ชีวสมมูลของยาฉีดเข้ากล้ามเซโฟเพอราโซนและซัลแบคแทมที่มีจำหน่ายในประเทศไทย

นางสาวศิริพร ฉวานนท์

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BIOEQUIVALENCE OF CEFOPERAZONE AND SULBACTAM INTRAMUSCULAR INJECTIONS COMMERCIALLY AVAILABLE IN THAILAND

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ศึกษาชีวสมมูลของยาฉีดเข้ากล้ามเซโฟเพอราโซนและซัลแบคแทม 500/500 มิลลิกรัมที่มีจำหน่ายในประเทศไทย 2 ผลิตภัณฑ์ ผลการทดสอบในหลอดทดลองพบว่า ยาทั้งสองผลิตภัณฑ์ได้มาตรฐานตามข้อกำหนดทั่วไปและมีความเท่าเทียมกันทางเภสัชกรรม การเปรียบเทียบชีวปริมาณออกฤทธิ์ของผลิตภัณฑ์ยาที่ผลิตภายในประเทศและผลิตภัณฑ์ยาต้นแบบดำเนินการในอาสาสมัครชายไทยสุขภาพดีจำนวน 22 คน แต่ละคนได้รับยาฉีดเข้ากล้ามเซโฟเพอราโซนและซัลแบคแทม 500/500 มิลลิกรัมครั้งเดียวตามแบบแผนการทดลองแบบสุ่มข้ามสลับชนิด 2 ทาง โดยเว้นระยะเวลา 1 สัปดาห์ระหว่างการบริหารยา 2 ผลิตภัณฑ์ เก็บตัวอย่างเลือดที่เวลาเฉพาะต่างๆหลังการให้ยา แยกพลาสมาและวัดความเข้มข้นในพลาสมาของเซโฟเพอราโซนและซัลแบคแทมโดยใช้วิธีไฮเพอฟอร์แมนซ์ลิขวิด-โครมาโตรกราฟฟีที่ได้พัฒนาและตรวจยืนยัน วิเคราะห์หาค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ที่เกี่ยวข้องจากข้อมูลความเข้มข้นของยาในพลาสมาและเวลาของยาทั้ง 2 ผลิตภัณฑ์ นำค่าพารามิเตอร์ทางเภสัช-จลนศาสตร์ที่จำเป็นสำหรับการประเมินชีวสมมูลของยาทั้ง 2 ผลิตภัณฑ์มาเปรียบเทียบกันโดยใช้วิธีทางสถิติ ค่าช่วงความเชื่อมั่นร้อยละ 90 ของสัดส่วนของแต่ละพารามิเตอร์ทางเภสัชจลนศาสตร์ที่แปลงข้อมูลเป็นลอการิทึมของเซโฟเพอราโซนและซัลแบคแทมของผลิตภัณฑ์ยาที่ผลิตในประเทศเทียบกับผลิตภัณฑ์ยาต้นแบบอยู่ภายในช่วงร้อยละ 80-125 จากการอนุมานทางสถิติสรุปได้ว่าผลิตภัณฑ์ยาที่ผลิตในประเทศมีชีวสมมูลกับผลิตภัณฑ์ยาต้นแบบทั้งในเชิงอัตราเร็วและปริมาณยาที่ถูกดูดซึมเข้าสู่ระบบการไหลเวียนของโลหิต ในการศึกษานี้ ค่าเฉลี่ยของพื้นที่ใต้เส้นโค้งระหว่างความเข้มข้นของยาในพลาสมากับเวลาของเซโฟเพอราโซนและซัลแบคแทม คือ 166.16 ± 42.83 และ 43.97 ± 10.42 ไมโครกรัมต่อมิลลิลิตรคูณชั่วโมง ตามลำดับ ค่าความเข้มข้นสูงสุดของยาในพลาสมาของเซโฟเพอราโซนและซัลแบคแทม คือ 32.4 ± 7.0 และ 18.57 ± 5.5 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ ค่าพารามิเตอร์นี้มีความสอดคล้องกันกับค่าเดียวกันที่ผู้ทำการศึกษาก่อนหน้านี้ได้รายงานไว้ อย่างไรก็ตามค่าครึ่งชีวิตของการขจัดยาของเซโฟเพอราโซน (4.1 ± 1.6 ชั่วโมง) และซัลแบคแทม (1.5 ± 0.4 ชั่วโมง) มีค่าสูงกว่าเล็กน้อย

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY/ CEFOPERAZONE/ SULBACTAM

SIRIPORN CHAWANON; BIOEQUIVALENCE OF CEFOPERAZONE AND SULBACTAM INTRAMUSCULAR INJECTIONS COMMERCIALLY AVAILABLE IN THAILAND. THESIS ADVISOR: ASSOC. PROF.UTHAI SUVANAKOOT, Ph.D. 151 pp. ISBN 974-53-2251-2.

Bioequivalence of two products of 500/500 mg cefoperazone/sulbactam intramuscular injection commercially available in Thailand were studied. *In vitro* tests indicated that both products completely complied general specification requirements and they were pharmaceutical equivalence. Comparative bioavailability of a local product relative to an innovator's product was conducted in 22 healthy Thai male volunteers. Each subject received a single dose of 500/500 mg cefoperazone/sulbactam injection intramuscularly in a randomized two way crossover design with 1 week washout period between dosing. Blood samples were collected at specified time intervals. Plasma was separated and analyzed for cefoperazone and sulbactam concentrations using a developed and validated HPLC method. The relevant pharmacokinetic parameters were determined from plasma concentration-time profiles of both products. The principal pharmacokinetic parameters (AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}) were statistically evaluated based on log-transformed data for bioequivalence between the two products. The 90% confidence intervals for the ratios of log-transformed data of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} for cefoperazone and sulbactam of local product to that of innovator's product were within 80-125%. Based on these statistical inferences, it was concluded that the two products were bioequivalence in terms of both the rate and extent of drug absorption into systemic circulation. In this study, the mean $AUC_{0-\infty}$ values of cefoperazone and sulbactam were 166.16 ± 42.83 and 43.97 ± 10.42 µg.hr/mL, respectively. The mean C_{max} values were 32.4 ± 7.0 and 18.57 ± 5.5 µg/mL for cefoperazone and sulbactam, respectively. These values agreed with results of previous studies. However, the mean elimination half-life ($t_{½}$) of cefoperazone (4.1 ± 1.6 hr) and sulbactam (1.5 ± 0.4 hr) appear to be slightly greater.

Department	Pharmacy	Student's signature……..……..…….
Field of study	Pharmacy	Advisor's signature……...……
Academic year	2004	

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LIST OF ABBREVIATIONS

°C	=	degree Celsius
µg	=	microgram
mg	=	milligram
kg	=	kilogram
µL	=	microliter
mL	=	milliliter
µm	=	micrometer
mm.	=	millimeter
m	=	meter
min	=	minute
hr	=	hour
EU	=	endotoxin unit
rpm	=	revolution per minute
%L.A.	=	percent labeled amount
M	=	molar
USP	=	United States Pharmacopoeia
UV	=	ultraviolet
AUC_{0-t}	=	area under the plasma concentration-time curve from time 0 to the last time of collecting the sample
$AUC_{0-\infty}$	=	area under the plasma concentration-time curve from time 0 to infinity
C_{max}	=	peak plasma concentration
t_{max}	=	time to peak plasma concentration
$t_{1/2}$	=	elimination half-life
CI	=	confidence interval
S.D.	=	standard deviation
C.V.	=	coefficient of variation
R.S.D.	=	relative standard deviation

CHAPTER I

INTRODUCTION

During the last two decades, the regulation authority, pharmaceutical industry and academy in Thailand have been very interesting in bioavailability and bioequivalence study. The dramatically growth of the generic pharmaceutical industry and a great increase in the use of generic drug products motivation by their lower cost have driven some questions about their quality and efficacy. The generic (multisource) products contain the same amount of the same therapeutically active ingredients in the same dosage form manufactured by the local brand and should meet all applicable pharmacopoeial standards of identity, strength, quality and purity. However, pharmaceutical equivalency in drug products does not assure clinical and therapeutics equivalency. Also the bioavailability of drug from dosage form can be affected by a variety of factors such as formulation and method of manufacturing. Therefore, the variation of bioavailability may lead to failure of therapy or development of some adverse reaction. Several studies have reported variations in the efficacy of generic drug compared with the corresponding innovator's drugs (Nuwar et al.,1990; Hope and Havrda, 2001; Borgherini, 2003). These reports give rise to doubts about the interchangeability of generic and innovator's product. The generic formulations may be widespread. Any loss of efficacy can have ethical and health outcome, as well as economic consequences. To ensure that the generic products is safe and effective, bioequivalence studies are used to compare the bioavailability of the same drug from various drug products. If the drug products are bioequivalent and therapeutically equivalent to innovator's product, then the clinical efficacy and the safety profiles of these products are assumed to be similar and may be substituted for each other (Chereson, 1996).

According to the FDA, to be interchangeable with the innovator's product, a generic drug product must be not only pharmaceutically equivalent but also bioequivalent. (Pharmaceutically equivalents are drug products that contain identical active ingredients and are identical in strength or concentration, dosage form, and route of administration. Pharmaceutically equivalent drug products are formulated to contain the same amount of active ingredient in the same dosage form and to meet the same or compendial or other applicable standards (i.e., strength, quality, purity,

and identity), but they may differ in characteristics such as shape, release mechanisms, packaging, inactive excipients, expiration time and within certain limits, labeling). For a generic drug product to be considered bioequivalent to an innovator's product, it must be shown to have the same rate and extent of absorption as the innovator's product when administered at the same molar dose of the active therapeutic moiety under similar experimental conditions. Bioequivalence thus plays a critical role in assuring the therapeutic quality of multisource drug products in the marketplace.

Cefoperazone/sulbactam is an antibacterial combination consisting of β-lactam antibiotic cefoperazone and the β-lactamase inhibitor sulbactam. It has been launched in combination 1:1 or 1:1.5. This combination exhibits synergistic antimicrobial activity against many β-lactamase producing bacteria. Furthermore, the combination shows potent antimicrobial activity against *Acinetobacter*, expanding the antimicrobial spectrum of cefoperazone (Yokota, Azuma, and Suzuki, 1984)

Cefoperazone is a third generation cephalosporin with a broad spectrum of activity against most gram-positive and gram-negative bacteria including *Pseudomonas aeruginosa* and most members of the family *Enterobacteriaceae* (Neu et al., 1979; Thornsberry and Jones, 1981), which acts against sensitive organisms during the stage of active multiplication by inhibiting biosynthesis of cell wall mucopeptide.

Sulbactam, a penicillanic acid sulfone, is a potent semi-synthetic β-lactamase inhibitor without real antibacterial activity, except against *Neisseriaceae* and *Acinetobacter*. The present combination prevents cefoperazone from being hydrolyzed by β-lactamase enzymes and exhibits antibacterial activity against cefoperazone-resistant bacteria.

Cefoperazone/sulbactam is used in the treatment of respiratory tract infections, urinary tract infections, intra-abdominal infections, septicemia, meningitis, skin and soft tissue infections, bone and joint infections, pelvic inflammatory disease, endometritis, gonorrhea and other infections of the genital tract. The combination of cefoperazone and sulbactam was given by intravenous or intramuscular injection due to its poor absorption orally. Usual adult dosage is 2-4 g /day (cefoperazone 1-2 g/day) given in equally divided dose every 12 hours. In children, the usual dose is 40-80 mg/kg/day (cefoperazone 20-40 mg/kg/day) in 2-4 equally divided dose (McEvoy, 2003).

In Thailand, the combination of cefoperazone 500 mg and sulbactam 500 mg was commercially available in two brands, one is an innovator's product "Sulperazon®" and another is a generic drug product "Sulcef® 1 g injection" manufactured by the local company. The previous study (มุมมองด้านการตลาดต่อการลงทุนด้านอุตสาหกรรมยาและเวชภัณฑ์, 2546) has revealed that the market price of sulperazon ®as high as 230 million bath ranking in level 13 from the best seller drug in 2003. This finding shows that cefoperazone/sulbactam is widely dispensed in hospital. Thus, using of a locally made generic drug-product resulted in a lowering health management costs. However, the quality of a locally made drug-product is the important factor to make a decision in drug product selection for the rational use of medicine in clinical practice. Pharmacists have the responsibility of correctly selecting and dispensing multisource products that will have the greatest possibility of achieving a positive therapeutic outcome in a cost effective manner. The more information about a product and bioequivalence study will be the most appropriate choice for decision making. To date, there is no studies that investigate the bioequivalence of cefoperazone/sulbactam intramuscular injection which is available in Thailand.

In this study, the comparative bioavailability of a local brand of cefoperazone/sulbactam 500/500 mg intramuscular injections (Sulcef® 1 g injection) commercially available in Thailand relative to the innovator's product (Sulperazon® 1 g injection) was performed in order to facilitate substitution of a brand-name (innovator) product with a generic product, in terms of efficacy and economic aspect.

Objectives: The purposes of this study were to;

1. Investigate the bioequivalence of a local brand of cefoperazone/sulbactam intramuscular injections commercially available in Thailand relative to the innovator's product.
2. Develop HPLC method for determination of combination drug (cefoperazone and sulbactam) in human plasma which applies to bioequivalence evaluation.
3. Compare the pharmacokinetic parameters of cefoperazone and sulbactam in healthy Thai male volunteers relative to the finding of previous studies that were published in the journals.

CHAPTER II

LITERATURE REVIEW

Bioavailability and bioequivalence

The bioavailability of a drug formulation often determines its therapeutic efficacy, as bioavailability affects the onset, intensity, and duration of the therapeutic response. In pharmacokinetics, the term bioavailability describes the rate of absorption of the active ingredient in a drug and the extent (AUC) to which the active drug ingredient is absorbed from a drug product and becomes available at the site of action. As the pharmacologic response is generally related to the concentration of drug at the receptor site, the availability of drug from a particular formulation is an important element in that product's clinical efficacy. However, drug concentrations cannot usually be measured easily at the site of action. Therefore, based on the premise that the drug concentration at the site of action is in equilibrium with that in the blood, it is therefore possible to obtain an indirect measure of drug response by monitoring drug levels in the blood or urine. Most bioavailability studies determine the drug concentration in blood or urine. Thus, bioavailability is concerned with how quickly and how much of a drug appears in the blood after a specific dose is administered. In most cases one is concerned with the extent of absorption of drug, (that is, the fraction of the dose that actually reaches the bloodstream) since this represents the effective dose of a drug. This is generally less than the amount of drug actually administered in the dosage form. In some cases, notably those where acute conditions are being treated, one is also concerned with the rate of absorption of a drug, since rapid onset of pharmacologic action is desired. Conversely, these are instances where a slower rate of absorption is desired, either to avoid adverse effects or to produce a prolonged duration of action. (Chereson, 1996)

Absolute bioavailability (F) is the fraction of an administered dose that reaches the systemic circulation and ranges from 0 (no drug absorption) to 1 (complete drug absorption). Because the total amount of drug reaching the systemic circulation is directly proportional to the AUC, F is determined by comparing the AUC of the product of interest following the

extravascular administration (e.g., oral, rectal, transdermal, intramuscular, subcutaneous) and the same dose of drug administered intravenously.

Relative bioavailability is the availability of a drug product compared with that of another dosage form or another formulation of the same drug given at the same dose. This measure expresses the effects on drug absorption of differences in drug formulations.

Bioavailability studies are used to define the effect of changes in the physicochemical properties of the drug substance and the effect of the drug product (dosage form) on the pharmacokinetics of the drug. The bioavailability of a generic product is generally expressed as relative bioavailability, based on a comparison of its AUC with that of the innovator's product (Shargel, Wupong and Yu, 2005).

Bioequivalence is a relative term which indicates that the drug substance in two or more dosage forms reaches the systemic circulation at the same relative rate and to the same relative extent (Abdou, 1989). In other words, the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives become available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study (Williams, 2003). Bioequivalence studies are used to compare bioavailability of the same drug and/or same therapeutic moiety from various drug products. If the drug products are bioequivalent, then efficacy of these drug products are assumed to be similar. When the drug products are bioequivalent and therapeutically equivalent to innovator's product, then the clinical efficacy and the safety profiles of these products are assumed to be similar and may be substituted for each other (Chereson, 1996).

In general, the FDA considers two products to be "therapeutic equivalent" if they each meet the following criteria (U.S. Department of Health and Human Service, Food and Drug Administration, 1990)

1) they are pharmaceutical equivalents
2) they are bioequivalent
3) they are in compliance with compendial standard for strength, quality, purity and identity
4) they are adequately labeled and

5) they have been manufactured in compliance with Good Manufacturing practices as established by FDA

Drug products with possible bioavailability and bioequivalence problems (Shargel, Wupong and Yu, 2005).

Biopharmaceutical properties of the active drug substance or the formulation of the drug product may indicate that the drug may have variable bioavailability and/or bioequivalence problem. Some of these biopharmaceutic properties include:

a) The active drug ingredient has low solubility in water (e.g., less than 5 mg/mL)
b) The dissolution rate of one or more such products is slow (e.g., less than 50% in 30 minutes when tested with a general method specified by US-FDA)
c) The particle size and/or surface area of the active drug ingredient is critical in determining its bioavailability.
d) Certain structural forms of the active ingredient (e.g., polymorphic forms, solvates, complexes, and crystal modification) dissolve poorly, thus affecting absorption.
e) Drug products that have a high ratio of excipients to active ingredients (e.g., greater than 5:1)
f) Specific inactive ingredients (e.g., hydrophilic or hydrophobic excipients and lubricants) either may be required for absorption of the active drug ingredient or therapeutic moiety or may interfere with such absorption.
g) The active drug ingredient, therapeutic moiety, or its precursor is absorbed in large part in a particular segment of the GI tract or is absorbed from a localized site.
h) The degree of absorption of the active ingredient, therapeutic moiety, or its precursor is poor (e.g., less than 50%, ordinarily in comparison to an intravenous dose), even when it is administered in pure form (e.g., in solution)
i) There is rapid metabolism of the therapeutic moiety in the intestinal wall or liver during the absorption process (first-pass metabolism), so that rate of absorption is unusually important in the therapeutic effect and/or toxicity of the drug product.
j) The therapeutic moiety is rapidly metabolized or excreted, so that rapid dissolution and absorption are required for effectiveness.

k) The active drug ingredient or the therapeutic moiety is unstable in specific portions of the GI tract and requires special coatings or formulations (e.g., buffers, enteric coating, and film coatings) to ensure adequate absorption.

l) The drug product is subject to dose-dependent kinetics in or near the therapeutic range, and the rate and extent of absorption are important to bioequivalence.

Methods of assessing equivalence

Assessment of equivalency of drug product will normally require in vivo study (approaches include bioequivalence studies, pharmacodynamic studies and clinical trial studies). In selected cases in vitro dissolution studies may be sufficient to provide some indication of equivalence.

Dighe and Adams (1991) suggested several test methods which are available for determining the bioequivalence of drug product including;

a) Comparative bioavailability studies (blood level or urinary excretion data), in which the active drug substance or one or more metabolites is measured in an accessible biological fluid such as plasma, serum, whole blood or urine.

b) Comparative pharmacodynamic studies in humans.

c) Comparative clinical studies

d) In vitro dissolution tests.

Blood level studies are the most common type of human bioavailability studies, and are based on the assumption that there is a direct relationship between the concentration of drug in blood or plasma and the concentration of drug at the site of action. By monitoring the concentration in the blood, it is thus possible to obtain an indirect measure of drug response. Following the administration of a single dose of a medication, blood samples are drawn at specific time intervals and analyzed for drug content. A profile is constructed showing the concentration of drug in blood at the specific times the samples were taken. The key parameters to note are:

1. $AUC_{0-\infty}$, the area under the plasma concentration-time curve. The $AUC_{0-\infty}$ is proportional to the total amount of drug reaching the systemic circulation, and thus characterizes the extent of absorption.

2. C_{max} , the maximum drug concentration**.** The maximum concentration of drug in the plasma is a function of both the rate and extent of absorption. C_{max} will increase with an increase in the dose, as well as with an increase in the absorption rate.

3. t_{max} , the time at which the C_{max} occurs. The t_{max} reflects the rate of drug absorption, and decreases as the absorption rate increases.

Bioavailability (the rate and extent of drug absorption) is generally assessed by the determination of these three parameters. Since the AUC is representative of, and proportional to, the total amount of drug absorbed into the circulation, it is used to quantitate the extent of drug absorption.

An alternative bioavailability study measures the cumulative amount of unchanged drug excreted in the urine. These studies involve collection of urine samples and the determination of the total quantity of drug excreted in the urine as a function of time. These studies are based on the premise that urinary excretion of the unchanged drug is directly proportional to the plasma concentration of total drug. Thus, the total quantity of drug excreted in the urine is a reflection of the quantity of drug absorbed from the gastrointestinal tract. This technique of studying bioavailability is most useful for those drugs that are not extensively metabolized prior to urinary elimination. Determination of bioavailability using urinary excretion data should be conducted only if at least 60% of a dose is excreted unchanged in the urine after an IV dose. Other conditions which must be met for this method to give valid results include:

1. The fraction of drug entering the bloodstream and being excreted intact by the kidneys must remain constant.

2. Collection of the urine has to continue until all the drug has been completely excreted (ten times the half-life).

Urinary excretion data are primarily useful for assessing extent of drug absorption, although the time course for the cumulative amount of drug excreted in the urine can also be used to estimate the rate of absorption. In practice, these estimates are subject to a high degree of variability, and are less reliable than those obtained from plasma concentration-time profiles. Thus, urinary excretion of drug is not recommended as a substitute for blood concentration data; rather, these studies should be used in conjunction with blood level data for confirmatory purpose (Chereson, 1996).

If quantitative analysis of the drug and/or metabolites in plasma or urine cannot be developed with sufficient accuracy and sensitivity, the pharmacodynamic studies may be used for establishing equivalence between two drug products. Furthermore, pharmacodynamic studies in humans are required if measurements of drug concentrations cannot be used as surrogate end points for the demonstration of efficacy and safety of the particular drug product e.g., for topical products without intended absorption of the drug into the systemic circulation. Pharmacodynamic studies compared the response which is measured should be a pharmacological or therapeutic effect which is relevant to the claims of efficacy and/or safety. The response should be measured quantitatively and be recordable in an instrument recorded on a repetitive basis to provide a record of the pharmacodynamic events which are substitutes for plasma concentration. The time course of the intensity of drug action can be described in the same way as in a study in which plasma concentrations were measured, and parameters can be derived which describe the area under the effect-time curve, the maximum response and the time when maximum response occurred.

In some of the cases pharmacodynamic studies cannot be performed because lack of meaningful pharmacodynamic parameters which can be measured and a comparative clinical trial has to be performed in order to demonstrate equivalence between two formulations. However, if clinical study is considered as being undertaken to prove equivalence the same statistical principles apply as for the bioequivalence studies. The number of patients to be included in the study will depend on the variability of the target parameters and the acceptance range, and is usually much higher than the number of subjects in comparative bioavailability.

In vitro dissolution studies should be reserved for rapidly dissolving drug product when generic product and reference products, both dissolve with sufficient rapidity (e.g., > 80% in 15 minutes). In vitro dissolution studies should be base on generation of comparative dissolution profiles rather than single point dissolution test, in multiple dissolution test condition and physiologically relevant media are recommended (WHO Expert committee on specifications for the pharmaceutical preparations, 1996).

Registration of generic drug product in Thailand.

Since 1990, drug registration may be classified as new drugs which include one or more of the following characteristics; a new chemical entity, a new indication, a new combination, a new delivery system, and non new drug which is considered the generic drug. Owing to political pressure concerning patent protection on pharmaceutical products, the Thai FDA has introduced an administrative measure to protect original novel drug products during the first period of introduction to Thai market by the introduction of a Safety Monitoring Program (SMP) or post marketing surveillance. New drugs are on conditional approval when first introduced into market. They are available only in medical institution or hospital where SMP study can be conducted. Before distribution, the company is requested to submit SMP protocol and conduct the program as approved by FDA. The SMP is estimated to take about two years. During this period, a SMP is conducted. Data are collected and evaluated for safety and efficacy. The collected data are submitted to FDA for unconditional approval. After a drug has received unconditional approval, the company can distribute it via normal channels. Generic products are allowed to be submitted for registration after the status of new drug product has changed to unconditional. Registration of generic product, the FDA may require a bioavailability/bioequivalence (BA/BE) study to prove its bioequivalence to the innovator's product characteristics and labeling, and the documentation of manufacturing (GMP) and quality control (Prakongpan, 1996).

In 1992, the Department of Medical Sciences, Ministry of Public Health, published Guidelines for Bioavailability/Bioequivalence Studies. These guidelines follow those of several selected country. They describe the study design, protocol and evaluating criteria pertaining to a bioequivalence study of known drug products. The procedures can be modified from these guidelines to suit specific purposes. However, a study specific protocol must be submitted and approved by authority prior to initiation of the test. Now the guidelines have been revised in many versions. The requirement for BA/BE study in Thailand is in early stage and require the review process to develop a suitable guidance.

Cefoperazone/sulbactam

Physicochemical properties

Cefoperazone/sulbactam is commercially available in Thailand as a fixed dose combination of cefoperazone sodium and sulbactam sodium (1:1). Cefoperazone is a semi synthetic cephalosporin antibiotic. The major structural difference between cefoperazone and other parenteral cephalosporins is that cefoperazone containes a piperazine side chain; the side chain results in antipseudomonal activity. Cefoperazone also contains an N-methylthiotetrazole (NMTT) side chain at position 3 of the cephalosporin nucleus. The NMTT side chain enhances antibacterial activity, prevents metabolism of the drug, and also may be associated with certain adverse effect (e.g., hypoprothrombinemia, disulfiram-like reactions).

Cefoperazone sodium occurs as a white or slightly yellow crystalline powder, hygroscopic, freely soluble in water and poorly soluble in alcohol. The drug has a pKa of 2.55.

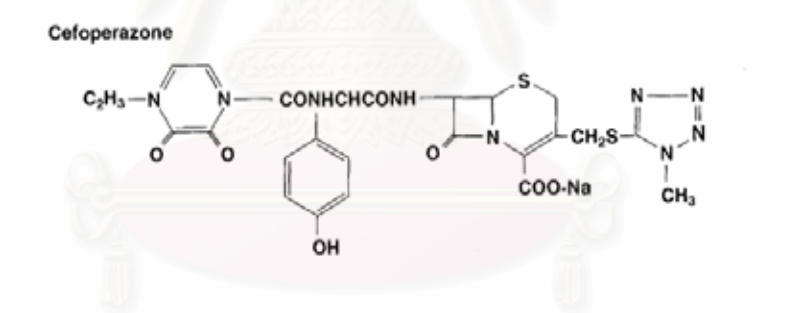


Figure 1 Chemical structure of cefoperazone

Cefoperazone sodium : $C_{25}H_{26}N_9NaO_8S_2$

Molecular weight = 667.65

Chemical name : 5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[[(4-ethyl-2,3-dioxo-1-piperazenyl)carbonyl]amino](4-hydroxyphenyl)acetyl]amino]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-,[6R-[6α,7β(R*)]] (The United States Pharmacopeial Convention, 2004)

Sulbactam is a penicillanic acid sulfone which is potent semisynthetic β-lactamase inhibitor commercially available as the sodium salt.

Sulbactam sodium occurs as a white to off-white crystalline powder. Freely soluble in water and in diluted acid; sparingly soluble in acetone, in chloroform, and in ethyl acetate.

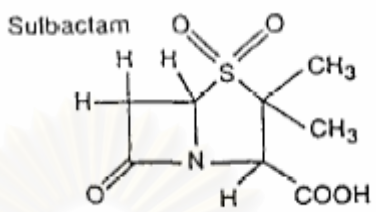


Figure 2 Chemical structure of sulbactam

Empirical formula: $C_8H_{10}NNaO_5S$

Molecular weight: 255.22

Chemical name: 4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,3,3-dimethyl-7-oxo-,4,4-dioxide,(2s-cis) (The United States Pharmacopeial Convention, 2004)

Mechanism of action

Cefoperazone usually is bactericidal in action. Like other cephalosporins the antibacterial activity of the drug results from inhibition of mucopeptide synthesis in bacterial cell wall. Sulbactam dose not posses any useful antibacterial activity, except against *Neisseriaceae* and *Acinetobacter*. However, biochemical studies with cell-free bacterial systems have shown it to be an irreversible inhibitor of most important β-lactamases produced by β-lactam antibiotic-resistant organisms. The potential for sulbactam's preventing the destruction of penicillins and cephaloporins by resistant organisms was confirmed in whole-organism studies using resistant strains, in which sulbactam exhibited marked synergy with penicillins and cephalosporins. As sulbactam also binds with some penicillin-binding proteins, sensitive strains are also often rendered more susceptible to cefoperazone/sulbactam than to cefoperazone alone (Scholar and Pratt, 2000).

Antibacterial activity

The combination of cefoperazone and sulbactam is active against all organisms sensitive to cefoperazone. In addition, it demonstrates synergistic activity (up to 4-fold reduction

in minimum inhibitory concentrations for the combination versus those for each component) in a variety of organisms most markedly the following; *Haemophilus influenzae*, *Bacteriodes* sp, *Staphylococcus* sp, *Acinetobacter calcoaceticus*, *Enterobacter aerogenes*, *Escherichia coli*, *Proteusmirabilis*, *Klebsiella pneumoniae*, *Morganella morganii*, *Citrobacter freundii*, *Enterobacter cloacae*, *Citrobacter diversus*. (Fu et al., 2003; Fu, D. W. et al., 2004)

Cefoperazone/sulbactam is active in vitro against a wide variety of clinically significant organism:

a) Gram-Positive Organisms: *Staphylococcus aureus*, penicillinase- and nonpenicillinase-producing strains, *Staphylococus epidermidiose*, *Streptococcus pneummoniae* , *Streptococcus pyogenes*, *Streptococcus agalactiae* , most other strains of β-hemolytic streptococci, many strains of *Streptococcus faecalis* (enterococcus).

b) Gram-Negative Organisms: *Escherichia coli*, *Klebsiella Enterobacter* and *Citrobacte*r spp, *Haemophilus influerzae*, *Proteus mirabilis*, *Proteus vulgaris*, *Moeganella morgani*, *Providencia rettgeri* , *Providencia*, *Serratia* (including *S. marcescens*), *Salmonella* and *Shigella* spp, *Pseudomonas aeruginosa* and some other *Pseudomonas* sp, *Acinetobacter calcoaceticus*, *Neisseria gonoeehoeae*, *Neissria meningitides*, *Bordetella pertussis, Yersinia enterrocolitica*.

c) Anaerobic Organisms: Gram-negative bacilli (including *Bacteroides fragilis*,other *Bacteroides* and *Fusobacterium* spp). Gram-positive and gram-negative cocci (including *Peptococcus*, *Peptostretococcus* and *Veillonella* spp). Gram-positive bacilli (including *Clostridium*, *Eubasterium* and *Lactobacillus* spp) (Brogden et al., 1981;Barry et al., 1990; Fass et al., 1990; Munoz, 1996; Yamaguchi et al., 1999).

Susceptibility testing

a) MIC (The minimum inhibitory concentrations) was determined by the standard agar dilution method.

b) Susceptibility disk zone size (Kirby-Bauer disk diffusion method)

Table 1 Susceptibility Ranges of Cefoperazone/Sulbactam

MIC (μg/mL-expressed as cefoperazone concentration		Susceptibility disk zone size (mm)	
Susceptible	≤ 16	Susceptible	≥ 21
Resistant	≥ 64	Intermediate	16-20
		Resistant	≤ 15

The recent study in Thailand has been shown that cefoperazone/sulbactam has good antibacterial activity against *Klebsiella pneumoniae* and *Eschericha coli*. These bacterial are important clinical pathogens because they are resistant to third generation cephalosporins, by having an extended-spectrum β-lactamase (ESBLs). Cefoperazone/sulbactam susceptibility against ESBL-producing and non-producing *K. pneumoniae* is 98% and 100%, respectively. For *E coli*, cefoperazone/sulbactam susceptibility is 96% and 100%, respectively. Cefoperazone/sulbactam was slightly less active against ESBL-producing strain than imipenam and less resistant than amoxicillin/clavulanic acid (Ingviya et al., 2003).

Pharmacokinetics

Cefoperazone

Absorption

Cefoperazone is not appreciably absorbed from the GI tract and must be given parenterally. The pharmacokinetics of cefoperazone after intramuscular and intravenous injection was reported. The peak serum concentration of biological active drug after intramuscular administration of 0.5 g,1 g or 2 g dose of cefoperazone in healthy adults was average 33 , 47-74 and 97-111 μg/mL, respectively. The t_{max} is within 1-2 hours. The AUC in 1 g or 2g dose average 284 and 485 μg-hr/mL, respectively (Brogden, 1981).

Following IV administration over 15 minutes of a single 1g, 2g, 3, or 4g dose of cefoperazone, serum concentrations of the drug average 138-158, 223-253, 331-340 and 506 μg/mL, respectively. The C_{max} of the drug following 1 g, 2g or 3g of IV bolus dose average 140-

200, 250-375 and 518 μg/mL, respectively. The average AUC is 200, 406 and 877 μghr/mL, respectively (Brogden, 1981).

Distribution

Following IM or IV administration, cefoperazone is widely distributed into body tissues and fluids including ascetic fluid, bile, sputum, endometrium, myometrium, tonsils, sinus mucous membrane, middle ear fluid, lungs, pleural fluid, prostatic tissue, adipose tissue, aqueous humor and bone . The apparent volume of distribution of cefoperazone is approximately 10-13 L in adults and 0.5 L/kg in neonates (McEvoy, 2003).

Cefoperazone concentrations in CSF are low following IM or IV administration of usual dosages in patients with uninflamed meaninges. CSF concentration of the drug is generally higher in patients with inflamed meninges. Concentration in bile following IM or IV injection of usual doses of the drug are generally up to 100 times higher than concurrent serum concentrations. Although concentrations of cefoperazone in bile are lower in patients with biliary or hepatocellular diseases, therapeutic biliary concentrations of the drug may be attained. Cefoperazone crosses the placenta and distributes in low concentrations into milk (McEvoy, 2003).

Cefoperazone is irreversibly bound to plasma proteins to the extent about 90-93%. The protein binding of cefoperazone depend on the concentrations of the drug (Brogden, 1981).

Elimination

The mean serum half-life of cefoperazone is 1.6-2.05 hrs. Cefoperazone is excreted principally in bile. Approximately 15-30% of the dose is excreted in urine as unchanged drug within 12-24 hrs, less than 1% of the dose excreted in urine as metabolites. Patient with hepatic impairment, the serum half-life of cefoperazone is prolonged and urinary excretion of the drug is increased. Cefoperazone is excreted in urine by glomerular filtration and a lesser extent is by tubular secretion. The serum half-life, peak serum concentrations and AUC of cefoperazone reported for patients with impaired renal function are not different than those of reported for patients with normal renal function (McEvoy, 2003).

Sulbactam

Absorption

Sulabctam is administerd parenterally as it is poorly absorbed when given orally. Following intravenous administration of sulbactam 0.5 and 1 g to healthy volunteers, peak serum plasma concentrations were approximately 20 and 40 μg/mL, respectively. The area under concentration-time curves (AUC) were 28.9 and 66.4 μg-hr/mL, respectively. After IM injection of sulbactam 0.5 and 1 g to healthy volunteers, the mean peak serum concentrations were 13-19 and 28-34 μg/mL, respectively. AUC after 0.5 g IM injection was 35 μg-hr/mL. Comparison of AUC and urinary recoveries with those obtained after IV dose indicated that the IM injection dose was completely bioavailable relative to IV administration (Foulds et al., 1983).

Distribution

Following IM or IV administration, sulbactam is distributed into body tissues and fluids including intra peritoneal fluid, bile and biliary tissue, sputum, myometrium, prostatic tissue, ovaries, intestinal mucosa, gall bladder tissue and cerebrospinal fluid. The mean volume of distribution of sulbactam in central or plasma compartment is within 7.5-12 L in healthy volunteers (Deborah, Campoli and Brogden, 1987).

