Bioequivalence Study of Risperidone 2 mg Tablet in Healthy Thai Male Volunteers

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ชื่อวิทยานิพนธ์	การศึกษาชีวสมมูลของยาเม็ดริสเพอริโดน ขนาด 2 มิลลิกรัม ในอาสาสมัครชายไทยสุขภาพดี
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บทคัดย่อ

ริสเพอริโดนเป็นยารักษาโรคจิตกลุ่มใหม่ ริสเพอริโดนถูกเมแทโบไลต์เป็น 9-ไฮดรอกซีริสเพอริโดน ซึ่งมีความแรงเทียบเท่ากับริสเพอริโดน ริสเพอริโดนมีประสิทธิภาพในการรักษาโรคจิตเภทโดยมีผลข้างเคียงด้าน extrapyramidal น้อยกว่ายาต้านโรคจิตในกลุ่มดั้งเดิม อย่างไรก็ตามยังไม่มีข้อมูลการศึกษาพารามิเตอร์ทางเภสัชจลนศาสตร์ของริสเพอริโดนและ 9-ไฮดรอกซีริสเพอริโดนรวมถึงการเปรียบเทียบชีวประสิทธิผลของยาเม็ดริสเพอริโดนในประชากรไทย วัตถุประสงค์ของการศึกษานี้คือการเปรียบเทียบชีวประสิทธิผล (Bioavailability) และพารามิเตอร์ทางเภสัชจลนศาสตร์ของยาสามัญริสเพอริโดนขนาด 2 มิลลิกรัม ที่ผลิตขึ้นในประเทศไทย กับยาต้นแบบที่ผลิตจากต่างประเทศ (Risperdal®) ในอาสาสมัครชายไทยสุขภาพปกติ โดยได้ศึกษาในอาสาสมัครจำนวน 22 คน โดยแบบแผนการทดลองเป็นแบบสุ่มไขว้สลับ แบบ 2 x 2 เว้นระยะห่างของการให้ยาเป็นเวลา 2 สัปดาห์ อาสาสมัครรับประทานยาต้นแบบหรือยาสามัญขนาด 2 มิลลิกรัม ครั้งเดียว และเก็บตัวอย่างเลือดของอาสาสมัครก่อนการบริหารยาและช่วงเวลาภายหลังการรับประทานยาจนถึง 96 ชั่วโมง ทำการแยกพลาสมาและเก็บไว้ที่อุณหภูมิ -20 องศาเซลเซียส จนกว่าจะนำมาวิเคราะห์ การวิเคราะห์หาระดับยาริสเพอริโดนและ9-ไฮดรอกซีริสเพอริโดน ในพลาสมาใช้วิธีโครมาโตกราฟฟีชนิดของเหลวประสิทธิภาพสูงและตรวจวัดด้วยแมสเปกโตรมิเตอร์ (LC-MS-MS) วิเคราะห์หาพารามิเตอร์ทางเภสัชจลนศาสตร์ของริสเพอริโดนและ 9-ไฮดรอกซีริสเพอริโดนโดยใช้แบบจำลองชนิด non-compartment พิจารณาชีวสมมูลของตำรับยาโดยการเปรียบเทียบช่วงความเชื่อมั่นที่ 90% ของค่าเฉลี่ยของอัตราส่วน (ยาสามัญ/ยาต้นแบบ) ในรูปลอการิทึม ของความเข้มข้นสูงสุดของยาในพลาสมา (C_{max}) และพื้นที่ใต้เส้นกราฟที่แสดงความสัมพันธ์ระหว่างความเข้มข้นของยาในพลาสมา (AUC) ซึ่งต้องอยู่ในช่วง 80 เปอร์เซ็นต์ ถึง 125 เปอร์เซ็นต์ ตามข้อกำหนดของคณะกรรมการอาหารและยาแห่งประเทศไทย ผลการศึกษาพบว่าช่วงความเชื่อมั่นที่ 90% ของค่าเฉลี่ยของอัตราส่วนC_{max}, AUC_{0-48} และ $AUC_{0-\infty}$ ในรูป

ลอการิทึม ของริสเพอริโดนมีค่าเท่ากับ 82.8% - 103.6%, 88.3% - 107.4% และ 88.1% - 106.8% ตามลำดับ สำหรับ 9-ไฮดรอกซีริสเพอริโดน ช่วงความเชื่อมั่นที่ 90% ของค่าเฉลี่ยของอัตราส่วน C_{max}, AUC_{0-96} และ $AUC_{0-\infty}$ มีค่าเท่ากับ 93.4% - 108.4%, 92.0% - 108.1%, และ 92.2% - 108.7% ตามลำดับ ไม่พบผลของลำดับการให้ยาต่อค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ที่ทำการศึกษา อาสาสมัครทุกคนสามารถทนต่อยาทั้ง 2 ตำรับ อาการข้างเคียงที่พบโดยทั่วไปคืออาการง่วงนอน ไม่พบความผิดปกติของการตรวจร่างกายและผลการตรวจเลือดทางห้องปฏิบัติการ ภายหลังจากเสร็จสิ้นการศึกษาและไม่มีอาสาสมัครถอนตัวออกจากการศึกษา

การศึกษาชีวสมมูลในอาสาสมัครชายไทยสุขภาพปกตินี้ พบว่ายาสามัญริสเพอริโดนขนาด 2 มิลลิกรัม ชีวสมมูลกับยาต้นแบบริสเพอริโดน ทั้งอัตราเร็วและปริมาณของการดูดซึมเข้าสู่ระบบการไหลเวียนของโลหิต

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ABSTRACT

Risperidone is an atypical antipsychotic drug. It is mainly metabolized to 9-hydroxyrisperidone, which is as potent as risperidone. Risperidone is effective in treatment of schizophrenia with less extrapyramidal side effects than typical antipsychotics. It has become more selected choice for the treatment of schizophrenia. However, information concerning pharmacokinetic properties of risperidone, including the relative bioavailability of the tablet formulations in the Thai population is not available. The aim of this study was to compare the relative bioavailability and other pharmacokinetic properties of a newly developed generic 2 mg risperidone tablet with those of an established branded formulation (Risperdal®) in healthy Thai male volunteers. This 2 × 2, crossover, randomized, double-blind study was performed in 22 healthy Thai male volunteers. A single dose of 2-mg risperidone was orally administered under fasting conditions, with a washout period of 14 days, blood samples were collected at predetermined time interval over a period of 96 hours. Using a validated liquid chromatography tandem mass spectrometric method, a plasma concentration of risperidone and 9-hydroxyrisperidone were determined and a corresponding plasma concentration-time curve for each volunteer was generated. A non-compartmental model was used for the analysis of pharmacokinetic parameters. The formulations were considered bioequivalent if the 90% confidence interval (CI) of logarithmically (ln)-transformed of the ratios (Test/Reference) of C_{max} and AUC were within the range of 80% to 125% according to the regulatory requirements set forth by Thailand FDA. Twenty-two subjects were completed in the study. The 90% CI for the ratios of the pharmacokinetic parameters (Test/Reference) of risperidone were 82.8% - 103.6% for C_{max}, 88.3% - 107.4% for AUC_{0-last} ,and 88.1% - 106.8% for $AUC_{0-\infty}$, respectively. For 9-

hydroxyrisperidone, the 90% CI for the ratio of C_{max} ,AUC_{0-last} , and $AUC_{0-\infty}$ were 93.4% - 108.4%, 92.0% - 108.1%, and 92.2% - 108.7%, respectively. No sequence effect was observed for both risperidone and 9-hydroxyrisperidone. Both formulations were well tolerated. The most commonly reported adverse event after intake of either formulation was somnolence. None of abnormal examination and blood chemistry could be found in all subjects at the end of the study. No subjects withdrew from the study.

In this study of selected healthy Thai male volunteers, a single, 2 mg dose of a Thailand locally made tablet was found to be bioequivalent to that of innovator's formulation based on the rate and extent of absorption.

CONTENTS

CONTENTS (Continued)

CONTENTS (Continued)

CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF ABBREVIATIONS AND SYMBOLS

9OHRIS	=	9-hydroxyrisperidone
%	=	Percent
μg	=	Microgram
μL	=	Microlitre
®	=	Trade name
°C	=	Degree Celsius
ALB	=	Albumin
ALP	=	Alkaline phosphatase
ANOVA	=	Analysis of Variance
AUC	=	Area under the plasma concentration-time curve
$AUC_{0-\infty}$	=	Area under the plasma concentration-time curve from zero to infinity
AUC_{0-t}	=	Area under the plasma concentration-time curve from zero to the last time point
BMI	=	Body mass index
BUN	=	Blood urea nitrogen
CBZ	=	Carbamazepine
CI	=	Confidence interval
CL	=	Clearance
cm	=	Centimeter
C_{max}	=	Maximum concentration
Cr	=	Creatinine
CV	=	Coefficient of variation
DBI	=	Direct bilirubin
df	=	Degree of freedom
E	=	Eosinophil

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

f_1	=	Difference factor
f_2	=	Similarity factor
FBS	=	Fasting blood sugar
FDA	=	Food and Drug Administration
h	=	Hour
Hb	=	Haemoglobin
Hct	=	Hematocrit
HPLC	=	High performance liquid chromatography
i.e.	=	Id est = That is
IS	=	Internal standard
kg	=	Kilogram
L	=	Liter
LC-MS-MS	=	Liquid chromatography tandem mass spectrometry
LLOQ	=	Lower limit of quantification
ln	=	Natural logarithm
m	=	Meter
m/z	=	Mass to charge ratio
min	=	Minute
mL	=	Milliliter
MS	=	Mean of square
MTBE	=	Methyltertiarybuthylether
ng	=	Nanogram
No.	=	Number
P	=	*p*-value

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

QC	=	Quality control
r^2	=	Coefficient of determination
RIS	=	Risperidone
RSD	=	Relative standard deviation
SD	=	Standard deviation
SGOT	=	Serum glutamic oxaloacetic transaminase
SGPT	=	Serum glutamic pyruvic transaminase
SS	=	Sum of square
$T_{1/2}$	=	Half-life
TBL	=	Total bilirubin
T_{max}	=	Time to maximum concentration
TP	=	Total protein
Vd	=	Volume of distribution
WBC	=	White blood cell

CHAPTER 1

INTRODUCTION

1. 1 Background and Rationale

Even though a number of new effective pharmaceuticals in the world market are increasing, many people especially in poor and developing countries still have a limited access. The cost of drug is a main barrier to the drug accessibility. Supporting of a generic drug product is therefore important to increase the use of cheaper off-patent drugs and increase accessibility of needed medicines (Homedes *et al*, 2005). Since the generic drug products come from multi-source of manufacturers and are generally cheaper than the original ones, the equivalent tests are required to ensure that they can be used as substitutes and provide the same therapeutic effects. Although, the active ingredient(s) in a generic drug product and the original are the same, the other excipients may differ. In general, only pharmaceutical equivalence study cannot be thoroughly assured the therapeutic equivalence. The differences in clinical response among individuals may be due to differences in pharmacokinetic and/or pharmacodynamic behavior of the drug. Two drug products are therapeutically equivalent if they are pharmaceutically equivalent and, after administration in the same molar dose, their effects with respect to efficacy and safety are essentially the same, as determined by appropriate bioequivalence, pharmacodynamic, clinical or in vitro studies (Shagel and Yu, 1999). For the approval of a generic drug product in Thailand, Office of Food and Drug Administration (Thailand FDA) requires comparison of bioavailability between generic drug product and original drug product in healthy Thai volunteers to ensure the interchangeability in Thai people (Thailand FDA, 2005).

Bioequivalence study is based on the administration of two drug products in the same molar dose under similar experimental conditions. Drugs should be considered bioequivalent if their rate and extent of absorption are not significantly different. The pharmacokinetic parameters of interest are the observed area under the plasma concentration-time curve (AUC), the peak plasma concentration (C_{max}) and time to peak plasma concentration (T_{max}) (Thailand FDA, 2005).

Schizophrenia is a chronic, complex psychiatric disorder of mental and thought. It affects to patients with significant disturbance in interpersonal relationships and ability to function in society (Crismon and Buckley, 2005). In the year 2003, the survey of mental disorder in Thailand reported that the lifetime prevalence and point prevalence of schizophrenia are 1.17 and 0.59, respectively (พรเทพ ศิริวนารังสรรค์ และคณะ, 2546).

The symptoms of schizophrenia are classified into two categories, positive and negative symptoms. Recently, the third category of disorganized symptom has been identified. Antipsychotic medication is the main treatment of schizophrenia. This medication should be able to treat most of schizophrenia symptoms, consequently, improve not only patient's illness but also quality of their lives. (Crismon and Buckley, 2005; Baldessarini and Tarazi, 2005). Antipsychotic drugs are classified into two groups, typical and atypical. Although typical or conventional antipsychotic drugs, e.g., chlorpromazine and haloperidol, could reduce positive symptoms, but negative symptoms are resisted. In addition, the occurring side effects, especially extrapyramidal side effects, have been a highly limiting factor of drug use. Patients who experience these side effects are difficult to manage and intolerable. Alternatively, atypical antipsychotic drugs have similar or greater efficacy and fewer side effects than typical antipsychotics (Casey, 1996; Choovanichvong *et al*, 2000). Recently, newer atypical antipsychotic drugs including risperidone, olanzapine, quetiapine and ziprasidone have become more selected choice of schizophrenia treatment drugs.

Risperidone is a benzisoxazole derivative atypical antipsychotic drug. After oral administration, it is metabolized to the major therapeutically active metabolite, 9-hydroxyrisperidone, which has equipotent pharmacological effect to the parent. Both risperidone and 9-hydroxyrisperidone bind to serotonin and dopamine receptors with high affinity. Many clinical investigation of risperidone in schizophrenia treatment indicated its efficacy for treatment of both positive and negative symptoms with fewer extrapyramidal side effects comparing to other typical antipsychotics (Chouinard *et al*, 1993; Marder *et al*,1994; Trevitt *et al*, 1997; Yen *et al*, 2004).

Since most schizophrenia patients require lifelong treatment, the high-price original medicines may cause financial problem and affect the treatment's compliance. In other words, the use of affordable generic drug products may contribute to the success of treatments.

However, the quality of locally made generic drug products is an important issue for consideration in healthcare professionals. In this study, 2 mg risperidone tablet formulation from local Thai manufacturer was tested for bioequivalency to the original risperidone, Risperdal® in order to support the generic drug registration in Thailand. Furthermore, results from this bioequivalence study would provide medical professionals to use this affordable medication effectively.

1.2 Objectives

To compare the rate and extent of absorption of a generic risperidone 2-mg tablet formulation locally made in Thailand with that of an innovative formulation when given as an equal labeled dose.

CHAPTER 2

REVIEW OF LTERATURES

2.1 Bioavailability

Bioavailability is a pharmacokinetic term that describes the rate and extent to which the active moiety is absorbed from a drug product and becomes available at the site of drug action. However, drug concentrations cannot be directly measured 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 basis that the drug at the site of action is in equilibrium with drug in the blood. Data from these *in vivo* bioavailability studies are important to establish recommended dosage regimens and support the drug labeling.

Absolute bioavailability, F, is the fraction of an administered dose which actually reaches the systemic circulation. Since the total amount of drug achieved in the systemic circulation is directly proportional to concentration-time curve (AUC), the absolute bioavailability of drug is generally measured by comparing the respective AUCs of the same dose of extravascular and intravenous administrations as follows

$$absolute\ bioavailability = \frac{AUC_{ev} / Dose_{ev}}{AUC_{iv} / Dose_{iv}}$$

Where, AUC_{ev} and AUC_{iv} are the area under the plasma concentration-time curves following the extravascular and intravenous administration of a given dose of drug, respectively.

Relative bioavailability refers to the comparative of availability of a drug to another product with the same active ingredient when they are given in the same dose. These measurements determine the effects of formulation differences on drug absorption which obtained by comparing their AUCs.

$$relative\ \ bioavailability = \frac{AUC_A / Dose_A}{AUC_B / Dose_B}$$

Where AUC_A and AUC_B are the area under the plasma concentration-time curves following the test product and reference product of a given dose of drug, respectively (Chereson and Banakar, 2000).

2.2 Bioequivalence

The comparative bioavailability assessment of two or more formulations of the same active ingredient administered by the same route is termed bioequivalence. Bioequivalent drug products means pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose of the therapeutic moiety under similar experimental conditions, either single dose or multiple dose.

Pharmaceutical equivalent refers to drug products that contain identical amounts and form of active ingredient, (i.e., the salt or ester of the same therapeutic moiety), in identical dosage forms, but not necessarily containing the same inactive ingredients. Pharmaceutical alternative refers to drug products that contain the identical therapeutic moiety, or its precursor, but not necessarily in the same amount or dosage form or as the same salt or ester (Shargel and Yu, 1999).

Drug products are considered to be therapeutic equivalents only if they are pharmaceutical equivalents and if they can be expected to have the same clinical effect and safety profile after administration under the conditions specified in the labeling. In general, the FDA considers two products to be "therapeutic equivalents" if they meet the following criteria:

1. they are pharmaceutical equivalents,
2. they are bioequivalent (demonstrated either by a bioavailability measurement or an *in vitro* standard),
3. they are in compliance with compendial standards for strength, quality, purity and identity,
4. they are adequately labelled, and
5. they have been manufactured in compliance with Good Manufacturing Practices (GMP) (USFDA, 2007).

2.3 Method for assessing bioavailability and bioequivalence

Bioavailability experiment could be designed dependent upon the objectives of the study, the ability of drug analysis, the pharmacodynamics of drug substance, the route of drug administration and the nature of the drug product. Pharmacodynamic and pharmacokinetic parameters as well as clinical observations and *in vitro* studies may be used to determine drug bioavailability from drug products.

Investigation of clinical effect is one of methods for assessing the bioavailability of a drug product. However, clinical studies are complex, expensive, time-consuming and require a sensitive and quantitative measure of the observed response. 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. However, monitoring of pharmacologic data is often difficult to establish the precision and reproducibility. The common method for assessing bioavailability of a drug is the measurement of the drug concentrations in the blood (Shagel and Yu, 1999).

2.3.1 Plasma Drug Concentration

Measurement of drug concentration in blood, plasma or serum is the most direct data to determine systemic drug bioavailability. By appropriate blood sampling, an accurate plasma drug concentration-time curve can be obtained using validated bioanalytical assay. After

administration of a single dose of treatment, blood samples are collected at specific time points and analyzed for drug content. A profile of the relationship between the drug concentrations in blood and the specific times is created. The pharmacokinetics parameters, AUC, C_{max} and T_{max} are generally used for the evaluation of drug bioavailability.

AUC: The area under the plasma concentration-time curve is a measurement of the extent of drug bioavailability. The AUC reflects the total amount of drug reaching the systemic circulation.

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

T_{max}: The time at which the Cmax occurs. The **T_{max}** reflects the rate of drug absorption, and decreases as the absorption rate increases (Chereson and Banakar, 2000).

2.3.2 Urinary Drug Excretion Data

Urinary drug excretion data is an indirect method for bioavailability estimation. These studies are based on the principle that urinary excretion of the unchanged drug is directly proportional to the plasma concentration of total drug. Unchanged form of drug must be excreted in urine in significant quantities. Urine sample should be collected timely and total quantity of urinary drug excretion must be obtained. The cumulative amount of drug excreted in urine (D_u), rate of drug excretion in urine (dD_u/d_t) and time to maximum urinary excretion (t^{∞}) are the key parameters to estimate bioavailability.

D_u^{∞}: The cumulative amount of drug excreted in urine is directly related to the total amount of drug absorbed. Urinary sample are collected periodically after administration and each sample is analyzed by specific assay.

dD_u/d_t: The rate of drug excretion in urine is determined under the assumption that the elimination of most drugs is first-order process. The rate of drug excretion is dependent on the first-order elimination rate constant, k, and plasma drug concentration, C_p.

t^{∞} : The time to maximum urinary excretion is the total time for drug to be excreted in urine (Shargel and Yu, 1999).

Bioavailability using urinary data 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 data should be conducted only if at least 60% of dose is excreted unchanged in the urine after an IV dose. Other conditions are including:

- The fraction of drug entering the bloodstream and being excreted intact by the kidneys must remain constant.
- Collection of the urine has to continue until all the drug has been completely excreted approximate ten times of 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 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 substitute for blood concentration data (Chereson and Banakar, 2000).

2.3.3 Acute Pharmacodynamic Effect

An acute pharmacodynamic effect can be used as an index of drug bioavailability when the quantitative measurement of a drug is not available, or insufficient accuracy and/or reproducibility. In this case, an acute pharmacodynamic effect-time curve is created. Measurements of this effect should be made with sufficient frequency to permit a reasonable estimate of the total area under the curve for a time period at least three half-life of the drug. With this method, dose-response curve is characterized for the determination of bioavailability. Pharmacodynamic parameters of interest are the total area under the acute pharmacodynamic effect-time curve, peak pharmacodynamic effect, and time for peak

pharmacodynamic effect. Onset time and duration of the pharmacokinetic effect may also be included in the analysis of the data. However, precision and reproducibility of pharmacodynamics data are often difficult to establish, and there are only a limited number of pharmacologic effects that are applicable to this method (Shargel and Yu, 1999).

2.3.4 Clinical Observation

Clinical trial approach is the least accurate, least sensitive and least reproducible of the general approaches for bioavailability determination. This approach would be considered when analytical methods are not available to permit the use of any approaches described above.

2.3.5 *In vitro* studies

It would be possible to use *in-vitro* data to predict a drug's *in-vivo* bioavailability. Ideally, *in-vitro* drug dissolution rate should correlate with *in vivo* drug bioavailability. It would prefer to substitute a quick, inexpensive *in-vitro* test for *in-vivo* bioavailability studies if it tests could reliably and exactly estimate drug absorption and reflect the *in-vivo* performance of a drug in humans. However, there are problems with the *in-vitro* dissolution testing which should be considered. The first problem is related to instrument variance and the absence of a standard method. The tests described in the USP are, but a few of the large number of dissolution methods, proposed to predict bioavailability. Since the dissolution rate of a dosage form is dependent on the methodology used in the dissolution test**,** changes in the apparatus, dissolution medium, etc., can significantly modify the results. Another significant problem is related to the difference between the *in-vitro* and *in-vivo* environments in which dissolution occurs. The *in-vivo* environment is far more complex, variable, and unpredictable than any *in-vitro* test environment, making *in-vitro/in-vivo* correlations very difficult. A simple dissolution test in a standardized vehicle cannot reflect the *in vivo* absorption of a drug across a population (Shargel and Yu, 1999).

However, *in vivo* study can be waived to *in vitro* dissolution testing for immediate release solid oral dosage forms based on an approach termed the Biopharmaceutics Classification System (BCS) (USFDA, 2000).

2.3.6 Biopharmaceutic classification system (USFDA, 2000)

The BCS is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. When combined with the dissolution of the drug product, the BCS takes into account three major factors that govern the rate and extent of drug absorption from immediate release solid oral dosage forms: dissolution, solubility, and intestinal permeability. According to the BCS, drug substances are classified as follows:

Class 1: High Solubility – High Permeability

Class 2: Low Solubility – High Permeability

Class 3: High Solubility – Low Permeability

Class 4: Low Solubility – Low Permeability

In addition, immediate release solid oral dosage forms are categorized as having rapid or slow dissolution. Within this framework, when certain criteria are met, the BCS can be used as a drug development tool to help sponsors justify requests for biowaivers.

Observed *in vivo* differences in the rate and extent of absorption of a drug from two pharmaceutically equivalent solid oral products may be due to differences in drug dissolution *in vivo*. However, when the *in vivo* dissolution of an immediate release solid oral dosage form is rapid in relation to gastric emptying and the drug has high permeability, the rate and extent of drug absorption is unlikely to be dependent on drug dissolution and/or gastrointestinal transit time. Under such circumstances, demonstration of *in vivo* bioequivalence may not be necessary for drug products containing Class 1 drug substances, as long as the inactive ingredients used in the dosage form do not significantly affect absorption of the active ingredients. The drug substance for which a waiver is being requested should be highly soluble, highly permeable and rapid dissolving. The recommended methods for determining solubility, permeability, and in vitro dissolution are discussed below.

2.3.6.1 Solubility

The solubility class boundary is based on the highest dose strength of an immediate release product that is the subject of a biowaiver request. A drug substance is considered highly soluble when the highest dose strength is soluble in 250 ml or less of aqueous media over the pH range of 1-7.5. The volume estimate of 250 ml is derived from typical bioequivalence study protocols that prescribe administration of a drug product to fasting human volunteers with a glass of water.

2.3.6.2 Permeability

The permeability class boundary is based indirectly on the extent of absorption (fraction of dose absorbed, not systemic bioavailability) of a drug substance in humans and directly on measurements of the rate of mass transfer across human intestinal membrane. Alternatively, nonhuman systems capable of predicting the extent of drug absorption in humans can be used (e.g., in vitro epithelial cell culture methods). In the absence of evidence suggesting instability in the gastrointestinal tract, a drug substance is considered to be highly permeable when the extent of absorption in humans is determined to be 90% or more of an administered dose based on a mass balance determination or in comparison to an intravenous reference dose.

2.3.6.3 Dissolution

Drug product is considered rapidly dissolving when not less than 85% of the labeled amount of the drug substance dissolves within 30 minutes, using U.S. Pharmacopeia (USP) Apparatus I at 100 rpm (or Apparatus II at 50 rpm) in a volume of 900 ml or less in each of the following media: (1) 0.1 N HCl or Simulated Gastric Fluid USP without enzymes; (2) a pH 4.5 buffer; and (3) a pH 6.8 buffer or Simulated Intestinal Fluid USP without enzymes.

2.4 Design and analysis of bioequivalence.

Bioequivalence studies, in most cases, are performed by pharmacokinetic studies according to a cross-over design, in which all treatments are applied to a group of normal, healthy subjects. The two–treatment, two–period (2 x 2) crossover trial is routinely used to establish bioequivalence of two treatments. Subjects are randomly assigned into two groups, usually of equal number. Subjects in the first group receive treatment A followed by treatment B (sequence AB), and for the second group the sequence is reverse (sequence BA), as described in Table 2.1. An appropriate time of washout period is specified in order to avoid carry over effect after the first treatment. In general, it should have time-space from the first dosing approximately more than 5 half-lives. After administration of each treatment, blood samples are collected according to the trials schedule, and the concentration of the drug in the blood is quantified according to the validated analytical method. Samples of each subject from both first and second periods are analyzed in the same analytical run. The typical pharmacokinetic parameters of interest are AUC and C_{max}.

