากยา ผลการวิเคราะห์ข้อมูลทางสถิติ ที่ระดับความเชื่อมั่น 90% พบว่าค่าเฉลี่ยของสัดส่วนของ Ln C_{max} อยู่ในพิสัย 0.8377-1.1985 และค่าเฉลี่ยของสัดส่วนของ Ln $AUC_{0\text{-t last}}$ อยู่ในพิสัย 0.8610-1.1590 ซึ่งอยู่ในเกณฑ์มาตรฐานที่ถือว่ามีชีวสมมูลกันตามที่สำนักงานคณะกรรมการอาหารและยากำหนดไว้ (0.80-1.25)

สรุปผลการวิจัย: ผลการวิจัยครั้งนี้แสดงว่า Erec® ซึ่งเป็น sildenafil ตำรับยาใหม่ชนิดยาสามัญ มีชีวสมมูลกันกับ Viagra® ที่เป็นตำรับอ้างอิงเมื่อให้โดยการรับประทานขณะท้องว่างในขนาด 100 มก.

คำสำคัญ: sildenafil, ชีวสมมูล

Results: All subjects were well tolerated and presented no serious side effect. Statistical analysis revealed that the 90% confident intervals (CI) for the ratios between test and reference drugs of the log transformed the C_{max} (0.8377-1.1985) and $AUC_{0\text{-t last}}$ (0.8610-1.1590), are within the Food and Drug Administration Guideline range of bioequivalence (0.80 to 1.25).

Conclusions: It can be concluded that the 100 mg formulation of Test (Erec®) is bioequivalent to the Reference.

Keywords: sildenafil, bioequivalence

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Introduction

Sildenafil is a popular drug used for improving penile erectile function[1]. It has been registered with US-FDA since 1998. This drug selectively inhibits enzyme cyclic GMP phosphodiesterase type 5 (cGMP PDE5) which is chiefly responsible for metabolism of cGMP in penile corpus carvernosum, leaving a high level of cGMP in penis. As cGMP is a secondary messenger of nitric oxide (NO), it causes smooth muscle and blood vessels in penis to relax resulting in the dilatation of blood vessel dilation and penile erection[2].

Sildenafil is absorbed rapidly through gastrointestinal tract after oral administration. Oral bioavailability of this drug is about 40% when compared with the intravenous route. The majority of the drug (about 80%) is metabolized by CYP 3A4 in the liver to a less active compound, N-desmethyl sildenafil[3]. About 20% of sildenafil is metabolized by CYP 2C9, and less than 2% by CYP 2C19 and CYP 2D6[1,4]. Metabolites of sildenafil are mainly excreted through feces [2]. Complete excretion from the body takes 24 hours [5]. A study in twelve healthy volunteers found that after taking an oral single dose of 50 mg sildenafil, the maximum blood concentration (C_{max}) of 159 ng/ml can be observed within 1 hour (T_{max}) with the half-life of 4 hours and the absolute oral bioavailabilty of about 40 % [6]. The maximum plasma concentration of sildenafil 50 mg oral dose is increased by 70% in the elderly with more than 65 years of age; by 47% in patients with cirrhosis and by 90% in patients with severe abnormal kidney function[7].

Side effects of sildenafil are mostly resulting from its inhibitory actions on cGMP PDE5 and cGMP PDE6[8]. Common side effects are headache, flushing, nausea, dyspepsia, rhinitis, hypotension and abnormality in color perception. In addition, these side effects are dose-dependent[9, 10].

Generally, the recommended dose of sildenafil is 50 mg, orally taken 1 hour before sexual engagement. The dose can be increased up to 100 mg or reduced to 25 mg, depending on efficacy and tolerance to drugs side effects. However, it is recommended that the maximum dose should not exceed 100 mg, with maximum frequency of using at only once a day[6].

Despite the high price of the drug from the innovator, sildenafil has been clinically used worldwide including Thailand since its launching. Recently, some generic sildenafil formulations are locally produced at lower price. However, to ensure the efficacy and safety of these generic formulations, it is necessary to compare the bioavailability between the generic and the innovator formulations. This study was aimed to compare the oral bioavailability of Erec®, a generic sildenafil (Unison Laboratories, Thailand) with that of an innovator (Viagra®, Pfizer, Australia) after single oral administration of 100 mg sildenafil.

