ชีวสมมูลของยาเม็ดอะม็อกซิซิลลิน และคลาวูลานิกแอซิด

นางสาวยุวดี ศิริสมบูรณ์



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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# BIOEQUIVALENCE OF AMOXICILLIN AND CLAVULANIC ACID TABLETS

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ศึกษาชีวสมมูลของยาเม็ดเคลือบฟิล์มอะม็อกซิซิลลิน และคลาวูลานิกแอซิด 500/125 มิลลิกรัม 2 ผลิตภัณฑ์ ผลการศึกษาในหลอดทดลองพบว่า ยาทั้งสองผลิตภัณฑ์มีร้อยละของยาที่ระบุไว้ในฉลากความสม่ำเสมอของตัวยาสำคัญในผลิตภัณฑ์ได้มาตรฐานที่กำหนดในเภสัชตำรับสหรัฐอเมริกาฉบับที่ 25 การศึกษาการละลายของตัวยาพบว่า เส้นโค้งของผลิตภัณฑ์ยาที่ผลิตภายในประเทศไทยมีลักษณะเหมือนเส้นโค้งของผลิตภัณฑ์ยาต้นแบบ การเปรียบเทียบชีวปริมาณออกฤทธิ์ของยาเม็ดเคลือบฟิล์ม อะม็อกซิซิลลินและคลาวูลานิกแอซิด 500/125 มิลลิกรัมของผลิตภัณฑ์ยาที่ผลิตภายในประเทศไทยและผลิตภัณฑ์ยาต้นแบบกระทำในอาสาสมัครชายไทยสุขภาพดี 18 คนโดยใช้แบบแผนการทดลองข้ามสลับชนิด 2 ทาง อาสาสมัครได้รับยาเม็ดขนาด 500/125 มิลลิกรัมครั้งเดียว เก็บตัวอย่างเลือดที่เวลาต่างๆ ที่เหมาะสมหลังการให้ยาโดยการรับประทาน วัดระดับยาในพลาสมาโดยใช้เอชพีแอลซี จากการวิเคราะห์ข้อมูลทางสถิติพบว่า ค่าลอการิทึมของความเข้มข้นของยาสูงสุดในพลาสมา และลอการิทึมพื้นที่ใต้เส้นโค้งระหว่างความเข้มข้นของยาในพลาสมากับเวลาของยาทั้ง 2 ผลิตภัณฑ์ไม่มีความแตกต่างอย่างมีนัยสำคัญ ($p>0.05$) ค่าร้อยละ 90 ของช่วงความเชื่อมั่นของสัดส่วนของแต่ละพารามิเตอร์ทางเภสัชจลนศาสตร์ที่แปลงข้อมูลเป็นลอการิทึมของยาอะม็อกซิซิลลิน และคลาวูลานิกแอซิดของผลิตภัณฑ์ยาที่ผลิตภายในประเทศไทยเทียบกับผลิตภัณฑ์ยาต้นแบบอยู่ภายในช่วงร้อยละ 80–125 ยกเว้นพารามิเตอร์ความเข้มข้นของยาสูงสุดในพลาสมาของยาคลาวูลานิกแอซิดซึ่งพบว่าอยู่ในช่วงร้อยละ 80–126 ซึ่งเป็นไปตามที่คาดการณ์ เพราะคลาวูลานิกแอซิดเป็นยาที่มีความแปรปรวนเกี่ยวกับการดูดซึม ค่าร้อยละ 90 ของช่วงความเชื่อมั่นของสัดส่วนของค่าความเข้มข้นของยาสูงสุดสำหรับยาที่มีความแปรปรวนได้รับการยอมรับกำหนดไว้ระหว่างร้อยละ 75-133 ดังนั้นจึงสามารถสรุปได้ว่าผลิตภัณฑ์ยาเม็ดที่ผลิตภายในประเทศไทยมีชีวสมมูลกับผลิตภัณฑ์ยาเม็ดต้นแบบทั้งในเชิงอัตราเร็วและปริมาณยาที่ถูกดูดซึมเข้าสู่ระบบการไหลเวียนของโลหิต

ภาควิชา เภสัชกรรม ลายมือชื่อนิสิต.....
สาขาวิชา เภสัชกรรม ลายมือชื่ออาจารย์ที่ปรึกษา.....
ปีการศึกษา 2546

## 4476601233: MAJOR PHARMACY
KEY WORD: BIOEQUIVALENCE / BIOAVAILABILITY / AMOXICILLIN / CLAVULANIC ACID / TABLETS

YUWADEE SIRISOMBOON: BIOEQUIVALENCE OF AMOXICILLIN AND CLAVULANIC ACID TABLETS. THESIS ADVISOR: ASSOC. PROF. UTHAI SUVANAKOOT, Ph.D. 159 pp. ISBN 974-17-3982-6.

The bioequivalence of two brands of 500/125 mg amoxicillin/clavulanic acid film coated tablet were evaluated. *In vitro* studies indicated that both brands met the general requirements of the United States Pharmacopoeia 25 for content of active ingredients and uniformity of dosage units. Dissolution profile studies revealed that the curves of local product were similar to those of innovator's product. Comparative bioavailability of the local product relative to innovator's product was conducted in 18 healthy Thai male volunteers using an oral single dose of 500/125 mg tablets in a two way crossover design. Blood samples were collected at appropriate time interval. Plasma amoxicillin and clavulanic acid concentrations were determined by high performance liquid chromatography. Individual plasma amoxicillin and clavulanic acid concentration-time profile was analyzed for relevant pharmacokinetic parameters. Data analysis revealed that there were no statistically significant differences ($p>0.05$) in the corresponding logarithmically transformed pharmacokinetic parameters (AUC and $C_{max}$ values) between the local and innovator's products for both amoxicillin and clavulanic acid. The 90% confidence interval for the ratios of individual parameter based on log-transformed data of amoxicillin and clavulanic acid of local product to that of innovator's product were within 80-125% except only that of the parameter $C_{max}$ value for clavulanic acid which was found to be 80-126%. This was expected because of the variable nature of clavulanic acid absorption and an inherent variability of $C_{max}$. The 90% confidence interval for the $C_{max}$ ratio of such drug was recommended to be 75-133%. Thus, it could be concluded that both products were bioequivalent in terms of both the rate and the extent of drug absorption into systemic circulation.



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|---|---|---|
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## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| % | = | percent |
| °C | = | degree Celcius |
| µg | = | microgram |
| mg | = | milligram |
| kg | = | kilogram |
| µL | = | microliter |
| mL | = | milliliter |
| L | = | liter |
| nm | = | nanometer |
| µm | = | micrometer |
| mm | = | millimeter |
| cm | = | centimeter |
| m | = | meter |
| M | = | molar |
| N | = | normality |
| v/v | = | volume by volume |
| min | = | minute |
| hr | = | hour |
| rpm | = | revolution per minute |
| BMI | = | body mass index |
| AUC | = | area under the plasma concentration-time curve |
| $C_{max}$ | = | peak plasma concentration |
| $t_{max}$ | = | time to peak plasma concentration |
| $t_{1/2}$ | = | half-life |
| F | = | fraction of dose to be absorbed |
| V | = | apparent volume of distribution |
| $K_e$ | = | elimination rate constant |
| $r^2$ | = | coefficient of determination |
| %L.A. | = | percent labeled amount |

## LIST OF ABBREVIATIONS (cont.)

| | | |
|---|---|---|
| LLOQ | = | lower limit of quantification |
| CI | = | confidence interval |
| S.D. | = | standard deviation |
| C.V. | = | coefficient of variation |
| R.S.D. | = | relative standard deviation |
| ANOVA | = | analysis of variance |
| MSE | = | mean square error |
| S.E. | = | standard error |
| $f_1$ | = | difference factor |
| $f_2$ | = | similarity factor |
| ln | = | natural logarithms |
| IV | = | intravenous |
| MIC | = | minimum inhibitory concentration |
| HPLC | = | high performance liquid chromatography |
| UV | = | ultraviolet |
| WS | = | working standard |
| RS | = | reference standard |
| Mfg | = | manufacturing |
| Exp | = | expiration |
| CSF | = | cerebrospinal fluid |
| GI | = | gastrointestinal |
| AST | = | aspartate aminotransferase |
| ALT | = | alanine aminotransferase |
| FDA | = | Food and Drug Administration |
| USP | = | United States Pharmacopeia |
| PBE | = | population bioequivalence |
| IBE | = | individual bioequivalence |
| NDA | = | new drug application |
| ANDA | = | abbreviated new drug application |

# CHAPTER I

# INTRODUCTION

Bioavailability and bioequivalence of drug-products have emerged as critical issues in pharmacy and medicine during the last three decades. Concern about lowering health care costs has resulted in a tremendous increase in the use of generic drug-products. At the present, about one half of all prescriptions written are for drugs that can be substituted with a generic product. Over 80% of the approximately 10,000 prescription drugs available in 1990 were available from more than one source. With the increasing availability and use of generic drug-products, health care professionals are confronted with an ever-larger array of multisource products from which they must select those that are therapeutically equivalent. This phenomenal growth of the generic pharmaceutical industry and the abundance of multisource products have prompted some questions among many health professionals and scientists regarding the therapeutic equivalency of these products, particularly those in certain critical therapeutic categories such as anticonvulsant and cardiovascular drugs (Colaizzi and Lowenthal, 1986). Inherent in the currently accepted guidelines for product substitution is the assumption that a generic drug considered to be bioequivalent to a brand-name drug will elicit the same clinical effect. Numerous papers in the literature indicate that there is concern that the current standards for approval of generic drugs may not always ensure therapeutic equivalence (Levy, 1960; Dettelbach, 1986; Lamy, 1986). The availability of different formulations of the same drug substance given at the same strength and in the same dosage form poses a special challenge to health care professionals, making these issues very relevant to pharmacists in all practice settings. Since pharmacists play an important role in product-selection decisions, they must have an understanding of the principles and concepts of bioavailability and bioequivalence.

Bioequivalence study is a comparative study of bioavailability that assesses whether the drug of two or more similar dosage forms reach the systemic circulation at the same rate and extent. Usually, the comparison is performed between a generic drug-product and innovator's product. In 1984, the United States Food and Drug

Administration (USFDA) required that every generic drug-product had to pass the bioequivalence study before they could be sold in the market. In 2000, the office of Food and Drug Administration of Thailand also required the bioequivalence study and declared "Criteria and Guideline for the Bioequivalence Study of Generic Drugs" to provide a good quality of the generic drug-products in Thailand (Thai FDA, 2000).

Amoxicilllin/clavulanic acid is an antibacterial combination consisting of the β-lactam antibiotic amoxicillin and the β-lactamase inhibitor clavulanic acid. Oral preparations of amoxicillin alone are highly effective against most common community-acquired pathogens involved infections of the upper and lower respiratory tracts, urogenital tracts and gastrointestinal tracts. The increasing occurrence of bacterial resistance by the production of β-lactamase nowadays severely limits the clinical effectiveness of amoxicillin. Therefore, combining amoxicillin with the β-lactamase inhibitor clavulanic acid is a rational approach to overcome these clinical limitations (Sourgens et al., 2001).

Amoxicillin/clavulanic acid is well absorbed following oral administration. Peak plasma concentrations of amoxicillin and of clavulanic acid are generally attained within 1-2.5 hours following oral administration of a single dose of amoxicillin and clavulanate potassium in fasting adults. Following oral administration of a single tablet containing 500 mg of amoxicillin and 125 mg of clavulanic acid in fasting healthy adults, peak plasma concentrations of amoxicillin average 6.5-9.7 μg/mL and peak plasma concentrations of clavulanic acid average 2.1-3.9 μg/mL have been reported. Amoxicillin has an elimination half-life of 1-1.3 hours and clavulanic acid has a distribution half-life of 0.28 hours and an elimination half-life of 0.78-1.2 hours (McEvoy, 2001).

Amoxicillin/clavulanic acid is commercially available for oral administration as film coated tablet, powder for oral suspension and chewable tablets. In Thailand, amoxicillin/clavulanic acid film coated tablets are available through a variety of brand names from different manufacturers. Among such drug-products, the innovator's product (Augmentin®) which 2-3 times higher retail price than the locally made generic drug-product (Cavumox®) is included. Others are the imported generic drug-products such as Amoksiklav®, Curam® and Ranclav®. In Thailand, amoxicillin/clavulanic acid is also widely dispensed both in hospitals and drugstores. Therefore, using of a locally made

generic drug-product resulted in a lowering health care costs. However, the quality of locally made drug-product is the important factor that health care professionals are concerned. In this study, the comparative bioavailability of local brand of amoxicillin/clavulanic acid film coated tablets (Cavumox®) commercially available in Thailand relative to the innovator's product (Augmentin®) was conducted in order to facilitate drug-products selection, in terms of the drug's efficacy and economic aspect.

**Objectives:** The purposes of this study were to;

1. Compare the bioavailability of a local brand of amoxicillin/clavulanic acid film coated tablet commercially available in Thailand relative to the innovator's product.
2. Investigate the pharmaceutical equivalence of a local brand of amoxicillin/ clavulanic acid film coated tablet commercially available in Thailand relative to the innovator's product.

**Significance of the study:**

1. This study will provide bioavailability data of a local brand of amoxicillin/ clavulanic acid film coated tablet commercially available in Thailand as compared to the innovator's product.
2. This study will provide analysis method data of amoxicillin/clavulanic acid in plasma

# CHAPTER II

# LITERATURES REVIEW

## Bioavailability

Bioavailability is a term that describes the rate and extent to which the active drug ingredient is absorbed from a drug-product and becomes available at the site of drug action. Since pharmacologic response is generally related to the concentration of drug at the receptor site, the availability of a drug from a dosage form is a critical element of a drug-product's clinical efficacy. However, drug concentrations usually cannot be readily measured directly at the site of action. Therefore, most bioavailability studies involve the determination of drug concentration in the blood or urine. This is based on the premise that the drug at the site of action is in equilibrium with drug in the blood. It is therefore possible to obtain an indirect measure of drug response by monitoring drug levels in the 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. The bioavailability of a drug-product often determines the therapeutic efficacy of that product since it affects the onset, intensity and duration of therapeutic response of the drug. In most cases one is concerned with the extent of absorption of drug since this represents the "effective dose" of a drug (Chereson and Banakar, 2000).

"Absolute" bioavailability, F, is the fraction of an administered dose which actually reaches the systemic circulation, and ranges from F = 0 (no drug absorption) to F = 1 (complete drug absorption). Since the total amount of drug reaching the systemic circulation is directly proportional to the area under the plasma drug concentration as a function of time curve (AUC), F is determined by comparing the respective AUC of the test product and the same dose of drug administered intravenously. The intravenous route is the reference standard since the dose is, by definition, completely available.

$$\text{Absolute Bioavailability} = \frac{AUC_{ev}}{AUC_{iv}}$$

Where, $AUC_{ev}$ and $AUC_{iv}$ are, respectively, the area under the plasma concentration-time curve following the extravascular and intravenous administration of a given dose of drug. Knowledge of F is needed to determine an appropriate oral dose of a drug relative to an IV dose.

"Relative" or “Comparative” bioavailability refers to the availability of a drug-product as compared to another dosage form or product of the same drug given in the same dose. These measurements determine the effects of formulation differences on drug absorption. The relative bioavailability of product A compared to product B, both products containing the same dose of the same drug, is obtained by comparing their respective $AUC_s$.

$$\text{Relative Bioavailability} = \frac{AUC_A}{AUC_B}$$

Where, $AUC_A$ and $AUC_B$ are, respectively, the area under the plasma concentration-time curve of test product and reference product (Shargel and Yu, 1993).

**Bioequivalence**

Bioequivalence studies are designed to compare drug-products. The objective is to determine whether the drug substance in two or more similar dosage forms reaches the systemic circulation at the same relative rate and to the same relative extent. Two medicinal products are bioequivalents if they are pharmaceutical equivalents or alternatives and if their bioavailabilities after administration in the same molar doses are similar to such degree that their effects, with respect to both efficacy and safety, will be essentially the same. These studies may be necessary before a generic product may be marketed.

**Past Bioavailability Problems**

There were a number of examples of drug-products which have exhibited bioavailability problems in the past. Glazko et al., (1968) reported that the absorption of chloramphenical capsule from a generic product after oral administration was

significantly less than that of original product. Oxytetracycline and tetracycline from 13 different manufacturers were found to be significantly bioinequivalence, as well (Blair et al., 1971). In addition, four to seven fold differences in serum levels and serious bioinequivalence were demonstrated after administration of digoxin of different manufacturers and various lots of the same manufacturer (Lindenbaum et al., 1971). As digoxin has a low therapeutic index, the high fluctuation of serum level might lead to toxic effect.

In 1972, McGilveray et al. reported that nitrofurantoin's bioavailability was shown to be influenced highly by its particle size. In addition, a randomized crossover study of oral nitrofurantoin was conducted and the results were shown both bioinequivalence and therapeutic inequivalence (Ali, 1988) and several studies reported the bioinequivalence of marketed nitrofurantoin products (Meyer et al., 1974).

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

Other drugs with problems in the past included acetazolamide, aminosalicylate, ampicillin, aspirin, ascorbic acid, chloramphenicol, chlorothiazide, diazepam, levodopa, iron and furosemide (Bourne, 2003).

The clinical significance of these reported differences in bioavailability relates to the therapeutic index of the drug, the dose of the drug and the nature of the disease. In 1973, the Ad Hoc Committee on Drug Product Selection of the American Pharmaceutical Association published a list of drugs with a potential for therapeutic inequivalence based on reported evidence of bioinequivalence. The drugs fall in three categories: "high", "moderate" and "low" risk based on the clinical implications (Table 1).

**Table 1** Drugs with Various Risk Potential for Inequivalence

| High risk potential | Moderate risk potential | Low risk potential |
|---|---|---|
| Aminophylline | Amphetamine | Acetaminophen |
| Bishydroxycoumarin | Ampicillin | Codeine |
| Digoxin | Chloramphenicol | Hydrochlorothiazide |
| Phenytoin | Digitoxin | Ephedrine |
| Para-aminosalicylic acid | Erythromycin | Meprobamate |
| Prednisolone | Griseofulvin | Penicillin VK |
| Quinidine | Oxytetracycline | Sulfisoxazole |
| Warfarin | Pentobarbital | |
| | Phenylbutazone | |
| | Phenacetin | |
| | Salicylamide | |
| | Sulfadiazine | |
| | Tetracycline | |
| | Tolbutamide | |

**Methods of Assessing Bioavailability**

Bioavailability testing is a means of predicting the clinical efficacy of a drug and provides the most reliable method available for determining bioequivalence. The bioavailability of a drug substance formulated into a pharmaceutical product is fundamental to the goals of dosage form design and essential for the clinical efficacy of the medication. Thus, bioavailability testing, which measures the rate and extent of drug absorption, is a way to obtain evidence of the therapeutic utility of a drug-product. Bioavailability determinations are performed to ensure that a given drug-product will get the therapeutic agent to its site of action in an adequate concentration.

***In Vivo* Methods**

One method for assessing the bioavailability of a drug-product is through the demonstration of a clinically significant effect. However, such clinical studies are complex, expensive, time - consuming and require a sensitive and quantitative measure of

the desired response. Further, response is often quite variable, requiring a large test population. Quantification of pharmacologic effect is another possible way to assess a drug's bioavailability. This method is based on the assumption that a given intensity of response is associated with a particular drug concentration at the site of action e.g., variation of miotic response intensity can be directly related to the oral dose of chlorpromazine. However, monitoring of pharmacologic data is often difficult, precision and reproducibility are difficult to establish, and there are only a limited number of pharmacologic effects (e.g. heart rate, body temperature, blood sugar levels) that are applicable to this method. Because of these limitations, alternative methods have been developed to predict the therapeutic potential of a drug.