Table 2.1 Experiment design for two-way crossover study

	Period I	Period II
Sequence I	A	B
Sequence II	B	A

2.5 Statistical analysis in bioequivalence study

2.5.1 Confidence interval in bioequivalence study

The significant differences between pharmacokinetic parameters obtained from test formulation and the reference one are tested based on two one-sided assumption. The hypothesis tests are inappropriate in bioequivalence because two products that are very close, but with small variances, may be considered difference, whereas products that are widely different,

and with large variances, may be considered equivalent. The use of confidence interval is more appropriate and has better statistical properties (Bolton, 1997). The current practice is to carry out the two one-sided tests at the 0.05 level of significance. This confidence interval implies that we are 95% confident that these two limits, referred to as the lower and upper confidence limits, will cover the true value of the difference. One test verifies that the bioavailability of the test product is not too low and the other to show that it is not too high. The current FDA guidelines are that two formulations whose rate and extent of absorption differ by -20%/+25% or less are generally considered bioequivalent (Chereson and Banakar, 2000).

2.5.2 Analysis of Variance (ANOVA) in bioequivalence study

An analysis of variance (ANOVA) is a statistical procedure used to test the difference within and between the data of treatment and control groups. A bioequivalent product should produce no significant difference in all pharmacokinetic parameters tested (Shargel and Yu, 1999). In general, AUC and C_{max} from bioequivalence studies are subjected to ANOVA which separates the variance into four components: subjects, period, treatment and error (residual). In the absence of differential carryover effects, the statistic test of interest is applied for treatment differences. The subject and period variability are separated from error term which then represents “intra-subject” variation (Bolton, 1997). For a crossover trial with *n* subjects and *t* treatments, the results from ANOVA are presented as shown in Table 2.2.

Table 2.2 ANOVA table for t-period, t-treatment crossover design (Bolton, 2007)

Sources of variation	Degree of freedom (df)	Sum of squares (SS)	Mean squares (MS)	F Statistic
Sequence	q-1	SSQ	MSQ	MSQ/MSS
Subject (seq)	(n1-1)+(n2-1)	SSS	MSS	MSS/MSE
Treatment	t-1	SST	MST	MST/MSE
Period	p-1	SSP	MSP	MSP/MSE
Error	[(t-1)(n-1)]-(p-1)	SSE	MSE	
Total	tn-1			

t is number of treatments; n is number of subjects; n1 and n2 are number of subjects in sequence 1 and 2, respectively. q is number of sequences; p is number of periods

SSQ-Sum of squares due to sequence; SST-Sum of squares due to treatments; SSS-Sum of squares due to subjects; SSP-Sum of squares due to period; SSE-Sum of squares due to error;

MSQ- Mean sum of squares due to sequences; MST-Mean sum of squares due to treatments; MSS-Mean sum of squares due to subjects; MSP-Mean sum of squares due to period; MSE-Mean sum of squares due to error

2.5.3 Logarithmic transformation of pharmacokinetic parameters

In general, bioequivalence studies compare AUC, C_{max} and T_{max} of the formulations. The AUC and C_{max}, should be logarithmically transformed before further statistical analysis. The use of log transformed values of these parameters is recommended due to the following reasons:

2.5.3.1 **Clinical rationale**:

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.

2.5.3.2 Pharmacokinetic rationale:

In the crossover design, the usual assumption is that the observation is a function of additive effects due to subject, period and treatment. But pharmacokinetic equations are of multiplicative character, for example,

$$AUC = \frac{F.dose}{Clearance}$$

Where $0 < F < 1$ represents the fraction absorbed.

The multiplicative term "clearance" can be regarded as a function of the subject. Consequently, Westlake contended that the subject effect is not additive function if the data is analyzed on the original scale of measurement. Logarithmic transformation takes this pharmacokinetic equation into an additive model equation:

$$\ln AUC = \ln F + \ln dose - \ln Clearance$$

Where ln represents the natural logarithm

Similar arguments are given for C_{max}. Log transformation of C_{max} data results in additive treatment of the volume of distribution, V_d.

2.5.3.3 Statistical rationale:

It is commonly believed that the distributions of many biological parameters have much longer right tails than would be expected had the parameter come from a normal distribution. If an outcome random variable is affected by many random causes, each of which produces a small proportional effect, the resulting distribution can be represented by the log normal distribution (Rani and Pargal, 2004).

2.6 The role of metabolites in bioequivalence.

In general, bioequivalence is assessed by comparing the plasma concentrations of the parent drug after application of various preparations. However, a number of reasons for the use of metabolite data will be considered in the following cases:

1. The parent drug is prodrug.
2. The parent drug cannot be measured due to the limitation of assay's sensitivity.
3. The parent drug is conversed to metabolite rapidly.
4. The parent drug and metabolite are equipotent, with the measured metabolite level being higher than parent (Midha *et al*, 2004).

The role of computer simulation in bioequivalence studies of metabolite have become advantages that all of parameters can be specified, and the correct answers are known given the assumptions. The disadvantage is that biological complexity may be oversimplified. Chan and Jackson (1991) investigated the estimation of bioequivalence studies using metabolite data of immediate release drug products which exhibited linear pharmacokinetics and no first-pass effect. This study was completed by the simulated method using a simple pharmacokinetics model to generate plasma concentration-time profiles for the parent and metabolite. To simulate bioequivalence studies, bivariate distributions of the absorption rate constant were generated and varied. The values of formation and excretion rate constant were assumed to be constant for each subject. The results indicated that, in all cases, the within-subject variability and 90% confidence interval for C_{max} of the metabolite is always tighter than that of parent drug, respectively. Furthermore, the first pass effect on drug was studied. The key finding in this study was that the model was able to distinguish between C_{max} of parent drug and that of the metabolite. The results showed that in cases where within-subjects variability of absorption rate constant (WSV-K_a) was greater than within-subjects variability of first-pass metabolite formation rate constant (WSV-K_s), the width of the 90% confidence interval of parent is greater than metabolite in term of C_{max}. In reverse case when WSV-K_a was smaller than WSV-K_s, the 90% confidence interval of metabolite was wider. They concluded that, in the absence of known relative variability of absorption and first-pass process, both parent and metabolite data should be included for the establishment of

bioequivalence, if the metabolite(s) play the important role in efficacy and safety of drug (Chan and Jackson, 1995). The further study of Jackson (2000) was reported on simulation of pharmacokinetic data on highly variable drugs and determination the effect of intrinsic clearance on the probability of meeting bioequivalence criteria were studied. The result showed that the C_{max} of metabolite was insensitive to the changes inwith the 25% change in absorption rate constant when intrinsic clearance less than hepatic blood flow except where renal clearance was low. In reverse case when intrinsic clearance was less than hepatic blood flow, metabolite data was better (Jackson, 2000).

Rosenbaum and Lam (1997) carried out a study based on the fundamental pharmacokinetics parameters of intrinsic and hepatic clearances in which a parent drug and single first-pass effect metabolite were considered. Plasma concentrations of both species were simulated under conditions of between-subject variability in intrinsic hepatic clearance, within-subject variability in hepatic clearance, and/or a concentration-dependent model for assay error. The results indicated that the parent drug was higher sensitive than metabolite to within-subject variables. This is the reason they concluded that the parent drug was the more discriminating for bioequivalence. For the use of the sum of parent and metabolite in bioequivalence evaluation, Mahmood (1998) has been investigated. Four drugs with active metabolite were selected to assess bioequivalence. Bioequivalence criteria of 80% - 125% were applied to the parent compound, active metabolite, and the sum of parent compound and metabolite. The results indicated that the application of 80% -125% bioequivalence criteria to the sum of parent compound and metabolite might be misleading. The 90% confidence interval should be applied separately to the parent compound and each metabolite.

However, it could not indicate the simple rules about the potential use of metabolite data in any bioequivalence studies. Nevertheless, if possible, it should include C_{max} of both parent drug and metabolite for evaluation of bioequivalence whenever the metabolite data are important to evaluation of clinical efficacy and safety of drug product. Any decision to use metabolite data in a given bioequivalence study must be made a priori by a drug regulatory agency and should be communicated to the sponsor in the design stage of the study. It will also avoid submission of data on multiple analytes for a posteriori evaluation, which may lead to unacceptable changes in the consumer and producer risks. It is important to assure patients that all

drug products for oral administration, brand or generic, are high-quality formulations (Midha *et al*, 2004).

2.7 Drug information

Risperdal® is the brand name of the innovative product of risperidone from Janssen Pharmaceutica which was approved in United State in the year 1993. Risperdal® tablets are available in 0.25 mg (dark yellow), 0.5 mg (red-brown), 1 mg (white), 2 mg (orange), 3 mg (yellow), and 4 mg (green) strengths. Inactive ingredients are colloidal silicon dioxide, hypromellose, lactose, magnesium stearate, microcrystalline cellulose, propylene glycol, sodium lauryl sulfate, and starch (corn). Tablets of 0.25, 0.5, 2, 3, and 4 mg also contain talc and titanium dioxide. The 0.25 mg tablets contain yellow iron oxide; the 0.5 mg tablets contain red iron oxide; the 2 mg tablets contain FD&C Yellow No. 6 Aluminum Lake; the 3 mg and 4 mg tablets contain D&C Yellow No. 10; the 4 mg tablets contain FD&C Blue No. 2 Aluminum Lake.

Risperdal® is also available as a 1 mg/mL oral solution. The inactive ingredients of this solution are tartaric acid, benzoic acid, sodium hydroxide, and purified water (Janssen, 2008).

2.7.1 Physicochemical properties

Risperidone, [3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-a]pyrimidin-4-one (figure 2.1), is a benzisoxazol derivative. Its molecular formula is $C_{23}H_{27}FN_4O_2$ with a molecular weight of 410.49. Risperidone is a white to slightly beige powder. It is practically insoluble in water, freely soluble in methylene chloride, and soluble in methanol and 0.1 N HCl (Janssen, 2008).

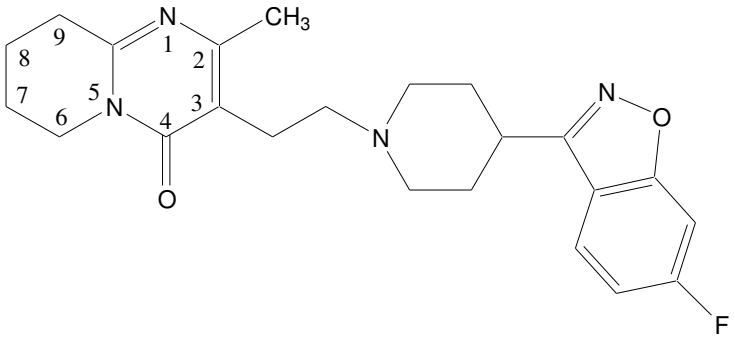


Figure 2.1 Structural formula of risperidone. (Budavari *et al*, 1996)

2.7.2 Mechanism of action

Mechanism of action of antipsychotic drugs in schizophrenia treatment is complex and unclear. Single theory cannot explain the real mechanisms of antipsychotic drugs in schizophrenia treatment. Three main theories of antipsychotic actions are purposed: (1) Dopamine receptor antagonist represents the mechanism of typical antipsychotic drugs, (2) Serotonin dopamine antagonists, and (3) Partial dopamine agonists. Atypical antipsychotic drugs act as both dopamine and serotonin receptor antagonists. Newer antipsychotics drugs have additive effect of partial dopamine agonist which advantages in decreasing of extrapyramidal side effects. (Yagcioglu, 2007) However, mechanisms of action of atypical antipsychotics have more details than described above. Various theories are considered and further studied to prove any consumption related to efficacy and safety of risperidone used.

Risperidone is a selective monoaminergic antagonist with high affinity (Ki of 0.12 to 7.3 nM) for the serotonin Type 2 ($5HT_2$), dopamine Type 2 (D_2), α_1 and α_2 adrenergic, and H_1 histaminergic receptors (Leysen *et* al, 1988). Risperidone was selected for treatment in patients with schizophrenia because of its potent antagonist effects to serotonin 5-HT_2 and dopamine D_2 receptor. Several published papers showed serotonin 5-HT_2 and dopamine D_2 antagonist of risperidone in schizophrenia patient using positron emission tomography imaging. Occupancy of risperidone to serotonin 5-HT_2 were greater than dopamine D_2 occupancy at all doses. (Kapur *et al,* 1999; Nyberg *et al*, 1999; Frankle *et al*, 2004) Risperidone displayed higher

binding affinity than haloperidol for alpha-1 adrenergic, histamine-H1 and alpha-2 adrenergic receptors and no affinity for cholinergic and muscarinic receptors. (Leysen *et al*, 1988)

2.7.3 Pharmacokinetic Properties

2.7.3.1 Absorption

The pharmacokinetics of risperidone has been investigated by manufacturer in healthy male volunteers. It is well absorbed from gastrointestinal tract in which the peak plasma concentration is within 1 hour in extensive metabolisers and within 2 hours in poor metabolisers. Oral absorption of the drug is not affected by food. The absolute oral bioavailability of risperidone in extensive metabolisers is approximately 66%. In consideration of active moiety, the absolute bioavailability is 70%. Plasma concentration of risperidone and 9-hydroxyrisperidone are dose proportional over the dosing range of 1 to 16 mg daily (Huang *et* al, 1993). Gutierrez *et al* (1997) compared oral bioavailability of a 1-mg tablet with a 1 mg/ml oral solution. The results demonstrated bioequivalence of these two drug products with relative oral bioavailability of 95%.

2.7.3.2 Metabolism

Risperidone is extensively metabolized in liver to a major therapeutically active metabolite, 9-hydroxyrisperidone (Figure 2.2), which displays similar pharmacological effect and potency to the parent compound (Van Beijsterveldt *et* al, 1994). Phase I metabolism of risperidone is mainly occurred by alicyclic hydroxylation at the 9-position of the pyrimidinone moiety. Metabolic pathway is mostly processed through liver microsomal enzyme, cytochrome P450 (CYP) subtype 2D6. 9-hydroxyrisperidone accounts for 32% of the dose in the extensive metabolisers, 22% in the intermediate metabolisers and 8% in poor metabolisers (Mannen *et al*, 1993). The minor metabolic pathways are: (1) oxidative *N*-dealkylation at the piperidine nitrogen, which results in two acidic non-pharmacologically active metabolites, one derived from risperidone itself and the other from the 9-hydroxy-metabolite, (2) hydroxylation at the 7-position of the pyrimidinone moiety and (3) the scission of the benzisoxazole moiety to a carbonyl and a

phenol function as shown in figure 2.3 (Mannen *et al*, 1993; Huang *et* al, 1993). Alicyclic hydroxylation at the 7-position is accounted for 1-5% of the dose (Mannen *et al,* 1993). Scordo *et al*. (1999) reported that steady-state plasma concentration of risperidone and the ratio of risperidone to 9-hydroxyrisperidone are highly dependent on the CYP2D6. Not only CYP2D6, but also CYP3A4 are responsible for the transformation of risperidone to 9-hydroxyrisperidone. *In vitro* enzyme activities study of Fang *et al* (1999) using the 9 isosomes of recombinant human cytochrome P450 (CYP) indicated that CYP2D6, 3A4 and 3A5 associated with the metabolism of risperidone to 9-hydroxyrisperidone. CYP2D6 has activity 18.8 times higher than CYP3A4 and 37.5 times higher than CYP3A5. CYP2D6 subjects to generic polymorphism. Therefore, some individuals are extensive metabolisers, approximately 90% of the Caucasian and 99% of the Oriental population, other are intermediate or poor metabolisers (Zanger *et* al, 2004). As a consequence, risperidone undergoes only minor metabolism to 9-hydroxyrisperidone in poor metabolisers of CYP2D6, and therefore its half-life is much longer in these subjects (Heykants *et al,* 1994).

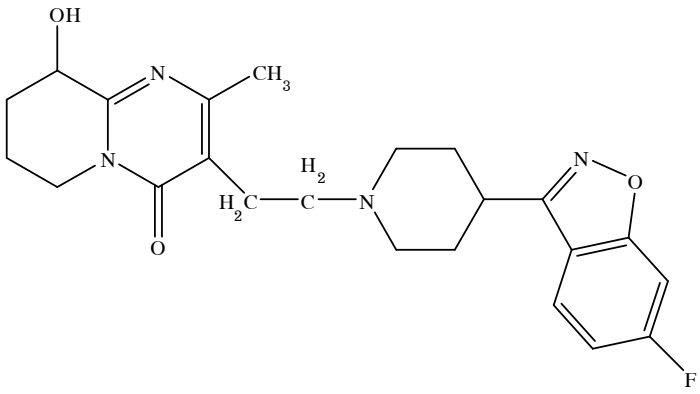
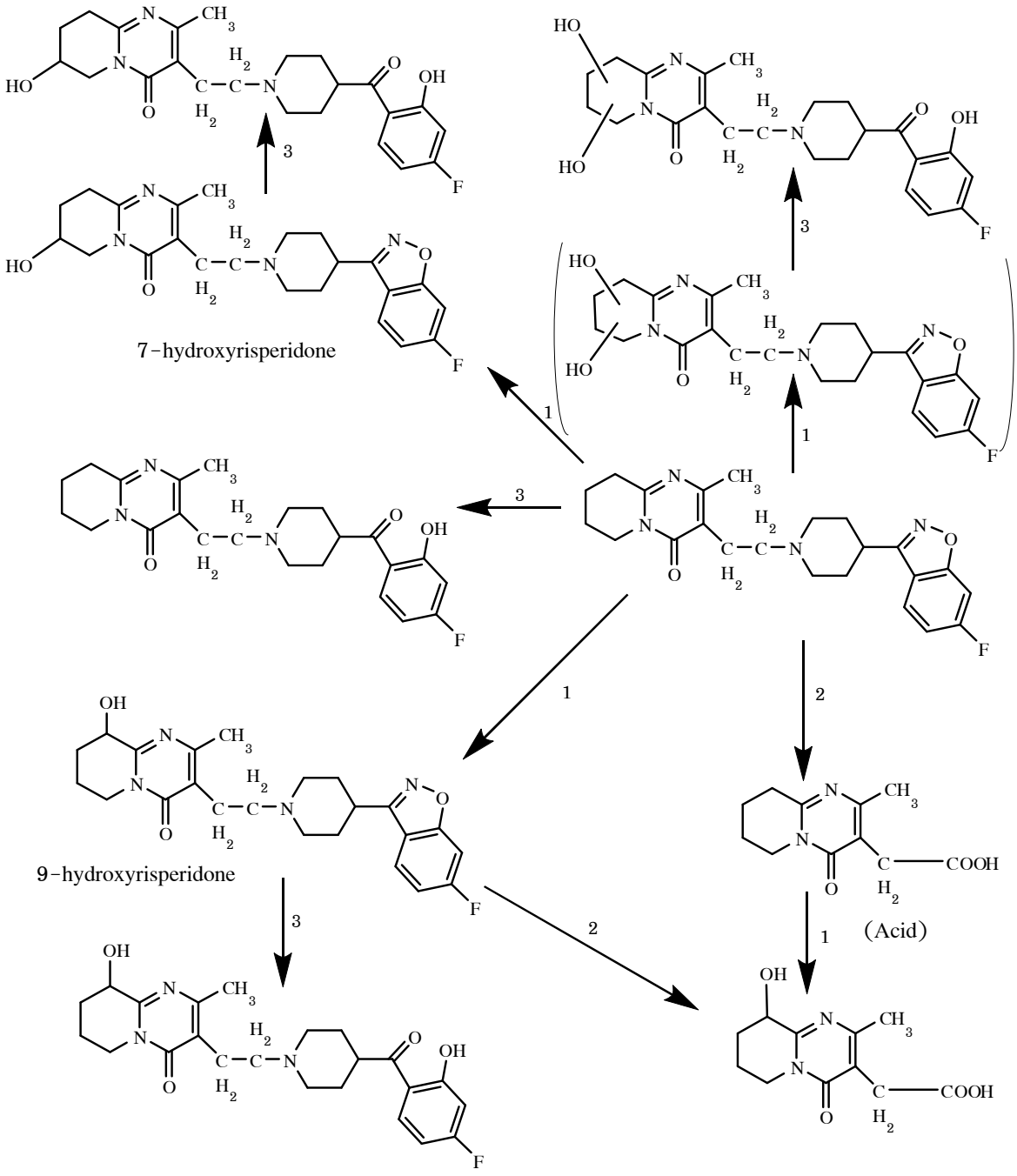


Figure 2.2 Structural formula of 9-hydroxyrisperidone (Mannen *et al*, 1993)



Metabolic pathways: 1 Aliphatic hydroxylation, 2 Oxidative *N*-dealkylation, 3 Benzisoxazole scission (Structure in parentheses is hypothetical)

Figure 2.3 Metabolic pathways of risperidone in humans (Mannen *et al*, 1993)

2.7.3.3 Distribution

Risperidone and 9-hydroxyrisperidone are rapidly distributed in body with apparent volume of distribution at steady state approximately 1.3 L/kg (Huang *et* al, 1993). Approximately 90% and 77% of risperidone and 9-hydroxyrisperidone, respectively binds to albumin and alpha-1 acid glycoprotein (Mannen *et al*, 1994).

2.7.3.4 Excretion

Within 1 week after oral administration, risperidone is excreted in the urine 70% and 15% in feces (Mannen *et al*, 1993). Apparent half-life, $t_{1/2}$, of risperidone is approximately 3 hours in extensive metabolisers and 16 hours in poor metabolisers. 9-hydroxyrisperidone has longer half-life than risperidone, approximately 20 hours in extensive metabolisers and 27 hours in poor metabolisers. Pharmacokinetic parameters of active moiety are not significantly difference from parent and metabolite. Overall half-life is approximately 20 hours (Huang *et* al, 1993).

2.7.4 Effects of age and disease states

Plasma protein binding: The pharmacokinetics of risperidone in healthy elderly volunteers and in patients with liver disease is similar, although the free fraction of risperidone in plasma was increased in the former group as a result of decreased levels of albumin and α1-acid glycoprotein (Mannen *et al*, 1994). Snoech *et al* (1995) reported that human serum albumin (HSA) decreased in patients with liver and renal disease. The decrease in HSA concentration in cirrhotic and renal disease patients was much more pronounced than that in elderly subjects. Plasma concentrations of α1-acid-glycoprotein (AAG) were increased in the elderly and in the renal disease patients, while lower AAG levels were determined in cirrhotic patients.

Pharmacokinetic parameter: In patients with renal impairment, the pharmacokinetic parameters of risperidone and 9-hydroxyrisperidone are presented similar to those of poor metabolisers subject. The decreasing in oral clearance and a prolonged elimination half-life of risperidone associated with lower concentrations of 9-hydroxyrisperidone. The total oral and renal clearance of risperidone in renal disease patients is reduced. The renal clearance of 9-hydroxy-risperidone was reduced, the elimination half-life prolonged and the AUC enlarged in patients with renal insufficiency and in the elderly. As the active moiety mainly consists of 9-hydroxyrisperidone of which the elimination rate was reduced in patients with a reduced creatinine clearance, the elimination rate of the active moiety also reduce in these patients. Therefore, a significantly reduced total oral clearance and a prolonged elimination half-life of the active moiety are observed in elderly and in renal disease patients. The mean total oral clearance of the active moiety was reduced by about 30% in the elderly and by about 50% in renal disease patients. The elimination half-life of the active moiety averaged about 18 h in young subjects and about 25 h in elderly and renal disease patients (Snoech *et al*, 1995).

2.7.5 Therapeutic Use

Schizophrenia: Risperidone is indicated for the treatment of schizophrenia. Risperidone is initially administered of 1 mg twice a day dosing (BID) in first day of treatment. Dosage may increase in increment of 1 mg BID in second and third day, as tolerated, to target dose of 3 mg BID (Janssen, 2008). Multicenter controlled clinical trials of effective dose with minimal side effects in chronic schizophrenia patients indicate that risperidone is effective against positive and negative symptoms in dose rang 2-6 mg daily. The optimal therapeutic dose of risperidone is 6 mg/day which has therapeutic effectiveness in both safety and efficacy (Chouinard *et al*, 1993; Marder *et al*, 1994; Nyberg *et al*, 1999).

Marder *et al*. (1994) reported the optimal therapeutic effects were 4 to 8 mg per day dose range. At endpoint of study, clinical improvement (20% reduction in total scores on the Positive and Negative Syndrome Scale for Schizophrenia, PANSS) was presented by 35% of patients receiving 2 mg daily of risperidone, 57% of receiving 6 mg, 40% of receiving 10 mg, 51% of receiving 16 mg; and by 30% receiving 20 mg daily haloperidol and 22% receiving

placebo. Extrapyramidal effects, evaluated with Extrapyramidal Symptom Rating Scale (ESRS), significantly increased in patients treated with 16 mg daily of risperidone and 20 mg daily of haloperidol. Clinical improvements were significantly found in patients receiving 6 and 16 mg of risperidone daily. Doses above 10 mg per day have not been shown to be more efficacious than lower doses and were associated with more extrapyramidal symptoms and other adverse events. The safety of risperidone has not been established above 16 mg total daily dose, administered twice daily. The dose of risperidone should be adjusted gradually over several days based on clinical response to a target dose of 4 to 6 mg per day. Some patients may benefit from lower initial doses and/or a shower adjustment schedule.

Karnchanathanalers (1998) studied the effective dose of risperidone in 10 chronic schizophrenia Thai patients for 6 months. The results indicated that the mean risperidone dose was 5.56 mg/day (modal risperidone dose 4 mg/day).

Bipolar mania: Risperidone is also indicated for the short-term treatment of acute manic or mixed episodes associated with bipolar I disorder. Once-daily dose of risperidone should be initially administered with 2 mg to 3 mg per day. Dosage should be adjusted at intervals of not less than 24 hours and in dosage increments/decrements of 1 mg per day, as studied by research group of Khanna. (Khanna *et al*, 2005) They investigated 3-week efficacy of risperidone in bipolar I disorder treatment using double-blind, placebo-controlled study in 290 acute mania associated with bipolar I disorder patients at dosage range of 1-6 mg per day. The results indicated that the mean modal dose of risperidone was 5.6 mg/day. At week 1 and 2, mean Young Mania Rating Scale (YMRS) scores were decrease significantly in risperidone than placebo group. No unexpected adverse events were reported. Extrapyramidal side effects were reported in risperidone group more than placebo group. However, extrapyramidal symptoms were mild in most case and the median ESRS change from baseline was 0 at endpoint of study in both groups.

An acute and continuation study of risperidone in bipolar I disorder treatment was further studied by Smulevich *et al*, (2005). They investigated the efficacy and safety of risperidone and haloperidol in a randomized, double-blind trial. 438 patients with acute bipolar mania received risperidone, haloperidol, or placebo for 3 weeks, followed by double-blind risperidone or haloperidol for 9 weeks. At week 3, mean YMRS score significantly reduced from

baseline greater in patients receiving risperidone than placebo. Furthermore, reductions in YMRS scores were seen in patients receiving risperidone or haloperidol during the subsequent 9 weeks. They concluded that risperidone monotherapy was an effective and well-tolerated treatment for bipolar mania and that efficacy was maintained over the long term.