Materials and methods

Drugs and reagents

Standard sildenafil, Reference material: Viagra 100 mg / tablet from Pfizer Pty Limited., Australia (Lot No. 314833322, Manufacturing date 7-2003, Expiry date 7-2007).

Local product: Erec 100 mg / tablet from Unison Laboratories Co., Ltd., Thailand (Lot No. T08/3-258, Manufacturing date 26-8-03).

Standard Rofecoxib, Internal standard were obtained from Cadila Health Care Limited, India. Acetonitrile and methanol (analytical Grade) were obtained from Lab Scan (Analytical Sciences, Bangkok Thailand) and Merck (Darmstadt, Germany), respectively.

Equipments

A high-performance liquid chromatography (HPLC) system was performed using an auto sampler (Intelligent sampler 851-AS, Jusco Corporation, Tokyo, Japan). and pump (Waters 510, Millipore, Milford MA, USA). The eluent was detected with UV-Visible spectrophotometer (UV1000, hermo Separation Product, USA) set at 256 nm. CSW32 Data Acquisition Software version 1.4.10.15 (Data Apex Ltd., Praque, The Czech Republic) was employed for chromatographic data collection.

Subjects

All volunteers in this experiment were healthy Thai male. The number of volunteers enrolled in the study was fifteen. Before participating in the study, the subjects were physically examined by a clinician including, measurement of blood pressure and heart rates, a 12-lead electrocardiogram and routine laboratory tests for kidney and liver functions were performed. Subjects were interviewed for medical history particularly concerning the cardiovascular functions. If any abnormality was detected, participant would be excluded from the experiment, to avoid any risk of hypotension. All subjects voluntarily participated in the project and were informed the details of processes and side effects of the drug that they encounter may occur, both verbally and in written document. All volunteers had signed the informed consent to participate in the experiment. This project was approved by The Khon Kaen University Medical School Ethics Committee for Human Research.

Study design

The study was conducted using a single-dose, randomized, two-way crossover design with a 2 week-washout period between the doses. Each subject was assigned to receive either Reference formulation or Test formulation by random sampling. Randomization code was blinded to the analyst. Subjects were housed one night before the study day. After an over night fast, subjects were given either one tablet of the Reference product or Generic product (which contained sildenafil 100 mg) with 240 ml water. The subjects were allowed to have normal activities while avoiding physical exertion. Blood samples (about 8 ml) were collected before dosing (0 h), at 10, 20, 40 minutes, then 1, 1.5, 2, 2.5, 4, 6, 9 and 12 hours. All blood samples were collected into the tube coated with heparin, to prevent blood clotting, then were centrifuged at 3,000 rpm for 15 minutes, and kept under -80 ^{o}C for later analysis.

At the study day, lunch and dinner were served at 4 and 10 h after drug dosing, respectively. The meals provided for subjects in period 1 and period 2 were identical. To reduce any risk, drug administration and blood sampling was conducted closely in Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Thailand. In case of the appearance of side effect, participant would be kept in the hospital for observation and treatment.

Determination of plasma sildenafil concentration

Plasma sildenafil concentrations were analysed by high performance liquid chromatography using a modified method of Lee and Min[5]. Rofecoxib was used as an internal standard. Under the chromatographic conditions used, the retention times for sildenafil and internal standard were 3.9 and 6.5 minutes. Standard curves were constructed in the sildenafil concentrations range from 10-1,500 ng/ml. The standard curve samples were treated in the same manner as the plasma samples collected from the volunteers. Sildenafil concentrations in quality control and study samples were quantified by comparison of the peak height ratios between sildenafil peak and internal standard peak with those of the standard curve. Validation of analytical method was assessed under Good Laboratory Practice and US-FDA Guidance for Industry: Bioanalytical Method Validation[13].

Data analysis

The pharmacokinetic parameters of sildenafil were determined from plasma concentration versus time profiles by non-compartmental method using Kinetica Software (InnaPhase, USA). The area under the plasma concentration versus time curve ($AUC_{0\text{-}t\ last}$) was calculated using the linear trapezoidal method from the zero time point to the last quantifiable concentration. The maximum plasma concentration (C_{max}) and the time to

reach the maximum plasma concentration (T_{max}) were taken directly from the observed data. The terminal elimination rate constant (K_e) was obtained from the terminal log-linear concentration time values. Elimination half-life was estimated as $0.693/K_e$.