Elimination

The mean elimination half-life of sulbactam was approximately 1 hour in healthy subject. Sulbactam is primarily eliminated by excretion into urine, mainly tubular secretion. The renal clearance was approximately 204 mL/min. The total clearance of drug from serum was 266 mL/min. The non renal clearance was 65 mL/min (Foulds et al., 1983).

Sulbactam coadministered with cefoperazone with combination 1:1 or 1:2, either by 1h infusion or IV bolus injection, twice a day for 5 day, did not significantly effect the peak serum concentration, AUC or urinary recoveries of cefoperazone. Furthermore, no major accumulation of cefoperazone or sulbactam was observed during either study. From this study, Foulds et al.(1983) has concluded that coadministration of sulbactam with cefoperazone will not effect pharmacokinetics of each other and not affect the usual dosing regimens for cefoperazone.

Therapeutic use

Cefoperazone/sulbactam is used for the treatment of respiratory tract infection (upper and lower), urinary tract infections (upper and lower), intra-abdominal infections, septicemia, meningitis, skin and soft tissue infections, bone and joint infections, pelvic inflammatory disease, endometritis, gonorrhea and other infections of the genital tract serious infection (Munoz, 1996).

In view of wide spectrum of activity of this combination, it has a potential to be used to treat severe infection caused by susceptible organism in hospitalized patients (Chytra and Herold, 2003). Study conducted in patients with haematological disease experiencing severe concomitant infections has shown that cefoperazone/sulbactam is effective superior to standard therapy (Horiuchi et al., 1989). Lazarus et al. (1996) demonstrated that cefoperazone/sulbactam appeared safe and effective for initial empiric treatment of the febrile, neutropenic bone marrow transplant patient. It has also been shown that cefoperazone-sulbactam (2 g of cefoperazone and 1 g of sulbactam every 8 hours) can be used effectively as initial empiric treatment of febrile granulocytopenic adult cancer patients with acute leukemia and lymphoma (El Zawahry, 1996). Naveen, Santosh and Aparna (2003) reported that cefoperazone/sulbactam has 86.6% susceptible to 60 recently isolated strains of *P. aeruginosa*, causing nosocomial outbreaks in burn ward as colonization of burn wound, this combination has highly potential to treatment of infections in burn wound patients.

Dosage and administration

Cefoperazone/sulbactam is administered parenterally, dosage of cefoperazone/sulbactam is a fix combination 1:1, presented in sterile powder for injection.

Adults dosage: The usual dosage is 2-4 g/day (1-2 g/day cefoperazone activity) given in equally divided doses every 12 hours. The manufacturer states that for the treatment of severe infections, the daily dosage may be increased up to 8 g (4 g cefoperazone activity) given in equally divided doses every 12 hours. The recommended maximum daily dose of sulbactam is 4 g (8 g of combination of cefoperazone and sulbactam). In case of where dose cefoperazone > 4 g/day. It may be necessary to administer additional cefoperazone separately (Drugs of today, 1987).

Pediatric dosage: The manufacturer recommends usual dosage 40-80 mg/kg/day (20-40 mg/kg/day cefoperazone activity) in 2-4 equally divided doses. In serious infection the daily dosage may be increased up to 160 mg/kg/day in 2-4 equally divided doses. The maximum daily dose of sulbactam in pediatric is 80 mg (160 mg of combination of cefoperazone and sulbactam)

In adults with hepatic disease dosage of cefoperazone should be not exceed than 4 g daily. Patients with renal impairment should receive a maximum 1 g/day (maximum dose of cefoperazone/sulbactam is 2 g) (McEvoy, 2003).

Adverse effect

Adverse effects reported with cofeperazone are similar to those reported with other cephalosporins. In addition, hypoprothrombinemia and disulfiram-like reactions also have been reported with cefoperazone (Foster, Raehl and Wilson, 1980).

Dermatologic and Sensitivity Reactions

Hypersensitivity reactions, including rash skin reactions, fever, eosinophilia, urticaria, and pruritus, have been reported in less than 2% of patients receiving cefoperazone. However, it is not clear whether the mechanism of this reaction is immunologic in nature, If a severe hypersensitivity reaction occurs during cefoperazone therapy, the drug should be discontinued and patient given appropriate therapy (e.g., epinephrine, corticosteroids, maintenance of an adequate airway, oxygen) as indicated.

Hematologic Effects

Slight decrease in hemoglobin concentration and hematocrit value has been reported in 5% or less of patients receiving cefeporazone. Reversible neutropenia has also been reported in about 2% of patients receiving prolonged administration of the drug.

Cefeporazone has caused hypoprothrombinemia, with or without bleeding, and vitamin K deficiency during cefoperazone therapy may be due in part form cefoperazone-induced reduction of vitamin K-producing bacteria in the GI tract. Hypoprothrombinemia also has been reported with other β-lactam antibiotics that contain an N-methylthiotetrazole (NMTT) side chain like that contained in cefoperazone (e.g., cefamandole, cefotetan), and it has been suggested that the NMTT side chain may interfere with hepatic synthesis of vitamin K-dependent clotting factors. Hypoprothronbinemia and bleeding during cefoperazone therapy have been reported most

frequently in geriatric or debilitated patients, patients with severe renal failure, or following radical GI surgery and have usually been reversed by administration of vitamin K. Cefoperazone-induced hypoprothrombinemia may be more likely to occur in the presence of hypoalbuminemia. Patients with poor nutritional status, malabsorption states (e.g., cystic fibrosis), or alcohol dependence or those receiving prolonged enteral or parenteral hyperalimentation are at particular risk of cefoperazone-induced vitamin K deficiency.

GI Effects

Adverse GI effects, including diarrhea or loose stools, nausea, and vomiting, have been reported in patients receiving cefoperazone. Diarrhea has occurred in 0.5-7% of patients receiving the drug. Most reported cases of diarrhea were mild or moderate in severity and responded to symptomatic therapy or discontinuance of the drug; however, severe diarrhea and colitis have been reported rarely. *Clostridium difficile* has been isolated from the feces of patients who developed diarrhea while receiving cefoperazone. Mild case of *C.difficile*-associated diarrhea and colitis may respond to discontinuance of cefoperazone alone, but diagnosis and management of moderate to severe cases should include sigmoidoscopy (or other appropriate endoscopic examination), appropriate bacteriologic studies, and treatment with fluid, electrolyte, and protein supplementation as indicated. If *C.difficile*-associated diarrhea and colitis is moderate to severe or is not relieved by discontinuance of cefoperazone, appropriate anti-infective therapy (e.g., oral metronidazole or vancomycin) should be administered. Isolation of the patient may be advisable. Other causes of colitis should be considered.

Hepatic Effects

Mild, transient elevation of serum AST (SGOT), ALT (SGPT), and alkaline phosphatase concentration have been reported. However, these elevations in liver enzymes were not accompanied by overt signs or symptoms of hepatic dysfunction and their clinical importance has not been established. One patient with a history of liver disease developed substantially elevated liver enzymes and clinical signs and symptoms of nonspecific hepatitis during therapy with cefoperazone ; however, the enzymes returned to pretreatment concentrations and the symptomatology resolved following discontinuance of the drug.

Renal Effects

Transient elevations in BUN and serum creatinine concentration have been reported.

Local Effects

Transient pain at the injection site reportedly occurs in patient receiving cefoperazone/sulbactam intramuscularally (Brogden et al., 1981, Munoz et al., 1996 and McEvoy, 2003).

Precautions and Contraindications

Prior to initiation of cefoperazone/sulbactam therapy, careful inquiry should be made concerning previous hypersensitivity reactions to cephalosporins, penicillins, or other drugs. There is clinical and other β- lactam antibiotics, including penicillins and cephamycins. Cefoperazone is contraindicated in patients who are hypersensitive to the drug or other cephalosporins and should be used with caution in patients with a history of hypersensitivity to penicillins. Use of cephalosporins should be avoided in patients who have had an immediate-type (anaphylactic) hypersensitivity reaction to penicillins. Although it has not been definitely proven that allergic reactions to antibiotics are more frequent in a topic individuals, the manufacturer states that cefoperazone should be used with caution in patients with a history of allergy, particularly to drugs.

Prolonged use of cefoperazone/sulbactam may result in overgrowth of nonsusceptible organisms. Careful observation of the patient during cefoperazone therapy is essential. If suprainfection or superinfection occurs, appropriate therapy should be instituted.

Cefoperazone/sulbactam should be used with caution in patients with a history of GI disease, particularly colitis. Because *C. Difficile*-associated diarrhea and colitis has been reported with the use of cephalosporins, it should be considered in the differential diagnosis of patients who develop diarrhea during cefoperazone therapy.

Because hypoprothrombinemia, with or without bleeding, and vitamin K deficiency have occurred rarely in patients receiving cefoperazone, prothrombin time (PT) should be monitored when the drug is used in patients receiving prolonged enteral or parenteral hyperalimentation or in patients with poor nutritional status, malabsorption states (e.g., cystic fibrosis), or alcohol dependence. Vitamin K should be administered if indicated. The manufacturer states that prophylactic vitamin K therapy in patients receiving cefoperazone is probably not warranted. However, some clinicians suggest that prophylactic vitamin K may be indicated when

cefoperazone is used in geriatric or debilitated patients or patients with impaired renal and/or liver function.

Patients should be warned to avoid ingestion of alcohol during and for 72 hours after cefoperazone therapy since disulfiram-like reactions have been reported with the drug.

Because serum concentrations of cefoperazone are higher and more prolonged in patients with hepatic disease and/or biliary obstruction, serum concentrations of cefoperazone should be monitored in these patients when dosage of the drug is greater than 4 g daily. Serum concentrations of cefoperazone should be also be monitored when dosage of the drug is greater than 1-2 g daily in patients with both hepatic and renal impairment.

Pregnancy, Fertility, and Lactation

Reproduction studies in mice, rats, and monkeys using cefoperazone dosage up to 10 times the usual human dosage have not revealed evidence of impaired fertility or harm to fetus. There are no adequate and controlled studies to date using cefoperazone in pregnant women, and the drug should be used during pregnancy only when clearly needed.

Cefoperazone has caused adverse effects on the testes of prepubertal rats. Reduced germinal cell population and vacuolation of Sertoli cell cytoplasm occurred following subcutaneous administration of cefoperazone in a dosage of 1000 mg/kg daily (approximately 16 times the average adult human dosage). The severity of lesions was dosage dependent in the range of 100-1000 mg/kg daily; the low dosage caused a minor reduction in spermatocytes. The effect on spermatocytes has not been observed in adult rats. The cefoperazone-induced lesions were histologically reversible at all but the highest dosage level; however, the studies did not evaluate subsequent development of reproductive function. Adverse testicular effects (e.g., reduced testicular weight, seminiferous tubule degeneration, delayed maturity of germinal epithelium) have occurred in prepubertal rats receiving other β-lactam antibiotics that contain an N-methylthiotetrazole (NMTT) side chain like that contained in cefoperazone (e.g., cefamandole, cefotetan). The relevance of these findings to humans is not known.

Because cefoperazone is distributed into milk, the drug should be used with caution in nursing women.

Drug Interactions

Alcohol

Disulfiram-like reactions characterized by flushing, headache, nausea, vomiting, sweating, and tachycardia have occurred when alcohol was ingested within 72 hours after administration of cefoperazone. Symptoms usually occur within 15-30 minutes after ingestion of alcohol and subside 1-2 hours later. These reactions do not occur if alcohol is ingested prior to the first does of cefoperazone. Disulfiram-like reactions have been reported with other β-lactam antibiotics that contain an N-methythiotetrazole (NMTT) side chain similar to that contained in cefoperazone (e.g., cefamandole, cefotetan) and appear to result from accumulation of acetaldehyde. Ingestion of alcohol should be avoided during and for 72 hours after the administration of cefoperazone (Brogden et al., 1981, Mcevoy, 2003).

Significance of the study: This study will provide the reliable information on the bioavailability and bioequivalence to facilitate drug product selection, in order for a generic drug product to be interchangeable with the innovator's product.

CHAPTER III

MATERIALS AND METHODS

Materials

A. Test Products

Two commercial brands of 500/500 mg cefoperazone/sulbactam intramuscular injections were tested in this study. One was Sulcef®, a test product, manufactured by Siam Bheasach Co., Ltd., and another was Sulperazon®, an innovator's product assigned as reference product, imported by Pfizer International Ltd. Other informations of these products were shown in Appendix A.

B. Reagent

1. Working standard cefoperazone sodium (Supplied by Siam Bhaesach Co., Ltd.) Lot No. 3037CJB1D; Potency: 90.29 %
2. Working standard sulbactam sodium (Supplied by Siam Bhaesach Co., Ltd.) Lot. No.20030603; Potency: 91.30 %
3. Salicylic acid (Supplied by Department of Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn Univerity) Lot. No. 4534683G
4. Working standard ranitidine hydrochloride (Supplied by Siam Bhaesach Co., Ltd.) Lot. No. R8012301; Potency: 89.09 %
5. Acetronitrile HPLC grade (Labscan, Ireland) Lot. No.02050157, 04070512
6. Monobasic potassium phosphate AR. (Merck KGaA, Germany) Lot. No. A585773443
7. Dibasic potassium phosphate AR. (Carlo Erba Reagent, Italy) Lot. No. 321A712801
8. Monobasic sodium phosphate AR. (Carlo Erba Reagent, Italy) Lot. No. 3E187123I
9. Dibasic sodium phosphate AR. (Carlo Erba Reagent, MI) Lot. No. 3C709163E
10. Tribasic sodium phosphste AR. (Fisher Scientific, UK) Lot. No. 0397432
11. Tetrabutylammonium hydroxide solutiom (40% in water) (Fluka, Switzerland) Lot. No. 1066837, 14603052
12. Tetrabutylammonium bromide (Fluka, Switzerland) Lot. No. 359995/1 34297
13. Imidazole (Fluka, Switzerland) Lot. No. 384580/1, 441169/1
14. Phosphoric acid (Carlo Erba Reagent, Italy) Lot. No. 2B322292B

15. Methanol anhydrous (Karl Fischer, Scharlau Chemie S.A., Spain) Lot. No. 63612
16. Karl Fischer reagent (Karl Fischer, Scharlau Chemie S.A., Spain) Lot. No. 43571
17. Endotoxin (*E.coli* 055.B5 endotoxin) (A Combrex Company, Bio Whittaker, USA) Lot. No. 325340
18. LAL pyrogen (A Combrex Company, Bio Whittaker, USA) Lot. No. 4L2964
19. LAL reconstitute buffer (A Combrex Company, Bio Whittaker, USA) Lot. No. 4L1600
20. LAL reagent water (A Combrex Company, Bio Whittaker, USA) Lot. No. 01104985
21. Fluid Thioglycollate medium (Bacto®, Becton Dickinson and Company, USA) Lot. No. 4201240
22. Tryptic soy broth; Soybean-Casein Digest medium (Difco®, Becton Dickinson and Company, USA) Lot. No. 4190440

C. Apparatus

1. High performance liquid chromatography (Series 1100, Agilent Technologies, UK)
2. Chemstation Plus program (Series 1100, Agilent Technologies, UK)
3. Analytical balance (A&D HR 120, A&D company Ltd., Japan)
4. Digital pH meter (Model 350, Beckman Coulter Ltd., USA)
5. Sonicator (Bransonic 221, USA)
6. Vortex mixer (Voltex-Genie2, Scientific Industries, Inc., USA)
7. Centrifuge (Br4i, Jouan, France)
8. Automatic tritator (Meterohm model 785 DMP, Metrohm Siam Ltd., Thailand)
9. Particle counter (HIAC/ROYCO, Model 9703, Pacific Scientific, USA)
10. Ultramicroplate reader (ELX 808 iu with program win KQCL, Bio-Tek instrument. Inc., USA)
11. Tissue culture plate, 96 well, flat bottom (Microtest ®96,Becton Dickinson Labware, USA)
12. Micropipette 100 µL (Gilson Medical Electronics S.A., France)
13. Micropipette 1000 µL (Oxford, Nichiryo, Japan)
14. Water bath

15. Freezer
16. Glassware

Methods

A. *In Vitro* Studies

Cefoperazone for injection and sulbactam powder are described in USP 27 for some characteristics but cefopearazone and sulbactam for injection are not available in any pharmacopoeias. In this study, both commercial brands of cefoperazone/sulbactam were determined following the validated methods as stated in local manufacturer's in-house specification. The tests were:

1. Identification: The identification of cefoperazone and sulbactam was determined by HPLC following the same method as analysis for content of active ingredient.

Acceptance criteria: The retention time of the major peak in the chromatogram of the assay preparation corresponds to that in the chromatogram of the standard preparation, as obtained in assay.

2. Constituted solution: Cefoperazone and sulbactam 1 g for injection from each brand were constituted with sterile water for injection 1 volume as directed in the labeleling supplied by the manufacturer

Acceptance criteria: The solid dissolves completely, leaving no visible residue as undissolved matter and the constituted solution is not significantly less clear than equal volume of sterile water for injection that contained in a similar container.

3. Bacterial endotoxins:

In this test, bacterial endotoxins were measured using turbidimetric method according to USP 27, which is based on the development of turbidity and spectrophotometric technique. It was described as follows.

Standard preparation: Standard curve of control standard endotoxin (CSE) was established using the standard endotoxin solution. Four-endotoxin concentrations (0.01, 0.1, 1.0 and 10 EU/mL) were prepared to generate the standard curve. The test was performed using at least three replicates of each standard endotoxin concentration.

Sample preparation: Ten vials of 500/500 mg cefoperazone/sulbactam IM injection from each brand were sampled and diluted with sterile water for injection (pyrogen free) to made concentration of 0.5 mg/mL of cefoperazone and 0.5 mg/mL of sulbactam.

Procedure: In the beginning of experiment, apparatus was set up and tested for suitable analysis. Next, sample solutions, positive sample control solutions (sample solution spike with standard endotoxin solution, negative control solutions (sterile water for injection) and control standard endotoxin solutions concentration 0.1 EU/mL were prepared. 100 μL of these solutions was spiked into the micro well plate in duplicate. Then, the well plate was incubated in the chamber of the Ultramicroplate reader for 10 minuets. Immediately after incubation, an aliquot 100 μL of *Limulus* Amebocyte Lysate (LAL) reagent was spiked into each micro well plate to start up the reaction. At the end point, standard curve parameter and recovery (%C.V.) of the added endotoxin in the solution was reported.

Acceptance criteria: The cefoperazone/sulbactam 500/500 mg IM injection contains not more than 0.0200 EU/mg according to Manufacturer's in-house specifications if the following conditions are met.

1) Standard curve parameters, namely, the coefficient of determination (r^2) must be more than 0.98; slope should be range from -0.40 to -0.1, Y- interception should be within 2.5 to 3.5 and %CV of each concentration must be less than 10%.
2) The endotoxin recovery, calculated from the concentration found in positive sample control solution after subtracting the endotoxin concentration found in sample solution is within 50 to 200%.
3) The result of negative control solution does not exceed the limit of the blank value required in the description of the LAL Reagent used.

4. Sterility test:

The sterility tests were determined according to membrane filtration method of USP 27. It was described as follows.

Sample preparation: Sample was prepared under laminar air flow, twenty vials of cefoperazone/sulbactam 500/500 mg for injections from each brand were sampled, accurately

weighed 300 mg of powder from each vial and introduced into a 500-mL volumetric flask. 500 mL of Diluting fluid A (0.1% Peptone solution) was added to the flask to dissolve the powder.

Sample testing: Prior to test, the filter apparatus and 0.45 μm, 47 mm cellulose ester membranes with hydrophobic edge were assembled and were sterilized. After sample preparation, the sample solution was transferred to upper chamber of filter unit under strict aseptic conditions. Vacuum was applied to pull solution through filter. When solution has been filtered, turned off vacuum. To remove residual portions of solution, Diluting fluid A was rinsed all surfaces efficiently. After all solution has been filtered, turned off vacuum and carefully removed top half of filter assembly. The membrane was then removed aseptically and was cut into two halves. One half of the membrane was placed in a sterile Soybean-casein digest medium, which was supplemented with cephalosporinase to inactivate the antibiotic in the sample. The other half was placed in a Fluid thioglycollate medium that was supplemented with cephalosporinase. All of these were incubated at prescribed temperatures for the specified time that was recommended in USP 27. After complete incubation, the media with membrane portion were observed for presence of microbial growth.

At the same time of sample testing, the control tests were performed to confirm sterilization condition, aseptic technique and the suitability of the test method. The types of control of sterility testing include the following tests:

4.1 Negative control test: 500 mL of Diluting fluid A was transferred to upper chamber of filter unit under aseptic conditions. Testing using the same procedure as described earlier in sample testing section.

4.2 Positive control test (Growth promotion test) : *Staphylococus aureus* strain ATCC 6538, *Pseudomonas aeruginosa* strain ATCC 9027 and *Bacteroides vulgatus* strain ATCC 8482 were inoculated to Soybean-casein digest medium that was supplement with cephalosporinase. *Bacillus subtilis* strain ATCC 6633, *Candida albicans* strain ATCC 10231 and *Aspergillus niger* strain ATCC 16404 were inoculated to Fluid thioglycollate medium, which was supplemented with cephalosporinase. Immediately after that the Soybean-casein digest medium and Fluid thioglycollate medium containing microorganisms and control (blank medium-no microbial inoculum) were incubated at 32.5± 2.5°C and 22.5± 2.5°C, for Soybean-casein digest

medium and Fluid thioglycollate medium, respectively for 5 days. After complete incubation, the evidence of microbial growth in inoculated media was compared with control (blank).

4.3 Positive sample control test (Bacteriostatic and fungistatic testing) : sample product was prepared as described above. After that, the sample solution was transferred to upper chamber of filter unit under strict aseptic conditions. Vacuum was applied to pull solution through filter. After all solution has been filtered, turned off vacuum and carefully removed top half of filter assembly. The membrane was removed aseptically and was cut into two halves. One half of the membrane was placed in a sterile Soybean-casein digest medium, which was filled with cephalosporinase. The other half was placed in a Fluid thioglycollate medium that was filled with cephalosporinase. Then *Staphylococus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027) and *Bacteroides vulgatus* (ATCC 8482) were added to Soybean-casein digest medium containing membrane sample. *Bacillus subtilis* (ATCC 6633), *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404) were added to Fluid thioglycollate medium containing membrane sample. Soybean-casein digest medium were incubated at 32.5± 2.5°C and Fluid thioglycollate medium were incubated at 22.5± 2.5°C for 5 days. After complete incubation, the presence of microbial growth in inoculated media with product (sample) was compared with culture media (positive control in 4.2)

Acceptance criteria: (a) Microbial growth was found in positive control (that is, media show growth-promoting quality); (b) no microorganisms growth in blank media (that is, media and environment for testing were sterility); (c) positive sample control did not show decreased or no microbial activity compared to control culture media and no visible evidence of microorganism in negative control test was observed (that is, the antimicrobial properties of sample was not inhibit the growth of testing microorganism). In addition, no microbial growth was found in sample product.

5. pH: Two vials of cefoperazone/sulbactam 500/500 mg for injections from each brand were dissolved with 20 mL deionized water. Then the pH of samples was measured with pH meter.

Acceptance criteria: pH is ranging between 4.5-6.5.

6. Water content: The water content was determined according to direct titration method. It was described as follows.

The sample preparation was prepared by accurately weighing drug powder about 100 mg into a 50 mL volumetric flask. Then, 40 mL of anhydrous methanol was added to the flask. This sample was titrated with Karl Fischer reagent by using the Karl Fischer autotitrator.

Acceptance criteria: Water content is not more than 4 %.

7. Particulate matter in injections: The particulate matter was determined according to light-obscuration particle count test of USP 27. It was described as follows.

This test was performed in an environment that does not contribute any significant amount of particulate matter to the injections. Glassware, closures and other equipment are particulate-free. Before proceeding the procedure, the test in an environment and blank count were performed. Sterile water for injection that passes through the filter having porosity of 1.2 μm to remove any particulate matter was used as blank. 50 mL of blank was swirled to suspend particles. Then, particle was determined using Liquid particle counter. If more than 10 particles of 10 μm or greater size, or more than 2 particles of 25 μm or greater size were observed in a 10 mL of blank, the environment is not suitable for particulate analysis.

Test preparation: Ten vials of 500/500 mg cefoperazone/sulbactam injections from each brand were dissolved with 4.0 mL of sterile water for injection (for each vial). All samples were pooled into clean, dry, particulate free beaker and degased by sonicating for 30 seconds. Then sample was determined by liquid particle counter.

Acceptance criteria: The average number of particles in sample does not exceed 6000 particles/vial for size ≥ 10 μm and does not exceed 600 particles/vial for size ≥ 25 μm

8. Content of active ingredient:

Cefoperazone for injection and sulbactam powder are described in USP 27 for some characteristics but cefopearazone and sulbactam for injection is not available in any pharmacopoeias. The amounts of cefoperzone and sulbactam in vials were determined according to method that was developed by the manufacturer. It was described as follows.

8.1 Assay of cefoperazone:

Mobile phase: A mixture of buffer and acetonitrile (85:15)

Triethylamine solution: - Triethylamine 1.4 mL and glacial acetic acid 0.57 mL were mixed in 10 mL of water.

To prepared buffer, 1.2 mL of triethylamine solution and 0.16 mL of glacial acetic acid were mixed in 880 mL of water.

Standard preparation: Cefoperazone standard solution was prepared by accurately weighing cefoperazone WS about 20 mg into a 50 mL volumetric flask, dissolved with water and made up to volume. 5.0 mL of this solution was transferred to a 100-mL volumetric flask and adjusts to volume with deionized water.

Assay preparation: Twenty vials of the injections were randomly selected. Drug powders were carefully and completely removed out of each vial and mixed. They were weighed and calculated for average weight per vial. An accurately weighed portion of drug powder, equivalent to about 20 mg of cefoperazone was transferred to a 50 mL volumetric flask, Then, water was added to dissolve the powder and made up to volume. 5.0 mL of this solution was transferred to a 100 mL volumetric flask and adjusted to volume with deionized water.

Chromatographic system: The high performance liquid chromatography was equipped with a 254-nm detector and 4.6 × 250 mm column that contain packing octadecylsilane (C_{18}), 5 µm. The flow rate was about 1.0 mL/min.

Procedure: 20 µL of the standard and assay preparation were separately injected into HPLC at chromatographic condition as described above. The major peak was measured for the response (peak area).

8.2 Assay of sulbactam :

Mobile phase: a mixture of buffer and methanol (95:5)

Buffer was prepared by dissolving monobasic sodium phosphate 7.8 g in 900 mL of water. Then, the solution was adjusted to pH 4.4 ± 0.05 with phosphoric acid and diluted to 1000 mL with water.

Standard preparation: Sulbactam standard solution was prepared by accurately weighing sulbactam WS about 20 mg into a 50 mL volumetric flask, dissolved with water and made up to volume.

Assay preparation: Twenty vials of the injections were randomly selected. Drug powders were carefully and completely removed out of each vial and mixed. They were weighed and calculated for average weight per vial. An accurately weighed portion of drug

powder, equivalent to about 20 mg of sulbactam was transferred to a 50 mL volumetric flask, water was added to dissolved and made up to volume.

Chromatographic system: The HPLC system was equipped with a 230 nm detector and 4.6 ×250 mm column that contains packing octadecylsilane (C_{18}), 5 μm. The column temperature was maintained at 40º C. The flow rate was about 1.0 mL/min.

Procedure: 20 μL of the standard and assay preparation were separately injected in to HPLC at chromatographic condition as described above. The major peak was measured for the response (peak area).

The % labeled amount (%L.A.) of cefoperazone and sulbactam in each vial was calculate by the formula:

$$\%L.A. = Ru/Rs \times Cs/Cu \times \frac{Avg.wt.(g)/vial}{0.5} \times Percent\ standard$$

In which: Ru = Peak response of cefoperazone or sulbactam obtained from assay preparation.

Rs = Peak response of cefoperazone or sulbactam obtained from standard preparation

Cu = Final concentration of cefoperazone or sulbactam in assay preparation

Cs = Final concentration of cefoperazone or sulbactam in standard preparation

Avg.wt/vial = Average weight (g)/ vial

Acceptance criteria: Cefoperazone/sulbactam 500/500 mg for injection contains an amount of cefoperazone not less than 90.0%, not more than 120.0% of labeled amount and sulbactam not less than 90.0% and not more than 120.0% of labeled amount. For bioequivalence study, % L.A. of active ingredient of test and innovator's product should not be different more than 5%.

9. Uniformity of dosage units:

Ten vials of 500/500 mg cefoperazone/sulbactam intramuscular injections from each brand were sampled and individually assayed for the percent labeled content of cefoperazone and sulbactam in each vial 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.

Acceptance criteria: Content uniformity of the dosage unit lies within the range of 85.0-115.0% of the label claim and the relative standard deviation (R.S.D.) is less than or equal to 6.0 %.

B. *In Vivo* Studies

This single-dose, randomized, 2-way crossover study was conducted at the Faculty of Pharmaceutical Sciences, Chulalongkorn University (Bangkok, Thailand). The protocol was approved by the Independent Ethical Committee of the faculty. The study strictly adhered to ICH-GCP guideline.

1. Test and reference products

Two drug-products were *in vivo* tested in this study. The test product was Sulcef® injection 1g Lot. No. J49CFA02/05 (Siam Bheasach Co., Ltd., Bangkok, Thailand). The reference product was Sulperazon® 1 g Lot. No.439272 (Pfizer Int'l Corp., Bangkok, Thailand).

2. Subjects

The exact number of subjects participated in bioequivalence study can be estimated using an equation of Liu and Chow (1992). However, that approach needs the coefficient of variation of AUC and/or C_{max} value calculated from ANOVA table which is not available for cefoperazone and sulbactam in the literatures. Therefore, the number of subjects used was figured out as recommended by US-FDA which was 22-24 subjects. Twenty two healthy Thai male volunteers, aged form 18 to 45 years participated in this study. Demographic data are presented in Table 20. Prior to study initiation, volunteers were selected after passing a clinical screening procedure including a physical examination and blood/urine biochemical laboratory tests. Inclusion/exclusion criteria 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.

Inclusion criteria:

1) Healthy Thai male volunteers with the age range from 18 to 45 years and body mass index between 18 and 24 kg/m^2

2) Normal physical and laboratory biochemical test

3) No history of gastrointestinal tract diseases, hepatic diseases, renal diseases, allergic diseases or others that affect bioavailability of the drug

4) Non-smokers and without history of alcohol or drug abuse

5) No history of allergic reaction to penicillin or cephalosporin

Exclusion criteria:

1) Refuse to finish the study

2) Allergic or having adverse drug reaction to cefoperazone and sulbactam

All subjects were asked to avoid taking other medications, smoking, alcoholic and caffeinated beverages for 1 week prior to receiving study medication and throughout the study period. Before each subject's participation in the trial, informed consent was obtained from the subjects after explaining purpose of the study, the risk/benefit and possible side effect of medication.

3. Study design

The study was conducted in a randomized two-way crossover design. A 500/500 mg cefoperazone/sulbactam intramuscular injection either test or reference product was administered to volunteer according to a single dose, two-treatment, two- period, two-sequence with a washout period of 1 week between each administration as shown in Table 2.

4. Drug administration and sample collection

Subjects were given single dose IM injection with one vial of 500/500 mg cefoperazone /sulbactam (reference or test) after reconstituting with 3.4 mL of sterile water for injection. Subjects were not permitted to lie down or sleep. Approximately 7 mL of blood samples were withdrawn from a forearm vein of each subject using disposable syringe at the following time: predose, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 6, 8 and 10 hours after dosing. All blood samples were collected in heparinized tubes, chilled at 0° C. The blood samples were centrifuged at 3000 rpm for 15 minutes. Plasma were removed using plastic pipette, divided into two aliquots and placed in glass tubes. They were immediately frozen at –20°C until analysis. After a washout period of 7 days, the same manners were repeated to complete the crossover design.

Table 2 Randomization Schedule

Sequence	Subjects no.	Period		
		1	washout	2
1	1, 4, 7, 9, 10, 13, 15, 16, 17, 19, 21	Reference product		Test product
2	2, 3, 5, 6, 8, 11, 12, 14, 18, 20, 22	Test product		Reference product

5. Subject monitoring

Subjects were continuously monitored and periodically questioned for any adverse events. Vital signs, such as blood pressure and heart rate, were periodically recorded to ensure well being of subjects. If adverse drug reactions occurred, subjects would be diagnosed and treated by doctor. The time of onset, duration of adverse event and subsequent treatments would be recorded in case record forms.

6. Analysis of plasma samples

6.1 HPLC assay of cefoperazone in plasma

The plasma concentrations of cefopearazone were determined by the method modified from that of Reitberg et al. (1988)

In 1988, Reitberg et al. reported a HPLC method for the determination of cefoperazone in human serum. The separation of the drug was rapid, needing only 11 min. The validated method was found to be specific, linear and reproducible. However, their method is not suitable for the large number of samples in bioequivalence study because it involves many extraction procedures and lack of sensitivity to determine a low concentration of cefoperazone in plasma. Therefore, in this study, cefoperazone was extracted from human plasma by protein precipitation using acetonitrile followed by centrifugation as in previous studies (Haginaka et al., 1985; Follette et al., 1995). The sample preparation and analysis was described as follows.

6.1.1 Sample preparation

An aliquot (1 mL) of plasma sample was transferred to a glass test tube, 100 μL of internal standard (0.2 mg/mL of salicylic acid in phosphate buffer) and 1000 μL of acetonitrile was added for protein precipitation. The mixture was shaken for 30 seconds in a

vortex mixer and centrifuged at 14,000 rpm 20°C for 45 minutes. Supernatant was then separated and 25 μL aliquot of the solution was injected into the HPLC.

6.1.2 Chromatographic systems

Apparatus : Beckman HPLC pump equipped with a degasser, an autoinjector, a column oven, a spectro UV detector and computerized integrator.

Column : The precolumn was Alltima C_{18} guard column, 5 μm, 7.5x4.6 mm (Alltech Associates, Inc., USA). The analytical column was a μ-Bondapak® C_{18}, stainless steel column, 300×3.9 mm (i.d.), 125 A 10 μm of dimethyloctadecylsilyl bond amorphouse silica. (Water Associates Pty-Ltd., Milford, MA, USA)

UV detector : 215 nm

Mobile phase : 0.02 M tetrabutylammonium hydroxide and 0.01 M tribasic sodium phosphate adjust pH to 3.5 and mix with acetonitrile (25:75, vol/vol).

Flow rate : 1.5 mL/min.

Temperature : Ambient (25°C)

Retention time : Cefoperazone was approximately 12 min

Salicylic acid was approximately 18 min

6.1.3 Preparation of standard solutions

Stock standard solutions were prepared. Cefoperazone W.S. was accurately weighed about 0.03320 g and dissolved in 10 mL of deionized water to give a nominal concentration of 3.0 mg/mL cefoperazone. Dilutions of this solution were made with deionized water to give working solutions of 30, 60, 120, 180, 240,300, 360 and 480 μg/mL, respectively.

Salicylic acid (internal standard) solution was prepared by accurately weighing 0.0200 g of salicylic acid and dissolving in 100 mL of potassium phosphate buffer to give a nominal concentration 0.2 mg/mL. Potassium phosphate buffer was prepared by dissolving 0.2 g of dipotassium hydrogen phosphate and 0.8 g of potassium dihydrogen phosphate with 100 mL of deionized water. The stock solution and working solutions for cefoperazone and salicylic acid were prepared on a daily basis.

6.1.4 Preparation of standard calibration curve

An aliquot (100 μL) of working standard solutions of cefoperazone was spiked to blank plasma to produce a set of calibration standards of 3, 6, 12, 18, 24, 30, 36 and 48 μg/mL, respectively. All these standard solutions were analyzed following the same procedure as described earlier. The peak area ratios of cefoperazone to that of internal standard were plotted against the known concentration of cefoperazone and the calibration curves were fitted to a straight line by linear regression analysis. Calibration standards were prepared on a daily basis.

6.2 HPLC assay of sulbactam in plasma

The plasma concentrations of sulbactam were determined by the method modified from those of Haginaka, Wakai and Uno (1985), Haginaka et al. (1985) and Bawdon and Madsen (1988).

