การศึกษาเภสัชจลนศาสตร์และชีวสมมูลของยาไดคลอกซาซิลินชนิดแคปซูล 500 มิลลิกรัม ในอาสาสมัครคนไทยสุขภาพดี

นางสาววีริสา บินเจ๊โซ๊ะ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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PHARMACOKINETICS AND BIOEQUIVALENCE STUDY OF 500 MILLIGRAM DICLOXACILLIN CAPSULES IN HEALTHY THAI VOLUNTEERS.

Miss Verisa Binjehsoh

A Thesis Submitted in Partial Fulfillment of the Requirements

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By	Miss Verisa Binjehsoh
Field of Study	Pharmacy
Thesis Advisor	Associate Professor Ubontip Nimmannit, Ph.D.
Thesis Co-Advisor	Associate Professor Uthai Suvanakoot, Ph.D.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

……. Dean of Faculty of Pharmaceutical Sciences

(Associate Professor Boonyong Tantisira, Ph.D.)

Thesis Committee

…….Chairman

(Associate Professor Porntip Nimmannitya, M.Sc. in Pharm.)

…….Thesis Advisor

(Associate Professor Ubontip Nimmannit,Ph.D.)

…….Thesis Co-advisor

(Associate Professor Uthai Suvanakoot, Ph.D.)

…….Member

(Associate Professor Parkpoom Tengamnuay, Ph.D.)

…….Member

(Wiyada Agkarawut, Ph.D.)

วีริสา บินเจ๊โซ๊ะ: การศึกษาเภสัชจลนศาสตร์และชีวสมมูลของยาไดคลอกซาซิลินชนิดแคปซูล 500 มิลลิกรัม ในอาสาสมัครคนไทยสุขภาพดี (PHARMACOKINETICS AND BIOEQUIVALENCE STUDY OF 500 MILLIGRAM DICLOXACILLIN CAPSULES IN HEALTHY THAI VOLUNTEERS.) อ.ที่ปรึกษา รศ. ดร. อุบลทิพย์ นิมมานนิตย์:, อ.ที่ปรึกษาร่วม : รศ.ดร.อุทัย สุวรรณกูฏ, 128 หน้า. ISBN 974-17-0733-9.

ศึกษาเภสัชจลนศาสตร์และชีวสมมูลของยาไดคลอกซาซิลินชนิดแคปซูล 500 มิลลิกรัม 3 ผลิตภัณฑ์ ผลการศึกษาในหลอดทดลองพบว่า ยาทุกผลิตภัณฑ์มีเปอร์เซ็นต์ยาที่ระบุไว้ตามฉลาก ความสม่ำเสมอของเภสัชภัณฑ์ ได้มาตรฐานที่กำหนดในเภสัชตำรับสหรัฐอเมริกาฉบับที่ 24 การศึกษาการละลายตัวยาพบว่า เส้นโค้งของผลิตภัณฑ์ยาที่ผลิตภายในประเทศ (B และ C) เหมือนเส้นโค้งของผลิตภัณฑ์ยาต้นแบบ (A)

เปรียบเทียบชีวสมมูลของยาไดคลอกซาซิลินชนิดแคปซูล 500 มิลลิกรัมของ ผลิตภัณฑ์ B และ C เทียบกับผลิตภัณฑ์ A กระทำในอาสาสมัครชายไทยสุขภาพดี 15 คน โดยใช้แบบแผนการทดลองข้ามสลับชนิด 3 ทาง อาสาสมัครได้รับยาขนาด 500 มิลลิกรัม 2 แคปซูล ครั้งเดียว เก็บตัวอย่างเลือดที่เวลาต่างๆ ที่เหมาะสมหลังการให้ยา วัดระดับยาในซีรัมโดยวิธี ไฮเพอฟอร์แมนซ์ลิขวิดโครมาโตกราฟฟี การวิเคราะห์ระดับยาของอาสาสมัครแต่ละคน ทางเภสัชจลนศาสตร์พบว่า ค่าลอการิทึมของความเข้มข้นของยาสูงสุดในซีรัม และลอการิทึมพื้นที่ใต้เส้นโค้งระหว่างความเข้มข้นของยาในซีรัมกับเวลาของทั้ง 3 ผลิตภัณฑ์ ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($p>0.05$) และอัตราส่วนของแต่ละพารามิเตอร์เภสัชจลนศาสตร์ที่แปลงเป็นข้อมูลลอการิทึมของยาแคปซูลผลิตภัณฑ์ B และ C เทียบกับยาแคปซูลผลิตภัณฑ์ A อยู่ภายในช่วง 80-125 เปอร์เซ็นต์ของช่วงระยะความเชื่อมั่นที่ 90 เปอร์เซ็นต์ แสดงว่ายาแคปซูลผลิตภัณฑ์ B และ C มีชีวสมมูลกับยาแคปซูลผลิตภัณฑ์ A ทั้งในด้านอัตราเร็วและปริมาณยาที่ถูกดูดซึมเข้าสู่ร่างกาย

เภสัชจลนศาสตร์ของยาไดคลอกซาซิลินชนิดแคปซูล 500 มิลลิกรัม อธิบายได้ด้วยแบบจำลองชนิดเปิดหนึ่งห้อง มีการดูดซึมยาและการขจัดยาเป็นแบบปฏิกิริยาอันดับหนึ่ง เวลาที่ความเข้มข้นของยาสูงสุดในซีรัม มีค่าระหว่าง 1.35 ถึง 1.60 ชั่วโมง อัตราเร็วคงที่ของการดูดซึมยามีค่าระหว่าง 1.44 ถึง 1.62 ต่อชั่วโมง อัตราเร็วคงที่ของการขจัดยามีค่าระหว่าง 0.530 ถึง 0.585 ต่อชั่วโมง ค่าเวลาดูดซึมยาเฉลี่ยมีค่าระหว่าง 0.73 ถึง 0.89 ชั่วโมง ค่าการกระจายตัวมีค่าระหว่าง 21.34 ถึง 28.91 ลิตร และค่าครึ่งชีวิตของยาวัดได้ระหว่าง 1.23 ถึง 1.34 ชั่วโมง

ภาควิชา	เภสัชกรรม	ลายมือชื่อนิสิต……..……..
สาขาวิชา	เภสัชกรรม	ลายมือชื่ออาจารย์ที่ปรึกษา…….
ปีการศึกษา	2544	ลายมือชื่ออาจารย์ที่ปรึกษาร่วม……..…..……..

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VERISA BINJEHSOH: PHARMACOKINETICS AND BIOEQUIVALENCE STUDY OF 500 MILLIGRAM DICLOXACILLIN CAPSULES IN HEALTHY THAI VOLUNTEERS. THESIS ADVISOR: ASSOC. PROF. UBONTIP NIMMANNIT, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. UTHAI SUVANAKOOT, Ph.D. 128 pp. ISBN 974-17-0733-9.

Three brands of 500 mg dicloxacillin capsules were evaluated. *In vitro* studies indicated that all brands met the general requirements of the United States Pharmacopoeia 24 for content of active ingredient and uniformity of dosage units. Dissolution profile studies revealed that each curve of local brand (B and C) was similar to that of innovator's product (A).

Comparative bioavailability of 500 mg dicloxacillin capsule of brands B and C relative to brand A was conducted in 15 healthy Thai male volunteers using a single dose of 2x500 mg capsules in a three way crossover design. Blood samples were collected at appropriate time interval. Serum dicloxacillin concentrations were determined via high performance liquid chromatography. Individual serum dicloxacillin concentration-time profile was analyzed for relevant pharmacokinetic parameters. Data analysis revealed that there were no statistically significant differences ($p>0.05$) among the corresponding logarithmically transformed pharmacokinetic parameters; AUC and C_{max} values of all three brands. Also, the ratios of individual parameter based on log-transformed data of brands B and C to that of brand A were within 80-125 % of 90% confidence interval. These implied that brands B and C were bioequivalent with brand A in terms of both the rate and amount (extent) of drug absorption.

The pharmacokinetics of 500 mg dicloxacillin capsule was described by one compartment open model with first order absorption and first order elimination. Time to peak serum concentrations was between 1.35 to 1.60 hr, the absorption rate constants were between 1.44 to 1.62 hr^{-1}, the eliminate rate constants were within 0.53 to 0.59 hr^{-1} , the mean absorption time were between 0.73 to 0.89 hr, the volume of distribution ranged from 21.34 to 28.91 L and the elimination half-life varied between 1.23 to 1.34 hr.

Department	Pharmacy	Student's signature…….……
Field of study	Pharmacy	Advisor's signature…….……...
Academic year	2001	Co-advisor's signature…….……..

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Verisa Binjehsoh

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LIST OF ABBREVIATIONS

^{O}C	=	degree Celcius
mcg	=	microgram
mg	=	milligram
kg	=	kilogram
mcL	=	microliter
mL	=	milliliter
L	=	liter
mm	=	millimeter
cm	=	centrimeter
nm	=	nanometer
min	=	minute
hr	=	hour
rpm.	=	revolution per minute
%L.A.	=	percent labeled amount
M	=	molar
USP	=	United States Pharmacopoeia
UV	=	ultraviolet
v/v	=	volume by volume
C_{max}	=	peak serum concentration
t_{max}	=	time to peak serum concentration
AUC	=	area under the serum concentration-time curve
K_a	=	absorption rate constant
K	=	elimination rate constant
$t_{1/2}$	=	half-life
MAT	=	mean absorption time

LIST OF ABBREVIATIONS (CON.)

V_d	=	volume of distribution
CI	=	confidence interval
S.D.	=	standard deviation
C.V.	=	coefficient of variation
F	=	fraction of dose to be absorbed

CHAPTER I

INTRODUCTION

Bioequivalence study is a comparative study of bioavailability that assesses whether the drug of two or more similar dosage forms reach the systemic circulation at the same rate and extent. Usually, the comparison is performed between the innovator's product and the locally made product. It is during the past 30 years, bioequivalence study became the subject of interest. Lindenbaum et al., 1971 investigated the bioavailability of digoxin tablets from four companies and found four to seven fold differences in serum levels in the same subjects taking products of different manufacturers and various lots of the same manufacturer. In 1984, the United States Food and Drug Administration (USFDA) required that every locally made products had to pass the bioequivalence study before they could be sold in the market. In 2000, the office of Food and Drug Administration of Thailand also required such data. The declaration intended to provide a good quality of the product which would result in the same clinical efficacy (Thai FDA, 2000).

Dicloxacillin is a semisynthetic penicillinase-resistant penicillin. Dicloxacillin, like cloxacillin and oxacillin, is an isoxazolyl penicillin. Dicloxacillin is resistant to inactivation by staphylococcal penicillinase and is active against many penicillinase-producing strains of *Staphylococcus aureus* and *Staphylococcus epidermis* that are resistant to other commercially available penicillins. Dicloxacillin should not be used orally for the initial treatment of severe, life-threatening infections, including endocarditis, but may be used as follow-up therapy after parenteral penicillinase-resistant penicillin therapy (McEvoy, 2000).

Dicloxacillin is about twice as well absorbed from the gastro-intestinal tract as cloxacillin sodium but absorption is also reduced by the presence of food in the stomach (Bennett, 1994). After an oral dose of 500 mg, peak plasma dicloxacillin

concentrations (C_{max}) of 10 to 18 mcg/mL in about 1 hour have been reported in fasting subjects. Doubling the dose can double the plasma concentration. About 97 percent of dicloxacillin in circulation is bound to plasma proteins. Dicloxacillin has been reported to have a plasma half-life of 0.5 to 1 hour. The half-life is prolonged in neonates (McEvoy, 2000).

Dicloxacillin capsules are available in Thailand through a variety of brand names from different manufacturers. Among such drug-products, the innovator's product (Diclocil®) with 2-3 times higher retail price than the locally made products is included. In Thailand where dicloxacillin is also widely prescribed, the differences in race and biological behavior may contribute to the bioavailability difference of the drug, the differences of bioavailability exist among the pharmaceutically equivalent products. In other words, compliance with *in vitro* testing can not guarantee bioequivalency. Payakachat et al., 1995 found bioinequivalence among four products of phenytoin capsule marketed in Thailand. Thus, the bioequivalence of these dicloxacillin capsules should be evaluated.

In this study, the comparative bioavailabilities of two local brands of dicloxacillin capsules commercially available in Thailand relative to the innovator's product were conducted in order to facilitate drug products selection, in terms of the drug's efficacy and economic aspect and to investigate the pharmacokinetics of dicloxacillin following an oral administration in healthy Thai male volunteers.

Objectives

1. To compare the bioavailability of two local brands of dicloxacillin capsules commercially available in Thailand relative to the innovator's product.
2. To investigate the pharmacokinetics of a single dose administration of dicloxacillin capsules in healthy Thai male volunteers.

3. To determine the *in vitro* quality of dicloxacillin capsules marketed in Thailand

Significance of the study

1. This study will provide bioavailability data of dicloxacillin capsules commercially available in Thailand as compared to the innovator's product.
2. This study will provide the pharmacokinetics of dicloxacillin following an oral administration in healthy Thai male volunteers which would be useful for clinical application including appropriate dosage regimens for the most effective therapy.

CHAPTER II

LITERATURES REVIEW

Bioavailability and bioequivalence

Definition

Bioavailability denotes the measurement of the rate and extent (amount) of the drug that reaches the systemic circulation following the administration of a dosage form (Abdou, 1989).

Absolute bioavailability indicates that the bioavalability is determined by comparing the rate and extent of absorption of the drug from its administered dosage form to those obtained following intravenous administration. In certain cases where parenteral administration is not advisable, the reference drug preparation is administered as an oral solution or intramuscular injection. Absolute bioavailability is expressed on a scale of 0 to100 percent (Abdou, 1989).

Relative bioavailability refers to the bioavailability of one drug product as compared to another standard dosage formulation having the same drug chemical entity, or to other established standard (Abdou, 1989).

Bioequivalence is a relative term which indicates that the drug substance in two or more similar dosage forms reaches the systemic circulation at the same relative rate and to the same relative extent (Abdou, 1989).

History

Early concerns over bioequivalence were directed mostly toward the disintegration of pills, sugar coated and enteric coated tablets. Many of these

pharmaceutical products were used to exhibit “all or none effect”. If a pill or coated tablet disintegrated in the gastrointestinal tract, the drug was assumed to have been absorbed and the expected biological response was obtained. On the other hand, if the product failed to disintegrate, then it was a clear case of bioinequivalence *in vivo*. However, serious deficiency in the physiological availability of the drug was noted to exist even though the tablet disintegrated fully *in vivo* (Wagner, 1971). The pioneering work of Melnick et al., (1945) was crucial in establishing the urinary drug recovery procedure as a reliable quantitative method for monitoring bioequivalence.

There were several studies clearly demonstrated that marketed products were prone to bioequivalence problem. The bioinequivalence of different brands of ampicillin were reported (Ali, 1981; Ali and Farouk, 1981). Nitrofurantoin’s bioavailability was shown to be influenced highly by its particle size and several studies reports the bioinequivalence of marketed nitrofurantoin products (McGilveray, Mattok and Dann, 1972). In addition, a randomized crossover study of oral nitrofurantoin was conducted and the results shown both bioinequivalence and therapeutic inequivalence (Ali, 1988).

In 1968, Glazko et al. reported that the absorption of chloramphenical capsule from a generic product after oral administration was significantly less than that of original product. Oxytetracycline and tetracycline from 13 different manufacturers were found to be significantly bioinequivalence, as well (Blair et al., 1971).

Several toxic episodes caused by overdosage of phenytoin, an extensively used antiepileptic drug with a narrow therapeutic range were reported in Australia. Further study revealed wide variation in the plasma level obtained after administration of phenytoin products marketed in Australia (Albert et al., 1974). Besides, Melikian et al.,1977 found significant bioinequivalence among 13 lots of phenytoin sodium capsules from eight different manufacturers that could lead to subtherapeutic or toxic level of the drug.

In addition, four to seven fold differences in serum levels and serious bioinequivalence were demonstrated after administration of digoxin of different manufacturers and various lots of the same manufacturers (Lindenbaum et al., 1971). As digoxin has a low therapeutic index, the high fluctuation of serum level might lead to toxic effect.

Determination of bioavailability

Bioavailability testing of drug products in humans provides the most reliable method available for determining bioequivalence. The testings normally are performed in healthy volunteers under restricted dietary conditions and fixed activity levels. Effect of gender and age of the volunteers on bioavailability are considered when there is a specific concern that they may affect drug safety or efficacy. In conducting bioavailability studies, drug blood levels or cumulative urinary excretion data following the administration of the test dosage form are compared to those following the administration of a standard or reference preparation. The best reference is an intravenous dose of the drug where bioavailability is assured to be 100 percent. In this case, absolute bioavailability is determined. The relative bioavailability is used to establish bioequivalence between same generic drug products from multiple sources and the innovator's formulation (original formulation) usually used as the reference preparation due to its approved clinical safety and efficacy.

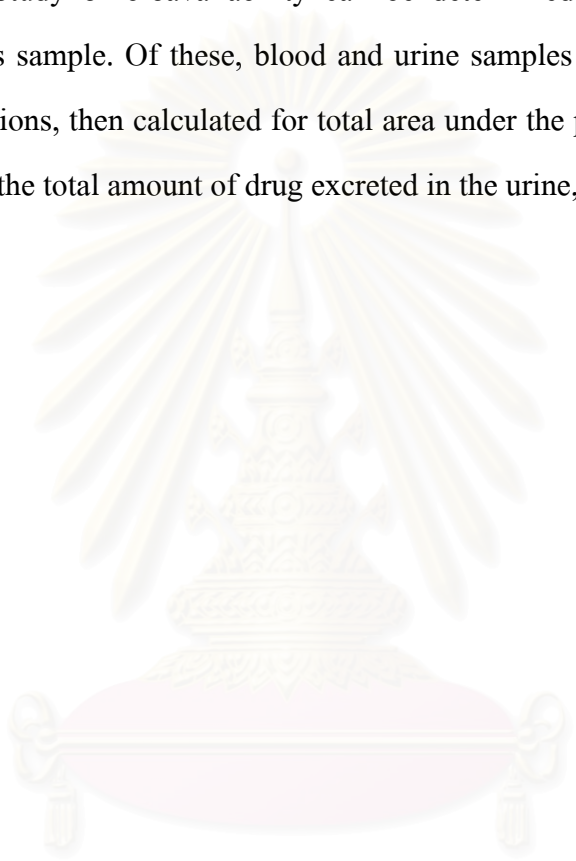
The bioavailability studies should be conducted with sensitivity for the moral and ethical issues involved in using human volunteers for the experiments. The possibility of adverse effects and the hazards in various blood sample collections must be highlighted. Special populations, such as children and pregnancy or breast – feeding women, must not be included for humanitarian reasons. Also, individuals with certain enzyme deficiencies or abnormal metabolism should be avoided, as should any individual requiring certain medications on a regular basis, to minimize any bias or source of variability. The guidelines for Biopharmaceutical Studies in Man, published

by the Academy of Pharmaceutical Sciences recommends that test volunteers should be normal healthy adult males, except where not applicable, such as in case of antifertility drugs where female volunteers have to be used. Generally, Thai FDA test volunteers range in age from 18-45 years and body mass index values range in 18-24 kg/m^2 (Thai FDA, 2000). Individuals with any past history of gastrointestinal tract, liver or kidney malfunctions must be excluded. Also, those with significant organ abnormality or diseases should be avoided. All participating volunteers should be subjected to a thorough physical examination and the following hematological and clinical chemistry tests. Participants for oral studies are asked to fast overnight prior to and for at least four hours after dosing. They also should abstain from any medication for a minimum of one week and should have taken no enzyme-inducing drug for one month prior to dosing. A standard diet can be specified as well as the type and volume of fluid intake. Strenuous physical activities and demanding sports should be avoided during the period of the study.

Regarding the number of subjects needed, there is no specific recommendation, as it depends on several factors. These may include the inherent subject-to-subject variability for the drug under study, the expected magnitude of the difference between the test dosage forms and the particular statistical design of the study. However, the American Pharmaceutical Association guidelines suggests general rules for deciding on the number of subjects required for bioavailability testing. After deciding on the appropriate pharmacokinetic parameters for which comparisons are to be made, the magnitude of differences among the test preparations should be specified. The design of the experiment, partial cross-over, total cross-over, Latin square, etc., should be specified and, accordingly, the statistician will be able to recommend the appropriate number of participants. A well designed protocol is a flexible one that assesses the overall variability of the results sequentially during the progress of the study and modifies the experimental plan accordingly. Study conditions should be adhered to as rigorously as possible as they are a major source

for variability. As a rules of thumb, a minimum number of 12 healthy subjects may be employed in a crossover bioequivalence study, provided that the testing conditions are strictly standardized and assay methodology utilized has been thoroughly validated to generate sufficiently accurate and reproducible results, The number should increase when the patients and / or the parallel design are used (Abdou, 1989).

Study of bioavailability can be determined in blood, urine, saliva, sweat and feces sample. Of these, blood and urine samples are mostly measured for drug concentrations, then calculated for total area under the plasma concentration and time curve and the total amount of drug excreted in the urine, respectively.



Dicloxacillin

1. **Physicochemical Properties**

Dicloxacillin is a semisynthetic penicillinase-resistant penicillin. Dicloxacillin, like cloxacillin and oxacillin, is an isoxazolyl penicillin. Figure 1 shows the chemical structure of dicloxacillin. The presence of two chloride ions on the phenyl group distinguishes this drug from cloxacillin (Reynolds, 1996). Dicloxacillin is commercially available as the monohydrate sodium salt. Potency of dicloxacillin sodium contains not less than 850 mcg of dicloxacillin (McEvoy, 2000). Dicloxaciilin sodium occurs as a white to off-white, crystalline powder. The drug is freely soluble in water, soluble in alcohol and in methyl alcohol. Dicloxacillin sodium has a pK_a of 2.7-2.8 (Reynolds, 1996).

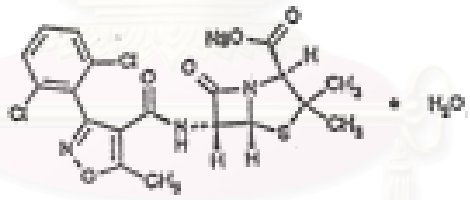


Figure 1 Chemical Structure of Dicloxacillin

Chemical name	:	3(2,6-dichlorphenyl)-5methyl-4-isoxazolyl penicillin
Empirical formula	:	$C_{19}H_{17}Cl_2N_3O_5S$
Molecular weight	:	470.3
Synonym	:	BRL-1702
Appearance	:	White crystalline powder

2. Antibacterial Activity

Dicloxacillin sodium is an antibacterial agent of the benzoyl penicillin series. It is resistant to enzymatic reaction by penicillinase (β-lactamase). It has been demonstrated to be efficacious in the treatment of penicillinase-producing staphylococcal infections and effective in the treatment of other commonly encountered gram-positive coccal infections.

In vitro sensitivity, the minimum inhibitory concentrations of dicloxacillin for some selected bacterial species are shown in Table 1.

Table 1 The Minimum Inhibitory Concentrations of Dicloxacillin for Some Selected Bacterial Species (Kucers, 1972 and Pachla, 1999).

Organism	MIC_s (mcg/mL)
Staph. pyogenes (nonpenicillinase- producer)	0.16
Staph. pyogenes (penicillinase- producer)	0.12
Strep. pyogenes (Group A)	0.05
Strep. pneumoniae (Dip. pneumoniae)	0.15
Strep. faecalis (Enterococcus, Group D)	>12.5
Diplococcus pneumoniae	0.1

3. Pharmacokinetics

3.1 Absorption and Plasma Concentrations

Dicloxacillin is resistant to inactivation in the presence of acidic gastric secretions and is rapidly but incompletely absorbed from gastrointestinal tract. In healthy, fasting adults, 35 to 76 percent of an orally administered dose of dicloxacillin is absorbed from the gastrointestinal tract and peak serum concentrations of the drug are generally attained within 0.5-2 hrs. Presence of food in the gastrointestinal tract generally decreases the rate and extent of absorption of dicloxacillin.