Statistical analysis

The statistical analysis was performed using Kinetica Software (InnaPhase, USA). Analysis of Variance (ANOVA) was performed on the log (natural)-transformed pharmacokinetic parameters C_{max}, and $AUC_{0\text{-t last}}$. The ANOVA model included sequence, formulation and period as fixed effects and subjects nested within sequence as random effect. According to the standard criteria of the Thai-FDA, bioequivalence of the two formulations was established when formulation or treatment effect of $AUC_{0\text{-t last}}$. and C_{max} should not be different at alpha level of 0.05 and the 90% confidence interval of the mean ratio of $AUC_{0\text{-t last}}$ and C_{max} between the Test product and the Reference product should fell within the 0.80 to 1.25 for log-transformed data.

Results and Discussion

Fifteen male volunteers with age ranging from 19 to 43 years (mean ± SD, 28.8 ± 7.49 and ranging in body mass index from 19.5 to 25.0 (mean ± SD, 22.5 ± 1.9) were enrolled in this study. All of participants were physically healthy based on their medical examination and results form clinical laboratory tests. None of these volunteer showed serious adverse effect of sildenafil. Only mild adverse drug events such as flushing, headache and abnormal color perception were observed and all of the study subjects were well tolerated to both Test and Reference products. It was found that, approximately 30 minutes after administration of both sildenafil products, 33%, 16% and 10% of the volunteers were experienced flushing, headache and abnormal color perception as a yellow color tinge to vision, respectively. These symptoms disappeared in an hour after drug administration. In the present study, all volunteers could tolerate sildenafil effects from both Test and Reference formulation. No subject was withdrawn from the trials.

Furthermore, it has been reported previously in erectile dysfunction patients after oral sildenafil administration that occurred headache (16%), red face and flushing (10%) and abnormality of eye sight (such as light sensitive, blurred vision, change in color) (3%) [9]. The study in 912 Singaporean men with ED[11] also found side-effects of sildenafil 25-100 mg in 13.9% of patients in the form of flushing, headache, blurred vision and giddiness. Moreover, it has been reported that these similar profile side effects were transient and mild in severity after administration of sildenafil at dosage range of 25-200 mg, and all adverse events resolved spontaneously [6, 12].

Sildenafil plasma concentration-time curves in the fifteen volunteers after oral administration of either Erec® and Viagra® are shown in Figure 1. The mean values of pharmacokinetic parameters of sildenafil after oral administration of 100 mg show no statistical difference between the the Test (Erec®) and Reference (Viagra®) formulation (Table 2 and Table 3). The mean ± SD of T_{max} for the Test and Reference formulations were 1.02 ± 0.47 and 0.99 ± 0.59 hr, respectively, while those of C_{max} for the Test and Reference formulations were 645.06 ± 220.97 and 661.54 ± 278.94 ng/ml, respectively. Furthermore, the mean ± SD of $AUC_{0\text{-t last}}$ for the Test and Reference formulations were 1861.35 ± 576.46 and 1876.36 + 650.27 ng*hr/ml, respectively. The mean ratio of Ln C_{max} and Ln $AUC_{0\text{-t last}}$ (F relative) and the mean ratio of Ln C_{max} ± SD and Ln $AUC_{0\text{-t last}}$ ± SD between between the Test and Reference were 1.0003 ± 0.0602 and 0.9999 ± 0.0458, respectively (Table 4). In the present study, the pharmacokinetic parameters in all healthy Thai male volunteers receiving a 100 mg, single dose of sildenafil, show T_{max}, C_{max} and $AUC_{0\text{-t last}}$ similar to Nichols' study[6] which

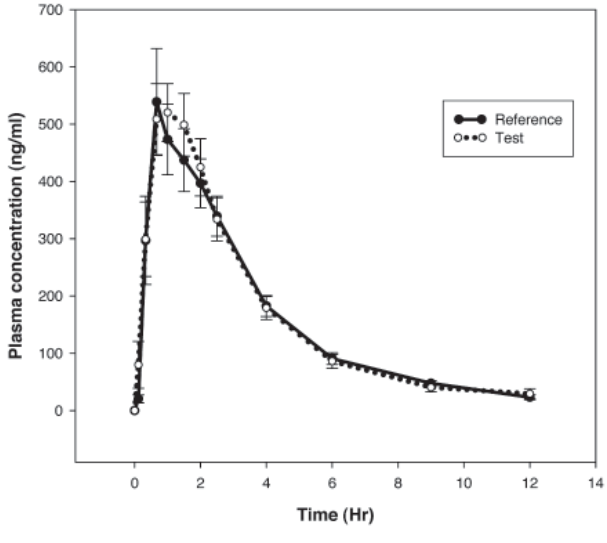

Figure 1 Comparative mean plasma concentration and time curve of sildenafil in fifteen volunteers after oral administration 100 mg of either Test or Reference (y-error bar = SE).