**Blood Level Studies**

The current method to assess the clinical performance of a drug involves measurement of the drug concentrations in the blood. These methods are relatively easy to conduct and require a limited number of subjects. The studies usually are performed in healthy human volunteers under tightly controlled fasting conditions and fixed activity levels. Effect of gender and age of the volunteers on bioavailability are considered when there is a specific concern that they may affect drug safety or efficacy. The bioavailability studies should be conducted with sensitivity for the moral and ethical issues involved in using human volunteers for the experiments. The possibility of adverse effects and the hazards in various blood sample collections must be highlighted. Special populations, such as children and pregnancy or breastfeeding women, must not be included for humanitarian reasons. Also, individuals with certain enzyme deficiencies or abnormal metabolism should be avoided to minimize any bias or source of variability. Normally, the study is conducted in a group of male subjects except where not applicable, such as in case of antifertility drugs which female volunteers have to be used. Thai FDA recommends that the subjects range in age from 18-45 years and body mass index values range in 18-24 $kg/m^2$ or within ± 10% of ideal body weight (Thai FDA, 2000). Individuals with any past history of gastrointestinal tract, liver or kidney malfunctions must be excluded. Also, those with significant organ abnormalities or diseases should be avoided. All participating volunteers should be subjected to a thorough physical

examination and the following hematological and clinical chemistry tests. Participants should abstain from any medication for a minimum of one week and should have taken no enzyme-inducing drug for one month prior to dosing. A standard diet can be specified as well as the type and volume of fluid intake. Strenuous physical activities and demanding sports should be avoided during the period of the study. The design of the experiment, partial crossover, total crossover, Latin square, etc., should be specified. A well designed protocol is a flexible one that assesses the overall variability of the results sequentially during the progress of the study and modifiers the experimental plan accordingly. Study conditions should be adhered to as rigorously as they are a major source for variability. Generally, a crossover study design is used. Using this method, both the test and the reference products are compared in each subject, so that intersubject variables, such as age, weight, differences in metabolism, etc., are minimized. Each subject thus acts as his/her own control. Also, with this design, subjects' daily variations are distributed equally among all dosage forms or drug-products being tested. Most bioavailability evaluations are made on the basis of single dose administration. Other important considerations in the methodology of a bioavailability study are sample size, period of trial, and sampling. For statistical purposes, twelve subjects are considered to be a minimum sample size. Otherwise there will not be enough data to draw valid conclusions. The bioavailability testing period should be of a sufficient length of time to ensure that drug absorption has been completed. This length of time is at least three times the half-life of the drug; generally a period of four to five times the half-life is used. The subjects are randomly selected for each group and the sequence of drug administration is randomly assigned.

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 which the samples were taken. The key parameters to note are:

1. AUC, the area under the plasma concentration-time curve, 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**,** 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, reflects the rate of drug absorption, and decreases as the absorption rate increases.

Bioavailability is generally assessed by the determination of these three parameters. Since the AUC is representative of the total amount of drug absorbed into the circulation, it is used to quantitate the extent of drug absorption. For $C_{max}$ and $t_{max}$, if all other factors are constant, such as the extent of absorption and rate of elimination, then $C_{max}$ is proportional to the rate of absorption and $t_{max}$ is inversely proportional to the absorption rate. Thus, the faster the absorption of a drug the higher the maximum concentration will be and the less time it will take to reach the maximum concentration.

**Urinary Excretion Data**

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. As a rule of thumb, 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 (Chereson and Banakar, 2000).

**Statistical Criteria**

After a bioequivalence study is conducted and these pharmacokinetic parameters are determined, the pharmacokinetic data must be examined according to a set of pre-determined criteria to confirm or refute the bioequivalence of the test and reference formulation. That is, one must determine whether the test and reference products differ within a predefined level of statistical significance. Since the statistical outcome of a bioequivalence study is the primary basis of the decision for accepting or against therapeutic equivalence of two products, it is critically important that the experimental data be analyzed by an appropriate statistical test. In the early 1970s, bioequivalence was usually determined only on the basis of mean data. Mean AUC and $C_{max}$ values for the generic product had to be within ±20% of those of the reference product (Dighe and Adams, 1991). Although the 20% value was somewhat arbitrary, it was felt that for most drugs, a 20% change in the dose would not result in significant difference in the clinical response to drugs (Meyer, 1991).

Westlake (1972) was the first to suggest the use of confidence intervals based on the two one-sided t-tests as a means of testing for bioequivalence. The current guidelines recommended that confidence intervals of two formulations whose rate and extent of absorption differ by -20%/+25% are generally considered to be bioequivalent. Therefore, a generic manufacturer must show that the 90% confidence interval for the ratio of the mean AUC and $C_{max}$ values of its product to that the innovator is within the limits of 0.80 to 1.25. Since these tests are carried out at the 0.05 level of significance, there is no more

than a 5% chance that they will be approved as equivalent if they differ by as more than is allowed by the equivalence criteria.

In addition, linear model procedures are recommended for the analysis of pharmacokinetic data derived from *in vivo* bioequivalence studies. An analysis of variance (ANOVA) should be performed on the pharmacokinetic parameters (AUC and $C_{max}$) which were transformed into logarithmic scales prior to the ANOVA. The USP 25 described the rational for log transformation as follows:

Clinical rational – Using log transformation, the general linear statistical model employed in the analysis of bioequivalence data allows inferences about the difference between the two means on the log scale, which can then be retransformed into inferences about the ratio of the two averages on the original scale. Log transformation thus achieves the general comparison based on the ratio rather than the difference.

Pharmacokinetic rational – The use of AUC and $C_{max}$ as measure of the amount and rate of drug absorbed, respectively, thus involves a multiplicative term, which might be regarded as a function of the subject. For this reason, the subject effect is not additive if the data is analyzed on original scale of measurement. Therefore, logarithmic transformation of the AUC and $C_{max}$ data will bring the general equation into the equation in an additive fashion as follows:

| | | | |
|---|---|---|---|
| AUC ; | $AUC_{0-\infty}$ | = | $FD/VK_e$ |
| | $\ln AUC_{0-\infty}$ | = | $\ln F + \ln D - \ln V - \ln K_e$ |
| $C_{max}$ ; | $C_{max}$ | = | $(FD/V) \times e^{(-K_e \times t_{max})}$ |
| | $\ln C_{max}$ | = | $\ln F + \ln D - \ln V - K_e t_{max}$ |
| where ; | F | = | Fraction absorbed |
| | D | = | Administered dose |
| | V | = | Apparent volume of distribution |
| | $K_e$ | = | Elimination rate constant |

Statistical rational – Logarithmic transformation of the data from bioequivalence studies can be used to circumvent the use of estimates of the reference product average for computation of the confidence interval for ratio of product averages. This is advantage for the cases where a least square estimate for the reference product mean is not well defined. Standard parametric methods are ill-suited to making inferences about the ratio of two averages, though some valid methods do exist. Log transformation changes the problem to one of making inferences about the difference of two averages, for which the standard methods are well suited. In addition, many biological data correspond more closely to a log-normal distribution than to a normal distribution.

Although the decision of bioequivalence is now made in a more statistically valid way and the associated concerns have diminished somewhat, some important questions and controversies in bioequivalence remain. However, a great deal of progress has been made in this area in the last twenty years. The improved design of the studies, the interpretation of the data, the increased scientific rigor of the acceptance criteria, as well as the more rigorous auditing and inspection program have made bioequivalence data an appropriate and valid means of approving generic drug products.

**Population and Individual Bioequivalence**

For physicians and patients, bioequivalent drug-products can and should be used interchangeably to achieve a similar therapeutic effect. Therefore, bioequivalence studies, in fact, serve as surrogates for clinical trials in evaluation of therapeutic equivalence in efficacy and safety between the innovator's product and its generic product. However, when a physician has the possibility of administering a generic drug-product, he or she needs to consider the anticipated therapeutic effect that may be obtained from the patient. If a new patient has just begun a drug regimen, the physician does not have any information about the patient's therapeutic response to any of the different formulation that he or she could prescribe. As a result, the only relevant information that the physician could have is the comparison of the marginal distributions for some pharmacokinetic responses between the generic and innovator drug- product from a population of subjects. If the marginal distributions follow an approximate normal distribution, the equivalence can be evaluated through inferences on population

parameters, such as average and intra-subject variability. This concept is referred to as the population bioequivalence (PBE). Given the information about population parameters, the physician can determine whether to prescribe an innovator drug-product or its generic drug-product to a new patient who is just beginning the drug regimen. Therefore, prescribability is the interchangeability for the new patient.

On the other hand, for the patients already receiving the long-term administration of an innovator formulation, the physician now has the information about patient's response to that formulation. To ensure the similar efficacy and safety by the generic drug-product switch, it follows that the concentration of the active ingredient for the generic drug-product must still be within the patient's same therapeutic window established by the innovator's drug-product. This concept is then referred to as switchability that requires bioequivalence within the same patient. Switchability, therefore, is exchangeability within the same subject. To assure drug switchability, it is suggested that bioequivalence be assessed within the same individuals. This concept of bioequivalence is known as individual bioequivalence (IBE).

The concept of individual bioequivalence has attracted the United States FDA's attention since introduced by Anderson and Hauck (1990), which has led to a significant change in regulatory requirement for assessment of bioequivalence. In 1997, a draft guidance "In Vivo Bioequivalence Studies Based on Population and Individual Bioequivalence Approaches" was recently distributed by the United States FDA for public comment. The draft guidance recommended that population bioequivalence (PBE) and individual bioequivalence (IBE) be used to assess drug prescribability and drug switchability, respectively. More specifically, this guidance recommends that PBE be applied to new formulations, additional strengths, or new dosage forms in new drug application (NDA), whereas IBE should be considered for abbreviated new drug application (ANDA) or generic drug (Chow and Liu, 2000).

**Amoxicillin/Clavulanic Acid**

**Physicochemical Properties**

Amoxicillin/clavulanic acid is commercially available as a fixed combination of amoxicillin trihydrate and the potassium salt of clavulanic acid. Amoxicillin is a semisynthetic aminopenicillin with a chemical structure of α-amino-β-hydroxy-benzyl penicillin (Figure 1). Clavulanic acid is a naturally occuring β-lactam produced by *Streptomyces clavuligerus*. Clavulanic acid contains a β-lactam ring and is structurally similar to penicillins and cephalosporins; however, the β-lactam ring in clavulanic acid is fused with an oxazolidine ring rather than with a thiazolidine ring as in penicillins or a dihydrothiazine ring as in cephalosporins (Figure 2). Although commercially available amoxicillin and clavulanic acid contains amoxicillin as the trihydrate and clavulanic acid as the potassium salt, potency of amoxicillin is calculated on the anhydrous basis and potency of clavulanate potassium is expressed in terms of clavulanic acid.

Amoxicillin occurs as a white, practically odorless, crystalline powder and is sparingly soluble in water. Clavulanic acid occurs as an off-white, crystalline powder and is very soluble in water and slightly soluble in alcohol at room temperature, clavulanic acid has a pKa of 2.7.

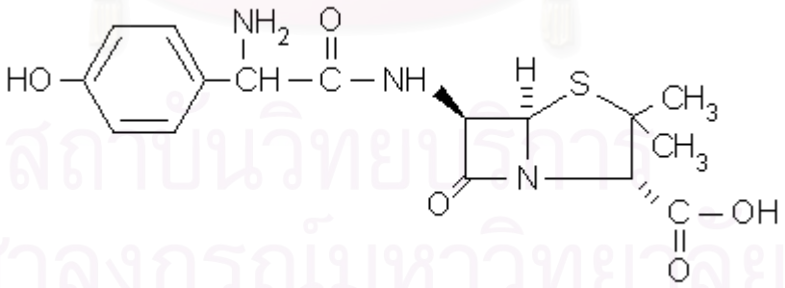



Figure 1 Chemical Structure of Amoxicillin

Chemical name : 4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,6-[[amino-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-,[2S-[2α,5α,6β(S*)]]

Empirical formula : $C_{16}H_{19}N_3O_5S$

Molecular weight : 365.41

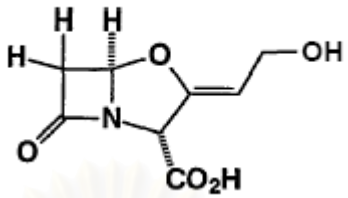



Figure 2 Chemical Structure of Clavulanic Acid

Chemical name : 4-Oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,3-(2-hydroxyethylidene)-7-oxo-,[2R-(2α,3Z,5α)]

Empirical formula : $C_8H_9NO_5$

Molecular weight : 199.2

**Antibacterial Activity**

Clavulanic acid is an irreversible inhibitor of intracellular and extracellular β-lactamase, effective against a wide variety of these enzymes. Clavulanic acid, therefore, protects amoxicillin from inactivation by many β-lactamase. As consequence the antibacterial activity of amoxicillin has been restored at a time when the spread of resistance due to β-lactamase production severely threatened its usefulness.

Clavulanic acid alone possesses only weak antibacterial activity, except against *Leginella spp*., and certain strains of *Branhamella catarrhalis*, *B. fragilis* and *Neisseria gonorrhoeae*. However, the addition of clavulanic acid to amoxicillin increases the susceptibility to amoxicillin of amoxicillin-resistant strains of Gram negative and Gram-positive aerobic and anaerobic bacteria where resistance is caused by β-lactamase production. These include *staphylococcus aureus* (but not methicillin-resistant strains), *Haemophilus spp., Branhamella catarrhalis, Neisseria gonorrhoeae, Escherichia coli, Proteus spp., Klebsiella pneumoniae, Citrobacter diversus, Salmonella spp., Shigella spp., Campylobacter jejuni, Bacteroides spp*., and *Mycobacterium spp*. The susceptibility of amoxicillin-sensitive strains is not generally affected by the addition of clavulanic acid (McEvoy, 2001).

**Table 2** *In Vitro* Antibacterial Activity of Amoxicillin/Clavulanic Acid against Gram-positive and Gram-negative Pathogens (Easton, Noble and Perry, 2003).

| Pathogens | $MIC_{90}$ (mg/L) |
|---|---|
| Gram-positive Bacteria | |
| *Streptococcus pneumoniae* (penicillin-susceptible) | 0.03 |
| *S. pneumoniae* (penicillin-intermediate) | 1 |
| *S. pneumoniae* (penicillin-resistant) | 4 |
| *Staphylococcus aureus* (methicillin-susceptible) | 2 |
| *S. aureus* (methicillin-resistant) | >16 |
| *Streptococcus pyogenes* | ≤ 0.03 |
| Gram-negative Bacteria | |
| *Haemophilus influenzae* | 2 |
| *H. influenzae* (β-lactamase positive) | 2 |
| *H. influenzae* (β-lactamase negative) | 1 |
| *Moraxella catarrhalis* | 0.25 |
| *M. catarrhalis* (β-lactamase positive) | 0.5 |
| *M. catarrhalis* (β-lactamase negative) | 0.03 |

$MIC_{90}$ = minimum concentration required to inhibit growth of 90% of test strains

Amoxicillin/clavulanic acid is bactericidal *in vitro*, usually at concentration no more than one dilution higher than *in vitro* inhibitory concentration. The *in vitro* synergy of clavulanic acid combined with amoxicillin has been confirmed *in vivo* in numerous experimental infections in animals. The combination of amoxicillin with clavulanic acid appears to suppress the development of resistance under experimental conditions.

**Pharmacokinetics**

**Absorption**

Amoxicillin and clavulanic acid are both generally stable in the presence of acidic gastric secretions and are well absorbed following oral administration. Peak serum concentrations of amoxicillin and of clavulanic acid are generally attained within 1-2.5

hours following oral administration of a single dose of amoxicillin and clavulanic acid in fasting adults.

Following oral administration of a single tablet containing 500 mg of amoxicillin and 125 mg of clavulanic acid in fasting healthy adults, peak serum concentrations of amoxicillin average 6.5-9.7 μg/mL and peak serum concentrations of clavulanic acid average 2.1-3.9 μg/mL, The concentrations of amoxicillin achieved following oral administration of amoxicillin and clavulanic acid are similar to those achieved following oral administration of equivalent doses of amoxicillin alone (McEvoy, 2001).

Studies in healthy adults using amoxicillin/clavulanic acid indicate that presence of food in GI tract does not affect oral absorption of either amoxicillin or clavulanic acid following administration of fixed combination preparations of the drugs (McEvoy, 2001).

**Distribution**

Following administration of amoxicillin and clavulanic acid, amoxicillin and clavulanic acid are both distributed into the lungs, pleural fluid, and peritoneal fluid. Low concentrations (i.e., less than 1 μg/mL) of each drug are attained in sputum and saliva (McEvoy, 2001).

Only minimal concentrations of amoxicillin or clavulanic acid are attained in cerebrospinal fluid (CSF) following oral administration of amoxicillin and clavulanic acid in patients with uninflamed meninges; higher concentrations may be attained when meninges are inflamed. In one study in patients with uninflamed meninges who received a single 250 mg oral dose of clavulanic acid as the sodium salt, concentrations of clavulanic acid in CSF obtained 1-6 hours after the dose ranged from 0-0.2 μg/mL. In 2 patients with continuous CSF drainage after neurosurgical procedures who received a similar oral dose of the drug, peak CSF concentrations of clavulanic acid were 2.4 and 0.4 μg/mL, respectively, and occurred approximately 4 hours after the dose; concurrent serum concentrations of clavulanic acid were 2.3 and 0.3 μg/mL, respectively (McEvoy, 2001).

Amoxicillin is 17-20% bound to serum proteins. *In vitro* or *in vivo* following oral administration, clavulanic acid is reportedly 22-30% bound to serum proteins at a concentration of 1-100 μg/mL. Amoxicillin and clavulanic acid readily crossed the

placenta. Amoxicillin and clavulanic acid are distributed into milk in low concentrations (McEvoy, 2001).

**Elimination**

Serum concentrations of amoxicillin and clavulanic acid both decline in a biphasic manner and half-lives of the drugs are similar. Following oral administration of amoxicillin and clavulanic acid in adults with normal renal function, amoxicillin has an elimination half-life of 1-1.3 hour and clavulanic acid has a distribution half-life of 0.28 hour and an elimination half-life of 0.78-1.2 hour. In one study in children 2-15 years of age, the elimination half-lives of amoxicillin and of clavulanic acid averaged 1.2 and 0.8 hours, respectively (McEvoy, 2001).

The metabolic fate of clavulanic acid has not been fully elucidated; however, the drug appears to be extensively metabolized. In rats and dogs, the major metabolite of clavulanic acid is 1-amino-4- hydroxybutan-2-one; this metabolite has also been found in human urine following administration of clavulanic acid. Clavulanic acid is excreted in urine principally by glomerular filtration. Studies in dogs and rats using radiolabeled clavulanic acid indicate that 34-52, 25-27 and 16-33% of a dose of the drug is excreted in urine, feces and respired air, respectively (McEvoy, 2001).

Following oral administration of a single oral dose of amoxicillin and clavulanic acid in adults with normal renal function, approximately 50-73 and 25-45% of the amoxicillin and clavulanic acid doses, respectively, are excreted unchanged in urine within 6-8 hours. In one study in healthy adults who received a single oral dose of 250 mg of amoxicillin and 125 mg of clavulanic acid, urinary concentrations of amoxicillin and of clavulanic acid averaged 381 and 118 μg/mL, respectively, in urine collected over the first 2 hours after the dose (McEvoy, 2001).

Serum concentrations of amoxicillin and of clavulanic acid are higher and the serum half-lives prolonged in patients with renal impairment. In one study in patients with creatinine clearances of 9 mL/min, the serum half-lives of amoxicillin and of clavulanic acid were 7.5 and 4.3 hours, respectively.