Irritability Associated with Autistic Disorder: Risperidone is indicated for the treatment of irritability associated with autistic disorder in children and adolescents aged 5–16 years, including symptoms of aggression towards others, deliberate self-injuriousness, temper tantrums, and quickly changing moods. (Pandina *et al*, 2006) Dosing should be initiated at 0.25 mg per day for patients who weigh less than 20 kg and 0.5 mg per day for patients who weight equal or more than 20 kg. After a minimum of four days from treatment initiation, the dose may be increased to the recommended dose of 0.5 mg and 1 mg per day for patients who weigh less than 20 kg and weigh equal or more than 20 kg, respectively. This dose should be maintained at least for 14 days. In patients not achieving sufficient clinical response, treatment should be prolonged more than 2 weeks and dosing should be adjusted by 0.25 mg per day for patients who weigh less than 20 kg or 0.5 mg per day for patients who weight equal or more than 20 kg.

2.7.6 Contraindication

Hypersensitivity reactions, including anaphylactic reactions and angioedema, have been observed in patients treated with risperidone. Therefore, risperidone is contraindicated in patients with a known hypersensitivity to the product (Janssen, 2008).

2.7.7 Drug interactions

Drug interactions of risperidone have not been systematically evaluated. Since risperidone affects on central nervous system, co-administration with other centrally acting drugs and alcohol should be careful. Risperidone has antagonistic effect to adrenergic receptor, so it may enhance the hypotensive effects of other therapeutic agents. Because of dopamine antagonistic effect of risperidone, drugs acting on dopamine receptor such as levodopa or other

dopamine agonistic drugs may be affected. Because risperidone is metabolized by CYP2D6, drug-drug interaction can occur from the inhibitory and induction effect of other drugs on CYP2D6. Inhibitors of CYP2D6 interfere with conversion of risperidone to 9-hydroxyrisperidone. Drug interactions that reduce the metabolism of risperidone to 9-hydroxyrisperidone would increase the plasma concentrations of risperidone and lower the concentrations of 9-hydroxyrisperidone (Janssen, 2008).

Carbamazepine: Ono *et al*. (2002) studied the effect of carbamazepine co-administration on plasma concentrations of risperidone and its active metabolite 9-hydroxyrisperidone. 11 schizophrenic in-patients were treated with 6 mg/day risperidone and carbamazepine 400 mg/day for 1 week. Plasma concentrations of risperidone and 9-hydroxyrisperidone during carbamazepine co-administration were significantly lower than baseline. They suggested that carbamazepine induces the metabolism of risperidone and 9-hydroxyrisperidone, and that the decrease in risperidone concentration is dependent on the CYP2D6 activity.

Paroxetine: Paroxetine, a potent inhibitor of CYP2D6, impair the elimination of risperidone, primarily by inhibiting CYP2D6 and lesser effect to the metabolism of 9-hydroxyrisperidone or other pathways of risperidone biotransformation. Spina *et* al, (2001) studied the effects of paroxetine (20 mg/day) on steady-state plasma concentrations of risperidone and its active metabolite 9-hydroxyrisperidone in 10 schizophrenia patients. During paroxetine administration, mean plasma concentrations of risperidone increased significantly, whereas levels of 9-hydroxyrisperidone decreased slightly but not significantly. The sum of active moiety increased significantly by 45% over baseline. One patient developed parkinsonian symptoms in the second week of combinative therapy.

Fluoxetine: Spina *et al* (2002) studied a clinically relevant pharmacokinetic drug interaction between risperidone and fluoxetine in 10 schizophrenia patients. Mean plasma concentrations of risperidone significantly increased during fluoxetine (20 mg/day) administration, while the levels of 9-hydroxyrisperidone were not significantly affected. The sum

of active moiety increased 75% compared with baseline. The mean plasma risperidone/9-hydroxyrisperidone ratio also increased significantly. These findings indicate that fluoxetine, a potent inhibitor of CYP2D6 and a less potent inhibitor of CYP3A4, reduces the clearance of risperidone by inhibiting its 9-hydroxylation or alternative metabolic pathways.

Bondolfi *et al* (2002) also studied effect of co-administer of risperidone (6 mg/day) and fluoxetine (20 mg/day) in 11 schizophrenia patients with addition of CYP2D6-genotypic specification. The results showed that AUC of risperidone increased when co-administered with fluoxetine in both extensive metaboliser and poor metaboliser groups, which correlated to the increasing of the AUC of the active moiety. In extensive metabolisers, the AUC of 9-hydroxy-risperidone remained similar, whereas it increased in poor metaboliser groups. Ten of the 11 patients showed a clinical improvement compared to baseline. The severity and incidence of extrapyramidal symptoms and adverse events did not significantly increase when fluoxetine was added.

Fluvoxamine: Fluvoxamine is a moderate inhibitor of CYP2C9 and CYP3A4, and slightly CYP2D6 inhibition activity. Co-administration of fluvoxamine (100 mg/day) and risperidone (6 mg/day), mean plasma concentrations of risperidone, 9-hydroxyrisperidone and the active moiety were not significantly modified following. Fluvoxamine co-administration with risperidone was well tolerated and no patient developed extrapyramidal side effects (D'Arrigo *et al*, 2005).

Donepezil: Donepezil is also metabolized by cytochrome P450 enzyme which mainly involve to CYP3A4 subtype and slightly to CYP2D6. Once-daily dosing of 5 mg donepezil HCl co-administered with risperidone (4-6 mg/day) does not alter the pharmacokinetics of risperidone in patients with schizophrenia. The combination of risperidone and donepezil HCl was well tolerated (Reyes *et al*, 2004).

Sertraline: Sertraline mainly undergoes CYP3A4 metabolism. Four weeks administered of sertraline (up to 100 mg/day) and risperidone (4-6 mg/day) in schizophrenia patient does not clinically significant changes in plasma risperidone and 9-hydroxyrisperidone concentrations (Spina *et al*, 2004).

Rifampin: Rifampin, a dual inducer of CYP3A and P-glycoprotein, is associated with a significant decrease in risperidone area under the curve. The apparent oral clearance of risperidone approximately doubled after rifampin treatment (Mahatthanatrakul *et al*, 2007; Kim *et al,* 2008).

Itraconazole: A studied of drug-drug interaction between risperidone (2-8 mg/day) and itraconazole (200 mg/day) in 19 schizophrenia patients was conducted by Jung *et al* (2005). The results showed that itraconazole, a CYP3A4 and P-glycoprotein inhibitor, increased the concentrations of risperidone by 69% and 9-hydroxyrisperidone by 71% in extensive metaboliser patients. For the poor metaboliser patients, 75% and 73% increasing of risperidone and 9-hydroxyrisperidone concentration were observed, respectively.

Lithium and Divalproex sodium

A prospective 6-month open trial examined the safety and efficacy of two combination therapies in 37 bipolar I disorder patients with age-range of 5–17 years: (1) divalproex sodium plus risperidone, or (2) lithium plus risperidone. The results indicated that both divalproex sodium plus risperidone and lithium plus risperidone showed strong effects coupled with safety and tolerability in treating children and adolescents with manic or mixed episodes associated with type I bipolar disorder (Pavuluri *et al*, 2004).

Ravindra *et al*. (2004) conducted the study of drug-drug interaction by comparing of pharmacokinetic parameters of divalproex (1000 mg daily) and risperidone (2-4 mg daily) or placebo. At the endpoint of study, they found that pharmacokinetic parameters of valproate did not change in both risperidone and placebo groups. This study confirmed that adjunctive risperidone treatment had no influence on the steady-state pharmacokinetics of valproate and this combination was safe and well tolerated.

2.7.8 Pregnancy

Risperidone is a Pregnancy Category C drug (Janssen, 2008). Coppola *et al*. (2007) evaluated the worldwide post-marketing experience of risperidone used during pregnancy. 713 pregnancies were identified in women who were receiving risperidone. The study indicated that in utero exposure to risperidone did not appear to increase the risk of spontaneous abortions, structural malformations and fetal teratogenic risk in general population. Self-limited extrapyramidal effects in neonates were observed after exposure to risperidone during the third trimester of pregnancy. However evidence of spontaneous abortion, structural malformations and fetal teratogenic could be found. Risperidone should only be used during pregnancy if the benefits outweigh the potential risks.

2.7.9 Side effects and adverse reactions

The adverse effect profile of risperidone reflects its antagonistic activity at adrenergic, histaminergic, serotoninergic and dopaminergic receptors. Overall, the drug is well tolerated and has low susceptibility to induce extrapyramidal symptoms. The common adverse events of risperidone reported by Janssen Pharmaceutica in the year 2008 are described in Table 2.3.

Table 2.3. Adverse Reactions in ≥ 1% of risperidone-treated adult patients with schizophrenia in double-blind, placebo-controlled trials (Janssen, 2008)

	Percentage of patients reporting event		
Adverse Reaction	**2-8 mg per day (N=366)**	**>8-16 mg per day (N=198)**	**Placebo (N=225)**
Body as a whole - general disorders			
Back pain	3	2	<1
Fatigue	3	1	0
Chest pain	3	1	2
Fever	2	1	1
Asthenia	1	1	<1
Syncope	<1	1	<1
Edema	<1	1	0
Cardiovascular disorders, general			
Hypotension postural	2	<1	0
Hypotension	<1	1	0
Central and peripheral nervous system disorders			
Parkinsonism*	12	17	6
Dizziness	10	4	2
Dystonia*	5	5	2
Akathisia*	5	5	2
Dyskinesia	1	1	<1
Gastrointestinal system disorders			
Dyspepsia	10	7	6
Nausea	9	4	4
Constipation	8	9	7
Abdominal pain	4	3	0
Mouth dry	4	<1	<1

* Parkinsonism includes extrapyramidal disorder, hypokinesia and bradykinesia. Dystonia includes dystonia, hypertonia, oculogyric crisis, muscle contractions involuntary, tetany, laryngismus, tongue paralysis, and torticollis. Akathisia includes hyperkinesia and akathisia.

Table 2.3. Adverse Reactions in ≥ 1% of risperidone-treated adult patients with schizophrenia in double-blind, placebo-controlled trials (Continued)

Adverse Reaction	Percentage of patients reporting event		
	2-8 mg per day (N=366)	>8-16 mg per day (N=198)	Placebo (N=225)
Gastrointestinal system disorders			
Saliva increased	3	1	<1
Diarrhea	2	<1	1
Hearing and vestibular disorders			
Earache	1	1	0
Heart rate and rhythm disorders			
Tachycardia	2	5	0
Arrhythmia	0	1	0
Metabolic and nutritional disorders			
Weight increase	1	<1	0
Creatine phosphokinase increased	<1	2	<1
Musculoskeletal system disorders			
Arthralgia	2	3	<1
Myalgia	1	0	0
Platelet, bleeding and clotting disorders			
Epistaxis	<1	2	0
Psychiatric disorders			
Anxiety	16	12	11
Somnolence	14	5	4
Anorexia	2	0	<1

Table 2.3. Adverse Reactions in ≥ 1% of risperidone-treated adult patients with schizophrenia in double-blind, placebo-controlled trials (Continued)

Adverse Reaction	Percentage of patients reporting event		
	2-8 mg per day (N=366)	>8-16 mg per day (N=198)	Placebo (N=225)
Red blood cell disorders			
Anemia	<1	1	0
Reproductive disorders, male			
Ejaculation failure	<1	1	0
Respiratory system disorders			
Rhinitis	7	11	6
Coughing	3	3	3
Upper respiratory tract infection	2	3	<1
Dyspnea	2	2	0

Extrapyramidal effects: Extrapyramidal symptoms are believed to the blockade of dopaminergic receptor in motor-control area in brain. Extrapyramidal symptoms is dose-depending side effect and the severity is linearly related to given dose over the range 1 to 16; at the range of 4 to 8 mg/day the severity of symptoms is comparable to that with placebo and haloperidol. Incidence of extrapyramidal event in patients treated with risperidone is less than patients treated with haloperidol. Compare with placebo risperidone is tiny more induce extrapyramidal (Chouinard *et al*, 1993; Marder *et al*., 1994).

Hypotension: Risperidone is potent α-adrenergic antagonist. In healthy volunteers found poor tolerance to hypotensive effect of risperidone. The dose-proportional decrease in blood pressure with reflex tachycardia was observed in schizophrenia patients receiving risperidone 2 – 16 mg daily (Marder *et al*, 1994). Patients with schizophrenia frequency exhibit postural hypotension on the long term therapy and appear more tolerating. Titration of dosing is the strategy to minimize the risk of clinically hypotension, dizziness and tachycardia.

Monitoring of orthostatic vital signs should be considered in patients. A dose reduction should be considered if hypotension occurs (Silver *et al*, 1990).

Hyperprolactinemia: At therapeutic dose of risperidone, the increasing of plasma prolactin has been studied. After single oral (≤ 4 mg) or intramuscular (≤ 1 mg) dose administration of risperidone in healthy volunteers, the plasma prolactin increased 5- to 10- fold within 1 to 4 hours before dropping to baseline within 24 hours (Huang *et al*, 1993).

Weight gain: Atypical antipsychotic medications may have an affinity for dopamine, serotonin, and histamine receptors that could lead to increased eating and weight gain (Richelson and Souder, 2000). Risperidone, have been associated with significant weight gain. Martin and L'Ecuyer (2002) investigated the changes of triglyceride, cholesterol and weight in 22 risperidone-treated in-patients with age range of 8.6 – 15.8 years old. The results showed that average (± SD) weight change is 7.0 (±4.7) kg and the triglyceride and cholesterol level in plasma did not changed. Bobes' *et al* (2002) conducted a retrospective of 636 patients who received monotherapy of risperidone or other antipsychotics drugs at least 4 weeks. 232 patients were treated with risperidone 1-15 mg per day. The results presented that significant change of body weight was found in 54% of patients.

2.8 Analytical method

The early methods for the quantification of risperidone (RIS) and 9-hydroxyrisperidone (9OHRIS) mostly used high performance liquid chromatography (HPLC) with ultraviolet (UV) or electrochemical detection (Aravagiri *et al*., 1998; Balant-Gorgia *et al*., 1999). Because of their high lower limit of quantitation (LLOQ), these methods are suitable for therapeutic drug monitoring of target concentrations in the range of 2-30 ng/mL for RIS and 9OHRIS, but not suitable for pharmacokinetic studies. In recent years, several LC-MS-MS methods have been developed and all have much improved sensitivity and specificity. The published papers for LC-MS-MS method are reviewed and presented in Table 2.4. These LC-MS methods can simultaneously determined RIS and 9OHRIS in human plasma with the LLOQ of less than 0.5 ng/mL.

Table 2.4 Review of LC-MS methods for the determination of RIS and 9OHRIS in human plasma

Instrumental Model	Sample volume (mL)	Sample Preparation	Analytical Column	Mobile Phase	Detection Mode	Reference
Triple quadrupole	0.5	Bond Elut Certify solid phase extraction column (Varian, Walnut Creek, USA)	3-µm, C-18 BDS-Hypersil column (100 x 4.6 mm I.D.) (Alltech, Deerfield, USA)	Step gradient elution 0.01 M ammonium formate pH 4.0 : Acetonitrile 67 :33 for 3.2 min, 15:85 to 4.7 min, 67:33 until 7.5 min	MRM	Remmerie *et al*, 2003
Triple quadrupole	0.025	Protein precipitate with acetonitrile	Zorbax SB C18 12.5 × 4.6 mm, 5 µm and Zorbax SB C18, 30 × 2.1 mm, 3.5 µm (Wilmington, DE, USA)	Analytical mobile phase: 10 mM ammonium acetate : acetonitrile (20:80)	MRM	Flarakos *et al*, 2004

Table 2.4 Review of LC-MS methods for the determination of RIS and 9OHRIS in human plasma (Continued)

Instrumental Model	Sample volume (mL)	Sample Preparation	Analytical Column	Mobile Phase	Detection Mode	Reference
Single quadrupole	0.5	Liquid-liquid extraction with Methy-*tert*-butyl ether (MTBE)	5 µm, C-18 Zorbax SB-column (150 x 2.1 mm I.D.) (Wilmington, DE, USA)	Step gradient elution 0.01 M ammonium acetate pH 4.0 : Acetonitrile 65 :35 to 26:74 within 5 min 26:74 for 4 min; 65:35 for 6 min.	SIM (LC-MS)	Zhang *et al* , 2005
Triple quadrupole	0.1	Protein precipitate with acetonitrile	3-µm, C-18 Betasil (100x3mm I.D.) (Thermo Electron Corporation, UK)	0.01 M ammonium acetate pH 4.6 : Acetonitrile (65 :35)	SRM	Bhatt *et al*, 2006
Triple quadrupole	0.3	Centrifugation at 15000 g and inject the supernatant	3-µm, C-18 Alltima-C18 column (100 x 2.1 mm I.D.) (Alltima HP, Germany)	0.1% formic acid:acetonitrile (40:60)	SRM	Huang *et al*, 2007

Table 2.4 Review of LC-MS methods for the determination of RIS and 9OHRIS in human plasma (Continued)

Instrumental Model	Sample volume (mL)	Sample Preparation	Analytical Column	Mobile Phase (v/v)	Detection Mode	Reference
Triple quadrupole	0.2	Liquid-liquid extraction with MTBE	50 x 4.6 mm I.D. Chiralcel OJ column (Chiral Technologies Inc.,West Chester, PA)	Gradient elution of 0.01 M ammonium acetate in ethanol/propanol mixture (50:50) : hexane 20:80 to 90:10 within 4.5 min. 90:10 for 2 min. 20:80 for 1.5 min.	MRM	Cabovska *et al*, 2007

Table 2.4 Review of LC-MS methods for the determination of RIS and 9OHRIS in human plasma (Continued)

Instrumental Model	Sample volume (mL)	Sample Preparation	Analytical Column	Mobile Phase (v/v)	Detection Mode	Reference
Triple quadrupole	0.3	Protein precipitate with acetonitrile	Oasis HLB extraction columns (S-25 µm, 20 × 2.1 mm i.d.) (Waters, Milford, USA) and Cyano (CN) analytical column (S-5 µm, 50 × 4.0 mm i.d.) (YMC, Schermbeck, Germany)	Analytical mobile phase: 0.01 M ammonium acetate pH 4.0 : Acetonitrile (25 :75, v/v)	MRM	Kousoulos *et al*, 2007

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemical Standards

Working standard risperidone (RIS) was purchased from Synfine Research Inc., Canada, Lot. No. A-1208-102, Potency 99.8%. Working standard 9-hydroxyrisperidone (9OHRIS) also purchased from Synfine Research Inc., Canada, Lot. No. S-1203-194A1, Potency 98.5%. Carbamazepine (CBZ, used as internal standard, Karindo, Italy, Lot. No. CB-14/06, Potency 99.92%) received from Regional Medical Sciences Center Songkhla, Thailand.

3.1.2 Chemicals and reagents

Acetonitrile (HPLC grade) and Diethyl ether were purchased from Labscan Co., Ltd., Thailand. Sodium hydroxide and acetic acid were obtained from Merck Co., Ltd. Analytical grade methanol was purchased from J.T. Baker, United States of America. Ammonium acetate was purchased from Ajax Finechem, Australia.

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3.1.3 Equipments

Liquid chromatography tandem mass spectrometer consisted of Agilent 1200 series of pump, autosampler and column oven (Agilent Technologies, UK). Ion-trap mass spectrometer (LC-MSD trap XCT, Agilent Technologies, UK) was connected to liquid chromatography system as a detector. The column was C-18 with particle size 5 micron and dimension of 2.1 mm x 150 mm (Alltima HP, Germany). A guard-pak precolumn module was used to reduce column degeneration.

3.1.4 Computerized software

WinNonlin® version 1.1 (Pharsight, USA) was used to compute pharmacokinetic parameters from plasma concentration-time curve of RIS and 9OHRIS.

3.2 Method

Protocol for in vivo bioequivalence study of 2-mg risperidone tablet was written in accordance with the Guideline for the Bioequivalence Study of Generic Drug established by Drug Control Department, Office of Food and Drug Administration, Thailand 2007. Consideration of ethical issue was approved by the Ethic Committee of Faculty of Pharmaceutical Sciences, Prince of Songkla University.

3.2.1 Investigated Products

Two products of 2-mg risperidone film-coated tablet, Product A being a test and Product B being a reference, were tested.

Product A	:	Risperidone 2 mg film-coated tablets locally made by the manufacturer in Thailand, Mfg. date 08-2007.
Product B	:	Risperidone 2 mg film-coated tablets from innovator's manufacturer, Batch no. : 5BL000, Mfg. date: 02-2005, Expiry : 01-2008.

3.2.2 Content of Active Ingredient

3.2.2.1 Chromatographic system

The liquid chromatograph is equipped with a 278 nm detector and a C-18 column (4.6 x 150 mm, 5 μm). The flow rate is 1.0 mL/min. An aliquot of 20 μL of standard preparation and samples were separately injected into the chromatograph. The mobile phase was acetonitrile and 50 mM ammonium acetate buffer pH 5.0 (30:70). The %labeled amount of risperidone was calculated from the standard calibration curve.

3.2.2.2 Standard calibration curve of risperidone

The stock solution of risperidone in methanol was prepared at the concentration of 0.1 mg/mL. The stock solution was then diluted with mobile phase to produce a set of standard solutions at concentration of 10, 20, 30, 40 and 50 μg/mL. The standard calibration curve was constructed by plotting peak area of risperidone versus concentration and analyzed using linear regression analysis.

3.2.2.3 Sample preparation

Ten tablets was transferred to a 200-mL volumetric flask, 100 mL of mobile phase was added and mechanically shaken for 10 minutes and sonicated for 10 minutes, then adjusted with mobile phase to the volume. The mixture was centrifuged for 10 minutes. 2.0 mL of clear supernatant was accurately pipetted to a 10-mL volumetric flask and then adjusted to volume with mobile phase.

3.2.3 Uniformity of dosage units

Ten film-coated tablets were individually assayed for the percent labeled amount of risperidone by the same method as described for the analysis of the content of active ingredient. A tablet was placed in a 100-mL volumetric flask and 50 mL of mobile phase was added. The mixture was shaken for 10 minutes and sonicated for 10 minutes. The mixture was centrifuged for 10 minutes. The clear supernatant was injected to HPLC system. The mean and standard deviation of percent labeled amount were calculated. The criteria of uniformity of dosage units established by the United State Pharmacopeia 28 (USP 28) were used.

3.2.4 Dissolution test

The dissolution test was conducted using the USP dissolution apparatus II. A tablet of risperidone was placed in each vessel containing 900 mL of dissolution medium at $37 \pm 0.5^{\circ}C$. The apparatus was operated at 50 rpm. Five milliliters of each sample was sampling at 5, 10, 15, 20, 30, 45 and 60 minutes. The dissolution medium (5 mL) was added immediately after each sampling to maintain the constant volume of dissolution medium. Twelve tablets of each product were tested in three dissolution media, i.e., 0.1 N HCl, acetate buffer pH 4.5 and phosphate buffer pH 6.8. The amount of dissolved risperidone was quantitated using HPLC according to the method described below. The dissolution profiles were constructed by plotting percent risperidone dissolved versus time for all dissolution media.

HPLC method for determining risperidone in dissolution medium

The liquid chromatograph was equipped with a 278 nm detector and a C-18 column (4.6 x 150 mm, 5 μm). The flow rate was 1.0 mL/min. An aliquot of 20 μL of standard preparation and dissolution samples were separately injected into the chromatograph. The concentrations of risperidone dissolved were calculated from the standard calibration curve.

3.2.4.1 Standard calibration curve of risperidone

The stock solution of risperidone in methanol was prepared at the concentration of 0.1 mg/mL. The stock solution was then diluted with mobile phase to produce a set of standard solutions at concentration of 0.5, 1, 2, 3 and 5μg/mL. The standard calibration curve was constructed by plotting peak area of risperidone versus concentration and analyzed using linear regression analysis.

3.2.4.3 Dissolution profiles comparison

The dissolution profiles of test product and reference product were compared using difference factor (f_1) and similarity factor (f_2) (Thailand FDA, 2000). They were calculated using the following equation:

$$f_1 = \frac{\left(\sum_{t=1}^{n} |R_t - T_t|\right) \times 100}{\sum_{t=1}^{n} R_t}$$

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

Where; n = Number of sampling points

R_t = Percent dissolved of reference product at time t

T_t = Percent dissolved of test product at time t

Acceptance criteria

The difference factor (f_1) should be within 0 – 15 and the similarity factor (f_2) should be within 50 – 100 (Thailand FDA, 2005).

3.2.5 Number of subjects

The number of subjects required for a study should be statistically significant. The sample size for each sequence group is determined from the following equation.

$$n \geq [t_{\alpha,2n-2} + t_{\beta,2n-2}]^2.[CV/(V-\delta)]^2$$

n	=	number of subjects per sequence
t	=	the appropriate value from the *t* distribution
α	=	the significant level (usually 0.10)
$1-\beta$	=	the power (usually 0.8)
CV	=	coefficient of variation
V	=	bioequivalence limit
δ	=	difference between product

According to the current USFDA guidelines (USFDA, 2001), V is usually set to be $\pm$ 20% of the average relative bioavailability in the bioequivalence studies. CV is the coefficient of variation, which is the intra-subject variability expressed as percentage of the average relative bioavailability. The intra-subject variations were derived from the 90% confidence intervals of $AUC_{0-\infty}$ and C_{max} data of the active moiety (RIS + 9OHRIS) from the study of van Schaick et al (2003), which were 16% and 17%, respectively. However, in this study, the higher value of intra-subject variation, 20%, was used to calculate the number of subjects. The following illustrates the computation of sample size to achieve an 80% power at the 5% nominal level of δ. With the initial guess of n = 12, the sample size for each sequence group is approximately:

$$n \geq [t_{\alpha,2n-2} + t_{\beta,2n-2}]^2 [CV/(V - \delta)]^2$$

$$n \geq [1.72 + 0.86]^2 [0.2/(0.2 - 0.05)]^2$$

$$n \geq 11.8$$

Thus n = 12 is required for sample size per sequence, a total of 2 × 12 = 24 subjects would be required in this study.

3.2.6 Subjects

Twenty four healthy Thai male volunteers with ages range from 18 to 45 years participated in the study. Information of this study was explained to all volunteers by the investigator. Before the physical examination and clinical laboratory testing, all volunteers were screened for use of medication, cigarettes, caffeine and alcohol preparation. All subjects were willing to sign the informed consent.