Table 1 Comparison of sildenafil pharmacokinetic parameters after single oral administration of 100 mg of the Test and the Reference.

Parameters	Test		Reference	
	Mean ± SD	%CV	Mean ± SD	%CV
C_{max} (ng/ml)	645.06 ± 220.97	34.26	661.54 ± 278.94	42.17
$AUC_{0\text{-t last}}$ (ng*hr/ml)	1861.35 ± 576.46	30.97	1876.36 ± 650.27	34.66
T_{max} (hr)	1.02 ± 0.47	46.08	0.99 ± 0.59	59.6
K_e (hr^{-1})	0.26 ± 0.14	50.00	0.26 ± 0.12	46.15
$t_{1/2}$ (hr)	3.36 ± 1.70	50.60	3.11 ± 1.14	36.66
Cl (L/hr)	58.84 ± 18.91	32.13	58.94 ± 18.62	31.59

Table 2 ANOVA comparison of Ln $AUC_{0\text{-t last}}$ between Test (Erec®, T) and Reference (Viagra®, R) for formulation, sequence, subject (within sequence) and period effect.

Source	Degree of Freedom	Sum of squares	Mean Squares	Computed F	p-values
Period	1	0.078225	0.078225	1.4806	0.2453 NS
Subject	13	2.107950	0.162150	3.0692	0.0265***
Formulation	1	0.000008	0.000008	0.0002	0.9902 NS
Sequence	1	0.015741	0.015741	0.0971	0.7603 NS
Error	13	0.686814	0.052832		
Total	29	2.88730			

NS, Not significant; ***, significantly different at P<0.05

Table 3 ANOVA comparison of Ln C_{max} between Test (Erec®, T) and Reference (Viagra®, R) for formulation, sequence, subject (within sequence) and period effect.

Source	Degree of Freedom	Sum of squares	Mean Squares	Computed F	p-values
Period	1	0.012531	0.012531	0.1634	0.6927 NS
Subject	13	3.036910	0.233609	3.0452	0.0273 ***
Formulation	1	0.000030	0.000030	0.0004	0.9846 NS
Sequence	1	0.481936	0.481936	2.0630	0.1745 NS
Error	13	0.997276	0.076714		
Total	29	4.528690			

NS, Not significant; *** significantly different at P<0.05

Table 4 The means and 90% CI of ratios of Ln C_{max} and $AUC_{0\text{-t last}}$ and F relative of Ln C_{max} and $AUC_{0\text{-t last}}$

Pharmacokinetic parameters	Means ratio of Ln tranformed	Means ratio of Frel	90% CI	FDA Acceptable range
C_{max} (Test/Reference)	6.4108/6.4088	1.0003	0.8377-1.1985	0.80-1.25
$AUC_{0\text{-t last}}$ (Test/Reference)	7.4841/7.4852	0.9999	0.8610-1.1590	0.80-1.25

reported in 32 healthy volunteers. Analysis of sildenafil pharmacokinetic parameters calculated from the Reference formulation after a single dose administration of 100 mg in healthy Thai male volunteers reveals the inter-individual difference in these parameters which similar to that reported by Milligan, et al[13]. In Milligan's report, a population pharmacokinetic analysis of 591 erectile dysfunction patients receiving a single dose of 100 mg sildenafil showed T_{max} of 1.16 $\pm$ 0.99 hours, C_{max} 328 $\pm$ 237 ng/ml and $AUC_{0\text{-t last}}$ of 1,963 $\pm$ 860 ng.hr.ml^{-1}. The mean of C_{max} and $AUC_{0\text{-t last}}$ of the Test formulation was very closed to that of the Reference formulation. In addition, the T_{max} of both formulations were not statistically significant different.