Amoxicillin and clavulanic acid are both removed by hemodialysis. Clavulanic acid is also removed by peritoneal dialysis. Only minimal amounts of amoxicillin appear to be removed by peritoneal dialysis (McEvoy, 2001).

**Therapeutic Use**

Amoxicillin and clavulanic acid is used orally for the treatment of lower respiratory tract infections, otitis media, sinusitis, skin and skin structure infections, and urinary tract infections caused by susceptible organisms. Amoxicillin and clavulanic acid also has been used orally for the treatment of chancroid and gonorrhea caused by susceptible organisms (McEvoy, 2001).

Amoxicillin and clavulanic acid is used principally for the treatment of infections caused by susceptible β-lactamase-producing strains of *Moraxella catarrhalis*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella*, and *Staphylococcus aureus*. Although amoxicillin and clavulanic acid also may be effective in the treatment of infections caused by non- β-lactamase-producing organisms susceptible to amoxicillin alone, most clinicians state that an aminopenicillin used alone is preferred to the combination drug for the treatment of these infections and that amoxicillin and clavulanic acid should be reserved for use in the treatment of infections caused by, or suspected of being caused by, β-lactamase-producing organisms when an aminopenicillin alone would be ineffective (McEvoy, 2001).

**Adverse Effects**

Adverse effects reported with amoxicillin/clavulanic acid are generally dose related and are similar to those reported with amoxicillin alone. With the exception of adverse gastrointestinal (GI) effects, which have been reported more frequently with amoxicillin and clavulanic acid than with amoxicillin alone, the frequency and severity of adverse effects reported with the fixed combination preparations are generally similar to those reported with amoxicillin alone (McEvoy, 2001).

GI effects are the most frequent adverse reactions to oral amoxicillin and clavulanic acid. Diarrhea or loose stools has been reported in about 9% of patients receiving the drug. Nausea and vomiting have been reported in 1-5% of patients.

Abdominal discomfort, anorexia, flatulence, dyspepsia, gastritis, stomatitis, glossitis, black or hairy tongue and enterocolitis also have been reported. The frequency of nausea and vomiting appears to be related to the dose of clavulanic acid since these effects have been reported in up to 40% of patients when a 250 mg dose of clavulanic acid rather than a 125 mg dose was used in conjunction with amoxicillin. Administration of oral amoxicillin and clavulanic acid with meals reportedly decreases the frequency and severity of adverse GI effects (McEvoy, 2001).

*Clostridium difficile*-associated diarrhea and colitis (antibiotic-associated pseudomembranous colitis) caused by toxin producing clostridia may occur during or following discontinuance of amoxicillin and clavulanic acid. Rash and urticaria have been reported in approximately 3% of patients. Other adverse effects that have been reported in 1% or less of patient receiving the drug include candidal vaginitis, dizziness, headache, fever, and slight thrombocytosis (McEvoy, 2001).

Moderate increases in serum concentrations of AST (SGOT) and/or ALT (SGPT), alkaline phosphatase, and/or bilirubin have been reported in patients receiving amoxicillin and clavulanic acid. Hepatic dysfunction has been reported most frequently in geriatric patients, males or patients receiving prolonged therapy with the drug (McEvoy, 2001).

**Drug Interactions**

Oral probenecid administered shortly before or concomitantly with amoxicillin and clavulanic acid slows the rate of renal tubular secretion of amoxicillin and produces higher and prolonged serum concentrations of amoxicillin (McEvoy, 2001).

**Administration**

Amoxicillin/clavulanic acid is administered orally. Chewable tablets should be thoroughly chewed before swallowing. Amoxicillin/clavulanic acid has also been given IV. Because GI absorption of amoxicillin and clavulanic acid is not affected by food, the drug may be administered orally without regard to meals. However, administration of oral amoxicillin and clavulanic acid with meals reportedly may minimize adverse GI effects (McEvoy, 2001).

Amoxicillin and clavulanic acid powder for oral suspension should be reconstituted at the time of dispensing by adding the amount of water specified on the bottle to provide a suspension containing 125 mg of amoxicillin and 31.25 mg of clavulanic acid per 5 mL, 200 mg of amoxicillin and 28.5 mg of clavulanic acid per 5 mL, or 250 mg of amoxicillin and 62.5 mg of clavulanic acid per 5 mL. After tapping the bottle to thoroughly loosen the powder for oral suspension, the water should be added to the powder in 2 portions and the suspension was agitated well after each addition. The suspension should be agitated well just prior to administration of each dose (McEvoy, 2001).

**Dosage**

Dosage of amoxicillin and clavulanic acid is generally expressed in terms of the amoxicillin content of the fixed combination. Although commercially available amoxicillin and clavulanic acid contains amoxicillin as the trihydrate and clavulanic acid as the potassium salt, potency of amoxicillin is calculated on the anhydrous basis and potency of clavulanate potassium is expressed in terms of clavulanic acid.

**Adult Dosage**

The usual adult oral dosage of amoxicillin and clavulanic acid is one 250/125 mg film coated tablet every 8 hours or one 500/125 mg film coated tablet every 12 hours. For more severe infections and infections of the respiratory tract, the usual adult oral dosage is one 500/125 mg every 8 hours or one 875/125 mg every 12 hours (McEvoy, 2001).

**Pediatric Dosage**

Children weighing 40 kg or more may receive the usual adult oral dosage of amoxicillin and clavulanic acid. The usual dosage of amoxicillin and clavulanic acid in neonates and infants younger than 12 weeks of age is 30 mg/kg of amoxicillin daily given in divided doses every 12 hours. Because experience with the oral suspension containing 200 mg of amoxicillin/5 mL is limited in this age group, the manufacturer recommends that the oral suspension containing 125 mg of amoxicillin/5 mL be used in neonates and infants younger than 12 weeks of age (McEvoy, 2001).

For the treatment of sinusitis, lower respiratory tract infections, and more severe infections in pediatric patients 12 weeks of age and older, the usual dosage of amoxicillin and clavulanic acid is 45 mg/kg of amoxicillin daily in divided doses every 12 hours administered as the oral suspension containing 200 or 400 mg of amoxicillin/5 mL or as chewable tablets containing 200 or 400 mg of amoxicillin. Alternatively, these infections in this age group can be treated with a dosage of 40 mg/kg of amoxicillin daily in divided doses every 8 hours administered as the oral suspension containing 125 or 250 mg of amoxicillin/5 mL or as chewable tablets containing 125 or 250 mg of amoxicillin.

For the treatment of less severe infections in pediatric patients 12 weeks of age or older, the usual dosage of amoxicillin and clavulanic acid is 25 mg/kg of amoxicillin daily in divided doses every 12 hours administered as the oral suspension containing 200 or 400 mg of amoxicillin/5 mL or as chewable tablets containing 200 or 400 mg of amoxicillin. Alternatively, less severe infections in this age group can be treated with a dosage of 20 mg/kg of amoxicillin daily in divided doses every 8 hours administered as the oral suspension containing 125 or 250 mg of amoxicillin/5 mL or as chewable tablets containing 125 or 250 mg of amoxicillin (McEvoy, 2001).

**Dosage in Renal Impairment**

In patients with renal impairment, dose and/or frequency of administration of amoxicillin and clavulanic acid should be modified in response to the degree of renal impairment. Some clinicians suggest that modification of usual dosage is unnecessary in adults with creatinine clearances greater than 30 mL/min. These clinicians recommend that adults with creatinine clearances of 15-30 mL/min receive the usual dose of the drug every 12-18 hours, adults with creatinine clearances of 5-15 mL/min receive the usual dose every 20-36 hours, and adults with creatinine clearances less than 5 mL/min receive the usual dose every 48 hours. However, other clinicians suggest that use of amoxicillin and clavulanic acid should be avoided in patients with creatinine clearances less than 30 mL/min until more data are available on use of the drug in these patients. Some clinicians suggest that adults undergoing hemodialysis receive a 500 mg tablet containing 500 mg of amoxicillin and 125 mg of clavulanic acid halfway through each dialysis period and an additional 500 mg tablet after each dialysis period (McEvoy, 2001).

# CHAPTER III

# MATERIALS AND METHODS

## Materials

### A. Test Products

Augmentin® manufactured by SmithKline Beecham Pharmaceuticals, was assigned as innovator's product, and Cavumox® manufactured by Siam Bheasach Co., Ltd., was used as test product. Each film coated tablet contains amoxicillin trihydrate equivalent to amoxicillin 500 mg and clavulanate potassium equivalent to clavulanic acid 125 mg. Other informations of these products were shown in Appendix A.

### B. Reagents

1. Working standard amoxicillin trihydrate (Antibioticos, Spain) Lot No. R5070001; Potency: 86.25%
2. Reference standard clavulanate lithium (The United States Pharmacopeial Convention, Inc., USA) Lot No. 13442; Potency: 95.20%
3. Theophylline (China) Lot No. S99123.
4. Salicylic acid (BDH Chemicals Ltd., England) Lot No. 4534683G
5. Acetronitrile HPLC grade (Labscan, Thailand) Lot No. 02100046
6. Methanol HPLC grade (Labscan, Thailand) Lot No. 03010087
7. Monobasic potassium phosphate AR. (Merck, Germany) Lot No. A397373247
8. Dibasic potassium phosphate AR. (Carlo Erba Reagent, MI) Lot No. 321 A712801
9. Monobasic sodium phosphate AR. (Carlo Erba Reagent, MI) Lot No. 10049-21-5
10. Dibasic sodium phosphate AR (Carlo Erba Reagent, MI) Lot No. 7558-79-4
11. Imidazole (Fluka, Switzerland) Lot No. 384580/1 33495
12. Perchloric acid (Merck, Germany) Lot No. H9C063

### C. Apparatus

1. Analytical balance (Sartorius, 1615MP; S/N 3209026, Germany)
2. Digital pH meter (Orion 420A, Germany)
3. High performance liquid chromatography (LC-10AD, Shimadzu, Japan)
4. Vacuum manifold processing station (Agilent Technologies, UK)
5. AccuBOND II ODS-$C_{18}$ Cartridges 500 mg 3 mL (Agilent Technologies, UK)
6. Sonicator (Bransonic 221, USA)
7. Dissolution apparatus (VK 7000, Vankel Technology Group, Inc., USA)
8. Vortex mixer (Vortex-Genie, Scientific Industries, Inc., USA)
9. Centrifuge (Sigma 302K, Sigma Lab, Centrifuge Gmbtt, Germany)
10. Micropipet (Gilson Medical Electronics S.A., France)
11. Glassware

## Methods

### A. *In Vitro* Studies

Both commercial brands of 500/125 mg amoxicillin and clavulanic acid were evaluated following the tests as stated in the United States Pharmacopoeia 25 (USP 25). The tests were:

#### 1. Content of Active Ingredient

The amounts of amoxicillin and clavulanic acid in tablets were determined according to method of USP 25. It was described as follows.

Diluent – Dissolve 7.8 g of monobasic sodium phosphate in 900 mL of water, adjust with phosphoric acid or 10 N sodium hydroxide to a pH of 4.4 ± 1, dilute with water to make 1000 mL, and mix.

Mobile phase – Prepare a suitable mixture of pH 4.4 sodium phosphate buffer and methanol (95:5), and filter through a membrane filter of 0.5 μm or finer porosity. Make adjustments if necessary.

Standard preparation – Dissolve accurately weighed quantities of amoxicillin WS and clavulanate lithium RS in water to obtain a solution having known concentrations of about 0.5 mg/mL and 0.2 mg/mL, respectively.

Assay preparation – Dissolve not less than 10 tablets, accurately counted, in water with the aid of mechanical stirring, transfer to a suitable volumetric flask, dilute with water to volume, and mix. Filter a portion of this solution, discarding the first 10 mL of the filtrate. Dilute an accurately measured volume of the filtrate quantitatively and stepwise with water to obtain a solution containing about 0.5 mg of amoxicillin per mL.

Chromatographic system – The liquid chromatograph is equipped with a 220 nm detector and 4 mm x 30 cm column that contains 10 μm packing L1. The flow rate is about 1 mL/min.

Procedure – Separately inject equal volumes (about 20 μL) of the standard preparation and the assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of amoxicillin and clavulanic acid in each tablet taken by the formula:

$$(L/D)(CP/1000)(r_u/r_s)$$

in which

L is the labeled quantity, in mg, of amoxicillin or clavulanic acid in each tablet.

D is the concentration, in mg/mL, of amoxicillin or clavulanic acid in the assay preparation on the basis of the number of tablets taken, the labeled quantity of amoxicillin or clavulanic acid in each tablet, and the extent of dilution.

C is the concentration, in mg/mL, of amoxicillin WS or clavulanic lithium RS in the standard preparation.

P is the potency, in μg of amoxicillin or clavulanic acid per mg, of amoxicillin WS or clavulanic lithium RS, respectively.

$r_u$ and $r_s$ are the amoxicillin or clavulanic acid peak response obtained from the assay preparation and standard preparation, respectively.

**2. Uniformity of Dosage Units**

Ten tablets of 500/125 mg amoxicillin/clavulanic acid tablets from each brand were individually assayed for the percent labeled content of amoxicillin and clavulanic acid in each tablets following the same method as analysis for content of active ingredient. The mean and standard deviation of percent labeled amount were calculated as well as the relative standard deviation.

**3. Dissolution Test**

The dissolution test was conducted using the USP dissolution apparatus II for amoxicillin/clavulanic acid tablets (USP 25, 2002). A tablet of amoxicillin/clavulanic acid was placed in each vessel of the dissolution tester containing 900 mL of double distilled water at 37 ± 0.5 °C as dissolution medium. The apparatus was operated at the rate of 75 rpm. Five mL of each sample was withdrawn after the apparatus was operated at time 5, 10, 15, 20, 30, 45, 60 minutes, respectively. The equivalent amount of double distilled water equilibrated at 37 ± 0.5 °C was added immediately after each sampling to maintain a constant volume of dissolution medium. Twelve tablets of each brand were tested. The amount of amoxicillin/clavulanic acid dissolved in each sample was quantitated using HPLC following the same chromatographic system as analysis for content of active ingredient and calculated using the calibration curve. The dissolution profiles were then constructed by plotting percent amoxicillin/clavulanic acid dissolved of each brand versus time.

**Standard calibration curve of amoxicillin**

Certain amount of standard amoxicillin was dissolved in double distilled water to produce a set of standard solutions of 0.04, 0.09, 0.17, 0.35, 0.44, and 0.87 mg/mL, respectively. All these standard solutions were analyzed using HPLC following the same chromatographic system as analysis for content of active ingredient. The peak heights of amoxicillin versus known amoxicillin concentrations were fitted to a straight line using linear regression analysis.

**Standard calibration curve of clavulanic acid**

Certain amount of standard clavulanic acid was dissolved in double distilled water to produce a set of standard solutions of 0.01, 0.03, 0.05, 0.10, 0.15, and 0.20 mg/mL, respectively. All these standard solutions were analyzed using HPLC following the same chromatographic system as analysis for content of active ingredient. The peak heights of clavulanic acid versus known clavulanic acid concentrations were fitted to a straight line using linear regression analysis.

**Dissolution profiles comparison**

The dissolution profiles of test product were compared to that of the innovator's product to assess their similarities using difference factor ($f_1$) and similarity factor ($f_2$) (Thai FDA, 2000) calculated from equations:

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

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

where; n = Number of sampling points

$R_t$ = Percent dissolved of innovator's product at time t

$T_t$ = Percent dissolved of test product at time t

**B. *In Vivo* Studies**

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

**1. Test Products**

Two commercial brands of 500/125 mg amoxicillin/clavulanic acid film coated tablets were *in vivo* tested in this experiment.

**2. Subjects**

Eighteen healthy Thai male volunteers with ages range from 18 to 44 years participated in this study. Demographic data are presented in Table 21. Prior to testing, all subjects had to pass physical examination and clinical laboratory tests. Information was provided to all subjects, explaining the risks and benefits. Written informed consents were obtained before study initiation. The protocol was approved by the Ethics Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. The volunteers were asked to take no medication, alcoholic preparations and cigarettes for at least two weeks preceding the study and during the experimental period.

Inclusion criteria:

1) Healthy Thai male volunteers with the ages range from 18 to 45 years and body mass index between 18 to 24 $kg/m^2$
2) Normal physical and laboratory biochemical test
3) No history of gastrointestinal tract disease, hepatic disease, renal disease, allergic disease or others that affecting to bioavailability of the drug
4) Non-smokers and without a history of alcohol or drug abuse
5) No history of allergic reaction to amoxicillin and clavulanic acid

Exclusion criteria:

1) Refuse to finish the study
2) Allergic or having adverse drug reaction to amoxicillin and clavulanic acid

**3. Dose and Drug Administration**

One tablet of 500/125 mg amoxicillin/clavulanic acid was given orally with 200 mL of water in a single dose. All subjects received each dose in the morning after 8 hours overnight fast. No food or drink was permitted until 4 hours after dosing.

**4. Subject Monitoring**

Blood pressure and pulse rate of each subject were monitored hourly during the first 4 hours of the study. Any unusual symptoms were observed and subjects

were periodically questioned for those throughout the study period. If adverse drug reactions occurred, they would be recorded in case record forms and these subjects would be diagnosed and treated by doctor.

**5. Experimental Design**

The study was conducted in a randomized two way crossover design. Each subject received the drug in a randomized order with 1 week washout period between each administration as shown in Table 3.

**Table 3** Randomization Schedule

| Sequence | Subject no. | Period | |
|---|---|---|---|
| | | 1 | 2 |
| 1 | 1 | A | B |
| | 2 | A | B |
| | 3 | A | B |
| | 4 | A | B |
| | 5 | A | B |
| | 6 | A | B |
| | 7 | A | B |
| | 8 | A | B |
| | 9 | A | B |
| 2 | 10 | B | A |
| | 11 | B | A |
| | 12 | B | A |
| | 13 | B | A |
| | 14 | B | A |
| | 15 | B | A |
| | 16 | B | A |
| | 17 | B | A |
| | 18 | B | A |

A and B: represent the brand name of 500/125 mg amoxicillin/clavulanic acid film coated tablets

**6. Sample Collection**

7 mL of blood samples were withdrawn from a forearm vein of each subject using a disposable syringe at the following time point: predose, at 0.15, 0.50, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7 and 9 hours postdose. All blood samples were collected in glass tubes containing lithium heparin, chilled at 0°C. Within 30 minutes after collection, blood samples were centrifuged at approximately 3000 rpm for 15 minutes. Following separation of the plasma, the samples were divided into two aliquots and stored at -20°C until analysis.

**7. Analysis of Amoxicillin in Plasma Samples**

**7.1 Sample preparation**

The method of sample preparation for amoxicillin in plasma samples was modified from that of Charles and Chulavatnatol (1993) method. The process was described as follows:

0.5 mL of plasma sample was mixed with 10 μL of internal standard solution (1 mg/mL of theophylline in water) and 170 μL of 10% perchloric acid solution. The mixture was vortexed for 10 seconds and centrifuged at 15000 rpm for 30 minutes. Supernatant was then separated and neutralized with 200 μL of 0.8 M $Na_2HPO_4$. Finally, 20 μL of the solution was injected into the HPLC.

**7.2 Chromatographic systems**

Apparatus : Shimadzu® LC-10A HPLC pump, equipped with a degasser (DGU-12A), a communication bus module (CBM-10A), an autoinjector (SIL-10A), a column oven (CTO-10A), a spectro UV detector (SPD-10A) and computerized integrator.

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

UV detector : 230 nm

Mobile phase : 0.067 M Monobasic potassium phosphate : Acetronitrile (95 : 5)

Flow rate : 1.0 mL/min.

Temperature : 35°C

Retention time: Amoxicillin ~ 4-5 min.

Theophylline ~ 15-16 min.

The concentrations of amoxicillin in plasma samples were computed using a standard calibration curve.