Following oral administration of a single 500-mg dose of dicloxacillin in fasting adults, peak serum concentrations of the drug range from 10 to 18 mcg/mL; serum concentrations of the drug decline rapidly and are generally low 6 hrs after the drug is administered. In fasting adults who receive a single 250-mg oral dose dicloxacillin as a capsule, serum concentrations of the drug average 2.9-3, 4.6-5.5, 3-5.6, and 1.5-1.7 mcg/mL at 30 mins., 1 hr, 2 hrs, and 4 hrs, respectively, after dose. When a single 250-mg oral dose of dicloxacillin is administered as an oral suspension in fasting adults, serum concentrations of the drug average 6, 7.4, 5.3, and 0.7 mcg/mL at 30 mins, 1 hr, 2 hrs, and 4 hrs, respectively after the dose.

In one study in children with acute osteomyelitis who received oral dicloxacillin in a dosage of 100 mg/kg daily given in divided doses every 6 hrs, serum concentrations of the drug ranged from 12-40 mcg/mL 1 hr after dosing and 6.5-20 mcg/mL 3 hrs after dosing (McEvoy, 2000).

3.2 Distribution

Dicloxacillin is distributed into bone, bile, pleural fluid, and synovial fluid. In children 2-16 years of age with acute osteomyelitis who received dicloxacillin intramuscularly in a dosage of 50 mg/kg daily, dicloxacillin concentrations in bone ranged from 1.8-21.6 mcg/g and concurrent serum concentrations of the drug ranged

from 7-9 mcg/mL in samples taken 1-3 hrs after dosing. In children 7 months to 14 years of age with suppurative arthritis who received a single oral dicloxacillin dose 25 mg/kg, dicloxacillin concentrations in synovial fluid obtained 2 hrs after the dose were 70 percent of concurrent serum concentrations; synovial fluid concentrations averaged 13.6 mcg/mL. Like other penicillins, only minimal concentrations of dicloxacillin are attained in cerebral spinal fluid . Dicloxacillin is 95-99 percent bound to serum proteins.

Dicloxacillin reportedly distributes into amniotic fluid in therapeutic concentrations following usual dosages. The drug also crosses the placenta and is distributed into milk. Following oral administration of single 250-mg dose of dicloxacillin in lactating women, milk concentrations of the drug were 0.1-0.3 mcg/mL 2 and 4 hrs after dosing and undetectable 6 hrs after the dose was given (McEvoy, 2000).

3.3 Metabolism and Elimination

The serum half-life of dicloxacillin in adults with normal renal function is 0.6-0.8 hr. In children 2-16 years of age, the serum half-life of the drug averaged 1.9 hrs.

Dicloxacillin is partially metabolized to active and inactive metabolites. Following administration of a single 500-mg oral dose, dicloxacillin 10 percent of the absorbed drug was hydrolyzed to penicilloic acids which are microbiologically inactive. Dicloxacillin is also hydroxylated to a small extent to a microbiologically active metabolite which appears to be slightly less active than dicloxacillin.

Dicloxacillin and its metabolites are rapidly excreted in urine mainly by tubular secretion and glomerular filtration. The drug is also partly excreted in feces via biliary elimination. Following oral administration of a single 250-mg, 500-mg, or 1-g dose of dicloxacillin in adults with normal renal function, 31 to 65 percent of the dose

is excreted in urine as unchanged drug and active metabolites within 6-8 hrs; approximately 10 to 20 percent of this is active metabolites.

The serum half-life of dicloxacillin is slightly prolonged in patients with impaired renal function and has been reported to range from 1-2.2 hrs in patients with severe renal impairment. Serum concentrations of dicloxacillin are higher and the serum half-life is longer in neonates than in older children.

Patients with cystic fibrosis eliminate dicloxacillin more rapidly than do healthy individuals. In these patients renal clearances of the drug average 282 mL/min. per 1.73 m^2 while healthy individuals had renal clearances averaging 95 mL/min. per 1.73 m^2. Following oral administration of a single 6.25-mg/kg dose of the drug, peak serum concentration-time curves (AUC) were, on average, 2.5 times lower in patients with cystic fibrosis than in healthy individuals. Dicloxacillin is only minimally removed by hemodialysis or peritoneal dialysis (McEvoy, 2000).

4. Therapeutic Use

Dicloxacillin shares the uses of other oral penicillinase-resistant penicillins and is generally used only in the treatment of infections caused by, or suspected of being caused by, penicillinase-resistant staphylococci. Dicloxacillin should not be used orally for the initial treatment of severe, life-threatening infections, including endocarditis, but may be used as a follow-up therapy after parenteral penicillinase-resistant penicillin treatment. The drug should not be used orally in the treatment of meningitis (McEvoy, 2000). However, dicloxacilin gives satisfactorily results in staphylococcal infections such as osteomyelitis when administered by the oral route (Kucers, 1987).

5. Adverse Effects

Adverse effects reported with dicloxacillin are similar to those reported with other penicillinase-resistant penicillins (McEvoy, 2000). Gastrointestinal disturbances, eg. nausea, vomiting, epigastric discomfort, flatulence and loose stools have been noted in some patients receiving dicloxacillin sodium (Pascual, 1999).

Pseudomembranous colitis has been reported with the use of dicloxacillin. Therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with dicloxacillin use. Treatment with antibiotics alters the normal flora of the colon and may allow overgrowth of *Clostridium difficile*. Cholestyramine and colestipol resins have been shown to bind *Clostridium difficile* toxin *in vitro*. These exchange resins have been reported to reduce bowel movement frequency and systemic symptoms in patients with pseudomembranous colitis. Mild case of colitis may respond to drug discontinuance alone. Modulate cases should be managed with fluid, electrolyte and protein supplement. For severe cases, the treatment of choice is vancomycin.

Pruritus, urticaria, skin rashes and allergic symptoms have been occasionally encountered as with all penicillins. Mildly elevated aspartate aminotransferase levels (<100 units) have been reported in a few patients for whom pre-therapeutic determinations were not made. Minor changes in the results of cephalin flocculation tests have been noted without other evidence of hepatic dysfunction. Eosinophilia, with or without overt allergic manifestations, has been noted in some patients during therapy (Pascual, 1999).

6. Drug Interactions

Dicloxacillin decreases effects of oral contraceptives and warfarin. Disulfiram and probenecid may increase penicillin levels (Lacy et al., 2000).

7. **Administration**

Dicloxacillin sodium is administered orally. The drug has also been given by slow intravenous injection or infusion or by intramuscular injection.

Oral dicloxacillin should not be used for initial treatment of severe infections and should not be relied on in patients with nausea, vomiting, gastric dilatation, esophageal achalasia, or intestinal hypermotility. Since food interferes with gastrointestinal tract absorption of dicloxacillin, the drug should be administered at least 1 hr before or 2 hrs. after meals.

Dicloxacillin sodium powder for oral suspension should be reconstituted at the time of dispensing by adding the amount of water specified on the bottle to provide a suspension containing 62.5 mg of dicloxacillin per 5 mL. To avoid the formulation of lumps that may not be dispersible, the water should be added to the powder for oral suspension in 2 portions and the suspension should be agitated vigorously immediately after each addition (McEvoy, 2000).

8. **Dosage**

Dosage of dicloxacillin sodium is expressed in terms of dicloxacillin. Dosage of the drug should be adjusted according to the type and severity of infection.

Adult Dosage

The usual oral dosage of dicloxacillin for the treatment of mild to moderate upper respiratory tract infections or localized skin and skin structure infections caused by susceptible organisms in adults is 125 mg every 6 hrs. For more severe infections such as those of the lower respiratory tract or for disseminated infections in adults, the usual oral dosage of dicloxacillin is 250 mg every 6 hrs; higher dosage may be necessary depending on the severity of the infection.

Pediatric Dosage

Children weighing 40 kg or more may receive the usual adult dosage of dicloxacillin.

In children older than 1 month of age who weigh less than 40 kg, the usual oral dosage of dicloxacillin for the treatment of mild to moderate upper respiratory tract infections or localized skin and skin structure infections is12.5 mg/kg daily given in divided doses every 6 hrs. The usual oral dosage for treatment of more severe infections such as those of the lower respiratory tract and for disseminated infections in children older than 1 month of age who weigh less than 40 kg is 25 mg/kg daily given in divided dose every 6 hrs; higher dosage may be necessary depending on the severity of the infection. Some clinicians recommend that children older than 1 month of age receive 25-50 mg/kg daily in divided doses every 6 hrs for the treatment of mild to moderate infections.

When dicloxacillin is used in children as follow-up therapy to parenteral penicillinase- resistant penicillin therapy in the treatment of acute or chronic osteomyelitis caused by susceptible staphylococci, the usual oral dosage of the drug is 50-100 mg/kg daily given in divided doses every 6 hrs. If oral anti-infective therapy is used in the treatment of osteomyelitis, compliance must be assured and many clinicians suggest that serum bactericidal titers (SBT) be used to monitor adequacy of therapy and to adjust dosage.

Dosage in Renal Impairment

Adjustment of dicloxacillin dosage in patients with renal impairment is generally unnecessary (McEvoy, 2000 and Pascual, 1999).

Duration of Therapy

The duration of dicloxacillin therapy depends on the type and severity of infection and should be determined by the clinical and bacteriologic response of the patient. For most staphylococcal infections, therapy should be continued for at least 14 day; more prolonged therapy is necessary for the treatment of osteomylitis, endocarditis, or other metastatic infections. When oral dicloxacillin is used as follow-up therapy to parenteral penicillinase-resistant therapy in the treatment of acute osteomylitis, the drug is generally given for 3-6 weeks or until the total duration of parentral and oral therapy is at least 6 weeks; when used as follow-up therapy in the treatment of chronic osteomylitis, the drug is generally given for at least 1-2 months and has been given for as long as 1-2 years.

Although natural penicillins are generally preferred, if dicloxacillin is used in the treatment of infections caused by group A β-hemolytic streptococci, therapy should be continued for at least 10 days to decrease the risk of rheumatic fever and glomerulonephritis.

CHAPTER III

MATERIALS AND METHODS

Materials

A. Test Products

Three commercial brands of dicloxacillin capsules were bought from various drugstores. Each capsule contains Dicloxacillin Sodium equivalent to Dicloxacillin 500 mg. One was the innovator's product that was assigned as the reference standard against the other two leading locally manufactured brands. The letters A, B and C were given to represent the brand names of each product. Other informations of these products were shown in Appendix A.

B. Reagents

1. Working standard dicloxacillin sodium (Donated from Siam Bhaesach Co., Ltd) potency : 93.45%
2. Working standard cloxacillin sodium (Donated from Siam Bhaesach Co., Ltd.) potency : 98.75%
3. Acetonitrile HPLC grade (Lab-Scan, Thailand) Batch No. 01020099
4. Methanol HPLC grade (Lab-Scan, Thailand) Lot No. 97070068
5. Monobasic potassium phosphate AR. (E-Merck, Germany) Lot No. K23775573
6. Absolute ethanol (Lab-Scan, Thailand) Lot No. 97090059

C. Apparatus

1. Analytical balance (Sartorius, 1615MP ; S/N 3209026, Germany)
2. Digital pH meter (Orion, Germany)
3. High performance liquid chromatography (LC-10AD, Shimadzu, Japan)
4. Sonicator (Bransonic 221, USA)
5. Dissolution apparatus (VK 7000, Vankel Technology Group, Inc., USA)
6. Vortex mixer (Vortex-Genie, Scientific Industries, Inc., USA)
7. Centrifuge (Sigma 302K, Sigma Lab, Centrifuge Gmbtt, Germany)
8. Micropipet (Socorex, Switzerland)
9. Glassware

Methods

A. *In Vitro* Studies

Three commercial brands of 500 mg dicloxacillin capsules were evaluated following the tests as stated in the USP 24. The tests were:

1. Content of Active Ingredient

The amount of dicloxacillin in capsules was determined according to the method of USP 24. It was described as follow.

Diluent – Dissolve 5.44 g of monobasic potassium phosphate in water to make 2000 mL of solution, and adjust with 8N potassium hydroxide to a pH of 5.0±0.1.

Mobile phase – Prepare a suitable filtered mixture of diluent and acetonitrile (1500:500). Make adjustments if necessary.

Standard preparation – Dissolve an accurately weighed quantity of dicloxacillin sodium Working Standard in diluent to obtain a solution having a known concentration of about 1.1 mg/mL.

Chromatographic system – The liquid chromatograph is equipped with a 225 nm detector and a 4.6 mm x 25 cm column containing packing L1. The flow rate is about 2 mL/min.

Assay preparation – Remove, as completely as possible, the contents of not less than 10 capsules, and weigh. Mix, and transfer an accurately weighed portion of powder, equivalent to about 200 mg of dicloxacillin, to a 200 mL volumetric flask, dilute with diluent to volume, and mix for 10 mins. Filter about 25 mL of the resulting solution, discarding the first 5 mL of the filtrate. Use the clear filtrate as the assay preparation.

Procedure – Separately inject equal volumes (about 10 mcL) of the standard preparation and the assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of dicloxacilllin in the portion of capsule contents taken by formula

$$0.2CE(r_u/r_s)$$

in which

C is the concentration, in mg/mL, of dicloxacillin sodium working standard in standard preparation.

E is the dicloxacillin equivalent, in mcg per mg, of dicloxacillin sodium WS

r_u and r_s are the dicloxacillin peak responses obtained from the assay preparation and the standard preparation, respectively.

2. Uniformity of Dosage Units

Ten capsules of 500 mg dicloxacillin capsules from each brand were individually assayed for the percent labeled content of dicloxacillin in each capsules following the same method as analysis for content of active ingredient. The mean and standard deviation of percent labeled amount were calculated as well as the relative standard deviation.

3. Dissolution Test

The dissolution test was conducted using the USP dissolution apparatus I for dicloxacillin capsules (USP 24, 2000). A capsule of dicloxacillin was placed in each vessel of the dissolution tester containing 900 mL of water at 37 $\pm$ 0.5°C as dissolution medium . The apparatus was operated at the rate of 100 rpm. Five mL of each sample was withdrawn after the apparatus was operated at time 10, 20, 25, 30, 45, 60 and 90 min., respectively. The equivalent amount of water equilibrated at 37 $\pm$ 0.5°C was added immediately after each sampling to maintain a constant volume of dissolution medium. Twelve capsules of each brand were tested. The amount of dicloxacillin dissolved in each sample was quantitated using spectrophotometer by measuring its absorbance at the maximum wavelength of 274 nm and calculated using the calibration curve. The dissolution profiles were then constructed by plotting percent dicloxacillin dissolved of each brand versus time.

Calibration Curve

Stock standard solutions of dicloxacillin (1 mg/mL) were prepared in water. 1, 2, 3, 4, 5, 6, and 7 mL of dicloxacillin stock solution were transferred into 7 separated 10 mL flasks. Each of them was diluted with the dissolution medium to volume and mix. The final concentrations of dicloxacillin in standard solutions were 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 mg/mL. They were assayed by spectrophotometry at the maximum wavelength of 274 nm. The absorbance of dicloxacillin versus the

known dicloxacillin concentrations were fitted to the straight line using linear regression.

4. *In Vitro* Evaluation

The *in vitro* data of all three brands of dicloxacillin capsules were examined and evaluated to determine whether each brand conformed to the general standard requirements of USP 24 in order to conclude their pharmaceutical equivalences. In addition, the dissolution profile of each generic product was compared to that of the innovator's product to assess their similarities using difference (f_1) and similarity factors (f_2) (Thai FDA, 2000).

$$f_1 = [\{ \sum_{t=1}^{n} | R_t - T_t | \} / \sum_{t=1}^{n} R_t \}] \times 100$$

$$f_2 = 50 \times \log [\{ 1 + (1/n) \sum_{t=1}^{n} (R_t - T_t)^2 \}^{-0.5} \times 100]$$

n = the number of sampling points

R_t = percent dissolved of innovator's product at time t

T_t = percent dissolved of tested product at time t

B. *In Vivo* Studies

The methods used for *in vivo* studies were those as specified in the Criteria and Guideline for the Bioequivalence Study of Generic Drugs of Drug Control Department, Office of Food and Drug Administration, Thailand, 2000. The details was described as follows:

1. Test Products

All three commercial brands of 500 mg dicloxacillin capsules, were *in vivo* tested in this study.

2. Subjects

Fifteen healthy Thai male volunteers with the ages range from 18 to 45 years participated in this study. Demographic data are presented in Table 13. Prior to testing, all subjects had to pass the physical examination and clinical laboratory tests. The methods of the study were clearly explained to all subjects. They gave written informed consents before participating the study and they were asked to take no medication, alcoholic preparations and cigarettes for at least two weeks preceding the study and during the experimental period.

Inclusion criteria

1. Fifteen healthy Thai male volunteers with the ages range from 18 to 45 years with body mass index between 18 to 24 kg/m^2.
2. Non smoker
3. Non alcoholic drinker
4. No history of allergic reactions to a pennicillin antibacterial agent and related compound
5. No drug use at least two weeks prior to and during the study
6. Having written informed consent
7. Normal physical and laboratory biochemical test

Exclusion criteria

1. Refuse to finish the study
2. Allergic or having adverse drug reaction to dicloxacillin

3. Dose and Drug Administration

Two capsules of 500 mg dicloxacillin were given orally with 200 mL of water in a single dose. All subjects received each dose in the morning after 8 hrs overnight fast. No food or drink was permitted until 4 hrs after dosing.

4. Experimental Design

The study was conducted in a three way randomized complete crossover design. Each subject received the drug in a randomized order with 1 week washout period between each administration as shown in Table 2.

5. Sample Collection

5 mL of blood sample were collected from a forearm vein of each subject using a disposable syringe before drug administration and at 0.0, 0.5, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.50, 5.00 and 7.00 hrs after dosing. They were immediately transferred into tubes without any anticoagulants. These tubes were allowed to stand upright until the blood was clotted. Afterward, the serum was separated by micropipette and kept at -20°C until subsequent analysis.

6. Analysis of Dicloxacillin in Serum Samples

6.1 Sample preparation

1 mL of serum sample was mixed with 30 mcL of the internal standard solution (cloxacillin 0.2 mcg/mL in water) for 10 seconds using vortex mixer. The samples were then extracted employing an equal volume of acetonitrile. They were agitated using a mixer for 10 seconds and centrifuged at 5,000 rpm for 10 min. 20 mcL of clear supernatant liquid were injected into HPLC system.

6.2 Chromatographic systems

Apparatus : Shimadzu® LC-10A HPLC pump, equipped with Spectro Monitor 4100 variable wavelength UV detector and Shimadzu® C-R1A File No. 2 integrator.

Column : μ - Bondapak® (C_{18}), stainless steel column, 300 x 3.9 mm (i.d.), 125 A 10 μm of dimethyloctadecylsilyl bond amorphous silica. (Waters Associates Pty-Ltd., Milford, MA, USA)

UV detector : 230 nm

Mobile phase : 0.05 M Potassium dihydrogen phosphate : Acetronitrile (73.5 : 26.5)

Flow rate : 1.7 mL/min.

Pressure : 130 kg/cm^2

Injection volume : 20 mcL

Retention time : Dicloxacillin ~ 4 - 5 min.

Cloxacillin ~ 7 - 8 min.

The concentrations of dicloxacillin in serum samples were computed using a standard calibration curve.

6.3 Standard calibration curve

Nine standard concentrations of dicloxacillin in pooled serum were prepared. They were 0.9, 1.0, 1.5, 2.5, 5.0, 10.0, 25.0, 50.0 and 75.0 mcg/mL, respectively. These solutions were analyzed following the method described earlier. The peak area ratios of dicloxacillin to that of the internal standard versus known concentrations of dicloxacillin were fitted to straight line using linear regression (Thaijssen, 1980).

Table 2 Randomization Schedule

Sequence	Subject No.	Period		
		1	2	3
1	1	A	B	C
	2	A	B	C
	3	A	B	C
	4	A	B	C
	5	A	B	C
2	6	B	C	A
	7	B	C	A
	8	B	C	A
	9	B	C	A
	10	B	C	A
3	11	C	A	B
	12	C	A	B
	13	C	A	B
	14	C	A	B
	15	C	A	B

A, B, C : represent the brand name of 500 mg. dicloxacillin capsules

7. Assay Validation

The modified Pachla et al., (1986) method used for analyzing dicloxacillin in serum sample was validated under the following conditions.

7.1 Accuracy

Accuracy in term of percent recovery was done by computing the ratio of inversely estimated concentration obtained using linear regression of a standard dicloxacillin concentrations in serum (0.9, 20 and 75 mcg/mL) to known concentration of each standard dicloxacillin concentration in serum multiplied by one hundred.

7.2 Within-run precision

This precision was determined by analyzing three sets of standard dicloxacillin concentration in serum (0.9, 20 and 75 mcg/mL) on the same day. Peak area ratio of dicloxacillin to cloxacillin was compared and the percent coefficient of variation (%C.V.) for each concentration was determined.

7.3 Between-run precision

This precision was determined by comparing peak area ratio of three sets of standard dicloxacillin concentration in serum (0.9, 20 and 75 mcg/mL) on three different days and the percent coefficient of variation (%C.V.) for each concentration was calculated.

7.4 Linearity

Linearity in term of the coefficient of determination (r^2) was read from the linear regression line of the calibration curve.

Acceptance Criteria

The percent recovery was within ±20%. The percent coefficient of variations were less than 15 and the coefficient of determination was greater than 0.99 (Shah et al., 1991).

8. Pharmacokinetic Analysis

The pharmacokinetic analysis of individual serum dicloxacillin concentrations versus time profiles from each treatment were established using noncompartment method.

The peak serum concentration (C_{max}), and the time to reach the peak serum concentration (t_{max}) of dicloxacillin were directly obtained from the data meanwhile the area under the concentration-time curve (AUC) was calculated using trapezoidal rule and extended to infinity by adding with C*/K term, where C* was the last measurable serum dicloxacillin concentration and K is the first order elimination rate constant. Other pharmacokinetic parameters, the absorption rate constant (K_a) was obtained by residual method assuming that the kinetic of dicloxacillin followed one compartment open model with first order pharmacokinetic. The elimination rate constant (K) was obtained from slope of the concentration-time curve in semilogarithmic scale. The mean absorption time (MAT) was calculated using an equation: $MAT = 1/K_a$. The ratio of volume of distribution to the fraction of dose to be absorbed (V_d/F) was calculated using an equation: $V_d/F = X_0K_a$ / intercept (K_a-K) , and the elimination half-life ($t_{1/2}$) was calculated using an equation: $t_{1/2} = 0.693/ K$.

9. Evaluation of Bioequivalence

The bioequivalence of all three brands of 500 mg dicloxacillin capsules were evaluated using the corresponding pharmacokinetic parameters AUC and C_{max}. Both of them were transformed into logarithmic scales.

9.1 Statistical test

The differences of these two corresponding pharmacokinetic parameters in terms of log- transformed data among the three brands were determined by analysis of variance for three way crossover design at $\alpha = 0.05$.

9.2 Construction of 90% confidence interval

A 90% confidence interval of individual parameter ratio based on log-transformed data was constructed using an equation

$$90\%\ CI = (\bar{X}_T - \bar{X}_R) \pm (t_{0.1,df} \times S.E.)$$

where; $\bar{X}_T$ and $\bar{X}_R$ = Mean ln C_{max} and mean ln AUC values of tested and innovator's product, respectively.

$t_{0.1}$ = Tabulated t value at $\alpha = 0.1$, df of MSE

S.E. = $\sqrt{2MSE / n}$ Where; MSE is the mean square error obtained from the ANOVA table.

$$\% \text{ Lower limit} = \{e^{[(XT - XR) - (t0.1,df \times S.E.)]}\} 100$$

$$\% \text{ Upper limit} = \{e^{[(XT - XR) + (t0.1,df \times S.E.)]}\} 100$$

Each tested brand was considered to be bioequivalent to the innovator's product, when the 90% confidence interval of individual parameter of tested products relative to that of innovator's product was within 80-125%.