3.2.7 Subject Selection Criteria

Inclusion criteria:

- Healthy Thai male volunteers with the ages range from 18 to 45 years old and body mass index between 18 to 25 kg/m^2.
- Have normal physical and laboratory biochemical test.
- Willing to sign informed consent.

Exclusion criteria:

- Known to be allergic or have adverse drug reaction to risperidone or other inactive ingredients of risperidone products.
- Habit smoker or history of routine smoking within 1 year before study.
- Have history of seizure and allergic disease.
- Have physiological conditions that affect bioavailability, e.g., gastrointestinal tract disease, hepatic disease, renal disease, hypertension, and heart disease as described below:

  Gastrointestinal disease: Signs and symptoms that interfere with drug absorption.

  Hepatic disease: Raised hepatic enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), more than two times of the upper limit of normal range.

  Renal disease: Serum creatinine more than 2.0 mg/dL.

  Hypertension: Sitting diastolic blood pressure more than 95 mmHg.

  Heart disease: History of congestive heart failure, pericarditis, aortic stenosis, myocardial infarction, angina pectoris, cardiac arrhythmia or other heart disease.

3.2.8 Dose and Drug Administration

A single dose, one of 2-mg risperidone tablet, was given orally with 240 mL of water after at least 10 hours of overnight fasting. No food or drink was permitted until 4 hours after dosing. The volunteers were asked to take no medication, alcohol preparation for at least two weeks prior to the study and during the experimental period. Caffeine containing food and beverage were abstained at least three days before the drug administration of each period and during blood sample collection.

3.2.9 Subject Monitoring

Blood pressure, pulse rate and body temperature were monitored every 2 hours after administration. Subjects were asked for unusual symptoms periodically. Any occurred serious symptoms were immediately managed by the physician and recorded in the case report form.

3.2.10 Experimental Design

The experiment was conducted as randomized double-blind, two-way crossover design. Each subject received the drug in a randomized order with a 2-week washout period between each administration as shown in Table 3.1.

3.2.11 Sample Collection

Each 5 mL of blood samples were drawn from forearm vein at the time interval: pre-dose, 0.25, 0.5, .075, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72 and 96 hours after administration. Within 30 minutes after collection, blood samples were centrifuged at 3,000 rpm for 10 minutes under the temperature of 4 $^{\circ}$C. Collected plasma samples were stored at -20 $^{\circ}$C until analysis.

Table 3.1 Drug administration schedule.

Sequence	Subject No.	Period	
		1	2
1	SR01	A	B
	SR03	A	B
	SR05	A	B
	SR07	A	B
	SR09	A	B
	SR11	A	B
	SR13	A	B
	SR15	A	B
	SR17	A	B
	SR19	A	B
	SR21	A	B
	SR23	A	B
2	SR02	B	A
	SR04	B	A
	SR06	B	A
	SR08	B	A
	SR10	B	A
	SR12	B	A
	SR14	B	A
	SR16	B	A
	SR18	B	A
	SR20	B	A
	SR22	B	A
	SR24	B	A

3.2.12 Analysis of RIS and 9OHRIS in plasma

A LC-MS/MS method was developed and validated to determine RIS and 9OHRIS simultaneously, as described below.

3.2.12.1 Selection of extracting solvent

20 μL of internal standard solution (5,000 ng/mL carbamazepine in methanol) was added to 1 mL of plasma sample spiked with RIS and 9OHRIS at the concentration of 5 ng/mL. Ethyl acetate, ether, mixture of ether and isopropanol (90:10, v/v), mixture of ether and isopropanol (80:20, v/v), and methyl *tert*-butyl ether (MTBE) were chosen based on their polarity for the extraction of both RIS and 9OHRIS from untreated plasma. Furthermore, sample was treated with 0.1 N NaOH and 20 N NaOH before extracted with ether and MTBE. 6 mL of each extracting solvent was immediately added into plasma sample and reciprocally shaken for 2 minutes. The mixture was centrifuged at 6000 rpm for 10 minutes at 0°C. Organic phase was transferred into another glass tube and allowed to evaporate at room temperature. The residue was reconstituted with 150 μL of mobile phase and an aliquot of 40 μL was injected into LC-MS/MS system.

3.2.12.2 Sample preparation

From the results of extracting solvent selection, the sample was processed as follows. 20 μL of internal standard solution (5,000 ng/mL carbamazepine in methanol) was added to 1 mL of plasma sample. Then 150 μL of 20 N NaOH was added and vortex-mixed for 10 seconds. Ether (6 mL) was immediately added and reciprocally shaken for 2 minutes. The mixture was centrifuge at 6000 rpm for 10 minutes at 0°C. The ether phase was transferred into another glass tube and allowed to evaporate at room temperature. The residue was reconstituted with 150 μL of mobile phase and an aliquot of 40 μL was injected into LC-MS/MS system.

3.2.12.3 Chromatographic Conditions

Column : Alltima HP (dimethyloctadecylsilyl bonded-silica, C-18, particle size 5 μm), stainless steel column (2.1 x 150 mm)

Mobile phase : 50 mM ammonium acetate buffer pH 5.5 : Acetonitrile. (65 : 35)

Flow rate : 0.3 mL/min.

Temperature : 30 °C

3.2.12.4 Mass spectrometric detection

The electrospray ion trap mass spectrometer operated in the positive ionization mode. The tuning parameters for RIS, 9OHRIS and CBZ were optimized by infusing a mixture of standard RIS, 9OHRIS and CBZ each at 100 ng/mL in mobile phase at 20 μL/min. The following detection parameters were used: drying gas flow rate 10 L/min; nebulizer pressure 45 psi; and drying gas temperature 325 °C. Capillary energy was set at 4000 volts. All studied compounds were completely resolved with isocratic mobile phase before being introduced to electrospray ionization chamber. Transitions of positive ions were detected in multiple reaction monitoring (MRM) mode as listed in Table 3.2. Mass spectrometric detections were separated into three segments as shown in Table 3.3.

Table 3.2 *m/z* transition of RIS, 9OHRIS and CBZ

	Precursor ion (*m/z*)	Product ion (*m/z*)
RIS	411.4	191.4
9OHRIS	427.4	207.4
CBZ (Internal Standard)	237.3	194.0

Table 3.3 Detection of *m/z* transitions in MRM mode.

Run time (minute)	Detected *m/z* transition
~0 – 4.10	427.4 → 207.4
~4.11-6.50	411.4 → 191.4
~6.51-8.50	237.3 → 194.0

3.2.12.5 Calibration samples

Known quantitative amount of standard RIS and 9OHRIS were added to the pooled drug-free plasma to produce a mixture of RIS and 9OHRIS in series of concentration at 0.1, 0.5, 1, 5, 10, 20 and 30 ng/mL for RIS and 0.3, 0.5, 1, 5, 10, 20 and 30 ng/mL for 9OHRIS. All calibration samples were analyzed by the same procedure as described earlier. The peak area ratios of RIS or 9OHRIS to CBZ (y) versus known concentration (x) were fitted to straight line using weighted least square linear regression analysis with $1/x^2$ as a weighting factor.

3.2.12.6 Quality control samples

Quality control samples (QCs) are samples with known concentration prepared by spiking drug-free biological fluid with analyzed-drug. Known amount of standard RIS and 9OHRIS were added to the pooled drug-free plasma to produce a concentration of 0.5, 5 and 20 ng/mL for both RIS and 9OHRIS. The estimated concentrations of RIS and 9OHRIS in quality control samples were calculated using the respective peak area ratios.

3.2.13 Bioanalytical method validation

The developed LC-MS-MS method for the simultaneous determination of RIS and 9OHRIS in human plasma was validated according to the USFDA guidelines for bioanalytical method validation, USFDA, 2001 as described below:

3.2.13.1 Specificity

Blank human plasma samples from six different sources were analyzed according to the developed method. The results from blank samples should demonstrate that the peaks of interest were not interfered by endogenous compounds, degradation products, or other drugs.

3.2.13.2 Limit of quantification (LOQ)

The lowest concentration of calibration samples were analyzed in six replicates. The reliable LOQ should be established based on the signal to noise ratio of greater than 10 with acceptable accuracy (within 85 – 115%) and precision (%RSD <15%).

3.2.13.3 Range and Linearity

The quantitative relationship between concentration and response should be adequately characterized over the entire range of expected sample concentrations. Seven concentrations of calibration samples of the mixture of RIS and 9OHRIS at 0.1, 0.5, 1, 5, 10, 20, 30 ng/mL and 0.3, 0.5, 1, 5, 10, 20, 30 ng/mL, respectively were analyzed. The linear relationship between the peak area ratios of RIS and 9OHRIS to CBZ versus concentrations were calculated using regression analysis. The regression parameters for the slope and intercept were calculated by weighted least square linear regression using $1/x^2$ as a weighting factor (Bolton, 1997). The coefficient of determination (r^2) should be more than 0.99. The 15% deviation of the concentrations of RIS and 9OHRIS from the nominal concentrations should be met.

3.2.13.4 Accuracy

Accuracy is the degree to which the true value of the concentration of drug is estimated by the assay. The QC samples at three concentrations of 0.5, 5 and 20 ng/mL of RIS and 9OHRIS were analyzed in five replicates. The %accuracy was calculated as the percentage of the ratio between the estimated concentration using standard calibration curve and the theoretical concentration. The mean value of both compounds should be within the range of 85.0 % - 115.0%.

$$Accuracy\ \ (\%) = \frac{estimated\ concentration}{theoretical\ concentration} \times 100$$

3.2.13.5 Within-run precision

Five replicate determinations of QC samples containing RIS and 9OHRIS at three concentrations (0.5, 5 and 20 ng/mL) were analyzed within the same analytical run. The percentage of relative standard deviation (%RSD) of the estimated concentrations from five replications was calculated. Within-run precision expressed as %RSD should be not more than 15%.

3.2.13.6 Between-run precision

Five replicate determinations of QC samples containing RIS and 9OHRIS at three concentrations (0.5, 5 and 20 ng/mL) were analyzed on five different runs. The %RSD of the estimated concentrations from five replications was calculated. Between-run precision expressed as %RSD should be not more than 15%.

3.2.13.7 Recovery of extraction

The QC samples at low, medium and high concentrations (0.5, 5 and 20 ng/mL) were extracted and analyzed in five replicates. Recovery of extraction was calculated as the percentage of the ratios between the respective peak area from extracted QC sample and the peak area from un-extracted standard solution at the same concentration.

$$Extraction\ Recovery\ (\%) = \frac{Peak\ area\ of\ analyte\ from\ QC\ sample}{Peak\ area\ of\ analyte\ from\ un\text{-}extracted\ standard\ solution} \times 100$$

3.2.13.8 Freeze-thaw stability

The QC samples at low and high concentrations (0.5 and 20 ng/mL) were stored at -20°C for 24 hours and thawed unassisted at room temperature. One freeze-thaw cycle is defined as the sample being undergone freeze and thaw once. The thawed samples were refrozen for 12-24 hours under the same conditions for the next freeze-thaw cycle. After completing one, two and three freeze-thaw cycles, samples were analyzed in three replicates. The %deviation of the mean estimated concentrations of RIS and 9OHRIS in QC sample from that in freshly prepared sample should be within ±15%.

$$\%Deviation = \frac{Estimated\ concentration\ of\ QCsample - Estimated\ concentration\ of\ freshly\ prepared\ sample}{Estimated\ concentration\ of\ freshly\ prepared\ sample} \times 100$$

3.2.13.9 Short-term stability

QC samples at low and high concentrations (0.5 and 20 ng/mL) were prepared and stored at -20°C. After that, the samples were thawed unassisted at room temperature and analyzed after being kept at 0, 6, 10 and 24 hours at room temperature. The %deviation of the mean estimated concentration from the zero time should be within ± 15%.

3.2.13.10 Long-term stability

QC samples at low and high concentrations (0.5 and 20 ng/mL) were prepared and stored at -20°C for 0, 1, 2, 3 and 9 months. After that, the samples were thawed at room temperature and analyzed by the developed method. The %deviation of the mean estimated concentration of the QC sample from the freshly prepared sample should be within ± 15%.

3.2.13.11 Autosampler stability

In order to establish the autosampler stability of RIS and 9OHRIS in processed sample, three aliquots of processed QC sample at low (0.5 ng/ml), and high (20 ng/ml) were stored in autosampler at ambient temperature for 0, 12, 24, 48 and 72 h. Thereafter, samples were analyzed and concentrations were compared with the concentrations at zero hour. The processed sample is stable in autosampler if the deviation was within ± 15%.

3.2.13.12 Stock solution stability

RIS, 9OHRIS and CBZ were prepared by dissolving suitable amount of each pure standard in methanol and kept at room temperature for, 6, 14, and 24 h. After that they were stored at -20°C for 15 days, 1, 2, and 3 months. Stock solutions were diluted with mobile phase to the concentration of 10 µg/mL before being analysed and the concentrations were compared to that of freshly prepared solutions of the same concentration. The stock solution is stable if the deviation was within ± 2%.

The %deviation of short-term, long-term, autosampler and stock solution stability were calculated using the following equation.

$$\%Deviation = \frac{Estimated\ concentration\ at\ each\ time\ point - Estimated\ concentration\ of\ freshly\ prepared\ sample}{Estimated\ concentration\ of\ freshly\ prepared\ sample} \times 100$$

3.2.14 Statistical analysis for the evaluation of bioequivalence

The pharmacokinetic parameters, C_{max}, T_{max} and AUC were determined using WinNonlin version 1.1 on noncompartmental model. AUC from 0 to time t (AUC_{0-t}) was calculated using the linear trapezoidal method by the following equation.

$$AUC\Big|_{t_1}^{t_2} = \Delta t \times \frac{C_1 + C_2}{2}$$

where Δt is $t_2 - t_1$

C_1 is the concentration at time t_1

C_2 is the concentration at time t_2

AUC from time 0 to infinity ($AUC_{0-\infty}$) was calculated as the sum of AUC_{0-t} and the ratio of the last quantificable concentration over the elimination rate constant (C_{last}/K_e). K_e was obtained from the slope of the terminal log-linear phase of the semilog plot of concentration versus time. The $t_{1/2}$ was calculated as ln2/ K_e.

3.2.14.1 ANOVA

The pharmacokinetic parameters of interest (AUC, C_{max}) derived from the plasma concentration-time curve after natural logarithmically transformed were subjected to ANOVA in which subject, period, sequence and treatment variances were estimated. ANOVA were calculated manually with Microsoft Excel software (Microsoft®, USA) using the following equations (Bolton, 1997).

$$Sequence\ \ sum\ \ squares = \frac{(\sum Sequence\ \ 1)^2}{N_1} + \frac{(\sum Sequence\ \ 2)^2}{N_2} - C.T.$$

$$Subject\ \ sum\ \ squares = \frac{\sum\left(\sum S_i\right)^2}{2} - (C.T.)_1 - (C.T.)_2$$

$$Correction\ term\ \ 1\ \ \left(C.T.\right)_1 = \frac{(\sum Sequence\ \ 1)^2}{N_1}$$

$$Correction\ term\ \ 2\ \ \left(C.T.\right)_2 = \frac{(\sum Sequence\ \ 2)^2}{N_2}$$

$$Period\ \ sum\ \ squares = \frac{(\sum Period\ \ 1)^2}{N_1} + \frac{(\sum Period\ \ 2)^2}{N_2} - C.T.$$

$$Treatment\ \ sum\ \ squares = \frac{(\sum Pr oduct\ \ A)^2}{N_1} + \frac{(\sum Pr oduct\ \ B)^2}{N_2} - C.T.$$

$$Correction\ term\ \ (C.T.) = \frac{(\sum X_t)^2}{N_t}$$

$$Total\ \ sum\ \ squares = \sum X_T^2 - C.T.$$

Error sum of squares = Total SS – Sequence SS – Subject SS – Period SS –Treatment SS

Where $\sum X_i$ is the sum of all observations.

$\sum$ *Sequence 1* is the sum of observation for sequence 1.

$\sum$ *Sequence* 2 is the sum of observation for sequence 2.

$\sum S_i$ is the sum of observation for subject i.

$\sum$ *Period 1* is the sum of observation for period 1.

$\sum$ *Period* 2 is the sum of observation for period 2.

$\sum$ *Product* A is the sum of observation for Product A.

$\sum$ *Product* B is the sum of observation for Product B.

$\sum X^2_T$ is the sum of the squared observations

$C.T._1$ is the correction terms for sequence 1

$C.T._2$ is the correction terms for sequence 2

3.2.14.2 90% Confidence interval

The 90% confidence interval for the ratio of the geometric mean (Test/Reference) of natural log-transformed (ln) parameters, at the 10% significance level, was considered for the establishment of bioequivalence using the following equation (Bolton, 1997).

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

Where; X_T and X_R = Geometric mean of ln AUC and ln C_{max} values of the test and reference, 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 = $[e^{(X_T - X_R)-(t_{0.1,df} \times S.E.)}] \times 100$

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

3.2.15 Criteria for bioequivalence

To establish bioequivalence, the calculated 90% confidence interval for AUC and C_{max} of RIS and 9OHRIS should fall within the range of 80 – 125%.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Assay and Content Uniformity

The results of assay and content uniformity were presented in Table 4.1. The %labeled amount of risperidone of Product A and Product B were 98.9% and 98.5%, respectively. The %labeled amount of the individual 10 tablets was within 85.0 – 115.0% with the %RSD of less than 6.0%. According to USP 28, both products were conformed to the requirement of Uniformity of Dosage Units.

Table 4.1 Summary of the assay and content uniformity of Product A and Product B

Product	Assay		Content Uniformity	
	%labeled amount (mean±SD)	%RSD	%labeled amount (mean±SD)	%RSD
A	98.9±0.16	0.16	98.1±2.12	2.12
B	98.5±0.30	0.30	99.4±0.59	0.59

4.2 Dissolution testing

The dissolution profiles of Product A and Product B in three dissolution media were illustrated in Figure 4.1 - 4.3. The calculated f_1 and f_2 parameters were presented in Table 4.2. The %CV of the %dissolved of 12 tablets at time 10 - 15 minutes were less than 20%, in all three dissolution media. The %CV at 20 minutes until the last sampling time in all dissolution media were less than 10%. According to the criteria of Thailand FDA guideline for bioequivalence study (Thailand FDA, 2000), the %CV of dissolution results were in acceptable value, which is, in early

sampling time (e.g. 15 minute), the %CV should be less than 20% and others should be less than 10%.

RIS is insoluble in water (Janssen, 2008) but as it is a basic compound with a pKa of about 8 (Remmerie *et al*., 2003), it would be soluble in medium with pH of lower than approximately 6.5, e.g, 0.1 N HCl (Janssen, 2008), buffer pH 4.5 and buffer pH 6.8. As a result, the dissolution of these two products in 0.1 N HCl and phosphate buffer pH 6.8 gave the f_1 and f_2 values within the acceptable range. The disintegration time might be a factor that caused the small difference in the dissolution profiles in 0.1N HCl. In phosphate buffer pH 6.8, the solubility of RIS from the formulation would be a rate limiting step.

In acetate buffer pH 4.5, however, the dissolution profiles of these two products were different with the f_1 and f_2 of 14.63 and 44.66, respectively. Although f_1 was within the acceptance value of 0 -15, the f_2 was less than the acceptance value of 50-100. From Figure 4.2, it can be seen that the dissolution rate of Product A is higher than that of Product B. This might be because of solubilizing excipient (confidential) in Product A formulation that best exhibits its solubilizing ability at a pH of approximately 4.5.

From the results of assay, uniformity of dosage units and dissolution of Product A and Product B, these two products might be considered pharmaceutically equivalent.

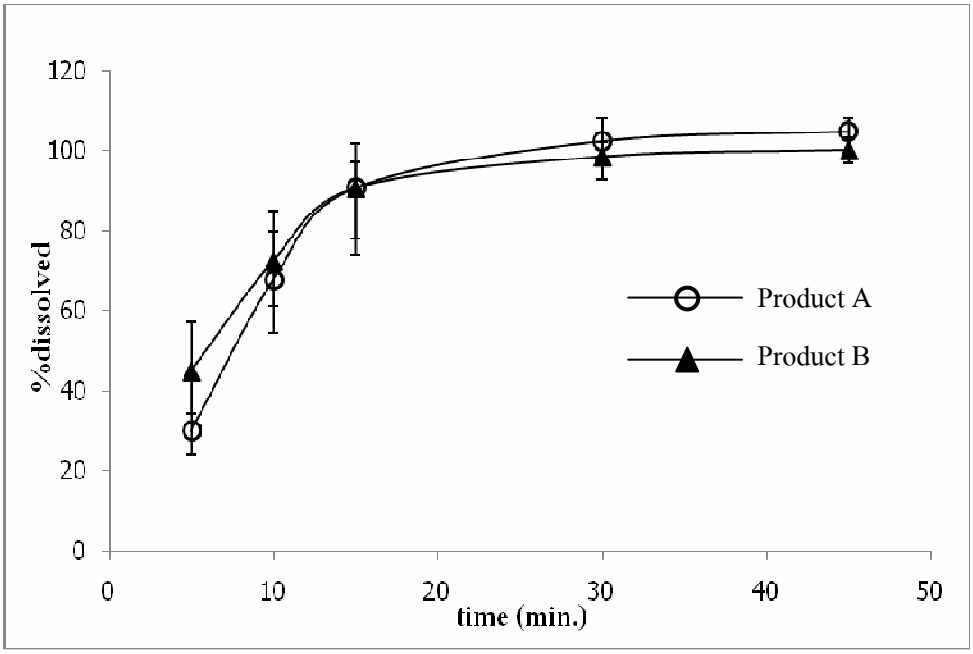


Figure 4.1. Dissolution profile of Product A and Product B in 0.1 N HCl

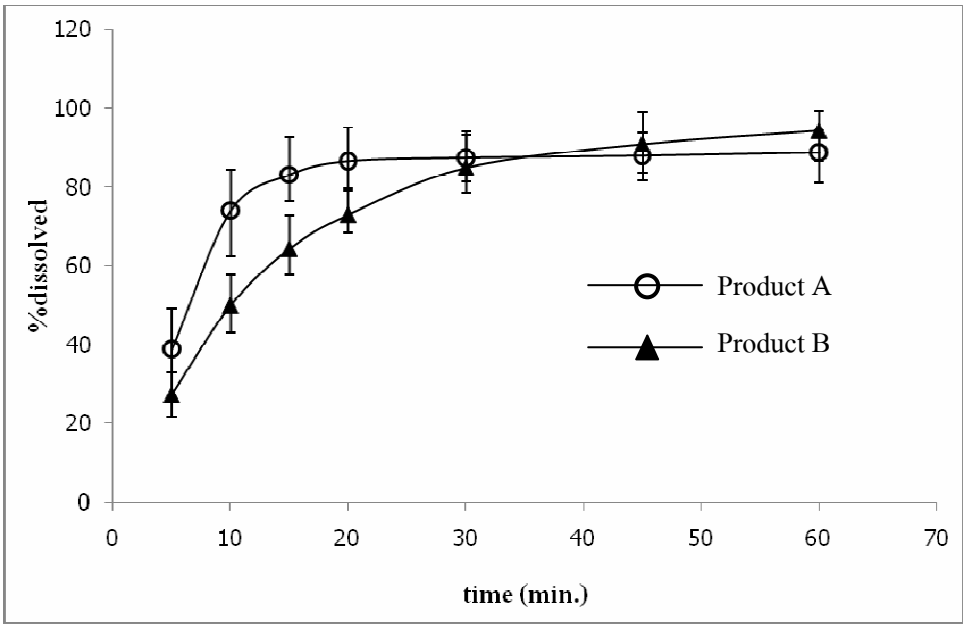


Figure 4.2 Dissolution profile of Product A and Product B in acetate buffer pH 4.5

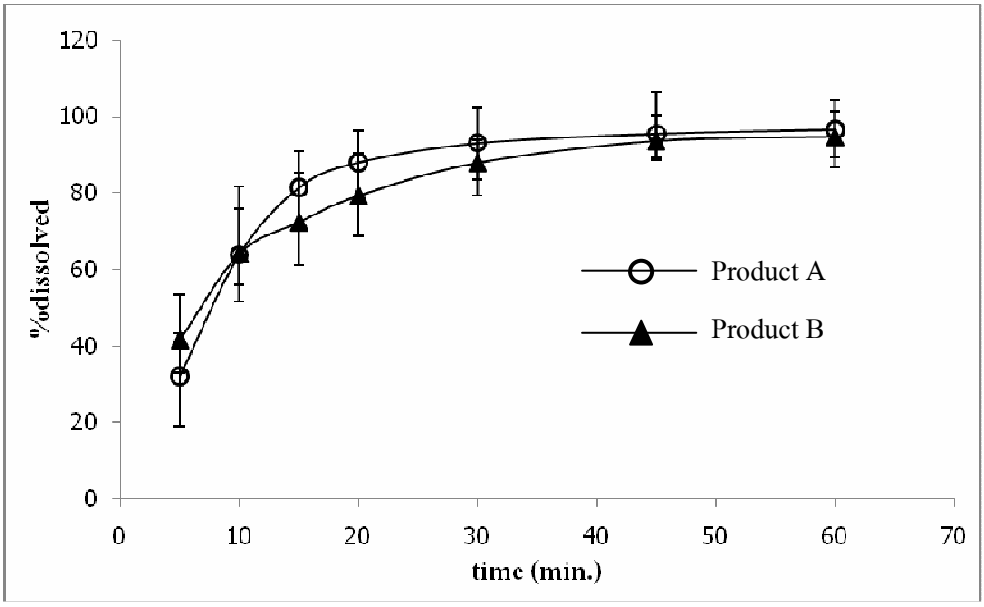


Figure 4.3 Dissolution profile of Product A and Product B in phosphate buffer pH 6.8

Table 4.2. The difference factor (f_1) and Similarity factor (f_2) of Product A and Product B in various dissolution medium

Dissolution medium	Parameter	
	f_1	f_2
0.1N HCl	7.26	56.31
acetate buffer pH 4.5	14.63	44.66
phosphate buffer pH 6.8	6.12	61.76

4.3 Bioanalytical method

4.3.1 Mass spectrometric detection

Due to the high efficiency of ionization and fragmentation of the interested compounds and the reason of specificity, the product ions were selected for their detection. Figure 4.4 showed the product ions of RIS, 9OHRIS and CBZ. With positive ion mode and electrospray ionization (ESI) conditions, RIS gave $M+H^+$ at *m/z* 411.4 as a precursor ion. This ion was selected to generate one major product ion at *m*/*z* 191.4. 9OHRIS gave $M+H^+$ at *m/z* 427.4 as a precursor ion. This ion was selected to generate one major product ion at *m*/*z* 207.4. RIS AND 9OHRIS are fragmented almost exclusively by cleavage of the ethyl-piperidinyl bond. CBZ, the internal standard, gave $M+H^+$ precursor ion at *m/z* 237.3, which was selected to generate one major product ion at *m*/*z* 194.0 from the cleavage of the $CONH_2$ group in the ring.