**7.3 Standard calibration curve**

Certain amount of standard amoxicillin was added to pooled drug free plasma to produce a set of standard solutions of 0.4, 0.8, 2.0, 4.0, 6.0, 10.0 and 12.0 μg/mL, respectively. All these standard solutions were analyzed following the same procedure as described earlier. The peak height ratio of amoxicillin to that of internal standard versus known amoxicillin concentrations were fitted to a straight line using linear regression analysis.

**8. Analysis of Clavulanic Acid in Plasma Samples**

**8.1 Sample preparation**

The method of sample preparation for clavulanic acid in plasma samples was modified from that of Wright, Durham, and Dunbar (1993) method. The process was described as follows:

1 mL of plasma sample was mixed with 250 μL of imidazole solution then let stand 1 hour to develop the clavulanic acid-imidazole reaction product. After 1 hour incubation of clavulanic acid-imidazole sample, the reaction product was added with 100 μL of internal standard solution (0.5 mg/mL of salicylic acid in potassium phosphate buffer) and mixed. Sample was loaded onto $C_{18}$ solid-phase extraction cartridge prepared by sequential rinse with 3 mL of methanol, 2 mL of water, and 3 mL of 0.07 M sodium phosphate buffer, pH 5. After sample was loaded, rinse with 3 mL of 0.01 M sodium phosphate buffer, pH 4 then elute clavulanic acid-imidazole reaction product and internal standard with 2 mL of methanol-0.07 M, pH 5 sodium phosphate buffer (55:45). Finally, 50 μL of the solution was injected into the HPLC.

**8.2 Chromatographic systems**

Apparatus : Shimadzu® LC-10A HPLC pump, equipped with a degasser (DGU-12A), a communication bus module (CBM-10A), an autoinjector (SIL-10A), a column oven (CTO-10A), a spectro UV detector (SPD-10A) and computerized integrator.

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

UV detector : 313 nm

Mobile phase : 0.07 M Monobasic potassium phosphate pH 4.35 : Methanol (95 : 5)

Flow rate : 1.0 mL/min.

Temperature : 35°C

Retention time: Clavulanic acid-imidazole reaction product ~ 13 -14 min.

Salicylic acid ~ 9-10 min.

The concentrations of clavulanic acid in plasma samples were computed using a standard calibration curve.

**8.3 Standard calibration curve**

Certain amount of standard clavulanic acid was added to pooled drug free plasma to produce a set of standard solutions of 0.1, 0.5, 1.0, 2.0, 4.0 and 6.0 μg/mL, respectively. All these standard solutions were analyzed following the same procedure as described earlier. The peak height ratios of clavulanic acid to that of internal standard versus known clavulanic acid concentrations were fitted to a straight line using linear regression analysis.

**9. Assay Validation**

The methods used for assay validation of amoxicillin and clavulanic acid were those as specified in 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:

**9.1 Selectivity**

Amoxicillin: Six sources of blank plasma samples were analyzed using the same procedure of amoxicillin as described earlier to ensure there is no interference the peaks of the drug and internal standard.

Clavulanic acid: Six sources of blank plasma samples were analyzed using the same procedure of clavulanic acid as described earlier to ensure there is no interference the peaks of the drug and internal standard.

**9.2 Lower limit of quantification (LLOQ)**

Amoxicillin: Five determinations of lowest concentration of standard amoxicillin (0.4 μg/mL) in plasma were analyzed. Analyte peak of these concentrations should be identifiable, discrete, and reproducible with a precision of not exceed 20% and accuracy of 80-120%.

Clavulanic acid: Five determinations of lowest concentration of standard clavulanic acid (0.1 μg/mL) in plasma were analyzed. Analyte peak of these concentrations should be identifiable, discrete, and reproducible with a precision of not exceed 20% and accuracy of 80-120%.

**9.3 Linearity and standard calibration curve**

Amoxicillin: Seven concentrations of standard solution of amoxicillin (0.4, 0.8, 2, 4, 6, 10 and 12 μg/mL) in plasma were analyzed. The peak height ratios of amoxicillin to that of the internal standard versus concentrations of amoxicillin were fitted to straight line using linear regression analysis. The coefficient of determination should be more than 0.99. The 20% deviation of the LLOQ from nominal concentration and 15% deviation of standards other than LLOQ from nominal concentration should be met.

Clavulanic acid: Six concentration of standard solution of clavulanic acid (0.1, 0.5, 1, 2, 4 and 6 μg/mL) in plasma were analyzed. The peak height ratios of

clavulanic acid to that of the internal standard versus concentrations of clavulanic acid were fitted to straight line using linear regression analysis. The coefficient of determination should be more than 0.99. The 20% deviation of the LLOQ from nominal concentration and 15% deviation of standards other than LLOQ from nominal concentration should be met.

**9.4 Accuracy**

Amoxicillin: Five determinations of three concentrations of standard amoxicillin (1.2, 5 and 11 μg/mL) in plasma were analyzed for the drug content. Accuracy in term of percent recovery was done by computing the ratio of estimated concentration obtained using linear regression of a standard amoxicillin in plasma to known concentration of each standard amoxicillin concentration in plasma multiplied by one hundred. The mean value should be within ±15% of the actual value.

Clavulanic acid: Five determinations of three concentrations of standard clavulanic acid (0.3, 3.0 and 5.0 μg/mL) in plasma were analyzed for the drug content. Accuracy in term of percent recovery was done by computing the ratio of estimated concentration obtained using linear regression of a standard clavulanic acid in plasma to known concentration of each standard clavulanic acid concentration in plasma multiplied by one hundred. The mean value should be within ±15% of the actual value.

**9.5 Precision**

**9.5.1 Within-run precision**

Amoxicillin: Five determinations of three concentrations of standard amoxicillin (1.2, 5 and 11 μg/mL) in plasma were analyzed within the same day. The percent coefficient of variation (C.V.) of estimated concentration was determined at each concentration level. The precision determined at each concentration level should not exceed 15% of the C.V.

Clavulanic acid: Five determinations of three concentrations of standard clavulanic acid (0.3, 3.0 and 5.0 μg/mL) in plasma were analyzed within the same day. The percent coefficient of variation (C.V.) of estimated concentration was

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

**9.5.2 Between-run precision**

Amoxicillin: Five determinations of three concentrations of standard amoxicillin (1.2, 5 and 11 μg/mL) in plasma were analyzed on five different days. The percent coefficient of variation of estimated concentration was determined at each concentration level for precision. The precision determined at each concentration level should not exceed 15% of the C.V.

Clavulanic acid: Five determinations of three concentrations of standard clavulanic acid (0.3, 3.0 and 5.0 μg/mL) in plasma were analyzed on five different days. The percent coefficient of variation of estimated concentration was determined at each concentration level for precision. The precision determined at each concentration level should not exceed 15% of the C.V.

**9.6 Recovery of extraction**

Amoxicillin: Five determinations of three concentrations of standard amoxicillin (1.2, 5 and 11 μg/mL) in plasma and in water were analyzed. Recovery of extraction was calculated by comparing the analytical results for extracted plasma samples at each concentration with unextracted standards that represent 100% recovery. 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.

Clavulanic acid: Five determinations of three concentrations of standard clavulanic acid (0.3, 3.0 and 5.0 μg/mL) in plasma and in potassium phosphate buffer were analyzed. Recovery of extraction was calculated by comparing the analytical results for extracted plasma samples at each concentration with unextracted standards that represent 100% recovery. 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.

### 9.7 Stability

#### 9.7.1 Freeze-thaw stability

Amoxicillin: Three aliquots of two concentrations of standard amoxicillin (1.2 and 11.0 μg/mL) in plasma were stored at -20°C for 24 hours and thawed unassisted at room temperature. When completely thawed, the samples were refrozen for 24 hours under the same conditions. This was one freeze-thaw cycle. The freeze-thaw cycle was repeated two more times then analyzed on the third cycle. The % deviation of the mean estimated concentration from the zero time should be within ± 15%.

Clavulanic acid: Three aliquots of two concentrations of standard clavulanic acid (0.3 and 5.0 μg/mL) in plasma were stored at -20°C for 24 hours and thawed unassisted at room temperature. When completely thawed, the samples were refrozen for 24 hours under the same conditions. This was one freeze-thaw cycle. The freeze-thaw cycle was repeated two more times then analyzed on the third cycle. The % deviation of the mean estimated concentration from the zero time should be within ± 15%.

#### 9.7.2 Long-term stability

Amoxicillin: Three aliquots of two concentrations of standard amoxicillin (1.2 and 11.0 μg/mL) in plasma were stored at -20 °C for 4 weeks and analyzed on two separate occasions. The % deviation of the mean estimated concentration from the zero time should be within ± 15%.

Clavulanic acid: Three aliquots of two concentrations of standard clavulanic acid (0.3 and 5.0 μg/mL) in plasma were stored at -20 °C for 4 weeks and analyzed on two separate occasions. The % deviation of the mean estimated concentration from the zero time should be within ± 15%.

#### 9.7.3 Short-term room temperature stability

Amoxicillin: Three aliquots of two concentrations of standard amoxicillin (1.2 and 11.0 μg/mL) in plasma were thawed at room temperature. The samples were analyzed after being kept at this temperature at 0, 4 and 12 hours. The %

deviation of the mean estimated concentration from the zero time should be within ± 15%.

Clavulanic acid: Three aliquots of two concentrations of standard clavulanic acid (0.3 and 5.0 μg/mL) in plasma were thawed at room temperature. The samples were analyzed after being kept at this temperature at 0, 4 and 12 hours. The % deviation of the mean estimated concentration from the zero time should be within ± 15%.

**9.7.4 Post-preparative stability**

Amoxicillin: Three aliquots of two concentrations of standard amoxicillin (1.2 and 11.0 μg/mL) in processed plasma sample were analyzed after keeping in the autosampler at 0, 2, 4, 12 and 24 hours. The % deviation of the mean estimated concentrations from the zero time should be within ± 15%.

Clavulanic acid: Three aliquots of two concentrations of standard clavulanic acid (0.3 and 5.0 μg/mL) in processed plasma sample were analyzed after keeping in the autosampler at 0, 6, 12 and 24 hours. The % deviation of the mean estimated concentrations from the zero time should be within ± 15%.

**10. Evaluation of Bioequivalence**

The bioequivalence of two brands of 500/125 mg amoxicillin/clavulanic acid film coated tablets were evaluated using the corresponding pharmacokinetic parameters AUC and $C_{max}$. Both of them were transformed into logarithmic scales.

**10.1 Statistical test**

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

**10.2 Construction of 90% confidence interval**

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

90% CI = $(X_T - X_R) \pm (t_{0.1,\,df} \times S.E.)$

where; $X_T$ and $X_R$ = Mean ln AUC and mean ln $C_{max}$ 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 = $[e^{(X_T - X_R) - (t_{0.1,df} \times S.E.)}]100$

% Upper limit = $[e^{(X_T - X_R) + (t_{0.1,df} \times S.E.)}]100$

The test product was considered to be bioequivalent to the innovator's product, when the 90% confidence interval of individual parameter of test product relative to that of innovator's product was within 80-125% for amoxicillin. However, for clavulanic acid, a high variable drug, the 90% confidence interval of the ln $C_{max}$ ratio was accepted to be within 75-133%.

# CHAPTER IV

# RESULTS AND DISCUSSION

## A. *In Vitro* Studies

### 1. Content of Active Ingredient

Both products were assayed for content of active ingredient and found that amoxicillin and clavulanic acid of both brands were within the limits of 90-120% as specified in the USP 25. However, the percent labeled amounts of both drugs of test product were slightly higher than those of the innovator's product as shown in Table 4. Naturally, both drugs were easily decomposed under various conditions such as exposing to light and humidity. Also, manufacturing date of innovator's product was 6-7 months earlier than that of test product. In addition, it was an imported product. The effects of aging and transportation could be contributed to decomposition of two drugs. Therefore, these factors might be resulted in less percent labeled amounts of innovator's product. However, it was still included in this study since it was the latest produced batch that was available in the market.

**Table 4** Content of Active Ingredient of 500/125 mg Amoxicillin/Clavulanic Acid Film Coated Tablets of the Test and Innovator's Products

| Assay no. | % Labeled Amount | | | |
|---|---|---|---|---|
| | Test Product | | Innovator's Product | |
| | Amoxicillin | Clavulanic Acid | Amoxicillin | Clavulanic Acid |
| 1 | 105.96 | 96.89 | 101.23 | 93.52 |
| 2 | 106.42 | 97.78 | 102.60 | 92.51 |
| Mean | 106.19 | 97.34 | 101.92 | 93.02 |
| S.D. | 0.32 | 1.34 | 0.97 | 0.71 |
| % R.S.D. | 0.30 | 1.37 | 0.95 | 0.76 |

**2. Uniformity of Dosage Units**

All two commercial brands of 500/125 mg amoxicillin/clavulanic acid film coated tablets were tested for uniformity of dosage units. Results were presented in Table 5. Each of them met the USP 25 specifications within the range of 85-115% of the label claim and the % R.S.D. was less than 6%.

**Table 5** Uniformity of Dosage Units of 500/125 mg Amoxicillin/Clavulanic Acid Film Coated Tablets of the Test and Innovator's Products

| Assay no. | % Labeled Amount | | | |
|---|---|---|---|---|
| | Test Product | | Innovator's Product | |
| | Amoxicillin | Clavulanic Acid | Amoxicillin | Clavulanic Acid |
| 1 | 105.35 | 97.12 | 100.88 | 91.83 |
| 2 | 106.09 | 97.80 | 100.97 | 91.91 |
| 3 | 106.42 | 98.11 | 101.96 | 92.81 |
| 4 | 108.22 | 99.77 | 101.21 | 92.12 |
| 5 | 105.95 | 97.68 | 101.41 | 92.31 |
| 6 | 106.99 | 98.63 | 102.10 | 92.94 |
| 7 | 107.12 | 98.75 | 101.81 | 92.68 |
| 8 | 106.11 | 97.82 | 101.06 | 91.99 |
| 9 | 107.22 | 98.84 | 102.24 | 93.06 |
| 10 | 107.43 | 99.04 | 101.34 | 92.24 |
| Mean | 106.69 | 98.35 | 101.50 | 92.39 |
| S.D. | 0.85 | 0.79 | 0.49 | 0.45 |
| % R.S.D. | 0.80 | 0.80 | 0.48 | 0.48 |

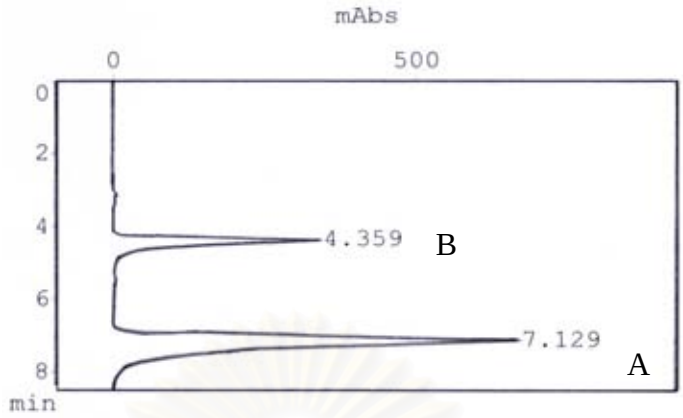



**Figure 3** Chromatogram of Analysis for Amoxicillin (A) and Clavulanic Acid (B) in Water

### 3. Dissolution Test

The amounts of amoxicillin and clavulanic acid dissolved at each sampling time were quantitated by HPLC at the wavelength of 220 nm and calculated using the standard calibration curve as shown in Appendix A. Chromatogram of analysis for amoxicillin and clavulanic acid in water was shown in Figure 3.

The dissolution data of amoxicillin and clavulanic acid of test and innovator's products were displayed in Tables 6 - 9, respectively. The data showed that both test and innovator's products dissolved rapidly. These were seen by 50% dissolution of amoxicillin and clavulanic acid of both brands could be reached within 10 minutes. Also, 100 % dissolution of amoxicillin could be attained within 20 and 30 minutes for innovator's and test products, respectively. In addition, more than 80% dissolution of clavulanic acid of those could be established within 15 minutes. For amoxicillin, their dissolution behaviors appeared to be similar. Also, those of clavulanic acid appeared to be. The mean plots of dissolution profiles of amoxicillin and clavulanic acid of both brands were shown in Figure 4.

None of twelve tablets dissolved less than 85% and 80% of labeled amount for amoxicillin and clavulanic acid, respectively, within 30 minutes. Therefore, both products of amoxicillin/clavulanic acid film coated tablets met the requirement of the United States Pharmacopoeia 25 as observed in Tables 6 - 9.

The dissolution profiles of amoxicillin and clavulanic acid of test product were compared to those of innovator's product to assess their similarities using difference factor ($f_1$) and similarity factor ($f_2$) as shown in Table 10. For amoxicillin, the difference factor ($f_1$) and similarity factor ($f_2$) of test product versus innovator's product were 4.68 and 63.29, respectively. For clavulanic acid, those were 8.97 and 55.39, respectively. These values were in the acceptance criteria ($f_1$ = 0 - 15, and $f_2$ = 50 - 100), referring that dissolution profiles of the test product were equivalent to that of the innovator's product.

***In Vitro* Evaluation**

All *in vitro* studies of both products revealed that they completely complied to the specification requirements as stated in the USP 25. Thus, it could be concluded that they were pharmaceutically equivalent to each other.