CHAPTER IV

RESULTS AND DISCUSSION

A. *In Vitro* studies

1. Content of active ingredient

All products were assayed for content of active ingredient and found that each brand was within the limits of 90-120 percent as specified in the USP 24. The %L.A. of brands A and C were quite similar meanwhile that of brand B was the highest values as shown in Table 3. The %L.A. of brand B relative to those of brands A and C was more than 5 percent difference. According to the Thai FDA guideline, brand B should be excluded. However, it was still included in this study since its earlier produced batch was unavailable in the market.

2. Uniformity of dosage units

All three commercial brands of 500 mg dicloxacillin capsules were tested for uniformity of dosage units. Results were presented in Table 4. Each of them met the USP 24 specifications within the range of 85-115 % of the labeled claim and the %C.V. was less than 6%. However, the precision of uniformity of dosage units of these three brands resulted in the same manner as those of content of active ingredients.

3. Dissolution test

The amount of dicloxacillin dissolved at each sampling time was quantitated by UV spectrophotometer at the maximum wavelength of 274 nm and the calibration curve plotted between the concentration of dicloxacillin and absorbance (Appendix A). The dissolution data of brand A, B and C were displayed in Tables 5-7,

respectively. Brand A dissolved rapidly and uniformity. This was seen by 50% and 100% dissolution of the drug could be reached at 10 and 20 mins, respectively, as well as their standard deviations were small and consistent. Brands B and C showed erratic slower dissolution as indicated in Tables 6 and 7. Their dissolution behaviors (B and C) appeared to be similar. Uniformity of dosage unit might contribute to these dissolution characteristics of each individual.

Three products of dicloxacillin capsules met the requirement of the United States Pharmacopeia 24 as observed in Tables 5-7. None of the twelve capsules dissolved less than 75% of labeled amount of dicloxacillin within 30 minutes. The mean plots of dissolution profiles of all brands were shown in Figure 2.

The dissolution profile of each generic brand was compared to that of innovator's product to assess their similarities using difference factor (f_1) and similarity factor (f_2). Results in Table 8 demonstrated that the difference factor (f_1) and similarity factor (f_2) of brands B vs A and brands C vs A were 9.74, 51.11 and 5.70, 59.67, respectively. These values were in the acceptance criteria (f_1 = 0-15, and f_2 = 50-100), referring that each of dissolution profile of the local brand was equivalent to that of the innovator's product.

4. *In vitro* evaluation

All *in vitro* studies of these three brands revealed that they completely complied the specification requirements as stated in the USP 24. These could be concluded that all of them were pharmaceutical equivalence.

Table 3 Content of Active Ingredient of 500 mg Dicloxaciilin Capsules of the Innovator's and the Tested Products

Assay No.	%Labeled Amount		
	A	B	C
1	104.35	113.77	105.80
2	105.57	111.27	108.03
3	103.67	115.27	104.40
Mean	104.53	113.58	106.08
S.D.	0.96	2.23	1.83
%C.V.	0.92	1.97	1.73

Table 4 Uniformity of Dosage Units of 500 mg Dicloxacillin Capsules of the Innovator's and the Tested Product

Dosage Unit	% Labeled Amount		
	A	B	C
1	105.99	114.97	112.58
2	105.92	114.65	112.81
3	105.00	113.46	111.10
4	103.24	114.17	106.87
5	104.52	113.98	111.14
6	104.62	114.43	106.18
7	103.70	114.86	112.03
8	105.45	113.63	106.90
9	104.07	113.58	107.65
10	105.49	104.81	105.39
Mean	104.80	113.25	109.27
S.D.	0.94	3.01	2.92
%C.V.	0.89	2.66	2.67

Table 5 Dissolution Data of 500 mg Dicloxacillin Capsules of Brand A

Flask	Time (min)						
No.	10	20	25	30	45	60	90
1	51.92	103.60	103.10	103.71	104.20	103.58	102.80
2	48.94	102.90	102.40	102.21	102.70	101.96	101.50
3	53.04	103.40	103.50	103.08	103.20	102.21	101.70
4	53.54	105.00	105.10	102.59	102.20	101.22	101.10
5	58.27	104.60	102.90	102.34	102.30	101.59	101.20
6	62.75	105.50	105.20	102.96	103.50	102.96	102.30
7	40.47	102.50	102.10	101.47	101.20	101.71	101.50
8	42.84	102.10	101.60	103.21	103.30	103.46	103.20
9	49.06	102.70	102.40	103.33	105.00	103.95	103.00
10	56.03	100.30	100.00	103.21	103.60	103.21	102.80
11	48.31	102.40	102.70	101.71	102.80	102.09	101.70
12	40.47	103.40	103.00	101.71	103.20	102.21	102.00
Mean	50.47	103.20	102.83	102.63	103.10	102.51	102.07
S.D.	6.93	1.41	1.41	0.73	0.98	0.89	0.73
%C.V.	13.73	1.36	1.37	0.72	0.95	0.86	0.72

Table 6 Dissolution Data of 500 mg Dicloxacillin Capsules of Brand B

Flask No.	Time (min)						
	10	20	25	30	45	60	90
1	30.09	78.60	101.02	113.83	114.53	114.29	113.54
2	41.94	105.70	102.01	107.67	108.50	107.44	106.39
3	38.76	96.58	105.68	112.10	114.78	114.53	113.91
4	47.47	105.09	117.53	115.78	114.29	113.91	113.54
5	36.71	96.09	108.78	113.26	115.07	114.29	113.17
6	37.55	75.76	101.76	112.48	113.09	112.92	112.67
7	43.73	100.90	110.75	103.58	102.20	101.96	101.50
8	31.08	95.11	113.46	110.18	113.91	113.29	113.04
9	34.48	91.16	108.54	111.55	112.42	112.92	112.67
10	36.10	85.37	105.33	107.69	110.43	109.93	111.30
11	50.78	107.06	114.57	115.03	117.27	116.03	115.66
12	37.09	87.59	110.51	110.30	110.30	109.43	109.81
Mean	38.82	93.75	108.33	111.12	112.23	111.75	111.43
S.D.	6.20	10.37	5.34	3.49	3.98	3.95	3.91
%C.V.	15.98	11.06	4.93	3.14	3.55	3.54	3.51

Table 7 Dissolution Data of 500 mg Dicloxacillin Capsules of Brand C

Flask No.	Time (min)						
	10	20	25	30	45	60	90
1	30.02	103.36	106.07	105.33	107.94	107.44	106.94
2	32.38	106.56	108.91	108.41	106.57	106.07	105.32
3	41.22	95.23	106.2	108.91	107.44	106.39	106.07
4	33.5	71.82	101.64	107.55	109.8	109.43	108.93
5	38.36	88.82	101.14	103.24	105.57	105.57	105.32
6	37.98	87.47	100.16	102.75	111.17	110.18	109.43
7	34.12	105.95	106.93	106.32	106.32	109.18	106.44
8	25.54	106.32	106.69	105.95	105.82	105.45	105.2
9	64.38	95.97	102.62	102.99	107.94	107.44	107.19
10	43.46	90.92	102.5	104.84	109.8	109.43	109.18
11	27.4	90.18	99.79	101.02	106.69	106.69	106.44
12	27.9	88.95	101.14	105.33	107.56	106.94	106.44
Mean	36.36	94.30	103.65	105.22	107.72	107.52	106.91
S.D.	10.46	10.25	3.11	2.41	1.74	1.64	1.51
%C.V.	28.78	10.87	3.00	2.29	1.62	1.53	1.41

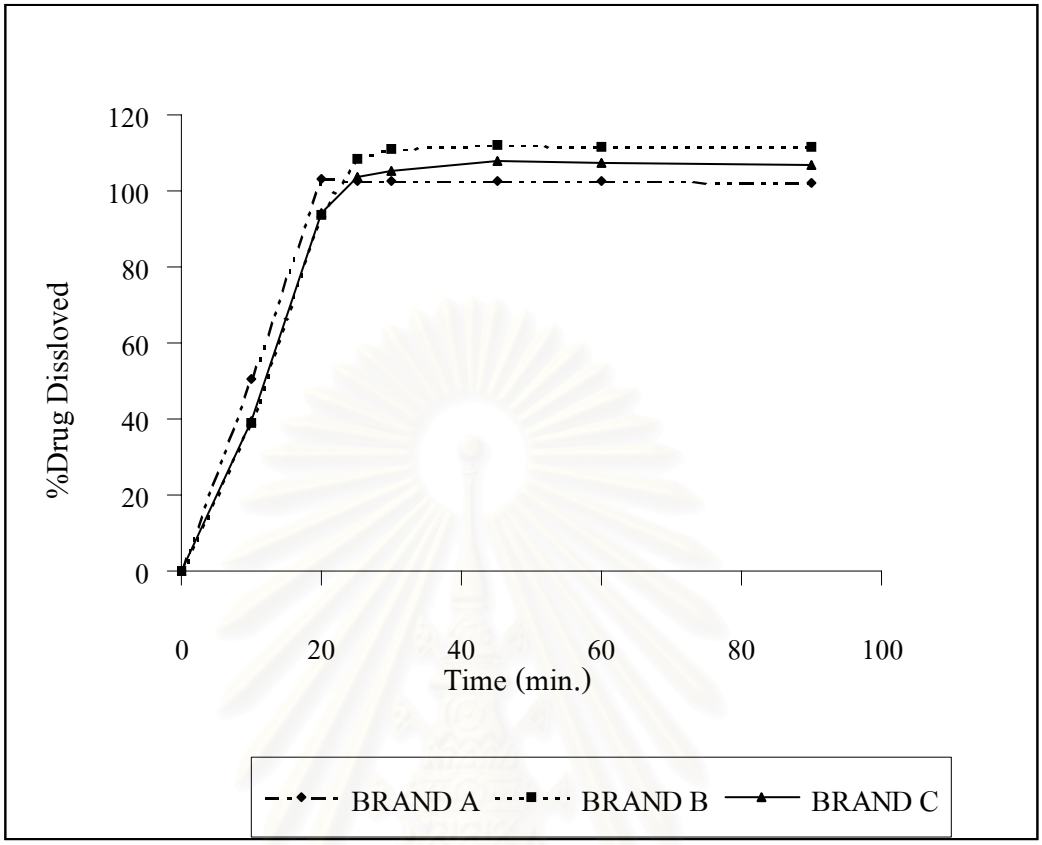


Figure 2 The Mean Dissolution Profiles of Three Brands of 500 mg Dicloxacillin Capsules in Water. Values are mean of 12 units. Error bars were omitted for clarity.

Table 8 The Difference Factor (f_1) and Similarity Factor (f_2) of Brands B and C Relative to Brand A.

Parameters	Values	
	B vs A	C vs A
Difference factor (f_1)[a]	9.74	5.70
Similarity factor (f_2)[b]	51.11	59.67

[a] Acceptance value : 0-15

[b] Acceptance value : 50-100

B. *In Vivo* Studies

1. Analysis of Dicloxacillin in Serum

Chromatograms of blank serum and serum spiked with cloxacillin (internal standard) were shown in Figure 3. Chromatograms of serum spiked with dicloxacillin and cloxacillin were shown in Figure 4. The retention times of cloxacillin and dicloxacillin were 4.7 and 7.6 mins, respectively. No any interference peaks due to the presence of plasma protein and/or endogenous substances were observed, indicating the specificity or selectivity of the analytical method used in this study.

The method of analysis was validated by determining the accuracy, the within run and between run precisions. The percent recovery for accuracy was 99.50 to 118.73 percent as report in Table 9. The %C.V. in the within-run and between-run precision were 3.81 to 12.70 percent and 5.69 to 13.68 percent, respectively and they were displayed in Tables 10 and 11. These results were within acceptance criteria for accuracy and precisions. The calibration curve of peak area ratio of dicloxacillin to cloxacillin versus serum dicloxacillin concentrations as shown in appendix was linear covered the range of concentrations used with the coefficient of determination (r^2) of 0.9988. The data including linear regression equation were reported in Table 12. The lower limit of quantitation was 0.9 mcg/mL.

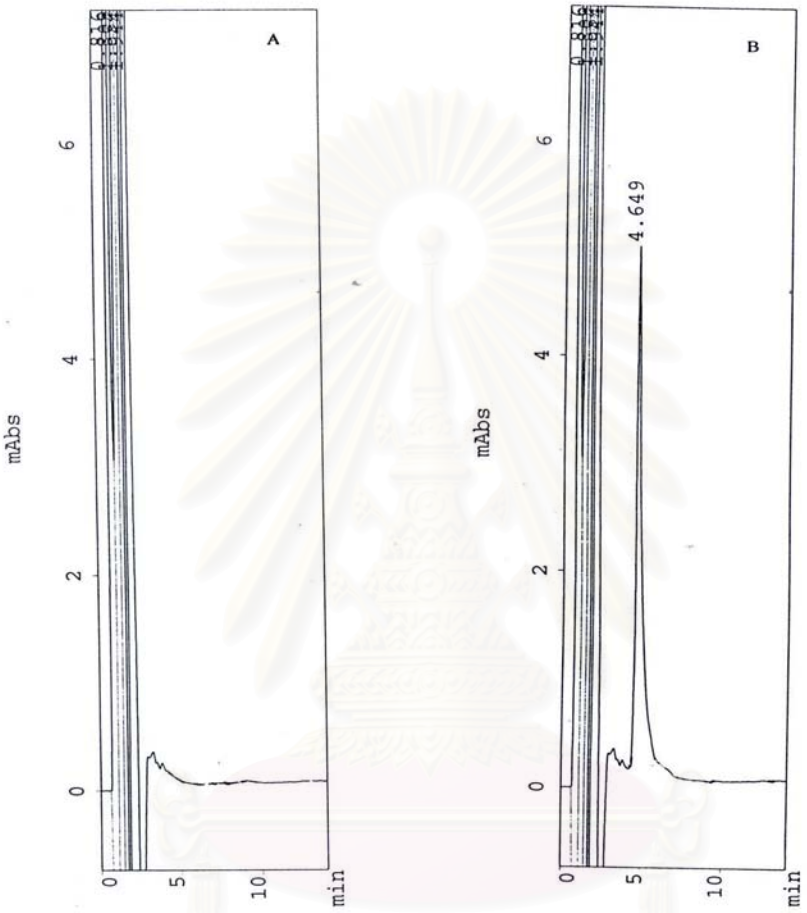


Figure 3 Chromatogram of Blank Serum (A) and Serum Spiked with Cloxacillin(internal standard) 6 mcg/mL (B)

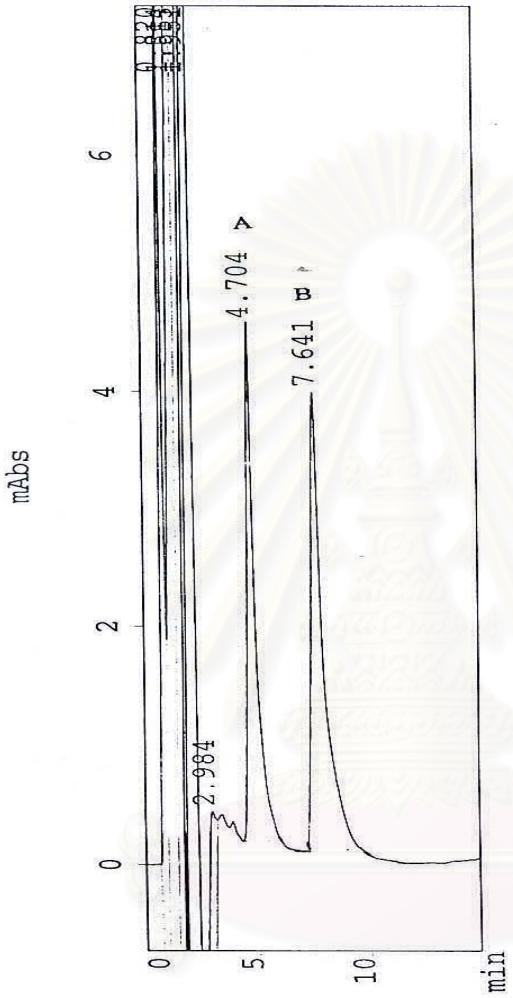


Figure 4 Chromatogram of Serum Spiked with Cloxacillin 6 mcg/mL (A) and Dicloxacillin 10 mcg/mL (B)

Peak A = cloxacillin, retention time 4.7 minutes

Peak B = dicloxacllin, retention time 7.6 minutes

Table 9 Percent Recovery of Analytical Method for Determination of Dicloxacillin in Serum.

Standard No.	Concentration (mcg/mL)	Peak Area Ratio*	Inversely Estimated Concentration* (mcg/mL)	%Recovery*
1	0.9	0.01487	1.068	118.73
2	20	3.06635	19.901	99.50
3	75	12.10551	75.664	100.89

* Results are mean of triplicate determinations.

Table 10 Within-Run Precision of Analytical Method for Determination of Dicloxacillin in Serum.

Concentration (mcg/mL)	Peak Area Ratio			Mean ± S.D.	%C.V.
	1	2	3		
0.9	0.01700	0.01341	0.01420	0.015 ± 0.0019	12.70
20	3.22757	2.90675	3.06473	3.066 ± 0.160	5.23
75	11.80267	12.63688	11.87697	12.106 ± 0.4617	3.81

Table 11 Between-Run Precision of Analytical Method for Determination of Dicloxacillin in Serum.

Concentration (mcg/mL)	Peak Area Ratio			Mean ± S.D.	%C.V.
	1	2	3		
0.9	0.1700	0.01373	0.01335	0.015 ± 0.0020	13.68
20	3.22757	2.87588	2.90607	3.003±0.1949	6.49
75	11.80267	13.22730	12.60091	12.540 ± 0.7140	5.69

Table 12 Linearity of Analytical Method for Determination of Dicloxacillin in Serum.

Standard No.	Concentration (mcg/mL)	Peak Area of Dicloxacillin*	Peak Area of Internal Standard*	Peak Area Ratio*
1	0.90	1875	110265	0.01700
2	25.00	402373	109760	3.66593
3	75.00	1337620	112623	11.87697

* Each data point is mean triplicate determation.

r^2 = 0.9988, y = 0.16213x - 0.1551

where: y = Peak area ratio

x = Concentration

r^2 = Coefficient of determination

2. Serum Dicloxacillin Concentrations

Fifteen male subjects were enrolled in this study. They were healthy based on passing physical examination as well as clinical blood biochemistry laboratory tests which presented in Appendix C. Their demographic data were shown in Table 13. The mean $\pm$ S.D. of age, weights and body mass index (BMI) values of them were of 28.20 $\pm$ 4.44 years , 61.93 $\pm$ 7.73 kg and 21.16 $\pm$ 1.92 kg/m^2, respectively. All BMI values were in the normal range (18-24 kg/m^2) as recommended (FDA, 2000).

The serum concentration of dicloxacillin at each sampling time interval ranging from 0 to 7 hours after oral administration of two 500 mg dicloxacillin capsules of brands A, B and C were presented in Tables 14 to 16, respectively. It was clearly observed that wide variations of serum dicloxacillin concentrations among subjects were resulted. This was due to intra and intersubjects variations of the drug. Moreover the serum dicloxacillin concentration in some subjects of all three brands showed irregular absorption phase as seen by occurring two or three peak concentrations. This might be explained that dicloxacillin underwent enterohepatic cycling resulted in reabsorption of drug repeatedly until it was totally eliminated. The serum dicloxacillin concentrations in all subjects were equal zero at the time zero. Individual serum dicloxacillin concentration-time profile of all brands for each of fifteen subjects were shown graphically from Figures 5 to 19. Comparisons of the mean serum dicloxacillin concentration profile of each brand from fifteen subjects were illustrated in Figure 20.

None was withdrawn from the study or exhibited signs of allergy and adverse drug reactions to dicloxacillin throughout the study period.

Table 13 Demographic Data of Subjects Participated in This Study.

Subject No.	Age (year)	Height (m)	Weight (kg)	BMI[a] (kg/m^2)
1	35	1.72	68	22.99
2	25	1.72	54	18.25
3	29	1.65	65	23.88
4	30	1.61	52	20.06
5	29	1.80	60	18.52
6	24	1.82	79	23.85
7	30	1.80	76	23.46
8	23	1.70	61	21.11
9	28	1.74	56	18.50
10	29	1.66	55	19.96
11	29	1.65	58	21.30
12	26	1.68	57	20.20
13	23	1.70	64	22.15
14	24	1.70	64	22.15
15	39	1.69	60	21.01
Mean	28.20	1.71	61.93	21.16
S.D.	4.44	0.06	7.73	1.92
%C.V.	0.16	0.04	0.13	0.09

$$\text{Body Mass Index} = \frac{\text{Weight (kg)}}{\text{Height (m)}^2}$$

Table 14 Serum Dicloxacillin Concentration (mcg/mL) from 15 Subjects Following Oral Administration of 2x500 mg Dicloxacillin Capsules of Brand A

Subject No.	Time (hr.)										
	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.50	3.50	5.00	7.00
1	8.25	8.08	9.48	9.54	9.60	9.58	9.92	15.08	21.97	7.12	1.55
2	26.18	29.27	29.99	27.23	26.52	23.65	22.69	21.00	17.76	8.62	2.62
3	23.54	33.66	34.90	33.59	35.39	33.02	28.04	22.20	12.25	6.11	2.35
4	8.24	8.68	9.47	13.24	15.83	18.54	15.71	13.98	10.54	1.11	0.00
5	40.01	42.61	50.47	33.11	28.28	27.24	29.34	21.36	9.75	4.06	1.12
6	67.29	48.73	44.01	42.84	29.36	29.32	27.20	17.27	9.21	3.50	1.35
7	3.59	4.49	4.62	4.67	5.50	5.54	14.51	15.45	17.98	6.83	1.87
8	20.04	28.63	25.88	21.82	14.95	13.47	12.74	11.69	15.19	10.36	2.52
9	15.05	14.14	13.25	13.10	26.42	36.16	33.16	26.25	12.25	5.93	1.88
10	39.75	49.14	42.88	37.89	37.24	31.32	40.01	39.52	19.33	7.53	1.71
11	35.30	29.57	18.52	18.67	17.10	25.59	27.89	27.94	13.67	4.78	1.32
12	27.33	39.92	35.71	33.54	26.77	21.05	17.34	14.01	6.68	3.89	1.66
13	40.75	56.21	42.25	31.65	23.73	13.81	16.41	9.98	5.02	2.23	0.00
14	13.75	54.12	49.91	36.01	27.41	21.16	16.01	10.94	7.59	4.38	2.20
15	12.94	23.86	34.57	36.12	36.48	35.90	29.46	28.63	17.24	7.69	2.35
Mean	25.47	31.41	29.73	26.20	24.04	23.02	22.70	19.69	13.10	5.61	1.88
Min	3.59	4.49	4.62	4.67	5.50	5.54	9.92	9.98	5.02	1.11	1.12
Max	67.29	56.21	50.47	42.84	37.24	36.16	40.01	39.52	21.97	10.36	2.62
S.D	16.92	17.12	15.40	11.81	9.58	9.49	8.73	8.19	5.04	2.50	0.49
%C.V	66.43	54.50	51.80	45.08	39.85	41.23	38.46	41.59	38.47	44.56	25.90

Concentrations in all subjects = 0 at t = 0

Table 15 Serum Dicloxacillin Concentration (mcg/mL) from 15 Subjects Following Oral Administration of 2x500 mg Dicloxacillin Capsules of Brand B