Analysis of variance (ANOVA) of the ln $AUC_{0\text{-t last}}$ and ln C_{max} obtained from the Test and Reference formulations revealed that the sequence, period or formulation was not significantly different at $p<0.05$. However, inter-individual variations of these pharmacokinetic parameters among subject were noted (Table 3 and Table 4). The 90% confident interval (CI) for the mean value of the mean for Ln $AUC_{0\text{-t last}}$ (0.8610-1.1590) and Ln C_{max} (0.8377-1.1985) were within the FDA Guideline range of bioequivalence (0.80 to 1.25)). It is noteworthy that none of the values obtained from 15 subjects were outside the range of 0.8-1.25 (Data not shown).

Conclusions

All volunteers participated in this study were well tolerated to both Test product and Reference product. The 90% confidence interval of the mean proportion of Ln $AUC_{0\text{-t last}}$ and Ln C_{max} are within the acceptable range of 0.80-1.25 according to the Thai FDA guideline. This indicates that the Test formulation (Erec®, Unison Laboratories, Co., Ltd, Thailand) was bioequivalent to the Reference formulation (Viagra®, Pfizer Pty Limited., Australia) in term of both rate and extent of absorption.

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Conflict of interest

The authors declare that they have no conflict of interest. None of the authors of this manuscript have received reimbursements, fee, funding or salary from an organization that has applied for the content of the manuscript.

References

1. Boyce EG, Umland EM. Sildenafil citrate: a therapeutic update. Clin Ther 2001; 23: 2-23.
2. Cheitlin MD, Hutter AM Jr, Brindis RG, Ganz P, Kaul S, et al. Use of sildenafil (Viagra) in patients with cardiovascular disease. Technology and Practice Executive Committee. Circulation 1999; 99: 168-77.
3. Walker DK, Ackland MJ, James GC, Muirhead GJ, Rance DJ, Wastall P, et al. Pharmacokinetics and metabolism of sildenafil in mouse, rat, rabbit, dog and man. Xenobiotica 1999; 29: 297-310.
4. Warrington JS, Shader RI, von Moltke LL, Greenblatt DJ. In vitro biotransformation of sildenafil (Viagra): identification of human cytochromes and potential drug interactions. Drug Metab Dispos 2000; 28: 392-7.
5. Lee M, Min DI. Determination of sildenafil citrate in plasma by high-performance liquid chromatography and a case for the potential interaction of grapefruit juice with sildenafil citrate. Ther Drug Monit 2001; 23: 21-6.
6. Nichols DJ, Muirhead GJ, Harness JA. Pharmacokinetics of sildenafil after single oral doses in healthy male subjects: absolute bioavailability, food effects and dose proportionality. Br J Clin Pharmacol 2002; 53 Suppl 1: 5S-12S.

7. Muirhead GJ, Wilner K, Colburn W, Haug-Pihale G, Rouviex B. The effects of age and renal and hepatic impairment on the pharmacokinetics of sildenafil. Br J Clin Pharmacol 2002; 53 Suppl 1: 21S-30S.
8. Corbin JD, Francis SH, Webb DJ. Phosphodiesterase type 5 as a pharmacologic target in erectile dysfunction. Urology 2002; 60: 4-11.
9. Goldenberg MM. Safety and efficacy of sildenafil citrate in the treatment of male erectile dysfunction. Clin Ther 1998; 20: 1033-48.
10. Glossmann H, Petrischor G, Bartsch G. Molecular mechanisms of the effects of sildenafil (VIAGRA). Exp Gerontol 1999; 34: 305-18.
11. Lim PH, Li MK, Ng FC, Chia SJ, Consigliere D, Gooren L, et al. Clinical efficacy and safety of sildenafil citrate (Viagra) in a multi-racial population in Singapore: A retrospective study of 1520 patients. Int J Urol 2002; 9: 308-15.
12. Boshier A, Wilton LV, Shakir SA. Evaluation of the safety of sildenafil for male erectile dysfunction: experience gained in general practice use in England in 1999. BJU Int 2004; 93: 796-801.
13. Milligan PA, Marshall SF, Karlsson MO. A population pharmacokinetic analysis of sildenafil citrate in patients with erectile dysfunction. Br J Clin Pharmacol 2002; 53 Suppl 1: 45S-52S.
14. Food and Drug Administration. Guidance for Industry: Bioanalytical Method Validation. Rockville, MD: US Department of Health and Human Services, FDA, Center for Drug Evaluation and Research; 2001.
15. Drug Control Division Food and Drug administration Ministry of Public Health, Thailand, Thailand guidelines for the conduct of bioavailability and bioequivalence studies; 2001.