**Table 6** Dissolution Data of Amoxicillin of 500/125 mg Amoxicillin/Clavulanic Acid Film Coated Tablets of the Test Product

| Vessel no. | Time (min) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 30 | 45 | 60 |
| 1 | 36.37 | 80.15 | 90.27 | 90.70 | 101.14 | 112.27 | 102.94 |
| 2 | 16.76 | 66.93 | 90.62 | 94.02 | 110.04 | 103.79 | 104.86 |
| 3 | 23.76 | 57.70 | 79.34 | 89.32 | 96.40 | 103.41 | 109.02 |
| 4 | 28.26 | 73.04 | 94.91 | 92.95 | 105.87 | 109.06 | 108.40 |
| 5 | 20.94 | 57.68 | 77.26 | 87.52 | 99.71 | 98.60 | 106.85 |
| 6 | 12.61 | 56.52 | 88.95 | 86.18 | 103.15 | 104.49 | 112.96 |
| 7 | 14.72 | 81.96 | 91.81 | 90.82 | 112.32 | 115.49 | 117.10 |
| 8 | 25.06 | 68.30 | 92.75 | 104.50 | 109.76 | 112.46 | 105.41 |
| 9 | 34.59 | 73.93 | 106.04 | 108.82 | 106.81 | 117.26 | 117.80 |
| 10 | 29.30 | 73.32 | 101.01 | 109.46 | 115.72 | 121.67 | 122.66 |
| 11 | 46.13 | 89.54 | 102.84 | 107.49 | 110.66 | 115.63 | 116.20 |
| 12 | 39.65 | 94.64 | 109.61 | 118.47 | 122.33 | 122.98 | 122.64 |
| Mean | 27.35 | 72.81 | 93.78 | 98.35 | 107.83 | 111.43 | 112.24 |
| S.D. | 10.40 | 12.33 | 9.86 | 10.74 | 7.24 | 7.68 | 6.94 |
| % C.V. | 38.04 | 16.94 | 10.51 | 10.92 | 6.72 | 6.89 | 6.18 |

**Table 7** Dissolution Data of Amoxicillin of 500/125 mg Amoxicillin/Clavulanic Acid Film Coated Tablets of the Innovator's Product

| Vessel no. | Time (min) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 30 | 45 | 60 |
| 1 | 25.59 | 59.45 | 85.21 | 95.01 | 96.76 | 99.81 | 102.06 |
| 2 | 17.48 | 55.72 | 72.62 | 87.80 | 96.64 | 96.67 | 98.11 |
| 3 | 27.01 | 55.94 | 88.81 | 94.73 | 95.61 | 106.92 | 109.37 |
| 4 | 24.11 | 56.92 | 84.12 | 85.95 | 107.27 | 103.39 | 104.45 |
| 5 | 20.96 | 60.50 | 87.39 | 97.87 | 102.68 | 105.49 | 102.49 |
| 6 | 26.92 | 59.50 | 94.64 | 95.61 | 101.43 | 106.52 | 103.93 |
| 7 | 22.91 | 59.03 | 98.72 | 108.78 | 112.98 | 112.19 | 111.61 |
| 8 | 26.50 | 62.41 | 91.97 | 101.64 | 106.93 | 110.55 | 109.33 |
| 9 | 22.94 | 62.53 | 94.62 | 108.10 | 112.05 | 111.42 | 112.20 |
| 10 | 25.00 | 63.30 | 99.37 | 112.19 | 117.43 | 117.91 | 117.84 |
| 11 | 25.18 | 62.21 | 95.89 | 109.15 | 115.87 | 116.20 | 117.54 |
| 12 | 25.29 | 66.76 | 100.00 | 110.16 | 114.18 | 115.79 | 115.70 |
| Mean | 24.16 | 60.36 | 91.11 | 100.58 | 106.65 | 108.57 | 108.72 |
| S.D. | 2.77 | 3.28 | 7.97 | 9.04 | 7.92 | 6.64 | 6.52 |
| % C.V. | 11.46 | 5.43 | 8.74 | 8.99 | 7.43 | 6.11 | 6.00 |

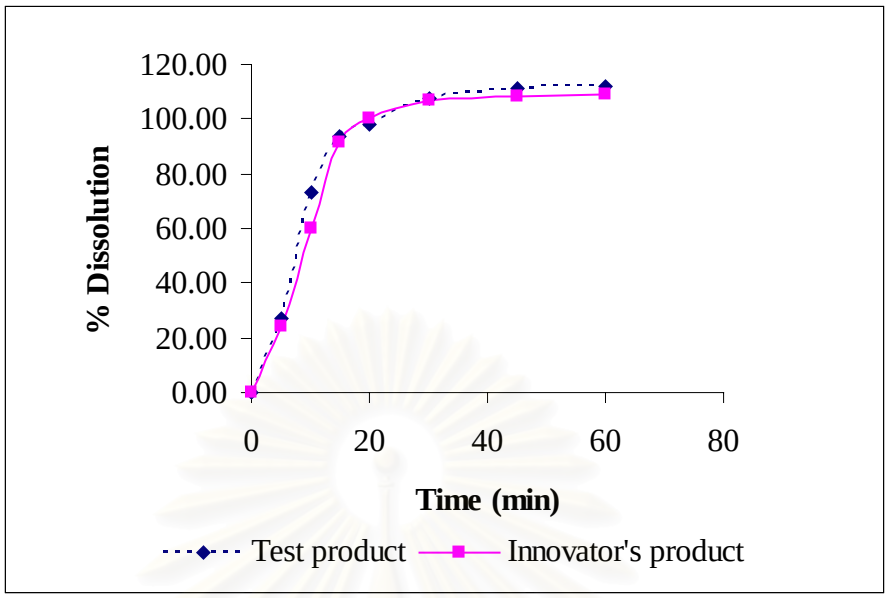
**Table 8** Dissolution Data of Clavulanic Acid of 500/125 mg Amoxicillin/Clavulanic Acid Film Coated Tablets of the Test Product

| Vessel no. | Time (min) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 30 | 45 | 60 |
| 1 | 38.36 | 81.41 | 87.53 | 85.04 | 90.49 | 95.09 | 83.08 |
| 2 | 18.03 | 65.59 | 89.03 | 89.11 | 100.27 | 87.46 | 87.55 |
| 3 | 24.13 | 57.17 | 74.56 | 83.88 | 86.15 | 88.35 | 91.96 |
| 4 | 28.43 | 73.46 | 91.55 | 85.92 | 91.62 | 89.59 | 89.03 |
| 5 | 20.13 | 54.58 | 70.30 | 77.72 | 83.56 | 98.83 | 100.39 |
| 6 | 14.01 | 54.77 | 90.30 | 82.34 | 91.17 | 86.31 | 91.00 |
| 7 | 15.97 | 87.39 | 91.97 | 89.38 | 102.22 | 100.54 | 100.39 |
| 8 | 27.23 | 71.11 | 95.25 | 101.78 | 100.70 | 98.83 | 90.07 |
| 9 | 39.76 | 76.65 | 103.23 | 101.02 | 100.96 | 99.26 | 98.44 |
| 10 | 32.15 | 75.47 | 101.39 | 103.76 | 103.15 | 103.74 | 102.18 |
| 11 | 50.46 | 95.35 | 105.87 | 106.25 | 104.67 | 104.10 | 102.74 |
| 12 | 43.29 | 97.00 | 100.86 | 107.60 | 106.72 | 104.76 | 103.43 |
| Mean | 29.33 | 74.16 | 91.82 | 92.82 | 96.81 | 96.40 | 95.02 |
| S.D. | 11.67 | 14.58 | 10.88 | 10.52 | 7.76 | 6.84 | 6.98 |
| % C.V. | 39.78 | 19.65 | 11.85 | 11.34 | 8.01 | 7.10 | 7.34 |

**Table 9** Dissolution Data of Clavulanic Acid of 500/125 mg Amoxicillin/Clavulanic Acid Film Coated Tablets of the Innovator's Product

| Vessel no. | Time (min) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 30 | 45 | 60 |
| 1 | 26.89 | 55.41 | 79.18 | 81.35 | 80.79 | 80.44 | 83.18 |
| 2 | 19.84 | 54.41 | 65.32 | 75.59 | 82.68 | 90.31 | 90.40 |
| 3 | 30.18 | 54.44 | 79.04 | 83.92 | 82.20 | 89.36 | 90.27 |
| 4 | 27.91 | 56.40 | 77.91 | 77.66 | 90.82 | 84.56 | 84.09 |
| 5 | 23.63 | 59.59 | 80.99 | 87.65 | 86.29 | 87.76 | 82.96 |
| 6 | 30.37 | 56.82 | 88.44 | 83.31 | 83.57 | 86.91 | 82.69 |
| 7 | 25.63 | 56.75 | 90.15 | 94.65 | 94.75 | 91.68 | 92.11 |
| 8 | 29.68 | 61.87 | 86.08 | 90.43 | 92.18 | 94.21 | 92.31 |
| 9 | 26.59 | 61.72 | 86.61 | 94.73 | 94.64 | 91.97 | 91.66 |
| 10 | 28.57 | 63.58 | 92.32 | 99.64 | 98.82 | 95.93 | 95.47 |
| 11 | 29.46 | 63.28 | 90.61 | 98.72 | 100.85 | 97.10 | 97.23 |
| 12 | 29.13 | 67.94 | 94.80 | 100.57 | 99.55 | 97.03 | 95.66 |
| Mean | 27.32 | 59.35 | 84.29 | 89.02 | 90.59 | 90.61 | 89.84 |
| S.D. | 3.10 | 4.32 | 8.20 | 8.71 | 7.30 | 5.14 | 5.32 |
| % C.V. | 11.36 | 7.27 | 9.73 | 9.79 | 8.06 | 5.68 | 5.92 |

A




B

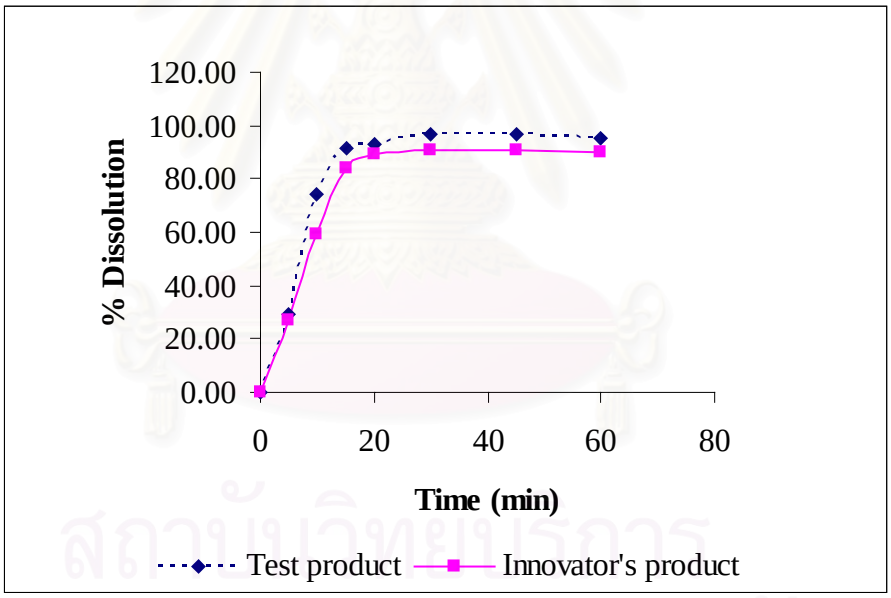



**Figure 4** The Mean Dissolution Profiles of 500/125 mg Amoxicillin/Clavulanic Acid Film Coated Tablet of the Test and Innovator's Products;

A : Amoxicillin

B : Clavulanic Acid

**Table 10** The Difference Factor ($f_1$) and Similarity Factor ($f_2$) of the Test Product Relative to the Innovator's Product

| Parameters | Values | |
|---|---|---|
| | Amoxicillin | Clavulanic Acid |
| Difference factor ($f_1$)[a] | 4.68 | 8.94 |
| Similarity factor ($f_2$)[b] | 63.27 | 55.39 |

[a] Acceptance value : 0-15%

[b] Acceptance value : 50-100%

**B. *In Vivo* Studies**

**1. Analysis of Amoxicillin and Clavulanic Acid in Plasma**

Amoxicillin: Chromatograms of blank plasma, plasma spiked with theophylline (internal standard) and plasma spiked with amoxicillin and theophylline were shown in Figure 5. The retention time of amoxicillin and theophylline were about 4.8 and 15.7 minutes, respectively as displayed in Figure 5C. There were no any interference peaks due to the presence of plasma protein and/or endogenous substance, amoxicillin and theophylline peaks were well separated. Also, there was no interference due to the presence of clavulanic acid.

Clavulanic acid: Chromatograms of blank plasma, plasma spiked with imidazole reagent and salicylic acid (internal standard) and plasma spiked with clavulanic acid, imidazole reagent and salicylic acid were shown in Figure 6. The retention time of clavulanic acid-imidazole reaction product and salicylic acid were 14.7 and 10.4 minutes respectively as shown in Figure 6C. No any interference peaks due to the presence of plasma protein and/or endogenous substance were observed, except the imidazole reagent peak. However, clavulanic acid-imidazole reaction product and salicylic acid peaks were well separated. In addition, there was no effect of amoxicillin to those.

**2. Assay Validation of Amoxicillin and Clavulanic Acid in Plasma**

Figures 7 and 8 showed that there was selectivity of the analysis method of both amoxicillin and clavulanic acid because of no interference peaks due to the presence of plasma protein and/or endogenous substance from six different blank plasma samples. These results illustrated the ability of the analytical method to differentiate and quantify amoxicillin and clavulanic acid in the presence of other components in the plasma sample.

As presented in Appendix B, the standard calibration curves of amoxicillin and clavulanic acid showed linear response over the range of concentrations used in the assay procedure with the coefficient of determination ($r^2$) of 0.9995 and 0.9997, respectively. The lower limit of quantification of amoxicillin was 0.4 μg/mL, and that of clavulanic acid was 0.1 μg/mL. These were 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%). The data including linear regression equation of amoxicillin and clavulanic acid were reported in Tables 11 and 12, respectively.

The accuracy, within- and between-run precision of analytical method for amoxicillin and clavulanic acid in plasma were shown in Tables 13-15, respectively. The percent recovery for accuracy of amoxicillin was 95.34 to 106.20%, and that of clavulanic acid was 101.89 to 113.13%. The %C.V. for the within- and between-run precision of amoxicillin were 2.22 to 7.91 and 8.89 to 11.66, and those of clavulanic acid were 2.11 to 6.40 and 7.38 to 12.65, respectively. These results were within acceptance criteria for accuracy (±15%) and precision (<15%).

As displayed in Table 16, the recovery of extraction for amoxicillin ranged between 81.36 and 85.65% with a %C.V. between 1.74 and 8.43, and that of clavulanic acid ranged between 49.63 and 55.45% with a %C.V. between 2.40 and 3.95. For internal standard of both, the recovery of extraction for theophylline and salicylic acid were 35.03 and 16.79% with 1.36 and 3.38 of %C.V., respectively. Regarding to the recovery of extraction of clavulanic acid and internal standard, it could be seen that these values were less than that of amoxicillin, especially internal standards. This result might be dependent on the extraction procedure of internal standards from plasma was not specific for them. However, recovery of extraction for internal standards was negligible effect to the accuracy or precision of analytical method for amoxicillin and clavulanic acid because of using ratio of peak height for estimation. For clavulanic acid, the process of sample preparation like developing the clavulanic acid-imidazole reaction product as well as extracting by solid phase extraction might be contributed to this result. According to the Guidance for validation (CDER, 2001), recovery of extraction of the analytes need not be 100%, but the extent of recovery of those should be consistent, precise, and reproducible. Therefore, these results were an acceptable range for the purpose of study.

In the freeze-thaw stability study as shown in Table 17, the percent deviation from the zero time of amoxicillin was -10 .41 to -13.49%, and that of clavulanic acid was -8.82 to -11.16%. These results indicated that no tendency of degradation of them after three

freeze-thaw cycles was observed because these could be still determined with the acceptable value (±15%).

Long-term stability studies of amoxicillin and clavulanic acid were displayed in Table 18. For amoxicillin, after storing at -20 °C for 4 weeks, plasma samples showed no loss of amoxicillin when reassayed two times. The percent deviation of amoxicillin was -9.24 to -13.49% from the zero time. For clavulanic acid, also no tendency of degradation of this after storing at -20 °C for 4 weeks was observed and that of clavulanic acid was -2.15 to -12.12%. These results were within acceptance criteria (±15%). Hence, these storage times in long-term stability could be established for sample analysis.

Table 19 displayed the short-term room temperature stability of amoxicillin and clavulanic acid. Both of them were tended to degrade after they were thawed at room temperature and kept at this temperature from 4 to 12 hours. The percent deviation of amoxicillin from the zero time was -3.51 to -20.17% and that of clavulanic acid was -5.88 to -58.96% after keeping at room temperature from 4 to 12 hours. These results illustrated that the samples should be rapidly extracted and analyzed after thawing at room temperature.

In addition, Table 20 showed that the processed plasma sample of amoxicillin and clavulanic acid should be analyzed within 4 hours and 6 hours after being kept in autosampler, respectively. Since the results were not within acceptance criteria (±15%) after they were kept in autosampler longer than those.

In this assay validation study indicated that the analysis methods of amoxicillin and clavulanic acid in plasma samples had been proven to be simple, specific, accurate and precise with the need of internal standard. The lower limit of quantification and stability data of them allowed to be successfully applied in a bioequivalence study of two brands of amoxicillin/clavulanic acid film coated tablets.

# CHAPTER V

# CONCLUSIONS

The bioequivalence of a local brand of 500/125 mg amoxicillin/clavulanic acid film coated tablets commercially available in Thailand as compared to the innovator's product was established. The related results were concluded as follows:-

*In vitro* studies:

1. The two brands of 500/125 mg amoxicillin/clavulanic acid film coated tablets met the general requirements of the United States Pharmacopoeia 25 for content of active ingredient, uniformity of dosage units. In addition, dissolutions of two brands were more than 85% and 80% of labeled amount for amoxicillin and clavulanic acid, respectively, within 30 minutes. These indicated that they were pharmaceutically equivalent.

2. Dissolution profiles comparison of amoxicillin and clavulanic acid for both brands were performed in water. The difference factor ($f_1$) and similarity factor ($f_2$) of local brand relative to those of the innovator's product were in the acceptance range, ensuring their equivalences.

*In vivo* studies:

The comparative bioavailability of a local brand of 500/125 mg amoxicillin/ clavulanic acid film coated tablet relative to the innovator's product was studied in eighteen healthy Thai male volunteers. A single dose of 500/125 mg amoxicillin/ clavulanic acid film coated tablets was administered to each subject in a crossover manner. Plasma concentration of amoxicillin and clavulanic acid were determined by high performance liquid chromatography. Individual plasma concentration-time profile was analyzed using graphical method. The observed values of relevant pharmacokinetic parameters (AUC and $C_{max}$) were used for bioavailability comparison.

Amoxicillin:

The area under the plasma concentration-time curves (AUC) of both brands ranged from 25.30 to 26.46 μg.hr/mL. The mean peak plasma concentrations ($C_{max}$) of both brands ranged from 7.25 to 7.83 μg/mL. The average times to peak plasma concentrations ($t_{max}$) of both brands ranged from 1.53 to 2.08 hours. There were no statistically significant difference of the corresponding pharmacokinetic parameters between the values of both brands studied ($p>0.05$) and 90% confidence interval of individual parameter based on log transformed data of local product relative to that of innovator's product was contained within 80-125%.

Clavulanic acid:

The area under the plasma concentration-time curves (AUC) of both brands were 4.58 μg/mL. The mean peak plasma concentrations ($C_{max}$) of both brands ranged from 1.48 to 1.49 μg/mL. The average times to peak plasma concentrations ($t_{max}$) of both brands ranged from 1.44 to 1.60 hour. There were no statistically significant difference of the corresponding pharmacokinetic parameters between the values of both brands studied ($p>0.05$) and 90% confidence interval of AUC parameter based on log transformed data of local product relative to that of innovator's product was contained within 80-125%. Because of the variable nature of clavulanic acid absorption and an inherent variability of $C_{max}$, 90% confidence interval of $C_{max}$ parameter of those were found to be slightly outside the acceptance range of 80-125%. It is advisable to use the acceptance range of 75-133% instead.

Hence, these results could be concluded that the local brand product was bioequivalent with the innovator's product.