Sulbactam may be assayed in plasma by high-pressure liquid chromatography by using a simple extraction procedure and detected with UV absorption at 225 nm (Sulochana et al., 1995) and 230 nm (Fredj et al., 1986). However, measurement at trace levels in human plasma by HPLC with direct UV detection was not practical due to chromatographic interference by endogenous substances in biological fluid and lack of sensitivity. Early studies indicated that sulbactam reacted with imidazole reagent to form an imidazole derivative. The product having UV absorption maxima at 313 nm (Bawdon and Madsen, 1988) and 320 nm (Haginaka et al., 1985) was separated using reversed-phase HPLC from the regular components of human plasma with an ion-pair buffer (Haginaka, Wakai and Uno, 1985; Haginaka et al., 1985; Bawdon and Madsen, 1988). These methods resulted in a highly sensitive assay and free of interfering products. In this study, the determination of sulbactam in human plasma using pre-column derivatization, sulbactam was reacted with immidazole reagent pH 9.0 at 60ºC for 50 min followed by protein precipitated with acetonitrile. The sample preparation and analysis was described as follows.

6.2.1 Sample preparation

A 2 M imidazole reagent was prepared by dissolving 1.3616 g of imidazole in water and adjusted the volume to 10 mL with deionized water. The derivatization procedure for sulbactam in plasma consisted of adding 200 μL of imidazole reagent to 0.5 mL of each plasma sample and the mixture was shaken on a vortex-mixer for 30 seconds. The mixture

was kept at 60°C for 50 minutes to allow the derivatization process to be completed and incubated at room temperature for 15 minutes. The samples were mixed with 700 µL of acetonitrile and 100 µL of internal standard (0.2 mg/mL of ranitidine). The precipitated protein was removed by centrifugation for 30 minutes at 14,000 rpm. 20 µL of supernatant was injected into HPLC.

6.2.2 Chromatographic systems

Apparatus : Beckman HPLC pump equipped with a degasser, an autoinjector, a column oven, a spectro UV detector and computerized integrator.

Column : A precolumn was Alltima C_{18} guard column, 5 µm, 7.5x4.6 mm (Alltech Associates, Inc., USA). The analytical column was a Hypersil® C_{18}, stainless steel column, 150× 4.6 mm, 5 µm ,sphere 120 A of dimethyloctadecylsilyl bond silica (Thermo Electron Corporation, England)

UV detector : 320 nm

Mobile phase : 5 mM tetrabutylammoniumbromide +1mM disodium hydrogen phosphate + 1 mM sodium dihydrogen phosphate solution: acetonitrile (75:25, vol/vol)

Flow rate : 1 mL/min.

Temperature : 50°C

Retention time : Ranitidine was approximately 3 min, Sulbactam-imidazole reaction product was approximately 7 min.

6.2.3 Preparation of standard solutions

Sulbactam stock standard solutions were prepared. Approximately 0.02191 g of sulbactam W.S. was accurately weighed and dissolved in 10 mL of deionized water to give a nominal concentration of 3 mg/mL sulbactam. Dilutions of this solution were made with deionized water to give working solutions of 10, 60, 90, 120, 240, 300 and 400 µg/mL, respectively. The ranitidine (internal standard) solution was prepared by accurately weighing 0.0200 g of ranitidine W.S. and dissolving in 10 mL of deionized water to give a nominal concentration 2 mg/mL. A 0.2 mg/mL working solution was prepared by taking 1000 µL of 2 mg/mL ranitidine solution and making up to 10 mL with deionized water. The stock solution and working solutions for sulbactam and ranitidine were prepared on a daily basis.

6.2.4 Preparation of standard calibration curve

50 μL of working standard solutions of sulbactam were spiked to blank plasma to produce a set of calibration standards of 1, 6, 9, 12, 24,30 and 40 μg/mL, respectively. The series of standard solutions were reacted with imidazole reagent and analyzed following the same procedure as described earlier. The peak area ratios of sulbactam-imidazole reaction product to that of internal standard were plotted against the known concentration of sulbactam and the calibration curves were fitted to a straight line by linear regression analysis. Calibration standards were prepared on a daily basis.

6.3 Method validation

The methods were validated following the Guidance for Industry: Bioanalytical Method Validation of Center for Drug evaluation and Research (CDER) and Center for Veterinary Medicine (CVM), U.S. Department of Health and Human Services, Food and Drug Administration, 2001. The details were described as follows:

1) Selectivity

Cefoperazone: Control blank human plasma from six different sources were analyzed using the same procedure of cefoperazone as described earlier. In all cases chromatograms were visually examined for potential interfering peaks. Sulbactam coadministered with cefoperazone were tested for interference to ensure that there is no interference to the peaks of cefoperazone and internal standard (salicylic acid).

Sulbactam : Control blank human plasma from six different sources were analyzed using the same procedure of sulbactam as described earlier. In all cases chromatograms were visually examined for potential interfering peaks. Cefoperazone coadministered with sulbactam were tested for interference to ensure that there is no interference to the peaks of sulbactam and internal standard (ranitidine).

2) Lower limit of quantification (LLOQ)

Five determinations of lowest concentration of standard cefoperazone in plasma and those of sulbactam were analyzed. The LLOQ were established by examination of the accuracy and precision data. Analyte peak of these concentrations should be identifiable, discrete and reproducible with an accuracy not exceeding ± 20% , together with a precision not exceeding 20%.

3) Linearity and standard calibration curve

For cefoperazone, eight concentrations of standard solution of cefoperazone (3, 6, 12, 18, 24, 30, 36 and 48 μg/mL,) in plasma were analyzed. For those of sulbactam seven concentrations of standard solution of sulbactam (1, 6, 9, 12, 24, 30, and 40 μg/mL) in plasma were analyzed. The peak area ratios of cefoperazone to that of internal standard were plotted against the corresponding concentration of the analyte and the calibration curves were constructed by linear regression analysis. The coefficient of determination (r^2) should be more than 0.99. The 20% deviation of LLOQ from nominal concentration and 15% deviation of standards other than LLOQ from nominal concentration should be met.

4) Accuracy

The accuracy of the method was determined by assessing the agreement between the estimated and nominal concentrations of three quality control samples (low (3×LLOQ), medium, high). The estimated concentration was the mean of the concentrations obtained from five replicates of three concentrations of quality control samples (QC samples).

For cefoperazone, three concentrations of QC samples were 9.0, 21.0 and 33.0 μg/mL for low, medium and high concentrations, respectively. For those of sulbactam, the corresponding concentrations were 3.0, 18.0 and 36.0 μg/mL. These QC samples were analyzed in five replicates for the drug content. Accuracy in term of percent recovery was done by computing the ratio of estimated concentration obtained from standard calibration of cefoperazone or sulbactam in plasma to known concentration of each standard cefoperazone or sulbactam in plasma multiplied by one hundred. The mean value should be within ±15% of actual value.

5) Precision

The precision of the method was determined by assessing the agreement between replicate measurements of three QC samples (low(3×LLOQ), medium, high).

5.1) Within-run precision

For cefoperazone, three concentrations of QC samples were 9.0, 21.0 and 33.0 μg/mL for low, medium and high concentrations, respectively. For those of sulbactam, the corresponding concentrations were 3.0, 18.0 and 36.0 μg/mL. These QC samples were analyzed in five replicates on the same day. The percent coefficient of variation (C.V.) of

estimated concentration was determined as each concentration level. The precision determined at each level should not exceed 15% of the C.V.

5.2) Between-run precision

For cefoperazone, three concentrations of QC samples were 9.0, 21.0 and 33.0 μg/mL for low, medium and high concentrations, respectively. For those of sulbactam, the corresponding concentrations were 3.0, 18.0 and 36.0 μg/mL. These QC samples were analyzed in five replicates on five different days. The percent coefficient of variation (C.V.) of estimated concentration was determined as each concentration level. The precision determined at each level should not exceed 15% of the C.V.

6) Extraction recovery

Cefoperazone: Five determinations of three concentration of standard cefoperazone (9.0, 21.0 and 33.0 μg/mL) in plasma and in water were analyzed. For sulbactam similar to that of cefoperazone, five determinations of three concentration of standard sulbactam (3.0, 18.0 and 36.0 μg/mL) in plasma and in water were analyzed. Percentage recovery was calculated by comparing the peak area of extracted plasma samples at each concentration with unextracted standards that represent 100% recovery. The percent recovery was determined as follows

$$\% \text{ Recovery} = \frac{\text{Peak area of analyte extracted from plasma}}{\text{Peak area of analyte unextracted in water}} \times 100$$

Recovery of analyte need not be 100%, but the extent of recovery of an analyte and of the internal standard should be consistent, precise and reproducible.

Internal standard: Five determinations of one concentration of salicylic acid and ranitidine in plasma and in water were analyzed. Percentage recovery was calculated by comparing the peak area of extracted plasma samples at each concentration with unextracted standards that represent 100% recovery.

7) Stability studies

7.1) Short-term room temperature stability

For cefoperazone, two concentrations of QC samples were 9.0 and 33.0 μg/mL for low and high concentrations, respectively. For those of sulbactam, the corresponding concentrations were 3.0 and 36.0 μg/mL. Three aliquots of these QC samples were

analyzed and stored at –20°C for 24 hours. Samples were thawed at ambient temperature. After being kept at this temperature at 4, 8 and 12 hours, samples were extracted and analyzed. The %deviation of the mean estimated concentration from the zero time should be within ±15%.

7.2) Long-term stability

For cefoperazone, two concentrations of QC samples were 9.0 and 33.0 μg/mL for low and high concentrations, respectively. For those of sulbactam, the corresponding concentrations were 3.0 and 36.0 μg/mL respectively. Three aliquots of these QC samples were analyzed and stored at –20°C and they were analyzed periodically over a period of 2 months. The %deviation of the mean estimated concentration from the zero time should be within ±15%.

7.3) Freeze-thaw stability

For cefoperazone, two concentrations of QC samples were 9.0 and 33.0 μg/mL for low and high concentrations, respectively. For those of sulbactam, the corresponding concentrations were 3.0 and 36.0 μg/mL, respectively. Three aliquots of these QC samples were analyzed and stored at –20°C for 24 hours and thawed unassisted at ambient temperature (25°C). This procedure was one freeze-thaw cycle. When completely one freeze and thaw cycle, the samples were refrozen for 24 hours under the same conditions.. The freeze-thaw cycle was repeated two more times then analyzed on the third cycle. The concentrations of freeze-thaw sample were compared with those of freshly prepared sample. The % deviation of the mean estimated concentration from the zero time should be within ±15%.

7.4) Post-preparative stability

For cefoperazone, two concentrations of QC samples were 9.0 and 33.0 μg/mL for low and high concentrations, respectively. For those of sulbactam, the corresponding concentrations were 3.0 and 36.0 μg/mL, respectively. Three aliquots of these QC samples in the processed sample extracts were analyzed after freshly prepared, and after kept in the autosampler at 4, 8, 12, and 16 hours. The % deviation of the mean estimated concentration from the zero time should be within ±15%.

7. Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis was employed to analyze plasma drug concentration–time data. The maximum plasma drug concentration (C_{max}) and the corresponding peak times (t_{max}) were obtained directly from experimental observations.

The AUC_{0-t} was calculated using linear trapezoidal rule from zero to the last measurable plasma drug concentration. $AUC_{0-\infty}$ was calculated as $AUC_{0-\infty} = AUC_{0-t} + C_t/k$, where C_t is the last concentration evaluated in plasma greater than LLOQ and k is the elimination rate constant at terminal phase calculated from slope of the terminal plasma concentration-time curve by linear regression of at least the last three data points. Elimination half-life ($t_{1/2}$) of the terminal log linear phase was calculated utilizing the equation 0.693/k. The mean residence time (MRT) was also calculated as AUMC/AUC; where AUMC is the area under the moment curve.

8. Statistical analysis

The bioequivalence of two brands of 500/500 mg cefoperazone/sulbactam IM injections was evaluated using the corresponding pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}. This was evaluated using the two one-sided tests procedure for logarithmic transformed data. Analysis of variance (ANOVA) for two way crossover design at α=0.05 was performed using logarithmically transformed data of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} to assess formulation, sequence, period and subject effects and obtain the residual error which was used to evaluate 90% confidence intervals. The difference of t_{max} values from both formulations was calculated.

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

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

where; $\overline{X}_T$ and $\overline{X}_R$ = Mean log AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} values of test and innovator's product respectively.

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

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

$$\%\ Lower\ limit = [\ antilog\ (\overline{X}_T - \overline{X}_R) - (t_{0.1,df} \times S.E.)\ ] \times 100$$

$$\%\ Upper\ limit = [\ antilog\ (\overline{X}_T - \overline{X}_R) + (t_{0.1,df} \times S.E.)\ ] \times 100$$

The test product is considered to be bioequivalent to the innovator's product, when the 90% confidence interval of individual parameters of test product relative to that of innovator's product was within 80-125% for cefoperazone and sulbactam.

9. Comparison of pharmacokinetic parameters of cefoperazone and sulbactam

Pharmacokinetic parameters of cefoperazone and sulbactam established in this study were compared to those previously published reports. Information found will be discussed.

CHAPTER IV

RESULTS AND DISCUSSION

A. *In Vitro* Studies

All two commercial brands of 500/500 mg cefoperazone/sulbactam intramuscular injection were determined for pharmaceutical equivalence following the tests as stated in manufacturer's in-house specification. Results were:

1. Identification

Chromatograms of analysis for cefoperazone and sulbactam are shown in Figures 3 and 4, respectively. As seen, the retention time of the major peak (cefoperazone or sulbactam) in the chromatogram of the assay preparation corresponds to that in the chromatogram of the standard preparation, as obtained in assay indicating both cefoperazone and sulbactam were found in the injections.

a

b

c

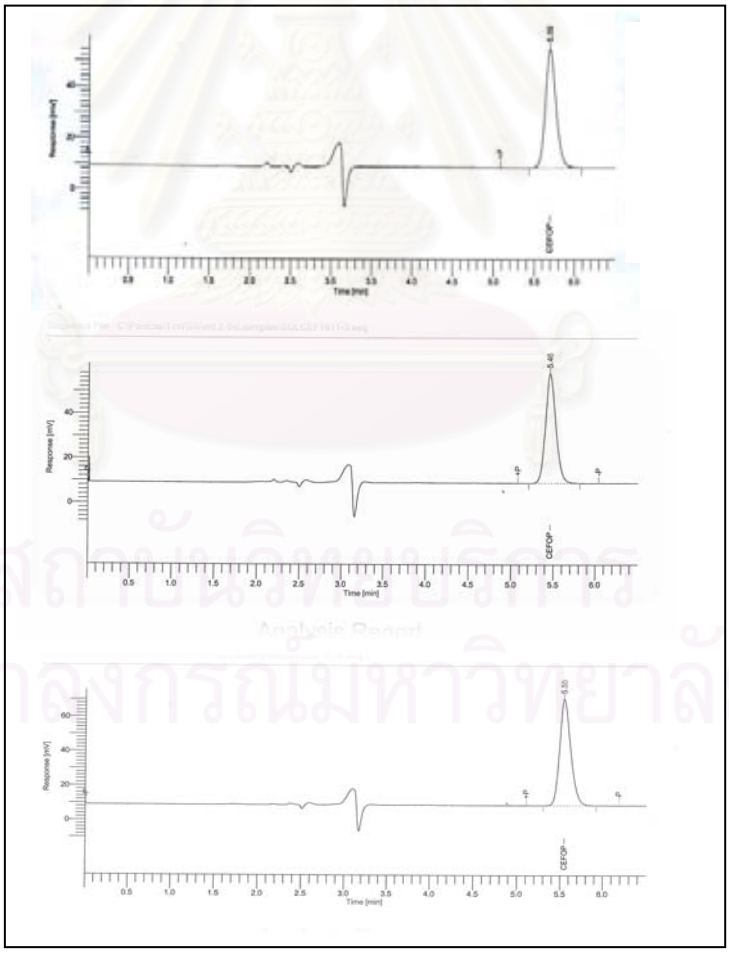


Figure 3 Chromatogram of analysis of cefoperazone; standard preparation (a), assay preparation of test product (b), assay preparation of innovator's product (c)

a

b

c

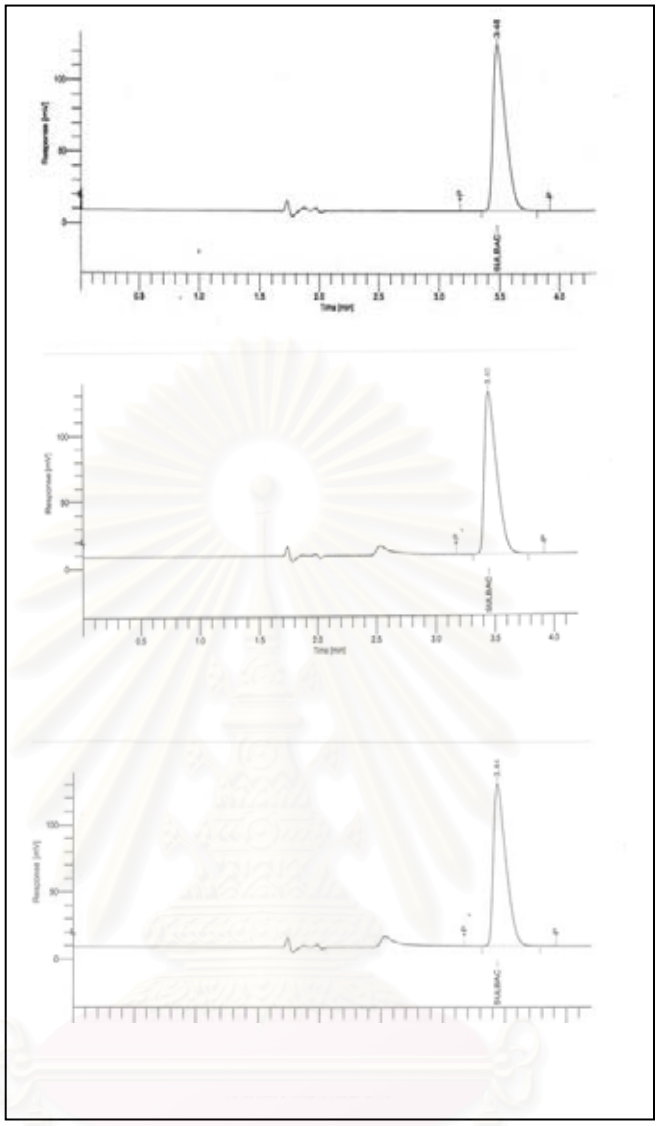


Figure 4 Chromatogram of analysis of sulbactam; standard preparation (a), assay preparation of test product (b), assay preparation of innovator's product (c)

2. Constituted solution

When powder dissolved completely, no visible residue as undissolved matter was observed and the constituted solution for both products was as clear as sterile water for injection that contained in a similar container.

3. Bacterial endotoxin test

From the data as shown in Table 3, standard curve parameter; coefficient of determination (r^2) was greater than 0.98, slope ranged from -0.40 to -0.1, Y- interception was within 2.5-3.5 and %C.V. of each concentration was less than 10%. The endotoxin recovery of

test and innovator's product was between 50 to 200%. These could be concluded that both products contain not more than 0.0200 EU/mg and they met the requirement of specification.

Table 3 Bacterial Endotoxin Test

Data	Value	Specification	Status
Standard concentration (EU/mL)	% C.V.	% C.V.	
0.01	3.43%	< 10%	Passed
0.1	0.75%	< 10%	Passed
1.0	3.14%	< 10%	Passed
10.0	5.09%	< 10%	Passed
Standard parameters			
Coefficient of determination	0.997	0.980 to 1.000	Passed
Slope	-0.203	-0.400 to -0.100	Passed
Y intercept	2.923	2.500 to 3.500	Passed
Positive sample control	% Recovery	% Recovery	
Test product	118.00%	50-200 %	Passed
Innovator's product	78.80%	50-200 %	Passed
Sample	EU/mg	EU/mg	
Test product	< 0.0100	< 0.0200	Passed
Innovator's product	< 0.0100	< 0.0200	Passed

4. Sterility test

In this study, it was found that there was no microbial growth in positive control. There was also no microorganisms growth in blank media. Positive sample control showed marked increase of microbial growth compared to control culture media and no visible evidence of microorganism in negative control test was observed. In addition, no microbial growth was found in both sample products. These referred that sterility test method (procedure, media, equipment, environment and personnel technique) was suitable for sterility testing. Both products are sterile and safe to use in volunteer whom participated in this study.

5. pH

pH of test product (5.29) was slightly higher than that of innovator's product (4.97). This is because the sources of raw materials of the two products are different and/or difference in formulation. However, both products complied the acceptance criteria of the specification.

6. Water content

Both test and innovator's product had water content of 2.06% and 2.51%, respectively. They passed the quality standard of in-house specification.

7. Particulate matter in injections

The environment was suitable for particulate analysis and both products met the requirements of the test as observed in Table 4.

Table 4 Particulate Matter Count of The Environment, Test and Innovator's Products

Assay no.	Particles per container*					
	Sterile water for injection		Test product		Innovator's product	
	≥ 10 μm	≥ 25 μm	≥ 10 μm	≥ 25 μm	≥ 10 μm	≥ 25 μm
1	1.00	0.00	191.20	8.00	8.00	0.80
2	0.00	0.00	189.60	7.20	10.40	0.00
3	0.00	0.00	183.20	2.40	20.80	0.80
Mean	0.33	0.00	188.00	5.87	13.07	0.53

* Calculated from sample volume: 5.00 mL, Total volume: 40.00 mL

8. Content of active ingredient

Both products were assayed for content of active ingredient and found that cefoperazone and sulbactam of both brands were within the limits of 90.0-120.0% as specified in the manufacturer's in-house specification as shown in Table 5. In addition, the differences in percent content of active ingredients of both products were less than 5%. This referred that the molar dose of both products are similar to each other and they could be included in bioequivalence study.

Table 5 Content of Active Ingredient of 500/500 mg Cefoperazone/Sulbactam Intramuscular Injections of Test and Innovator's Products

Assay no.	% Labeled amount			
	Cefoperazone		Sulbactam	
	Test product	Innovator's product	Test product	Innovator's product
1	116.51	110.91	106.24	109.36
2	115.10	112.29	104.87	109.24
Mean	115.81	111.60	105.56	109.30
Difference*	4.20		3.74	

Difference* = different in mean % L.A. of active ingredient (cefoperazone/sulbactam) of test and innovator's product

9. Uniformity of dosage units

The two commercial brands of 500/500 mg cefoperazone/sulbactam IM injections were tested for uniformity of dosage units. Results were presented in Table 6. All of them met the requirement of the manufacturer's in-house specification within the range of 85.0-115.0% label claim and R.S.D (relative standard deviation) was less than 6.0%.

Table 6 Content Uniformity of 500/500 mg Cefoperazone/Sulbactam Intramuscular Injections of Test and Innovator's Products

Assay no.	% Labeled amount			
	Test product		Innovator's product	
	Cefoperazone	Sulbactam	Cefoperazone	Sulbactam
1	108.73	103.64	109.98	109.24
2	108.57	109.14	113.44	109.35
3	114.38	111.74	110.16	108.20
4	114.52	108.69	112.54	110.35
5	108.75	110.95	109.80	107.67
6	112.06	107.98	110.70	107.49
7	105.82	104.74	111.55	108.78
8	107.29	107.90	112.13	109.99
9	109.30	106.98	112.51	108.90
10	105.46	107.70	111.18	108.61
Mean	109.49	107.95	111.40	108.86
S.D.	3.20	2.47	1.25	0.93
% R.S.D.	2.92	2.29	1.12	0.85

Table 7 Results of *In Vitro* Analysis of 500/500 mg Cefoperazone/Sulbactam Intramuscular Injections of Test and Innovator's Products

Category	Acceptance criteria	Result	Conclusion
Bacterial endotoxin	< 0.0200 EU/mg	Test : < 0.0100 EU/mg	Passed
		Innovator's: < 0.0100 EU/mg	Passed
Sterility	Sample showed no microbial growth and the control test was suitable for analysis.	Test: No microbial growth	Passed
		Innovator's: No microbial growth	Passed
pH	4.5-6.5	Test : 5.29	Passed
		Innovator's: 4.97	Passed
Water content	NMT* 4.0%	Test : 2.06%	Passed
		Innovator's: 2.51%	Passed
Particulate matter			
≥10 μm	NMT* 6000 particles/vial	Test : 188 particles/vial	Passed
		Innovator's: 13 particles/vial	Passed
≥ 25 μm	NMT* 600 particles/vial	Test : 6 particles/vial	Passed
		Innovator's: 1 particles/vial	Passed
Assay (% L.A.)			
Cefoperazone	90.00-120.00 %L.A.	Test : %L.A. 115.81	Passed
		Innovator's: %L.A. 111.60	Passed
Sulbactam	90.00-120.00 %L.A.	Test : %L.A. 105.56	Passed
		Innovator's: %L.A. 109.30	Passed
Content uniformity			
Cefoperazone	85.00-115.00%L.A. ; %R.S.D. ≤ 6	Test : %L.A. 105.46-114.52; R.S.D % 2.92	Passed
		Innovator's: %L.A. 109.80-113.44 ; R.S.D. 1.12%	Passed
Sulbactam	85.00-115.00%L.A. ; %R.S.D. ≤ 6	Test : %L.A. 103.64-111.74; R.S.D 2.29%	Passed
		Innovator's: %L.A. 107.49-110.35 ; R.S.D. 0.85%	Passed

NMT* = Not more than

10. *In Vitro* Evaluation

Both commercial brands of 500/500 mg cefoperazone and sulbactam for injection were determined following the validated test methods as stated in manufacturer's in-house specification. All *in vitro* studies of both products revealed that they contain the same molar dose of active ingredients in the same dosage form and completely complied the specification requirements (i.e., strength, quality, purity, and identity) as displayed in Table 7. These could be concluded that both products were pharmaceutical equivalence and they were safe to used in subjects whom participated in this study.

B. *In Vivo* Studies

1. Development of the HPLC method

1.1 Cefoperazone

The determination of cefoperazone in human plasma sample has been developed. Cefoperazone and salicylic acid (internal standard) were extracted from plasma by protein precipitation using acetonitrile followed by centrifugation. Aliquots of the supernatant were analysed by reversed-phase HPLC. The analysis was performed on a μ-Bondapak® C_{18} column, using a mixture of acetonitrile, tetrabutyl ammonium hydroxide and phosphate buffer (pH 3.5) as mobile phase with UV detection at 215 nm. The total assay can be performed in about an hour. The assay was rapid, simple and applicable to bioequivalence study.

1.2 Sulbactam

The reaction times and temperatures were chosen as optimum studies in which times were varied from 10-90 min and temperatures were varied from 40-60ºC. The reaction and temperature were selected in terms of high yield of sulbactam-imidazole product. It was found that sulbactam reacting with imidazole at 60ºC for 50 min yielded a product having an ultraviolet absorption maximum at 320 nm. The product was separated using reverse-phased HPLC from regular components of plasma with an ion-paired buffer at 50ºC. This study agreed with a previous one (Haginaka et al., 1985) in that *cis* and *trans* isomers of sulbactam-imidazole product co eluted on reversed-phase HPLC column. The broad peak with shoulder was observed when sulbactam derivative was analysis using an ion-paired mobile phase at room temperature. The isomers co eluted as a single peak by elevating the column temperature to 50 ºC.

2. Assay validation of cefoperazone and sulbactam in plasma

2.1 Selectivity/Specificity

Figure 5 (a, b, c, and d) represents typical chromatograms of blank plasma , blank plasma spiked with internal standard (salicylic acid), blank plasma spiked with cefoperazone together with internal standard and blank plasma spiked with cefoperazone and sulbactam together with internal standard, respectively. The mean retention times of cefoperazone and salicylic acid were 12 and 18 min, respectively. Peaks of drug and internal standard were well separated from other interfering peaks from six different blank plasma samples (pre-dose plasma of volunteers). In addition, there was no effect of sulbactam to those.

Figure 6 (a, b, c, and d) represents the chromatograms of blank plasma, the internal standard (ranitidine) and immidazole reagent in blank plasma, the sulbactam-imidazole reaction product together with internal standard in plasma, and the sulbactam-imidazole reaction product and cefoperazone together with internal standard in plasma, respectively. The mean retention times of ranitidine and sulbactam-imidazole product were 3 and 7 min, respectively. There were no interference peaks due to presence of plasma protein and/or endogenous substances from six different blank plasma samples. Ranitidine and sulbactam-imidazole product were well separated. Also, there was no any interfering due to the presence of cefoperazone.

a

b

c

d

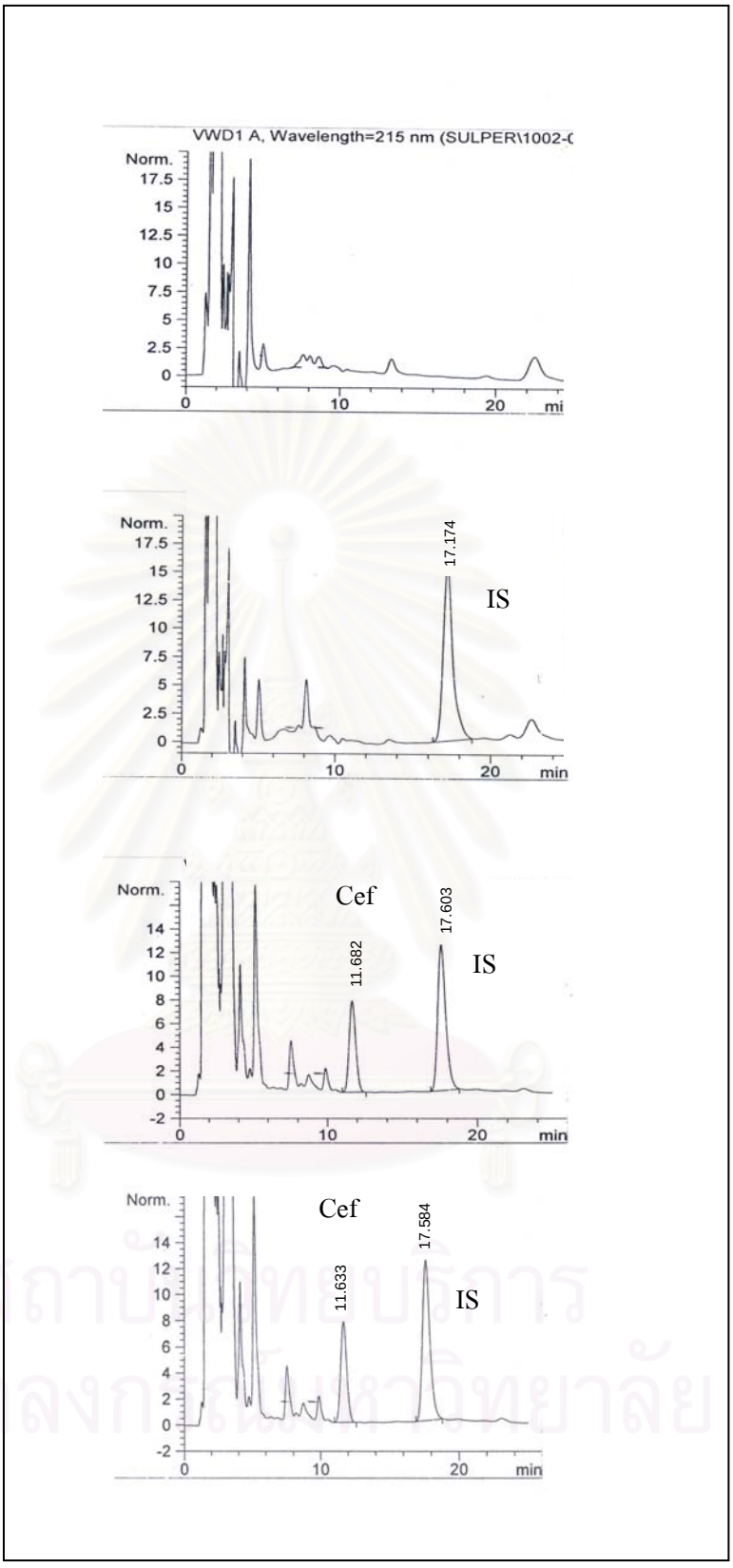


Figure 5 Chromatogram of blank plasma (a), blank plasma spiked with internal standard (salicylic acid) (b), blank plasma spiked with cefoperazone together with the internal standard (c),and blank plasma spiked with cefoperazone and sulbactam together with internal standard (d)

a

b

c

d

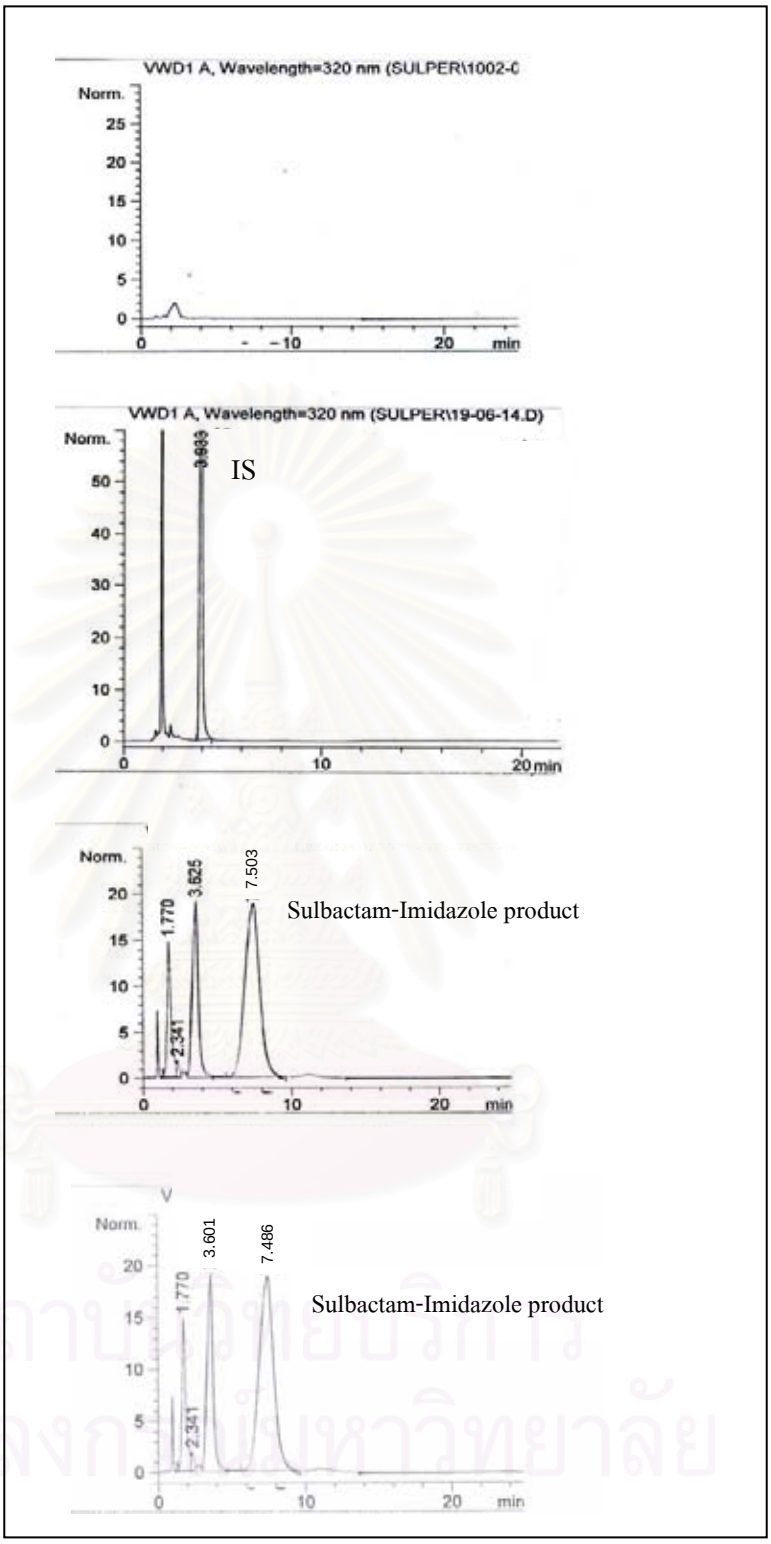


Figure 6 Chromatogram of blank plasma (a), the internal standard (ranitidine) and immidazole reagent in blank plasma (b), the sulbactam-imidazole reaction product together with internal standard in plasma (c), and the sulbactam-imidazole reaction product and cefoperazone together with internal standard in plasma (d)

2.2 The lower limit of quantification (LLOQ)

The lower limit of quantification of the analysis method of cefoperazone was found to be 3 μg/mL, and that of sulbactam was 1 μg/mL. The accuracy of cefoperazone at 3 μg/mL was 107.91% with a precision of 7.95%. The accuracy of sulbactam at 1 μg/mL was 105.11% with a precision of 8.80%. These findings was accepted taking into account to the fact that this level is the lowest on the standard calibration curves and its concentration can be still determined with acceptable accuracy (±20%) and precision (<20%). All data are presented in Tables 8 and 9.