Subject No.	Time (hr.)										
	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.50	3.50	5.00	7.00
1	12.87	17.46	25.86	37.12	40.86	37.14	30.84	21.91	11.13	2.34	0.00
2	26.28	30.54	48.28	45.38	35.22	30.41	26.35	17.44	8.35	3.39	0.00
3	3.20	4.73	5.69	5.33	4.97	3.65	2.70	4.06	28.09	13.91	4.06
4	1.07	4.83	5.54	6.04	7.70	7.78	10.20	10.29	23.97	5.64	1.07
5	32.66	36.39	37.87	36.03	28.43	23.95	20.78	15.87	10.63	3.45	1.58
6	14.69	28.37	39.23	47.40	51.58	47.10	38.55	28.41	18.53	7.93	2.45
7	26.88	44.85	51.67	46.46	33.83	25.38	19.08	14.52	5.70	2.11	0.00
8	8.91	10.06	18.28	24.04	23.84	19.94	20.98	18.45	14.30	7.60	2.84
9	4.44	4.50	4.16	3.26	2.61	2.59	3.12	4.94	25.13	11.51	3.05
10	11.64	23.08	25.86	38.62	39.40	42.61	37.51	41.85	17.84	7.59	2.01
11	58.77	52.73	33.68	30.94	24.85	23.77	20.00	14.89	7.33	3.07	0.90
12	34.22	33.57	30.95	30.46	26.55	22.20	17.45	12.72	6.29	2.63	0.00
13	16.62	20.33	22.99	33.44	39.18	34.52	27.19	24.53	10.38	3.63	1.14
14	21.09	50.48	46.85	38.44	29.36	23.30	20.92	16.47	10.11	6.60	2.28
15	20.10	34.63	39.19	41.13	39.23	37.39	33.58	26.10	16.99	7.25	3.55
Mean	19.56	26.44	29.07	30.94	28.51	25.45	21.95	18.16	14.32	5.91	2.27
Min	1.07	4.50	4.16	3.26	2.61	2.59	2.70	4.06	5.70	2.11	0.90
Max	58.77	52.73	51.67	47.40	51.58	47.10	38.55	41.85	28.09	13.91	4.06
S.D	14.92	16.18	15.58	14.88	14.15	13.39	10.95	9.57	7.16	3.50	1.05
%C.V	76.28	61.20	53.59	48.09	49.63	52.61	49.89	52.70	50.00	59.22	46.16

Concentrations in all subjects = 0 at t = 0

Table 16 Serum Dicloxacillin Concentration (mcg/mL) from 15 Subjects Following Oral Administration of 2x500 mg Dicloxacillin Capsules of Brand C

Subject No.	Time (hr.)										
	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.50	3.50	5.00	7.00
1	12.31	14.21	15.64	14.66	13.33	11.42	13.08	24.92	14.69	3.74	1.27
2	38.39	39.74	39.64	35.38	33.18	29.85	23.71	16.36	8.02	2.53	0.00
3	13.53	17.93	20.22	24.63	28.53	23.08	20.31	16.11	10.09	4.09	1.82
4	38.52	32.15	22.73	17.77	14.58	14.29	13.10	11.97	7.97	3.48	1.15
5	24.90	38.30	50.77	34.40	28.87	24.82	26.41	21.46	10.71	4.08	1.12
6	58.10	59.46	45.38	38.99	35.27	28.41	24.90	17.67	9.85	4.80	1.60
7	3.03	4.75	5.09	5.30	12.98	13.52	15.24	11.02	13.62	5.20	1.79
8	9.68	14.50	19.99	17.77	14.30	12.58	15.12	15.73	9.84	7.23	2.18
9	16.10	23.51	24.05	22.18	20.04	25.40	23.08	17.86	12.55	8.82	4.97
10	15.81	31.49	49.84	55.77	52.21	50.95	45.01	34.29	14.14	5.92	1.55
11	28.05	25.52	26.98	25.46	15.92	14.93	16.16	18.56	13.06	6.54	1.67
12	15.41	20.56	17.53	18.25	18.24	16.00	18.68	22.95	16.79	6.24	1.22
13	23.28	50.95	49.21	35.45	26.22	25.99	20.04	12.84	5.96	1.77	0.00
14	41.10	46.53	43.42	40.76	31.35	28.48	20.64	15.00	6.93	4.09	1.23
15	12.47	14.78	29.35	32.37	31.41	37.15	33.22	28.46	18.48	9.82	3.78
Mean	23.38	28.96	30.66	27.94	25.10	23.79	21.91	19.01	11.51	5.22	1.95
Min	3.03	4.75	5.09	5.30	12.98	11.42	13.08	11.02	5.96	1.77	1.12
Max	58.10	59.46	50.77	55.77	52.21	50.95	45.01	34.29	18.48	9.82	4.97
S.D	14.91	15.56	14.57	12.75	10.95	10.75	8.42	6.42	3.65	2.23	1.15
%C.V	63.77	53.73	47.52	45.63	43.63	45.19	38.43	33.77	31.71	42.72	58.78

Concentrations in all subjects = 0 at t = 0

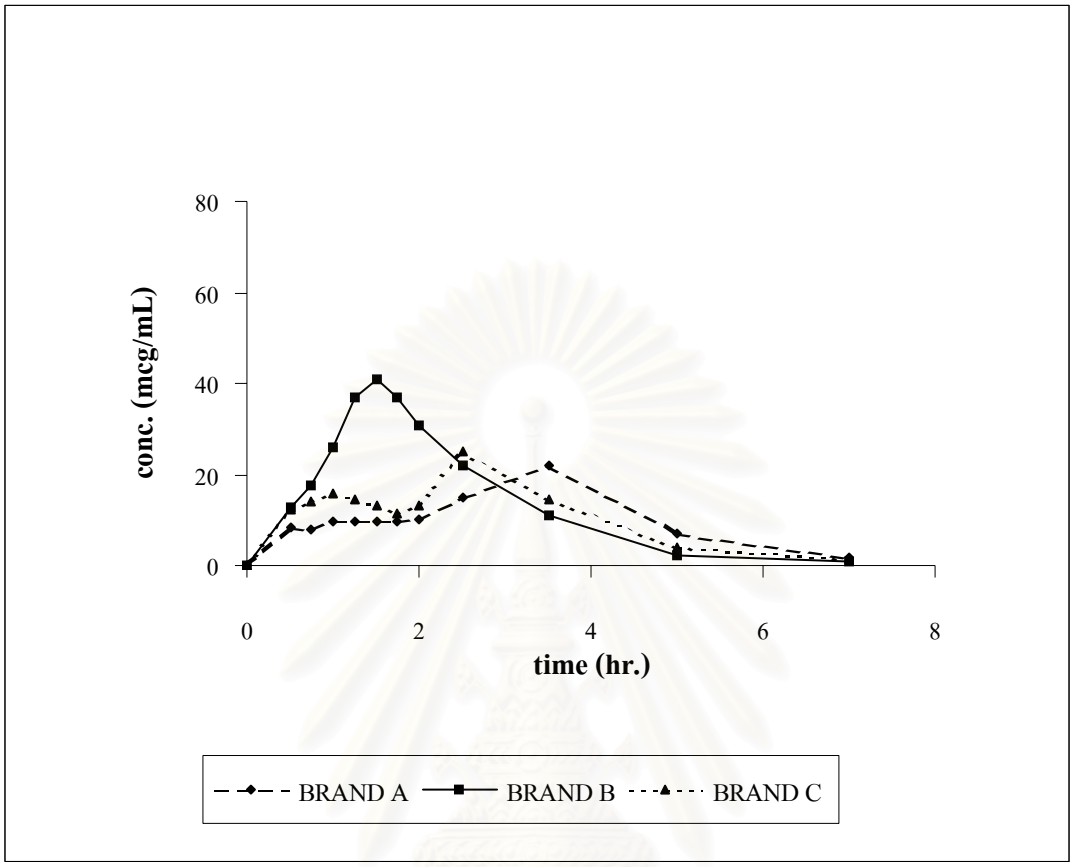


Figure 5 Serum Dicloxacillin Concentration-time Profile of Subject No.1 Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

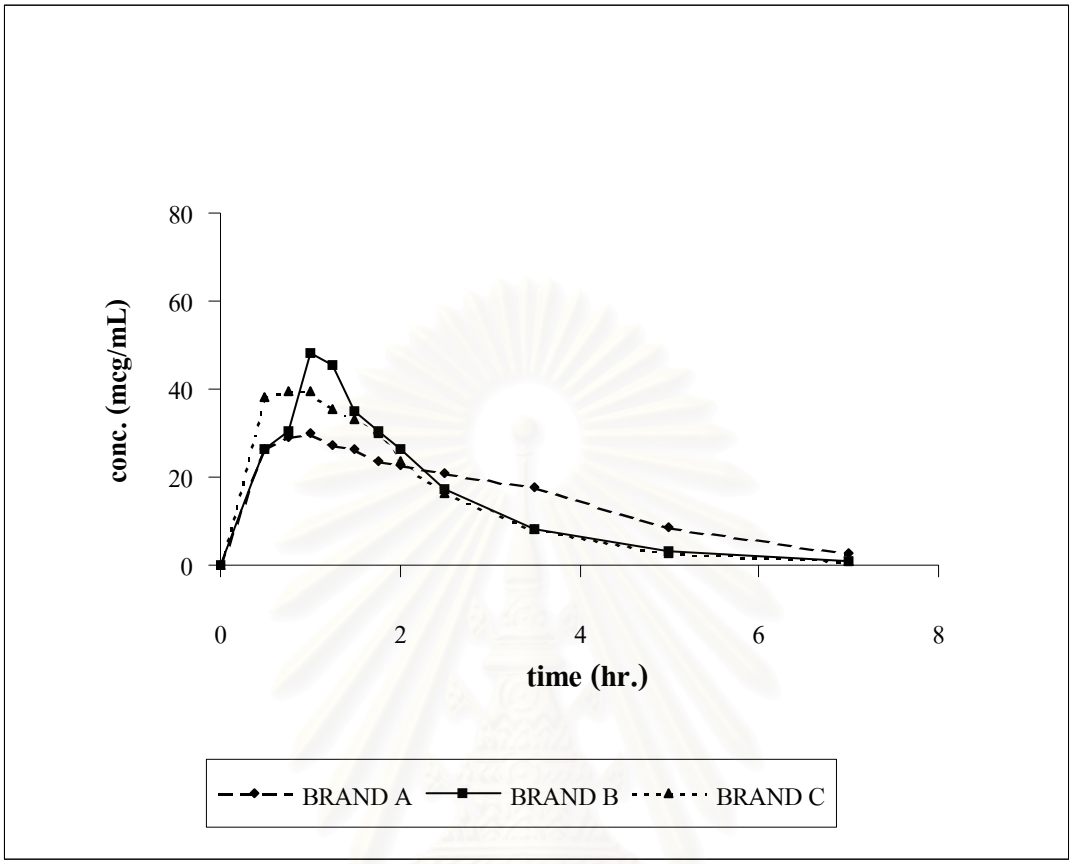


Figure 6 Serum Dicloxacillin Concentration-time Profile of Subject No.2 Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

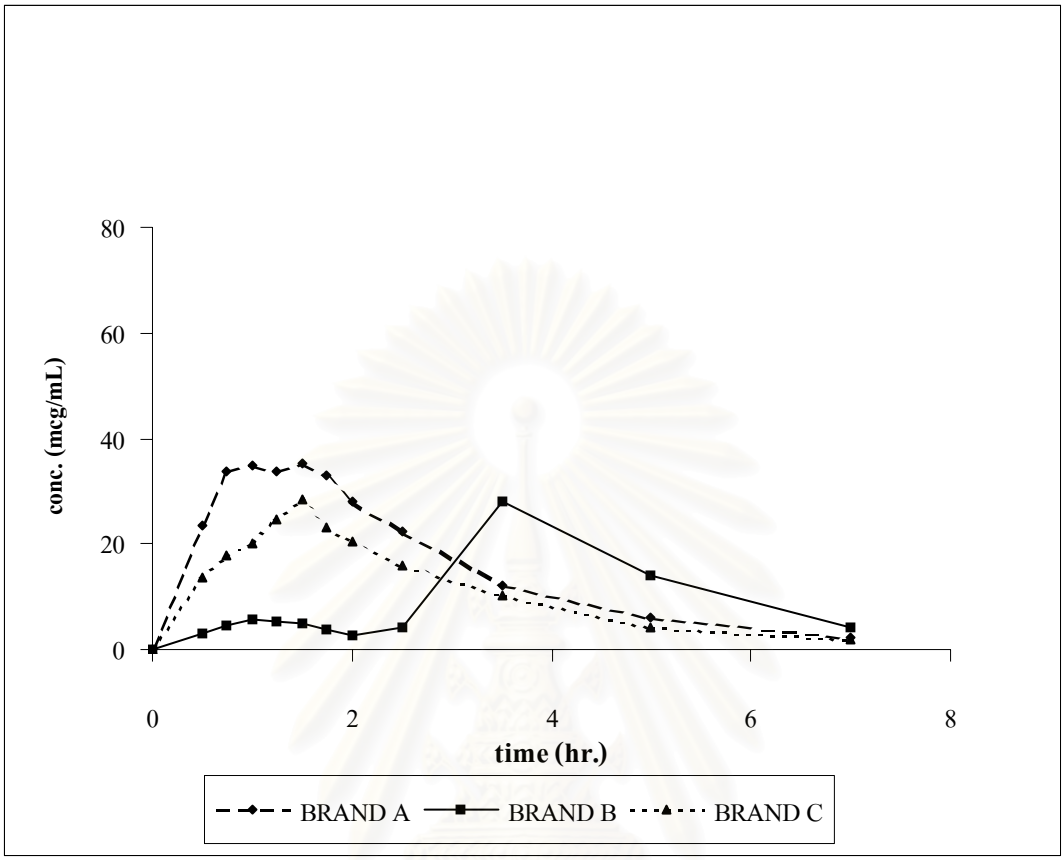


Figure 7 Serum Dicloxacillin Concentration-time Profile of Subject No.3 Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

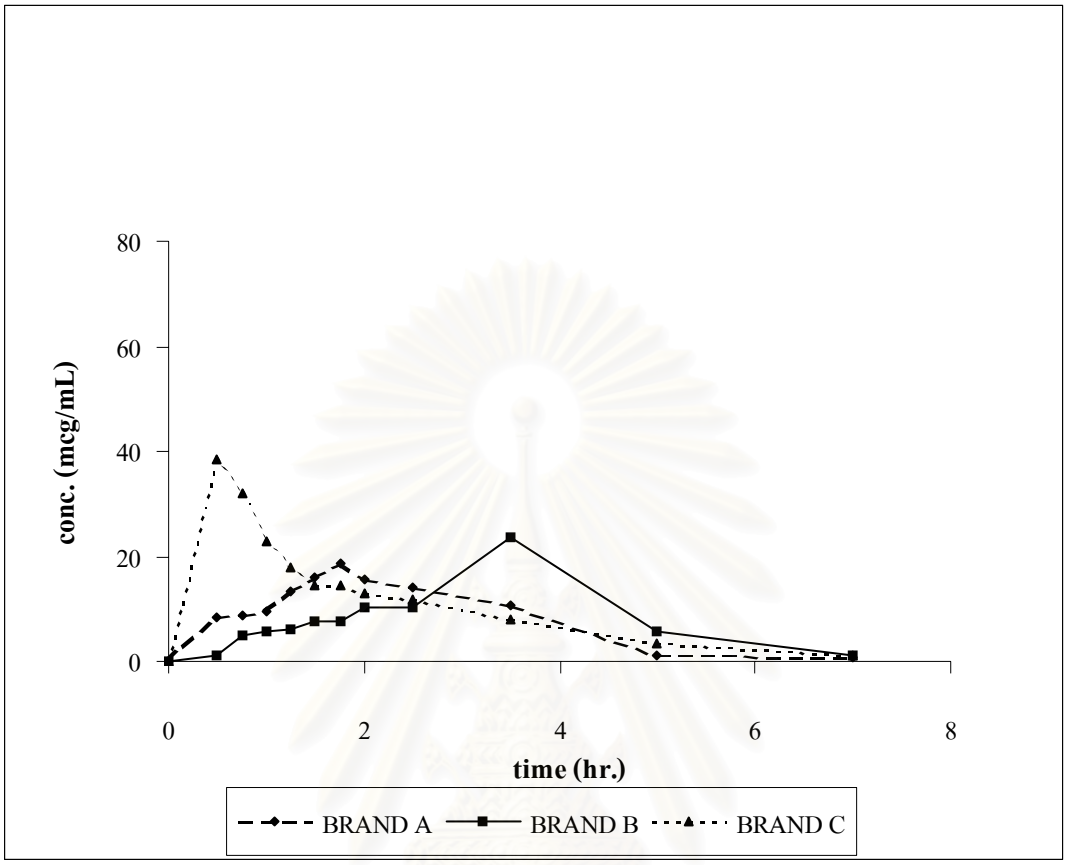


Figure 8 Serum Dicloxacillin Concentration-time Profile of Subject No.4 Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

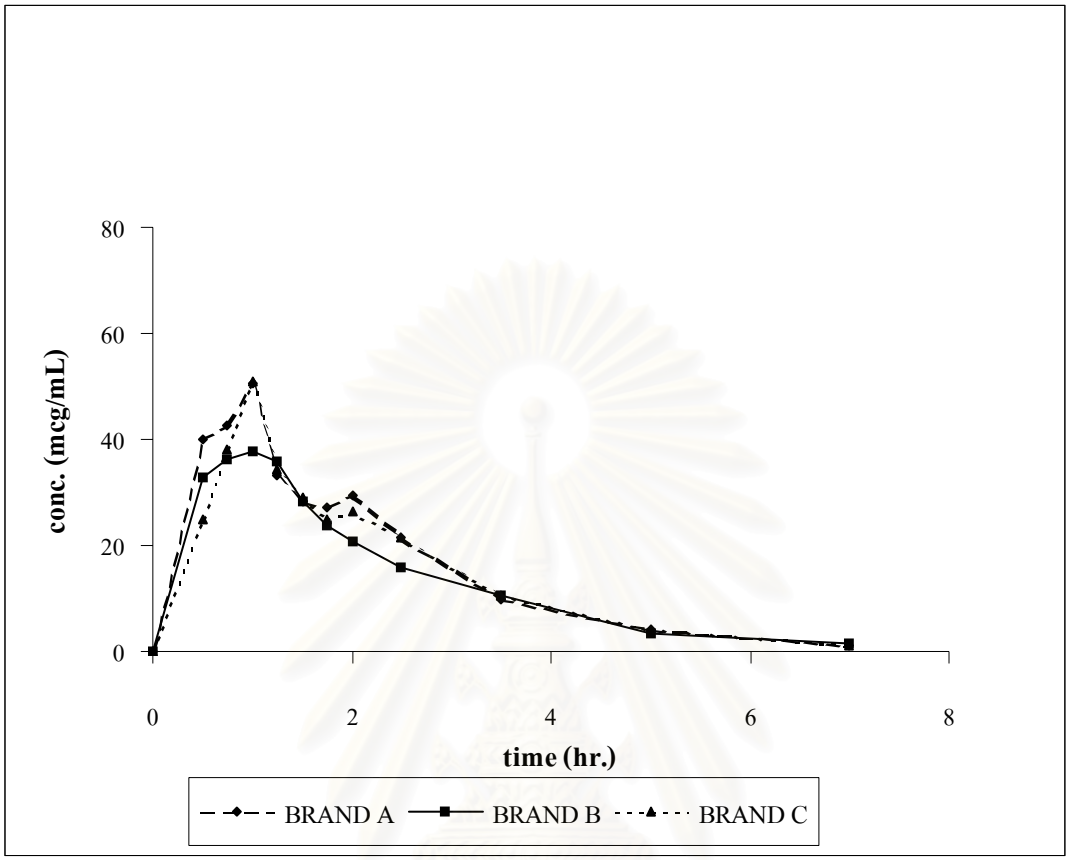


Figure 9 Serum Dicloxacillin Concentration-time Profile of Subject No.5 Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

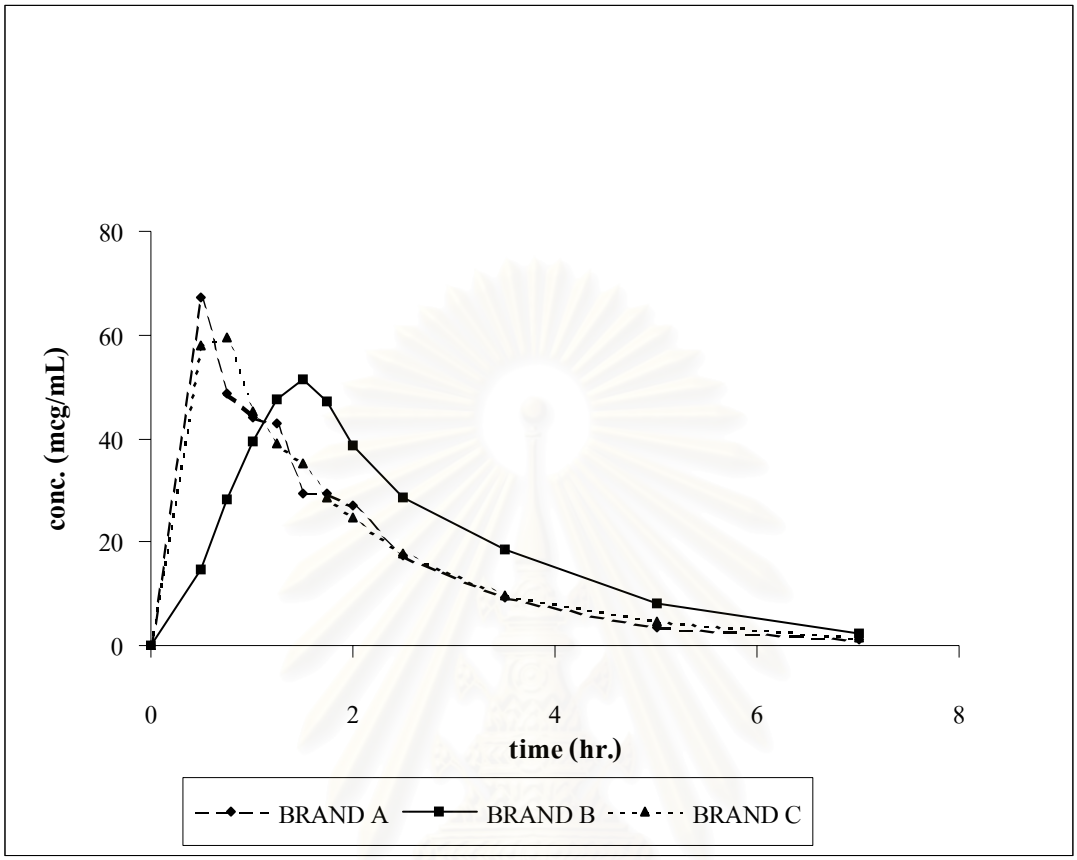


Figure 10 Serum Dicloxacillin Concentration-time Profile of Subject No.6 Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