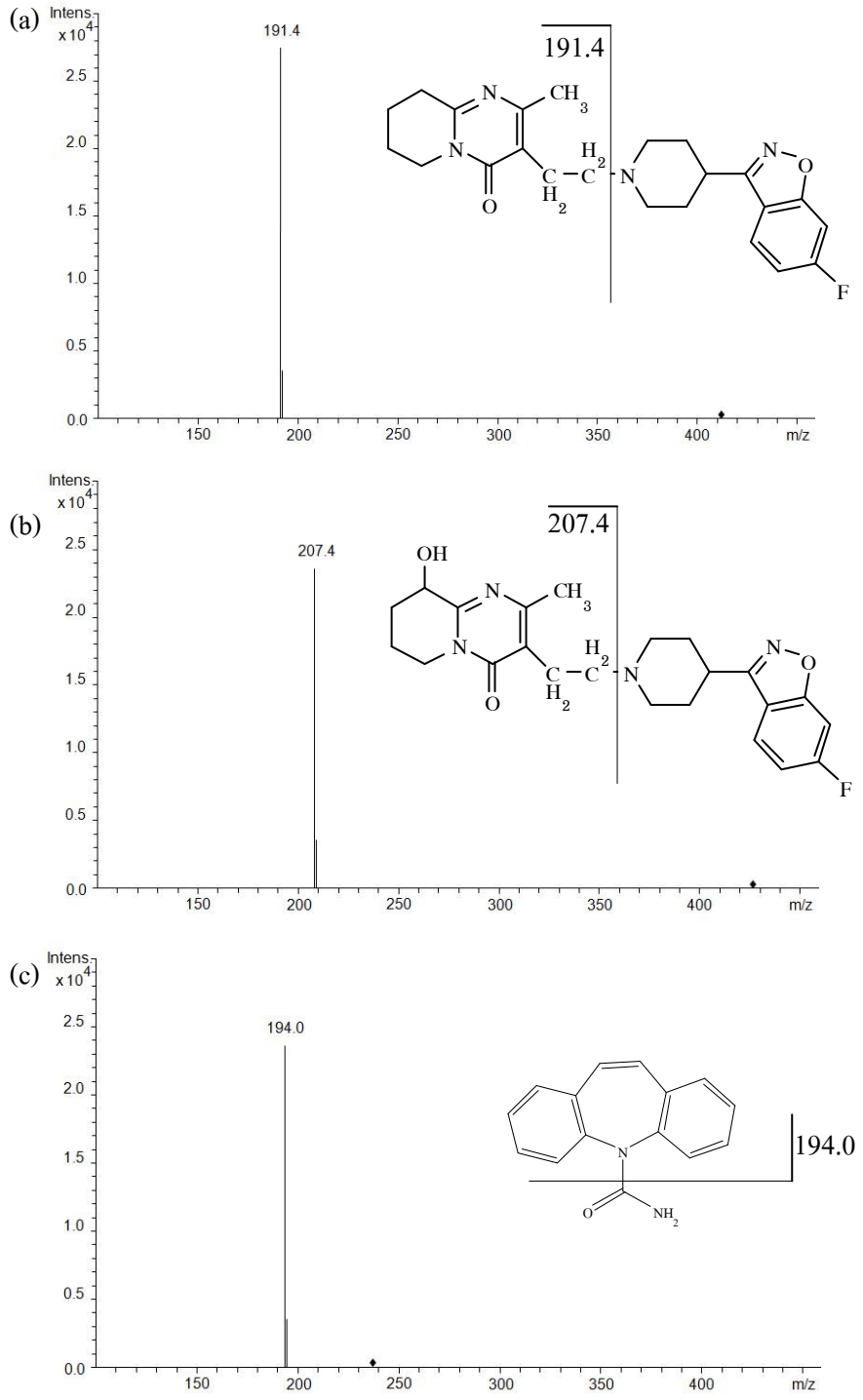


Figure 4.4 Electrospray ionization mass spectrum of product ion of RIS (a), 9OHRIS (b), and CBZ(c)

4.3.2 Optimization of extraction solvent

Sample preparation was achieved by adjusting the sample pH to more than 10 so that the RIS and 9OHRIS (pKa ~ 8) were converted into their neutral forms, and extracted into an organic solvent. Pure solvents and the mixture of solvents were investigated based on their polarity for extracting RIS and 9OHRIS from plasma. In this step, the RIS and 9OHRIS spiked plasma samples at concentration of 5 ng/mL were extracted with each solvent. The extraction efficiency was then evaluated by comparing peak area of RIS, 9OHRIS including CBZ obtained from extracted plasma samples with that from un-extracted standard solution at the same concentration. Results shown in Figure 4.5 indicated that RIS and CBZ can be extracted with all solvent systems with extraction recovery of greater than 80%. However, for 9OHRIS, a polar solvent was required to obtain reasonable recovery. It was found that with ether as extraction solvent, using 20 N NaOH in sample preparation gave higher recovery of 9OHRIS than that obtained from 0.1N NaOH. This might be due to the effects of both conversion of the analyte into its neutral form and the salting-out like-effect of NaOH at higher concentration. The use of isopropanol as the composition of extraction solvent gave high extraction recovery of 9OHRIS; however, many basic endogenous plasma components were also extracted and presented as dirty residues. Furthermore, the use of polar solvent such as isopropanol and ethyl acetate, as the component of extraction solvent made the evaporation process more difficult due to their high-boiling point. In this study, therefore, ether was chosen as the extraction solvent.

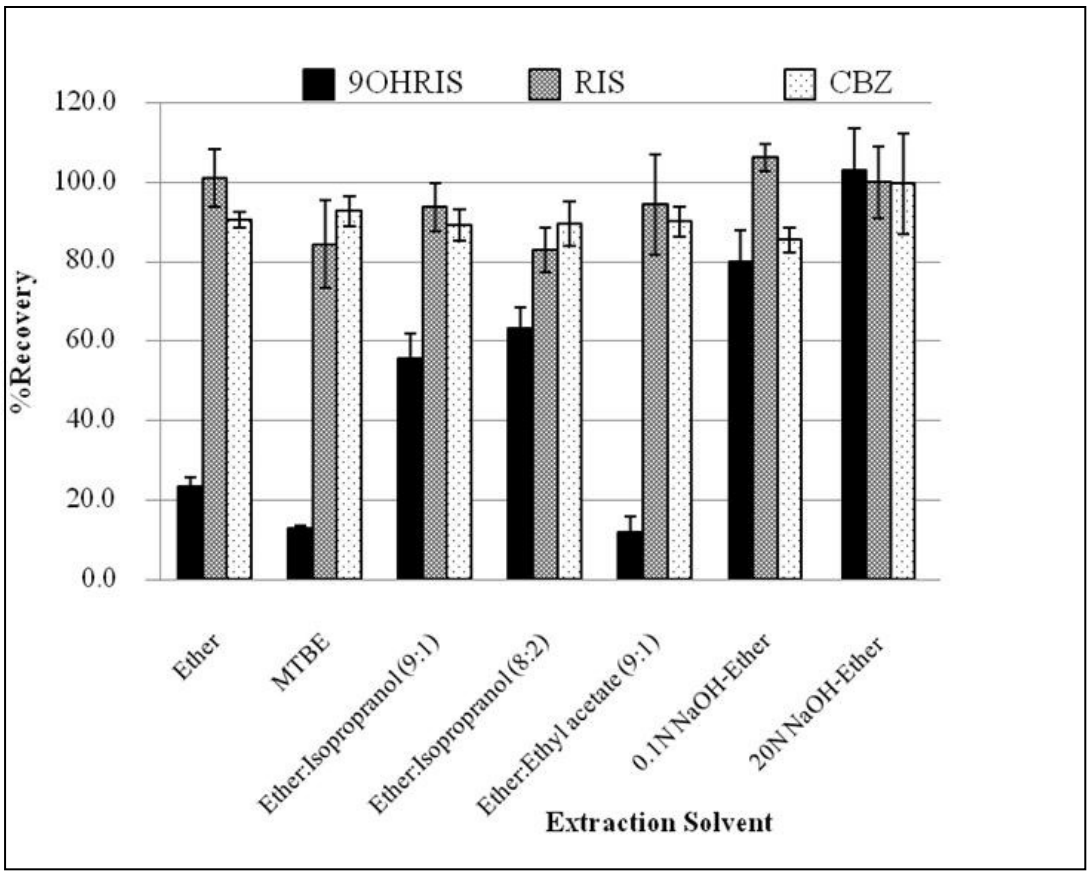


Figure 4.5 Comparison of extraction recovery (n=3) of RIS (5 ng/mL), 9OHRIS (5 ng/mL) and CBZ (100 ng/mL) obtained from various sample preparations and extraction solvent.

0.1N NaOH-Ether : 0.1N NaOH (150 µL) was added and then extracted with ether.

20 N NaOH-Ether : 20 N NaOH (150 µL)was added and then extracted with ether.

4.3.3 Specificity

Six blank samples from six different donors were analyzed to ensure that no endogenous compounds or other impurities interfered with the assay. Figure 4.6 and Figure 4.7 demonstrate the specificity of the method. It can be seen that no interferences from endogenous substances were observed. 9OHRIS, RIS, and CBZ can be separated with the C18-chromatographic column and eluted at retention time 3.3, 4.3 and 7.2 min, respectively. All interested peaks were completely resolved with isocratic system. The real subject sample at 6 h after oral administration was shown in Figure 4.8. A small peak appeared before the peak of 9OHRIS was proposed as 7-hydroxyrisperidone (7OHRIS) which is the minor metabolite of RIS (Remmirie *et al*, 2003). 7OHRIS gives the same *m/z* transition as 9OHRIS which cannot be discriminated by MS-MS. However, these two compounds can be resolved by reverse phase chromatographic system used in this study.

4.3.4 Limit of Quantitation (LOQ)

The LOQ with acceptable accuracy and precision of RIS was 0.1 ng/mL and that of 9OHRIS was 0.3 ng/mL as shown in Table 4.3 and 4.4. To obtain high specificity and sensitivity, mass spectrometric detection was set to detect the *m/z* transitions with MRM mode. The mass spectrometric detection of *m/z* transition was separated into three segments according to chromatographic separation of each peak.

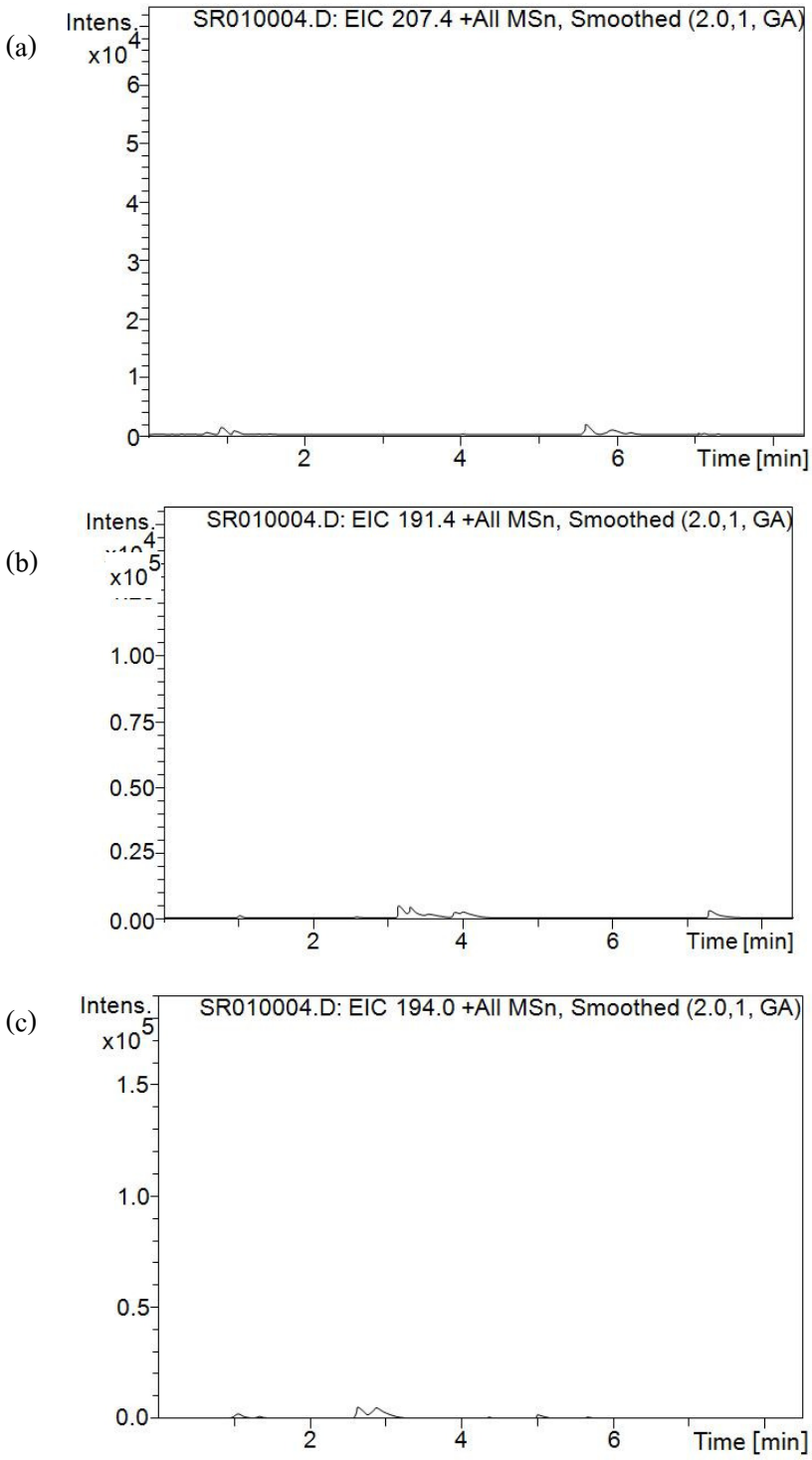


Figure 4.6 Chromatograms of blank plasma showing the segment of 9OHRIS (a), RIS (b) and CBZ (c)

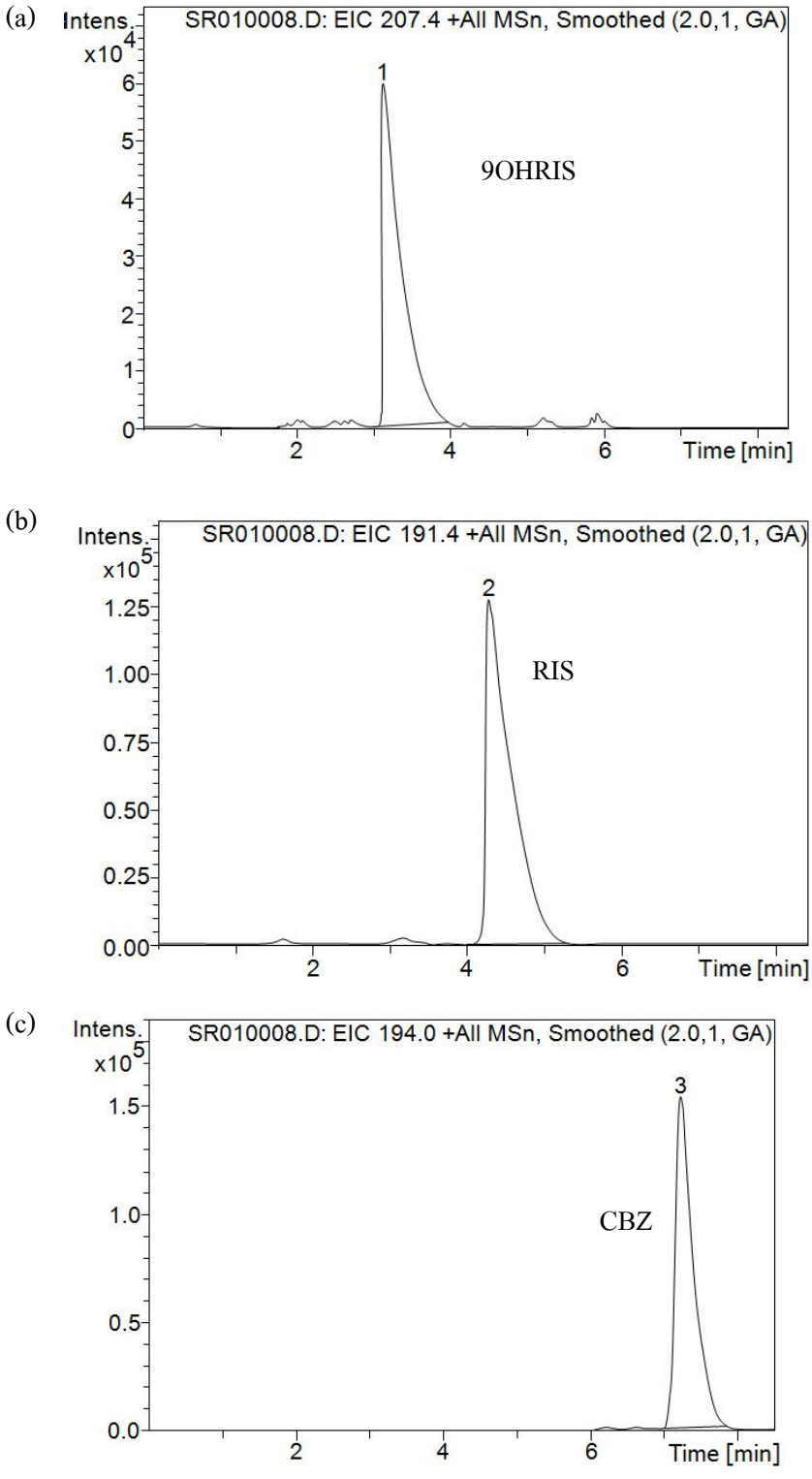


Figure 4.7 Chromatograms of 9OHRIS (a), RIS (b) at LOQ and CBZ (c) at100 ng/mL

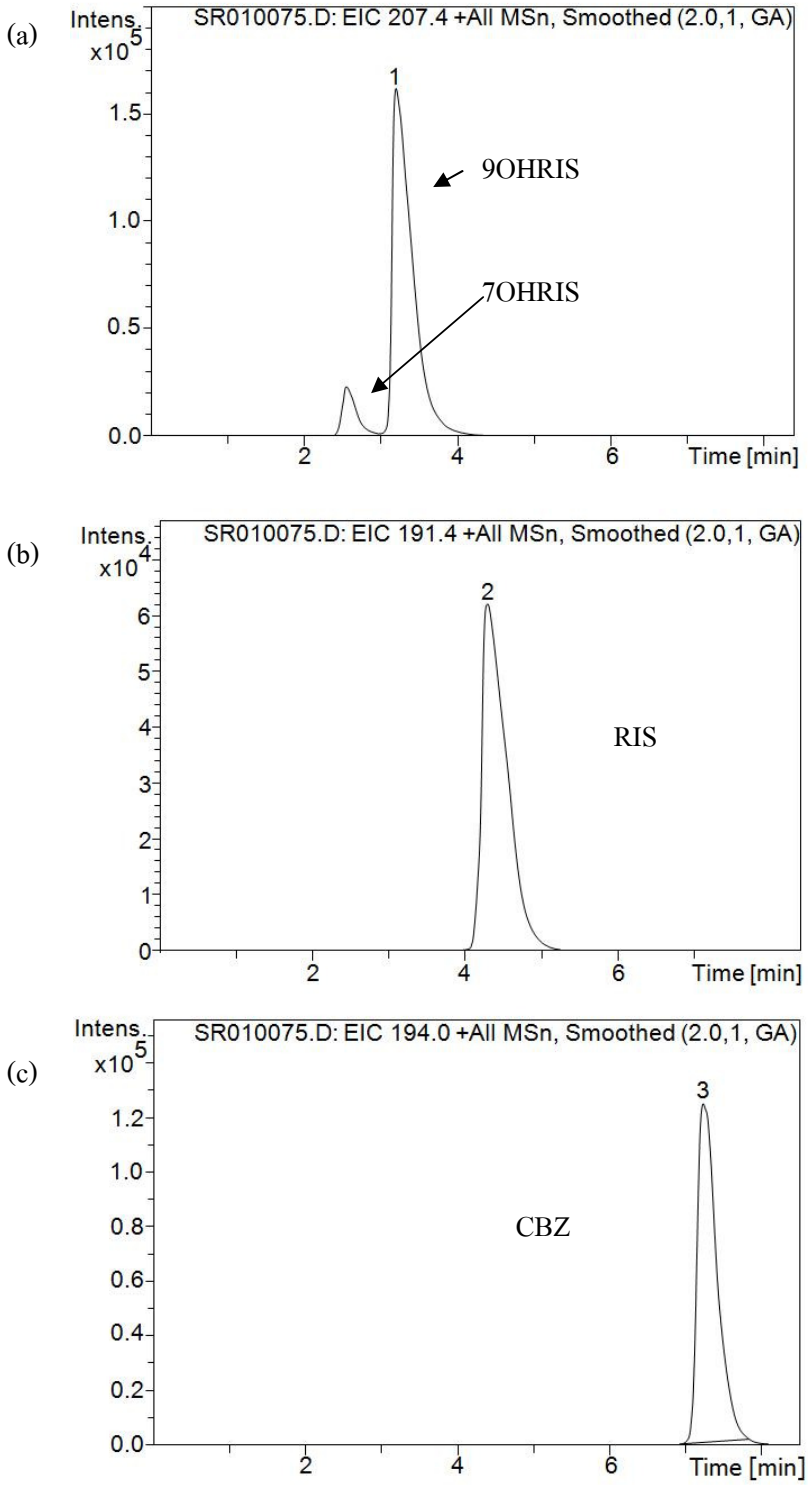


Figure 4.8 Chromatograms of 9OHRIS (a), RIS (b) and CBZ (c) of real subject sample at 6 h after oral administration

Table 4.3 LOQ of RIS in plasma

Experiment	Peak area		Peak Ratio	Estimated Concentration (ng/mL)	%Accuracy
	RIS	CBZ			
1	480681	3855978	0.12466	0.0980	98.00
2	399006	4500401	0.08866	0.1010	100.96
3	355455	4069339	0.08735	0.1002	100.19
4	281883	3341848	0.08435	0.1005	100.53
5	339837	3860929	0.08802	0.1020	102.04
6	327583	3726655	0.08790	0.1018	101.79
			Average	0.1006	100.6
			%RSD	1.44	1.44

Table 4.4 LOQ of 9OHRIS in plasma

Experiment	Peak area		Peak Ratio	Estimated Concentration (ng/mL)	%Accuracy
	9OHRIS	CBZ			
1	336259	3855978	0.08720	0.2943	98.11
2	301290	4500401	0.06695	0.3017	100.57
3	310656	4069339	0.07634	0.3117	103.89
4	230982	3341848	0.06912	0.2935	97.83
5	271092	3860929	0.07021	0.2883	96.11
6	221488	3726655	0.05943	0.2693	89.76
			Average	0.2931	97.71
			%RSD	4.85	4.85

4.3.5 Linearity

Linearity of RIS and 9OHRIS were examined by analyzing calibration curves containing seven standard concentrations of RIS (0.1, 0.5, 1, 5, 10, 20, and 30 ng/mL) and 9OHRIS (0.3, 0.5, 1, 5, 10, 20, and 30 ng/mL) by weighted least square linear regression with $1/x^2$ as a weighting factor. The equations for mean of five calibration curves for each analyte were: RIS; y=0.3474x+0.0654, (r^2=0.9981), 9OHRIS; y= 0.1631x+0.0317 (r^2 = 0.9983).

4.3.6 Accuracy and precision

The within- and between-run accuracy and precision were shown in Table 4.5 – 4.8. The accuracy of within- and between-run of these two compounds was within 85.0 – 115.0%. For the precision, the %RSD of within- and between –run of both RIS and 9OHRIS were less than 15%.

Table 4.5 Within-run accuracy and precision of RIS

Theoretical Concentration (ng/mL)	n	Average Concentration (ng/mL)	%RSD	%Accuracy
0.496	5	0.48 ± 0.04	7.63	97.5±5.89
4.960	5	5.18 ± 0.17	3.36	104.5±3.49
19.840	5	20.47 ± 0.69	3.37	103.2±3.48

Table 4.6 Within-run accuracy and precision of 9OHRIS

Theoretical Concentration (ng/mL)	n	Average Concentration (ng/mL)	%RSD	%Accuracy
0.505	5	0.48 ± 0.04	8.33	95.05±7.72
5.050	5	4.94 ± 0.34	6.88	97.82±6.71
20.200	5	19.29 ± 0.59	3.06	95.49±3.04

Table 4.7 Between-run accuracy and precision of RIS

Theoretical Concentration (ng/mL)	n	%Accuracy	%RSD
0.5	5	102.82±4.60	3.92
5.0	5	100.81±3.43	3.40
20.0	5	99.29±3.61	3.60

Table 4.8 Between-run accuracy and precision of 9OHRIS

Theoretical Concentration (ng/mL)	n	%Accuracy	%RSD
0.5	5	100.99±5.16	5.88
5.0	5	97.43±4.65	4.88
20.0	5	95.20±2.35	2.44

4.3.7 Extraction recovery

Mean extraction recoveries of RIS and 9OHRIS from spiked plasma at concentration 0.5, 5, and 20 ng/mL were greater than 86.5% and 86.3%, respectively, as shown in Table 4.9. For CBZ, the mean extraction recovery from spiked plasma at concentration of 100 ng/mL was 96.4%

Table 4.9 Extraction recovery of RIS, 9OHRIS and CBZ from spiked plasma

Compound	Concentration (ng/mL)	n	Average % Extraction Recovery	%RSD
RIS	0.5	5	86.5±7.8	9.02
	5	5	90.5±11.6	12.80
	20	5	104.1±9.5	9.10
9OHRIS	0.5	5	101.0±4.4	4.38
	5	5	87.1±4.9	5.65
	20	5	86.3±7.8	9.02
CBZ	100	5	96.4±8.1	8.37

4.3.8 Stability

4.3.8.1 Freeze-thaw stability

The stability of RIS and 9OHRIS at low (0.5 ng/mL) and high (20 ng/mL) concentrations was studied after sample being subjected to, one, two and three freeze-thaw cycles. Both RIS and 9OHRIS were stable after three freeze–thaw cycles. From Table 4.10 and 4.11, the percent deviation from freshly prepared sample of RIS was -9.91 to 3.52%, and that of 9OHRIS was -3.85 to 11.54%. The CV of all measured concentrations was less than 15%. These results indicated that no significant degradation after three freeze-thaw cycles was observed.