Table 8 Lower Limit of Quantification of Analysis Method for Determination of Cefoperazone in Plasma

Analysis no.	Known Concentration (μg/mL)	Estimated Concentration (μg/mL)	% Recovery
1	3.0	3.086	102.87
2	3.0	3.388	112.93
3	3.0	2.893	96.43
4	3.0	3.265	108.83
5	3.0	3.554	118.47
Mean		3.237	107.91
S.D.		0.257	8.58
%C.V.		7.94	7.95

Table 9 Lower Limit of Quantification of Analysis Method for Determination of Sulbactam in Plasma

Analysis no.	Known Concentration (μg/mL)	Estimated Concentration (μg/mL)	% Recovery
1	1.0	0.905	90.54
2	1.0	1.122	112.20
3	1.0	1.133	113.30
4	1.0	1.023	102.30
5	1.0	1.072	107.20
Mean		1.051	105.11
S.D.		0.093	9.24
%C.V.		8.85	8.80

2.3 Linearity and standard calibration curve

Concentration ranges for cefoperazone and sulbactam were 3.0-48.0 and 1.0-40.0 μg/mL, respectively. Standard calibration curves showed linear response over the range of concentrations used in the assay procedure. Linear regressions of peak area ratios versus concentrations give a typical coefficient of determination (r^2) of 0.9993 and 0.9990, respectively. The calibration curve data for cefoperazone and sulbactam in plasma are displayed in Tables 10 and 11.

Table 10 Linearity of Analytical Method for Determination of Cefoperazone in Plasma

Standard no.	Known Concentration (μg/mL)	Peak Area Ratio*	Estimated Concentration* (μg/mL)	S.D.	% C.V.	% Recovery*
1	3.0	0.0950	3.188	0.601	18.85	106.27
2	6.0	0.1526	5.660	0.820	14.49	94.33
3	12.0	0.3011	12.035	1.299	10.79	100.29
4	18.0	0.4411	18.045	1.318	7.31	100.25
5	24.0	0.5995	24.839	0.961	3.87	103.50
6	30.0	0.7075	29.478	0.782	2.65	98.26
7	36.0	0.8553	35.819	0.219	0.61	99.50
8	48.0	1.1454	48.271	0.595	1.22	100.56

* Each data point is mean of triplicate determinations

where ; $y = 0.0233x+0.0207$

$r^2 = 0.9993$

y = Peak area ratio

x = Concentration

r^2 = Coefficient of determination

Table 11 Linearity of Analytical Method for Determination of Sulbactam in Plasma

Standard no.	Known Concentration (μg/mL)	Peak Area Ratio*	Estimated Concentration* (μg/mL)	S.D.	% C.V.	% Recovery*
1	1.0	0.0778	1.137	0.120	10.50	113.74
2	6.0	0.4301	6.229	0.512	8.22	103.82
3	9.0	0.5738	8.305	0.187	2.25	92.28
4	12.0	0.8621	12.471	0.113	0.91	103.93
5	24.0	1.6219	23.451	1.255	5.35	97.71
6	30.0	2.0972	30.320	0.140	0.46	101.07
7	40.0	2.7687	40.023	0.278	0.69	100.06

* Each data point is mean of triplicate determinations

where ; $y = 0.0692x-0.0009$

$r^2 = 0.9990$

y = Peak area ratio

x = Concentration

r^2 = Coefficient of determination

2.4 Accuracy and precision

The accuracy, within- and between-run precisions of the analysis method for cefoperazone and sulbactam were assessed by analyzing quality control samples spiked with known amount of cefoperazone or sulbactam. Results are shown in Tables 12-14, respectively. It was seen that percent recovery of cefoperazone was 98.62 to 102.39 %, and that of sulbactam was 91.21 to 106.52%. The %C.V. for within- and between-run precisions of cefoperazone were 3.00 to 8.59 and 2.67 to 7.52, and those of sulbactam were 1.27 to 1.85 and 3.45 to 4.83 ,respectively. These results were within acceptance criteria for accuracy (recovery ±15%) and precision (%C.V.<15%).

Table 12 Accuracy of Analytical Method for Determination of Cefoperazone and Sulbactam in Plasma

Active Ingredient	Known Concentration (μg/mL)	Estimated Concentration* (μg/mL)	S.D.	% C.V.	% Recovery*
Cefoperazone	9.0	8.876	0.76	8.59	98.62
	21.0	21.240	0.64	3.00	101.14
	33.0	33.790	1.25	3.71	102.39
Sulbactam	3.0	2.736	0.05	1.85	91.21
	18.0	19.174	0.37	1.91	106.52
	36.0	37.185	0.47	1.27	103.29

* Results are mean of five determinations

$$\text{Where; \% Recovery} = \frac{\text{Estimated concentration}}{\text{Known concentration}} \times 100$$

Table 13 Within-Run Precision of Analytical Method for Determination of Cefoperazone and Sulbactam in plasma

Active Ingredient	Known Concentration (μg/mL)	Estimated Concentration* (μg/mL)	S.D.	% C.V.
Cefoperazone	9.0	8.876	0.76	8.59
	21.0	21.240	0.64	3.00
	33.0	33.790	1.25	3.71
Sulbactam	3.0	2.736	0.05	1.85
	18.0	19.174	0.37	1.91
	36.0	37.185	0.47	1.27

* Results are mean of five determinations

Table 14 Between-Run Precision of Analytical Method for Determination of Cefoperazone and Sulbactam in plasma

Active Ingredient	Known Concentration (μg/mL)	Estimated Concentration* (μg/mL)	S.D.	% C.V.
Cefoperazone	9.00	9.555	0.70	7.32
	21.00	22.008	0.59	2.67
	33.00	34.240	2.57	7.52
Sulbactam	3.0	2.959	0.14	4.83
	18.0	18.006	0.62	3.45
	36.0	35.546	1.71	4.81

* Results are mean of five determinations

2.5 Extraction recovery

As presented in Table 15, The recovery of extraction for cefoperazone ranged between 66.50 to 73.50 % with %C.V. between 3.56 to 7.98 and that of sulbactam ranged between 48.81 to 59.05 % with a %C.V. between 4.21-6.87. For internal standards, the recovery of extraction for salicylic acid and ranitidine were 66.43 and 54.66 % with 2.14 and 3.32 of % C.V. , respectively. Regarding to the recovery of extraction of sulbactam and ranitidine, it could be seen that these value were rather low. This result might be dependent on extraction procedure. For sulbactam, the process of sample preparation like developing the sulbactam-imidazole reaction product as well as extracting by protein precipitation. In this process the high volume of acetonitrile was used to precipitate protein resulting in dilution of drug concentration in the extracted plasma samples. However, according to the Guidance for Bioanalytical Validation (CDER, 2001), recovery of extraction need not be 100%, but the extent of recovery of analyte and internal standard should be consistent, precise, and reproducible. Therefore, these results were acceptable for the purpose of study.

Table 15 Recovery of Extraction of Analytical Method for Determination of Cefoperazone and Sulbactam in Plasma

Active Ingredient	Known Concentration (μg/mL)	Peak Area*		% Recovery of Extraction*	%C.V.
		Extracted	Unextracted		
Cefoperazone	9.0	131.23239	69.92	187.68352	7.98
	21.0	323.99106	73.50	440.82770	3.96
	33.0	476.50348	66.50	716.58557	5.44
Salicylic Acid	50.0	423.07742	66.43	636.87463	2.14
Sulbactam	3.0	115.34234	52.47	219.81536	6.12
	18.0	609.23413	48.81	1248.10258	6.87
	36.0	1345.33008	59.05	2278.16154	4.21
Ranitidine	50.0	510.5957	54.66	934.0588	3.32

* Results are mean of five determinations

2.6 Stability studies

In order to determine the stability of cefoperazone and sulbactam in plasma four studies were carried out: a short-term room temperature, a long-term, a freeze–thaw and processed samples stability studies.

As displayed in Table 16, a short-term room temperature stability of cefoperazone and sulbactam in plasma showed that both of them were tended to degrade after they were thawed at room temperature and kept at this temperature from 4 to12 hours. The percent deviation of cefoperazone from the zero time was -4.27 to –36.91% and that of sulbactam was -0.96 to –18.24% after keeping at room temperature from 4 to 12 hours. This indicated that cefoperazone and sulbactam were stable for 4 and 8 hours at room temperature, respectively. These results illustrated that the samples should be rapidly extracted and analyzed after thawing at room temperature.

The long-term stability of cefoperazone and sulbactam in plasma data are presented in Table 17. The results revealed that cefoperazone samples were stable for 6 weeks and sulbactam samples were stable for 4 weeks. The percent deviation of cefoperazone from the zero time for 6 weeks was-14.10% and that of sulbactam was –11.55% for 4 weeks. These results were within acceptance criteria (± 15%). Hence, these storage times were sufficient for completion of drug analysis.

The freeze-thaw stability was also determined. Quality control samples were analyzed immediately after preparation and after finishing three freezing-thawing cycles. As shown in Table 18, the percent deviation from the zero time of cefoperazone was –13.27 to -13.48%, and that of sulbactam was –10.47 to -14.04 %. These results indicated that no tendency of degradation of both drugs after three freeze-thaw cycles was observed, referring samples could withstand to this stress condition.

Finally, the stability of the processed plasma samples ready for injection were analyzed after freshly preparing, and after being kept in the autosampler at 4, 8, 12, and 16 hours. Table 19 showed that cefoperazone samples are stable upto 6 hours, meanwhile sulbactam samples are stable at least 16 hours after storing in autosampler. The loss was less than acceptance criteria (± 15%). These results illustrated that each run of sample analysis must be finished within 6 and 16 hours for cefoperazone and sulbactam, respectively.

In this assay validation study indicated that the analysis methods of cefoperazone and sulbactam in plasma samples had been proven to be reliable, specific, accurate and precise with the need of internal standard. The lower limit of quantification and stability data of this finding allowed to be successfully applied in a bioequivalence study of cefoperazone/sulbactam intramuscular injection.

Table 16 Short-Term Room Temperature Stability of Analytical Method for Determination of Cefoperazone and Sulbactam in Plasma

Active Ingredient	Hour	Known Concentration (μg/mL)	Estimated Concentration* (μg/mL)	S.D.	% Deviation*
Cefoperazone	0	9.0	8.623	0.265	-
		33.0	30.571	1.886	-
	4	9.0	8.255	0.223	-4.27
		33.0	29.155	1.361	-4.63
	8	9.0	8.087	0.437	-6.21
		33.0	25.336	0.812	-17.13
	12	9.0	7.249	0.918	-15.93
		33.0	19.289	2.755	-36.91
Sulbactam	0	3.0	2.676	0.068	-
		36.0	33.648	1.350	-
	4	3.0	2.640	0.878	-1.35
		36.0	33.325	2.457	-0.96
	8	3.0	2.434	0.068	-9.04
		36.0	32.396	0.325	-3.72
	12	3.0	2.188	0.052	-18.24
		36.0	27.574	0.159	-18.05

* Results are mean of triplicate determinations

where ; % Deviation $= \dfrac{\text{Est.conc.}_{\text{Hour n}} - \text{Est.conc.}_{\text{Hour 0}}}{\text{Est.conc.}_{\text{Hour 0}}} \times 100$

Table 17 Long-Term Stability of Analytical Method for Determination of Cefoperazone and Sulbactam in Plasma

Active Ingredient	Week	Known Concentration (μg/mL)	Estimated Concentration* (μg/mL)	S.D.	% Deviation
Cefoperazone	0	9.0	10.532	0.741	-
		33.0	34.964	3.585	-
	2	9.0	9.823	0.221	-6.41
		33.0	33.986	0.595	-2.80
	4	9.0	9.694	0.044	-7.96
		33.0	33.771	0.293	-3.41
	6	9.0	9.047	0.164	-14.10
		33.0	31.910	1.046	-8.73
Sulbactam	0	3.0	2.687	0.090	-
		36.0	37.698	0.069	-
	2	3.0	2.620	0.024	-2.72
		36.0	35.026	0.566	-7.07
	4	3.0	2.509	0.092	-6.63
		36.0	33.342	1.964	-11.55
	6	3.0	2.351	0.053	-12.51
		36.0	31.952	0.369	-15.24

* Results are mean of triplicate determinations

where ; $$\% \text{ Deviation} = \frac{\text{Est.conc.}_{\text{week n}} - \text{Est.conc.}_{\text{week 0}}}{\text{Est.conc.}_{\text{week 0}}} \times 100$$

Table 18 Freeze-Thaw Stability of Analytical Method for Determination of Cefoperazone and Sulbactam in Plasma

Active Ingredient	Cycle	Known Concentration (μg/mL)	Estimated Concentration* (μg/mL)	S.D.	% Deviation
Cefoperazone	0	9.0	9.540	1.061	-
		33.0	30.041	0.682	-
	3	9.0	8.275	0.347	-13.27
		33.0	25.993	1.785	-13.48
Sulbactam	0	3.0	2.780	0.033	-
		36.0	37.868	1.020	-
	3	3.0	2.390	0.260	-14.04
		36.0	33.905	0.391	-10.47

* Results are mean of triplicate determinations

where ; $$\% \text{ Deviation} = \frac{\text{Est.conc.}_{\text{cycle3}} - \text{Est.conc.}_{\text{cycle0}}}{\text{Est.conc.}_{\text{cycle0}}} \times 100$$

Table 19 Post-Preparative Stability of Analytical Method for Determination of Cefoperazone and Sulbactam in Plasma

Active Ingredient	Hour	Known Concentration (μg/mL)	Estimated Concentration* (μg/mL)	S.D.	% Deviation
Cefoperazone	0	9.0	9.957	0.817	-
		33.0	32.816	0.931	-
	3	9.0	9.257	0.084	-7.03
		33.0	30.281	2.017	-7.72
	6	9.0	8.650	0.339	-13.13
		33.0	29.290	1.338	-10.74
Sulbactam	0	3.0	2.976	0.532	-
		36.0	35.428	1.861	-
	4	3.0	3.015	0.502	1.32
		36.0	35.915	2.291	1.37
	8	3.0	3.045	0.512	2.32
		36.0	36.132	2.322	1.99
	12	3.0	3.072	0.520	3.25
		36.0	36.269	2.387	2.37
	16	3.0	3.080	0.541	3.51
		36.0	36.429	2.329	2.82

* Results are mean of triplicate determinations

where ; $$\% \text{ Deviation} = \frac{\text{Est.conc.}_{\text{Hour n}} - \text{Est.conc.}_{\text{Hour 0}}}{\text{Est.conc.}_{\text{Hour 0}}} \times 100$$

3. Plasma cefoperazone and sulbactam concentrations

Twenty-Two male subjects participated in the study. They were healthy based on passing physical examination as well as clinical blood/urine biochemistry laboratory tests (Appendix C). Their demographic data are shown in Table 20. None withdrew from the study or exhibited signs of allergy and adverse drug reactions to cefoperazone and/or sulbactam.

The plasma concentration–time profiles of cefoperazone and sulbactam from 22 subjects following IM injection of 500/500 mg of cefoperazone and sulbactam injection of test and innovator's product are summarized inTables 21-24, and the mean plasma concentration–time profiles are shown in Tables 25 and 26. No predose detectable levels were found in any of the subjects in either treatment period. As shown in Tables 21-24, there was rapid absorption of cefoperazone and sulbactam taking place within 15 minutes. Cefoperazone and sulbactam concentrations from both formulation were reached to maximum within 0.5-2 hours and 30-90 minutes, respectively. After reaching C_{max}, plasma concentrations of the drugs declined slowly until the end of 10 hours. Individual plasma cefoperazone and sulbactam concentration-time profiles of two brands for each of twenty two subjects were displayed graphically from Figures 7 to 28. Comparisons of the mean plasma cefoperazone and sulbactam concentration-time profiles of each brand of twenty two subjects were illustrated in Tables 25 and 26, and Figures 29 and 30.

4. Pharmacokinetics analysis

Primary pharmacokinetics parameter estimates of cefoeprazone and sulbactam after intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of test and innovator's product for all subjects are summarized in Tables 27-29. Analysis of variance of these corresponding pharmacokinetics parameters (log AUC_{0-t}, log $AUC_{0-\infty}$, log C_{max} and t_{max}) are reported in Tables 30-37. 90% confidence intervals for the ratio of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} of cefoperazone and sulbacatm of test to innovator's product based on log-transformed data are also displayed in Tables 30-35. All these intervals were within 80-125%. Other related pharmacokinetic parameters; elimination rate constant (k), elimination half-life ($t_{1/2}$), and mean residence time (MRT) of cefoperazone and sulbactam of all subjects participated in this study were also obtained and presented in Tables 38-39. Regarding the difference of these parameters

obtained from test product relative to those of innovator's product, they were tested based on analysis of variance for two way crossover design. Results are shown in Tables 40-45.

Table 20 Demographic Data of Subjects Participated in This Study

Subject no.	Age (year)	Height (m)	Weight (kg)	BMI* (kg/m^2)
1	31	169	63	22.06
2	26	170	71	24.57
3	27	173	69	23.05
4	28	168	58	20.55
5	36	170	69	23.38
6	43	165	59	21.67
7	31	166	65	23.59
8	38	168	63	22.32
9	33	170	60	20.76
10	33	166	55	19.96
11	33	170	60	20.76
12	38	166	58	21.05
13	28	172	66	22.31
14	34	175	65	21.22
15	48	170	66	22.84
16	37	168	70	24.80
17	33	169	69	24.16
18	40	172	70	23.66
19	42	167	57	20.44
20	44	165	61	22.41
21	52	177	73	23.30
22	23	160	52	20.31
Mean	35.36	168.91	63.59	22.26
S.D.	7.35	3.69	5.76	1.49
%C.V.	0.21	2.18	9.06	6.69

* BMI (Body Mass Index) = Weight (kg)/ $Height^2$ (m^2)

Table 21 Plasma Cefoperarazone Concentration (μg/mL) of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injection of Test Product

Subject . no.	P1	P2	Time (hr)														
			0	0.25	0.5	0.75	1	1.25	1.5	1.75	2	2.5	3	4	6	8	10
1		/	0.0	14.87	25.37	25.49	25.64	25.84	23.60	20.43	21.32	18.97	16.46	12.06	6.11	3.25	<LLOQ
2	/		0.0	17.46	39.21	41.55	46.80	43.74	36.03	34.24	34.30	27.10	20.60	15.67	7.34	4.53	3.84
3	/		0.0	28.54	37.56	37.34	36.08	31.24	31.70	31.99	31.88	27.75	20.33	27.52	10.58	9.14	4.39
4		/	0.0	3.71	8.40	12.52	14.00	14.45	17.48	18.74	18.43	17.31	16.66	14.00	9.10	5.41	3.18
5	/		0.0	31.05	38.62	33.53	33.12	31.55	29.19	29.26	27.12	24.71	18.04	12.01	7.04	4.37	<LLOQ
6	/		0.0	30.16	25.21	31.27	34.52	37.81	38.56	28.17	30.01	30.41	23.74	30.57	16.78	11.18	5.81
7		/	0.0	12.63	24.63	30.53	34.72	32.05	27.64	27.17	26.24	23.55	22.23	15.65	9.46	5.09	<LLOQ
8	/		0.0	13.52	20.96	17.00	21.88	22.97	24.69	20.30	20.68	20.76	16.04	13.74	10.79	6.21	3.51
9		/	0.0	14.37	16.08	20.41	26.00	24.76	27.27	29.54	29.38	28.43	26.90	22.90	17.52	12.92	7.67
10		/	0.0	22.08	31.98	43.04	45.26	45.74	41.52	39.30	36.82	34.82	26.87	22.46	13.33	8.44	5.50
11	/		0.0	13.10	6.23	10.26	14.28	18.16	21.34	25.40	25.90	34.01	32.11	29.90	29.25	12.15	10.72
12	/		0.0	<LLOQ	8.18	10.64	15.99	16.52	26.85	19.90	20.97	24.99	23.38	19.48	16.10	15.17	10.10
13		/	0.0	15.37	23.98	36.69	32.97	37.08	35.46	35.03	32.95	23.99	25.36	22.90	11.89	6.85	4.32
14	/		0.0	7.44	6.39	10.08	12.59	12.66	14.99	15.90	15.55	15.48	15.50	17.16	12.83	8.70	7.59
15		/	0.0	18.25	22.16	27.68	26.19	27.02	26.05	29.90	27.13	18.02	16.63	11.83	5.34	3.14	<LLOQ
16		/	0.0	41.54	43.72	39.67	46.85	40.38	37.89	43.65	36.40	33.54	26.70	23.46	12.89	9.53	6.35
17		/	0.0	<LLOQ	2.99	6.62	10.69	12.24	14.68	15.74	17.12	19.02	17.89	15.63	14.27	10.68	6.76
18	/		0.0	10.41	14.11	16.32	17.47	16.89	17.29	18.21	17.58	15.07	13.64	9.11	6.87	4.73	3.38
19		/	0.0	22.72	34.78	37.71	40.83	40.31	39.09	36.23	29.78	30.31	29.00	21.61	13.33	7.23	4.61
20	/		0.0	26.00	38.00	41.87	43.00	38.42	40.53	39.83	32.36	36.30	29.00	26.96	14.98	10.31	6.73
21		/	0.0	12.07	22.89	26.81	27.75	25.12	23.57	23.05	23.02	19.07	29.00	16.69	13.29	7.72	4.42
22	/		0.0	29.39	38.51	39.00	41.68	40.08	31.76	36.87	35.19	28.00	29.00	14.51	6.41	<LLOQ	<LLOQ
Mean			ND	19.23	24.09	27.09	29.47	28.86	28.51	28.13	26.82	25.07	29.00	18.90	12.07	7.94	5.82
S.D.			ND	10.62	12.62	12.16	11.89	10.67	8.42	8.35	6.66	6.52	29.00	6.25	5.30	3.67	3.17
%C.V.			ND	55.22	52.38	44.87	40.34	36.98	29.53	29.67	24.84	26.02	29.00	33.05	43.90	46.18	54.58

P1= period 1, P2= period 2, LLOQ = 3.0 μg/mL

Table 22 Plasma Cefoperarazone Concentration (μg/mL) of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injection of Innovator's Product

Subject no.	P1	P2	Time (hr)														
			0	0.25	0.5	0.75	1.00	1.25	1.5	1.75	2.00	2.5	3	4	6	8	10
1	/		0.0	12.49	24.41	23.88	25.10	24.86	23.85	23.91	26.14	24.95	16.19	9.68	5.30	2.28	<LLOQ
2		/	0.0	13.94	26.40	33.26	31.60	32.26	31.81	32.42	27.27	23.37	21.54	16.47	9.15	5.51	3.17
3		/	0.0	31.28	32.94	32.97	37.13	35.48	34.58	34.27	30.37	25.00	23.56	17.94	17.63	8.72	5.39
4	/		0.0	5.18	19.82	25.90	23.01	29.44	28.98	21.24	19.49	19.05	18.63	15.57	9.89	5.10	<LLOQ
5		/	0.0	9.98	17.38	25.51	26.44	29.95	30.97	28.37	24.37	23.63	20.78	15.05	7.97	4.65	2.89
6		/	0.0	17.57	32.41	33.03	37.11	32.45	34.11	34.29	37.05	30.05	30.96	24.94	14.67	10.32	7.90
7	/		0.0	26.56	33.44	35.90	35.11	36.52	34.35	31.17	27.48	21.36	14.04	11.44	7.25	4.29	<LLOQ
8		/	0.0	16.79	23.94	26.99	24.41	27.32	26.46	24.81	23.53	19.14	11.99	17.60	8.12	4.76	3.65
9	/		0.0	22.41	27.23	36.89	37.67	35.99	34.17	33.47	32.54	28.96	19.27	14.05	8.91	4.09	<LLOQ
10	/		0.0	16.42	26.19	28.67	28.36	31.02	33.88	35.37	33.08	23.34	21.27	21.73	13.30	8.96	6.99
11		/	0.0	14.06	16.10	20.57	21.91	24.98	26.98	25.40	26.46	26.17	24.06	22.10	19.19	12.74	6.07
12		/	0.0	13.98	20.97	25.25	26.24	25.83	26.64	26.15	29.24	23.73	22.64	20.50	15.00	11.47	8.88
13	/		0.0	12.18	25.46	27.62	31.67	39.87	29.96	29.91	22.23	23.10	20.67	13.97	5.77	<LLOQ	<LLOQ
14		/	0.0	3.81	13.23	18.87	22.02	22.30	25.79	24.15	25.17	21.20	20.62	16.31	10.61	7.13	4.27
15	/		0.0	10.48	28.93	31.86	33.48	34.40	32.02	28.87	27.59	23.27	19.27	13.06	4.63	<LLOQ	<LLOQ
16	/		0.0	20.59	31.47	33.55	41.14	40.99	32.81	29.08	29.23	30.99	26.21	22.34	13.12	7.50	<LLOQ
17	/		0.0	<LLOQ	6.27	8.10	11.19	13.83	21.78	14.68	17.46	16.38	18.22	15.25	10.43	9.12	3.55
18		/	0.0	3.57	9.49	11.54	14.06	15.27	15.45	15.17	17.15	15.39	16.17	14.68	10.74	6.57	3.86
19	/		0.0	17.27	25.83	32.58	30.09	30.98	29.64	27.26	29.28	27.54	24.63	12.86	12.82	7.15	<LLOQ
20		/	0.0	32.39	38.11	47.65	45.44	47.56	43.98	37.27	32.34	27.42	25.74	20.24	15.94	8.02	6.00
21	/		0.0	21.56	27.53	27.75	25.35	25.10	24.79	24.94	23.70	20.95	17.62	15.43	9.02	5.44	3.91
22		/	0.0	24.80	33.77	37.29	36.14	33.10	28.49	30.20	25.45	21.65	18.26	11.26	5.23	<LLOQ	<LLOQ
Mean			ND	16.54	24.60	28.44	29.30	30.43	29.61	27.84	26.66	23.48	20.56	16.48	10.67	7.04	3.33
S.D.			ND	8.65	8.22	8.72	8.41	7.90	5.76	5.93	5.00	4.07	4.32	4.04	4.11	3.53	2.96
%C.V.			ND	52.28	33.41	30.66	28.70	25.96	19.44	21.30	18.76	17.32	21.00	24.54	38.51	50.17	88.79

P1= period 1, P2= period 2, LLOQ = 3.0 μg/mL

Table 23 Plasma Sulbactam Concentration (μg/mL) of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injection of Test Product

Subject no.	P1	P2	Time (hr)													
			0	0.25	0.5	0.75	1.00	1.25	1.5	1.75	2.00	2.5	3	4	6	8	10
1		/	0.0	9.01	15.13	14.99	14.71	12.76	9.82	7.81	6.84	7.33	3.93	2.41	1.05	<LLOQ	<LLOQ
2	/		0.0	29.65	34.42	31.42	22.49	17.04	14.69	14.48	12.58	9.19	6.79	3.96	1.90	<LLOQ	<LLOQ
3	/		0.0	26.39	23.24	20.15	15.49	14.42	11.91	12.37	10.89	8.78	7.61	4.11	2.10	<LLOQ	<LLOQ
4		/	0.0	3.60	7.24	10.04	10.46	11.28	12.49	11.42	11.21	9.77	7.00	5.52	3.26	1.74	<LLOQ
5	/		0.0	25.76	25.89	22.88	11.06	11.54	11.50	10.51	9.40	4.89	4.23	2.95	<LLOQ	<LLOQ	<LLOQ
6	/		0.0	18.63	20.17	13.68	12.06	12.61	12.53	10.46	9.19	9.28	8.39	6.50	1.06	<LLOQ	<LLOQ
7		/	0.0	14.12	17.80	17.47	16.35	13.86	12.12	10.87	9.38	6.75	5.39	3.34	1.67	<LLOQ	<LLOQ
8	/		0.0	13.13	15.01	17.02	13.95	12.47	10.94	9.82	7.50	6.90	4.37	3.20	1.54	<LLOQ	<LLOQ
9		/	0.0	8.97	11.01	11.44	12.95	11.26	10.46	9.88	8.90	6.71	5.14	4.22	2.38	<LLOQ	<LLOQ
10		/	0.0	12.02	14.55	14.37	11.66	9.90	9.32	7.66	7.47	5.31	3.82	3.79	1.48	<LLOQ	<LLOQ
11	/		0.0	1.39	3.78	6.88	7.02	9.28	10.31	11.39	11.00	11.26	9.43	5.45	1.90	<LLOQ	<LLOQ
12	/		0.0	3.51	7.48	11.10	13.95	15.54	15.48	15.25	15.26	15.15	13.42	10.05	5.20	<LLOQ	<LLOQ
13		/	0.0	8.48	12.02	13.14	12.88	11.58	10.91	9.24	8.18	6.31	5.13	2.44	1.16	<LLOQ	<LLOQ
14	/		0.0	7.32	9.53	12.45	10.96	10.74	10.33	10.02	10.00	7.79	6.69	5.31	2.75	<LLOQ	<LLOQ
15		/	0.0	8.40	14.39	13.07	12.54	11.91	10.37	8.68	6.77	6.00	3.96	2.45	1.06	<LLOQ	<LLOQ
16		/	0.0	26.80	23.00	16.27	15.50	12.71	10.47	9.20	5.98	4.97	3.67	2.74	1.21	<LLOQ	<LLOQ
17		/	0.0	1.16	4.34	5.68	7.32	7.58	8.13	6.92	6.90	6.45	3.47	2.97	2.21	<LLOQ	<LLOQ
18	/		0.0	9.96	13.46	13.11	11.35	11.34	10.59	8.47	8.30	5.98	4.51	3.68	1.70	<LLOQ	<LLOQ
19		/	0.0	19.49	20.29	15.43	15.34	13.16	10.76	9.13	7.91	6.97	5.67	2.55	<LLOQ	<LLOQ	<LLOQ
20	/		0.0	23.67	25.49	19.55	15.88	12.69	11.21	10.25	8.25	7.93	5.79	3.28	<LLOQ	<LLOQ	<LLOQ
21		/	0.0	11.97	13.79	12.79	10.88	8.97	8.62	7.18	6.06	5.13	3.59	2.72	1.04	<LLOQ	<LLOQ
22	/		0.0	16.32	13.78	13.31	10.82	9.40	7.47	7.35	5.77	5.61	5.02	1.85	<LLOQ	<LLOQ	<LLOQ
Mean			ND	13.62	15.72	14.83	12.98	11.91	10.93	9.92	8.81	7.48	5.77	3.89	1.93	ND	ND
S.D.			ND	8.65	7.58	5.45	3.31	2.20	1.87	2.17	2.34	2.41	2.37	1.83	1.20	ND	ND
%C.V.			ND	63.51	48.21	36.72	25.46	18.45	17.08	21.89	26.53	32.22	41.07	46.99	62.33	ND	ND

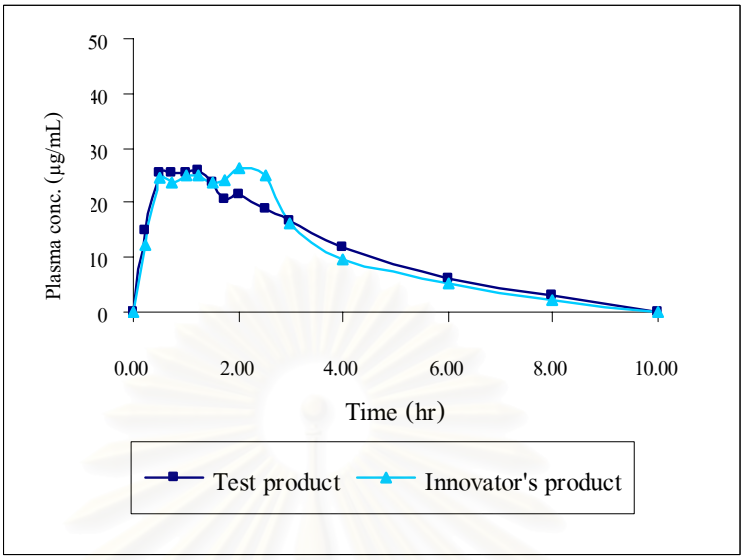
P1= period 1, P2= period 2, LLOQ = 1.0 μg/mL , ND = Not determined

Table 24 Plasma Sulbactam Concentration (μg/mL) of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injection of Innovator's Product

Subject no.	P1	P2	Time (hr)														
			0	0.25	0.5	0.75	1.00	1.25	1.5	1.75	2.00	2.5	3	4	6	8	10
1	/		0.0	12.81	18.81	18.20	15.59	11.83	10.04	10.15	7.70	5.46	4.27	3.17	1.49	<LLOQ	<LLOQ
2		/	0.0	16.50	27.09	21.60	18.41	18.75	14.35	11.64	9.68	6.57	5.88	3.36	1.53	<LLOQ	<LLOQ
3		/	0.0	15.57	21.69	21.50	19.03	16.71	14.34	13.59	11.31	8.57	7.49	4.88	2.00	<LLOQ	<LLOQ
4	/		0.0	14.08	17.68	17.78	16.51	14.02	11.46	11.50	9.45	7.92	5.11	3.69	1.46	<LLOQ	<LLOQ
5		/	0.0	11.84	14.58	16.32	14.02	12.83	10.82	9.59	7.38	5.27	3.62	2.02	<LLOQ	<LLOQ	<LLOQ
6		/	0.0	16.34	15.05	13.81	11.25	9.27	7.68	6.51	5.98	4.20	3.25	1.42	<LLOQ	<LLOQ	<LLOQ
7	/		0.0	31.66	22.11	15.20	14.35	10.99	7.28	6.66	5.47	3.88	3.11	1.90	1.88	<LLOQ	<LLOQ
8		/	0.0	8.66	11.49	11.47	9.38	8.14	7.21	6.41	5.19	3.91	3.06	2.04	<LLOQ	<LLOQ	<LLOQ
9	/		0.0	19.63	23.81	17.54	18.19	17.08	14.59	9.38	11.65	10.19	7.48	5.31	1.11	<LLOQ	<LLOQ
10	/		0.0	4.05	20.46	20.19	17.30	15.28	13.81	13.04	11.28	9.60	7.54	5.08	1.97	<LLOQ	<LLOQ
11		/	0.0	7.36	9.12	10.62	10.36	8.66	9.76	7.54	6.51	5.18	3.75	2.55	1.30	<LLOQ	<LLOQ
12		/	0.0	9.52	13.19	13.69	12.61	10.52	9.95	8.66	7.69	6.03	4.73	3.41	2.00	<LLOQ	<LLOQ
13	/		0.0	13.58	19.25	14.73	13.60	15.18	12.58	10.51	8.88	6.51	5.19	3.58	1.32	<LLOQ	<LLOQ
14		/	0.0	5.33	11.64	15.78	16.49	14.57	13.46	11.80	9.37	7.54	6.58	4.42	2.14	<LLOQ	<LLOQ
15	/		0.0	9.35	13.41	14.68	12.52	12.76	12.58	10.86	10.48	8.42	7.65	6.04	1.38	<LLOQ	<LLOQ
16	/		0.0	11.70	15.62	16.62	12.15	9.80	7.95	8.53	7.99	8.48	4.80	3.15	1.06	<LLOQ	<LLOQ
17	/		0.0	3.38	7.03	16.21	10.14	10.86	11.77	10.91	8.88	8.89	6.31	4.50	1.91	<LLOQ	<LLOQ
18		/	0.0	3.51	7.26	8.94	9.30	9.22	9.52	8.82	8.26	6.50	5.76	3.77	1.71	<LLOQ	<LLOQ
19	/		0.0	22.08	24.42	20.70	20.11	15.10	13.05	12.35	11.06	9.98	8.16	5.82	3.00	<LLOQ	<LLOQ
20		/	0.0	13.32	18.59	15.87	13.79	10.94	8.81	7.23	6.21	4.18	2.84	1.80	<LLOQ	<LLOQ	<LLOQ
21	/		0.0	23.73	25.43	21.58	17.30	12.38	11.49	11.21	9.11	7.32	6.07	3.89	1.47	<LLOQ	<LLOQ
22		/	0.0	15.54	17.56	16.69	14.16	11.49	8.71	7.63	6.86	4.57	3.34	2.07	<LLOQ	<LLOQ	<LLOQ
Mean			ND	13.16	17.06	16.35	14.39	12.56	10.96	9.75	8.47	6.78	5.27	3.54	1.69	ND	ND
S.D.			ND	7.00	5.78	3.48	3.24	2.94	2.43	2.17	1.96	2.04	1.73	1.36	0.83	ND	ND
%C.V.			ND	53.16	33.86	21.29	22.50	23.38	22.14	22.30	23.18	30.12	32.80	38.45	49.31	ND	ND