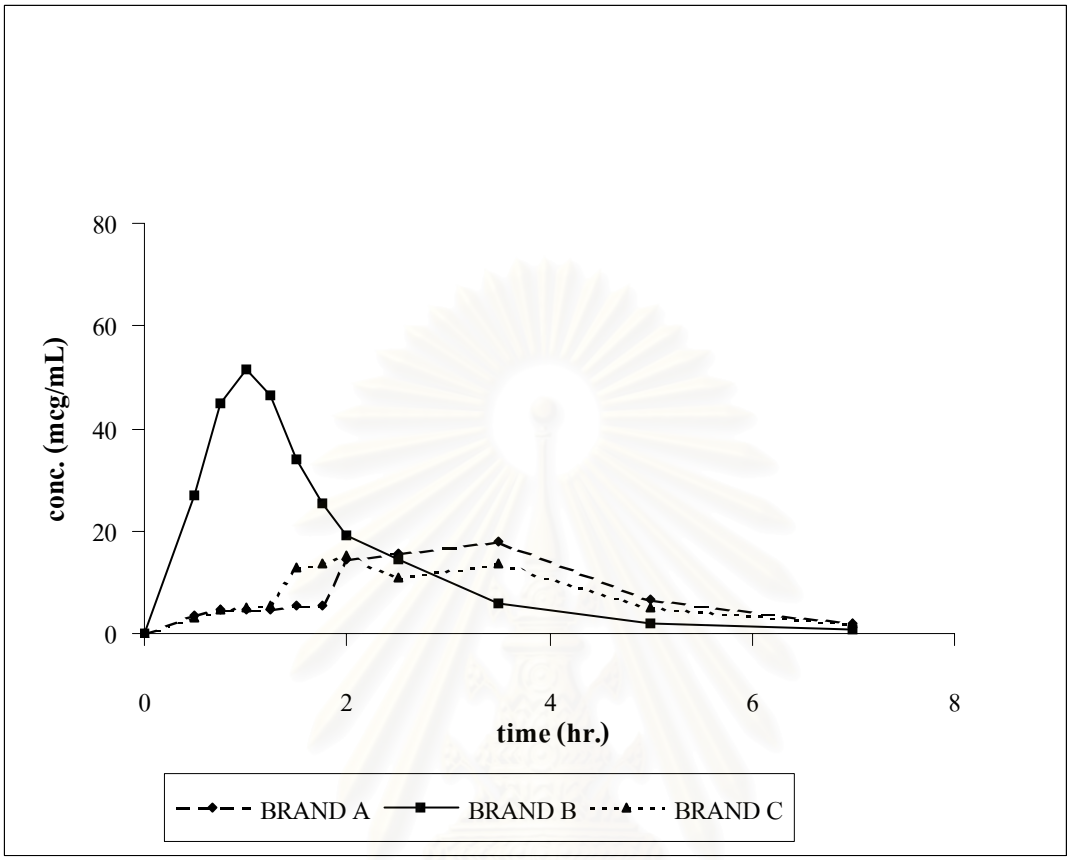


Figure 11 Serum Dicloxacillin Concentration-time Profile of Subject No.7 Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

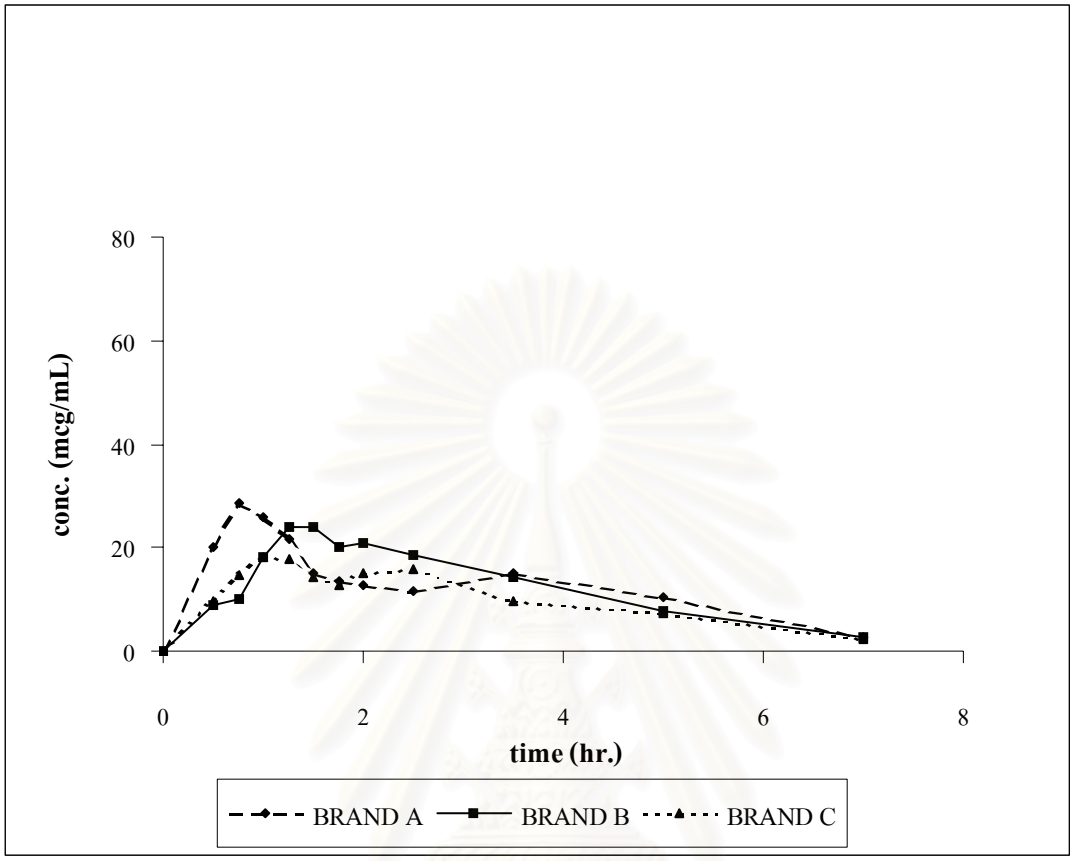


Figure 12 Serum Dicloxacillin Concentration-time Profile of Subject No.8 Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

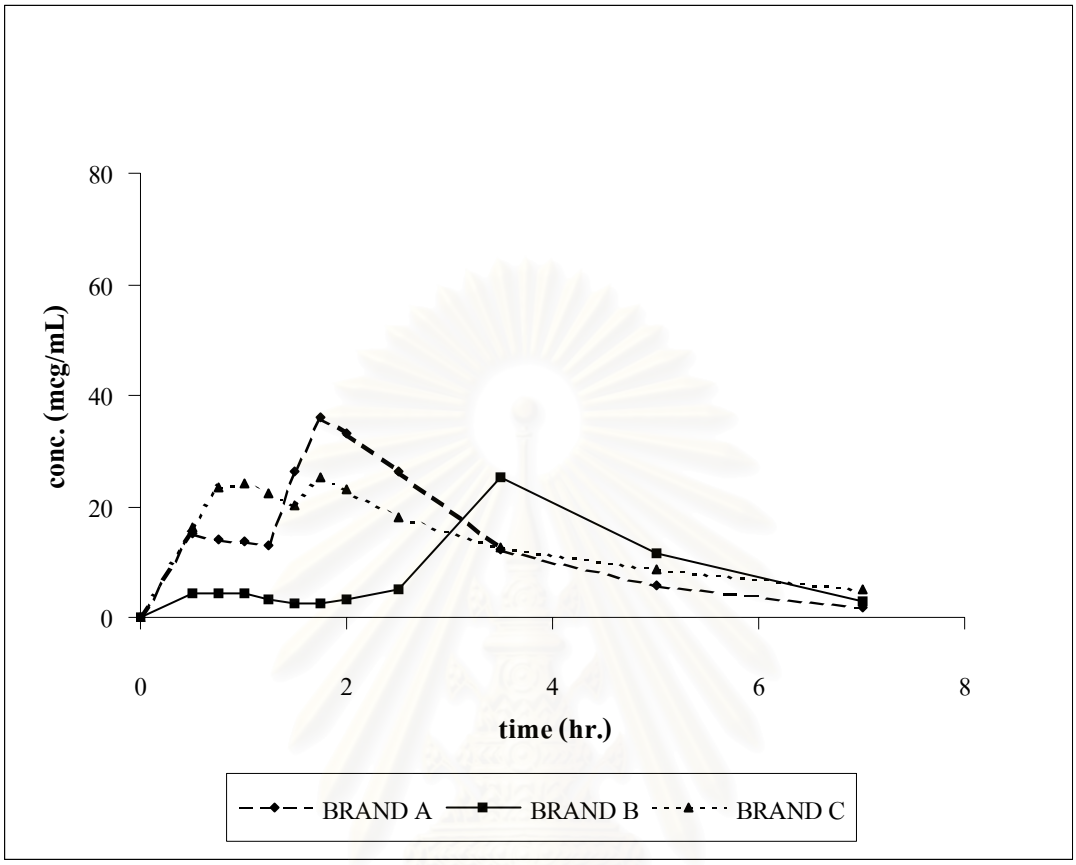


Figure 13 Serum Dicloxacillin Concentration-time Profile of Subject No.9 Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

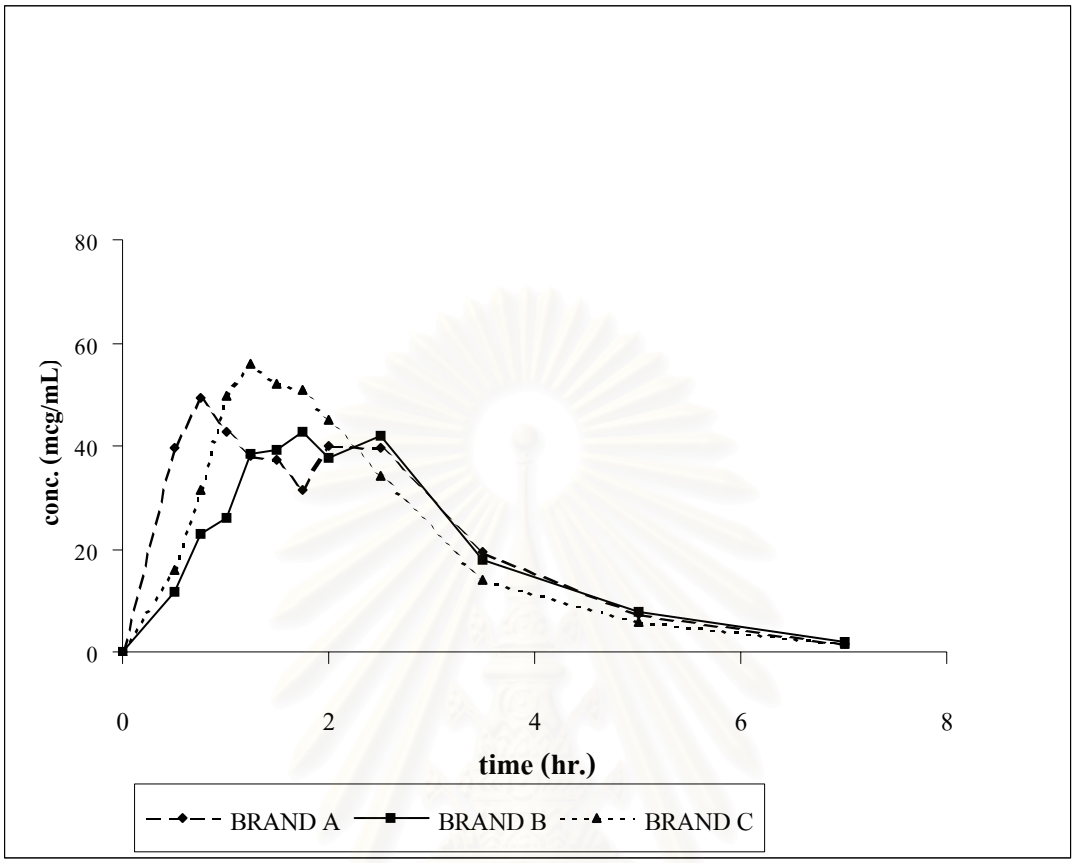


Figure 14 Serum Dicloxacillin Concentration-time Profile of Subject No.10 Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

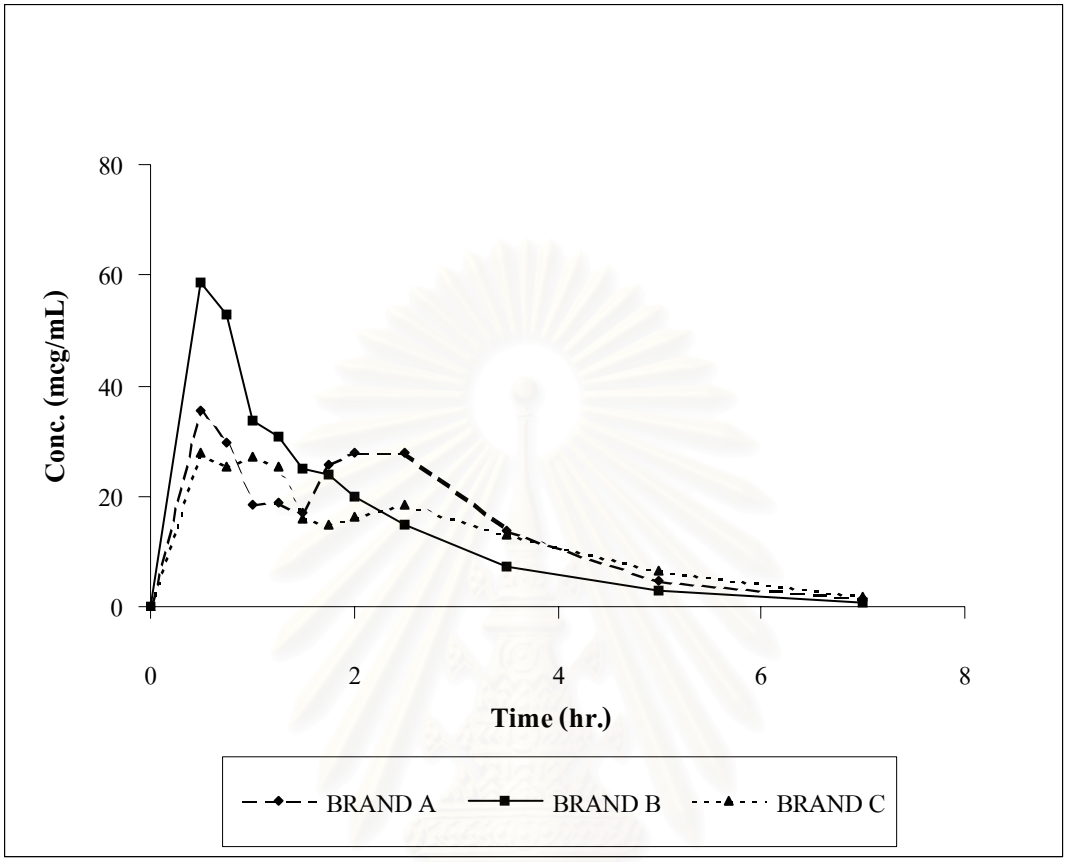


Figure 15 Serum Dicloxacillin Concentration-time Profile of Subject No.11 Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

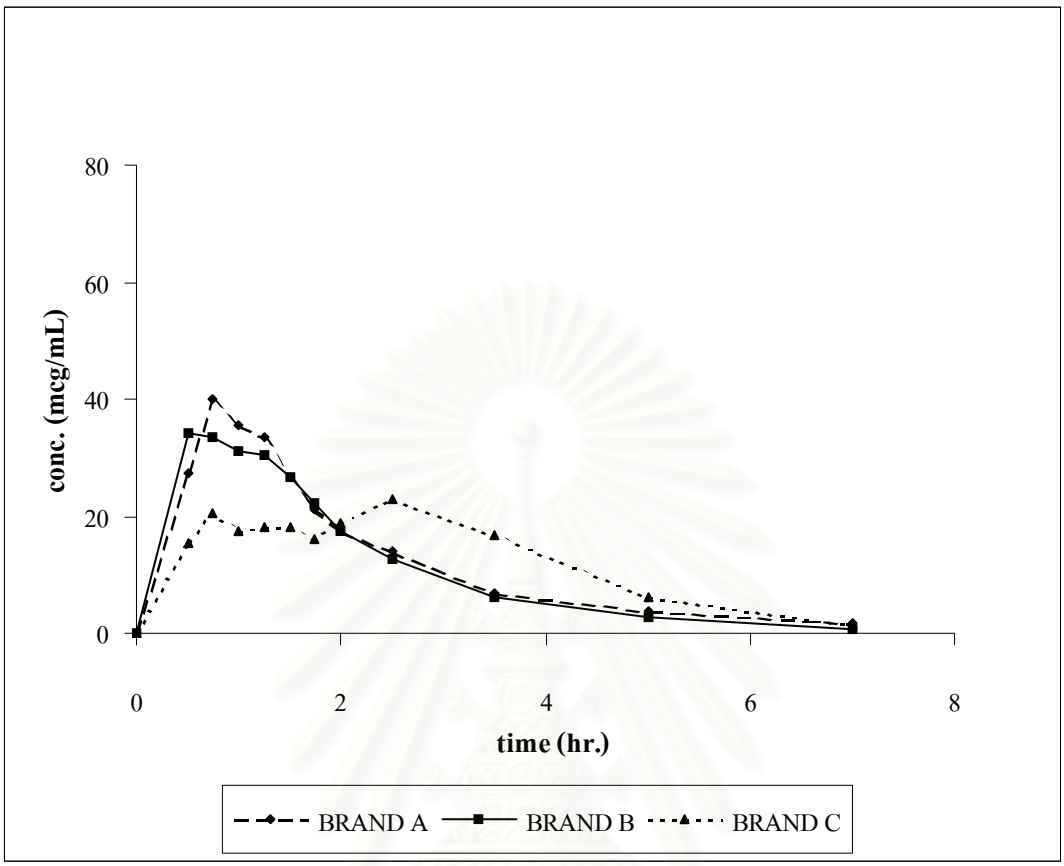


Figure 16 Serum Dicloxacillin Concentration-time Profile of Subject No.12 Following Oral Administration of Three Brands of 2x500mg Dicloxacillin Capsules.

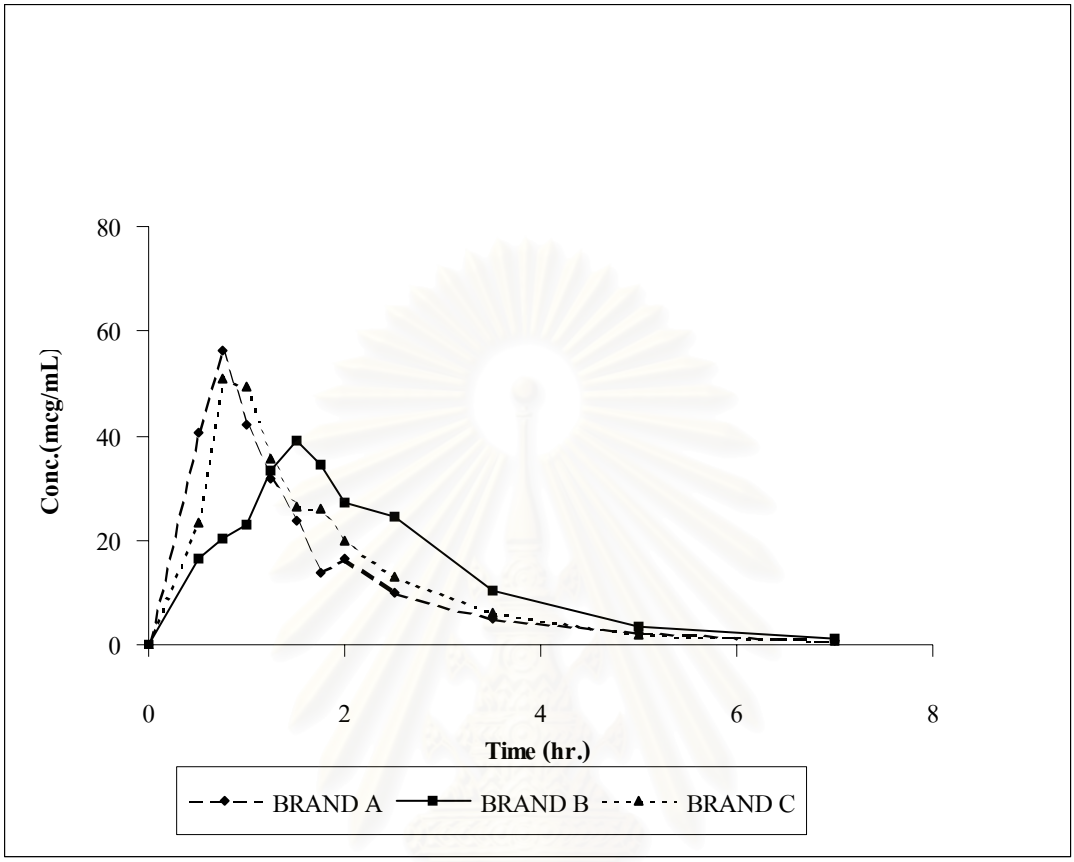


Figure 17 Serum Dicloxacillin Concentration-time Profile of Subject No.13 Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

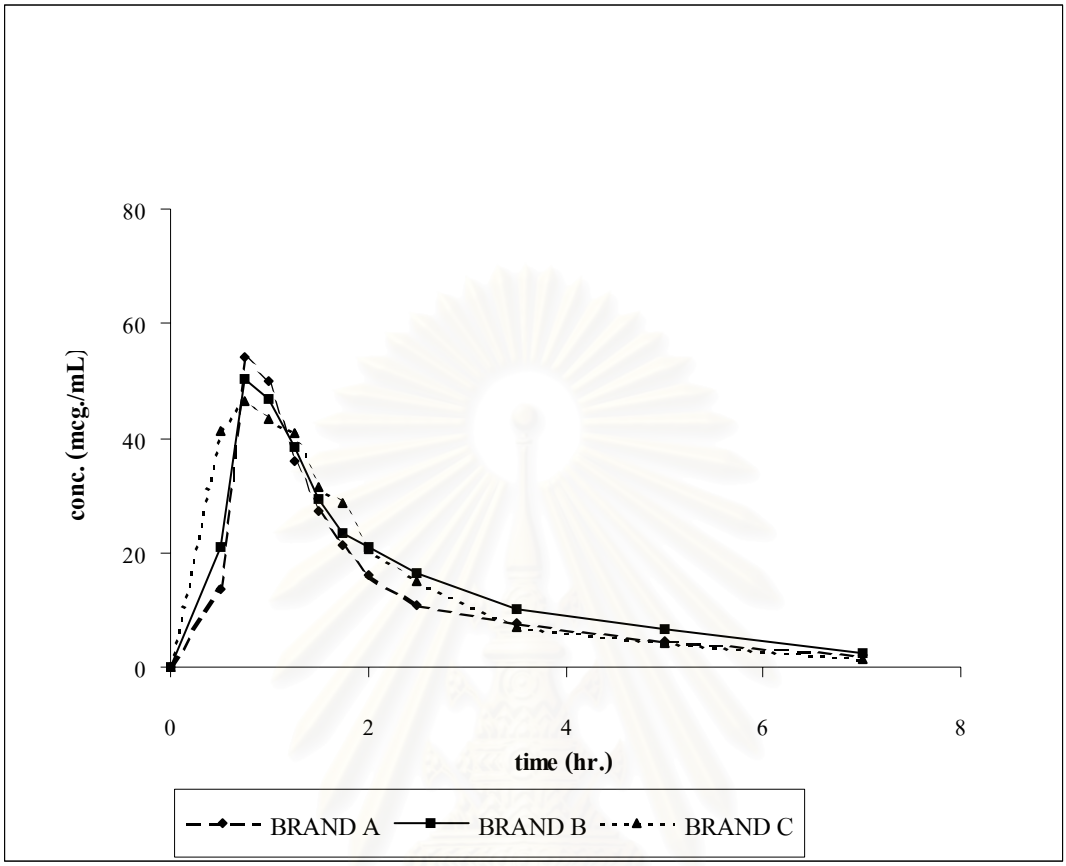


Figure 18 Serum Dicloxacillin Concentration-time Profile of Subject No.14 Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