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# APPENDICES

# APPENDIX A

Test Products

**Table 44** Test Products

| Brand Name | Manufacturer | Batch no. | Mfg.Date | Exp.Date |
|---|---|---|---|---|
| Augmentin® | SmithKline Beecham Pharmaceuticals, England | 75155 A | 24-04-02 | 24-4-05 |
| Cavumox® | Siam Bheasach Co., Ltd., Thailand | 92D054 | 22-11-02 | 22-11-04 |

# APPENDIX B

**Table 45** Typical Calibration Curve for Determination of Amoxicillin Concentrations in Water Estimated Using Linear Regression Analysis[1]

| Standard no. | Known Concentration (mg/mL) | Peak Height | Estimated Concentration[2] (mg/mL) | % Recovery[3] |
|---|---|---|---|---|
| 1 | 0.04 | 50207 | 0.044 | 101.48 |
| 2 | 0.09 | 90930 | 0.084 | 96.87 |
| 3 | 0.17 | 185007 | 0.177 | 101.72 |
| 4 | 0.35 | 355414 | 0.345 | 99.12 |
| 5 | 0.44 | 449408 | 0.438 | 100.59 |
| 6 | 0.87 | 887759 | 0.871 | 99.95 |

* Each data point was triplicately determined

[1] $r^2 = 0.9999$ $y = 1013363x + 5417$

[2] Estimated concentration $= \frac{[\text{Peak height} - 5417]}{1013363}$

[3] % Recovery $= \frac{\text{Estimated concentration}}{\text{Known concentration}} \times 100$

**Table 46** Typical Calibration Curve for Determination of Clavulanic Acid Concentrations in Water Estimated Using Linear Regression Analysis[1]

| Standard no. | Known Concentration (mg/mL) | Peak Height | Estimated Concentration[2] (mg/mL) | % Recovery[3] |
|---|---|---|---|---|
| 1 | 0.01 | 33812 | 0.010 | 104.04 |
| 2 | 0.03 | 85392 | 0.030 | 106.24 |
| 3 | 0.05 | 134660 | 0.050 | 104.76 |
| 4 | 0.10 | 263150 | 0.101 | 105.86 |
| 5 | 0.15 | 382405 | 0.148 | 98.69 |
| 6 | 0.20 | 516238 | 0.201 | 100.53 |

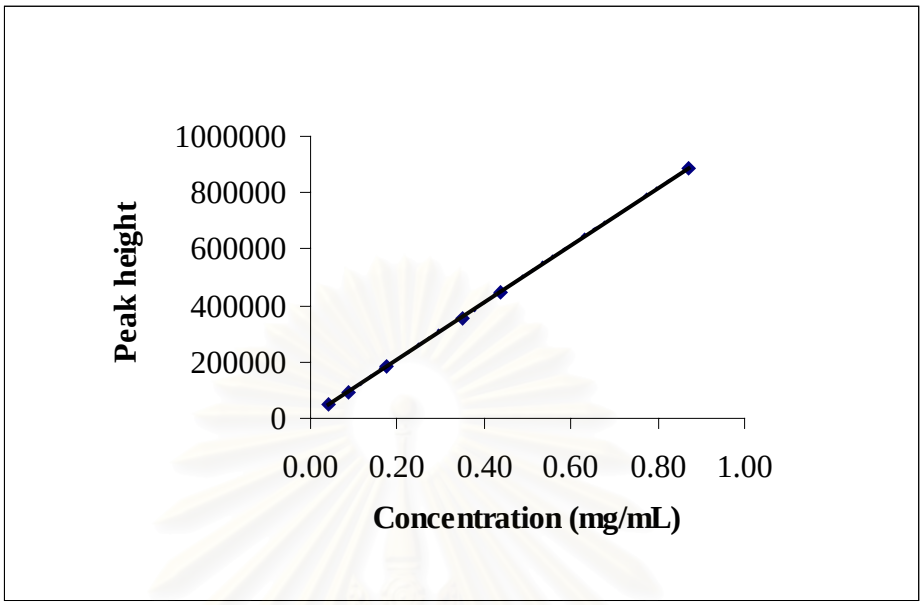
* Each data point was triplicately determined

[1] $r^2 = 0.9998$ $y = 2523617x + 8817$

[2] Estimated concentration $= \frac{[\text{Peak height} - 8817]}{2523617}$

[3] % Recovery $= \frac{\text{Estimated concentration}}{\text{Known concentration}} \times 100$

**A**




**B**

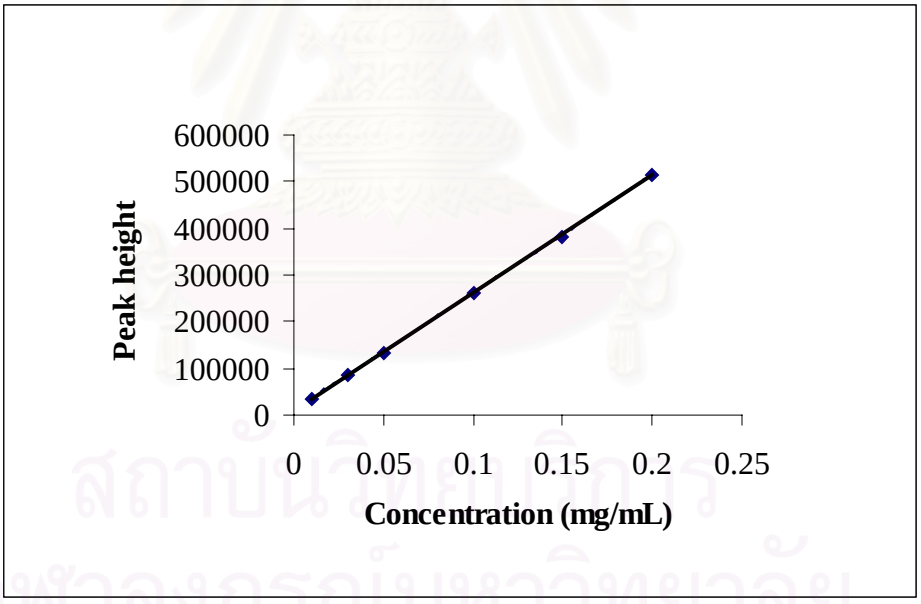



**Figure 29** Calibration Curve for Determination of Amoxicillin (A) and Clavulanic Acid (B) in Water

**Table 47** Typical Calibration Curve for Determination of Amoxicillin Concentrations in Plasma Estimated Using Linear Regression Analysis[1]

| Standard no. | Known Concentration (µg/mL) | Peak Height Ratio | Estimated Concentration[2] (µg/mL) | % Recovery[3] |
|---|---|---|---|---|
| 1 | 0.4 | 0.0263 | 0.415 | 103.79 |
| 2 | 0.8 | 0.0549 | 0.834 | 104.23 |
| 3 | 2.0 | 0.1322 | 1.939 | 96.96 |
| 4 | 4.0 | 0.2770 | 4.008 | 100.19 |
| 5 | 6.0 | 0.4115 | 5.930 | 98.84 |
| 6 | 10.0 | 0.7109 | 10.201 | 102.01 |
| 7 | 12.0 | 0.8274 | 11.870 | 98.92 |

* Each data point was triplicately determined

[1] $r^2 = 0.9995$ $y = 0.0700x - 0.0035$

[2] Estimated concentration $= \frac{[\text{Peak height ratio} + 0.0035]}{0.0700}$

[3] % Recovery $= \frac{\text{Estimated concentration}}{\text{Known concentration}} \times 100$

**Table 48** Typical Calibration Curve for Determination of Clavulanic Acid Concentrations in Plasma Estimated Using Linear Regression Analysis[1]

| Standard no. | Known Concentration (µg/mL) | Peak Height Ratio | Estimated Concentration[2] (µg/mL) | % Recovery[3] |
|---|---|---|---|---|
| 1 | 0.1 | 0.0157 | 0.119 | 119.29 |
| 2 | 0.5 | 0.1162 | 0.529 | 105.83 |
| 3 | 1 | 0.2316 | 0.998 | 99.84 |
| 4 | 2 | 0.4685 | 1.963 | 98.13 |
| 5 | 4 | 0.9554 | 3.942 | 98.56 |
| 6 | 6 | 1.4699 | 6.048 | 100.80 |

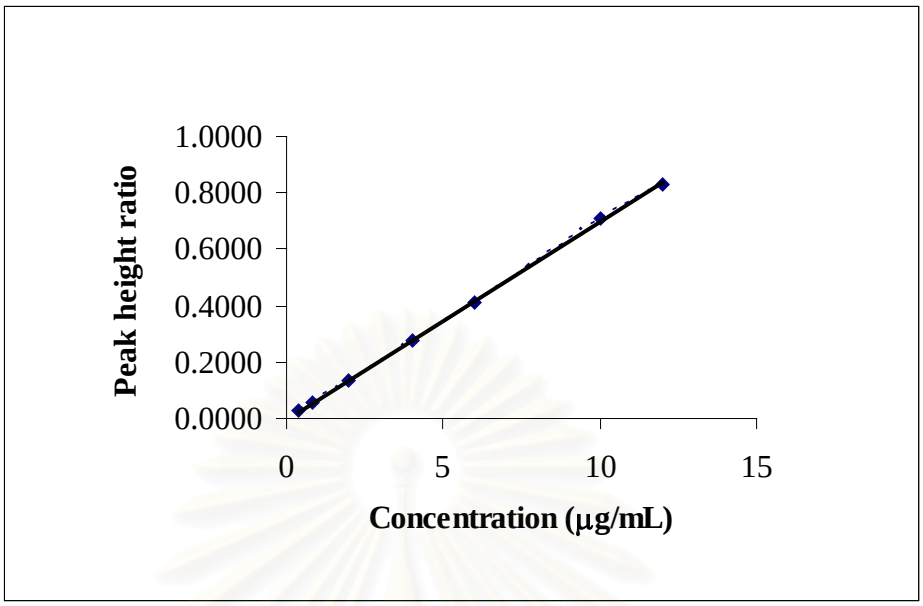
* Each data point was triplicately determined

[1] $r^2 = 0.9997$ $y = 0.2458x - 0.0136$

[2] Estimated concentration $= \frac{[\text{Peak height ratio} + 0.0136]}{0.2458}$

[3] % Recovery $= \frac{\text{Estimated concentration}}{\text{Known concentration}} \times 100$

**A**




**B**

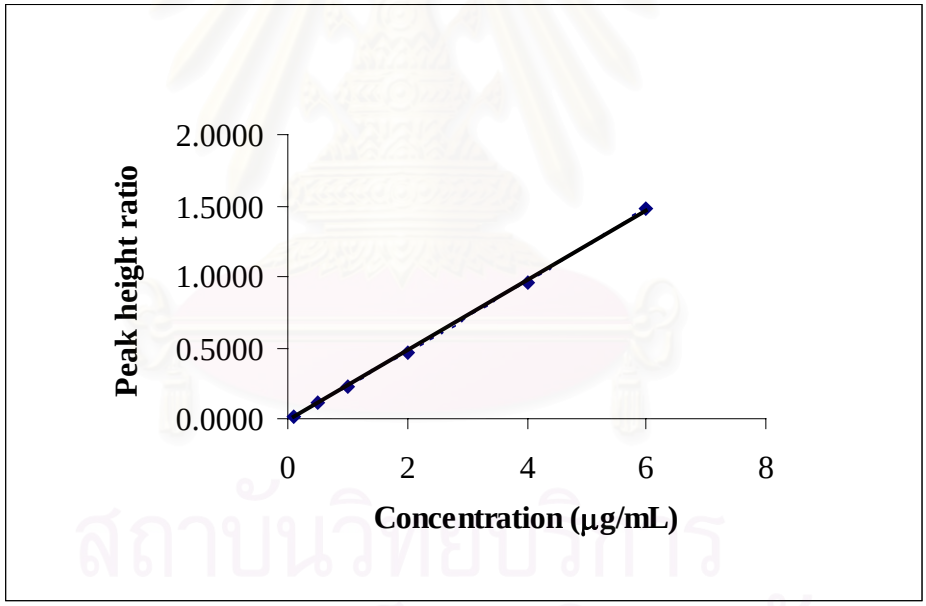



**Figure 30** Calibration Curve for Determination of Amoxicillin (A) and Clavulanic Acid (B) in Plasma

**APPENDIX C**

# Study Protocol Approval

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

| | |
|---|---|
| **Study Title** | : Bioequivalence of Amoxicillin and Clavulanic Acid Tablets |
| **Study Code** | : - |
| **Centre** | : Chulalongkorn University |
| **Principal Investigator** | : Miss Yuwadee Sirisomboon |
| **Protocol Date** | : January 22, 2003 |

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

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

Chairman of Ethics Committee : .....

(Signature)

Boonyong Tantisirs, Ph.D.

Secretary of Ethics Committee : .....

(Signature)

Poj Kulvanich, Ph.D.

**Date of Approval** : August 19, 2003

**หนังสือแสดงความยินยอม**

**การวิจัยเรื่อง ชีวสมมูลของยาเม็ดอะม็อกซิซิลลิน และคลาวูลานิกแอซิด**

**วันให้คำยินยอม วันที่ .....เดือน.....พ.ศ.....**

ข้าพเจ้า (นาย/นาง/นางสาว).....นามสกุล.....

อยู่บ้านเลขที่.....ซอย.....ถนน.....แขวง/ตำบล.....

เขต/อำเภอ.....จังหวัด.....รหัสไปรษณีย์.....

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับเอกสาร และการอธิบายจากผู้วิจัย ให้ทราบวัตถุประสงค์ของการวิจัย วิธีวิจัย อันตราย หรืออาการข้างเคียงที่อาจเกิดขึ้นจากการวิจัยหรือจากยาที่ใช้ รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว ผู้วิจัยได้ตอบคำถามต่างๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจ ไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ

ข้าพเจ้าเข้าร่วมโครงการนี้โดยสมัครใจ และมีสิทธิ์ที่จะบอกเลิกการเข้าร่วมโครงการวิจัยนี้เมื่อใดก็ได้ โดยการบอกเลิกจะไม่มีผลต่อการรักษาโรคที่ข้าพเจ้าจะได้รับต่อไป

ข้าพเจ้าอนุญาตให้ผู้วิจัยเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าในหน่วยงานที่เกี่ยวข้องได้ตามที่ผู้วิจัยเห็นสมควร ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับ และจะเปิดเผยได้เฉพาะในรูปที่เป็นการสรุปผลการวิจัย

ในการวิจัยครั้งนี้มีการเจาะเลือดครั้งละ....7....มิลลิลิตร เป็นเวลา.....2....วันๆ ละ.....14.....ครั้ง

ผู้วิจัยได้อธิบายให้ข้าพเจ้าทราบ และเข้าใจแล้วว่าการเจาะเลือดเพียงเล็กน้อย โดยทั่วไปจะไม่เกิดอันตรายใดๆ แก่ข้าพเจ้าเลย นอกจากอาจมีรอยช้ำบริเวณเจาะเล็กน้อย ซึ่งอาจหายได้เองภายใน 7 วัน

ผู้วิจัยรับรองว่าหากเกิดอันตรายใดๆ จากการวิจัยดังกล่าว ข้าพเจ้าจะได้รับการรักษาพยาบาลโดยไม่คิดมูลค่า และได้รับการชดเชยรายได้ที่สูญเสียไประหว่างการรักษาพยาบาลดังกล่าวตลอดจนเงินทดแทนความพิการที่อาจจะเกิดขึ้น และรายละเอียดเกี่ยวกับการรักษาพยาบาล หรือเงินชดเชยดังกล่าว

ข้าพเจ้าสามารถติดต่อได้ที่.....รศ.ดร. อุทัย สุวรรณกูฏ, ภญ. ยุวดี ศิริสมบูรณ์..... โดยบุคคลที่รับผิดชอบเรื่องนี้คือ.....บริษัท สยามเภสัช จำกัด.....

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ จึงได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม.....ผู้ยินยอม

ลงนาม.....ผู้รับผิดชอบการวิจัย

ลงนาม.....พยาน

**แบบบันทึกอาการไม่พึงประสงค์จากการใช้ยาอะม็อกซิซิลลิน และคลาวูลานิกแอซิด 500/125 มิลลิกรัม**

**(Case Record Form)**

ชื่อโครงการวิจัย: ชีวสมมูลของยาเม็ดอะม็อกซิซิลลิน และคลาวูลานิกแอซิด

ชื่อ/นามสกุลอาสาสมัคร: ..... อายุ: .....ปี เพศ: .....

ประวัติการแพ้ยา: ❒ ไม่มี ❒ มี (ระบุ).....

การศึกษาครั้งที่: ..... วัน/เดือน/ปี: ..... ได้รับยารหัส: .....

ชื่อ/นามสกุลแพทย์ผู้ดูแล: .....พญ. พรเลขา บรรหารศุภวาท.....

อาการไม่พึงประสงค์: ❒ พบ ❒ ไม่พบ

| อาการที่พบหรือปรากฏ | เวลาหลังรับประทานยา | วัน/เดือน/ปี |
|---|---|---|
| | | |

ความรุนแรง: ❒ น้อย ❒ ปานกลาง ❒ มาก

ภายหลังเกิดอาการ: ❒ ให้การรักษาทันที ❒ เฝ้าระวังอาการ ❒ ให้ถอนตัว

❒ ให้ทดลองต่อ ❒ อื่นๆ (ระบุ).....

ผลลัพธ์ที่เกิดขึ้น: ❒ หายเป็นปกติ ❒ ยังมีอาการอยู่ ❒ ไม่สามารถติดตามผล

ผลการประเมินความสัมพันธ์ของยาอะม็อกซิซิลลิน และคลาวูลานิกแอซิดกับอาการไม่พึงประสงค์

❒ ใช่แน่นอน ❒ น่าจะใช่ ❒ อาจจะใช่ ❒ สงสัย

หมายเหตุ: .....

ลงชื่อ.....

(พญ. พรเลขา บรรหารศุภวาท)

ผู้วินิจฉัยอาการ/ผู้ประเมินและบันทึก

ลงชื่อ.....

(รศ.ดร. อุทัย สุวรรณกูฏ)

ผู้วิจัยหลัก

**Table 49** 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 |
| WBC | 5-10 x $10^3$ cells/mL | 9.5 | 6.5 | 9.4 | 7.1 | 8.6 | 7.5 | 5.4 | 6.6 | 4.4 | 5.5 | 9.6 | 9.3 | 4.3 | 5.8 | 5.2 | 6.6 | 7.0 | 6.6 |
| Hemoglobin | 13-18 gm/dl | 14.7 | 11.9 | 14.4 | 14.9 | 15.1 | 12.5 | 13.6 | 14.9 | 13.6 | 13.2 | 15.9 | 14 | 14.9 | 13.6 | 15.3 | 15.3 | 16.1 | 15.2 |
| Hematocrit | 35-4 % | 44 | 36 | 44 | 45 | 45 | 37 | 41 | 45 | 41 | 40 | 47 | 41 | 43 | 40 | 45 | 45 | 47 | 45 |
| Plt. Count. | 1.5-4.0 x $10^5$ cells/mL | 3.2 | 1.8 | 2.5 | 2.9 | 1.9 | 2.5 | 2.9 | 2.3 | 1.9 | 2.0 | 1.9 | 2.1 | 1.7 | 2.6 | 1.8 | 1.6 | 2.3 | 2.3 |
| RBC. Count. | 4.7-6.1 x $10^6$ cells/mL | - | 4.2 | - | 5.3 | - | - | - | - | 5.2 | 4.5 | - | - | 4.7 | 4.3 | 5.0 | 5.6 | 5.4 | 5.1 |
| Monocyte | 2-6% | - | - | - | 1 | - | 2 | - | 1 | 1 | - | 1 | 2 | 1 | - | - | 1 | 1 | 2 |
| Eosinophil | 1-3% | - | 2 | 3 | 3 | 3 | 2 | 1 | - | 1 | 5 | 3 | 3 | - | - | 1 | 1 | 1 | - |
| Lymphocyte | 20-35% | 32 | 27 | 31 | 32 | 38 | 38 | 45 | 40 | 40 | 47 | 19 | 30 | 40 | 45 | 38 | 39 | 35 | 37 |

where; WBC = White Blood Cell
Plt. Count = Platelet Count
RBC Count = Red Blood Cell Count