P1= period 1, P2= period 2, LLOQ = 1.0 μg/mL, ND = Not determined

A



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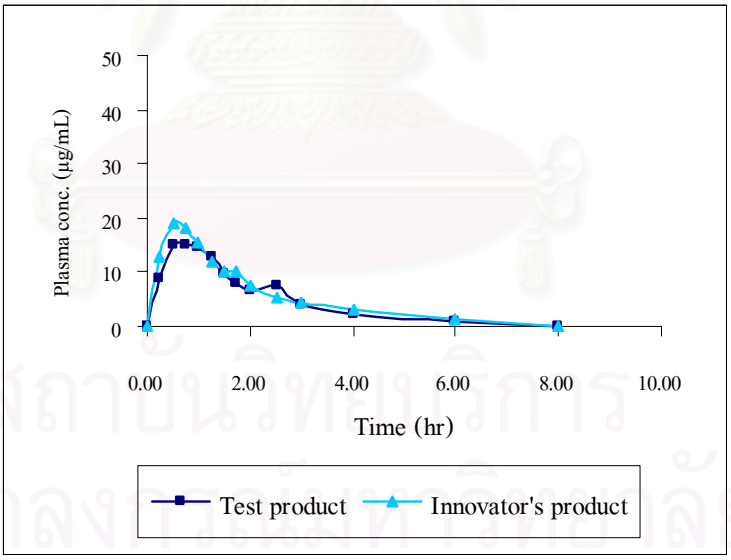
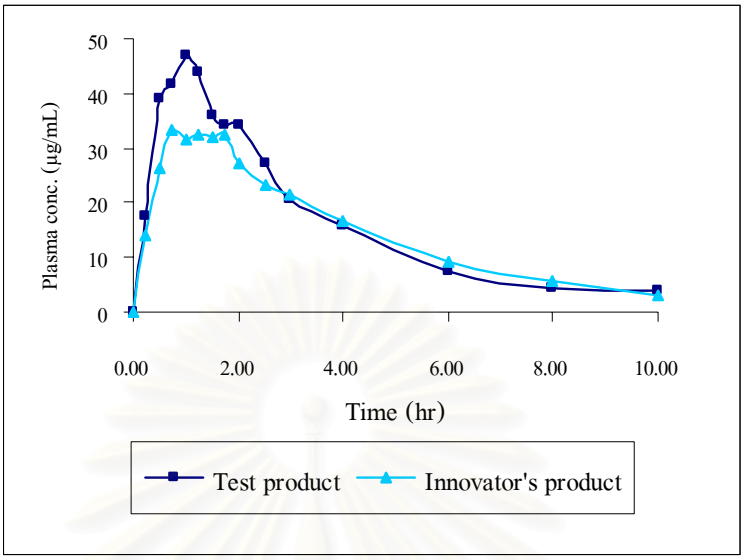


Figure 7 Plasma concentration vs. time profiles of subject No.1 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



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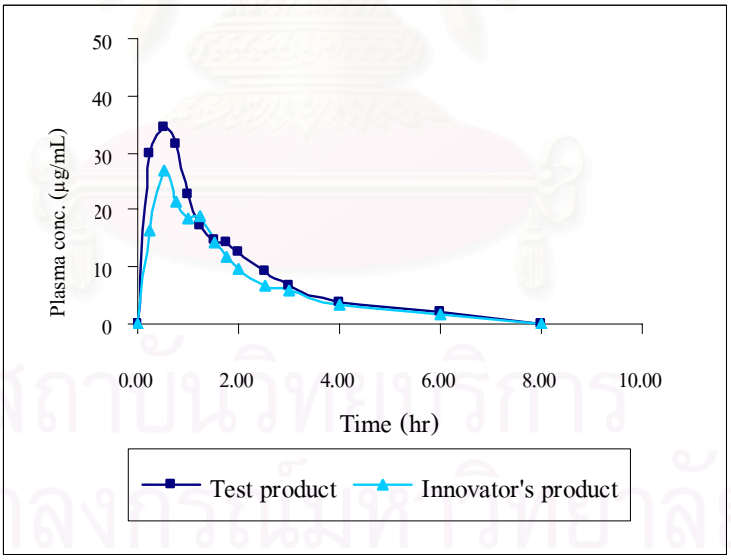
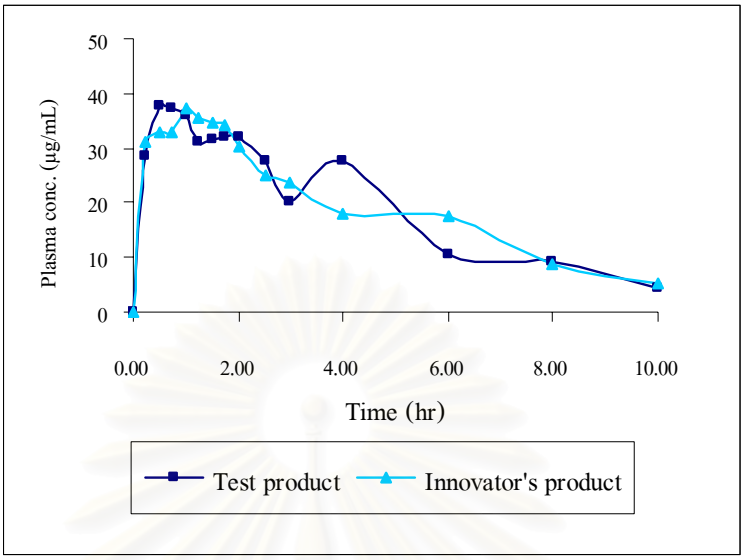


Figure 8 Plasma concentration vs. time profiles of subject No.2 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

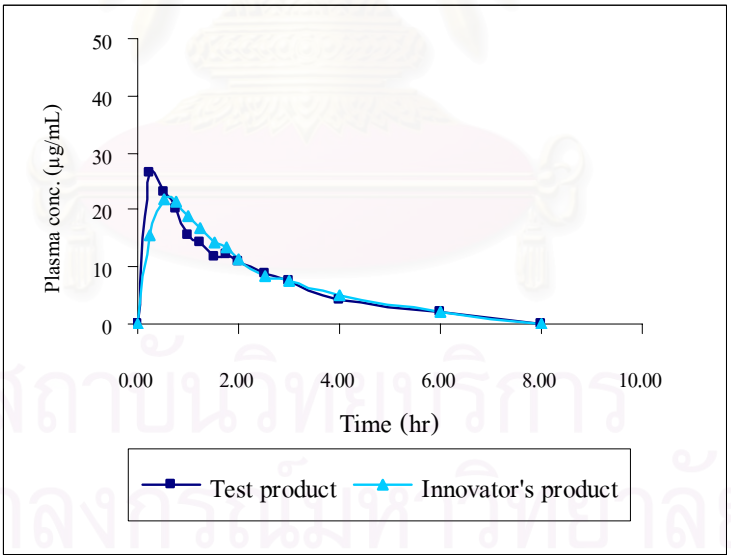
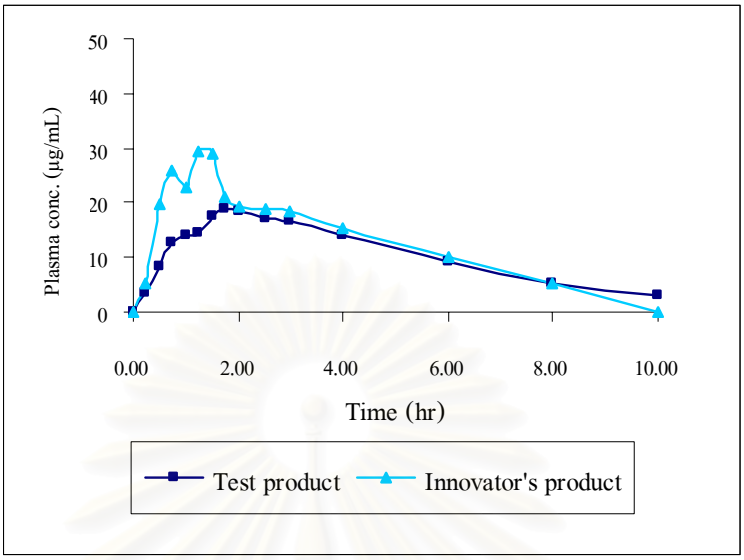


Figure 9 Plasma concentration vs. time profiles of subject No.3 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

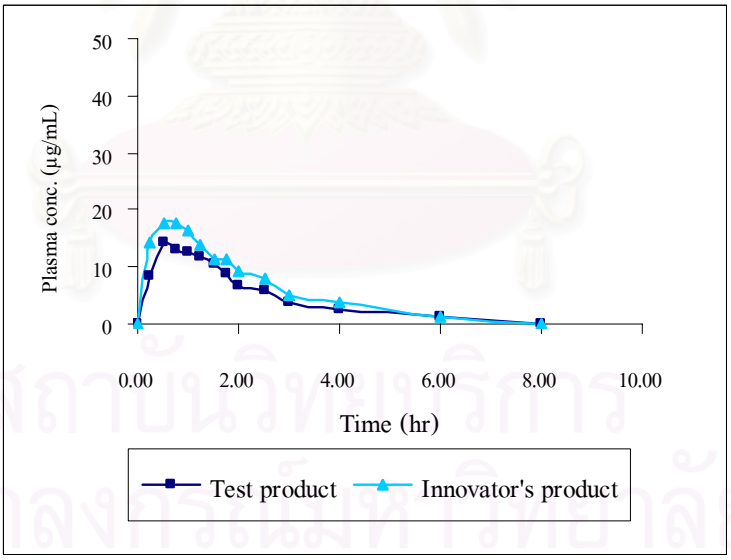
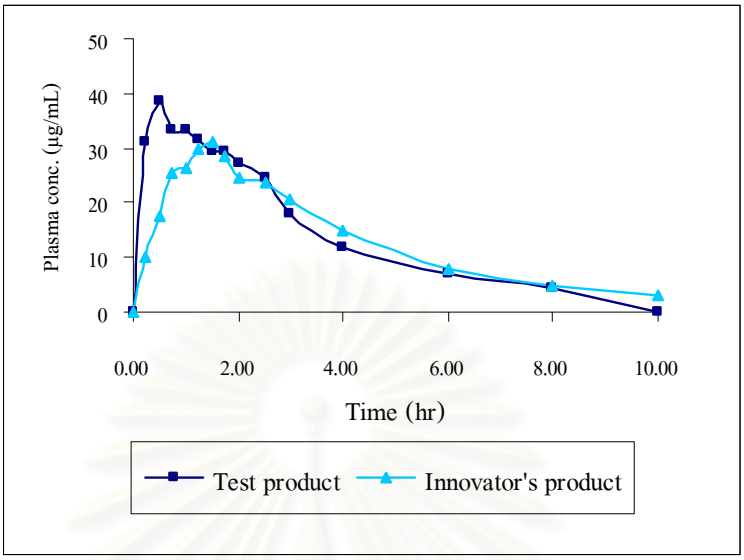


Figure 10 Plasma concentration vs. time profiles of subject No.4 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

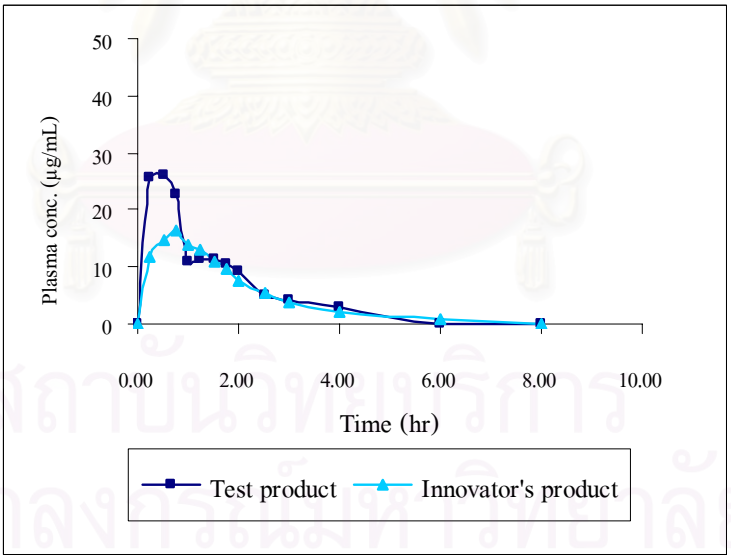
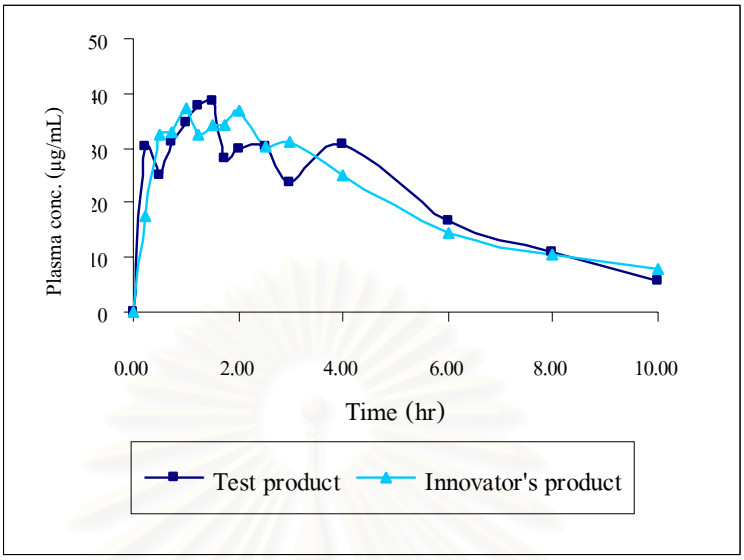


Figure 11 Plasma concentration vs. time profiles of subject No.5 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

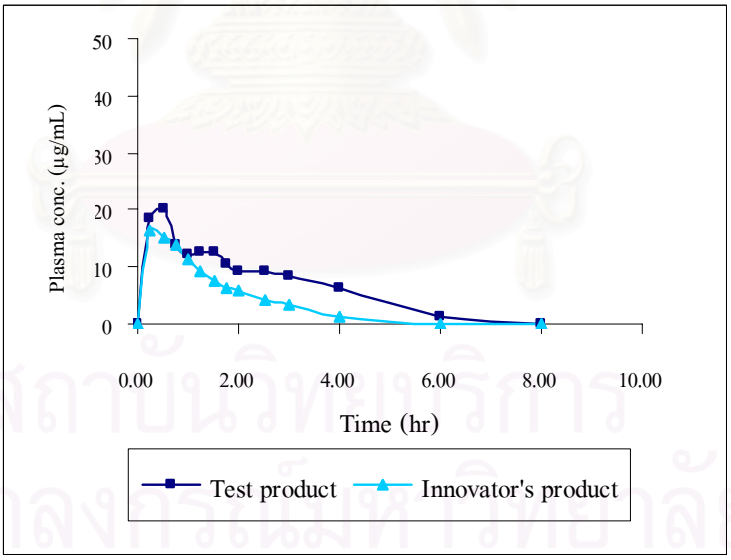
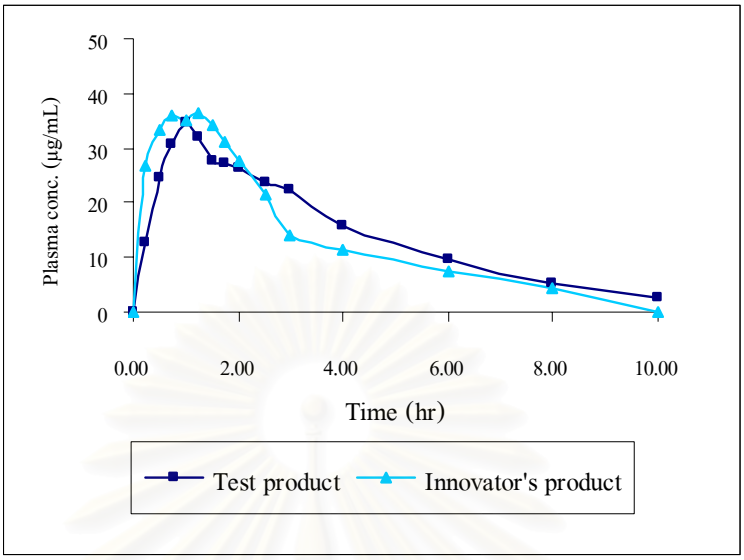


Figure 12 Plasma concentration vs. time profiles of subject No.6 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



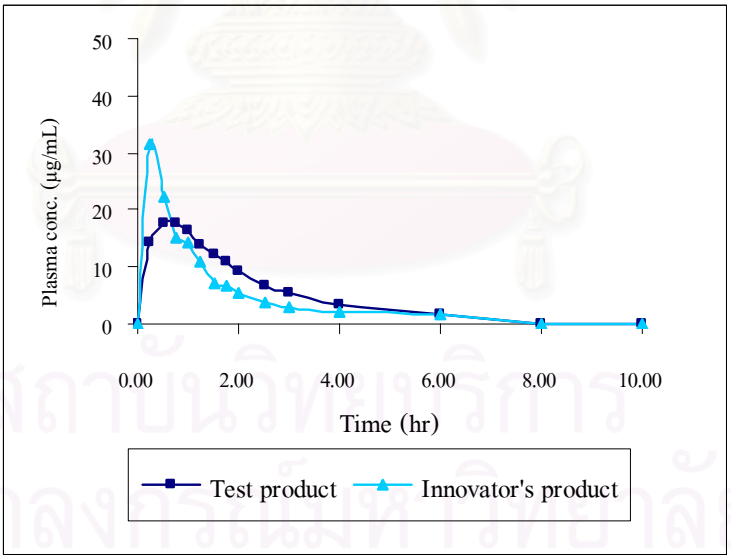
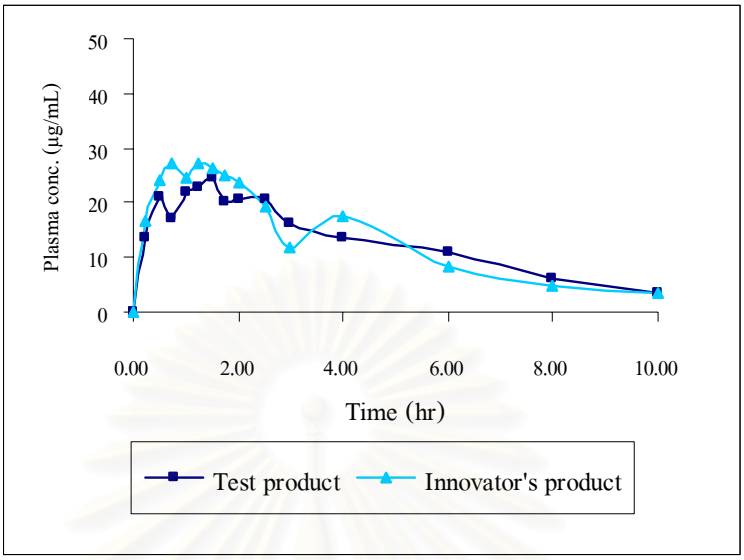


Figure 13 Plasma concentration vs. time profiles of subject No.7 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

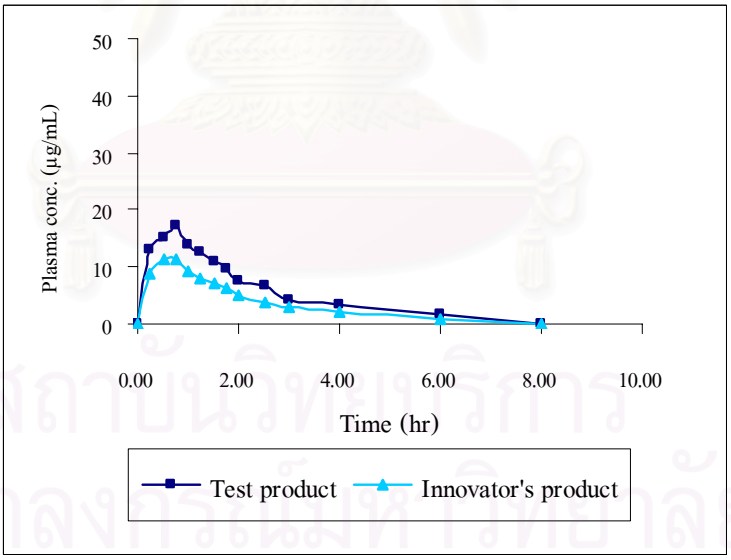
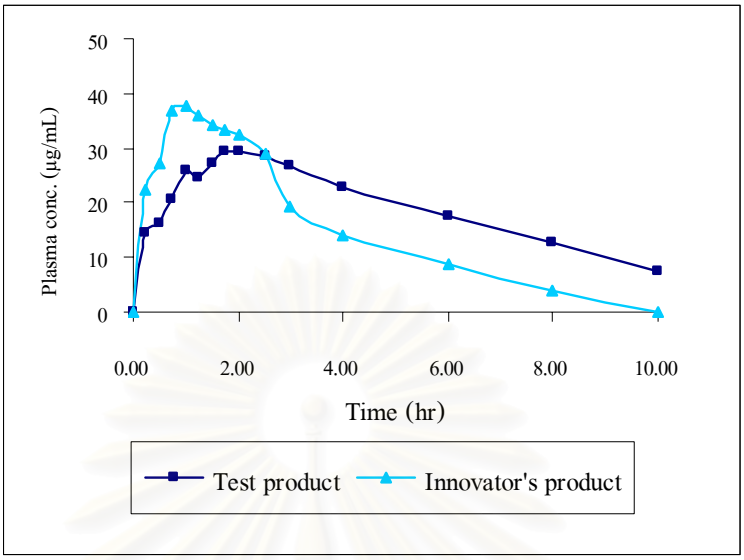


Figure 14 Plasma concentration vs. time profiles of subject No.8 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

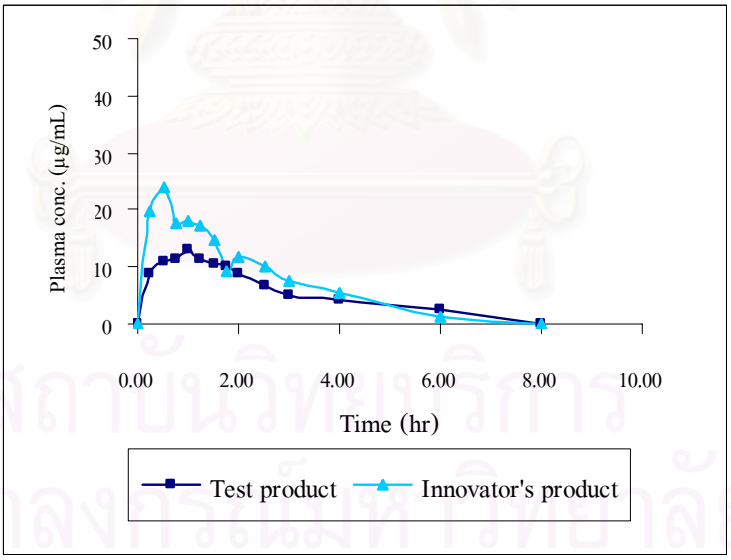
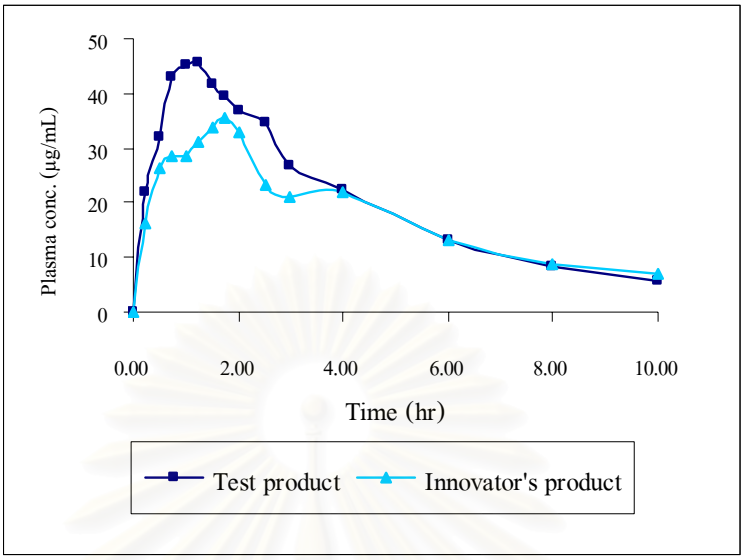


Figure 15 Plasma concentration vs. time profiles of subject No.9 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

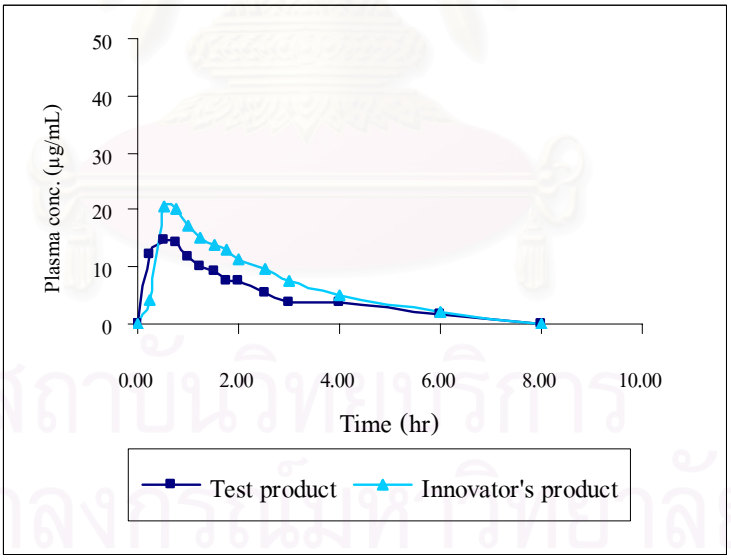
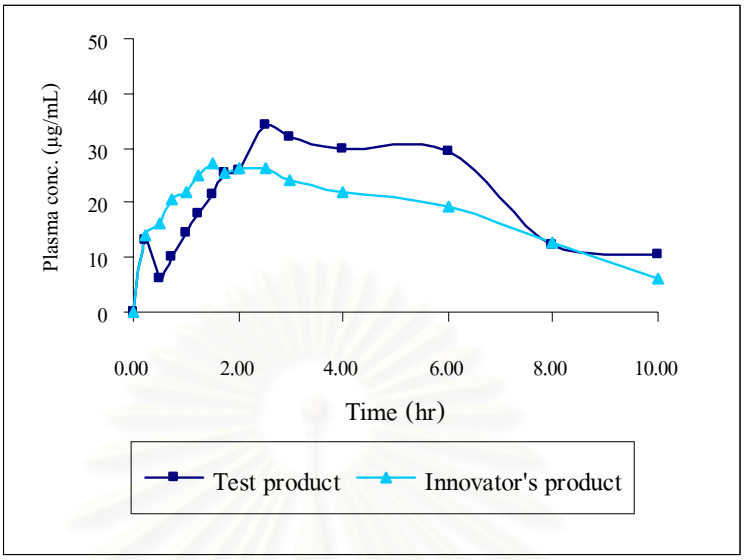


Figure 16 Plasma concentration vs. time profiles of subject No.10 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

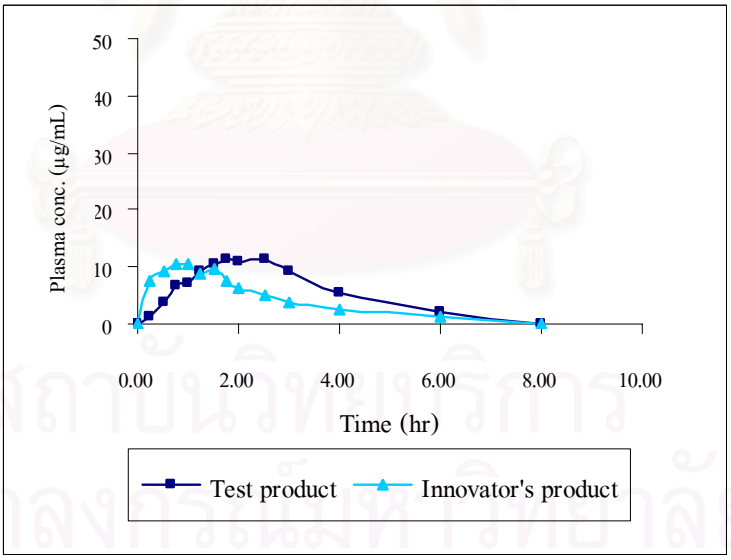
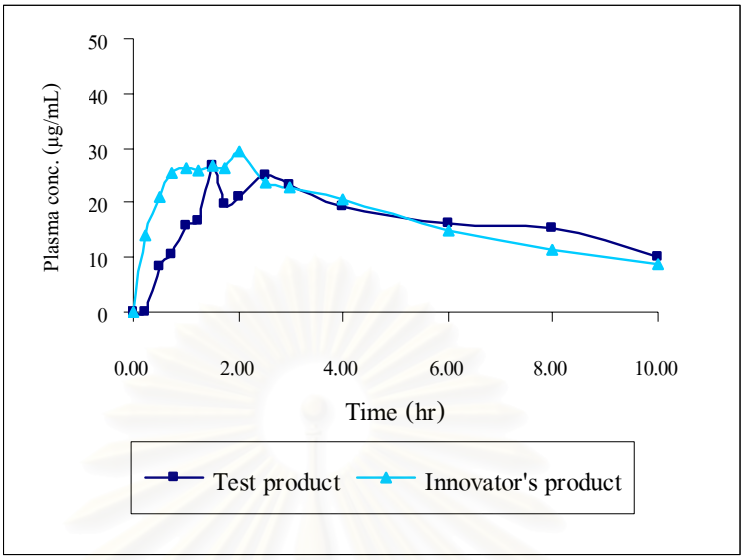


Figure 17 Plasma concentration vs. time profiles of subject No.11 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

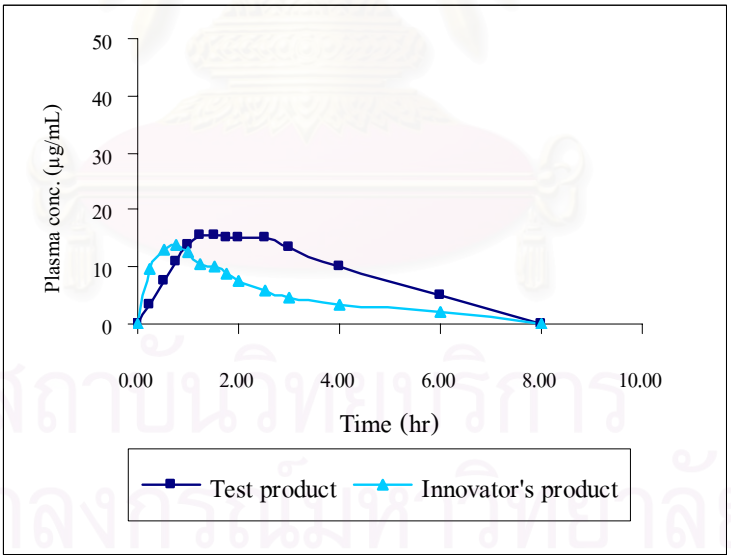
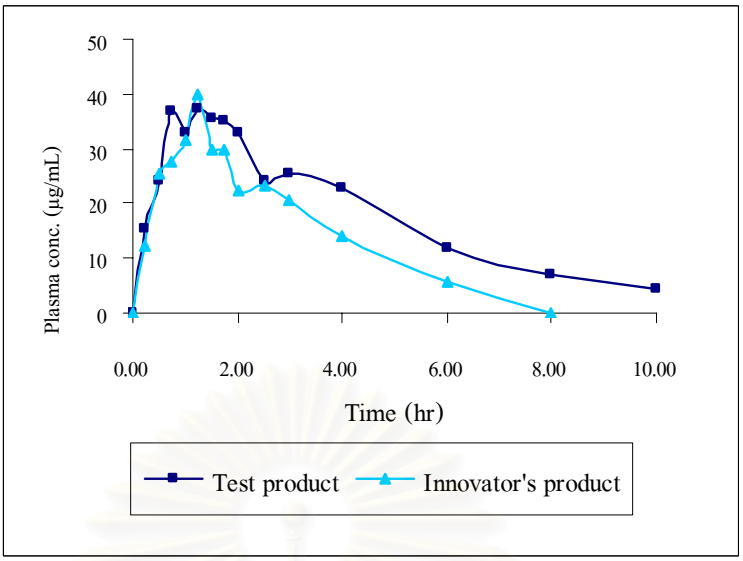


Figure 18 Plasma concentration vs. time profiles of subject No.12 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

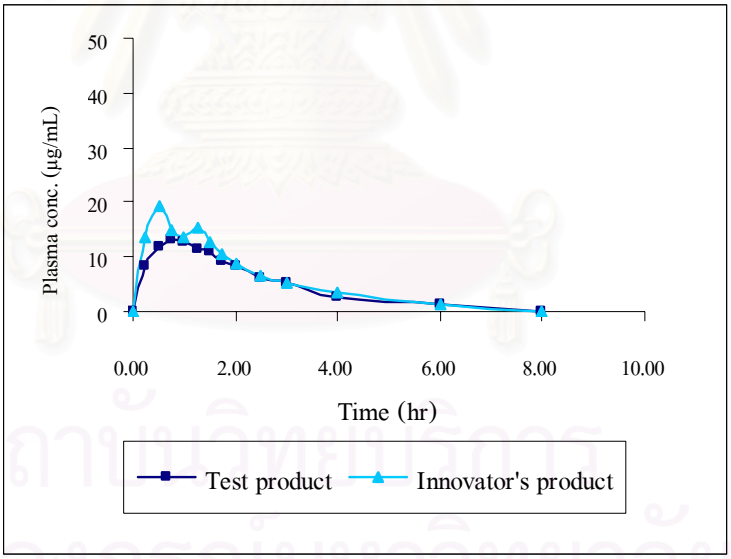
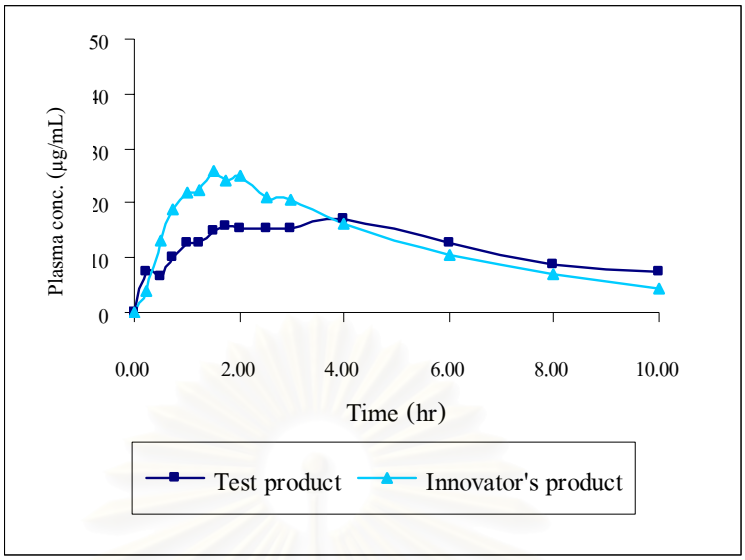