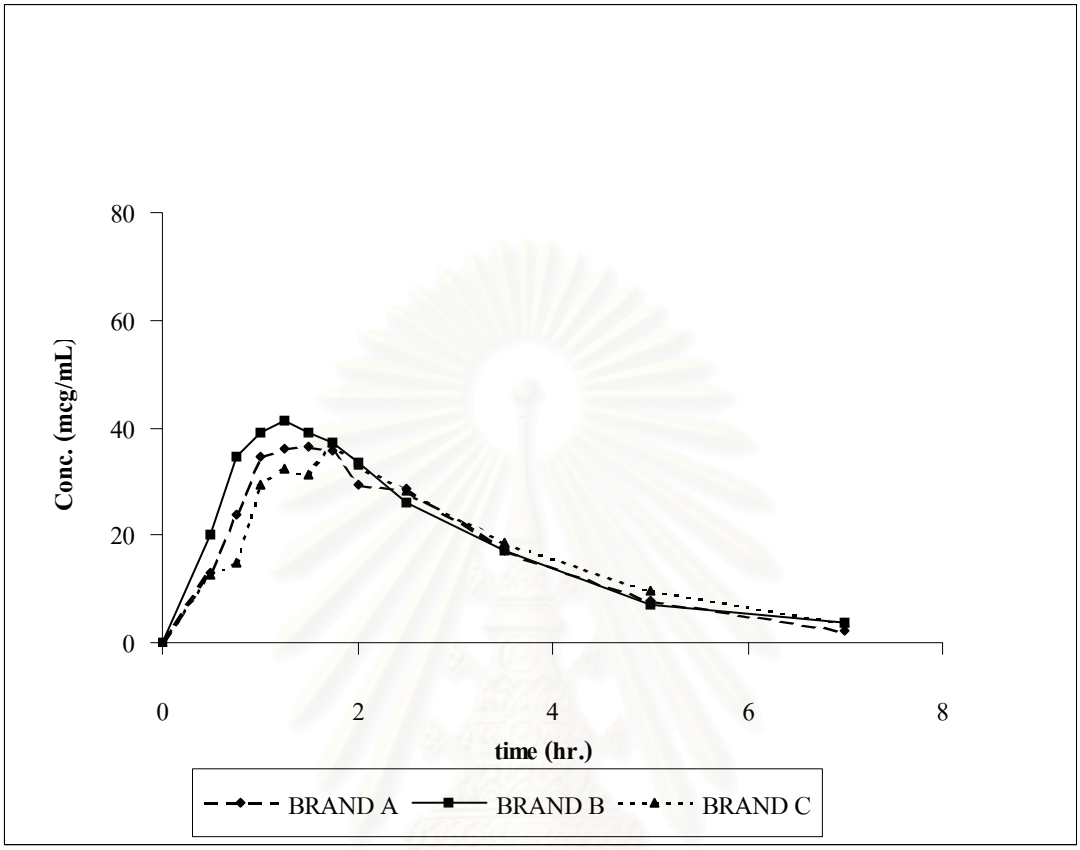


Figure 19 Serum Dicloxacillin Concentration-time Profile of Subject No.15 Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

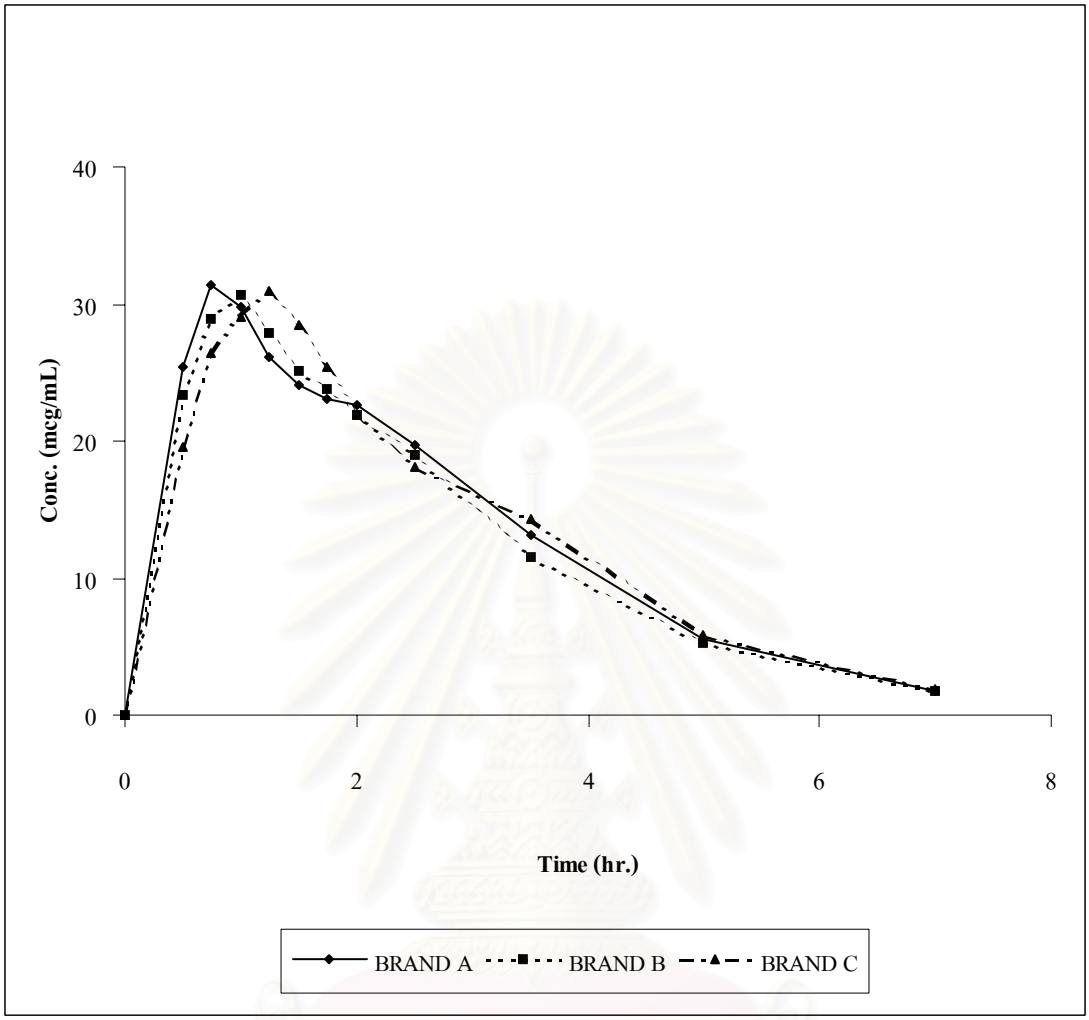


Figure 20 Mean Concentration-time Profile of 15 Subjects Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

1. Pharmacokinetic and bioequivalence study

3.1 Bioavalability Evaluation

The pharmacokinetic parameters, C_{max} and AUC are used to characterize the bioavailability of pharmaceutical formulation after administration. The parameters, C_{max} represented the rate of drug reaching the systemic circulation while the AUC value indicated the extent of drug absorption. These parameters were derived from serum drug concentration-time profiles. In bioequivalence study, drug products that are pharmaceutically equivalent are accepted to be bioequivalence when the ratios of the C_{max} and AUC values based on log-transformed data of the test product relative to the reference product were contained within 80-125% of 90% confidence interval.

The relevant pharmacokinetic parameters obtained for bioavailability comparison were as follows :

3.1.1. Area Under the Serum versus Time Curve (AUC)

The mean AUC values from each treatment of all brands were 97.73, 98.42 and 94.24 mcg.hr/mL for brands A, B and C, respectively, as shown in Table 17. These values were quite similar eventhough their differences in % L.A. This referred that absorption of the drug might be dependent on some specific factors like formulation as well as manufacturing processes. As seen, brand A with smallest quantity of drug, it could produce the AUC comparably to that of brand B, a highest %L.A. brand. Analysis of variance in Table 18 showed that there were no statistically significant differences ($p>0.05$) in AUC among three brands for formulation, period and sequence effects except subject effect ($p<0.05$), indicating intra-and inter-subject variability. The wide variation of serum dicloxacillin concentration might be responsible for this.

The 90% confidence interval for ratio of ln AUC of each brand to that of the innovator's product was calculated and reported in Table 19. Results were within

the acceptance criteria, referring brands B and C were bioequivalent to brand A with respect to the extent of drug absorption.

3.1.2. Peak Serum Concentration (C_{max})

Previous reports indicated that the mean peak serum concentration achieved following oral administration of 500 mg dicloxacillin capsules was about 10 to 18 mcg/mL (Reynolds, 1996). In this study, the mean peak serum dicloxacillin concentration for brands A, B and C were 38.51, 39.91 and 36.15 mcg/mL, respectively as seen in Table 20. These values were approximately as much as twice of those mentioned earlier, notifying dose proportionality. This finding agreed with previously published data (Pascual, 1999). The rank orders of these values were those of brand B, A and C. Again, results was correlated to drug content in dosage forms and absorption factor as discussed in the AUC section. Analysis of variance in Table 21 showed that there were no statistically significant difference ($p>0.05$) among the C_{max} values of all three brands for all effects tested expect that of subjects. The variations of serum dicloxacillin concentrations in individual due to both intra- and inter-subjects variability might contribute to this difference. The ratio of C_{max} of brands B and C relative to brand A based on log-transformed data were calculated following the 90% confidence interval equation and found to be 80.25-110.52% and 104.50-124.61%, respectively indicating, they were bioequivalent in term of the rate of drug absorption. They were reported in Table 22.

3.1.3. Time to Peak Serum Concentration (t_{max})

The times to peak serum dicloxacillin concentration from this experiment were found to be 1.35, 1.60, and 1.42 hr for brands A, B, and C, respectively and they were presented in Table 23. This indicated that all brands were rapidly absorbed as evidence by their t_{max} about 1.5 hr. However, absorption of the drug form brand A was fastest followed by those of brands C and B, respectively. Factors influenced these

results might be due to the differences of raw materials to be used as active ingredient and diluents as well as manufacturing process which individual factory used in the formulation. Usually, this parameter was meaningless for bioequivalence study. However, the difference of them should not exceed 20%. Comparisons were made and found that the differences of t_{max} of brands B, and C relatively to that of brands A were only 18.52 and 5.19%, respectively as displayed in Table 24.

3.2 Bioequivalence Evaluation

The corresponding pharmacokinetic parameters for bioavailability comparison were summarized in Table 25 as well as the 90% confidence interval of parameter ratio of tested brand relative to innovator's product (brand A). Results obtained could be concluded that brands B and C were bioequivalent with brand A with respect to both the rate and the amount of drug absorption into systemic circulation.

Table 17 Area Under the Serum Dicloxacillin Concentration-Time Curve(AUC) Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

Subject No.	AUC (mcg.hr/mL)		
	Brand A	Brand B	Brand C
1	73.18	92.18	73.62
2	114.60	98.83	96.67
3	111.75	88.57	78.57
4	53.71	62.61	73.04
5	109.36	96.50	103.02
6	118.17	138.90	121.31
7	63.77	91.52	57.85
8	93.34	90.72	73.63
9	94.55	70.50	109.00
10	151.41	133.24	140.66
11	101.41	99.02	90.41
12	86.57	79.39	88.28
13	82.73	94.67	84.91
14	90.17	106.29	101.09
15	121.18	133.39	121.47
Mean	97.73	98.42	94.24
Min	53.71	62.61	57.85
Max	151.41	138.90	140.66
S.D.	24.80	22.07	22.33
%C.V.	25.38	22.42	23.69

Table 18 Analysis of Variance for Three-Way Crossover Study at $\alpha = 0.05$ of ln Area Under the Serum Dicloxacillin Concentration-Time Curve Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]	F[e]	Significance Level
Total	44	2.490	--	--	--	
Sequences	2	0.167	0.083	0.499	3.88	NS
Subjects (sequence)	12	2.001	0.167	14.676	2.15	S
Periods	2	0.008	0.004	0.377	3.37	NS
Formulations	2	0.017	0.008	0.739	3.37	NS
Error	26	0.296	0.011	--	--	

NS = Not significant difference at $p > 0.05$

S = Significant difference at $p < 0.05$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Table 19 The 90% Confidence Interval for Ratio of ln AUC of Each Local Brand to That of Innovator' s Product.

Brand	Average	90% Confidence Interval
A	4.550	--
B	4.520	--
C	4.566	--
Ratio of ln AUC B/A	0.016	95.22 - 108.44 %
Ratio of ln AUC C/A	-0.030	90.94 - 103.56 %

Table 20 Peak Serum Dicloxacillin Concentration (C_{max}) Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

Subject No.	C_{max} (mcg/mL)		
	Brand A	Brand B	Brand C
1	21.97	40.86	24.92
2	29.99	48.28	39.74
3	35.39	29.09	28.53
4	18.54	23.67	38.52
5	50.47	37.87	50.77
6	67.29	51.58	59.46
7	17.98	51.67	15.24
8	28.63	24.04	18.26
9	36.16	25.13	25.40
10	49.14	42.61	55.77
11	35.30	58.77	28.05
12	39.92	34.22	22.95
13	56.21	39.18	50.95
14	54.12	50.48	46.53
15	36.48	41.13	37.15
Mean	38.51	39.91	36.15
Min	17.98	23.67	15.24
Max	67.29	58.77	59.46
S.D.	14.49	11.05	14.11
%C.V.	37.63	27.69	39.03

Table21 Analysis of Variance for Three-Way Crossover Study at $\alpha = 0.05$ of ln Peak Serum Dicloxacillin Concentration Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]	F[e]	Significance Level
Total	44	5.9590	--	--	--	--
Sequences	2	0.3831	0.1915	0.6341	3.88	NS
Subjects (sequence)	12	3.6242	0.3020	4.5953	2.15	S
Periods	2	0.1180	0.0590	0.8980	3.37	NS
Formulations	2	0.1251	0.0625	0.9516	3.37	NS
Error	26	1.7090	0.0657	--	--	--

NS = Not significant difference at $p > 0.05$

S = Significant difference at $p < 0.05$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Table 22 The 90% Confidence Interval for Ratio of ln C_{max} of Each Local Brand to That of Innovator's Product.

Brand	Average	90% Confidence Interval
A	3.58	--
B	3.52	--
C	3.64	--
Ratio of ln C_{max} B/A	0.06	104.50 – 124.61 %
Ratio of ln C_{max} C/A	-0.06	80.25 – 110.52 %

Table 23 Time to Peak Serum Dicloxacillin Concentration (t_{max}) Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

Subject No.	t_{max}(hr.)		
	A	B	C
1	3.50	1.50	2.50
2	1.00	1.00	0.75
3	1.50	3.50	3.50
4	1.75	3.50	0.50
5	1.00	1.00	1.00
6	0.50	1.50	0.75
7	3.50	1.00	2.00
8	0.75	1.25	1.00
9	1.75	3.50	1.75
10	0.75	1.75	1.25
11	0.50	0.50	0.50
12	0.75	0.50	2.50
13	0.75	1.50	0.75
14	0.75	0.75	0.75
15	1.50	1.25	1.75
Mean	1.35	1.60	1.42
Min	0.50	0.50	0.50
Max	3.50	3.50	3.50
S.D.	0.97	1.05	0.89
%C.V.	71.85	65.63	62.68

Table 24 Analysis of Variance for Three-Way Crossover Study at $\alpha = 0.05$ of Time to Peak Serum Concentration (t_{max}) Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]	F[e]	Significance Level
Total	44	40.0361	--	--	--	
Sequences	2	5.3444	2.6722	1.49	3.88	NS
Subjects (sequence)	12	21.5361	1.7947	3.85	2.15	S
Periods	2	0.5444	0.2722	0.58	3.37	NS
Formulations	2	0.5028	0.2514	0.54	3.37	NS
Error	26	12.1083	0.4657	--	--	

NS = Not significant difference at $p > 0.05$

S = Significant difference at $p < 0.05$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Difference of t_{max} of Brands B vs A $= \frac{(1.60 - 1.35)}{1.35} \times 100$

$= 18.52\ \%$

Difference of t_{max} of Brands C vs A $= \frac{(1.42 - 1.35)}{1.35} \times 100$

$= 5.19\ \%$

Table 25 Principal Pharmacokinetic Parameters (Mean ± S.D.) of Dicloxacillin from Fifteen Subjects Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

Parameter	Brand		
	A	B	C
AUC (mcg hr/mL)	97.73 ± 24.80	98.42 ± 22.07 (95.22 - 108.44)*	94.24 ± 22.33 (90.94-103.56)*
C_{max} (mcg/mL)	38.51 ± 14.49	39.91 ± 11.05 (104.50 - 124.61)*	36.15 ± 14.11 (80.25 - 110.52)*
t_{max} (hr)	1.35 ± 0.97	1.42 ± 0.89	1.60 ± 1.05

* 90% Confidence interval of parameter ratio of tested product to innovator's product

3.3 Pharmacokinetics of Dicloxacillin Capsules

From the plots of the mean serum dicloxacillin concentration versus time, the pharmacokinetics of dicloxacillin following oral administration of two 2x500 mg dicloxacillin capsules in healthy Thai male volunteers were described by mean of a one compartment open model. This was decided by after the concentration of the drug reached the maximum point, it apparently declined at the same rate over and elimination phase. This was a specific characteristics of one compartment open model plot after the drug was orally given. Relevant pharmacokinetic parameters were calculated using residual methods. They were as follows.

3.3.1. Absorption Rate Constant (K_a)

The average absorption rate constants for determined by residual method brands A, B and C were 1.51 $\pm$0.82, 1.44 $\pm$ 0.69 and 1.62 $\pm$ 0.87 hr^{-1}, respectively as shown in Table 26. The rank order of these values were those of brands C > A > B. Statistical analysis results in Table 27 indicated that there were no significantly differences ($p>0.05$) among these values. Absorption of drug appeared to be uncorrelated with drug dissolution. This was seen by even though brand B showed higher dissolution values than those of brands C and A , the rate of drug absorption from brand B appeared to be slower.

Table 26 Absorption Rate Constant (K_a) of Dicloxacillin Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules

Subject No.	$K_a(hr^{-1})$		
	A	B	C
1	0.67	1.14	0.86
2	1.04	1.66	2.08
3	1.74	0.53	1.44
4	1.01	0.90	1.21
5	2.15	2.47	1.81
6	2.94	0.85	2.71
7	0.67	2.49	1.16
8	1.33	0.99	1.32
9	0.99	0.78	0.96
10	1.40	1.05	1.43
11	1.31	2.08	1.18
12	1.42	2.65	0.96
13	3.58	1.09	1.75
14	0.94	1.39	4.21
15	1.39	1.58	1.21
Mean	1.51	1.44	1.62
Min.	0.67	0.53	0.86
Max	3.58	2.65	4.21
S.D.	0.82	0.69	0.87
%C.V.	54.40	47.50	53.56

Table 27 Analysis of Variance for Three-Way Crossover Study at $\alpha = 0.05$ of Absorption Rate Constant (K_a) Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]	F[e]	Significance Level
Total	44	26.7434	--	--	--	
Sequences	2	1.5229	0.7614	1.05	3.88	NS
Subjects (sequence)	12	8.6979	0.7248	1.16	2.15	NS
Periods	2	0.0701	0.0350	0.06	3.37	NS
Formulations	2	0.2384	0.1192	0.19	3.37	NS
Error	26	16.2143	0.6236	--	--	

NS = Not significant difference at $p > 0.05$

S = Significant difference at $p < 0.05$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

3.3.2. Elimination Rate Constant (K)

The average elimination rate constants obtained by regression analysis of at least three last data points of elimination phase from individual plot of serum dicloxacillin concentration versus time data of brands A, B and C were 0.53 ±0.09, 0.59 ± 0.12 and 0.56 ±0.11 hr^{-1}, respectively (Table 28). There were statistical differences among these values ($p<0.05$) with respect to subject effect while all other effects were not significant difference as shown in Table 29, indicating that the drug was eliminated from the body with different elimination rate in individual. However, the study was valid because the decisive index was based on formulation effect which was not statistical significance, indicating the same value of K.

3.3. 3. The Mean Absorption Time (MAT)

The mean absorption time is the pharmacokinetic parameter derived from statistical moment analysis in noncompartment method. It is the reciprocal value of the absorption rate constant designated as the time required for completion of absorption phase. In this investigation, the average mean absorption time obtained from individual treatment of brands A, B and C were 0.82 ± 0.36, 0.89 ±0.46 and 0.73 ± 0.26 hr, respectively (Table 30). There were no statistical difference among these values of all three brands ($p>0.05$) as presented in Table 31, providing that the drug was absorbed from the gastrointestinal tract into the body with the same rates although the drug demonstrated intra and intersubject variations.

3.3.4. Ratio of Volume of Distribution to Fraction of Dose to Be Absorbed (V_d/F)

The approximate average ratio volume of distribution to the fraction of dose to be absorbed computed from the data for brands A, B and C were 28.91 ± 36.79, 22.31 ± 7.95 and 21.34 ± 8.48 L., respectively (Table 32). There were no statistical difference among these values of all three brands ($p>0.05$) as shown in

Table 33. These values were quite three times greater than that reported by Nauta and Mattie, 1976 of differences of dosage form as well as route of administration of the drug.

3.3.5. Elimination half-life ($t_{1/2}$)

The mean elimination half-life of dicloxacillin determined for brands A, B and C were 1.34 $\pm$ 0.222, 1.23 $\pm$ 0.27 and 1.31 $\pm$ 0.33 hr, respectively (Table 34). Statistical analysis showed no significant difference among these values (Table 35). The values were higher than the results found by other investigators which was about 0.62 hr (Nauta and Mattie, 1976). This might be due to the difference in route of administration of the drug as well as the race of the subjects. In Thai subjects, the drug might be well distributed providing longer stayed in the body as compared to what obtained from foreigners.

Some of the pharmacokinetic parameters of dicloxacillin obtained from the present study were compared with those obtained from previous study. Results were presented in Table 36. It was clearly observed that there were wide variations of the corresponding values. The contributive differences could be dose, route of administration, formulation, and subjects variability. The differences of subject according to the difference in race, age, weight and normal habits had influenced for all.

Table 28 Elimination Rate Constant (K) of Dicloxacillin Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules

Subject No.	K(hr^{-1})		
	A	B	C
1	0.51	0.73	0.66
2	0.41	0.67	0.62
3	0.49	0.46	0.50
4	0.45	0.80	0.54
5	0.64	0.53	0.64
6	0.60	0.55	0.58
7	0.65	0.69	0.58
8	0.39	0.37	0.39
9	0.56	0.60	0.31
10	0.54	0.58	0.62
11	0.51	0.64	0.51
12	0.51	0.59	0.69
13	0.70	0.64	0.63
14	0.51	0.50	0.61
15	0.50	0.43	0.44
Mean	0.53	0.59	0.56
Min.	0.39	0.37	0.31
Max.	0.70	0.80	0.69
S.D.	0.09	0.12	0.11
%C.V.	16.41	19.95	19.48

Table 29 Analysis of Variance for Three-Way Crossover Study at $\alpha = 0.05$ of Elimination Rate Constant (K) Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]	F[e]	Significance Level
Total	44	0.4835	--	--	--	
Sequences	2	0.0130	0.0065	0.33	3.88	NS
Subjects (sequence)	12	0.2381	0.0198	2.51	2.15	S
Periods	2	0.0035	0.0018	0.22	3.37	NS
Formulations	2	0.0231	0.0115	1.46	3.37	NS
Error	26	0.2058	0.0079	--	--	

NS = Not significant difference at $p > 0.05$

S = Significant difference at $p < 0.05$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Table 30 Mean Absorption Time (MAT) of Dicloxacillin Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules

Subject No.	MAT (hr)		
	A	B	C
1	1.49	0.88	1.17
2	0.96	0.60	0.48
3	0.57	1.89	0.69
4	0.99	1.11	0.83
5	0.47	0.41	0.55
6	0.34	1.18	0.37
7	1.49	0.40	0.86
8	0.75	1.01	0.76
9	1.01	1.74	1.05
10	0.71	0.95	0.70
11	0.77	0.48	0.85
12	0.71	0.38	1.04
13	0.28	0.92	0.57
14	1.06	0.72	0.24
15	0.72	0.63	0.83
Mean	0.82	0.89	0.73
Min.	0.28	0.38	0.24
Max	1.49	1.89	1.17
S.D.	0.36	0.46	0.26
%C.V.	43.51	51.87	35.25

Table 31 Analysis of Variance for Three-Way Crossover Study at $\alpha = 0.05$ of Mean Absorption Time (MAT) Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]	F[e]	Significance Level
Total	44	5.8590	--	--	--	
Sequences	2	0.4055	0.2027	1.16	3.88	NS
Subjects (sequence)	12	2.0998	0.1750	1.51	2.15	NS
Periods	2	0.1527	0.0764	0.66	3.37	NS
Formulations	2	0.1814	0.0907	0.78	3.37	NS
Error	26	3.0196	0.1161	--	--	

NS = Not significant difference at $p > 0.05$

S = Significant difference at $p < 0.05$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Table 32 Ratio of Volume of Distribution to Fraction of Dose to Be Absorbed (V_d/F) of Dicloxacillin Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules

Subject No.	V_d/F (L)		
	A	B	C
1	31.33	22.24	33.74
2	16.40	17.76	13.53
3	17.43	45.43	27.85
4	35.09	23.26	33.48
5	13.53	18.18	14.68
6	13.23	22.38	16.10
7	16.77	12.56	20.40
8	16.83	21.59	30.10
9	21.16	31.94	36.11
10	12.47	18.71	10.14
11	10.18	18.10	17.93
12	25.57	18.07	15.10
13	17.26	19.52	17.20
14	26.81	28.29	15.82
15	16.97	16.69	17.97
Mean	19.40	22.31	21.34
Min.	10.18	12.56	10.14
Max	35.09	45.43	36.11
S.D.	7.21	7.95	8.48
%C.V.	37.14	35.64	39.74

Table 33 Analysis of Variance for Three-Way Crossover Study at $\alpha = 0.05$ of Ratio of Volume of Distribution to Fraction of Dose to Be Absorbed (V_d/F) Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules.