4.3.8.2 Short-term stability

The short term stability of RIS and 9OHRIS was shown in Table 4.12 and 4.13. For RIS, the percent deviation from freshly prepared spiked plasma sample was -10.78 to 7.69% and that of 9OHRIS was -10.69% to 4.65%. Although the results indicated that plasma sample could be left at room temperature for up to 24 hours, all plasma samples were thawed at room temperature within 2 hour before extraction.

4.3.8.3 Long-term stability

The long-term stability at -20 °C of RIS and 9OHRIS in plasma was studied using QC samples at the concentration of 0.5 and 20 ng/mL. The last plasma sample had to be analyzed after 7 months of storage, the 9-month -20°C-stored QCs were analyzed to ensure the stability of RIS and 9OHRIS. Both RIS and 9OHRIS in plasma samples showed no significant degradation for at least 9 months. The percent deviation of RIS from freshly prepared spiked plasma sample was -6.95% to 6.78% and that of 9OHRIS was -11.24% to 9.02%, as shown in Table 4.14 and 4.15.

4.3.8.4 Autosampler stability

Stability of processed QC samples at concentration of 0.5 and 20 ng/mL stored in the autosampler over a period of 72 h was evaluated. The results showed in Table 4.16 and 4.17. RIS and 9OHRIS seemed to be unstable at 72 h and 48 h, respectively. The results indicated that the processed samples should be kept in autosampler for not more than 24 hour. Therefore, a 85-sample analytical batch consisted of 1 blank-plasma sample, 68 duplicate subject plasma samples, 7 calibration-curve samples, 6 QC samples and 3 recovery samples, was analyzed in each run. This would take approximately 15 hours, which less than the maximum limit allowed to leave processed sample in autosampler, to complete the analysis.

4.3.8.5 Stock solution stability

Stability of stock solutions of RIS (0.1 µg/mL), 9OHRIS (0.1 µg/mL) and CBZ (0.1 µg/mL) in methanol was investigated. The results presented in Table 4.18 and Table 4.19 indicated that the stock solution of RIS, 9OHRIS and CBZ were stable in methanol for at least 24 h at room temperature and 3 months at -20^{O}C with the percent deviation less than 2.0%.

The validation results indicated that this method for simultaneous determination of RIS and 9OHRIS in human plasma is simple, specific, accurate and precise. The proposed validated method was applied for determination of RIS and 9OHRIS in plasma samples from 24 healthy Thai adult male subjects who received 2 mg test and reference products of risperidone under the fast condition.

Table 4.10 Freeze-thaw stability RIS (n=3)

Theoretical Concentration (ng/mL)	t=0	Cycle 1		Cycle 2		Cycle 3	
	Estimated Concentration (ng/mL)	Estimated Concentration (ng/mL)	%Deviation	Estimated Concentration (ng/mL)	%Deviation	Estimated Concentration (ng/mL)	%Deviation
0.5	0.52±0.04	0.51±0.02	2.52	0.54±0.02	3.52	0.53±0.06	2.84
20	19.25±1.7	17.34±0.77	-9.90	18.18±0.71	-5.57	18.31±0.88	-4.89

Table 4.11 Freeze-thaw stability of 9OHRIS (n=3)

Theoretical Concentration (ng/mL)	t=0	Cycle 1		Cycle 2		Cycle 3	
	Estimated Concentration (ng/mL)	Estimated Concentration (ng/mL)	%deviation	Estimated Concentration (ng/mL)	%deviation	Estimated Concentration (ng/mL)	%deviation
0.5	0.52±0.05	0.50±0.06	-3.85	0.58±0.05	11.54	0.58±0.05	11.54
20	18.80±1.56	19.94±0.44	6.06	20.83±1.11	10.80	20.82±0.7	10.74

Table 4.12 Short term stability of RIS (n=3)

Theoretical Concentration (ng/mL)	t=0	6 hr		10 hr		24 hr	
	Estimated Concentration (ng/mL)	Estimated Concentration (ng/mL)	%deviation	Estimated Concentration (ng/mL)	%deviation	Estimated Concentration (ng/mL)	%deviation
0.5	0.52±0.05	0.48±0.02	7.69	0.49±0.05	-5.47	0.49±0.05	-5.47
20	19.99±1.2	18.20±0.57	-8.97	18.79±0.32	-5.99	17.83±0.32	-10.78

Table 4.13 Short term stability of 9OHRIS (n=3)

Theoretical Concentration (ng/mL)	t=0	6 hr		10 hr		24 hr	
	Estimated Concentration (ng/mL)	Estimated Concentration (ng/mL)	%deviation	Estimated Concentration (ng/mL)	%deviation	Estimated Concentration (ng/mL)	%deviation
0.5	0.50±0.04	0.50±0.04	1.03	0.52±0.03	4.52	0.52±0.03	4.65
20	20.48±0.73	18.76±0.87	-8.41	18.94±0.8	-7.55	18.29±0.45	-10.69

Table 4.14 Long term stability of RIS (n=3)

Theoretical Concentration (ng/mL)	t = 0	t = 1 month		t = 2 months		t = 3 months		t = 9 months (n=2)	
	Estimated Concentration (ng/mL)	Estimated Concentration (ng/mL)	%deviation	Estimated Concentration (ng/mL)	%deviation	Estimated Concentration (ng/mL)	%deviation	Estimated Concentration (ng/mL)	%deviation
0.5	0.52±0.03	0.56±0.03	6.78	0.52±0.03	0.17	0.52±0.03	-0.01	0.50±0.02	-4.79
20	19.03±0.24	18.07±0.46	-5.09	18.29±0.57	-3.89	19.89±0.47	4.52	17.71±0.38	-6.95

Table 4.15 Long term stability of 9OHRIS (n=3)

Theoretical Concentration (ng/mL)	t=0	1 month		2 months		3 months		9 months (n=2)	
	Estimated Concentration (ng/mL)	Estimated Concentration (ng/mL)	%deviation	Estimated Concentration (ng/mL)	%deviation	Estimated Concentration (ng/mL)	%deviation	Estimated Concentration (ng/mL)	%deviation
0.5	0.55±0.01	0.56±0.03	1.82	0.49±0.02	-11.24	0.53±0.05	-3.90	0.51±0.02	-7.09
20	19.19±1.01	20.92±0.37	9.02	19.10±0.63	-0.45	19.65±0.37	2.41	17.72±0.34	-7.67

Table 4.16 Autosampler stability of RIS

Theoretical Concentration (ng/mL)	t=0	12 hour		24 hour		48 hour		72 hour	
	Estimated Concentration (ng/mL)	Estimated Concentration (ng/mL)	%deviation	Estimated Concentration (ng/mL)	%deviation	Estimated Concentration (ng/mL)	%deviation	Estimated Concentration (ng/mL)	%deviation
0.5	0.52±0.03	0.57±0.04	9.62	0.50±0.05	-3.85	0.48±0.03	-7.69	0.46±0.02	-11.54
20	19.06±0.24	18.78±0.91	-1.47	17.61±1.05	-7.61	18.03±1.13	-5.40	17.17±0.8	-9.92

Table 4.17 Autosampler stability of 9OHRIS

Theoretical Concentration (ng/mL)	t=0	12 hour		24 hour		48 hour		72 hour	
	Estimated Concentration (ng/mL)	Estimated Concentration (ng/mL)	%deviation	Estimated Concentration (ng/mL)	%deviation	Estimated Concentration (ng/mL)	%deviation	Estimated Concentration (ng/mL)	%deviation
0.5	0.55±0.01	0.50±0.03	-9.09	0.49±0.05	-10.91	0.46±0.05	-16.36	0.43±0.01	-21.82
20	19.19±1.01	18.77±0.99	-2.19	18.29±1.49	-4.69	17.20±0.9	-10.37	14.90±1.02	-22.36

Table 4.18 Stock solution stability of RIS, 9OHRIS and CBZ (n=3) at room temperature

Compounds	Theoretical concentration (µg/mL)	t=0	6 hr		14 hr		24 hr	
		Estimated Concentration (µg/mL)	Estimated Concentration (µg/mL)	%deviation	Estimated Concentration (µg/mL)	%deviation	Estimated Concentration (µg/mL)	%deviation
RIS	10	9.96±0.033	10.05±0.004	0.90	10.09±0.005	1.31	10.08±0.019	1.20
9OHRIS	10	9.96±0.002	10.10±0.001	1.41	10.00±0.028	0.40	10.06±0.028	1.00
CBZ	10	10.07±0.001	10.09±0.009	0.20	9.98±0.002	-0.89	10.00±0.021	-0.70

Table 4.19 Stock solution stability of RIS, 9OHRIS and CBZ at -20 °C

Compounds	Theoretical concentration (µg/mL)	15 days		1 month		2 months		3 months	
		Estimated Concentration (µg/mL)	%deviation	Estimated Concentration (µg/mL)	%deviation	Estimated Concentration (µg/mL)	%deviation	Estimated Concentration (µg/mL)	%deviation
RIS	10	9.95±0.014	-0.10	10.01±0.021	0.50	9.97±0.032	0.10	9.96±0.015	-0.02
9OHRIS	10	10.03±0.02	0.70	10.07±0.011	1.10	9.98±0.015	0.20	10.00±0.045	0.40
CBZ	10	9.98±0.020	-0.89	10.04±0.003	-0.30	10.04±0.019	-0.30	10.00±0.034	-0.70

4.4 Bioequivalence study

4.4.1 Subjects

Twenty-four healthy male Thai volunteers were enrolled in the study with age ranged from 20 to 44 years (29 $\pm$ 8.6 years), weight from 55 to 76 kg (61.9 $\pm$ 4.9 kg), and height from 155 to 185 cm (169.1$\pm$7.5 cm). The sample size of 24 subjects was sufficient to ensure a power of 80% for correctly concluding bioequivalence. Medical history, physical, hematological and biochemical profiles were examined for the inclusion of healthy volunteers as shown in appendix.

4.4.2 Bioequivalence assessment

Figures 4.11 - 4.32 showed plasma concentration-time profiles of the RIS, 9OHRIS of each subject after oral administration of 2-mg risperidone tablet of Product A and Product B. Plasma samples from subject SR10 and SR11 could not be analyzed since the LC-MS-MS was out of order during the analysis. The mean plasma concentration-time curves of RIS and 9OHRIS were shown in Figure 4.9 and 4.10, respectively. The pharmacokinetic parameters C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, extrapolated AUC (%), T_{max} and $T_{1/2}$ of test and reference products were calculated for RIS and 9OHRIS as presented in Table 4.21 and 4.23, respectively.

RIS was rapidly absorbed and reached the C_{max} value of 15.70$\pm$4.98 and 17.00$\pm$5.81 ng/mL for test and reference products, respectively. For 9OHRIS, C_{max} was 11.06$\pm$5.13 (test) and 11.47$\pm$5.21 (reference). The minimum and maximum values of T_{max} for RIS and 9OHRIS were 0.75–5.0 h (test) vs 0.5–1.5 h (reference) and 1.5–12.0 h (test) vs 0.75–12.0 h (reference), respectively.

The absorption of the parent compound, RIS, is generally affected by drug formulation. For the metabolite, 9OHRIS, inter-subject variation mainly due to the variation on metabolism of RIS. The inter-subject variations (%CV) of the observed C_{max} and T_{max} of 9OHRIS were higher than those of RIS. These results indicated that the inter-subject variation was more affected by metabolism than by drug formulation factor.

The mean AUC_{0-t} and SD of RIS, and 9OHRIS were 100.67±48.89 ng/mL.h^{-1} (test) vs 97.74±45.78 ng/mL.h^{-1} (reference) and 277.32±120.86 ng/mL.h^{-1}(test) vs 273.16±121.23 ng/mL.h^{-1} (reference), respectively. The terminal half-life, $T_{1/2}$, of RIS and 9OHRIS were 4.39±2.74 h (test) vs 4.01±1.71 h (reference) and 21.29+8.87 h (test) vs 22.52±8.92 h (reference), respectively.

The $T_{1/2}$ of RIS and 9OHRIS for both formulations were noticeably varied among individuals, with ranged of 1.5 – 13 h and 8 – 40 h, respectively. The large inter-individual variations in $T_{1/2}$ and other pharmacokinetic parameters were also observed by Mannens *et* al (1993), Huang *et* al (1993) and van Schaick *et* al (2003). The large inter-subject variability of pharmacokinetic parameters is due to genetic polymorphism of CYP2D6 responsible for the metabolism of RIS to 9OHRIS (Mannens *et* al, 1993; Huang *et* al, 1993).

The mean extrapolate portion of plasma concentration-time profile curve of RIS and 9OHRIS were less than the acceptable value of 20%. These results indicated that the blood sampling period, including the LOQ of the method is suitable to follow the pharmacokinetic characteristic of risperidone.

4.4.3 Statistical analysis of bioequivalence

Table 4.20 showed the 90% confidence interval (CI) of the geometric mean test/reference ratio of RIS and 9OHRIS. The 90% CIs for C_{max}, AUC_{0-48}, and $AUC_{0->\infty}$ were 82.8% - 103.6%, 88.3% - 107.4% and 88.1% - 106.8%, respectively for RIS and 93.4% - 108.4%, 92.0% - 108.1% and 92.2% - 108.7%, respectively for 9OHRIS.

ANOVA of the ln transformed data was presented in Table 4.22 and 4.24 No significant difference (p>0.05) from sequence effect was found for all of the pharmacokinetic parameters of all interested compounds. These results indicated that the washout period in the crossover design was properly performed. Also, there were no statistically significant difference from drug formulation and period on C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. However, as expected, there was statistically significant difference in studied pharmacokinetic parameters among individuals.

The results form ANOVA confirmed the bioequivalence of 2 mg risperidone test product with the reference product in terms of rate and extent of absorption.

4.5 Adverse events

Data of adverse event was shown in Table 4.25. The most commonly reported adverse event after intake of either formulation was somnolence. All subjects were somnolent within 1 h after administration of both test and reference 2 mg risperidone tablets which is similar to the report of Van Schaick *et al* (2003) and Borison *et al* (1994). Three subjects experienced orthostatic hypotension with both formulations. The orthostatic hypotension, which is presumably an early effect of risperidone's alpha-blocking activity, was also reported by Borison *et* al (1994). One subject reported headache with both reference and test products. Syncope was found in one subject after 9 h of administration of reference product but the symptom can be recovered to normal status within 1 hour. Intensity of these adverse events was mild and disappeared within 1 day. None of abnormal examination and blood chemistry was found in all subjects at the end of the study. No subjects withdrew from the study.

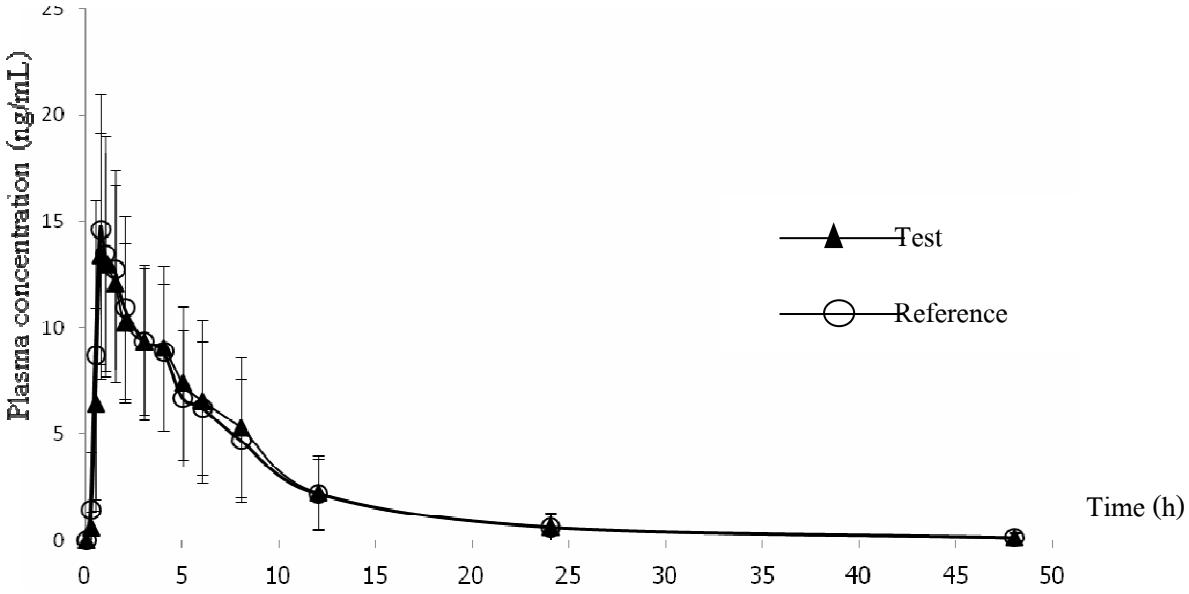


Figure 4.9 Average plasma concentration-time curves of RIS after single oral administration of 2 mg test and reference risperidone tablets.

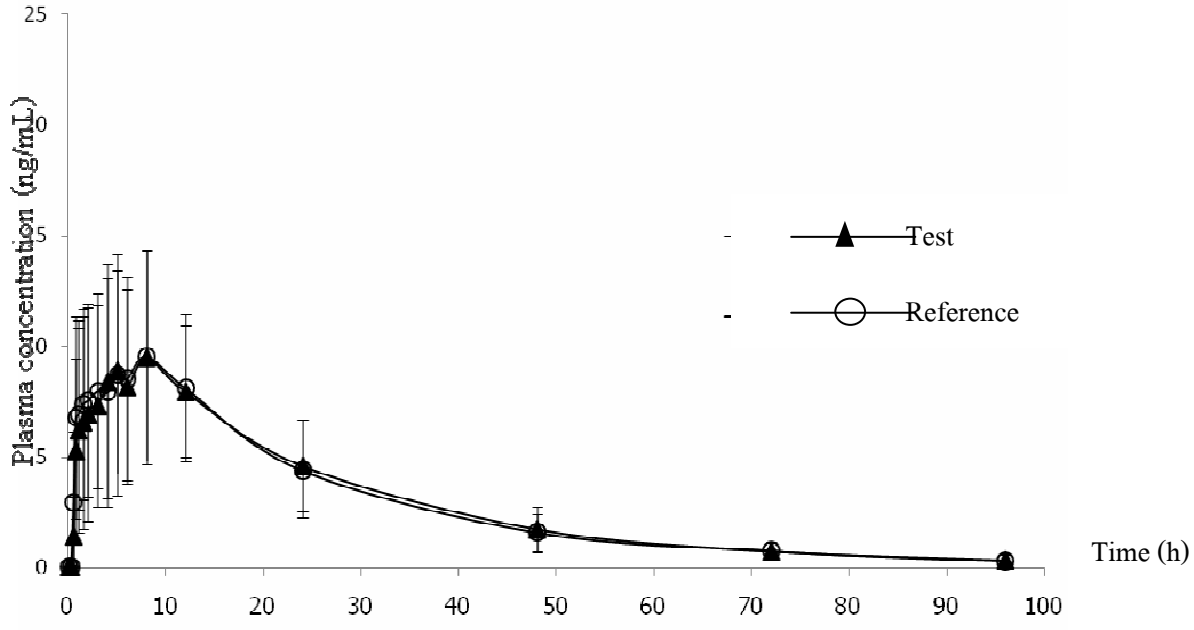


Figure 4.10 Average plasma concentration-time curves of 9OHRIS after single oral administration of 2 mg test and reference risperidone tablets.

Table 4.20 90% CIs of the Test/Reference ratio for C_{max}, $AUC_{0->t}$ and $AUC_{0->\infty}$ of RIS and 9OHRIS after single oral administration of 2 mg of risperidone tablets to 22 healthy male volunteers

Compound	Pharmacokinetic parameters	Geometric mean ± SD		90% CI	Power
		Test	Reference		
RIS	C_{max}	14.92±4.98	16.02±5.81	82.8% - 103.6%	84.5%
	$AUC_{0->48}$	87.95±48.89	85.68±45.78	88.3% - 107.4%	91.8%
	$AUC_{0->\infty}$	90.98±50.54	89.07±48.01	88.1% - 106.8%	92.5%
9OHRIS	C_{max}	9.92±5.14	10.35±5.20	93.4% - 108.4%	98.8%
	$AUC_{0->96}$	250.88±120.86	245.68±121.23	92.0% - 108.1%	97.7%
	$AUC_{0->\infty}$	268.67±123.61	262.82±123.61	92.2% - 108.7%	97.3%

Table 4.21 Individual pharmacokinetic parameters of RIS

Subject	Product A (Test)						Product B (Reference)					
	C_{max}	AUC_{0-48}	$AUC_{0-\infty}$	%AUC extrapolate	T_{max}	$T_{1/2}$	C_{max}	AUC_{0-48}	$AUC_{0-\infty}$	%AUC extrapolate	T_{max}	$T_{1/2}$
SR01	13.64	62.68	70.27	10.81	1.00	10.31	13.33	43.49	44.66	2.64	0.75	2.39
SR02	23.82	59.72	61.73	3.27	0.75	3.93	25.46	64.46	65.86	2.13	0.75	2.69
SR03	13.56	52.40	53.39	1.84	1.00	2.48	13.60	57.01	58.05	1.78	1.50	2.61
SR04	9.17	28.07	32.05	12.42	0.75	5.08	8.14	32.26	37.62	14.24	0.75	7.95
SR05	23.37	153.62	157.57	2.51	1.50	3.30	23.53	171.58	172.01	0.25	1.00	5.27
SR06	12.91	179.51	180.68	0.65	1.50	5.59	13.78	156.37	156.76	0.25	1.00	5.82
SR07	11.94	55.73	56.27	0.96	1.00	2.83	13.86	73.71	74.25	0.73	0.75	2.58
SR08	8.24	25.09	25.40	1.23	0.75	1.63	8.50	22.45	22.72	1.18	0.50	1.52
SR09	19.64	158.74	158.97	0.14	0.75	7.44	21.31	142.15	147.58	3.68	0.75	3.47
SR12	17.85	74.59	75.08	0.65	0.75	1.96	22.96	75.75	77.88	2.74	0.50	4.73
SR13	7.81	47.05	47.70	1.35	0.75	2.17	10.38	48.40	48.75	0.71	0.75	2.08
SR14	15.57	79.63	80.38	0.94	0.75	2.51	14.06	89.00	90.22	1.35	1.50	2.79
SR15	17.08	119.54	124.18	3.74	1.00	4.23	25.09	139.61	149.50	6.62	0.50	6.70
SR16	24.82	198.23	201.16	1.45	1.00	2.62	23.84	136.85	139.36	1.80	1.00	2.93
SR17	16.07	106.41	108.79	2.19	1.00	3.85	13.77	55.91	57.25	2.33	0.75	4.01
SR18	12.07	100.11	102.71	2.53	2.00	2.75	14.80	88.07	89.89	2.03	1.00	3.55
SR19	15.21	136.98	158.22	13.43	1.00	6.66	14.64	146.40	155.72	5.98	1.50	4.01
SR20	15.98	95.31	98.17	2.92	1.00	3.23	21.53	105.88	109.93	3.69	0.75	3.54
SR21	19.31	85.97	89.32	3.76	0.75	3.09	10.28	75.64	82.74	8.59	1.00	6.43
SR22	22.48	120.24	124.48	3.41	0.75	4.92	25.31	140.14	143.95	2.65	0.75	4.23
SR23	10.78	117.85	125.47	6.07	5.00	3.40	21.23	137.95	143.06	3.57	0.75	2.92
SR24	13.99	157.19	165.22	4.86	0.75	12.55	14.56	147.29	169.66	13.18	1.00	6.05
Mean	15.70	100.67	104.42	3.69	1.16	4.39	17.00	97.74	101.70	3.73	0.89	4.01
SD	4.98	48.89	50.30	3.78	0.91	2.74	5.81	45.78	48.01	3.84	0.30	1.71
%RSD	31.74	48.56	48.17	102.62	78.91	62.50	34.17	46.84	47.21	102.87	33.40	42.50

Table 4.22 Two-way ANOVA of ln-transformed C_{max}, $AUC_{0->48}$ and $AUC_{0->\infty}$ of RIS

Dependent variable	Source of Variation	df	Sum of squares	Mean Squares	F_{stat}	*P*-value
C_{max}	Sequence	1	0.0133	0.0133	0.0606	0.81
	Subjects	20	4.3738	0.2187	6.8654	0.00
	Period	1	0.0001	0.0001	0.0040	0.95
	Treatment	1	0.0548	0.0548	1.7203	0.20
	Error	20	0.6371	0.0319		
	Total	43	5.0791			
AUC_{0-48}	Sequence	1	0.0527	0.0527	0.0818	0.78
	Subjects	20	12.8790	0.6439	26.4789	0.00
	Period	1	0.0005	0.0005	0.0217	0.89
	Treatment	1	0.0076	0.0076	0.3106	0.58
	Error	20	0.4864	0.0243		
	Total	43	13.4261			
$AUC_{0-\infty}$	Sequence	1	0.0486	0.0486	0.0755	0.79
	Subjects	20	12.8619	0.6431	27.2650	0.00
	Period	1	0.0018	0.0018	0.0775	0.78
	Treatment	1	0.0050	0.0050	0.2104	0.65
	Error	20	0.4717	0.0236		
	Total	43	13.3890			