Figure 19 Plasma concentration vs. time profiles of subject No.13 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

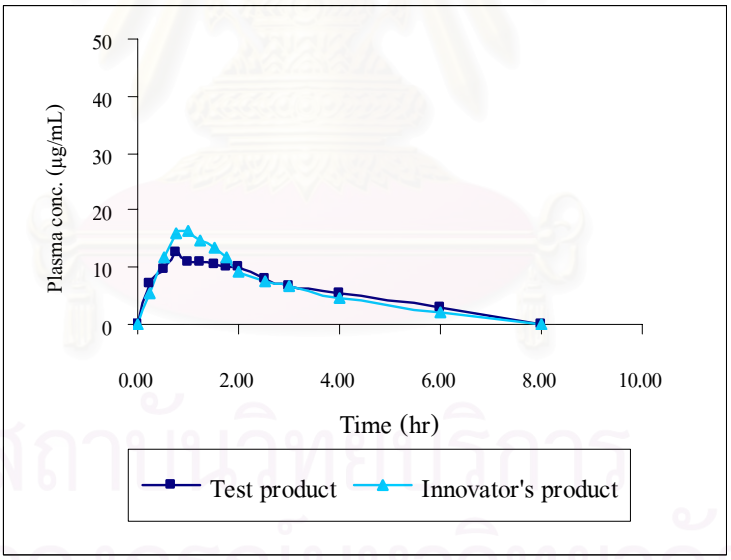
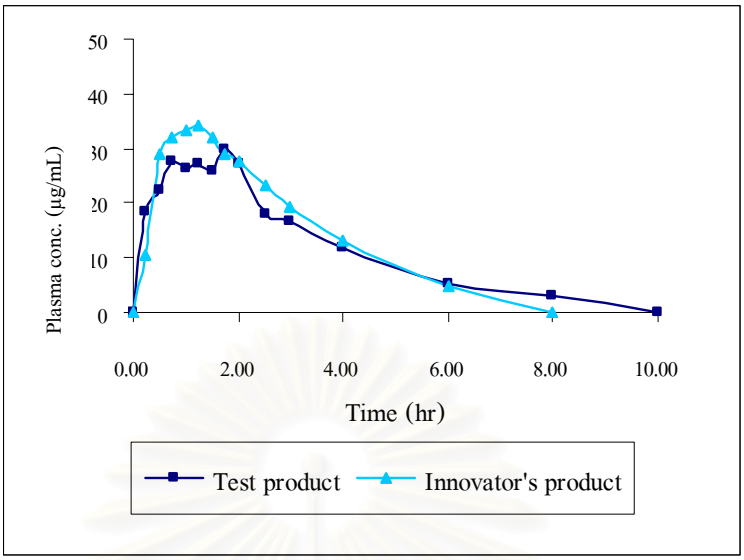


Figure 20 Plasma concentration vs. time profiles of subject No.14 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

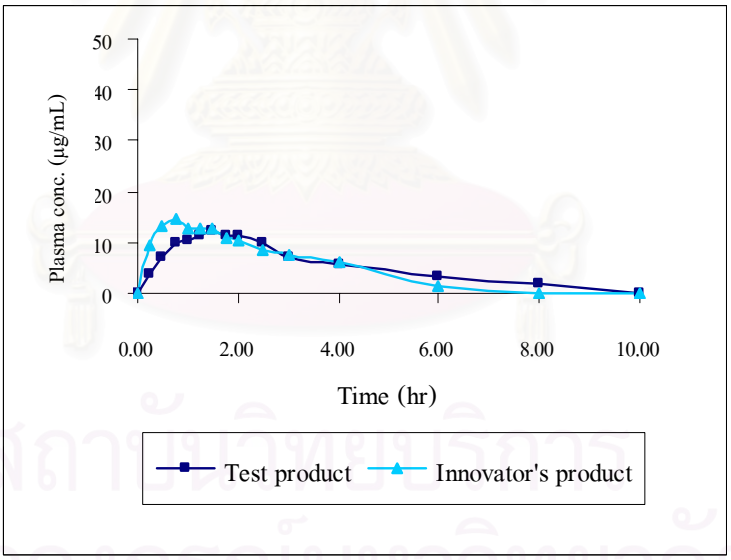
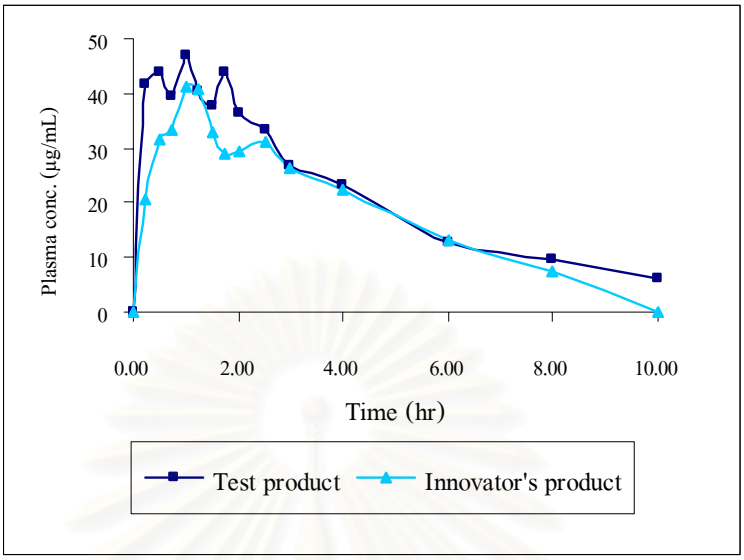


Figure 21 Plasma concentration vs. time profiles of subject No.15 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

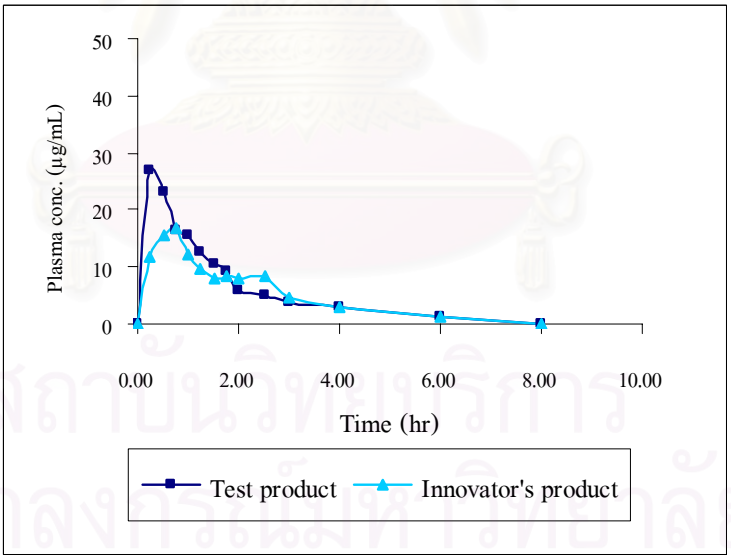
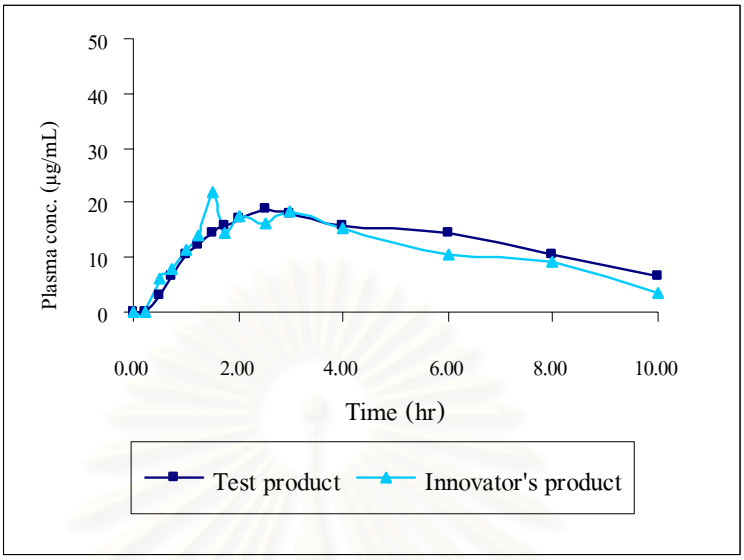


Figure 22 Plasma concentration vs. time profiles of subject No.16 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

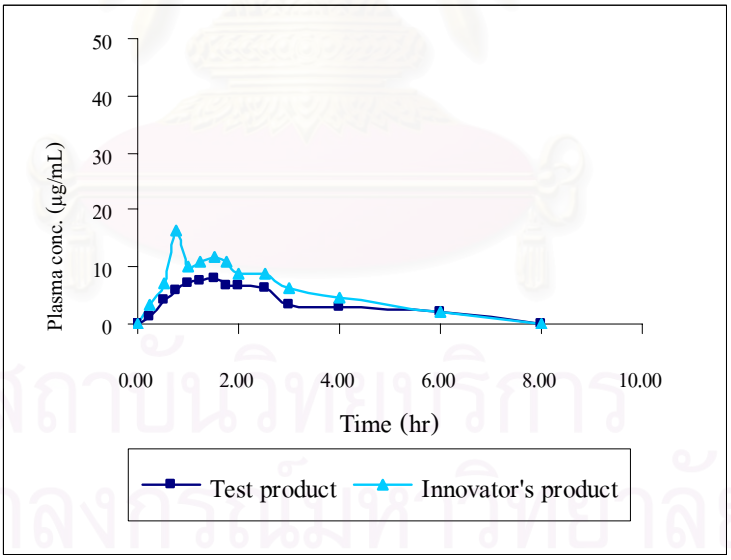
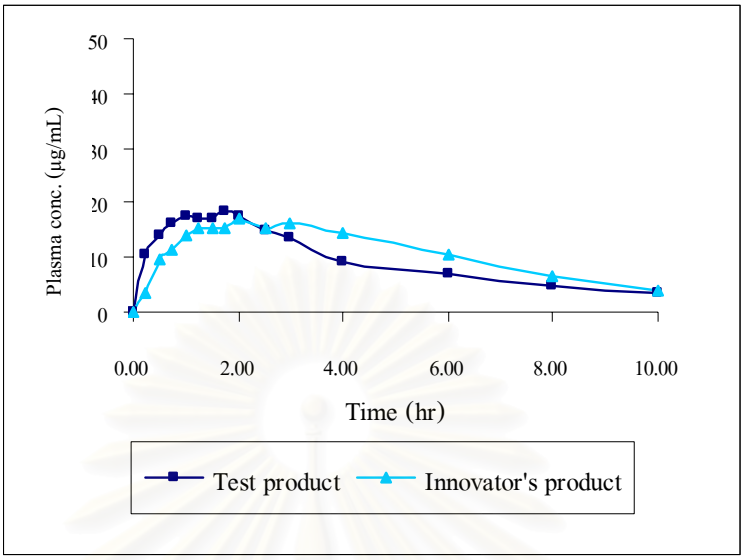


Figure 23 Plasma concentration vs. time profiles of subject No.17 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

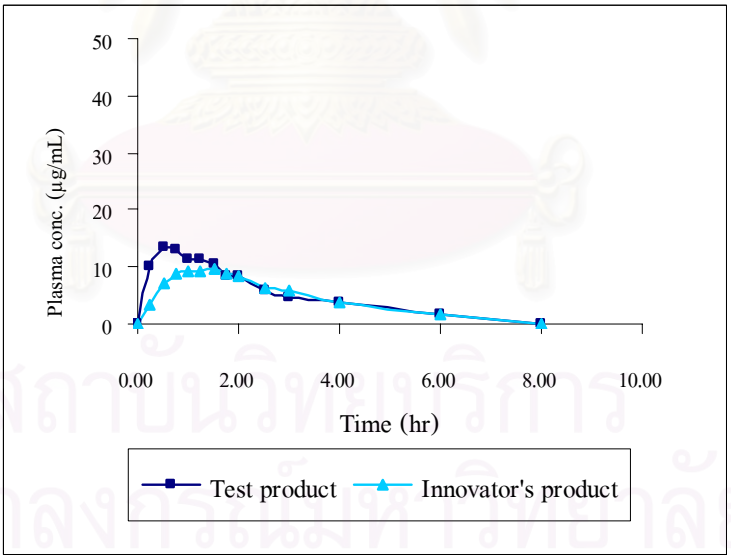
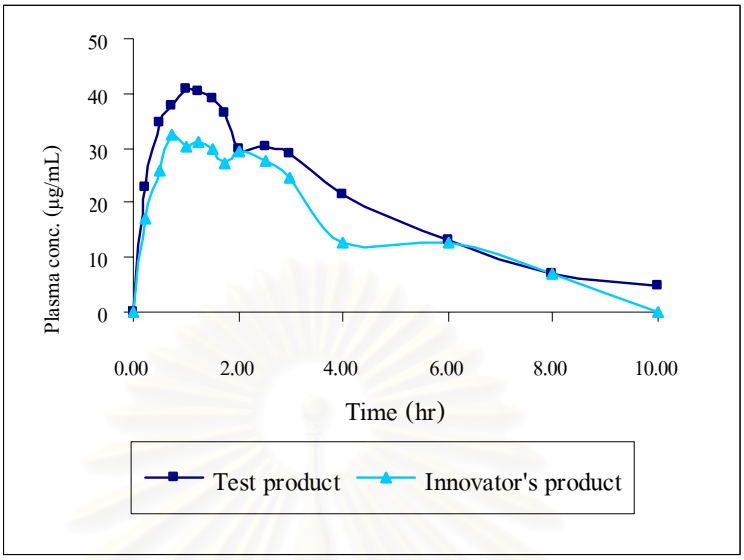


Figure 24 Plasma concentration vs. time profiles of subject No.18 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

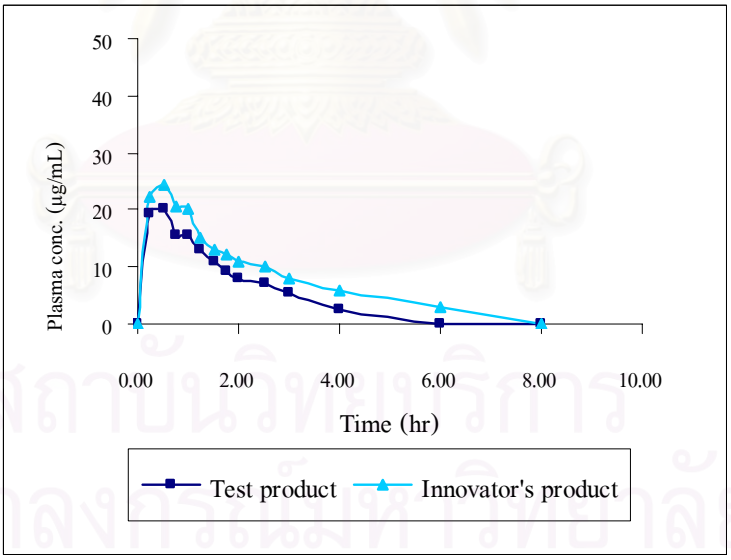
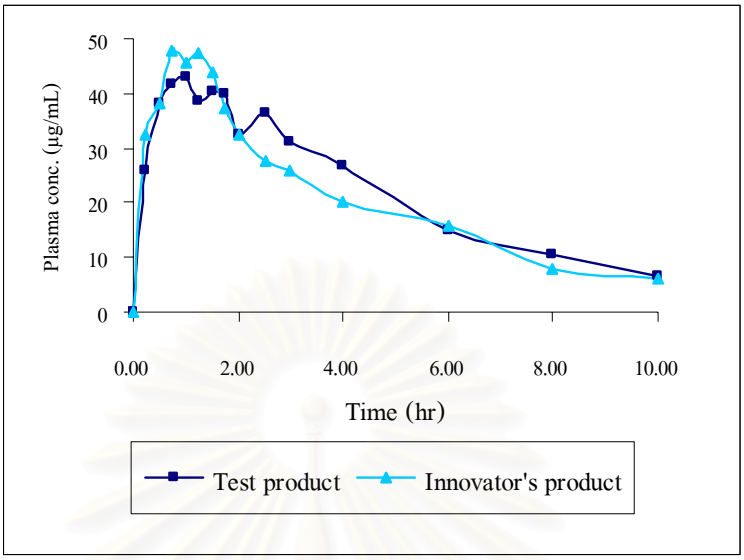


Figure 25 Plasma concentration vs. time profiles of subject No.19 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

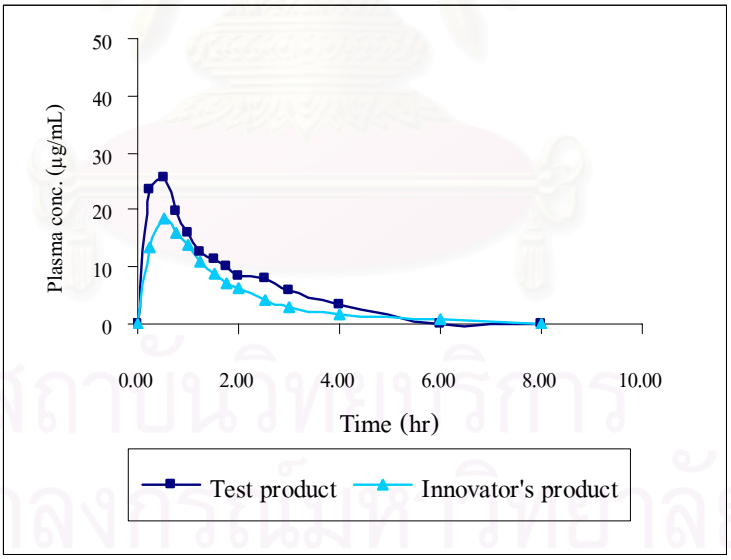
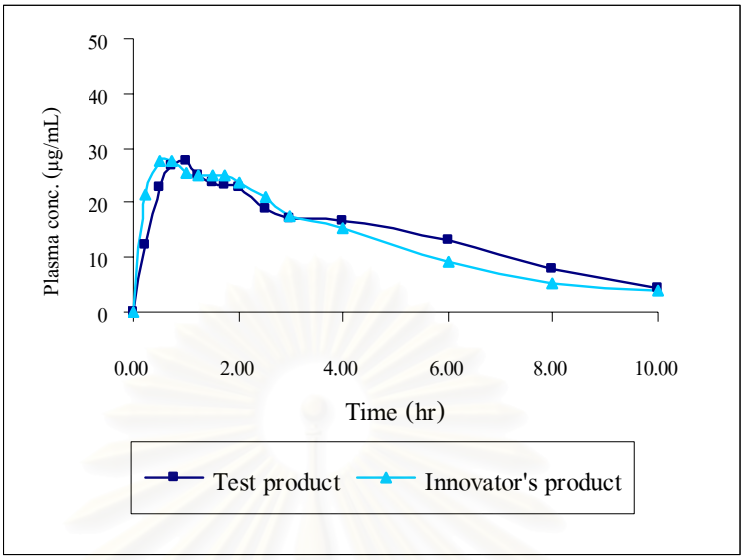


Figure 26 Plasma concentration vs. time profiles of subject No.20 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

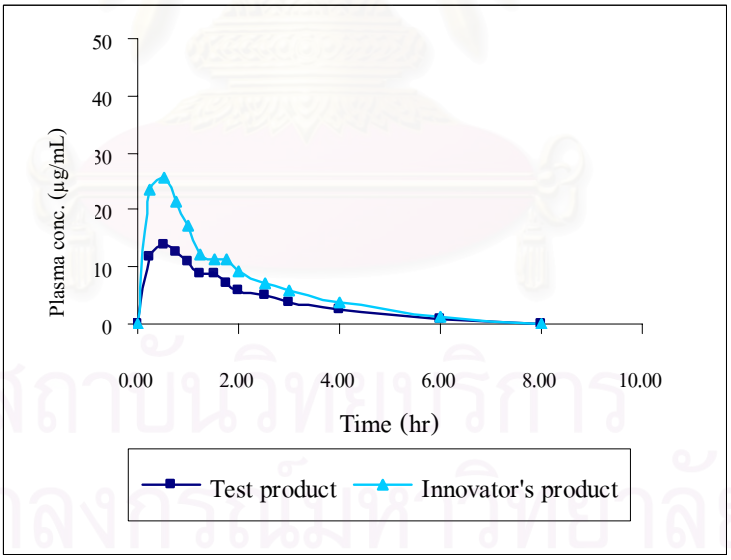
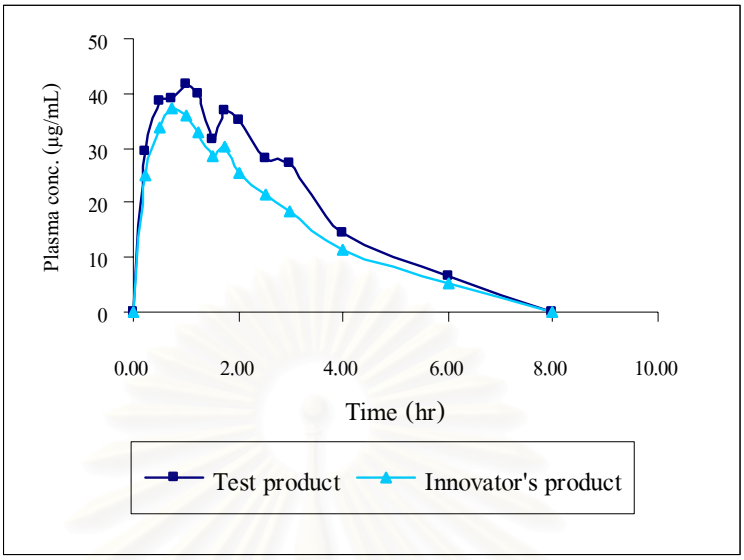


Figure 27 Plasma concentration vs. time profiles of subject No.21 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

A



B

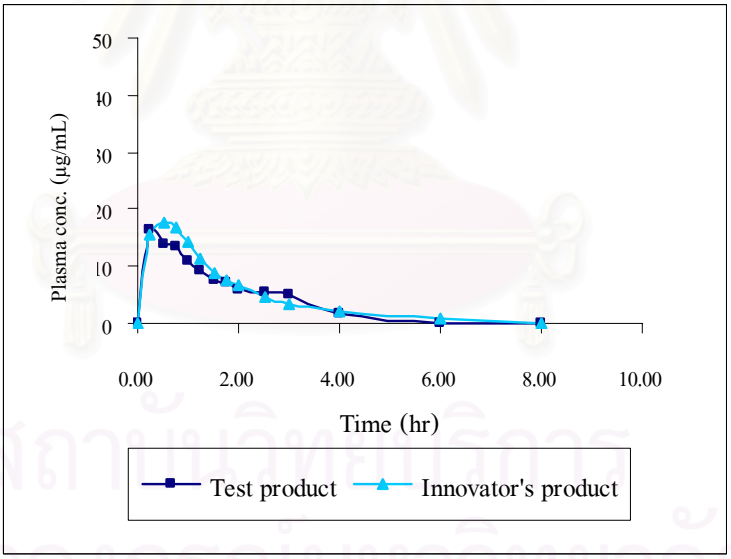


Figure 28 Plasma concentration vs. time profiles of subject No.22 following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of the test and innovator's products.

A: Cefoperazone

B: Sulbactam

Table 25 Mean Plasma Cefoperazone Concentration (μg/mL) of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's product

Time (hr)	Test Product	Innovator's Product
0	0.00	0.00
0.25	17.49	15.79
0.50	24.09	24.60
0.75	27.09	28.44
1.00	29.47	29.30
1.25	28.86	30.43
1.50	28.51	29.61
1.75	28.13	27.84
2.00	26.82	26.66
2.50	25.07	23.48
3	21.98	20.56
4	18.90	16.48
6	12.07	10.67
8	7.94	7.04
10	5.82	3.33

Table 26 Mean Plasma Sulbactam Concentration (μg/mL) of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's product

Time (hr)	Test Product	Innovator's Product
0	0	0
0.25	13.62	13.16
0.50	15.72	17.06
0.75	14.83	16.35
1.00	12.98	14.39
1.25	11.91	12.56
1.50	10.93	10.96
1.75	9.92	9.75
2.00	8.81	8.47
2.50	7.48	6.78
3	5.77	5.27
4	3.89	3.54
6	1.93	1.69
8	0	0
10	0	0

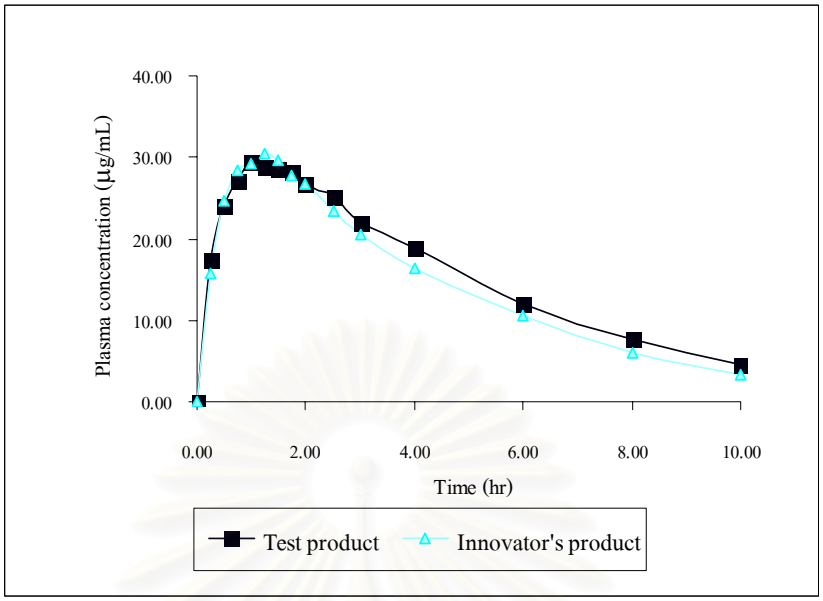


Figure 29 Mean plasma cefoperazone concentration-time profiles of 22 subjects following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of test and innovator's product

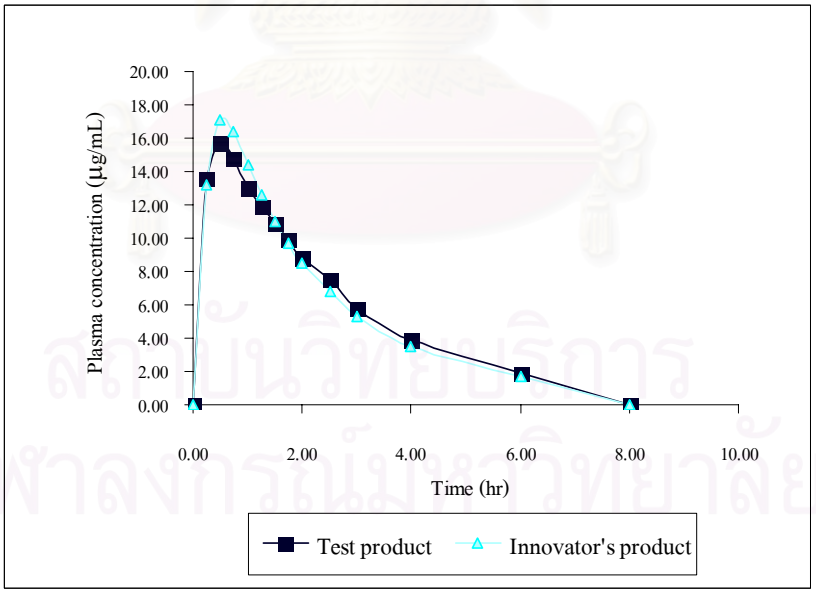


Figure 30 Mean plasma sulbactam concentration-time profiles of 22 subjects following intramuscular injection of 500/500 mg cefoperazone/sulbactam injections of test and innovator's product

5. Bioequivalence study

Bioequivalence of two formulations of the same drug reveals equivalence with respect to the rate and extent of drug absorption. AUC is accepted as a good indicator of the extent of absorption, whereas C_{max} and t_{max} are considered estimators of rate of absorption. The Thai FDA guideline have proposed that bioequivalence can only be assumed when characteristic parameters of bioavailability show no more than a defined difference (Thai FDA, 2000). When two formulations of the same drug or active moiety are pharmaceutically equivalent or pharmaceutical alternatives, bioequivalent in rate and extent of absorption, with respect to both efficacy and safety, adequately labeled and manufactured in compliance with Good Manufacturing Practice (GMP), it is assumed that they are therapeutically equivalent (Chow and Liu,1992).

In bioequivalence study, drug-products that are pharmaceutically equivalent are accepted to be bioequivalent when the ratios of the AUC and C_{max} values based on log-transformed data of test product relative to innovator's product are contained within 80-125% of 90% confidence interval. The time to peak plasma drug, a discontinuous variable, is analyzed, the comparison using mean proportional differences seems more appropriate. Table 46 summarized the results of statistical analysis for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}, respectively.

Elaborations of these principally relevant pharmacokinetic parameters obtained for bioavailability comparison were as follows:

5.1 Area under the plasma cefoperazone/sulbactam concentration-time curves (AUC_{0-t} and $AUC_{0-\infty}$)

The AUC_{0-t} and $AUC_{0-\infty}$ of cefoperazone and sulbactam for test and innovator's product from all subjects are presented in Table 27. Most subjects (20 from 22 subjects) had an AUC_{0-t} /$AUC_{0-\infty}$ ratio >80% for cefoperazone and sulbactam. To ensure a reliable estimation of the extent of absorption, a collection period of at least three half-lives is recommended by Thai Food and Drug Administration. This requisite was fulfilled, and the mean extrapolated area was well below 20% for both products, indicating sampling scheme was sufficiently long to ensure adequate description of the absorption phase and fully characterize the pharmacokinetic properties of cefoperazone and sulbactam. The extrapolated part of $AUC_{0-\infty}$ in some volunteers were over 20%, which might arise from error in evaluating elimination rate constant of the drug. Marzo et al.(1999) suggested that results obtained with AUC_{0-t} could conveniently support the

bioequivalence conclusion achieved with AUC when more than 20% of AUC is added to the extrapolation procedure in some volunteers.

For cefoperazone, the mean AUC_{0-t} values were 156.35 and 142.47 μg. hr/mL for test and innovator's products, respectively and those of sulbactam were 41.00 and 40.28 μg. hr/mL for test and innovator's products. The mean $AUC_{0-\infty}$ values of cefoperazone were 187.26 and 166.12 μg. hr/mL for test and innovator's products, respectively and those of sulbactam were 45.92 and 43.97 μg. hr/mL for for test and innovator's products as shown in Table 27. The AUC_{0-t} and $AUC_{0-\infty}$ of cefoperazone and sulbactam of test product were higher than those of innovator's product even though the percent content of sulbactam of test product was lower than that of innovator's product. This referred that the extent of drug absorption might be dependent on some specific factors like formulation as well as manufacturing process.

Analysis of variance for two way crossover designed based on log-transformed data of cefoperazone and sulbactam in Tables 28-31, showed that there were no statistically significant differences ($p>0.05$) in AUC_{0-t} and $AUC_{0-\infty}$ of cefoperazone of test and innovator's products for sequence and periods effects except subjects and formulation effects ($p<0.05$), indicating inter-subject variability. Although formulation effect was statistically significant difference from ANOVA, it had no effect on bioequivalent decision. For AUC_{0-t} and $AUC_{0-\infty}$ of sulbactam, there was no statistically significant differences ($p>0.05$) of test and innovator's products for sequence and formulation effects except subject and period effects ($p<0.05$), indicating inter-subject variability which were seen by wide variation of plasma sulbactam concentrations from plots of plasma drug concentrations versus time profiles.

Because of no information regarding the composition of ingredient in both formulations, significant difference in the extent of drug absorption for formulation effect was possibly dependent on physicochemical properties of the drug (particle size, crystal form), inactive excipients in formulation such as buffer, pH adjusting agents and tonicity adjusters. These critical factors affect absorption of drug. Cefoperazone and sulbactam ionized in sterile water for injection. After IM administration, the drug may precipitate at the injection site, resulting in prolonged absorption as the precipitated drug slowly redissoves in the tissue fluid. From in vitro evaluation, the pH of test product was different from that of innovator's product. This referred that they are different in purity of raw material and/or difference in formulation. Also, fraction of adipose tissue, variation in age and weight may contribute to these variations.

Two one-sided t-tests were performed on the ratios of mean of log AUC_{0-t} and log $AUC_{0-\infty}$ of cefoperazone and sulbactam of test product to those of innovator's products. 90% confidence interval for ratio of log AUC_{0-t} of cefoperazone and sulbactam of test product to those of innovator's product were 104.5-115.0 and 93.8-111.7%, respectively, as reported in Tables 28 and 29. The 90% confidence interval for ratio of log $AUC_{0-\infty}$ of cefoperazone and sulbactam of test product to those of innovator's product were 105.7-118.0% and 95.7-114.0%, respectively, as shown in Tables 30 and 31. These results were within the bioequivalence acceptance criteria. Therefore, with regard to cefoperazone and sulbactam, the results showed that the test product was bioequivalent to the innovator's product with respect to the extent of drug absorption.

5.2 Peak plasma cefoperazone/sulbactam concentration (C_{max})

The mean C_{max} of cefoperazone for test and innovator's products were 32.87 and 32.39 μg/mL, respectively and those of sulbactam were 17.64 and 18.57 μg/mL for both as shown in Table 32.

For cefoperazone, analysis of variance in Table 33 revealed that there were no statistically significant difference ($p>0.05$) between the log C_{max} values of both products for sequence, periods and formulation effects except subjects. The variations of plasma cefoperazone concentration in individual due to inter-subject variability were supported for these. For sulbactam, ANOVA in Table 34 showed that there was no statistically significant differences ($p>0.05$) of test and innovator's products for sequence and formulation effects except subject and period effects ($p<0.05$), indicating inter-subjects variability.

90% confidence interval for ratio of log C_{max} of cefoperazone and sulbactam of test product to those of innovator's product were 92.0-107.1 and 84.1-103.5%, respectively, as report in Tables 33 and 34. They also fell within the bioequivalence acceptance criteria. Thus, it could be concluded that the test product was bioequivalent with the innovator's product with respect to rate of drug absorption.

5.3 Time to peak plasma cefoperazone/sulbactam concentration (t_{max})

The mean time to peak plasma concentrations of cefoperazone from this study were 1.47 and 1.26 hours for test and innovator's product, respectively, and those of sulbactam were 0.69 and 0.63 hour, as presented in Table 35. This indicated that both products were rapidly absorbed. The t_{max} values of cefoperazone and sulbactam of test product were slightly higher than those of innovator's product. Factors responsible for these were the same as those for C_{max} values

Analysis of variance regarding t_{max} values of cefoperazone and sulbactam in Tables 36 and 37 showed that there were no statistically significant difference ($p>0.05$) from each other of both products for all effects. The difference of t_{max} of test relative to that of innovator's product was 16.26% and 10.88% for cefoperazone and sulbactam, respectively.

6. Bioequivalence evaluation

Statistical comparisons of AUC_{0-t}, $AUC_{0-\infty}$, C_{max} and t_{max} were summarized in Table 46 as well as the 90% confidence interval of principal parameter ratio of test product relative to innovator's product. Results were clearly indicated that no significant differences in the two brands of 500/500 mg cefoperazone/sulbactam intramuscular injection. 90% confidence intervals for bioavailability parameters were entirely within the Food and Drug Administration acceptance range. Both formulations of cefoperazone and sulbactam were well tolerated. No clinically relevant or drug-related side effects or dropouts of the subjects were encountered. We can conclude that the test product was bioequivalent to the innovator's product with respect to both the rate and the amount of drug absorption into systemic circulation. This finding suggests that the two products can be considered interchangeable in medical practice.

7. Related pharmacokinetic parameters

For cefoperazone, the mean elimination rate constant (k) were 0.225 and 0.253 hr^{-1}, the mean half-life ($t_{1/2}$) were 4.35 and 3.92 hr, and the mean residence time (MRT) were 5.37 and 4.70 hr for test and innovator's product, respectively, as shown in Table 38. For those of sulbactam, the corresponding values were 0.438 and 0.491 hr^{-1}, 1.68 and 1.46 hr, and 2.72 and 2.35 hr for test and innovator's product, respectively, as recorded in Table 39.