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]	F[e]	Significance Level
Total	44	2685.38	--	--	--	
Sequences	2	248.53	124.27	0.94	3.88	NS
Subjects (sequence)	12	1588.22	132.35	4.66	2.15	S
Periods	2	44.99	22.50	0.79	3.37	NS
Formulations	2	65.98	32.99	1.16	3.37	NS
Error	26	737.66	28.37	--	--	

NS = Not significant difference at $p > 0.05$

S = Significant difference at $p < 0.05$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Table 34 Elimination half-life ($t_{1/2}$) of Dicloxacillin Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules

Subject No	$t_{1/2}$ (hr)		
	A	B	C
1	1.37	0.95	1.05
2	1.71	1.04	1.12
3	1.41	1.51	1.38
4	1.55	0.86	1.28
5	1.09	1.31	1.09
6	1.15	1.25	1.20
7	1.07	1.01	1.19
8	1.78	1.86	1.79
9	1.23	1.15	2.23
10	1.29	1.19	1.11
11	1.37	1.08	1.36
12	1.36	1.19	1.00
13	0.99	1.08	1.10
14	1.35	1.40	1.13
15	1.39	1.63	1.59
Mean	1.34	1.23	1.31
Min.	0.99	0.86	1.00
Max.	1.78	1.86	2.23
S.D.	0.22	0.27	0.33
%C.V.	16.52	21.95	25.50

Table 35 Analysis of Variance for Three-Way Crossover Study at $\alpha = 0.05$ of Elimination half-life ($t_{1/2}$) Following Oral Administration of Three Brands of 2x500 mg Dicloxacillin Capsules

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]	F[e]	Significance Level
Total	44	3.3618	--	--	--	
Sequences	2	0.1240	0.0620	0.42	3.88	NS
Subjects (sequence)	12	1.7534	0.1461	2.80	2.15	S
Periods	2	0.0357	0.0178	0.34	3.37	NS
Formulations	2	0.0919	0.0460	0.88	3.37	NS
Error	26	1.3567	0.0522	--	--	

NS = Not significant difference at $p > 0.05$

S = Significant difference at $p < 0.05$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

e = F value obtained from the table

Table 36 Comparison of Some Pharmacokinetic Parameters of Dicloxacillin Obtained from This Study to Previous Reports.

	No. of subjects	Route	Dose (mg)	AUC (mcg.hr /mL)	C_{max} (mcg/mL)	t_{max} (hr.)	k_a (hr^{-1})	k (hr^{-1})	V_d (L)	$t_{1/2}$ (hr.)
The present study										
Brand A	15	oral	1000	97.7	38.5	1.4	1.5	0.5	28.9	1.34
Brand B	15	oral	1000	98.4	39.9	1.6	1.4	0.6	22.3	1.23
Brand C	15	oral	1000	94.2	36.2	1.4	1.6	0.6	21.3	1.31
Nauta and Mattie	4	IV	1000	114	-	-	-	1.13	7.07	0.62
	4	IV	2000	310	-	-	-	0.97	6.25	0.72
Bobey et al.	7	oral	250	-	7.8	1	-	-	-	-
	7	oral	500	-	15.1	1	-	-	-	-
Doluisio et al.	20	IM	250	21	5.07	1	0.53	2.1	-	2.0
Reynolds		oral	500	-	10-18	1	-	-	-	0.5-1
Lacy et al.		oral		-	-	0.5-2	-	-	-	0.6-0.8
McEvoy		oral	250	-	5.6	2	-	-	-	0.6-0.8

CHAPTER V

CONCLUSIONS

This study provided bioavailability data and pharmacokinetic parameters of dicloxacillin capsules commercially available in Thailand as compared to the innovator's product. The results were concluded as follows:-

In vitro studies:

1. The three brands of 500 mg dicloxacillin capsules met the general requirements of the United States Pharmacopeia 24 for content, and content of active ingredient. Dissolution of all brands was more than 75% of label claim within 30 mins. These indicated that they were pharmaceutically equivalent.

2. Dissolution profile for each brand was performed in water. The difference factor (f_1) and similarity factor (f_2) of each local brand relative to those of the innovator's product were in the acceptance range, ensuring their equivalences.

In vivo studies:

1. The comparative bioavailability of brands B and C relative to brand A were studied in fifteen healthy Thai male volunteers. A single dose of 2x500 mg dicloxacillin capsules was administered to each subject in a crossover manner. Serum dicloxacillin concentrations were determined by high performance liquid chromatography. Individual serum concentration-time profile was analyzed using graphical method. The observed values of relevant pharmacokinetic parameters (AUC and C_{max}) were used for bioavailability comparison.

The area under the serum dicloxacillin concentration-time curves of all brands ranged from 94.24 to 98.42 mcg hr/mL.

The mean peak serum dicloxacillin concentration of each treatment ranged from 36.15 to 39.91 mcg/mL.

The average times to peak serum dicloxacillin concentrations ranged from 1.35 to 1.60 hr.

There were no statistically significant differences of the corresponding pharmacokinetic parameters between the values of all three brands studied ($p>0.05$) and 90% confidence interval of individual parameter based on log-transformed data of brands B and C relatively to that of brand A were contained within 80-125%. These demonstrated that all of the products studied were bioequivalent with the same rates and amounts of drug absorption.

2. The pharmacokinetics of dicloxacillin following oral administration of 2x500 mg capsules were described by a mean of one compartment open model with first order absorption and elimination.

The average absorption rate constants obtained for brands A, B and C were 1.51, 1.44 and 1.62 hr^{-1} , respectively.

The average elimination rate constants obtained were 0.53 hr^{-1} for brand A, 0.59 hr^{-1} for brand B and 0.556hr^{-1} for brand C.

The average mean absorption time for brands A, B and C were 0.82, 0.89and 0.73 hr, respectively.

The mean volume of distribution of dicloxacillin ranged from 21.34 to 28.91 L.

The mean elimination half-life of dicloxacillin ranged from 1.23 to 1.34 hr.

REFERENCES

Abdou, H. M. Dissolution, bioavalability & bioequivalence. Easton, Pennsylvania: Mack Publishing Company, 1989.

Albert, K.; Sakmar, E.; Hallmark, M.; Weilder, D.; and Wagner, J. Bioavailability of diphenylhydantoin. Clin. Pharmaco. Ther. 16 (1974): 727-729.

Ali, A. A.; and Farouk, A. Comparative studies on the bioavalability of ampicillin anhydrate and trihydrate. Int. J. Pharm. 9 (1981): 239-43.

Ali, A. A.; Gorashi, A. S.; and Rao, Y. M. Bioavailability of five commercial nitrofurantoin products. East Pharm. 31 (1988): 125-127.

Ali, H. M. Comparative bioavailaility of 8 brandsof ampicillin. Int. J. Pharm. 7 (1981): 301-6.

Bennett, D. R.; Drug evaluations annual 1994. United States of America. 1994: 1401

Blair, D.; Barnes, R.; Wildner, E.; and Murray, W. Biological availability of oxytetracycline hydrochloride capsules, a comparison of all manufacturing sources supplying the United States market. J. Am. Med. Assoc. 215 (1971): 151-153.

Body, G.P.; Vallejos, C.; and Stewart, D. Flucloxacillin: A new semisynthetic isoxazolyl penicillin. Clin. Pharmacol. Ther. 27 (1972): 512-515.

Doluisio, J. T.; Lapiana, J. C.; and Dittert, L.W. Pharmacokinetics of ampicillin trihydrate, sodium ampicillin, and sodium dicloxacillin following intramuscular injection. J. Pharm. Sci. (1971): 715-719.

Ghosh, M. K.; HPLC methods on drug analysis. United States of America. 1992: 123-157.

Glazko, A.; Kinkel, A.; Alegnani, W.; and Holmes, E. An evaluation of the absorption characteristics of different chloramphenical preparation in normal human subject. Clin. Pharmacol. Ther. 9 (1968): 472-476.

Kucers, A.; and Bennett, M. M. The use of antibiotics: A comprehensive review with clinical emphasis. 4th ed.,. London. 1987: 109-120.

Kucers, A.; The use of antibiotics: A comprehensive review with clinical emphasis. Ed? Where? year? :54-60.

Lacy, C. F.; Armstrong, L. L.; Goldman, M. P.; and Lance, L. L. Drug information handbook. 8th ed. United States of America: 2000: 352-353.

Lindenbaum, J.; Mellow, M. H.; and Blackstone, M. O. Variation in biologic availability of digoxin from four preparations. N. Engl. J. Me. 285 (1971): 1344-7.

Lund, J. Clinical significance of generic inequivalence of three different pharmaceutical preparationa of phenytoin. Eur. J. Clin. Pharmacol. 7 (1974): 119-121.

McEvoy,G. K.; AHFS Drug Information. United States of America. 2000: 356-357.

McGilveray, I.; Mattok, G.; Hossie, R. A study of bioavalabilities and dissolution rates of commercial tablets of nitrofurantoin. J. Pharm. Pharmacol. 23 (1972): 246S-250S.

Melikian, A.; Straughn, A.; Slywka, G.; Whyatt, P.; and Meyer, M. Bioavailability of eleven phenytoin products. J. Pharmacokinet. Biopharm. 5 (1977): 133-136.

Menick, D.; Hochberg, M.; and Oser, B. Physiological availability of the vitamins. J. Nitr. 30 (1945): 47-50.

Meyers, M. C.; Slywka, G. W. A.; Dann, R. E.; and Whyatt, P. L. Bioavalability of fourteen nitrofurantoin products. J. Pharm. Sci. (1974): 1693-1698.

Nauta, E. H.; and Mattie, H. Dicloxacillin and cloxacillin: pharmacokinetics in healthy and hemodialysis subjects. Clin. Pharmacol. Ther. 20 (1976): 98-108.

Pachla, L. A.; Wright, D. S.; and Reynolds, D. L. Bioanalytic considerations for pharmacokinetic and biopharmaceutic studies. J. Clin. Pharmacol. 26 (1986): 332-335.

Pascual, R.D. MIMS annual. 1999: 231-232.

Payakachat, N.; Suthisisang, C. C.; Kraisintu, K.; Chulavatnato,l S.; and Towanabut, S. Pharmacokinetics and bioavailability of Phenytoin in healthy thais. A thesis submitted in partial fulfillment of the requirements for the degree of master of scince (Pharmacy). Faculty of graduated studies, Mahidol university. 1995.

Pitragool, W.; Determination of bioequivalence of 250 mg oral ciprofloxacin using urinary drug level. Master's Thesis, Department of Clinical Pharmacy, Graduate School, Mahidol University. 1998.

Reynolds, J. E. F. Martindale The Extra Pharmacopoeia. 31st ed. London: The Pharmaceutical Press, 1996.

Shah, V. P., and others.; Analytical methods validation: Bioavailability, bioequivalence and pharmacokinetic studies. Int. J. Pharm. 82 (1992): 1-7.

Shargel, L.; and Yu, A. B. C. Applied Biopharmaceutics and Pharmacokinetics. 3rded.,. London: Prentice Hall International(UK) Limited, 1993: 193-223.

The United States Pharmacopoeia XXIV /National Formulary XIX. U.S. Pharmacopoeia Convention Rockville, MD, 2000: 2000-2.

Thijssen, H. H. W.; Analysis of isoxazolyl penicillins and their metabolites in body fluids by high-performance liquid chromatography. J. Chromato. Biomed. Appl. 183(1980): 339-345.

Wagner, J.; Generic equivalence and inequivalence of oral products. Drug Intell. Clin. Pharm. 5 (1971): 115-117.

กองควบคุมยา. สำนักงานคณะกรรมการอาหารและยา. กระทรวงสาธารณสุข. หลักเกณฑ์และแนวปฏิบัติในการศึกษาชีวสมมูลของยาสามัญ. กรุงเทพมหานคร, 2543.

APPENDICES

APPENDIX A

Test products

Table37 Test Products

Brand name	Manufacturer	Batch No.	Mfg. Date	Exp. Date
Diclocil®	Bristol-Mayers Squibb(Thailand), Ltd.	002H03A	10-00	10-03
Dicloxacillin®	GPO.	DT001/44	03-01	03-04
Dixocillin®	Siam Beasach Co., Ltd	90A150	10-00	10-02

APPENDIX B

Table 38 Typical Calibration Curve for Determination of Dicloxacillin Concentrations in Water Estimated Using Linear Regression[1]

Standard No.	Concentration (mg/mL)	Absorbance	Inversely Estimated Concentration[2](mcg/mL)	%Recovery[3]
1	0.1	0.139	0.099	99.06
2	0.2	0.290	0.203	101.43
3	0.3	0.433	0.301	100.39
4	0.4	0.573	0.397	99.35
5	0.5	0.720	0.498	99.69
6	0.6	0.863	0.597	99.46
7	0.7	1.019	0.704	100.57

*Each data point was determined tripicately

1. $r^2 = 0.9998$, $y = 1.45464x - 0.00514$

2. Inversely estimated concentration $= \dfrac{(\text{absorbance} + 0.00514)}{1.45464}$

3. % Recovery $= \dfrac{\text{Inversely estimated concentration} \times 100}{\text{Known concentration}}$

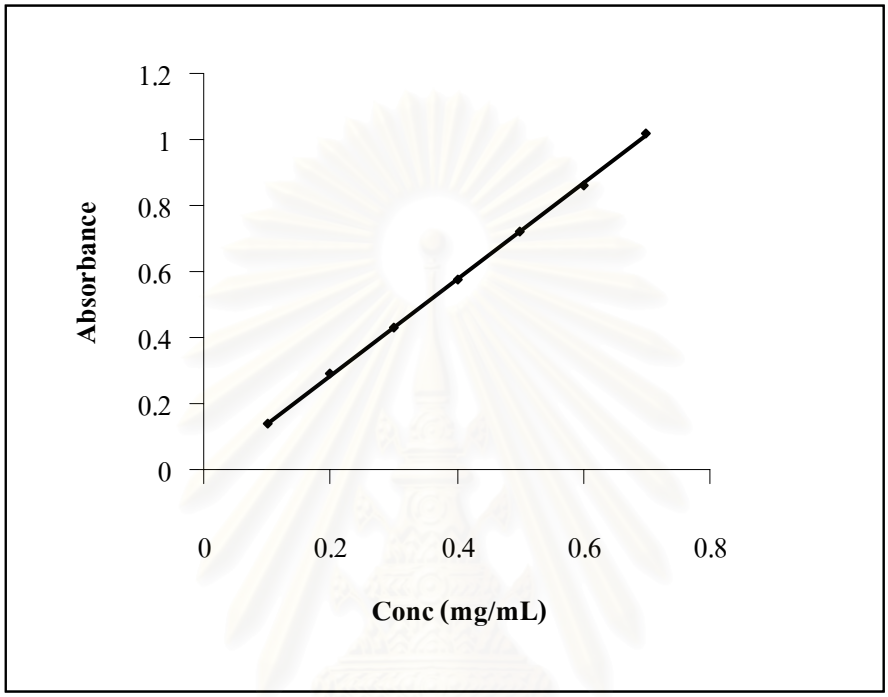


Figure 21 Calibration Curve for Determination of Dicloxacillin in Water

Table 39 Typical Calibration Curve for Determination of Dicloxacillin Concentrations in Serum Estimated Using Linear Regression[1]

Standard No.	Concentration (mcg/mL)	Peak Area Ratio	Inversely Estimated Concentration[2](mcg/mL)	%Recovery[3]
1	0.9	0.01700	1.062	117.97
2	1.0	0.03435	1.169	116.87
3	1.5	0.12888	1.752	116.79
4	2.5	0.27799	2.672	106.87
5	5.0	0.62223	4.795	95.91
6	10.0	1.42083	9.722	97.22
7	25.0	3.66593	23.572	94.29
8	50.0	8.26837	51.965	103.93
9	75.0	11.87697	74.226	98.97

*Each data point was determined tripicately

Mean	105.42
S.D.	9.65
%C.V.	9.21

1. r^2 = 0.9988, y = 0.16213 x - 0.1551

2. Inversely estimated concentration = $\frac{(\text{peak area ratio} + 0.1551)}{0.16213}$

3. % Recovery = $\frac{\text{Inversely estimated concentration}}{\text{Known concentration}} \times 100$

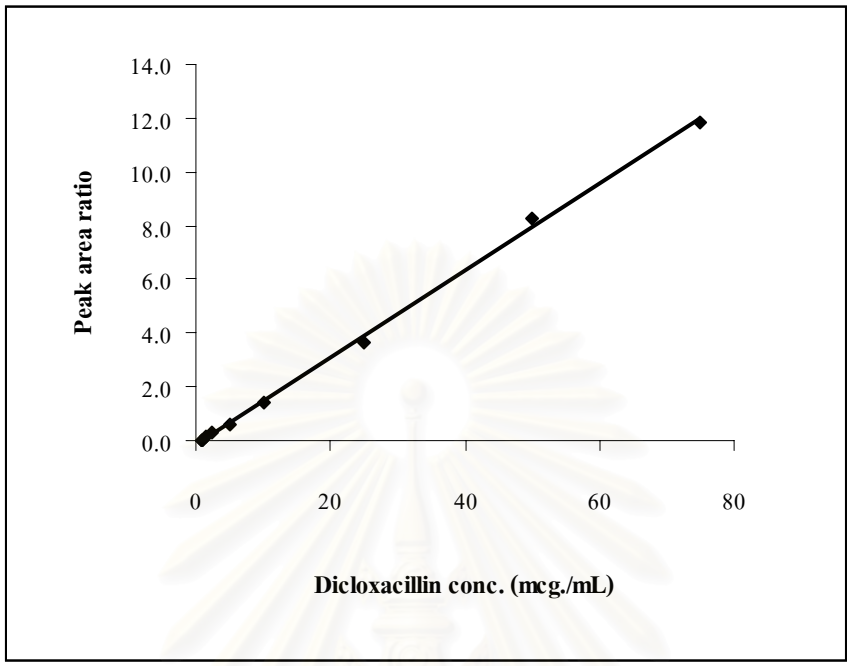


Figure 22 Calibration Curve for Determination of Dicloxacillin in Serum

APPENDIX C

NO. 3/ 2001

Study Protocol Approval

The Ethics Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand has approved the following study to be carried out according to the protocol dated and/ or amended as follows :

Study Title	: Pharmacokinetics and Bioequivalence Study of 500 mg Milligram Dicloxacillin Capsules in Healthy Thai Volunteers.
Study Code	: -
Centre	: Chulalongkorn University
Principal Investigator	: Miss Ubonthip Nimmannit
Protocol Date	: February 9, 2001

A list of the Ethics Committee members and positions present at the Ethics Committee meeting on the date of approval of this study has been attached.

This Study Protocol Approval Form will be forwarded to the Principal Investigator.

Chairman of Ethics Committee : .....

(Signature)

Sunibhond Pummangura, Ph.D.

Secretary of Ethics Committee : .....

(Signature)

Poj Kulvanich, Ph.D.

Date of Approval : May 15, 2001

Table40 Hematological Tests of Subjects

Clinical Test	Normal Range	Subject Number														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
White Blood Cell	5-10*10^3 cell / mL	4.3	5.2	7.0	5.6	6.3	4.9	7.0	5.8	4.4	5.5	8.3	6.7	6.6	6.6	5.8
Hemoglobin	13 – 18 g / dL	14.9	15.3	16.1	13.4	14.9	14.6	15	14.4	13.6	13.2	16.7	14.7	15.2	15.3	13.6
Hematocrit	35 – 49 %	43	45	47	41	45	43	44	43	41	40	49	44	45	45	40
Red Blood Cell	4.7-6.1*10^6 cell / mL	4.67	5.04	5.35	5.67	4.73	4.71	5.10	5.64	5.20	4.49	5.16	5.34	5.13	5.55	4.34
Platelet Count	150-400*10^3 cell / mL	169	183	234	208	175	252	189	199	189	195	241	182	230	158	262
Lymphocyte	20-35 %	40	38	35	40	42	37	35	40	40	47	45	35	37	39	45
Monocyte	2-6	1	-	1	1	2	-	3	-	1	-	-	2	-	1	-
Eosinophil	1-3	-	1	1	2	3	2	1	-	1	5	4	-	1	1	-
Basophil	0-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table41 Blood Chemical Tests of Subjects

Clinical Test	Normal Range	Subject Number														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
HBs Ag	Negative	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Anti HBs	Protective level >10	N	N	347	384	> 400	265	N	N	66	N	154	26	50	65	N
BUN	5 – 25 mg/dL	15	11	13	11	11	13	13	11	16	7	15	11	13	11	17
Creatinine	0.7 – 1.5 mg/dL	0.9	1.2	1.2	0.9	1.3	1.0	1.0	1.0	1.1	1.1	1.0	1.3	0.9	1.1	1.2
Total bilirubin	0 – 1.5 mg/dL	1.0	0.9	0.8	0.2	1.4	0.4	0.9	0.7	1.1	0.8	2.7	0.4	1.4	1.0	1.0
Direct bilirubin	0 – 0.5 mg/dL	0.2	0.1	0.1	0.1	0.3	0.1	0.1	0.1	0.3	0.1	0.1	0.1	0.2	0.2	0.2
AST	10 – 40 U/L	22	22	26	25	36	26	19	24	19	18	43	34	23	-	-
ALT	10 – 40 U/L	24	37	33	26	18	32	12	22	32	15	32	17	32	29	17
Alkaline phosphatase	35 - 100 U/L	57	52	44	48	64	82	79	72	66	82	56	71	68	44	61

HBs Ag = Hepatitis B Antigen

Anti HBs = Antibody Hepatitis B

BUN = Blood Urea Nitrogen

AST = Aspartate Aminotransferase

ALT = Alaline Aminotransferase

N = Negative

APPENDIX D

1. Means ($\bar{X}$)

$$\overline{X} = \Sigma X/n$$

2. Standard deviation (S.D.)

$$S.D. = \sqrt{\Sigma (X - \overline{X})^2 / n\text{-}1}$$

3. Coefficient of variation (C.V.)

$$C.V. = S.D. / Mean$$

4. Area under the concentration time curve ($AUC_{0\text{-}t}$)

$$[AUC]_0^t = \frac{\Sigma (C_{n-1} + C_n) (t_n - t_{n-1})}{2}$$

5. Area under the concentration time curve ($AUC_{0\text{-}\infty}$)

$$[AUC]_0^\infty = \frac{\Sigma (C_{n-1} + C_n) (t_n - t_{n-1})}{2} + C/K$$

6. Mean absorption time (MAT).

$$MAT = 1 / K_a$$

7. Elimination rate constant

$$K = \frac{\ln C_1 - \ln C_2}{t_2 - t_1}$$

8. Volume of distribution

$$V_d = \frac{FX_oK_a}{\text{intercept}(K_a - K)}$$

8. The elimination half-life

$$t_{1/2} = 0.693 / K$$

9. Analysis of variance for three way crossover design.

The experimental design is:

Sequence	Subject / Sequence	Period		
		I	II	III
I	1,2,3,4,5	A	B	C
II	6, 7,8,9,10	B	C	A
III	11,12,13,14,15	C	A	B

Where A = brand A

B = brand B

C = brand C

In statistical terms the calculations to set up an analysis of variance table are as follow:

Source of variation	d.f.	Sum of squares	Mean square
Total	g.n.t-1	SStotal	-
Sequence	g-1	SSsequence	MSsequence
Subjects (sequence)	g(n-1)	SSsubject	MSsubject
Period	p-1	SSperiod	MSperiod
Formulation	f-1	SSformulation	MSformulation
Error	(gn-2)(t-1)	SSerror	MSerror

Where

N = total number of subjects = gnt

f = number of formulation

g = number of sequence or group

p = number of time periods or week

n = number of subjects per sequence

C.T. = Correction term = $(\sum x)^2$ / g.n.t

Data presented are individual subject of the lnAUC of dicloxacillin following oral administration of three brands of 2x500 mg dicloxacllin capsule.