**Table 50** Blood Chemical Tests of Subjects Participated in This Study

| Blood Chemical Tests | Normal Range | Subject no. | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| HBS Ag | Negative | - | N | - | N | - | - | - | - | N | N | - | - | N | N | N | N | N | N |
| Sugar | 75-110 mg/dL | 81 | 92 | 96 | 89 | 92 | 114 | 90 | 98 | 80 | 91 | 100 | 96 | 78 | 89 | 92 | 114 | 90 | 98 |
| BUN | 8-20 mg/dL | 12 | 11 | 12 | 14 | 11 | 14 | 15 | 12 | 15 | 11 | 13 | 13 | 15 | 14 | 18 | 14 | 15 | 12 |
| Creatinine | 0.7-1.5 mg/dL | 1.0 | 0.9 | 1.0 | 1.2 | 1.2 | 0.9 | 1.1 | 1.3 | 0.8 | 0.7 | 1.2 | 1.0 | 1.0 | 1.2 | 1.4 | 0.7 | 1.1 | 1.0 |
| Uric Acid | 1.5-7.0 mg/dL | 4.5 | 7.8 | 4.1 | 6.9 | 6.1 | 5.1 | 5.0 | 4.7 | 4.0 | 4.4 | 4.8 | 5.9 | 4.1 | 6.9 | 6.1 | 5.1 | 5.2 | 4.7 |
| Cholesterol | 140-200 mg/dL | 165 | 166 | 193 | 150 | 194 | 177 | 165 | 199 | 160 | 150 | 161 | 150 | 193 | 121 | 194 | 177 | 165 | 199 |
| Triglyceride | 35-160 mg/dL | 75 | 123 | 125 | 104 | 47 | 47 | 88 | 74 | 80 | 80 | 104 | 59 | 111 | 104 | 47 | 47 | 88 | 145 |
| Total Protein | 6-8 gm/dL | 7.2 | 6.5 | 7.2 | 6.9 | 105 | 7.0 | 7.5 | 7.5 | 7.1 | 6.1 | 7.5 | 7.4 | 7.2 | 6.9 | 105 | 7.0 | 7.2 | 7.1 |
| Albumin | 3.5-5.3 gm/dL | 4.9 | 3.6 | 4.1 | 4.0 | 7.0 | 4.2 | 4.4 | 4.5 | 4.5 | 3.8 | 4.9 | 4.8 | 4.1 | 4.0 | 7.2 | 4.2 | 4.3 | 4.5 |
| Bilirubin Total | 0.3-1.0 mg/dL | 0.6 | 1.8 | 0.4 | 0.5 | 4.8 | 0.4 | 0.6 | 0.3 | 0.6 | 0.9 | 0.5 | 0.4 | 0.4 | 0.5 | 4.8 | 0.4 | 0.6 | 0.3 |
| Bilirubin Direct | 0-0.5 mg/dL | 0.3 | 0.8 | 0.2 | 0.3 | 0.4 | 0.2 | 0.3 | 0.1 | 0.2 | 0.1 | 0.2 | 0.2 | 0.2 | 0.3 | 0.4 | 0.2 | 0.3 | 0.1 |
| AST | 5.0-35 U/L | 50 | 24 | 20 | 30 | 8 | 21 | 32 | 21 | 22 | 22 | 34 | 33 | 20 | 25 | 10 | 21 | 12 | 21 |
| ALT | 5.0-45 U/L | 23 | 30 | 24 | 18 | 30 | 17 | 25 | 18 | 24 | 37 | 30 | 28 | 24 | 18 | 25 | 17 | 35 | 18 |
| Alk. Phos | 25-90 U/L | 62 | 71 | 41 | 41 | 28 | 33 | 58 | 40 | 57 | 52 | 39 | 39 | 41 | 39 | 29 | 33 | 32 | 36 |

where; N = Negative
HBS Ag = Antibody Hepatitis B
BUN = Blood Urea Nitrogen
AST = Aspartate Aminotransferase
ALT = Alanine Aminotransferase
Alk Phos = Alkaline Phosphatase

**Table 51** Adverse Drug Reactions of Subjects Participated in This Study

| Adverse Drug Reaction | Subject no. | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| History of allergy | No | No | No | No | No | No | No | No | No | No | No | No | No | No | No | No | No | No |
| GI tracts | | | | | | | | | | | | | | | | | | |
| Diarrhea | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Abdominal discomfort | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Anorexia | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Flatulence | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Dyspepsia | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Nausea/Vomiting | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Skin | | | | | | | | | | | | | | | | | | |
| Rash | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Urticaria | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Headache | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Fever | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Others | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |

where; No = No history
N = Not occurred

# APPENDIX D

1. Mean ( X )

$$X = \sum X/n$$

2. Standard deviation (S.D.)

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

3. Coefficeint of variation (C.V.)

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

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

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

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

$$[AUC]^\infty_o = \frac{\sum(C_{n-1} + C_n)}{2}(t_n - t_{n-1}) + C/K_e$$

where; $C$ = The last measurable plasma drug concentration

$K_e$ = Elimination rate constant

6. Elimination rate constant ($K_e$)

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

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

$$t_{1/2} = \frac{0.693}{K_e}$$

8. Analysis of variance for two way crossover design

The experimental design is:

| Sequence | Subject no. | Period | |
|---|---|---|---|
| | | I | II |
| I | 1,2,3,4,5,6,7,8,9 | A | B |
| II | 10,11,12,13,14,15,16,17,18 | B | A |

Where; A = Innovator's product

B = Test product

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

| Source of Variation | d.f. | Sum of Squares | Mean Squares |
|---|---|---|---|
| Total | 2n-1 | SStotal | - |
| Sequence | 1 | SSsequence | MSsequence |
| Subjects (sequence) | n-2 | SSsubject | MSsubject |
| Period | 1 | SSperiod | MSperiod |
| Formulation | 1 | SSformulation | MSformulation |
| Error | n-2 | SSerror | MSerror |

Where; n = Number of subjects

Data presented are individual subject of the ln AUC of amoxicillin following oral administration of 500/125 mg amoxicillin/clavulanic acid film coated tablet of test and innovator's products.

| Sequence | Subject | Innovator's Product | | Test Product | | Subject Total |
|---|---|---|---|---|---|---|
| I | 1 | 3.11 | | 2.98 | | 6.09 |
| | 2 | 3.14 | | 3.20 | | 6.34 |
| | 3 | 3.40 | | 3.21 | | 6.61 |
| | 4 | 3.53 | | 3.66 | | 7.19 |
| | 5 | 3.49 | | 3.63 | | 7.12 |
| | 6 | 3.20 | Period I | 3.32 | Period II | 6.52 |
| | 7 | 2.78 | SUM | 3.07 | SUM | 5.85 |
| | 8 | 2.74 | | 2.83 | | 5.57 |
| | 9 | 3.35 | 28.74 | 3.20 | 29.10 | 6.55 |
| II | 10 | 3.54 | | 3.54 | | 7.08 |
| | 11 | 2.90 | | 2.82 | | 5.72 |
| | 12 | 3.29 | | 3.12 | | 6.41 |
| | 13 | 3.34 | | 3.35 | | 6.69 |
| | 14 | 3.33 | | 3.41 | | 6.74 |
| | 15 | 3.07 | Period II | 3.17 | Period I | 6.24 |
| | 16 | 2.79 | SUM | 3.18 | SUM | 5.97 |
| | 17 | 3.19 | | 3.33 | | 6.52 |
| | 18 | 3.43 | 28.88 | 3.46 | 29.38 | 6.89 |
| Formulation total | | 57.62 | | 58.48 | | 116.10 |

Period I = 28.74 + 29.38 = 58.12

Period II = 29.10 + 28.88 = 57.98

Correction term = $(116.10)^2/36$ = 374.4225

SStotal = $[(3.11)^2 + (3.14)^2 + \ldots + (3.46)^2] - C.T.$ = 2.1529

SSsequence = $[(6.09 + 6.34 + \ldots + 6.55)^2 + (7.08 + 5.72 + \ldots + 6.89)^2]/18 - C.T.$ = 0.0049

SSsubject = $[(6.09)^2 + (6.34)^2 + \ldots + (6.89)^2]/2 - 0.0049 - C.T.$ = 1.9247

SSperiod = $[(58.12)^2 + (57.98)^2]/18 - C.T.$ = 0.0005

SSformulation = $[(57.62)^2 + (58.48)^2]/18 - C.T.$ = 0.0205

SSerror = 2.1529 – (0.0049 + 1.9247 + 0.0005 + 0.0205) = 0.2022

Analysis of variance data for two way crossover design

| Source of Variation | d.f. | SS | MS | $F_{ratio}$ | $F_{table}$ | Sig Level |
|---|---|---|---|---|---|---|
| Total | 35 | 2.1529 | -- | -- | -- | |
| Sequence | 1 | 0.0049 | 0.0049 | 0.0407 | 4.49 | NS |
| Subject (Seq) | 16 | 1.9247 | 0.1203 | 9.5183 | 2.33 | S |
| Period | 1 | 0.0005 | 0.0005 | 0.0431 | 4.49 | NS |
| Formulation | 1 | 0.0205 | 0.0205 | 1.6256 | 4.49 | NS |
| Error | 16 | 0.2022 | 0.0126 | -- | -- | |

Where; F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there were not significant differences for the AUC according to the formulation effect between the value of the test and innovator's products.

Data presented are individual subject of the ln AUC of clavulanic acid following oral administration of 500/125 mg amoxicillin/clavulanic acid film coated tablet of test and innovator's products.

| Sequence | Subject | Innovator's Product | | Test Product | | Subject Total |
|---|---|---|---|---|---|---|
| I | 1 | 1.60 | | 1.48 | | 3.08 |
| | 2 | 1.61 | | 1.68 | | 3.29 |
| | 3 | 1.82 | | 1.71 | | 3.53 |
| | 4 | 1.62 | | 1.86 | | 3.48 |
| | 5 | 1.50 | | 1.46 | | 2.96 |
| | 6 | 1.30 | Period I | 1.45 | Period II | 2.75 |
| | 7 | 1.46 | SUM | 1.50 | SUM | 2.96 |
| | 8 | 1.68 | | 0.96 | | 2.64 |
| | 9 | 1.85 | 14.44 | 1.29 | 13.39 | 3.14 |
| II | 10 | 1.75 | | 0.99 | | 2.74 |
| | 11 | 1.56 | | 1.38 | | 2.94 |
| | 12 | 1.17 | | 2.02 | | 3.19 |
| | 13 | 1.14 | | 1.46 | | 2.60 |
| | 14 | 1.73 | | 1.29 | | 3.02 |
| | 15 | 1.35 | Period II | 1.54 | Period I | 2.89 |
| | 16 | 1.18 | SUM | 1.17 | SUM | 2.35 |
| | 17 | 1.40 | | 1.86 | | 3.26 |
| | 18 | 1.23 | 12.51 | 1.60 | 13.31 | 2.83 |
| Formulation total | | 26.95 | | 26.70 | | 53.65 |

Period I = 14.44 + 13.31 = 27.75

Period II = 13.39 + 12.51 = 25.90

Correction term = $(53.65)^2/36$ = 79.9534

SStotal = $[(1.60)^2 + (1.61)^2 + \ldots + (1.60)^2] - C.T.$ = 2.2843

SSsequence = $[(3.08 + 3.29 + \ldots + 3.14)^2 + (2.74 + 2.94 + \ldots + 2.83)^2]/18 - C.T.$ = 0.1122

SSsubject = $[(3.08)^2 + (3.29)^2 + \ldots + (2.83)^2]/2 - 0.1122 - C.T.$ = 0.6921

SSperiod = $[(27.75)^2 + (25.90)^2]/18 - C.T.$ = 0.0951

SSformulation = $[(26.95)^2 + (26.70)^2]/18 - C.T.$ = 0.0017

SSerror = 2.2843 – (0.1122+ 0.6921 + 0.0951 + 0.0017) = 1.3831

Analysis of variance data for two way crossover design

| Source of Variation | d.f. | SS | MS | $F_{ratio}$ | $F_{table}$ | Sig Level |
|---|---|---|---|---|---|---|
| Total | 35 | 2.2843 | -- | -- | -- | |
| Sequence | 1 | 0.1122 | 0.1122 | 2.5943 | 4.49 | NS |
| Subject (Seq) | 16 | 0.6921 | 0.0433 | 0.5004 | 2.33 | S |
| Period | 1 | 0.0951 | 0.0951 | 1.0997 | 4.49 | NS |
| Formulation | 1 | 0.0017 | 0.0017 | 0.0201 | 4.49 | NS |
| Error | 16 | 1.3831 | 0.0864 | -- | -- | |

Where; F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there were not significant differences for the AUC according to the formulation effect between the value of the test and innovator's products.

Data presented are individual subject of the ln $C_{max}$ of amoxicillin following oral administration of 500/125 mg amoxicillin/clavulanic acid film coated tablet of test and innovator's products.

| Sequence | Subject | Innovator's Product | | Test Product | | Subject Total |
|---|---|---|---|---|---|---|
| I | 1 | 1.92 | | 1.52 | | 3.44 |
| | 2 | 1.93 | | 1.69 | | 3.62 |
| | 3 | 2.32 | | 1.79 | | 4.11 |
| | 4 | 2.31 | | 2.22 | | 4.53 |
| | 5 | 2.22 | | 2.63 | | 4.85 |
| | 6 | 2.10 | Period I | 2.12 | Period II | 4.22 |
| | 7 | 1.98 | SUM | 1.87 | SUM | 3.85 |
| | 8 | 1.43 | | 1.62 | | 3.05 |
| | 9 | 2.00 | 18.21 | 1.92 | 17.38 | 3.92 |
| II | 10 | 2.29 | | 2.30 | | 4.59 |
| | 11 | 1.86 | | 1.88 | | 3.74 |
| | 12 | 2.32 | | 2.06 | | 4.38 |
| | 13 | 2.06 | | 1.95 | | 4.01 |
| | 14 | 2.10 | | 2.00 | | 4.10 |
| | 15 | 1.79 | Period II | 1.86 | Period I | 3.65 |
| | 16 | 1.91 | SUM | 1.85 | SUM | 3.76 |
| | 17 | 1.91 | | 1.87 | | 3.78 |
| | 18 | 2.17 | 18.41 | 1.91 | 17.68 | 4.08 |
| Formulation total | | 36.62 | | 35.06 | | 71.68 |

Period I = 18.21 + 17.68 = 35.89

Period II = 17.38 + 18.41 = 35.79

Correction term = $(71.68)^2/36$ = 142.7228

SStotal = $[(1.92)^2 + (1.93)^2 + \ldots + (1.91)^2] - C.T.$ = 2.0752

SSsequence = $[(3.44 + 3.62 + \ldots + 3.92)^2 + (4.59 + 3.74 + \ldots + 4.08)^2]/18 - C.T.$ = 0.0069

SSsubject = $[(3.44)^2 + (3.62)^2 + \ldots + (4.08)^2]/2 - 0.0069 - C.T.$ = 1.6194

SSperiod = $[(35.89)^2 + (35.79)^2]/18 - C.T.$ = 0.0003

SSformulation = $[(36.62)^2 + (35.06)^2]/18 - C.T.$ = 0.0676

SSerror = 2.0752 – (0.0069 + 1.6194 + 0.0003 + 0.0676) = 0.3809

Analysis of variance data for two way crossover design

| Source of Variation | d.f. | SS | MS | $F_{ratio}$ | $F_{table}$ | Sig Level |
|---|---|---|---|---|---|---|
| Total | 35 | 2.0752 | -- | -- | -- | |
| Sequence | 1 | 0.0069 | 0.0069 | 0.0686 | 4.49 | NS |
| Subject (Seq) | 16 | 1.6194 | 0.1012 | 4.2513 | 2.33 | S |
| Period | 1 | 0.0003 | 0.0003 | 0.0117 | 4.49 | NS |
| Formulation | 1 | 0.0676 | 0.0676 | 2.8394 | 4.49 | NS |
| Error | 16 | 0.3809 | 0.0238 | -- | -- | |

Where; F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there were not significant differences for the $C_{max}$ according to the formulation effect between the value of the test and innovator's products.

Data presented are individual subject of the ln $C_{max}$ of clavulanic acid following oral administration of 500/125 mg amoxicillin/clavulanic acid film coated tablet of test and innovator's products.

| Sequence | Subject | Innovator's Product | | Test Product | | Subject Total |
|---|---|---|---|---|---|---|
| I | 1 | 0.36 | | 0.32 | | 0.68 |
| | 2 | 0.46 | | 0.26 | | 0.72 |
| | 3 | 0.68 | | 0.64 | | 1.32 |
| | 4 | 0.57 | | 0.80 | | 1.37 |
| | 5 | 0.18 | | 0.65 | | 0.83 |
| | 6 | 0.20 | Period I | 0.67 | Period II | 0.87 |
| | 7 | 0.15 | SUM | 0.43 | SUM | 0.58 |
| | 8 | 0.76 | | -0.42 | | 0.34 |
| | 9 | 1.19 | 4.55 | 0.32 | 3.67 | 1.51 |
| II | 10 | 0.42 | | 0.31 | | 0.73 |
| | 11 | 0.42 | | 0.34 | | 0.76 |
| | 12 | 0.12 | | 0.45 | | 0.57 |
| | 13 | 0.36 | | 0.06 | | 0.42 |
| | 14 | 0.49 | | 0.02 | | 0.51 |
| | 15 | -0.16 | Period II | 0.51 | Period I | 0.35 |
| | 16 | -0.09 | SUM | 0.15 | SUM | 0.06 |
| | 17 | -0.03 | | 0.63 | | 0.60 |
| | 18 | -0.04 | 1.49 | 0.34 | 2.81 | 0.30 |
| Formulation total | | 6.04 | | 6.48 | | 12.52 |

Period I = 4.55 + 2.81 = 7.36

Period II = 3.67 + 1.49 = 5.16

Correction term = $(12.52)^2/36$ = 4.3542

SStotal = $[(0.36)^2 + (0.46)^2 + \ldots + (0.34)^2] - C.T.$ = 3.4040

SSsequence = $[(0.68 + 0.72 + \ldots + 1.51)^2 + (0.73 + 0.76 + \ldots + 0.30)^2]/18 - C.T.$ = 0.4268

SSsubject = $[(0.68)^2 + (0.72)^2 + \ldots + (0.30)^2]/2 - 0.4268 - C.T.$ = 0.8320

SSperiod = $[(7.36)^2 + (5.16)^2]/18 - C.T.$ = 0.1344

SSformulation = $[(6.04)^2 + (6.48)^2]/18 - C.T.$ = 0.0054

SSerror = $3.4040 - (0.4268 + 0.8320 + 0.1344 + 0.0054)$ = 2.0054

Analysis of variance data for two way crossover design

| Source of Variation | d.f. | SS | MS | $F_{ratio}$ | $F_{table}$ | Sig Level |
|---|---|---|---|---|---|---|
| Total | 35 | 3.4040 | -- | -- | -- | |
| Sequence | 1 | 0.4268 | 0.4268 | 8.2088 | 4.49 | S |
| Subject (Seq) | 16 | 0.8320 | 0.0520 | 0.4149 | 2.33 | NS |
| Period | 1 | 0.1344 | 0.1344 | 1.0727 | 4.49 | NS |
| Formulation | 1 | 0.0054 | 0.0054 | 0.0429 | 4.49 | NS |
| Error | 16 | 2.0054 | 0.1253 | -- | -- | |

Where; F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there were not significant differences for the $C_{max}$ according to the formulation effect between the value of the test and innovator's products.