One-way analysis of variance of these values in Tables 40 -45, showed that there was no statistically significant differences ($p>0.05$) in k, $t_{1/2}$, and MRT values of cefoperazone and sulbactam, this result referred that clearance of cefoperazone and sulbactam was not difference between group of subject.

Table 27 Area Under the Plasma Concentration-time Curves (AUC_{0-t} and $AUC_{0-\infty}$) of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Products.

Subject no.	Cefoperazone				Sulbactam			
	AUC_{0-t} (μg.hr/mL)		$AUC_{0-\infty}$ (μg.hr/mL)		AUC_{0-t} (μg.hr/mL)		$AUC_{0-\infty}$ (μg.hr/mL)	
	Test Product	Innovator's Product	Test Product	Innovator's Product	Test Product	Innovator's Product	Test Product	Innovator's Product
1	103.70	93.86	114.37	106.47	34.90	39.42	36.92	42.52
2	157.70	145.68	170.55	157.73	63.30	49.98	66.88	52.85
3	184.81	186.22	204.94	212.40	53.44	54.07	58.25	58.56
4	103.59	117.43	118.32	139.13	47.51	44.09	53.25	47.11
5	129.10	129.47	148.62	139.71	40.41	31.63	45.22	34.67
6	208.30	202.67	238.35	246.39	50.22	27.47	52.62	29.64
7	133.12	125.73	152.31	139.05	43.26	38.12	46.88	41.78
8	123.62	125.88	140.15	141.22	38.97	22.91	42.31	26.77
9	187.92	141.09	236.44	153.81	38.25	54.21	45.17	56.28
10	206.33	174.11	259.90	210.87	35.36	50.31	38.90	54.92
11	216.37	181.61	281.31	220.96	39.42	28.82	43.76	31.87
12	164.27	173.65	263.78	236.61	64.22	36.23	85.59	41.52
13	173.68	111.18	191.06	126.78	34.45	42.03	36.80	44.76
14	121.60	132.26	175.13	152.59	41.22	43.26	50.94	48.48
15	107.53	110.66	116.28	121.77	33.08	45.86	35.20	4918
16	210.25	170.77	238.60	203.50	41.29	37.21	44.55	39.42
17	125.19	112.89	178.42	133.96	25.36	38.74	32.65	43.88
18	92.28	107.89	108.66	129.15	36.28	32.18	36.96	36.63
19	189.14	143.72	207.83	177.54	37.88	58.94	42.51	67.02
20	218.38	202.52	250.76	228.59	42.73	29.59	48.76	32.15
21	142.92	132.81	166.82	150.54	31.20	49.71	33.41	52.67
22	139.96	112.12	157.09	125.81	29.27	31.34	32.77	34.53
$\overline{X}$	156.35	142.47	187.26	166.12	41.00	40.28	45.92	43.97
S.D.	41.30	32.63	53.66	42.83	9.82	9.85	12.43	10.42
%C.V	26.42	22.90	28.66	25.78	23.95	24.45	27.07	23.69
$\overline{G}$	151.03	139.05	179.76	161.16	39.97	39.10	44.59	52.74
S.D.	0.12	0.10	0.13	0.11	0.10	0.11	0.10	0.45

$\overline{X}$ = Arithmetic Mean, $\overline{G}$ = Geometric Mean

Table 28 Analysis of Variance for Two-Way Crossover Study at $\alpha = 0.05$ of Log Area Under the Plasma Cefoperazone Concentration –time Curves (Log AUC_{0-t}) of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Products and 90% Confidence Interval for the Ratio of Log AUC_{0-t} Means

Source of Variation	d.f. [a]	SS [b]	MS [c]	F ratio [d]	F table [e]	Significance Level
Total	43	0.50952				
Sequence	1	0.02273	0.02273	1.05537	4.350	NS
Subject (seq)	20	0.43069	0.02153	12.81548	2.120	S
Period	1	0.00713	0.00713	4.24405	4.350	NS
Formulation	1	0.01528	0.01528	9.09523	4.350	S
Error	20	0.03369	0.00168			

Where; NS = Not significant difference at $p > 0.05$

S = Significant difference at $p < 0.05$

[a] = Degree of freedom

[b] = Sum of squares

[c] = Mean square

[d] = Variance ratio

[e] = F value obtained from the table

Products	Mean Log AUC_{0-t}	90% Confidence Interval
Test (T)	2.1941	104.5-115.0%
Innovator's (R)	2.1537	
T/R	1.0188	
Power = 99.90%		

Table 29 Analysis of Variance for Two-Way Crossover Study at $\alpha = 0.05$ of Log Area Under the Plasma Sulbactam Concentration –time Curves (Log AUC_{0-t}) of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's products and 90% Confidence Interval for the Ratio of Log AUC_{0-t} Means

Source of Variation	d.f. [a]	SS [b]	MS [c]	F ratio [d]	F table [e]	Significance Level
Total	43	0.45882				
Sequence	1	0.00218	0.00218	0.18940	4.350	NS
Subject (seq)	20	0.23029	0.01151	2.13148	2.120	S
Period	1	0.11711	0.11711	21.68704	4.350	S
Formulation	1	0.00120	0.00120	0.22222	4.350	NS
Error	20	0.10804	0.00540			

Where; NS = Not significant difference at $p > 0.05$

S = Significant difference at $p < 0.05$

[a] = Degree of freedom

[b] = Sum of squares

[c] = Mean square

[d] = Variance ratio

[e] = F value obtained from the table

Products	Mean Log AUC_{0-t}	90% Confidence Interval
Test (T)	1.6128	93.8-111.7%
Innovator's (R)	1.6051	
T/R	1.0048	
Power = 99.90%		

Table 30 Analysis of Variance for Two-Way Crossover Study at $\alpha = 0.05$ of Log Area Under the Plasma Cefoperazone Concentration –time Curves (Log $AUC_{0-\infty}$) of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Products and 90% Confidence Interval for the Ratio of Log $AUC_{0-\infty}$ Means

Source of Variation	d.f. [a]	SS [b]	MS [c]	F ratio [d]	F table [e]	Significance Level
Total	43	0.61096				
Sequence	1	0.03164	0.03164	1.24616	4.350	NS
Subject (seq)	20	0.50782	0.02539	11.80930	2.120	S
Period	1	0.00401	0.00401	1.86512	4.350	NS
Formulation	1	0.02458	0.02458	11.43256	4.350	S
Error	20	0.04291	0.00215			

Where; NS = Not significant difference at $p > 0.05$

S = Significant difference at $p < 0.05$

[a] = Degree of freedom

[b] = Sum of squares

[c] = Mean square

[d] = Variance ratio

[e] = F value obtained from the table

Products	Average Log $AUC_{0-\infty}$	90% Confidence Interval
Test (T)	2.255	105.7-118.0%
Innovator's (R)	2.207	
T/R	1.022	
Power = 99.90%		

Table 31 Analysis of Variance for Two-Way Crossover Study at $\alpha = 0.05$ of Log Area Under the Plasma Sulbactam Concentration–time Curves (Log $AUC_{0-\infty}$) of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Products and 90% Confidence Interval for the Ratio of Log $AUC_{0-\infty}$ Means

Source of Variation	d.f. [a]	SS [b]	MS [c]	F ratio [d]	F table [e]	Significance Level
Total	43	0.46396				
Sequence	1	0.00058	0.00058	0.04825	4.350	NS
Subject (seq)	20	0.24058	0.01202	2.25094	2.120	S
Period	1	0.11201	0.11201	20.97566	4.350	S
Formulation	1	0.00401	0.00401	0.75094	4.350	NS
Error	20	0.10678	0.00534			

Where; NS = Not significant difference at $p > 0.05$

S = Significant difference at $p < 0.05$

[a] = Degree of freedom

[b] = Sum of squares

[c] = Mean square

[d] = Variance ratio

[e] = F value obtained from the table

Products	Mean Log $AUC_{0-\infty}$	90% Confidence Interval
Test (T)	1.650	95.7-114.0%
Innovator (R)	1.631	
T/R	1.012	
Power = 99.90%		

Table 32 Peak Plasma Cefoperazone/Sulbactam Concentrations (C_{max}) of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Products.

Subject no.	C_{max} (μg/mL)			
	Cefoperazone		Sulbactam	
	Test Product	Innovator's Product	Test Product	Innovator's Product
1	25.84	26.14	15.13	18.81
2	46.80	33.26	34.42	27.09
3	37.56	37.13	26.39	21.69
4	18.74	29.44	12.49	17.78
5	38.62	30.97	25.98	16.32
6	38.56	37.11	20.17	16.34
7	34.72	36.52	17.80	31.66
8	24.69	27.32	17.02	11.49
9	29.54	37.67	12.96	23.81
10	45.74	35.37	14.55	20.46
11	34.01	26.98	11.39	10.62
12	26.85	29.24	15.54	13.69
13	37.08	39.87	13.14	19.25
14	17.16	25.79	12.45	16.49
15	29.90	34.40	14.39	14.68
16	46.85	41.14	26.80	16.62
17	19.02	21.78	8.13	16.21
18	18.21	17.15	13.46	9.52
19	40.83	32.58	20.29	24.42
20	43.00	47.65	25.49	18.59
21	27.75	27.75	13.79	25.43
22	41.68	37.29	16.32	17.56
$\overline{X}$	32.87	32.39	17.64	18.57
S.D.	9.66	6.96	6.46	5.46
% C.V.	29.39	21.49	36.61	29.40
$\overline{G}$	31.37	31.63	16.64	17.81
S.D.	0.14	0.10	0.15	0.13

$\overline{X}$ = Arithmetic Mean, $\overline{G}$ = Geometric Mean

Table 33 Analysis of Variance for Two-Way Crossover Study at $\alpha = 0.05$ of Log Peak Plasma Cefoperazone Concentrations(Log C_{max}) of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Products and 90% Confidence Interval for the Ratio of Log C_{max} Means

Source of Variation	d.f. [a]	SS [b]	MS [c]	F ratio [d]	F table [e]	Significance Level
Total	43	0.63292				
Sequence	1	0.00058	0.00058	0.02121	4.350	NS
Subject (seq)	20	0.54684	0.02734	6.58800	2.120	S
Period	1	0.00233	0.00233	0.56145	4.350	NS
Formulation	1	0.00008	0.00008	0.01928	4.350	NS
Error	20	0.08309	0.00415			

Where; NS = Not significant difference at $p > 0.05$

S = Significant difference at $p < 0.05$

[a] = Degree of freedom

[b] = Sum of squares

[c] = Mean square

[d] = Variance ratio

[e] = F value obtained from the table

Products	Mean Log C_{max}	90% Confidence Interval
Test (T)	1.497	92.0-107.1%
Innovator (R)	1.500	
T/R	0.998	
Power = 99.90%		

Table 34 Analysis of Variance for Two-Way Crossover Study at $\alpha = 0.05$ of Log Peak Plasma Sulbactam Concentration (Log C_{max}) of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Products and 90% Confidence Interval for the Ratio of Log C_{max} Means

Source of Variation	d.f. [a]	SS [b]	MS [c]	F ratio [d]	F table [e]	Significance Level
Total	43	0.83364				
Sequence	1	0.00038	0.00038	0.01404	4.350	NS
Subject (seq)	20	0.54131	0.02707	3.54784	2.120	S
Period	1	0.12982	0.12982	17.01442	4.350	S
Formulation	1	0.00960	0.00960	1.25820	4.350	NS
Error	20	0.15253	0.00763			

Where; NS = Not significant difference at $p > 0.05$

S = Significant difference at $p < 0.05$

[a] = Degree of freedom

[b] = Sum of squares

[c] = Mean square

[d] = Variance ratio

[e] = F value obtained from the table

Products	Mean Log C_{max}	90% Confidence Interval
Test (T)	1.221	84.1-103.5%
Innovator (R)	1.251	
T/R	0.976	
Power = 99.90%		

Table 35 Time to Peak Plasma Cefoperazone/Sulbactam Concentration (t_{max}) of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Products.

Subject no.	t_{max} (hr)			
	Cefoperazone		Sulbactam	
	Test Product	Innovator's Product	Test Product	Innovator's Product
1	1.25	2.00	0.50	0.50
2	1.00	0.75	0.50	0.50
3	0.50	1.00	0.25	0.50
4	1.75	1.25	1.50	0.75
5	0.50	1.50	0.50	0.75
6	1.50	1.00	0.50	0.25
7	1.00	1.25	0.50	0.25
8	1.50	1.25	0.75	0.50
9	1.75	1.00	1.00	0.50
10	1.25	1.75	0.50	0.50
11	2.50	1.50	1.75	0.75
12	1.50	2.00	1.25	0.75
13	1.25	1.25	0.75	0.50
14	4.00	1.50	0.75	1.00
15	1.75	1.25	0.50	0.75
16	1.00	1.00	0.25	0.75
17	2.50	1.50	1.25	0.75
18	1.75	2.00	0.50	1.50
19	1.00	0.75	0.50	0.50
20	1.00	0.75	0.50	0.50
21	1.00	0.75	0.50	0.50
22	1.00	0.75	0.25	0.50
Mean	1.47	1.26	0.69	0.63
S.D.	0.77	0.42	0.41	0.26
% C.V.	52.44	33.21	58.82	42.31

Table 36 Analysis of Variance for Two-Way Crossover Study at $\alpha = 0.05$ of Time to Peak Plasma Cefoperazone Concentrations (t_{max}) of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Products and Their Differences.

Source of Variation	d.f. [a]	SS [b]	MS [c]	F ratio [d]	F table [e]	Significance Level
Total	43	16.55682				
Sequence	1	0.05114	0.05114	0.09912	4.350	NS
Subject (seq)	20	10.31818	0.51591	1.80875	2.120	NS
Period	1	0.02273	0.02273	0.07969	4.350	NS
Formulation	1	0.46023	0.46023	1.61354	4.350	NS
Error	20	5.70454	0.28523			

Where; NS = Not significant difference at $p > 0.05$

[a] = Degree of freedom

[b] = Sum of squares

[c] = Mean square

[d] = Variance ratio

[e] = F value obtained from the table

Difference of t_{max} values of test vs. innovator's product $= \frac{(1.466-1.261)}{1.261} \times 100$

$= 16.26\%$

Table 37 Analysis of Variance for Two-Way Crossover Study at $\alpha = 0.05$ of Time to Peak Plasma Sulbactam Concentrations (t_{max}) of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Products and Their Differences.

Source of Variation	d.f. [a]	SS [b]	MS [c]	F ratio [d]	F table [e]	Significance Level
Total	43	5.01136				
Sequence	1	0.02273	0.02273	0.15536	4.350	NS
Subject (seq)	20	2.92614	0.14631	1.49281	2.120	NS
Period	1	0.05113	0.05113	0.52168	4.350	NS
Formulation	1	0.05113	0.05113	0.52168	4.350	NS
Error	20	1.96023	0.09801			

Where; NS = Not 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} values of test vs. innovator's product $= \frac{(0.693-0.625)}{0.625} \times 100$

$= 10.88\%$

Table 38 Pharmacokinetic Parameters; k, $t_{1/2}$, MRT of Cefoperazone of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Products

Subject no.	k (hr^{-1})		$t_{1/2}$ (hr)		MRT (hr)	
	Test Product	Innovator's Product	Test Product	Innovator's Product	Test Product	Innovator's Product
1	0.305	0.420	3.58	1.65	3.58	3.05
2	0.249	0.370	4.44	1.87	4.44	3.15
3	0.359	0.417	3.28	1.66	3.28	2.85
4	0.216	0.235	5.29	2.95	5.29	4.61
5	0.224	0.229	4.71	3.02	4.71	4.60
6	0.127	0.169	8.70	4.11	8.70	6.21
7	0.265	0.322	4.10	2.15	4.10	3.42
8	0.247	0.211	4.36	3.28	4.36	4.96
9	0.158	0.322	6.71	2.15	6.71	3.44
10	0.196	0.190	5.95	3.65	5.95	5.79
11	0.185	0.221	5.50	3.14	5.50	4.73
12	0.102	0.141	6.83	7.60	10.46	7.60
13	0.299	0.263	2.32	4.10	3.74	4.10
14	0.142	0.210	4.89	5.32	8.41	5.32
15	0.219	0.206	3.17	5.03	4.58	5.03
16	0.301	0.282	2.30	4.08	3.38	4.08
17	0.193	0.181	3.59	5.85	5.24	5.85
18	0.206	0.182	3.36	6.06	5.36	6.06
19	0.212	0.238	3.27	4.58	5.03	4.58
20	0.208	0.230	3.33	4.66	5.04	4.66
21	0.165	0.154	4.20	6.44	7.26	6.44
22	0.374	0.382	1.85	2.86	2.94	2.86
Mean	0.225	0.253	4.35	3.92	5.37	4.70
S.D.	0.071	0.083	1.65	1.65	1.91	1.28
%C.V.	31.69	32.81	37.99	42.10	35.57	27.23

Table 39 Pharmacokinetic Parameters; k, $t_{1/2}$, MRT of Sulbactam of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Products

Subject no.	k (hr^{-1})		$t_{1/2}$ (hr)		MRT (hr)	
	Test Product	Innovator's Product	Test Product	Innovator's Product	Test Product	Innovator's Product
1	0.519	0.481	1.34	1.44	2.17	2.29
2	0.493	0.483	1.40	1.44	2.32	2.27
3	0.501	0.415	1.38	1.67	2.24	2.64
4	0.303	0.483	2.28	1.44	3.87	2.29
5	0.371	0.480	1.87	1.44	2.12	2.26
6	0.303	0.372	2.28	1.86	4.04	3.06
7	0.462	0.513	1.50	1.35	2.37	2.11
8	0.551	0.371	1.26	1.87	1.93	2.85
9	0.344	0.510	2.02	1.36	3.25	2.15
10	0.418	0.427	1.66	1.62	3.58	2.64
11	0.472	0.497	1.47	1.39	2.28	2.12
12	0.232	0.378	2.98	1.83	4.75	2.92
13	0.530	0.533	1.31	1.30	2.01	2.08
14	0.283	0.410	2.45	1.69	3.76	2.85
15	0.437	0.445	1.59	1.56	2.40	2.45
16	0.614	0.665	1.13	1.04	1.77	1.80
17	0.442	0.652	1.57	1.06	2.38	1.65
18	0.393	0.384	1.76	1.80	3.03	3.11
19	0.461	0.529	1.50	1.31	2.40	2.13
20	0.543	0.701	1.28	0.99	1.97	1.66
21	0.438	0.427	1.58	1.62	3.18	2.64
22	0.528	0.650	1.31	1.07	1.95	1.74
Mean	0.438	0.491	1.68	1.46	2.72	2.35
S.D.	0.099	0.099	0.46	0.27	0.83	0.44
%C.V.	22.60	20.16	27.58	18.44	30.51	18.72

Table 40 One-Way Analysis of Variance at $\alpha = 0.05$ of Elimination Rate Constant (k) of cefoperazone of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Products.

Source of Variation	d.f. [a]	SS [b]	MS [c]	F ratio [d]	F table [e]	Significance Level
Total	43	0.261				
Fomulation	1	0.009	0.009	1.50	4.070	NS
Error	42	0.252	0.006			

Table 41 One-Way Analysis of Variance at $\alpha = 0.05$ of Elimination Rate Constant (k) of sulbactam of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Products.

Source of Variation	d.f. [a]	SS [b]	MS [c]	F ratio [d]	F table [e]	Significance Level
Total	43	0.441				
Formulation	1	0.031	0.031	3.100	4.070	NS
Error	42	0410	0.010			

Where; NS = Not significant difference at $p > 0.05$

[a] = Degree of freedom

[b] = Sum of squares

[c] = Mean square

[d] = Variance ratio

[e] = F value obtained from the table

Table 42 One-Way Analysis of Variance at $\alpha = 0.05$ of Elimination Half-Life ($t_{1/2}$) of Cefoperazone of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Products.

Source of Variation	d.f. [a]	SS [b]	MS [c]	F ratio [d]	F table [e]	Significance Level
Total	43	116.611				
Formulation	1	2.060	2.060	0.755	4.070	NS
Error	42	114.552	2.727			

Table 43 One-Way Analysis of Variance at $\alpha = 0.05$ of Elimination Half-Life ($t_{1/2}$) of Sulbactam of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Products.

Source of Variation	d.f[a].	SS [b]	MS [c]	F ratio [d]	F table [e]	Significance Level
Total	43	6.539				
Formulation	1	0.517	0.517	3.615	4.070	NS
Error	42	6.022	0.143			

Where; NS = Not significant difference at p> 0.05

[a] = Degree of freedom

[b] = Sum of squares

[c] = Mean square

[d] = Variance ratio

[e] = F value obtained from the table

Table 44 One-Way Analysis of Variance at $\alpha = 0.05$ of Mean Residence Time (MRT) of Cefoperazone of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Products.

Source of Variation	d.f. [a]	SS [b]	MS [c]	F ratio [d]	F table [e]	Significance Level
Total	43	116.327				
Formulation	1	4.891	4.891	1.844	4.070	NS
Error	42	111.436	2.653			

Table 45 One-Way Analysis of Variance at $\alpha = 0.05$ of Mean Residence Time (MRT) of Sulbactam of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Products.

Source of Variation	d.f. [a]	SS [b]	MS [c]	F ratio [d]	F table [e]	Significance Level
Total	43	20.050				
Formulation	1	1.476	1.476	3.339	4.070	NS
Error	42	18.574	0.442			

Where; NS = Not significant difference at $p > 0.05$

[a] = Degree of freedom

[b] = Sum of squares

[c] = Mean square

[d] = Variance ratio

[e] = F value obtained from the table

Table 46 Pharmacokinetic Parameters (Mean ± S.D.) of Cefoperazone and Sulbactam of 22 Subjects Following Intramuscular Injection of 500/500 mg Cefoperazone/Sulbactam Injections of Test and Innovator's Product.

Active Ingredients	Pharmacokinetic Parameters	Test Product	Innovator's Product	90% Confidence Interval
Cefoperazone	AUC_{0-t} (μg.hr/mL)	156.35 ± 41.30	142.47 ± 32.63	104.5-115.0%
	$AUC_{0-\infty}$ (μg.hr/mL)	187.26 ± 53.66	166.12 ± 42.83	105.7-118.0%
	C_{max} (μg/mL)	32.87 ± 9.66	32.39 ± 6.96	92.0-107.1%
	t_{max} (hr)	1.47 ± 0.77	1.26 ± 0.42	-
	k (hr^{-1})	0.225 ± 0.07	0.253 ± 0.08	-
	$t_{1/2}$ (hr)	4.35 ± 1.65	3.92 ± 1.65	-
	MRT (hr)	5.37 ± 1.91	4.70 ± 1.28	-
Sulbactam	AUC_{0-t} (μg.hr/mL)	41.00 ± 9.82	40.28 ± 9.85	93.8-111.7%
	$AUC_{0-\infty}$ (μg.hr/mL)	45.92 ± 12.43	43.97 ± 10.42	95.7-114.0%
	C_{max} (μg/mL)	17.64 ± 6.46	18.57 ± 5.46	84.1-103.5%
	t_{max} (hr)	0.69 ± 0.41	0.63 ± 0.26	-
	k (hr^{-1})	0.438 ± 0.10	0.491 ± 0.10	-
	$t_{1/2}$ (hr)	1.68 ± 0.46	1.46 ± 0.27	-
	MRT (hr)	2.72 ± 0.83	2.35 ± 0.44	-

8. Comparison of pharmacokinetic parameters of cefoperazone and sulbactam

Previous reports revealed that when cefoperazone and sulbactam in combination were administered in the anticipated clinical dosing regimen, pharmacokinetic and safety profiles are similar to those for the individual agents administered separately (Reitberg et al., 1988). The pharmacokinetic parameters obtained from this study were compared to those of previous studies. Comparisons are displayed in Table 47. It is clearly seen that $AUC_{0-\infty}$ values of cefoperazone and sulbactam from this study agree with those of Foulds et al., 1983 and Reitberg et al., 1988. These values are somewhat higher than the values found by Brogden et al. (1981). For C_{max} values, this study agrees with previous studies by Craig and Gerber (1981) and Foulds et al. (1983) for cefoperazone and sulbactam from, respectively. In contrast, C_{max} value of cefoperazone was lower than that of reported by Brogden et al.,1981; Foulds et al., 1983; Reitberg et al.,1988 and Schwartz et al., 1988 due to the difference of route of administration. The IV bolus injection and IV infusion of a drug are directly administered into blood circulation resulting in rapid and high peak plasma of drug concentration.

The elimination half-life ($t_{1/2}$) of cefoperazone and sulbactam from this study were longer than those of previous studies. This result is probably related to variability of metabolism in difference nation. From this finding, the maximum plasma drug concentrations as well as the clearances of cefoperazone and sulbactam many provide information to adjust drug dosage regimens in Thai patients.

Table 47 Summary of Some Pharmacokinetic Parameters of Cefoperazone and Sulbactam Obtained from the Present Study and Previously Published Reports.

Drug	C_{max} (μg/mL)	$AUC_{0-\infty}$ (μg.hr/mL)	$t_{1/2}$ (hr)	Remark	Reference
cefoperazone 0.5 g +	32.4 ± 7.0	166.16 ± 42.83	4.1 ± 1.6	healthy volunteers	Present study
sulbactam 0.5 g IM	18.57 ± 5.5	43.97 ± 10.42	1.5 ± 0.4		
cefoperazone 1g IV[a]	140-200	200	1.6-2.1	healthy volunteers	Brogden et al.,
cefoperazone 1g IV[b]	138-158	-			1981
cefoperazone 2 g IV[a]	250-375	406			
cefoperazone 2 g IV[b]	223-253	-			
cefoperazone 1g IV[b]	153	-	1.6-2.4	healthy volunteers	Craig and Gerber,
cefoperazone 1 g IM	65	-			1981
cefoperazone 2 g IM	111	-			
cefoperazone 2 g IV[a]	266	449	-	healthy volunteers	Foulds et al., 1983
sulbactam 0.5 g IV[a]	31.8	39.6	-		
sulbactam 0.5 g IM	14.2	35.5	-		
cefoperazone 2 g +	254	439.0	-		
sulbactam 1 g IV[a]	38.3	53.5	-		
cefoperazone 1 g +	91.4 ± 36.6	-	-	patients with benign	Bawdon and
sulbactam 0.5 g IV[b]	29.6 ± 10.3	-	-	prostate hyperplasia or	Madsen, 1986
			-	cancer	
sulbactam 0.5 g IV[a]	-	43.67	0.99	healthy volunteers	Deborah et al., 1987
cefoperazone 1 g +	150.7	208.6	1.57	healthy volunteers	Drugs of today,
sulbactam 1 g IV [b]	65.5	52.1	0.78		1987
cefoperazone 3 g IV [b]	430.9 ± 42.1	782.9 ± 104.7	1.8 ± 0.3	healthy volunteers	Reitberg et al.,
cefoperazone 3 g +	416.1 ± 50	756.7 ± 121.6	1.8 ± 0.3		1988
sulbactam 1.5 g IV [b]	88.3 ± 27.6	88.6 ± 21.8	1.0 ± 0.3		
sulbactam 1.5 g IV [b]	83.4 ± 25.3	77.7 ± 22.9	1.1 ± 0.3		
cefoperazone 2 g +	280.9 ± 21.2	564.9 ± 254	2.1 ± 0.8	patients on CAPD	Johnson et al.,
sulbactam 1 g IV [b]	82.2 ± 16.2	521.9 ± 86.5	6.9 ± 1.7		1988
cefoperazone 2 g +	298 ± 145	1247 ± 353	7.0 ± 3.5	infected elderly patients	Schwartz et al.,
sulbactam 1 g IV [b]	110 ± 77	228 ± 115	3.4 ± 1.2		1988
cefoperazone 2 g +	-	356 ± 52	1.6 ± 0.3	normal subjects	Reitberg et al.,
	-	672 ± 333	2.4 ± 1.1	impaired renal subjects	1988
	-	542 ± 457	2.8 ± 2.4	end stage renal subjects	
sulbactam 1 g IV [b]	-	64 ± 11	1.0 ± 0.2	normal subjects	
	-	411 ± 182	4.6 ± 2.2	impaired renal subjects	
	-	709 ± 271	9.7 ± 5.3	end stage renal subjects	
cefoperazone 1 g IM	65-75	-	1.6-2.4	healthy volunteers	USP DI, 2003

[a] = Bolus dose , [b] = Infusion

CHAPTER V

CONCLUSIONS

The bioequivalence of a local brand of 500/500 mg cefoperazone/sulbactam intramuscular injection commercially available in Thailand as compared to innovator's product was established. The results were concluded as follows;

1. *In Vitro* Evaluation:

Both commercial brands of cefoperazone and sulbactam for injection were determined following the validated methods and found that they completely complied the specification requirements. These could be concluded that they were pharmaceutical equivalence.

2. *In vivo* studies:

2.1 Development of HPLC method

A simple and selective high-performance liquid chromatographic method for the determination of cefoperazone and sulbactam in human plasma samples has been developed and validated. Cefoperazone and salicylic acid (internal standard) were extracted from human plasma by protein precipitation using acetonitrile followed by centrifugation. Aliquots of the supernatant were analysed by reversed-phase high-performance liquid chromatography (HPLC). The analysis was performed on a μ- Bondapak® C_{18} column, using a mixture of acetonitrile, tetrabutyl ammonium hydroxide and phosphate buffer (pH3.5) as mobile phase with ultraviolet detection at 215 nm. For the determination of sulbactam, it was reacted with imidazole to yield a product having an ultraviolet absorption maximum at 320 nm. The product was separated using reversed-phase HPLC from regular components of plasma with an ion-paired buffer at 50ºC. Coexisting cefoperazone did not interfere in the sulbactam assay. The validation of these analytical methods demonstrated specificity, good precision and accuracy. These validated methods were successfully applied for bioequievalance studies in human.

2.2 Bioequivalence study

The comparative bioavailability of local brand of 500/500 mg cefoperazone/ sulbactam intramuscular injection relative to the innovator's product was studied in 22 healthy Thai male volunteers. A single dose of 500/500 mg cefoperazone/sulbactam injection was IM

injection to each subject in a crossover manner with 1 week washout period between each administration. Plasma concentrations of cefoperazone and sulbactam were determined by validated HPLC method. Individual plasma concentration-time profiles were analyzed using graphical method. The observed value of relevant pharmacokinetic parameters; area under the plasma concentration-time curve (AUC) and peak plasma concentration (C_{max}) of cefoperazone and sulbactam were used for bioavailability comparison.

For cefoperazone, the mean AUC_{0-t} values were 156.35 and 142.47 μg. hr/mL, the mean $AUC_{0-\infty}$ values were 187.26 and 166.12 μg. hr/mL, and the mean C_{max} were 32.87 and 32.39 μg/mL, The mean time to peak plasma concentrations (t_{max}) were 1.47 and 1.26 hours for test and innovator's products, respectively. Analysis of variance of these pharmacokinetic parameters of test and innovator's product presented statistically significant difference in subject and formulation effects, indicating inter-subject variability. These referred that the extent and rate of cefoperazone absorption varies widely and depend on formulation and biopharmaceutical factors. 90% confidence interval of log transformed data of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} of local brand product relative to those of innovator's product were entirely within the Food and Drug Administration acceptance range (80-125%).

For sulbactam, the mean AUC_{0-t} values were 41.00 and 40.28 μg. hr/mL, the mean $AUC_{0-\infty}$ values were 45.92 and 43.97 μg. hr/mL, and the mean C_{max} were 17.64 and 18.57 μg/mL, The mean t_{max} were 0.69 and 0.63 hour for test and innovator's products, respectively. Analysis of variance of these pharmacokinetic parameters of test and innovator's product presented no statistically significant difference in formulation effects, 90% confidence interval of log transformed data of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} of local brand product relative to those of innovator's product were entirely within the Food and Drug Administration acceptance range (80-125%).

Subjects well tolerated to both products; none withdrew from the study or exhibited signs of allergy and adverse drug reactions to cefoperazone and/or sulbactam. Based on the pharmacokinetic parameters, statistical results and safety profiles of both drugs from this study, it can be concluded that local brand product was bioequivalent to the innovator's product and the two products could be considered interchangeably in medical practice.

2.3 Comparison of pharmacokinetic parameters of cefoperazone and sulbactam in healthy Thai male volunteers relative to the finding of previous studies that were published in the journals:

The plasma concentration-time curve ($AUC_{0-\infty}$) and the mean peak plasma concentrations (C_{max}) of cefoperazone and sulbactam obtained from this study were consistent with previous studies. It was concluded that pharmacokinetic parameters due to rate and extent of cefoperazone/sulbactam absorption did not show any unusual pharmacokinetics values except the elimination half-life which was higher than that of earlier studies due to variability of metabolism in difference nation.

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APPENDICES

APPENDIX A

Table 48 Drug-Products

Brand name	Manufacturer	Batch No.	Mfg. date	Exp. date
Sulcef®	Siam Bhesach Co.,Ltd., Thailand	J49CFA02/05	09-11-04	09-11-07
Sulperazon®	Pfizer Italia S.r.l., Latina, Italy	439272	01-2004	09-2006

APPENDIX B

Table 49 Analysis of Variation for Two Way Crossover Design

Source of variation	d.f.	Sum of squares	Mean squares	F_{ratio}
Total	2n-1	SStotal	-	-
Sequence	1	SSsequence	MSG = SSsequence/d.f.	MSG/MSS
Subjects (seq)	n-2	SSsubject	MSS = SSsubject/d.f.	MSS/MSE
Period	1	SSperiod	MSP = SSperiod/d.f.	MSP/MSE
Formulation	1	SSformulation	MSF = SSformulation/d.f.	MSF/MSE
Error	n-2	SSerror	MSE = SSerror/d.f.	-

Where; d.f. = Degree of freedom

F_{ratio} = F calculation

n = number of subjects

SStotal = Sum of square total

SSsequence = Sum of square sequence

SSsubject = Sum of square subject

SSperiod = Sum of square period

SSformulation= Sum of square formulation

SSerror = Sum of square error

MSG = Mean square sequence

MSS = Mean square subject

MSP = Mean square period

MSF = Mean square formulation

MSE = Mean square error

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

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

where; $\overline{X}_T$ and $\overline{X}_R$ = Mean log AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} values of test and innovator's product respectively.

$t_{0.1,df}$ = 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

$$\%\ Lower\ limit = [\ antilog\ (\overline{X}_T - \overline{X}_R) - (t_{0.1,df} \times S.E.)\ ] \times 100$$

$$\%\ Upper\ limit = [\ antilog\ (\overline{X}_T - \overline{X}_R) + (t_{0.1,df} \times S.E.)\ ] \times 100$$

Table 50 Example of ANOVA Calculation for Log C_{max} of Cefoperazone

Sequence	Subject	Innovator's Product		Test Product		Subject Total
I	1	1.42		1.41		2.83
	4	1.47		1.27		2.74
	7	1.56		1.54		3.10
	9	1.58	Period I	1.47	Period II	3.05
	10	1.55		1.66		3.21
	13	1.60		1.60		3.20
	15	1.54		1.48		3.02
	16	1.61		1.67		3.28
	17	1.34		1.28		2.62
	19	1.51	SUM	1.61	SUM	3.12
	21	1.44	16.62	1.44	16.43	2.88
II	2	1.52		1.67		3.19
	3	1.57		1.57		3.14
	5	1.49		1.59		3.08
	6	1.57		1.59		3.16
	8	1.44	Period II	1.39	Period I	2.83
	11	1.43		1.53		2.96
	12	1.47		1.43		2.90
	14	1.41		1.23		2.64
	18	1.23		1.26		2.49
	20	1.68	SUM	1.63	SUM	3.31
	22	1.57	16.38	1.62	16.51	3.19
Formulation Total		33.00		32.94		65.94
Mean of Formulation		1.500		1.497		

Period I total = 33.13 (16.62+16.51)

Period II total = 32.81 (16.43+16.38)

Correction term = $(65.94)^2/44$ = 98.82008

SStotal = $[(1.42)^2+(1.60)^2+\ldots+(1.62)^2]$ – C.T. = 0.63292

SSsequence = $[(2.83+3.20+\ldots+2.88)^2+ (2.90+3.19+\ldots+3.19)^2]/22$ – C.T= 0.00058

SSsubject = $[(2.83)^2+ (3.20)^2+\ldots+ (3.19)^2]/2$ - 0.00058 – C.T. = 0.54684

SSperiod = $[(33.13)^2+ (32.81)^2]/22$ – C.T. = 0.00233

SSformulation = $[(33.00)^2+ (32.94)^2]/22$ – C.T. = 0.00008

SSerror = 0.63292 – 0.00058 – 0.54684 – 0.00233 – 0.00008 = 0.08309

Analysis of variance for two way crossover design

Source of Variation	d.f.	SS	MS	F ratio	F table	Significance Level
Total	43	0.63292				
Sequence	1	0.00058	0.00058	0.02121	4.350	NS
Subject (seq)	20	0.54684	0.02734	6.58800	2.120	S
Period	1	0.00233	0.00233	0.56145	4.350	NS
Formulation	1	0.00008	0.00008	0.01928	4.350	NS
Error	20	0.08309	0.00415			

Where; F table obtained from the table of F ratio for 0.05 level of significance.