Sequence	Subject	Brand A		Brand B		Brand C		Subject total
I	1	4.2930		4.5238		4.2989		13.12
	2	4.7414		4.5934		4.5713		13.91
	3	4.7163	period I	4.4838	period II	4.3640	period III	13.56
	4	3.9836	sum	4.1370	sum	4.2910	sum	12.41
	5	4.6947	} 22.429	4.5695	} 22.308	4.6349	} 22.160	13.90
II	6	4.7721		4.9338		4.7983		14.50
	7	4.1553		4.5166		4.0578		12.73
	8	4.5362	period III	4.5078	period I	4.2991	period II	13.34
	9	4.5491	sum	4.2556	sum	4.6913	sum	13.50
	10	5.0200	} 23.033	4.8921	} 23.106	4.9463	} 22.793	14.86
III	11	4.6192		4.5953		4.5043		13.72
	12	4.4608		4.3744		4.4805		13.32
	13	4.4156	period II	4.5504	period III	4.4416	period I	13.41
	14	4.5017	sum	4.6661	sum	4.6160	sum	13.78
	15	4.7972	} 22.795	4.8933	} 23.080	4.7997	} 22.842	14.49
Formulation total		68.3		68.5		68.5		204.54

Period I = 22.429+23.106+22.842 = 68.377

Period II = 22.308+22.793+22.795 = 68.272

Period III = 22.160+23.033+23.080 = 67.895

1. Correction term = $(204.54)^2$ /45 = 929.741

2. SStotal = $[(4.2930)^2+(4.7414)^2+\ldots.+(4.7997)^2]$ – C.T. = 2.49

3. SSsequence =$[(13.12+13.91+13.56+12.41+13.90)^2+\ldots\ldots..+$
$(13.72+13.32+13.41+13.78+14.49)^2]/15$ – C.T.= 0.167

4. SSsubject = $[(13.12)^2+(13.91)^2+\ldots.+(14.19)^2]/3$ – C.T. = 2.002

5. SSperiod = $[(68.377)^2+(68.272)^2+(67.895)^2]$ /15– C.T. = 0.00857

6. SSformulation = $[(68.3)^2+(67.8)^2+(68.5)^2]/15$ – C.T. = 0.0168

7. SSresidual = [2.49-(0.166664+2.002+0.00857+0.0168)] = 0.296

8. MS = SS/df

Analysis of variance table for three way crossover design.

Source of variation	d.f.	SS	MS	Fratio	Ftable	Sig.level
Total	44	2.4895				
Sequence	2	0.1666	0.0833	0.50	3.88	NS
Subject (Seq)	12	2.0019	0.1668	14.68	2.15	S
Period	2	0.0086	0.0043	0.38	3.37	NS
Formulation	2	0.0168	0.0084	0.74	3.37	NS
Error	26	0.2955	0.0114			

Where: F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there are not significant differences for the AUC among three brands.

Data presented are individual subject of the ln C_{max} of dicloxacillin following oral administration of three brands of 2x500 mg dicloxacllin capsule.

Sequence	Subject	Brand A		Brand B		Brand C		Subject total
I	1	3.089		3.710		3.216		10.015
	2	3.401		3.877		3.682		10.960
	3	3.566	period I	3.336	period II	3.351	period III	10.253
	4	2.920	sum	3.164	sum	3.651	sum	9.735
	5	3.921	} 16.898	3.634	} 17.721	3.927	} 17.827	11.483
II	6	4.209		3.943		4.085		12.238
	7	2.889		3.945		2.724		9.558
	8	3.354	period III	3.180	period I	2.995	period II	9.529
	9	3.588	sum	3.224	Sum	3.235	sum	10.047
	10	3.895	} 17.935	3.752	} 18.044	4.021	} 17.060	11.668
III	11	3.564		4.074		3.334		10.971
	12	3.687		3.533		3.133		10.353
	13	4.029	period II	3.668	period III	3.931	period I	11.628
	14	3.991	sum	3.922	Sum	3.840	sum	11.753
	15	3.597	} 18.868	3.717	} 18.913	3.615	} 17.853	10.929
Formulation total		53.701		54.678		52.741		161.120

Period I = 16.898+18.044+17.853 = 52.796

Period II = 17.721+17.060+18.868 = 54.675

Period III = 17.827+17.935+18.913 = 53.649

1. Correction term = $(161.12)^2$ /45 = 576.88

2. SStotal = $[(3.089)^2+(3.401)^2+\ldots.+(3.615)^2]$ – C.T. = 5.959

3. SSsequence =$[(10.015+10.960+10.253+9.735+11.483)^2+\ldots\ldots..+$
$(10.971+10.353+11.628+11.753+10.929)^2]/15$ – C.T.= 0.3831

4. SSsubject = $[(10.015)^2+(10.960)^2+\ldots.+(10.929)^2]/3$ – C.T. = 3.6242

5. SSperiod = $[(52.796)^2+(54.675)^2+(53.649)^2]$ /15– C.T. = 0.118

6. SSformulation = $[(53.701)^2+(54.678)^2+(52.741)^2]/15$ – C.T. = 0.0168

7. SSresidual = [2.49-(0.166664+2.002+0.00857+0.0168)] = 0.296

8. MS = SS/df

Analysis of variance table for three way crossover design.

Source of variation	d.f.	SS	MS	Fratio	Ftable	Sig.level
Total	44	5.9592				
Sequence	2	0.3831	0.1915	0.63	3.88	NS
Subject (Seq)	12	3.6242	0.3020	4.60	2.15	S
Period	2	0.1180	0.0590	0.90	3.37	NS
Formulation	2	0.1251	0.0625	0.95	3.37	NS
Error	26	1.7088	0.0657			

Where: F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there are not significant differences for the C_{max} among three brands.

Data presented are individual subject of the t_{max} of dicloxacillin following oral administration of three brands of 2x500 mg dicloxacllin capsule.

Sequence	Subject	Brand A		Brand B		Brand C		Subject total
I	1	3.50		1.50		2.50		7.50
	2	1.00		1.00		0.75		2.75
	3	1.50	period I	3.50	period II	3.50	period III	8.50
	4	1.75	sum	3.50	sum	0.50	sum	5.75
	5	1.00	} 8.75	1.00	} 10.50	1.00	} 8.25	3.00
II	6	0.50		1.50		0.75		2.75
	7	3.50		1.00		2.00		6.50
	8	0.75	period III	1.25	period I	1.00	period II	3.00
	9	1.75	sum	3.50	Sum	1.75	Sum	7.00
	10	0.75	} 7.25	1.75	} 9.00	1.25	} 6.75	3.75
III	11	0.50		0.50		0.50		1.50
	12	0.75		0.50		2.50		3.75
	13	0.75	period II	1.50	period III	0.75	period I	3.00
	14	0.75	sum	0.75	Sum	0.75	Sum	2.25
	15	1.50	} 4.25	1.25	} 4.50	1.75	} 6.25	4.50
Formulation total		20.25		24.00		21.25		65.50

Period I = 8.75+9.00+6.25 = 24.00

Period II = 10.50+6.75+4.25 = 21.50

Period III = 8.25+7.25+4.50 = 20.20

1. Correction term = $(65.50)^2$ /45 = 95.3389

2. SStotal = $[(3.50)^2+(1.00)^2+\ldots.+(1.75)^2]$ – C.T. = 40.0361

3. SSsequence =$[(7.50+2.75+8.50+5.75+3.00)^2+\ldots\ldots..+$
$(1.50+3.75+3.00+2.25+4.50)^2]/15$ – C.T.= 5.3444

4. SSsubject = $[(7.50)^2+(2.75)^2+\ldots.+(4.50)^2]/3$ – C.T. = 21.5361

5. SSperiod = $[(24.00)^2+(21.50)^2+(20.00)^2]$ /15– C.T. = 0.5444

6. SSformulation = $[(20.25)^2+(24.00)^2+(21.25)^2]/15$ – C.T. = 0.0168

7. SSresidual = [40.0361-(5.344+21.536+0.5444+0.5028)] = 12.1083

8. MS = SS/df

Analysis of variance table for three way crossover design.

Source of variation	d.f.	SS	MS	Fratio	Ftable	Sig.level
Total	44	40.0361				
Sequence	2	5.3444	2.6722	1.49	3.88	NS
Subject (Seq)	12	21.5361	1.7947	3.85	2.15	S
Period	2	0.5444	0.2722	0.58	3.37	NS
Formulation	2	0.5028	0.2514	0.54	3.37	NS
Error	26	12.1083	0.4657			

Where: F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there are not significant differences for the t_{max} among three brands.

Data presented are individual subject of the K_a of dicloxacillin following oral administration of three brands of 2x500 mg dicloxacllin capsule.

Sequence	Subject	Brand A		Brand B		Brand C		Subject total
I	1	0.672		1.139		0.857		2.669
	2	1.040		1.663		2.084		4.788
	3	1.743	period I	0.529	period II	1.442	period III	3.715
	4	1.014	sum	0.899	sum	1.210	sum	3.123
	5	2.146	} 6.62	2.468	} 6.70	1.811	} 7.40	6.424
II	6	2.944		0.845		2.707		6.496
	7	0.673		2.490		1.162		4.324
	8	1.329	period III	0.994	period I	1.322	period II	3.645
	9	0.987	sum	0.784	Sum	0.957	sum	2.728
	10	1.402	} 7.34	1.050	} 6.16	1.427	} 7.58	3.879
III	11	1.308		2.079		1.184		4.571
	12	1.419		2.653		0.959		5.031
	13	3.584	period II	1.091	period III	1.747	period I	6.421
	14	0.940	sum	1.386	Sum	4.214	sum	6.540
	15	1.386	} 8.64	1.584	} 8.79	1.208	} 9.31	4.178
Formulation total		22.59		21.65		24.29		68.53

Period I = 6.62+6.16+9.31 = 22.09

Period II = 6.70+7.58+8.64 = 22.91

Period III = 7.40+7.34+8.79 = 23.59

1. Correction term = $(68.53)^2$ /45 = 104.3676

2. SStotal = $[(0.672)^2+(1.040)^2+\ldots+(1.208)^2]$ – C.T. = 26.7434

3. SSsequence =$[(2.669+4.788+3.715+3.123+6.424)^2+\ldots\ldots..+$
$(4.571+5.031+5.421+6.540+4.178)^2]/15$ – C.T.= 1.5229

4. SSsubject = $[(2.669)^2+(4.788)^2+\ldots+(4.178)^2]/3$ – C.T. = 8.6979

5. SSperiod = $[(22.09)^2+(22.91)^2+(23.53)^2]$ /15– C.T. = 0.0701

6. SSformulation = $[(22.59)^2+(21.65)^2+(24.29)^2]/15$ – C.T. = 0.2384

7. SSresidual = [26.7434-(1.5229+8.6979+0.0701+0.2384)] = 16.2143

8. MS = SS/df

Analysis of variance table for three way crossover design.

Source of variation	d.f.	SS	MS	Fratio	Ftable	Sig.level
Total	44	26.7434				
Sequence	2	1.5229	0.7614	1.05	3.88	NS
Subject (Seq)	12	8.6979	0.7248	1.16	2.15	NS
Period	2	0.0701	0.0350	0.06	3.37	NS
Formulation	2	0.2384	0.1192	0.19	3.37	NS
Error	26	16.2143	0.6236			

Where: F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there are not significant differences for the K_a among three brands.

Data presented next page are individual subject of the $t_{1/2}$ of dicloxacillin following oral administration of three brands of 2x500 mg dicloxacllin capsule.

Sequence	Subject	Brand A		Brand B		Brand C		Subject total
I	1	1.37		0.95		1.05		3.37
	2	1.71		1.04		1.12		3.87
	3	1.41	period I	1.51	period II	1.38	period III	4.29
	4	1.55	sum	0.86	sum	1.28	sum	3.70
	5	1.09	} 7.12	1.31	} 5.67	1.09	} 5.93	3.49
II	6	1.15		1.25		1.20		3.60
	7	1.07		1.01		1.19		3.27
	8	1.78	period III	1.86	period I	1.79	period II	5.43
	9	1.23	sum	1.15	Sum	2.23	Sum	4.61
	10	1.29	} 6.53	1.19	} 6.46	1.11	} 7.52	3.59
III	11	1.37		1.08		1.36		3.81
	12	1.36		1.19		1.00		3.54
	13	0.99	period II	1.08	period III	1.10	period I	3.17
	14	1.35	sum	1.40	Sum	1.13	Sum	3.88
	15	1.39	} 6.47	1.63	} 6.36	1.59	} 6.18	4.61
Formulation total		20.11		18.49		19.62		58.23

Period I = 7.12+6.46+6.18 = 19.76

Period II = 5.67+7.52+6.47 = 19.66

Period III = 5.93+6.53+6.36 = 18.82

1. Correction term = $(58.23)^2$ /45 = 75.3522

2. SStotal = $[(1.37)^2+(1.71)^2+\ldots.+(1.59)^2]$ – C.T. = 3.3618

3. SSsequence =$[(3.37+3.87+4.29+3.70+3.49)^2+\ldots\ldots..+$
$(3.81+3.54+3.17+3.88+4.61)^2]/15$ – C.T.= 0.1240

4. SSsubject = $[(3.37)^2+(3.87)^2+\ldots.+(4.61)^2]/3$ – C.T. = 1.7534

5. SSperiod = $[(19.76)^2+(19.66)^2+(18.82)^2]$ /15– C.T. = 0.0357

6. SSformulation = $[(20.11)^2+(18.49)^2+(19.62)^2]/15$ – C.T. = 0.0919

7. SSresidual = [3.3618-(0.1240+1.7534+0.0357+0.0919)] = 1.3567

8. MS = SS/df

Analysis of variance table for three way crossover design.

Source of variation	d.f.	SS	MS	Fratio	Ftable	Sig.level
Total	44	3.3618				
Sequence	2	0.1240	0.0620	0.42	3.88	NS
Subject (Seq)	12	1.7534	0.1461	2.80	2.15	S
Period	2	0.0357	0.0178	0.34	3.37	NS
Formulation	2	0.0919	0.0460	0.88	3.37	NS
Error	26	1.3567	0.0522			

Where: F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there are not significant differences for the $t_{1/2}$ among three brands.

Data presented are individual subject of the V_d /F of dicloxacillin following oral administration of three brands of 2x500 mg dicloxacllin capsule.

Sequence	Subject	Brand A		Brand B		Brand C		Subject total
I	1	31.33		22.24		33.74		87.31
	2	16.40		17.76		13.53		47.69
	3	17.43	period I	45.43	period II	27.85	period III	90.71
	4	35.09	sum	23.26	sum	33.48	sum	91.83
	5	13.53	} 113.78	18.18	} 126.87	14.68	} 123.28	46.39
II	6	13.23		22.38		16.10		51.71
	7	16.77		12.56		20.40		49.73
	8	16.83	period III	21.59	period I	30.10	period II	68.52
	9	21.16	sum	31.94	Sum	36.11	Sum	89.21
	10	12.47	} 80.46	18.71	} 107.18	10.14	} 112.85	41.32
III	11	10.18		18.10		17.93		46.21
	12	25.57		18.07		15.10		58.74
	13	17.26	period II	19.52	period III	17.20	period I	53.98
	14	26.81	sum	28.29	Sum	15.82	Sum	70.92
	15	16.97	} 96.79	16.69	} 100.67	17.97	} 84.02	51.63
Formulation total		291.03		334.72		320.15		945.90

Period I = 113.78+107.18+84.02 = 304.98

Period II = 126.87+112.85+96.79 = 336.51

Period III = 123.28+80.46+100.67 = 304.41

1. Correction term = $(945.90)^2$ /45 = 19882.28

2. SStotal = $[(31.33)^2+(16.40)^2+\ldots.+(17.97)^2]$ – C.T. = 2685.38

3. SSsequence =$[(87.31+47.69+90.71+91.83+46.39)^2+\ldots\ldots..+$
$(46.21+58.74+53.98+70.92+51.63)^2]/15$ – C.T.= 248.53

4. SSsubject = $[(87.31)^2+(47.69)^2+\ldots.+(51.63)^2]/3$ – C.T. = 1588.22

5. SSperiod = $[(304.98)^2+(336.51)^2+(304.41)^2]$ /15– C.T. = 44.99

6. SSformulation = $[(291.03)^2+(334.72)^2+(320.15)^2]/15$ – C.T. = 65.98

7. SSresidual = [2685.38-(248.53+1588.22+44.99+65.98)] = 737.66

8. MS = SS/df

Analysis of variance table for three way crossover design.

Source of variation	d.f.	SS	MS	Fratio	Ftable	Sig.level
Total	44	2685.38	--	--	--	
Sequence	2	248.53	124.27	0.94	3.88	NS
Subject (Seq)	12	1588.22	132.35	4.66	2.15	S
Period	2	44.99	22.50	0.79	3.37	NS
Formulation	2	65.98	32.99	1.16	3.37	NS
Error	26	737.66	28.37	--	--	

Where: F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there are not significant differences for the V_d/F among three brands.

Data presented are individual subject of the MAT of dicloxacillin following oral administration of three brands of 2x500 mg dicloxacllin capsule.

Sequence	Subject	Brand A		Brand B		Brand C		Subject total
I	1	1.49		0.88		1.17		3.53
	2	0.96		0.60		0.48		2.04
	3	0.57	period I	1.89	period II	0.69	period III	3.16
	4	0.99	sum	1.11	sum	0.83	sum	2.92
	5	0.47	}4.48	0.41	}4.89	0.55	}3.72	1.42
II	6	0.34		1.18		0.37		1.89
	7	1.49		0.40		0.86		2.75
	8	0.75	period III	1.01	period I	0.76	period II	2.51
	9	1.01	sum	1.74	Sum	1.05	sum	3.80
	10	0.71	}4.30	0.95	}5.29	0.70	}3.73	2.37
III	11	0.77		0.48		0.85		2.09
	12	0.71		0.38		1.04		2.13
	13	0.28	period II	0.92	period III	0.57	period I	1.77
	14	1.06	sum	0.72	Sum	0.24	sum	2.02
	15	0.72	}3.53	0.63	}3.13	0.83	}3.53	2.18
Formulation total		12.31		13.30		10.98		36.59

Period I = 4.48+5.29+3.53 = 13.29

Period II = 4.89+3.73+3.53 = 12.15

Period III = 3.72+4.30+3.13 = 11.15

1. Correction term = $(36.59)^2$ /45 = 29.7469

2. SStotal = $[(1.49)^2+(0.96)^2+\ldots.+(0.83)^2]$ – C.T. = 5.859

3. SSsequence =$[(3.53+2.04+3.16+2.92+1.42)^2+\ldots\ldots..+$
$(2.09+2.13+1.77+2.02+2.18)^2]/15$ – C.T.= 0.4055

4. SSsubject = $[(3.53)^2+(2.04)^2+\ldots.+(2.18)^2]/3$ – C.T. = 2.0998

5. SSperiod = $[(13.29)^2+(12.15)^2+(11.15)^2]$ /15– C.T. = 0.1527

6. SSformulation = $[(12.31)^2+(13.30)^2+(10.98)^2]/15$ – C.T. = 0.1814

7. SSresidual = [5.859-(0.4055+2.0998+0.1527+0.1814)] = 3.0196

8. MS = SS/df

Analysis of variance table for three way crossover design.

Source of variation	d.f.	SS	MS	Fratio	Ftable	Sig.level
Total	44	5.8590				
Sequence	2	0.4055	0.2027	1.16	3.88	NS
Subject (Seq)	12	2.0998	0.1750	1.51	2.15	NS
Period	2	0.1527	0.0764	0.66	3.37	NS
Formulation	2	0.1814	0.0907	0.78	3.37	NS
Error	26	3.0196	0.1161			

Where: F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there are not significant differences for the MAT among three brands.

Data presented are individual subject of the K_e of dicloxacillin following oral administration of three brands of 2x500 mg dicloxacllin capsule.

Sequence	Subject	Brand A		Brand B		Brand C		Subject total
I	1	0.506		0.730		0.662		1.90
	2	0.406		0.667		0.617		1.69
	3	0.493	period I	0.461	period II	0.501	period III	1.45
	4	0.447	sum	0.802	sum	0.541	sum	1.79
	5	0.635	} 2.49	0.529	} 3.19	0.636	} 2.96	1.80
II	6	0.601		0.554		0.579		1.73
	7	0.646		0.688		0.581		1.92
	8	0.389	period III	0.372	period I	0.388	period II	1.15
	9	0.563	sum	0.602	Sum	0.311	Sum	1.48
	10	0.537	} 2.74	0.582	} 2.80	0.623	} 2.48	1.74
III	11	0.505		0.643		0.510		1.66
	12	0.509		0.585		0.694		1.79
	13	0.700	period II	0.644	period III	0.631	period I	1.97
	14	0.512	sum	0.495	Sum	0.614	Sum	1.62
	15	0.498	} 2.72	0.426	} 2.79	0.435	} 2.88	1.36
Formulation total		7.95		8.78		8.32		25.05

Period I = 2.49+2.80+2.88 = 8.17

Period II = 3.19+2.48+2.72 = 8.39

Period III = 2.96+2.74+2.79 = 8.49

1. Correction term = $(25.05)^2$ /45 = 13.9496

2. SStotal = $[(0.506)^2+(0.406)^2+\ldots.+(0.435)^2]$ – C.T. = 0.4835

3. SSsequence =$[(1.90+1.69+1.45+1.79+1.80)^2+\ldots\ldots..+$
$(1.66+1.79+1.97+1.62+1.36)^2]/15$ – C.T.= 0.0130

4. SSsubject = $[(1.90)^2+(1.69)^2+\ldots.+(1.36)^2]/3$ – C.T. = 0.2381

5. SSperiod = $[(8.17)^2+(8.39)^2+(8.49)^2]$ /15– C.T. = 0.0035

6. SSformulation = $[(7.95)^2+(8.78)^2+(8.32)^2]/15$ – C.T. = 0.0231

7. SSresidual = [0.4835-(0.0130+0.2381+0.0035+0.0231)] = 0.2058

8. MS = SS/df

Analysis of variance table for three way crossover design.

Source of variation	d.f.	SS	MS	Fratio	Ftable	Sig.level
Total	44	0.4835				
Sequence	2	0.0130	0.0065	0.33	3.88	NS
Subject (Seq)	12	0.2381	0.0198	2.51	2.15	S
Period	2	0.0035	0.0018	0.22	3.37	NS
Formulation	2	0.0231	0.0115	1.46	3.37	NS
Error	26	0.2058	0.0079			

Where: F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there are not significant differences for the K_e among three brands.

VITAE

Miss. Verisa Binjehsoh was born on February 16, 1974 in Bangkok. She received a Bachelor of Pharmacy degree in 1995 from Faculty of Pharmacy, Rungsit University. She is a lecturer in Rungsit University, Pathum Thani, Thailand.