Table 4.23 Individual pharmacokinetic parameters of 9OHRIS

Subject	Product A (Test)						Product B (Reference)					
	C_{max}	AUC_{0-96}	$AUC_{0-\infty}$	%AUC extrapolate	T_{max}	$T_{1/2}$	C_{max}	AUC_{0-96}	$AUC_{0-\infty}$	%AUC extrapolate	T_{max}	$T_{1/2}$
SR01	19.98	429.45	440.39	2.48	8.00	12.02	17.75	422.96	432.13	2.12	8.00	11.31
SR02	16.74	352.59	362.38	2.70	4.00	22.98	18.56	379.57	389.74	2.61	4.00	12.53
SR03	19.25	508.96	522.05	2.51	4.00	14.24	20.14	467.40	479.83	2.59	5.00	15.46
SR04	8.82	155.97	161.57	3.46	8.00	7.67	8.01	151.66	160.14	5.29	8.00	9.97
SR05	6.13	202.52	214.27	5.48	4.00	19.47	5.80	190.72	204.39	6.68	5.00	18.14
SR06	8.44	229.51	244.04	5.96	8.00	20.76	7.55	212.20	227.16	6.59	8.00	32.45
SR07	5.79	106.39	112.66	5.56	2.00	22.31	5.99	117.40	126.10	6.90	2.00	18.76
SR08	18.41	313.70	323.19	2.94	1.50	14.64	15.87	309.81	323.97	4.37	0.75	14.71
SR09	9.13	289.46	313.26	7.60	6.00	20.85	9.63	275.93	299.45	7.85	6.00	17.96
SR12	11.30	261.95	290.07	9.70	5.00	37.46	13.29	287.87	312.03	7.74	5.00	44.52
SR13	12.28	305.05	313.09	2.57	8.00	10.82	13.06	312.95	327.87	4.55	12.00	20.50
SR14	6.32	150.87	175.06	13.82	5.00	28.77	5.40	136.30	141.37	3.59	2.00	34.61
SR15	5.39	189.49	202.31	6.34	5.00	25.95	7.33	215.69	235.97	8.59	8.00	29.95
SR16	4.94	124.90	129.11	3.26	12.00	17.28	4.64	88.23	98.65	10.56	6.00	14.92
SR17	9.13	198.78	238.92	16.80	1.50	38.61	8.36	138.27	157.56	12.24	1.00	30.85
SR18	15.88	404.41	425.53	4.96	4.00	26.41	19.35	423.59	445.09	4.83	5.00	26.31
SR19	4.39	114.67	130.19	11.92	12.00	23.49	7.68	169.32	204.28	17.11	12.00	32.84
SR20	11.11	444.39	468.00	5.05	8.00	15.45	10.41	359.48	387.38	7.20	1.00	20.68
SR21	16.44	435.63	447.82	2.72	8.00	18.26	16.95	327.27	344.11	4.89	3.00	27.18
SR22	10.26	303.51	373.20	18.67	12.00	39.81	12.28	339.30	361.96	6.26	8.00	25.14
SR23	16.93	390.23	415.21	6.02	8.00	19.60	17.96	503.73	530.76	5.09	8.00	21.45
SR24	6.16	188.69	193.44	2.46	12.00	11.50	6.43	179.89	188.15	4.39	6.00	15.21
Mean	11.06	277.32	295.26	6.50	6.64	21.29	11.47	273.16	289.91	6.46	5.63	22.52
SD	5.13	120.86	123.61	4.78	3.37	8.87	5.21	121.23	123.61	3.45	3.23	8.92
%RSD	46.45	43.58	41.87	73.60	50.79	41.69	45.37	44.38	42.64	53.46	57.47	39.60

Table 4.24 Two-way ANOVA of ln-transformed C_{max}, $AUC_{0->96}$ and $AUC_{0->\infty}$ of 9OHRIS

Dependent variable	Source of Variation	df	Sum squares	Mean Squares	F_{stat}	*P*-value
C_{max}	Sequence	1	0.0176	0.0176	0.0378	0.85
	Subjects	20	9.3299	0.4665	33.1868	0.00
	Period	1	0.0164	0.0164	1.1641	0.29
	Treatment	1	0.0198	0.0198	1.4105	0.25
	Error	20	0.2811	0.0141		
	Total	43	9.6648			
AUC_{0-96}	Sequence	1	0.0432	0.0432	0.0923	0.76
	Subjects	20	9.3482	0.4674	28.2250	0.00
	Period	1	0.0067	0.0067	0.4046	0.53
	Treatment	1	0.0048	0.0048	0.2912	0.60
	Error	20	0.3312	0.0166		
	Total	43	9.7341			
$AUC_{0-\infty}$	Sequence	1	0.0520	0.0520	0.1193	0.73
	Subjects	20	8.7142	0.4357	25.1380	0.00
	Period	1	0.0128	0.0128	0.7392	0.40
	Treatment	1	0.0053	0.0053	0.3076	0.56
	Error	20	0.3467	0.0173		
	Total	43	9.1310			

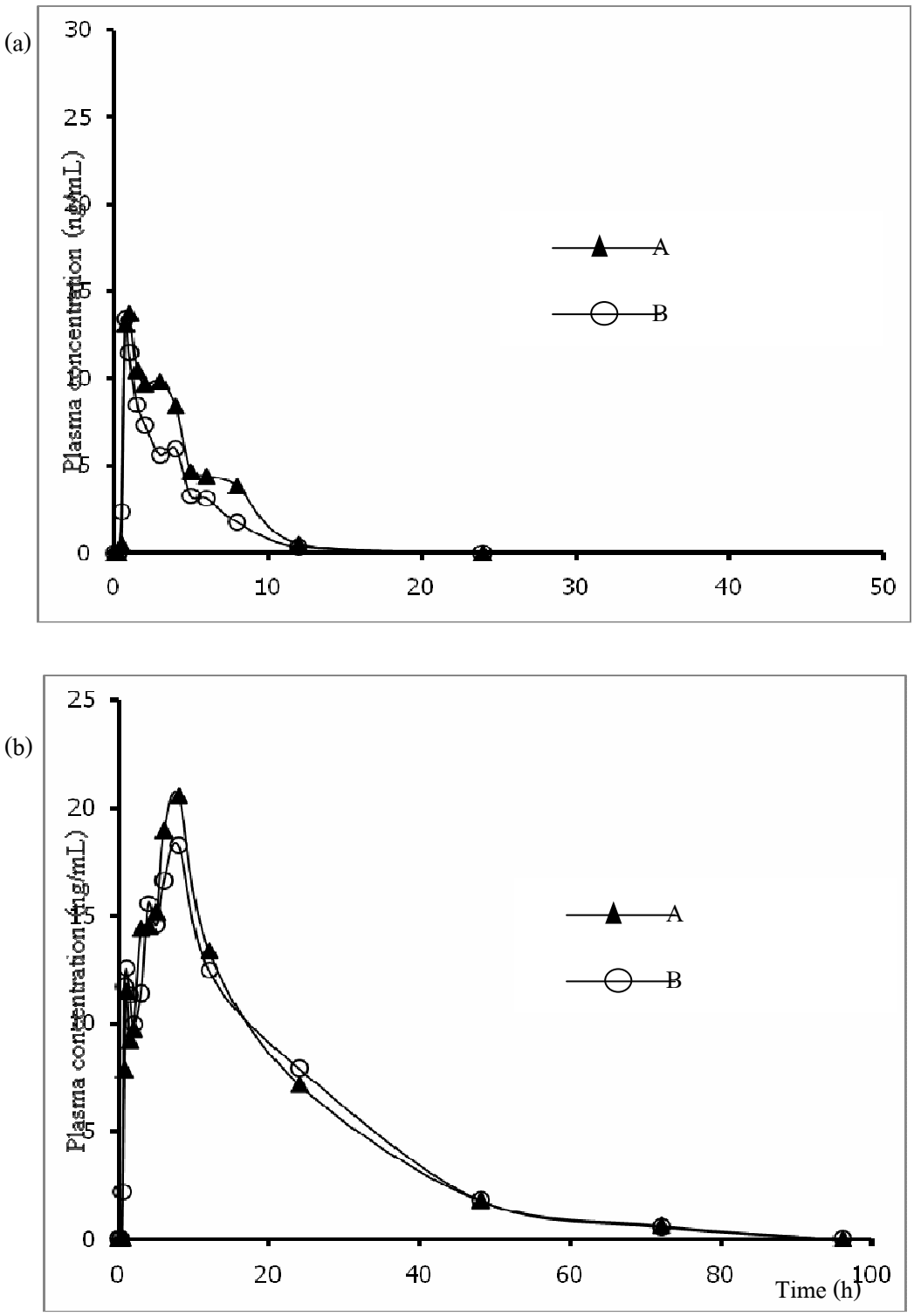


Figure 4.11 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 1

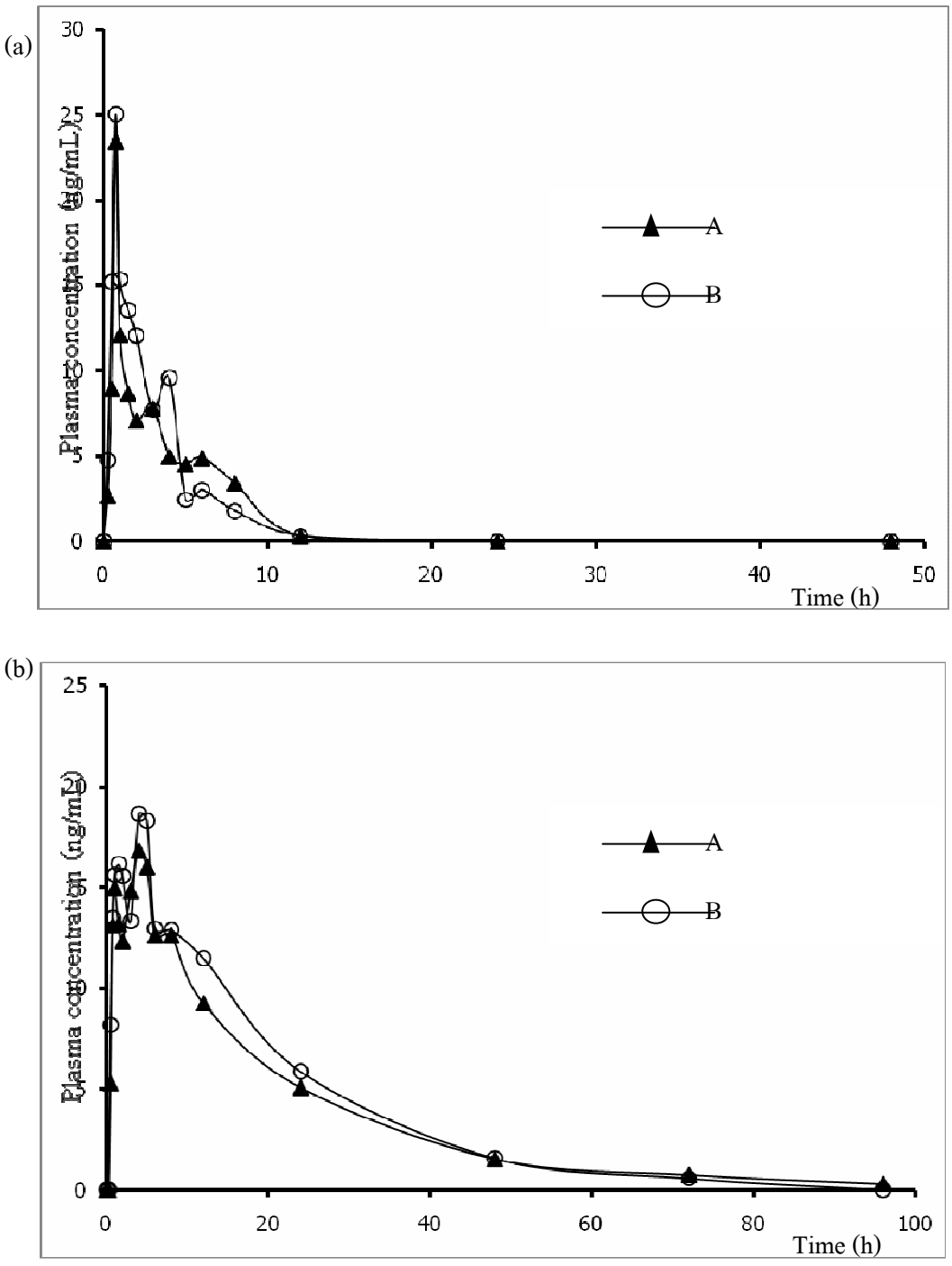


Figure 4.12 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 2

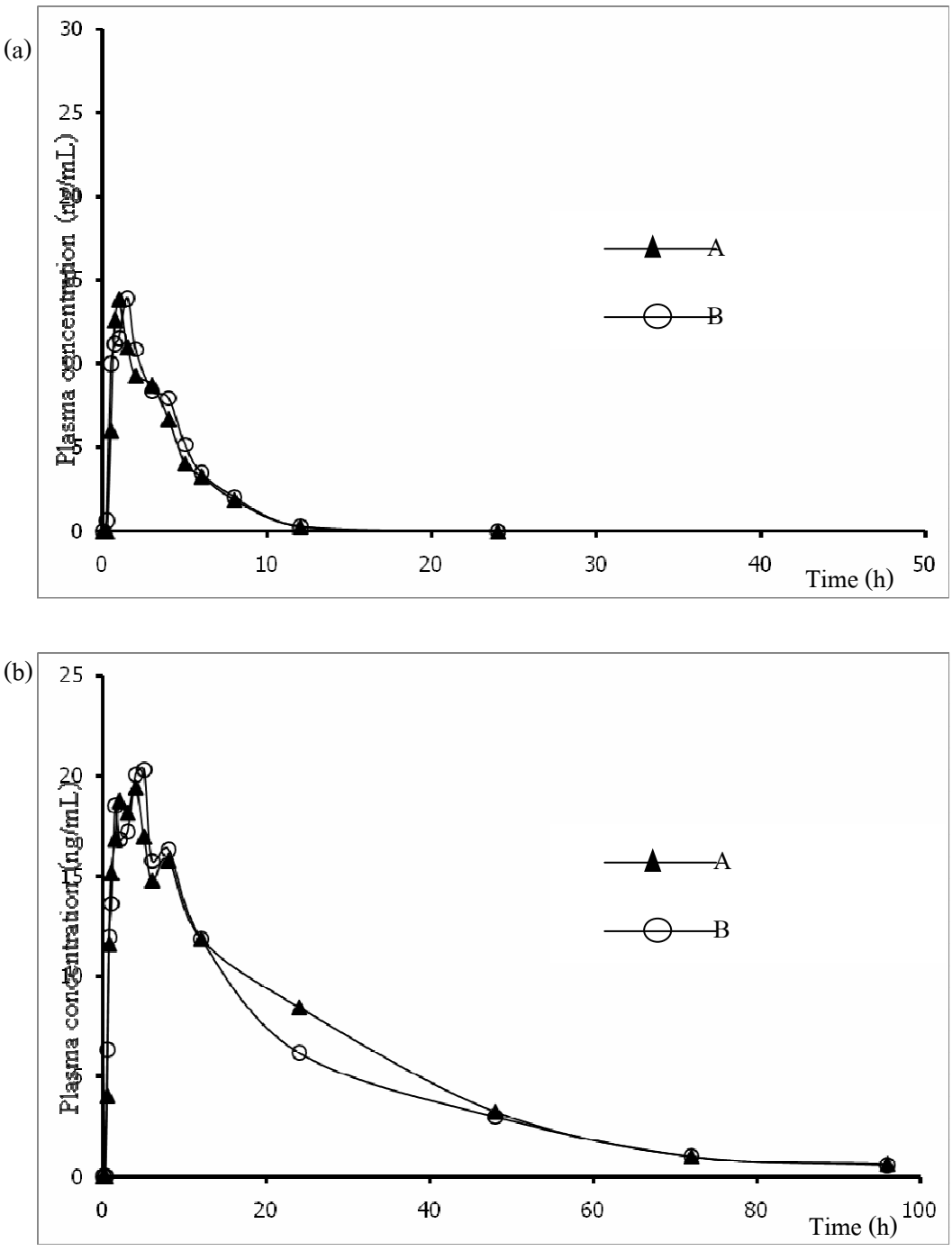


Figure 4.13 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 3

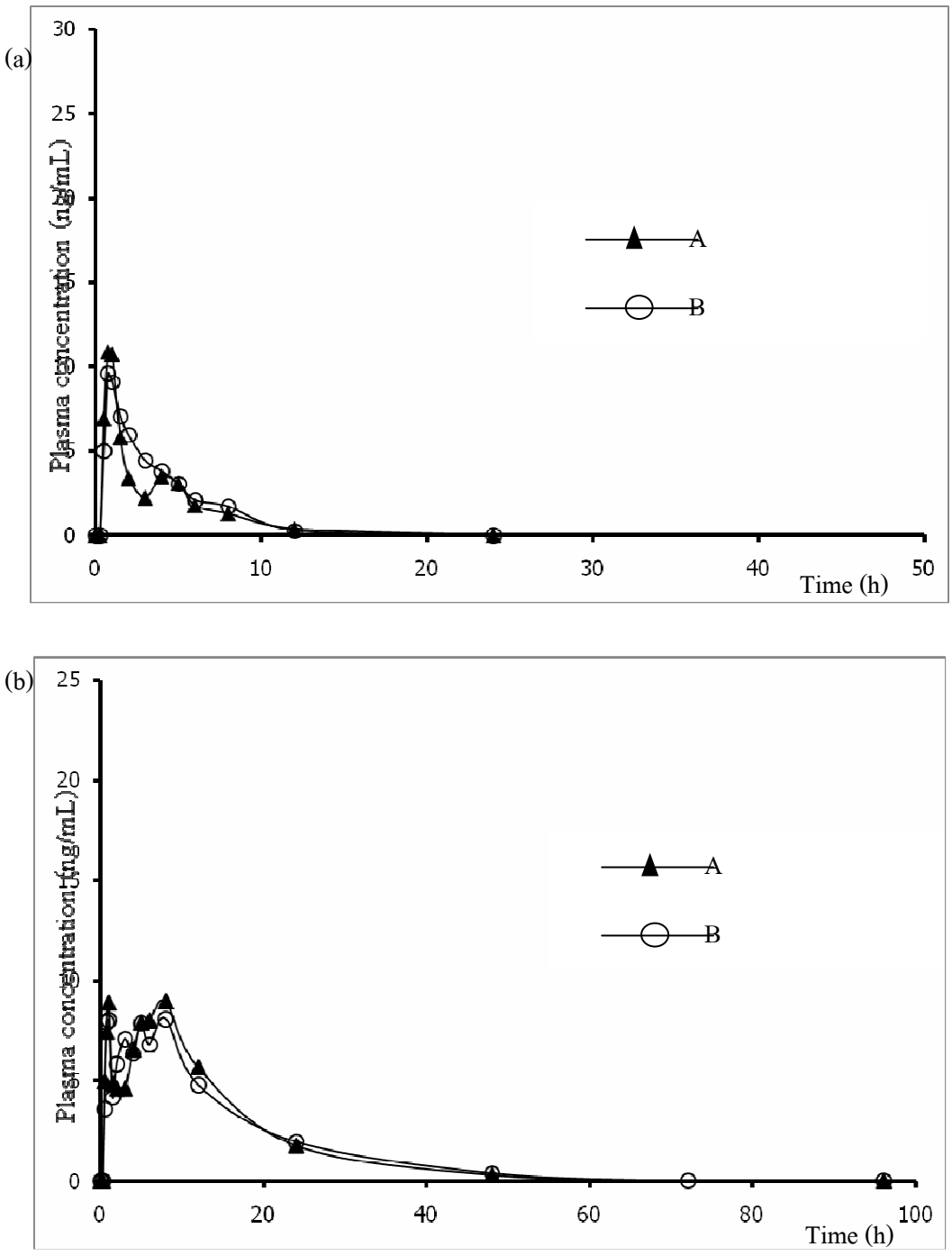
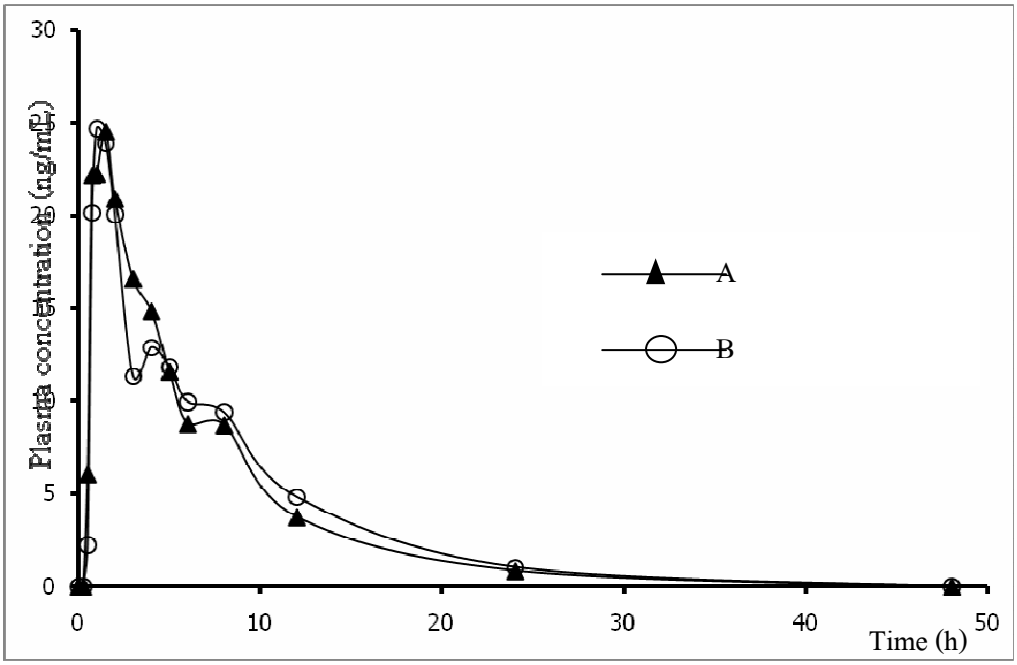


Figure 4.14 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 4

(a)



(b)

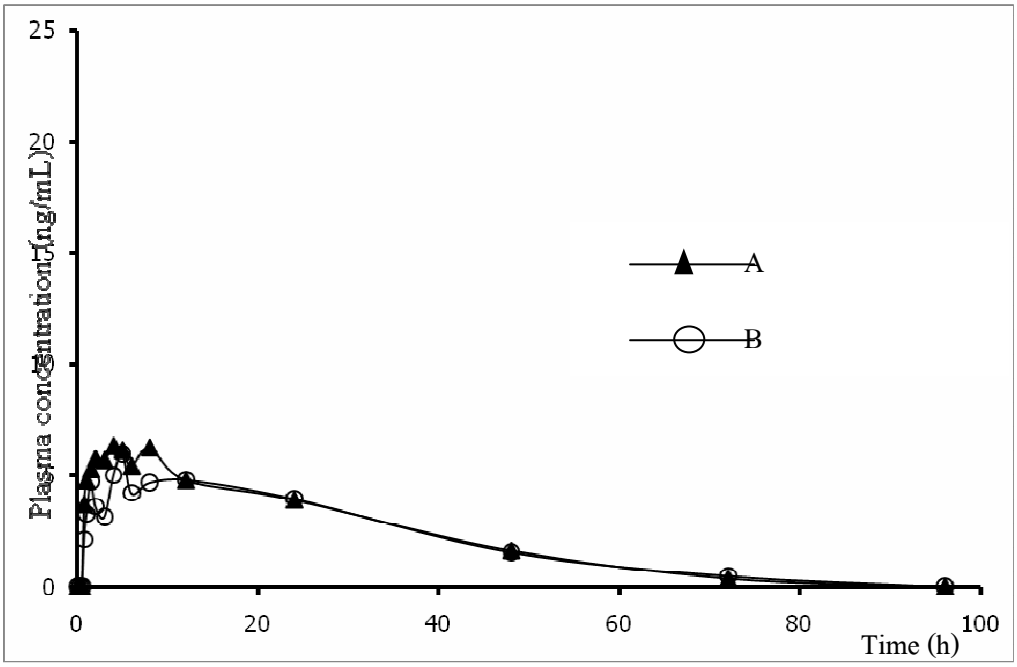


Figure 4.15 Plasma concentration-time profiles of 9OHRIS (a) and RIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 5