The 90% confidence interval was constructed as follow;

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

Lower limit = Antilog $[(\overline{X}_T-\overline{X}_R) - (t_{0.1,df} \times S.E.)] \times 100$

= Antilog $[(1.497-1.500) - (1.725 \times \sqrt{(2\times0.00415)/22})] \times 100$

= 92.0%

Upper limit = Antilog $[(\overline{X}_T-\overline{X}_R) + (t_{0.1,df} \times S.E.)] \times 100$

= Antilog $[(1.497-1.500) + (1.725 \times \sqrt{(2\times0.00415)/22})] \times 100$

= 107.1%

Table 51 Example of ANOVA Calculation for Log C_{max} of Sulbactam

Sequence	Subject	Innovator's Product		Test Product		Subject Total
I	1	1.27		1.18		2.45
	2	1.28		1.12		2.40
	3	1.17		1.16		2.33
	4	1.25		1.10		2.35
	5	1.22		1.43		2.65
	6	1.21		0.91		2.12
	7	1.50		1.25		2.75
	8	1.39	Period I	1.31	Period II	2.70
	9	1.38	SUM	1.11	SUM	2.49
	10	1.31		1.16		2.47
	11	1.41	14.39	1.14	12.87	2.55
II	12	1.14		1.19		2.33
	13	1.43		1.54		2.97
	14	1.22		1.10		2.32
	15	1.34		1.42		2.76
	16	1.21		1.41		2.62
	17	1.21		1.30		2.51
	18	0.98		1.13		2.11
	19	1.06	Period II	1.23	Period I	2.29
	20	1.27	SUM	1.41	SUM	2.68
	21	1.03		1.06		2.09
	22	1.24	13.13	1.21	14.00	2.45
Formulation Total		27.52		26.87		54.39
Mean of Formulation		1.251		1.221		

Period I total = 28.39 (14.39+14.00)

Period II total = 26.00 (12.87+13.13)

Correction term = $(54.39)^2/44$ = 67.23346

SStotal = $[(1.27)^2+(1.28)^2+\ldots+(1.21)^2]$ – C.T. = 0.83364

SSsequence = $[(2.45+2.40+\ldots+2.55)^2+ (2.33+2.97+\ldots+2.45)^2]/22$ – C.T= 0.00038

SSsubject = $[(2.45)^2+ (2.40)^2+\ldots+ (2.45)^2]/2$ - 0.00038 – C.T. = 0.54131

SSperiod = $[(28.39)^2+ (26.00)^2]/22$ – C.T. = 0.12982

SSformulation = $[(27.52)^2+ (26.87)^2]/22$ – C.T. = 0.00960

SSerror = 0.83364 – 0.00038 – 0.54131 – 0.12982 – 0.00960 = 0.15253

Analysis of variance for two way crossover design

Source of Variation	d.f.	SS	MS	F ratio	F table	Significance Level
Total	43	0.83364				
Sequence	1	0.00038	0.00038	0.01404	4.350	NS
Subject (seq)	20	0.54131	0.02707	3.54784	2.120	S
Period	1	0.12982	0.12982	17.01442	4.350	S
Formulation	1	0.00960	0.00960	0.01928	4.350	NS
Error	20	0.15253	0.00763	1.2582		

Where; F table obtained from the table of F ratio for 0.05 level of significance.

The 90% confidence interval was constructed as follow;

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

Lowerlimit = Antilog $[(\overline{X}_T-\overline{X}_R) - (t_{0.1,df} \times S.E.)] \times 100$

= Antilog $[(1.221-1.251) - (1.725 \times \sqrt{(2\times0.00763)/22})] \times 100$

= 84.1%

Upper limit = Antilog $[(\overline{X}_T-\overline{X}_R) + (t_{0.1,df} \times S.E.)] \times 100$

= Antilog $[(1.221-1.251) + (1.725 \times \sqrt{(2\times0.00763)/22})] \times 100$

= 103.5%

Table 52 Example of ANOVA Calculation for Log $AUC_{0-\infty}$ of Cefoperazone

Sequence	Subject	Innovator's Product		Test Product		Subject Total
I	1	2.03		2.06		4.09
	2	2.10		2.28		4.38
	3	2.09		2.07		4.16
	4	2.14		2.07		4.21
	5	2.31		2.38		4.69
	6	2.13		2.25		4.38
	7	2.14		2.18		4.32
	8	2.25	Period I	2.32	Period II	4.57
	9	2.19	SUM	2.37	SUM	4.56
	10	2.32		2.41		4.73
	11	2.18	23.88	2.22	24.61	4.40
II	12	2.37		2.42		4.79
	13	2.20		2.23		4.43
	14	2.18		2.24		4.42
	15	2.33		2.31		4.64
	16	2.15		2.17		4.32
	17	2.39		2.38		4.77
	18	2.11		2.04		4.15
	19	2.15	Period II	2.15	Period I	4.30
	20	2.36	SUM	2.40	SUM	4.76
	21	2.34		2.45		4.79
	22	2.10	24.68	2.20	24.99	4.30
Formulation Total		48.56		49.60		98.16
Mean of Formulation		2.207		2.255		

Period I total = 48.87 (23.88 + 24.99)

Period II total = 49.29 (24.68 + 24.61)

Correction term = $(98.16)^2/44$ = 218.98604

SStotal = $[(2.03)^2+(2.10)^2+\ldots+(2.20)^2]$ – C.T. = 0.61096

SSsequence = $[(4.09+4.38+\ldots+4.40)^2+ (4.79+4.43+\ldots+4.30)^2]/22$ – C.T= 0.03164

SSsubject = $[(4.09)^2+ (4.38)^2+\ldots+ (4.30)^2]/2$ – 0.03164 – C.T. = 0.50782

SSperiod = $[(48.87)^2+ (49.29)^2]/22$ – C.T. = 0..00401

SSformulation = $[(48.56)^2+ (49.60)^2]/22$ – C.T. = 0.02458

SSerror = 0.61096 – 0.03164 – 0.50782 – 0.00401 – 0.02458 = 0.04291

Analysis of variance for two way crossover design

Source of Variation	d.f.	SS	MS	F ratio	F table	Significance Level
Total	43	0.61096				
Sequence	1	0.03164	0.03164	1.24616	4.350	NS
Subject (seq)	20	0.50782	0.02539	11.80930	2.120	S
Period	1	0.00401	0.00401	1.86512	4.350	NS
Formulation	1	0.02458	0.02458	11.43256	4.350	S
Error	20	0.04291	0.00215			

Where; F table obtained from the table of F ratio for 0.05 level of significance.

The 90% confidence interval was constructed as follow;

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

Lowerlimit = Antilog $[(\overline{X}_T-\overline{X}_R) - (t_{0.1,df} \times S.E.)] \times 100$

= Antilog $[(2.255-2.207) - (1.725 \times \sqrt{(2\times0.00215)/22})] \times 100$

= 105.7%

Upper limit = Antilog $[(\overline{X}_T-\overline{X}_R) + (t_{0.1,df} \times S.E.)] \times 100$

= Antilog $[(2.255-2.207) + (1.725 \times \sqrt{(2\times0.00215)/22})] \times 100$

= 118.0%

Table 53 Example of ANOVA Calculation for Log $AUC_{0-\infty}$ of Sulbactam

Sequence	Subject	Innovator's Product		Test Product		Subject Total
I	1	1.63		1.57		3.20
	2	1.65		1.57		3.22
	3	1.69		1.55		3.24
	4	1.67		1.73		3.40
	5	1.60		1.65		3.25
	6	1.64		1.51		3.15
	7	1.62		1.67		3.29
	8	1.83	Period I	1.63	Period II	3.46
	9	1.75	SUM	1.65	SUM	3.40
	10	1.74		1.59		3.33
	11	1.72	18.54	1.52	17.64	3.24
II	12	1.62		1.93		3.55
	13	1.72		1.83		3.55
	14	1.69		1.71		3.40
	15	1.77		1.77		3.54
	16	1.54		1.66		3.20
	17	1.47		1.72		3.19
	18	1.56		1.57		3.13
	19	1.43	Period II	1.63	Period I	3.06
	20	1.51	SUM	1.69	SUM	3.20
	21	1.50		1.64		3.14
	22	1.54	17.35	1.52	18.67	3.06
Formulation Total		35.89		36.31		72.20
Mean of Formulation		1.631		1.650		

Period I total = 37.21 (18.54+18.67)

Period II total = 34.99 (17.64+17.35)

Correction term = $(72.20)^2/44$ = 118.47364

SStotal = $[(1.63)^2+(1.65)^2+\ldots+(1.52)^2]$ – C.T. = 0.46396

SSsequence = $[(3.20+3.22+\ldots+3.24)^2+ (3.55+3.55+\ldots+3.06)^2]/22$ – C.T= 0.00058

SSsubject = $[(3.20)^2+ (3.22)^2+\ldots+ (3.06)^2]/2$ - 0.00058 – C.T. = 0.24058

SSperiod = $[(37.21)^2+ (34.99)^2]/22$ – C.T. = 0.11201

SSformulation = $[(35.89)^2+ (36.31)^2]/22$ – C.T. = 0.00401

SSerror = 0.46396 – 0.00058 – 0.24058 – 0.11201 – 0.00401 = 0.10678

Analysis of variance for two way crossover design

Source of Variation	d.f.	SS	MS	F ratio	F table	Significance Level
Total	43	0.46396				
Sequence	1	0.00058	0.00058	0.04825	4.350	NS
Subject (seq)	20	0.24058	0.01202	2.25094	2.120	S
Period	1	0.11201	0.11201	20.97566	4.350	S
Formulation	1	0.00401	0.00401	0.75094	4.350	NS
Error	20	0.10678	0.00534			

Where; F table obtained from the table of F ratio for 0.05 level of significance.

The 90% confidence interval was constructed as follow;

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

Lower limit = Antilog $[(\overline{X}_T-\overline{X}_R) - (t_{0.1,df} \times S.E.)] \times 100$

= Antilog $[(1.650-1.631) - (1.725 \times \sqrt{(2\times0.00534)/22})] \times 100$

= 95.7%

Upper limit = Antilog $[(\overline{X}_T-\overline{X}_R) + (t_{0.1,df} \times S.E.)] \times 100$

= Antilog $[(1.650-1.631) + (1.725 \times \sqrt{(2\times0.00534)/22})] \times 100$

= 114.0%

APPENDIX C

Table 54 Hematological Tests of Subjects Participated in This Study

Hematological Tests	Normal Range	Subject no.																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
WBC	5-10 x 10^3 cells/mL	8.1	8.9	8.3	9.3	8.6	8.6	5.8	8.3	7.6	7.5	6.5	9.2	11.2	8.7	6.9	7.3	7.9	8.3	7.1	7.8	10.4	9.6
Hemoglobin	13-18 g/dL	10.9	13.8	14.6	14.6	14.8	15.1	15.2	14.9	13.2	14.2	11.9	14.5	15.4	16.7	14.5	14.5	13.8	14	14.6	15.4	14.5	15.7
Hematocrit	35-40%	33	42	44	44	45	45	45	45	39	42	36	43	47	50	44	43	42	41	44	46	45	46
Platelet Count	1.5-4.0 x 10^5 cells/mL	1.69	2.43	20.8	2.48	2.68	2.84	1.62	2.81	2.69	2.65	1.83	2.16	3.2	2.96	1.87	2.89	2.88	2.59	2.78	2.59	2.27	2.28
Lymphocyte	20-35%	23	44	22	25	26	33	25	37	21	37	27	33	18	30	31	41	30	27	26	34	32	27
RBC Count	4.7-601x 10^6 cells/mL	3.82	4.85	5.28	5.18	5.29	5.3	*	5.29	4.56	4.86	4.15	4.95	5.5	5.7	4.95	4.88	4.95	4.79	*	5.38	5.24	*

* Not determined

Table 55 Blood Biochemistry Tests of subjects Participated in This study

Blood Biochemistry Tests	Normal Range	Subject no.																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
HBS Ag	Negative	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Anti-HIV	Negative	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sugar	75-110 mg/dL	95	100	106	86	86	86	104	92	95	94	92	98	100	78	85	100	92	95	90	116	130	75
BUN	8.0-20.0 mg/dL	12	14	13	12	10	14	12	12	13	12	11	11	16	15	14	14	15	13	14	14	13	18
Creatinine	0.7-1.5 mg/dL	0.9	1.2	1.1	1	0.8	1	0.92	0.9	1.1	0.9	0.9	0.8	1.2	1	1.2	0.9	1.2	1	0.95	1.1	1.1	1.1
Uric Acid	1.5-7.0 mg/dL	6.1	6.5	5.4	6	5.4	6.9	6.4	5.8	5.7	5	7.8	6.9	6.7	6.8	6.8	6.5	7.2	5	6	7.9	7.6	5
Cholesterol	140-200 mg/dL	196	186	196	210	186	173	216	175	189	176	166	198	169	212	235	1195	171	204	219	258	270	190
Triglyceride	35-160 mg/dL	94	145	244	107	446	175	125	191	127	168	123	193	145	270	495	190	114	151	122	164	253	126
Total Protein	6.0-8.0 g/dL	7.3	6.3	6.8	6.5	6.4	7	7.4	7.4	6.5	6.5	6.5	7	6.9	6.5	6.4	7	6.4	7.1	6.9	6.9	6.4	7.4
Albumin	3.5-5.3 g/dL	4.6	4.5	4	4.2	3.6	4.7	4.3	4.9	3.5	3.6	3.6	3.8	4.4	4.2	3.7	4.9	3.6	4.6	4.2	3.8	3.6	4.5
Birilubin Total	0.3-1.0 mg/dL	0.45	0.4	0.35	0.48	0.25	0.41	*	0.3	0.4	0.3	1.8	0.5	0.35	0.42	0.45	0.4	0.3	0.4	*	0.5	1.5	*
Birilubib Direct	0-0.5 mg/dL	0.24	0.2	0.1	0.26	0.1	0.26	*	0.15	0.25	0.15	0.8	0.2	0.14	0.42	0.2	0.2	0.16	0.2	*	0.3	0.7	*
AST	5-35 U/L	37	31	24	17	20	37	30	24	28	32	32	43	21	40	30	24	20	32	31	24	41	21
ALT	5-45 U/L	14	23	15	14	18	41	22	17	22	30	29	53	15	51	28	21	12	25	25	18	49	18
Alk. Phos.	25-90 U/L	35	56	40	39	62	38	57	45	40	39	71	49	56	50	69	48	54	60	48	45	51	65

* Not determined

APPENDIX D

หนังสือยินยอมโดยได้รับการบอกกล่าว

ชื่อโครงการ : ชีวสมมูลของยาฉีดเข้ากล้ามเซโฟเพอราโซนและซัลแบคแทม

ชื่อผู้วิจัย : รศ.ดร. อุทัย สุวรรณกูฏ และ นางสาวศิริพร ฉวานนท์

อาสาสมัคร : อาสาสมัครชายไทยสุขภาพดี 20 คน

อายุ : 18-45 ปี

คำยินยอมของอาสาสมัคร

ข้าพเจ้า นาย.....ได้ทราบรายละเอียดของโครงการวิจัยตลอดจนประโยชน์และข้อเสี่ยงที่จะเกิดขึ้นต่ออาสาสมัครจากผู้วิจัยแล้วอย่างชัดเจน ไม่มีสิ่งใดปิดบังซ่อนเร้น และยินยอมให้ทำการวิจัยในโครงการที่มีชื่อข้างต้น และข้าพเจ้ารู้ว่าถ้ามีปัญหาหรือข้อสงสัยเกิดขึ้นข้าพเจ้าสามารถถามผู้วิจัยได้ และข้าพเจ้าสามารถไม่เข้าร่วมโครงการวิจัยนี้เมื่อใดก็ได้

ลงชื่อ.....(ผู้ให้ความยินยอม/อาสาสมัคร)

.....(พยาน)

.....(พยาน)

วันที่ .....

คำอธิบายของผู้ดำเนินการวิจัย

ข้าพเจ้าได้อธิบายรายละเอียดของโครงการตลอดจนประโยชน์ของการวิจัย รวมทั้งข้อเสี่ยงที่อาจจะเกิดขึ้นแก่อาสาสมัครทราบแล้วอย่างชัดเจน โดยไม่มีสิ่งใดปิดบังซ่อนเร้น

ลงชื่อ .....ผู้ดำเนินการวิจัย

วันที่ .....

แพทย์ผู้รับผิดชอบดูแลความปลอดภัยของอาสาสมัคร :

ชื่อ : พ.ญ. พรเลขา บรรหารศุภวาท ใบประกอบวิชาชีพเวชกรรม เลขที่ ว.25980

สถานที่ติดต่อ : 80/1 ซอย ลาดพร้าว 71 ถนน ลาดพร้าว เขต ลาดพร้าว กรุงเทพฯ 10230

โทรศัพท์ : 0-1870-0012, 0-2932-5819

เอกสารชี้แจงข้อมูล/คำแนะนำผู้เข้าร่วมโครงการ

(Patient Information Sheet)

ชื่อโครงการวิจัย : ชีวสมมูลของยาฉีดเข้ากล้ามเซโฟเพอราโซนและซัลแบคแทม

ชื่อผู้วิจัย : รศ.ดร. อุทัย สุวรรณกูฏ, นางสาวศิริพร ฉวานนท์

สถานที่วิจัย : คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ผู้สนับสนุนการวิจัย : บริษัท สยามเภสัช จำกัด

ความป็นมาของโครงการ :

ชีวสมมูล (Bioequivalence) ของยา เป็นข้อมูลสำคัญที่แสดงถึงประสิทธิภาพการรักษาของยาที่ผลิตในประเทศมีความเท่าเทียมกันกับยาต้นแบบที่ผลิตจากต่างประเทศ ซึ่งการศึกษาชีวสมมูลมีขึ้นเพื่อสนับสนุนให้ประชาชน และ บุคลากรทางการแพทย์ หันมาเลือกใช้ ยาที่ผลิตในประเทศซึ่งมีคุณภาพดี ราคาถูก สนองตอบนโยบายหลักประกันสุขภาพถ้วนหน้า หรือ นโยบาย 30 บาท รักษาทุกโรคของรัฐบาล ซึ่งหลักการสำคัญของนโยบายนี้คือ การให้ประชาชนทุกคนมีโอกาสได้รับการรักษาพยาบาลที่เท่าเทียมกัน โดยประชาชนทุกคนจ่ายค่ารักษาพยาบาล 30 บาทเท่านั้น ส่วนที่เหลือรัฐจะเป็นผู้รับผิดชอบ อาจกล่าวอีกนัยหนึ่งว่ารัฐซื้อประกันสุขภาพให้แก่ประชาชน โดยจัดสรรเงินให้แก่โรงพยาบาลรัฐบาล จำนวนหนึ่ง ขึ้นอยู่กับจำนวนประชากรที่มีทะเบียนบ้านในเขตรับผิดชอบของโรงพยาบาลนั้นๆ ซึ่งงบประมาณที่จัดสรรให้นั้น โรงพยาบาลจะนำมาเป็นค่าใช้จ่ายด้านการดำเนินการต่างๆ ค่าตอบแทนบุคลากร และค่าใช้จ่ายด้านการรักษาพยาบาล เช่น ค่ายา ค่าเวชภัณฑ์ อุปกรณ์การแพทย์ต่างๆ ดังนั้นโรงพยาบาลจะต้องบริหารเงินงบประมาณที่ได้รับจัดสรรมาให้เกิดประโยชน์สูงสุด โดยลดต้นทุนค่าใช้จ่ายคงที่ต่างๆลง ขณะเดียวกันก็พัฒนาประสิทธิภาพการรักษาโดยเลือกใช้ยาที่มีประสิทธิภาพการรักษาที่ดี และมีราคาถูก ลดการใช้ยาที่นำเข้าจากต่างประเทศและหันมาใช้ยาที่ผลิตในประเทศ โดยพิจารณาจากข้อมูลการศึกษาชีวสมมูลของยา ดังนั้นการศึกษาชีวสมมูลจึงสนับสนุนอุตสาหกรรมการผลิตยาในประเทศ ลดการนำเข้ายาจากต่างประเทศและช่วยพัฒนาเศรษฐกิจของชาติให้ดีขึ้น

การศึกษาชีวสมมูลคือการศึกษาเปรียบเทียบชีวปริมาณออกฤทธิ์ (Bioavailability) ของยา นั่นคือ การประเมินความเท่าเทียมของอัตราเร็วและปริมาณยาที่ถูกดูดซึมเข้าสู่ระบบการไหลเวียนโลหิตของยาที่มีขนาด ความแรง รูปแบบ และสภาวะการทดลองที่เหมือนกันทุกประการ โดยทั่วไปจะศึกษาเปรียบเทียบชีวปริมาณการออกฤทธิ์ ระหว่างยาต้นแบบ (innovator's product) ซึ่งหมายถึง ยาตัวแรกที่ได้รับการจดสิทธิบัตร และยาสามัญ (generic product) ซึ่งหมายถึง ยาที่

ผลิตขึ้น โดยมีตัวยาสำคัญ (active ingredient) ตัวเดียวกันและปริมาณเท่ากันกับยาต้นแบบ แต่แหล่งผลิตหรือกระบวนการผลิตต่างจากยาต้นแบบ

เซโฟเพอราโซน และ ซัลแบคแทม (Cefoperazone/Sulbactam) เป็นยาต้านเชื้อแบคทีเรียชนิดหนึ่งที่ออกฤทธิ์ฆ่าเชื้อแบคทีเรียได้อย่างกว้างขวางทั้งต่อแบคทีเรียกรัมบวก และกรัมลบ ประกอบด้วยตัวยาสำคัญสองตัวคือ Cefoperazone และ Sulbactam มีข้อบ่งใช้ในการรักษาโรคติดเชื้อที่ระบบทางเดินหายใจทั้งส่วนบนและส่วนล่าง, โรคติดเชื้อระบบทางเดินปัสสาวะทั้งส่วนบนและส่วนล่าง ,โรคติดเชื้อในช่องท้อง , ท่อน้ำดีอักเสบ ,การติดเชื้อที่ผิวหนังและ Soft tissue , ภาวะติดเชื้อที่กระดูกและข้อ , เยื่อหุ้มสมองอักเสบ ,septicemia , การติดเชื้ออุ้งเชิงกราน มดลูกอักเสบ และการติดเชื้อในระบบสืบพันธุ์

ขนาดยาที่ใช้โดยทั่วไปในผู้ใหญ่คือ เซโฟเพอราโซน/ซัลแบคแทม(อัตราส่วน 1:1) 2.0-4.0 กรัมต่อวัน ฉีดเข้าเส้นเลือดดำหรือเข้ากล้าม ทุก 12 ชั่วโมง โดยแบ่งให้ครั้งละเท่าๆกัน ในโรคติดเชื้อร้ายแรงหรือเชื้อที่ดื้อต่อการรักษา ขนาดการใช้เซโฟเพอราโซน/ซัลแบคแทม อาจเพิ่มขึ้นเป็น 8 กรัมต่อวัน

ปฏิกิริยาที่ไม่พึงประสงค์ของการใช้เซโฟเพอราโซน/ซัลแบคแทม อาการที่พบบ่อยได้แก่ คลื่นไส้ อาเจียน ท้องเดิน สำไส้อักเสบ เกิดภาวะ Hypoprothrombinemia และภาวะขาดวิตามิน K ดังนั้นจึงต้องให้วิตามิน K เสริมร่วมกับการฉีดเซโฟเพอราโซน/ซัลแบคแทม อาจพบปฏิกิริยาการแพ้ยาได้ นอกจากนี้การดื่มแอลกอฮอล์ภายหลังการให้เซโฟเพอราโซน จะเกิดปฏิกิริยาคล้าย Disulfiram reaction ซึ่งจะแสดงอาการคลื่นไส้ อาเจียน ปวดศีรษะ หัวใจเต้นเร็ว เหงื่อออก เป็นผื่นแดง ดังนั้นจึงไม่ควรดื่มหรือรับประทานอาหารที่มีแอลกอฮอล์ภายใน 72 ชั่วโมงหลังการได้รับเซโฟเพอราโซน

เภสัชจลนศาสตร์ของเซโฟเพอราโซน ดูดซึมได้น้อยในทางเดินอาหาร ต้องให้โดยการฉีดเข้ากล้ามหรือหลอดเลือดดำ เมื่อให้เซโฟเพอราโซนขนาด 1 กรัมโดยการฉีดเข้ากล้าม มีระดับยาสูงสุดในเลือด (C_{max}) 64.2 ไมโครกรัมต่อมิลลิลิตร ในระยะเวลา 15 นาทีถึง 2 ชั่วโมง (t_{max}) มีค่าครึ่งชีวิต ($t_{½}$) 1.6-2.6 ชั่วโมง ขับออกทางน้ำดีเป็นส่วนใหญ่ ขับออกทางไตประมาณ 25% ของขนาดยาที่ให้ ซัลแบคแทมมีความคงตัวต่อกรดในกระเพาะอาหารจึงสามารถใช้รับประทานได้ แต่ดูดซึมในทางเดินอาหารได้น้อย เมื่อให้ซัลแบคแทมขนาด 500 มิลลิกรัมโดยการฉีดเข้ากล้ามจะได้ระดับยาสูงสุดในเลือด (C_{max}) ประมาณ 13-19 ไมโครกรัมต่อมิลลิลิตร ค่าครึ่งชีวิต ($t_{1/2}$) 1-1.2 ชั่วโมง ระยะเวลาที่ระดับยาในเลือดสูงสุด (t_{max}) ประมาณ 15 นาที

ภายใต้นโยบาย 30 บาทรักษาทุกโรค โรงพยาบาลต้องให้บริการทางสุขภาพแก่ประชาชนอย่างมีประสิทธิภาพ โดยเสียค่าใช้จ่ายให้น้อยที่สุด ดังนั้นโรงพยาบาลจำเป็นต้องเปลี่ยนรูปแบบ

การบริหารจัดการด้านการรักษาและการจัดซื้อยา โดยเฉพาะการคัดเลือกยาที่จะใช้ในโรงพยาบาล พิจารณาเลือกยาที่ผลิตในประเทศเนื่องจากมีราคาถูกในขณะเดียวกันการรักษาก็ต้องมีประสิทธิภาพด้วย ดังนั้นข้อมูลที่แสดงว่ายาที่ผลิตในประเทศมีประสิทธิภาพในการรักษาโรคเท่าเทียมกับยาต้นแบบที่นำเข้าจากต่างประเทศจึงเป็นเครื่องมือสำคัญในการคัดเลือกยา และสร้างความมั่นใจให้แพทย์และเภสัชกรซึ่งเป็นผู้สั่งจ่ายยาให้แก่ผู้ป่วย ดังนั้นผู้วิจัยจึงมีความสนใจที่จะศึกษาชีวสมมูลของเซโฟเพอราโซนและซัลแบคแทมที่ผลิตในประเทศคือตำรับยา Sulcef ® ที่ผลิตโดยบริษัท สยามเภสัช จำกัด และยาต้นแบบที่นำเข้าจากต่างประเทศ คือตำรับยา Sulperazon ® ของบริษัท ไฟเซอร์ (ประเทศไทย) จำกัด โดยศึกษาในรูปแบบของยาฉีดเข้ากล้าม ขนาด 1 กรัม (เซโฟเพอราโซน 500 มิลลิกรัม/ ซัลแบคแทม 500 มิลลิกรัม)

วัตถุประสงค์ : เพื่อ

1. ศึกษาชีวสมมูลของเซโฟเพอราโซนและซัลแบคแทมของผลิตภัณฑ์ยาฉีดเข้ากล้ามที่ผลิตในประเทศกับผลิตภัณฑ์ยาต้นแบบ (Sulperazon ®)
2. เปรียบเทียบค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ของเซโฟเพอราโซนและซัลแบคแทมของผลิตภัณฑ์ยาฉีดเข้ากล้ามที่ผลิตในประเทศกับผลิตภัณฑ์ยาต้นแบบ ในอาสาสมัครชายไทยสุขภาพดี

รายละเอียดที่จะปฏิบัติต่อผู้เข้าร่วมโครงการ :

อาสาสมัครที่ได้รับคัดเลือกเข้าโครงการจะได้รับการฉีดยาเซโฟเพอราโซนและซัลแบคแทม ขนาด 1 กรัม เข้ากล้าม คนละ 1 เข็ม (ยาที่ผลิตในประเทศ 1 ครั้ง และยาต้นแบบ 1 ครั้ง) ตามแผนการทดลองแบบข้ามสลับด้วยระยะห่างของการให้ยาแต่ละครั้งประมาณ 7 วัน หลังจากการได้รับยาแต่ละครั้ง ทำการเจาะที่เส้นเลือดดำบริเวณแขนครั้งละประมาณ 7 มิลลิลิตร ก่อนการให้ยา และที่เวลา 15 , 30, 45 นาที, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 6, 8, 10 ชั่วโมง หลังการให้ยา อาสาสมัครจะได้รับการดูแลอย่างดีจากแพทย์ผู้ร่วมวิจัย พร้อมจัดอาหารและเครื่องดื่มบริการให้เมื่อครบกำหนดเวลาที่สามารถรับประทานได้

ประโยชน์และผลข้างเคียงที่จะเกิดแก่ผู้เข้าร่วมโครงการ :

นอกจากค่าตอบแทนที่อาสาสมัครจะได้รับ ผลการวิจัยนี้ยังมีประโยชน์ต่อส่วนรวม เพราะทำให้ทราบถึงคุณภาพและมาตรฐานของยาเซโฟเพอราโซน/ซัลแบคแทมที่ผลิตขึ้นในประเทศ เพื่อให้แพทย์ เภสัชกร มีความมั่นใจในการสั่งจ่ายยาให้แก่ผู้ป่วย และผู้ป่วยเองก็จะได้รับยาที่มีมาตรฐานและราคาถูกด้วย ผลข้างเคียงที่อาจเกิดแก่อาสาสมัครคาดว่าน้อยมาก เพราะการวิจัยนี้เป็นการให้ยาแบบครั้งเดียว อาสาสมัครจะได้รับยาคนละ 2 ครั้ง ตำรับละ 1 ครั้ง ในระยะเวลาห่างกัน 7 วัน จากรายงานพบว่าผู้ได้รับยาสามารถทนต่อยาได้ดี อย่างไรก็ตามอาจมีอาการคลื่นไส้อาเจียนบ้าง และอาสาสมัครไม่ควรดื่มหรือรับประทานอาหารที่มีแอลกอฮอล์ภายใน 72 ชั่วโมง

หลังการได้รับเซโฟเพอราโซน เนื่องจากจะแสดงอาการคลื่นไส้ อาเจียน ปวดศีรษะ หัวใจเต้นเร็ว เหงื่อออก เป็นผื่นแดง นอกจากนี้อาสาสมัครอาจเจ็บตอนฉีดยาเซโฟเพอราโซน/ซัลแบคแทมเข้ากล้ามและตอนเก็บตัวอย่างเลือด

การเฝ้าระวังความปลอดภัยของอาสาสมัคร :

อาสาสมัครทุกคนหลังได้รับยาและถูกเจาะเลือดที่เวลาต่างๆ แพทย์ผู้ดูแลจะประเมินผลอาการที่ไม่พึงประสงค์ทุกชั่วโมงของการศึกษา โดยการวัดความดันโลหิต, ชีพจร และซักถามถึงอาการไม่พึงประสงค์เนื่องจากยาเพื่อเฝ้าระวังความปลอดภัยระหว่างการทดสอบ อาการต่างๆที่ปรากฏจะถูกจดบันทึกในรายงาน case record form พร้อมให้การวินิจฉัยและรักษาจากแพทย์ โดยมียาและเวชภัณฑ์เตรียมพร้อมสำหรับการรักษาอย่างทันท่วงที

การเก็บข้อมูลเป็นความลับ :

ผู้วิจัยจะเก็บข้อมูลของผู้เข้าร่วมโครงการวิจัยเป็นความลับ จะเปิดเผยเฉพาะในรูปที่เป็นสรุปผลการวิจัย

แพทย์ผู้รับผิดชอบ :

ชื่อ : พ.ญ. พรเลขา บรรหารศุภวาท ใบประกอบวิชาชีพเวชกรรม เลขที่ ว.25980

สถานที่ติดต่อ : 80/1 ซอย ลาดพร้าว 71 ถนน ลาดพร้าว เขต ลาดพร้าว กรุงเทพฯ 10230

โทรศัพท์ : 0-1870-0012, 0-2932-5819

รายงานการเฝ้าระวังอาการไม่พึงประสงค์ของการใช้ยาเซโฟเพอราโซน/ซัลแบคแทม 1 กรัม

ชื่อโครงการวิจัย : ชีวสมมูลของยาฉีดเข้ากล้ามเซฟโฟเพอราโซนและซัลแบคแทม

ชื่อ-นามสกุล..... อายุ..... ปี เพศชาย น้ำหนัก…… ส่วนสูง……..

เลขที่.....การศึกษาครั้งที่ ..... วัน/เดือน/ปี ..... รับยารหัส .....

ประวัติการแพ้ยา ☐ กลุ่ม Penicillin ☐ กลุ่ม Cephalosporin ☐ อื่นๆ.....

1. การตรวจร่างกาย

	เวลา	อัตราการเต้นของหัวใจ (ครั้ง/นาที)	ความดันโลหิต (มิลลิเมตรปรอท)
ก่อนได้รับการฉีดยา			
หลังได้รับการฉีดยา			

2. อาการไม่พึงประสงค์ ☐ พบ ☐ ไม่พบ

อาการไม่พึงประสงค์ที่พบ	ลักษณะอาการ	เวลา	การปฏิบัติหลังเกิดอาการ
ระบบทางเดินอาหาร ผิวหนัง ระบบอื่นๆ	☐ คลื่นไส้ ☐ อาเจียน ☐ ท้องเสีย ☐ ปวดท้อง ☐ อื่นๆ……..……..… ☐ มีผื่นแดง ☐ มีอาการคัน ☐ อื่นๆ ..... ☐ ปวดศีรษะ ☐ เวียนศีรษะ ☐ มึนงง ☐ หายใจไม่สะดวก ☐ อื่นๆ.....		

หมายเหตุ :

.....

3. ผลการประเมินอาการไม่พึงประสงค์ □ เกิดจากยา □ ไม่ได้เกิดจากยา □ ไม่แน่ใจ

หมายเหตุ : .....

4. อาสาสมัคร □ เข้าร่วมการทดลองต่อไป □ ถอนตัว

ลงชื่อ .....

(พ.ญ. พรเลขา บรรหารศุภวาท)

แพทย์ผู้ดูแลและประเมินอาการไม่พึงประสงค์

ลงชื่อ .....

(รศ.ดร. อุทัย สุวรรณกูฏ)

ผู้วิจัยหลัก

VITA

Miss Siriporn Chawanon was born on June 7, 1975 in Bangkok. She received a Bachelor of Pharmacy degree in 1995 from Faculty of Pharmacy, Mahidol University. She is a pharmacist in Narcotic Control Division, Food and Drug Administration, Ministry of Public Health, Thailand.