Data presented are individual subject of the $t_{max}$ of amoxicillin following oral administration of 500/125 mg amoxicillin/clavulanic acid film coated tablet of test and innovator's products.

| Sequence | Subject | Innovator's Product | | Test Product | | Subject Total |
|---|---|---|---|---|---|---|
| I | 1 | 0.75 | | 4.00 | | 4.75 |
| | 2 | 2.50 | | 3.00 | | 5.50 |
| | 3 | 0.75 | | 1.50 | | 2.25 |
| | 4 | 1.00 | | 3.00 | | 4.00 |
| | 5 | 1.00 | | 1.50 | | 2.50 |
| | 6 | 1.50 | Period I | 1.00 | Period II | 2.50 |
| | 7 | 2.00 | SUM | 1.50 | SUM | 3.50 |
| | 8 | 3.00 | | 2.50 | | 5.50 |
| | 9 | 1.00 | 13.50 | 3.00 | 21.00 | 4.00 |
| II | 10 | 1.00 | | 1.00 | | 2.00 |
| | 11 | 2.00 | | 1.00 | | 3.00 |
| | 12 | 1.00 | | 2.50 | | 3.50 |
| | 13 | 1.00 | | 2.00 | | 3.00 |
| | 14 | 2.00 | | 1.50 | | 3.50 |
| | 15 | 1.50 | Period II | 2.00 | Period I | 3.50 |
| | 16 | 2.00 | SUM | 1.50 | SUM | 3.50 |
| | 17 | 1.00 | | 2.50 | | 3.50 |
| | 18 | 2.50 | 14.00 | 2.50 | 16.50 | 5.00 |
| Formulation total | | 27.50 | | 37.50 | | 65.00 |

Period I = 13.50 + 16.50 = 30.00

Period II = 21.00 + 14.00 = 35.00

Correction term = $(65.00)^2/36$ = 117.3611

SStotal = $[(0.75)^2 + (2.50)^2 + \ldots + (2.50)^2] - C.T.$ = 23.0139

SSsequence = $[(4.75 + 5.50 + \ldots + 4.00)^2 + (2.00 + 3.00 + \ldots + 5.00)^2]/18 - C.T.$ = 0.4444

SSsubject = $[(4.75)^2 + (5.50)^2 + \ldots + (5.00)^2]/2 - 0.4444 - C.T.$ = 8.7569

SSperiod = $[(30.00)^2 + (35.00)^2]/18 - C.T.$ = 0.6944

SSformulation = $[(27.50)^2 + (37.50)^2]/18 - C.T.$ = 2.7778

SSerror = 23.0139 – (0.4444 + 8.7569 + 0.6944 + 2.7778) = 10.3403

Analysis of variance data for two way crossover design

| Source of Variation | d.f. | SS | MS | $F_{ratio}$ | $F_{table}$ | Sig Level |
|---|---|---|---|---|---|---|
| Total | 35 | 23.0139 | -- | -- | -- | |
| Sequence | 1 | 0.4444 | 0.4444 | 0.8121 | 4.49 | NS |
| Subject (Seq) | 16 | 8.7569 | 0.5473 | 0.8469 | 2.33 | NS |
| Period | 1 | 0.6944 | 0.6944 | 1.0745 | 4.49 | NS |
| Formulation | 1 | 2.7778 | 2.7778 | 4.2982 | 4.49 | NS |
| Error | 16 | 10.3403 | 0.64627 | -- | -- | |

Where; F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there were not significant differences for the $t_{max}$ according to the formulation effect between the value of the test and innovator's products.

Data presented are individual subject of the $t_{max}$ of clavulanic acid following oral administration of 500/125 mg amoxicillin/clavulanic acid film coated tablet of test and innovator's products.

| Sequence | Subject | Innovator's Product | | Test Product | | Subject Total |
|---|---|---|---|---|---|---|
| I | 1 | 2.00 | | 1.50 | | 3.50 |
| | 2 | 1.50 | | 2.50 | | 4.00 |
| | 3 | 2.50 | | 0.75 | | 3.25 |
| | 4 | 1.00 | | 2.00 | | 3.00 |
| | 5 | 1.50 | | 0.75 | | 2.25 |
| | 6 | 1.00 | Period I | 1.00 | Period II | 2.00 |
| | 7 | 1.00 | SUM | 1.50 | SUM | 2.50 |
| | 8 | 1.50 | | 2.00 | | 3.50 |
| | 9 | 1.00 | 13.00 | 1.00 | 13.00 | 2.00 |
| II | 10 | 1.00 | | 1.50 | | 2.50 |
| | 11 | 2.00 | | 0.75 | | 2.75 |
| | 12 | 1.00 | | 3.00 | | 4.00 |
| | 13 | 1.00 | | 1.50 | | 2.50 |
| | 14 | 2.50 | | 2.00 | | 4.50 |
| | 15 | 1.50 | Period II | 1.50 | Period I | 3.00 |
| | 16 | 1.50 | SUM | 1.50 | SUM | 3.00 |
| | 17 | 1.50 | | 2.00 | | 3.50 |
| | 18 | 1.00 | 13.00 | 2.00 | 15.75 | 3.00 |
| Formulation total | | 26.00 | | 28.75 | | 54.75 |

Period I = 13.00 + 15.75 = 28.75

Period II = 13.00 + 13.00 = 26.00

Correction term = $(54.75)^2/36$ = 83.2656

SStotal = $[(2.00)^2 + (1.50)^2 + \ldots + (2.00)^2] - C.T.$ = 11.1719

SSsequence = $[(3.50 + 4.00 + \ldots + 2.00)^2 + (2.50 + 2.75 + \ldots + 3.00)^2]/18 - C.T.$ = 0.2101

SSsubject = $[(3.50)^2 + (4.00)^2 + \ldots + (3.00)^2]/2 - 0.2101 - C.T.$ = 3.9931

SSperiod = $[(28.75)^2 + (26.00)^2]/18 - C.T.$ = 0.2101

SSformulation = $[(26.00)^2 + (28.75)^2]/18 - C.T.$ = 0.2101

SSerror = 11.1719 – (0.2101 + 3.9931 + 0.2101 + 0.2101) = 6.5486

Analysis of variance data for two way crossover design

| Source of Variation | d.f. | SS | MS | $F_{ratio}$ | $F_{table}$ | Sig Level |
|---|---|---|---|---|---|---|
| Total | 35 | 11.1719 | -- | -- | -- | |
| Sequence | 1 | 0.2101 | 0.2101 | 0.8417 | 4.49 | NS |
| Subject (Seq) | 16 | 3.9931 | 0.2496 | 0.6098 | 2.33 | NS |
| Period | 1 | 0.2101 | 0.2101 | 0.5133 | 4.49 | NS |
| Formulation | 1 | 0.2101 | 0.2101 | 0.5133 | 4.49 | NS |
| Error | 16 | 6.5486 | 0.40929 | -- | -- | |

Where; F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there were not significant differences for the $t_{max}$ according to the formulation effect between the value of the test and innovator's products.

Data presented are individual subject of the $K_e$ of amoxicillin following oral administration of 500/125 mg amoxicillin/clavulanic acid film coated tablet of test and innovator's products.

| Sequence | Subject | Innovator's Product | | Test Product | | Subject Total |
|---|---|---|---|---|---|---|
| I | 1 | 0.61 | | 0.54 | | 1.15 |
| | 2 | 0.56 | | 0.41 | | 0.97 |
| | 3 | 0.15 | | 0.75 | | 0.90 |
| | 4 | 0.20 | | 0.66 | | 0.86 |
| | 5 | 0.21 | | 0.30 | | 0.51 |
| | 6 | 0.32 | Period I | 0.24 | Period II | 0.56 |
| | 7 | 0.74 | SUM | 0.27 | SUM | 1.01 |
| | 8 | 0.37 | | 0.51 | | 0.88 |
| | 9 | 0.20 | 3.36 | 0.61 | 4.29 | 0.81 |
| II | 10 | 0.18 | | 0.23 | | 0.41 |
| | 11 | 0.79 | | 0.51 | | 1.30 |
| | 12 | 0.48 | | 0.65 | | 1.13 |
| | 13 | 0.43 | | 0.24 | | 0.67 |
| | 14 | 0.44 | | 0.36 | | 0.80 |
| | 15 | 0.52 | Period II | 0.77 | Period I | 1.29 |
| | 16 | 0.86 | SUM | 0.36 | SUM | 1.22 |
| | 17 | 0.52 | | 0.42 | | 0.94 |
| | 18 | 0.38 | 4.60 | 0.23 | 3.77 | 0.61 |
| Formulation total | | 7.96 | | 8.06 | | 16.02 |

Period I = 3.36 + 3.77 = 7.13

Period II = 4.29 + 4.60 = 8.89

Correction term = $(16.02)^2/36$ = 7.1289

SStotal = $[(0.61)^2 + (0.56)^2 + \ldots + (0.23)^2] - C.T.$ = 1.3655

SSsequence = $[(1.15 + 0.97 + \ldots + 0.81)^2 + (0.41 + 1.30 + \ldots + 0.61)^2]/18 - C.T.$ = 0.0144

SSsubject = $[(1.15)^2 + (0.97)^2 + \ldots + (0.61)^2]/2 - 0.0144 - C.T.$ = 0.5914

SSperiod = $[(7.13)^2 + (8.89)^2]/18 - C.T.$ = 0.0860

SSformulation = $[(7.96)^2 + (8.06)^2]/18 - C.T.$ = 0.0003

SSerror = 1.3655 – (0.0144 + 0.5914 + 0.0860 + 0.0003) = 0.6734

Analysis of variance data for two way crossover design

| Source of Variation | d.f. | SS | MS | $F_{ratio}$ | $F_{table}$ | Sig Level |
|---|---|---|---|---|---|---|
| Total | 35 | 1.3655 | -- | -- | -- | |
| Sequence | 1 | 0.0144 | 0.0144 | 0.3896 | 4.49 | NS |
| Subject (Seq) | 16 | 0.5914 | 0.0370 | 0.8783 | 2.33 | NS |
| Period | 1 | 0.0860 | 0.0860 | 2.0445 | 4.49 | NS |
| Formulation | 1 | 0.0003 | 0.0003 | 0.0066 | 4.49 | NS |
| Error | 16 | 0.6734 | 0.04209 | -- | -- | |

Where; F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there were not significant differences for the $K_e$ according to the formulation effect between the value of the test and innovator's products.

Data presented are individual subject of the $K_e$ of clavulanic acid following oral administration of 500/125 mg amoxicillin/clavulanic acid film coated tablet of test and innovator's products.

| Sequence | Subject | Innovator's Product | | Test Product | | Subject Total |
|---|---|---|---|---|---|---|
| I | 1 | 0.71 | | 0.50 | | 1.21 |
| | 2 | 0.57 | | 0.22 | | 0.79 |
| | 3 | 0.36 | | 0.68 | | 1.04 |
| | 4 | 0.25 | | 0.42 | | 0.67 |
| | 5 | 0.35 | | 0.63 | | 0.98 |
| | 6 | 0.53 | Period I | 0.44 | Period II | 0.97 |
| | 7 | 0.48 | SUM | 0.24 | SUM | 0.72 |
| | 8 | 0.77 | | 0.22 | | 0.99 |
| | 9 | 0.28 | 4.30 | 0.60 | 3.95 | 0.88 |
| II | 10 | 0.52 | | 0.83 | | 1.35 |
| | 11 | 0.69 | | 0.48 | | 1.17 |
| | 12 | 0.24 | | 0.21 | | 0.45 |
| | 13 | 0.25 | | 0.45 | | 0.70 |
| | 14 | 0.28 | | 0.33 | | 0.61 |
| | 15 | 0.67 | Period II | 0.69 | Period I | 1.36 |
| | 16 | 0.28 | SUM | 0.39 | SUM | 0.67 |
| | 17 | 0.16 | | 0.23 | | 0.39 |
| | 18 | 0.12 | 3.21 | 0.42 | 4.03 | 0.54 |
| Formulation total | | 7.51 | | 7.98 | | 15.49 |

Period I = 4.30 + 4.03 = 8.33

Period II = 3.95 + 3.21 = 7.16

Correction term = $(15.49)^2/36$ = 6.6650

SStotal = $[(0.71)^2 + (0.57)^2 + \ldots + (0.42)^2] - C.T.$ = 1.2995

SSsequence = $[(1.21 + 0.79 + \ldots + 0.88)^2 + (1.35 + 1.17 + \ldots + 0.54)^2]/18 - C.T.$ = 0.0283

SSsubject = $[(1.21)^2 + (0.79)^2 + \ldots + (0.54)^2]/2 - 0.0283 - C.T.$ = 0.7022

SSperiod = $[(8.33)^2 + (7.16)^2]/18 - C.T.$ = 0.0380

SSformulation = $[(7.51)^2 + (7.98)^2]/18 - C.T.$ = 0.0061

SSerror = $1.2995 - (0.0283 + 0.7022 + 0.0380 + 0.0061)$ = 0.5248

Analysis of variance data for two way crossover design

| Source of Variation | d.f. | SS | MS | $F_{ratio}$ | $F_{table}$ | Sig Level |
|---|---|---|---|---|---|---|
| Total | 35 | 1.2995 | -- | -- | -- | |
| Sequence | 1 | 0.0283 | 0.0283 | 0.6456 | 4.49 | NS |
| Subject (Seq) | 16 | 0.7022 | 0.0439 | 1.3381 | 2.33 | NS |
| Period | 1 | 0.0380 | 0.0380 | 1.1593 | 4.49 | NS |
| Formulation | 1 | 0.0061 | 0.0061 | 0.1871 | 4.49 | NS |
| Error | 16 | 0.5248 | 0.0328 | -- | -- | |

Where; F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there were not significant differences for the $K_e$ according to the formulation effect between the value of the test and innovator's products.

Data presented are individual subject of the $t_{1/2}$ of amoxicillin following oral administration of 500/125 mg amoxicillin/clavulanic acid film coated tablet of test and innovator's products.

| Sequence | Subject | Innovator's Product | | Test Product | | Subject Total |
|---|---|---|---|---|---|---|
| I | 1 | 1.14 | | 1.28 | | 2.42 |
| | 2 | 1.24 | | 1.69 | | 2.93 |
| | 3 | 4.62 | | 0.92 | | 5.54 |
| | 4 | 3.47 | | 1.05 | | 4.52 |
| | 5 | 3.30 | | 2.31 | | 5.61 |
| | 6 | 2.17 | Period I | 2.89 | Period II | 5.06 |
| | 7 | 0.94 | SUM | 2.57 | SUM | 3.51 |
| | 8 | 1.87 | | 1.36 | | 3.23 |
| | 9 | 3.47 | 22.22 | 1.14 | 15.21 | 4.61 |
| II | 10 | 3.85 | | 3.01 | | 6.86 |
| | 11 | 0.88 | | 1.36 | | 2.24 |
| | 12 | 1.44 | | 1.07 | | 2.51 |
| | 13 | 1.61 | | 2.89 | | 4.50 |
| | 14 | 1.58 | | 1.93 | | 3.51 |
| | 15 | 1.33 | Period II | 0.90 | Period I | 2.23 |
| | 16 | 0.81 | SUM | 1.93 | SUM | 2.74 |
| | 17 | 1.33 | | 1.65 | | 2.98 |
| | 18 | 1.82 | 14.65 | 3.01 | 17.75 | 4.83 |
| Formulation total | | 36.87 | | 32.96 | | 69.83 |

Period I = 22.22 + 17.75 = 39.97

Period II = 15.21 + 14.65 = 29.86

Correction term = $(69.83)^2/36$ = 135.4411

SStotal = $[(1.14)^2 + (1.24)^2 + \ldots + (3.01)^2] - C.T.$ = 33.3956

SSsequence = $[(2.42 + 2.93 + \ldots + 4.61)^2 + (6.86 + 2.24 + \ldots + 4.83)^2]/18 - C.T.$ = 0.7021

SSsubject = $[(2.42)^2 + (2.93)^2 + \ldots + (4.83)^2]/2 - 0.7021 - C.T.$ = 14.9901

SSperiod = $[(39.97)^2 + (29.86)^2]/18 - C.T.$ = 2.8378

SSformulation = $[(36.87)^2 + (32.96)^2]/18 - C.T.$ = 0.4241

SSerror = 33.3956 – (0.7021 + 14.9901 + 2.8378 + 0.4241) = 14.4414

Analysis of variance data for two way crossover design

| Source of Variation | d.f. | SS | MS | $F_{ratio}$ | $F_{table}$ | Sig Level |
|---|---|---|---|---|---|---|
| Total | 35 | 33.3956 | -- | -- | -- | |
| Sequence | 1 | 0.7021 | 0.7021 | 0.7494 | 4.49 | NS |
| Subject (Seq) | 16 | 14.9901 | 0.9369 | 1.0380 | 2.33 | NS |
| Period | 1 | 2.8378 | 2.8378 | 3.1441 | 4.49 | NS |
| Formulation | 1 | 0.4241 | 0.4241 | 0.4699 | 4.49 | NS |
| Error | 16 | 14.4414 | 0.90259 | -- | -- | |

Where; F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there were not significant differences for the $t_{1/2}$ according to the formulation effect between the value of the test and innovator's products.

Data presented are individual subject of the $t_{1/2}$ of clavulanic acid following oral administration of 500/125 mg amoxicillin/clavulanic acid film coated tablet of test and innovator's products.

| Sequence | Subject | Innovator's Product | | Test Product | | Subject Total |
|---|---|---|---|---|---|---|
| I | 1 | 0.98 | | 1.39 | | 2.37 |
| | 2 | 1.22 | | 3.15 | | 4.37 |
| | 3 | 1.93 | | 1.02 | | 2.95 |
| | 4 | 2.77 | | 1.65 | | 4.42 |
| | 5 | 1.98 | | 1.10 | | 3.08 |
| | 6 | 1.31 | Period I | 1.58 | Period II | 2.89 |
| | 7 | 1.44 | SUM | 2.89 | SUM | 4.33 |
| | 8 | 0.90 | | 3.15 | | 4.05 |
| | 9 | 2.48 | 15.01 | 1.16 | 17.09 | 3.64 |
| II | 10 | 1.33 | | 0.83 | | 2.16 |
| | 11 | 1.00 | | 1.44 | | 2.44 |
| | 12 | 2.89 | | 3.30 | | 6.19 |
| | 13 | 2.77 | | 1.54 | | 4.31 |
| | 14 | 2.48 | | 2.10 | | 4.58 |
| | 15 | 1.03 | Period II | 1.00 | Period I | 2.03 |
| | 16 | 2.48 | SUM | 1.78 | SUM | 4.26 |
| | 17 | 4.33 | | 3.01 | | 7.34 |
| | 18 | 5.78 | 24.09 | 1.65 | 16.65 | 7.43 |
| Formulation total | | 39.10 | | 33.74 | | 72.84 |

Period I = 15.01 + 16.65 = 31.66

Period II = 17.09 + 24.09 = 41.18

Correction term = $(72.84)^2/36$ = 147.3796

SStotal = $[(0.98)^2 + (1.22)^2 + \ldots + (1.65)^2] - C.T.$ = 40.7796

SSsequence = $[(2.37 + 4.37 + \ldots + 3.64)^2 + (2.16 + 2.44 + \ldots + 7.43)^2]/18 - C.T.$ = 2.0736

SSsubject = $[(2.37)^2 + (4.37)^2 + \ldots + (7.43)^2]/2 - 2.0736 - C.T.$ = 20.0613

SSperiod = $[(31.66)^2 + (41.18)^2]/18 - C.T.$ = 2.5175

SSformulation = $[(39.10)^2 + (33.74)^2]/18 - C.T.$ = 0.7980

SSerror = $40.7796 - (2.0736 + 20.0613 + 2.5175 + 0.7980)$ = 15.3291

Analysis of variance data for two way crossover design

| Source of Variation | d.f. | SS | MS | $F_{ratio}$ | $F_{table}$ | Sig Level |
|---|---|---|---|---|---|---|
| Total | 35 | 40.7796 | -- | -- | -- | |
| Sequence | 1 | 2.0736 | 2.0736 | 1.6538 | 4.49 | NS |
| Subject (Seq) | 16 | 20.0613 | 1.2538 | 1.3087 | 2.33 | NS |
| Period | 1 | 2.5175 | 2.5175 | 2.6277 | 4.49 | NS |
| Formulation | 1 | 0.7980 | 0.7980 | 0.8330 | 4.49 | NS |
| Error | 16 | 15.3291 | 0.95807 | -- | -- | |

Where; F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there were not significant differences for the $t_{1/2}$ according to the formulation effect between the value of the test and innovator's products.

# VITA

Miss Yuwadee Sirisomboon was born on August 20, 1974 in Rachaburi. She received a Bachelor of Pharmacy degree in 1995 from Faculty of Pharmacy, Silpakorn University. She is a pharmacist in Drug Control Division, Food and Drug Administration, Ministry of Public Health, Thailand.