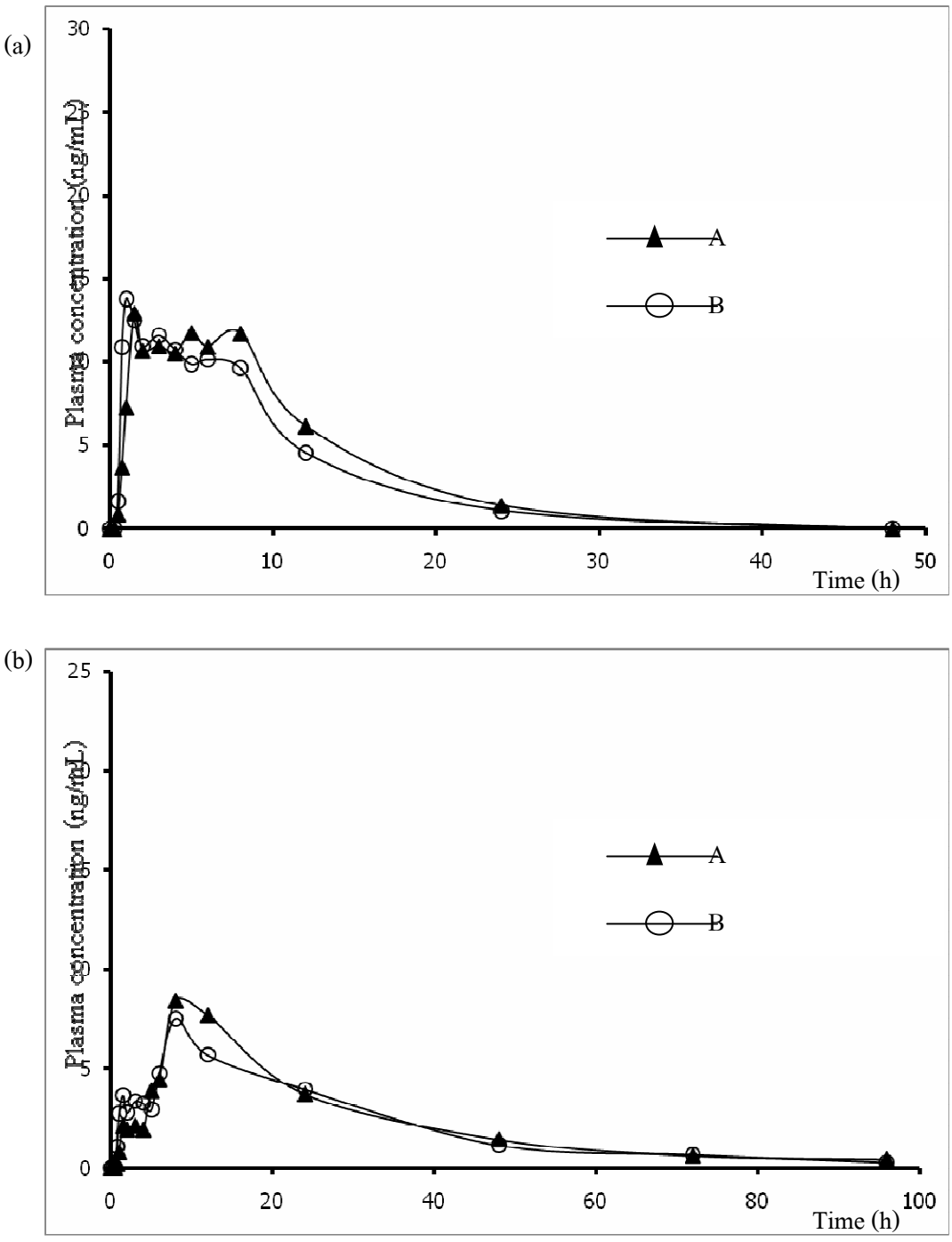


Figure 4.16 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 6

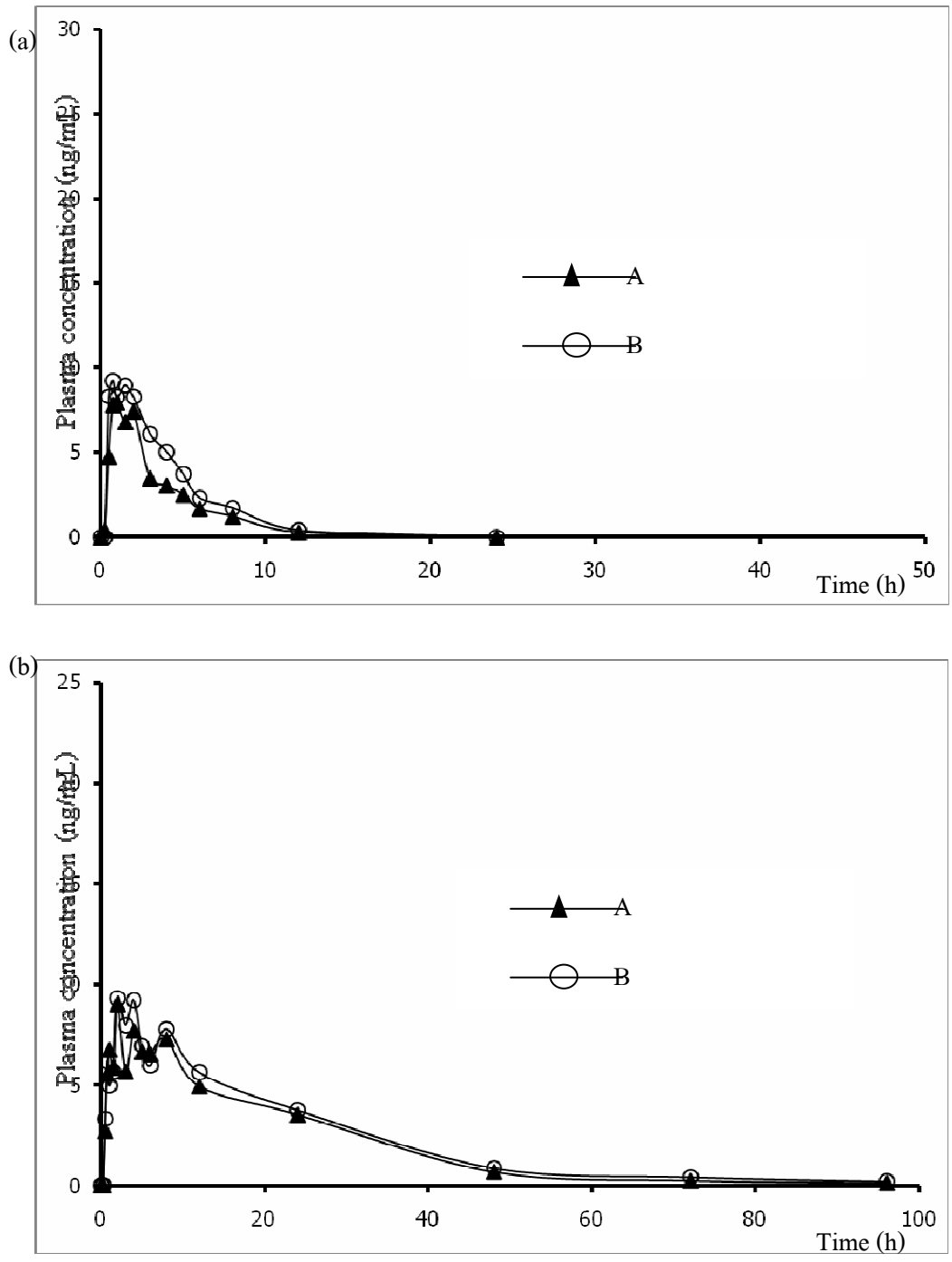


Figure 4.17 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 7

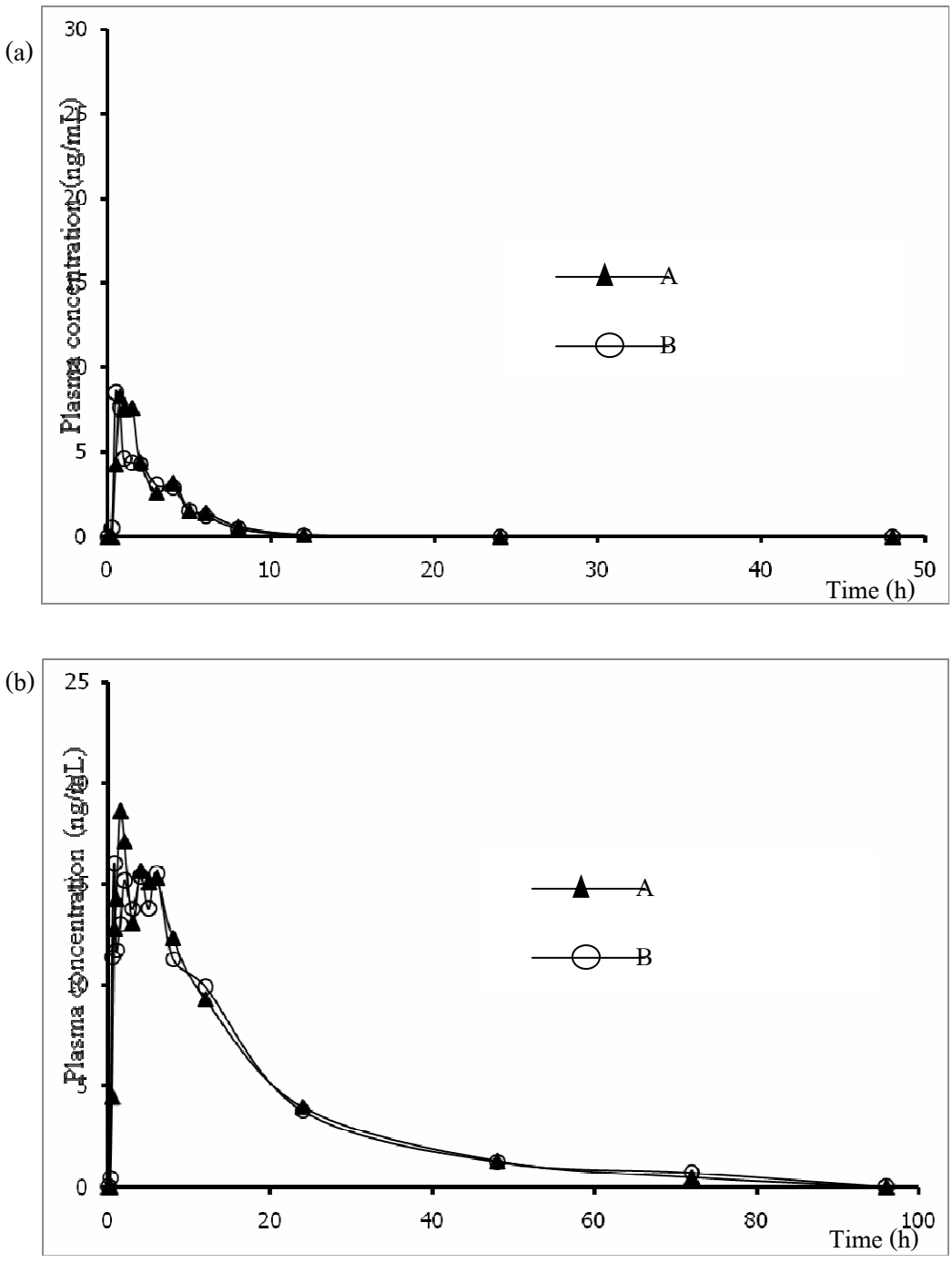


Figure 4.18 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 8

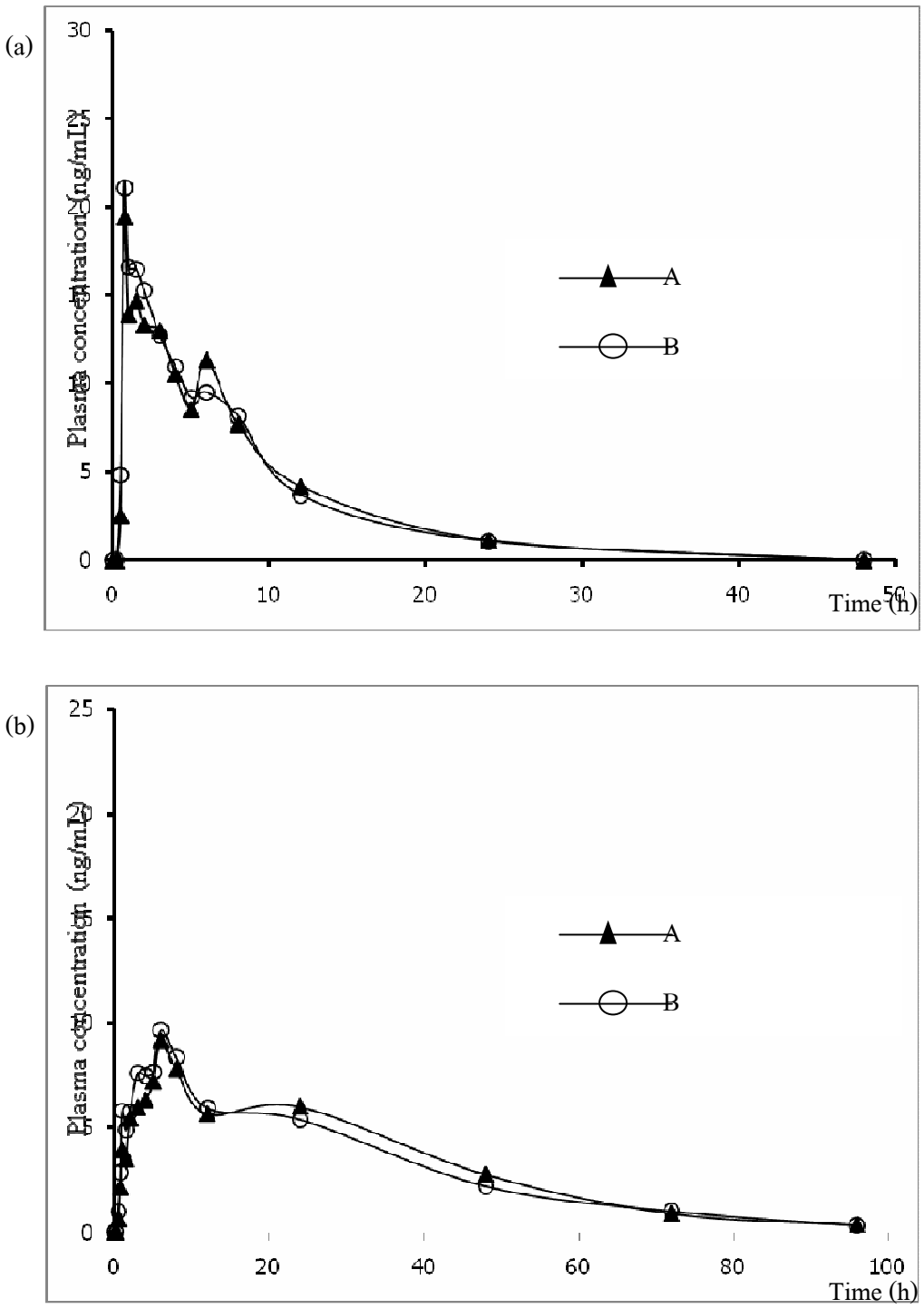


Figure 4.19 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 9

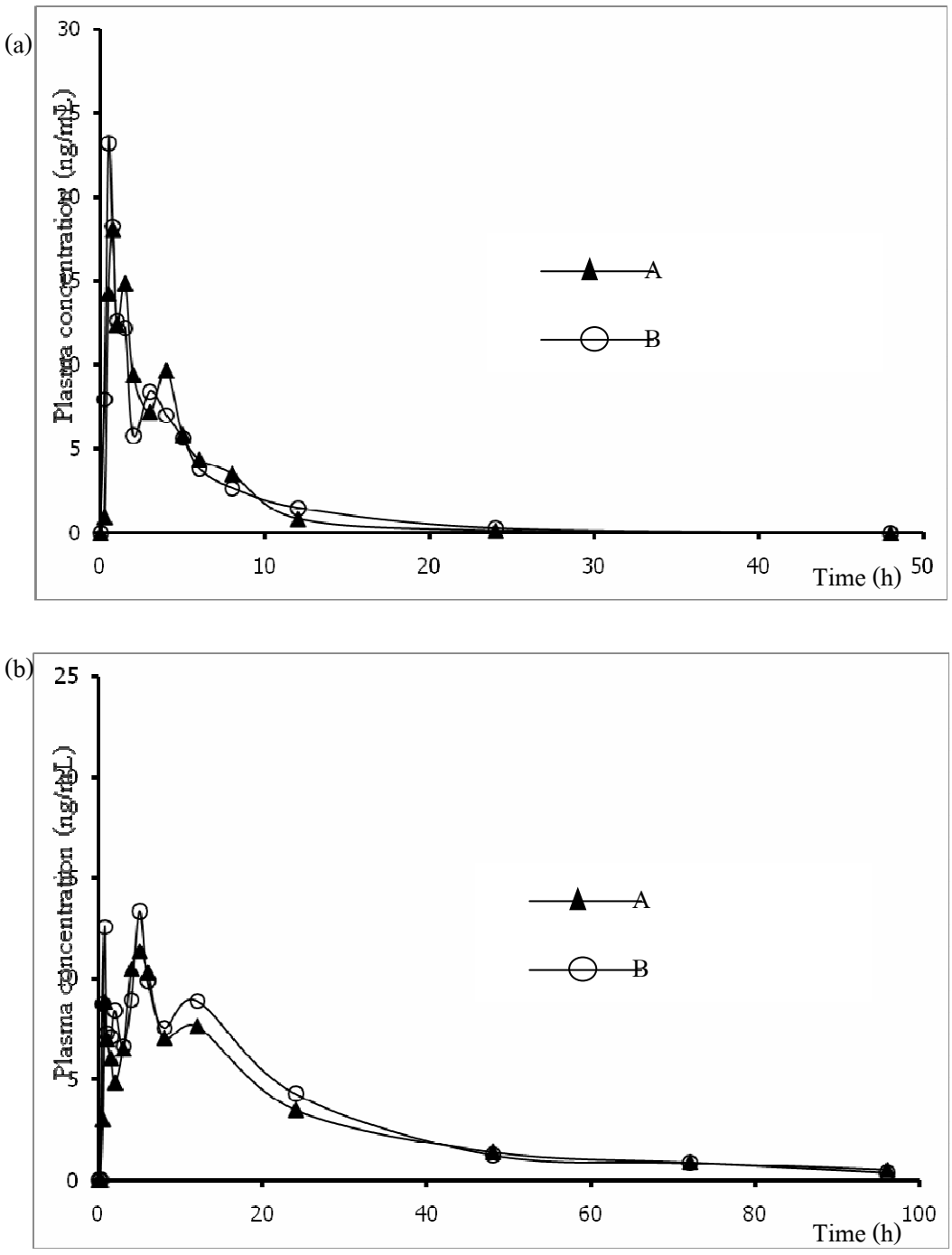


Figure 4.20 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 12

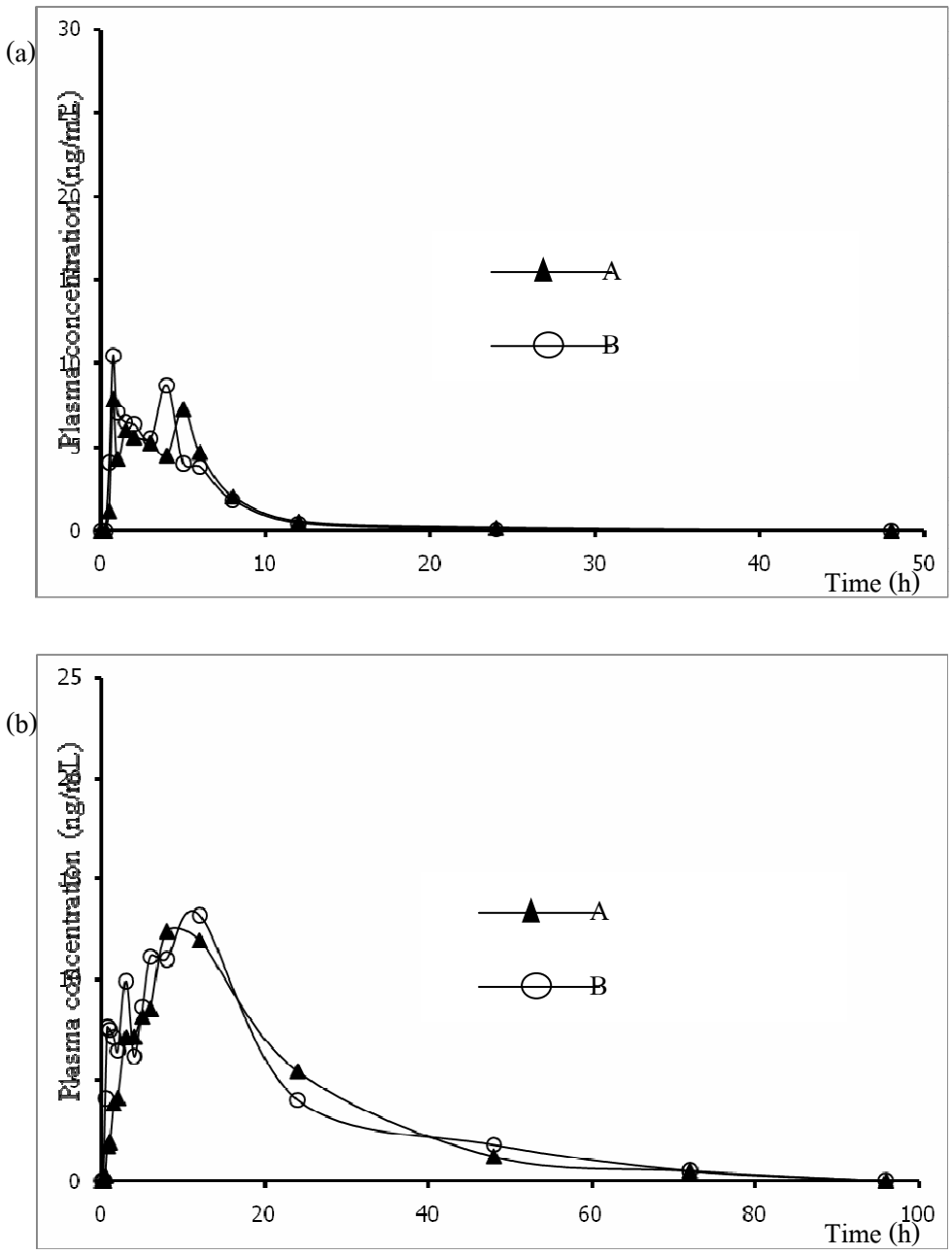


Figure 4.21 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 13

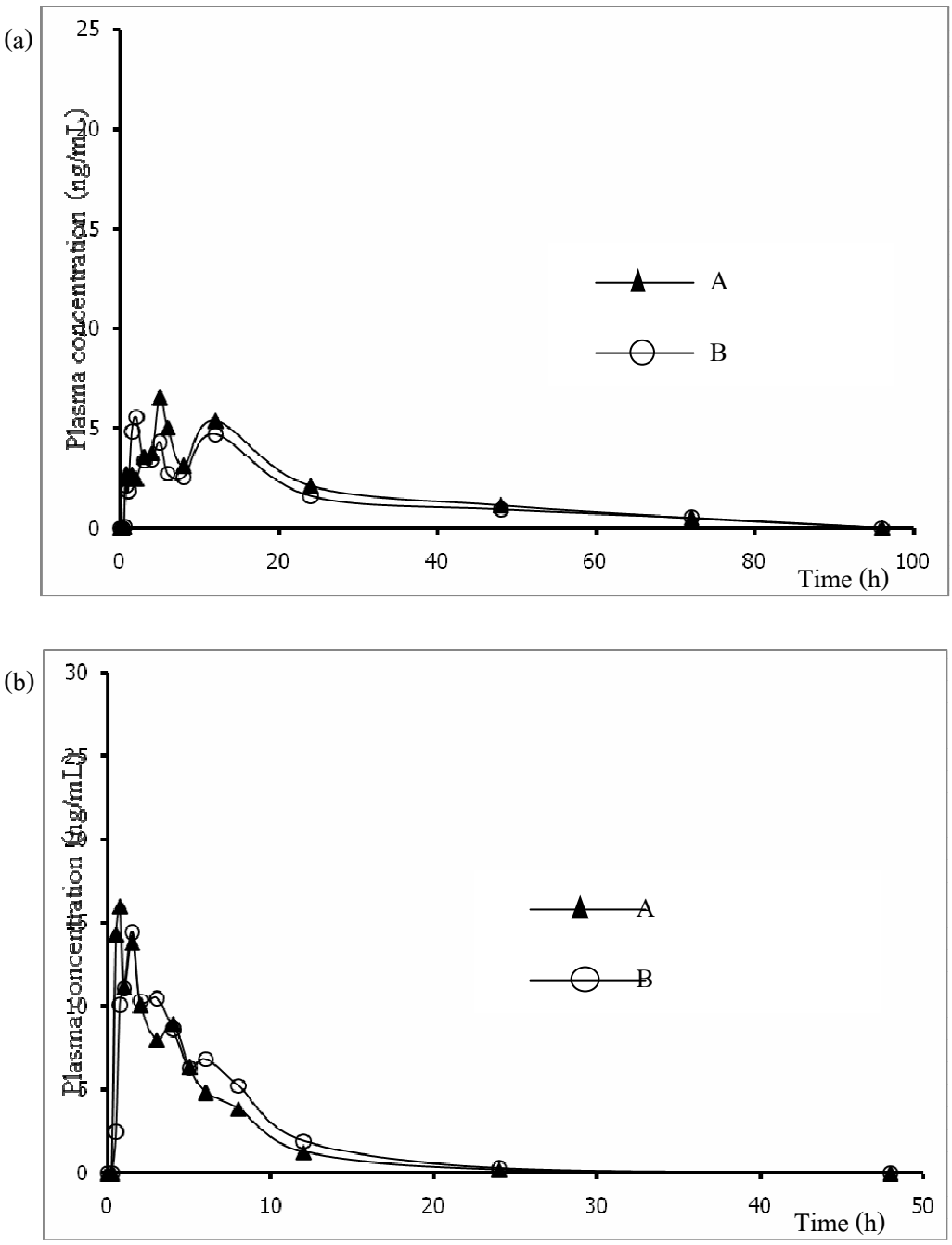


Figure 4.22 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 14

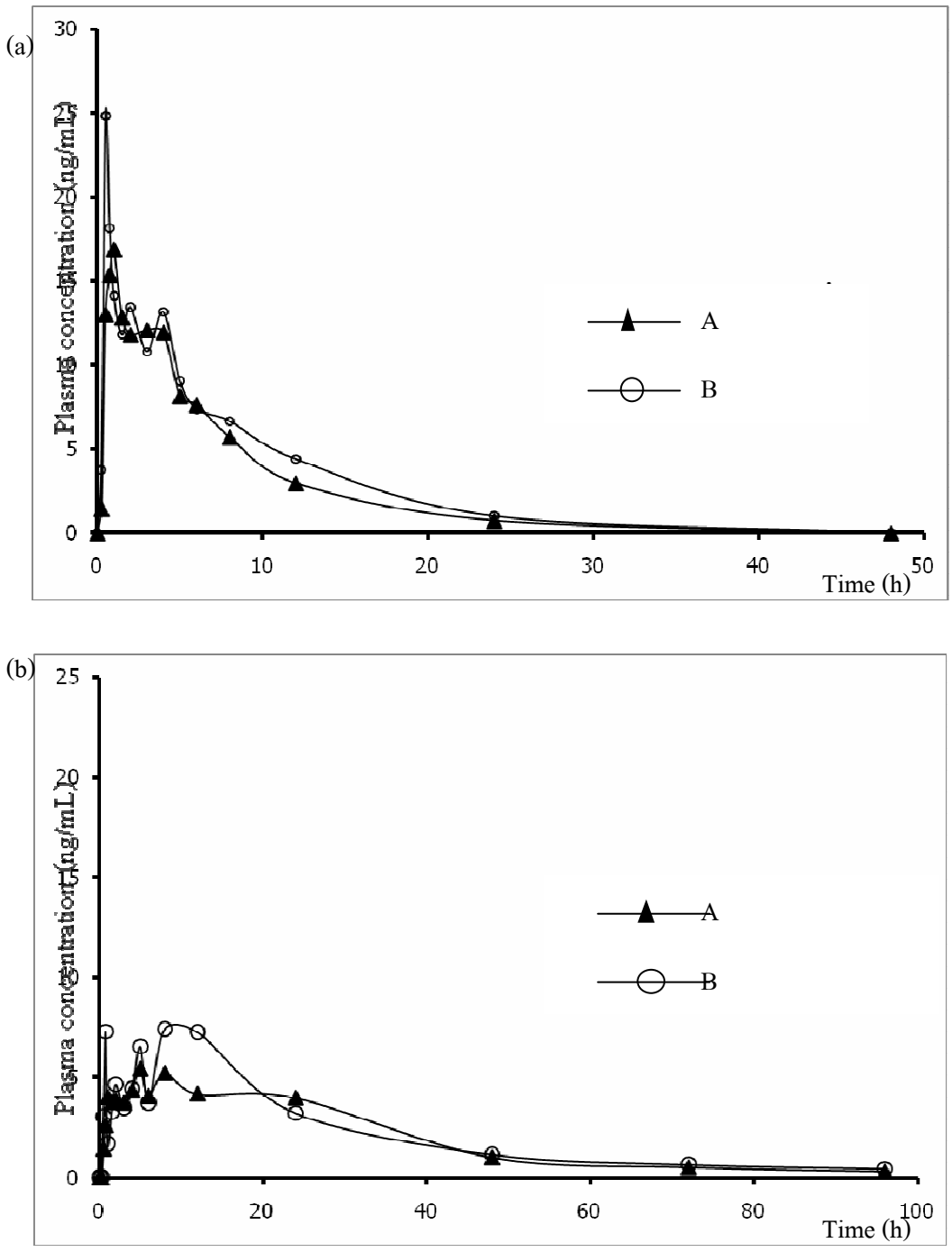


Figure 4.23 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 15

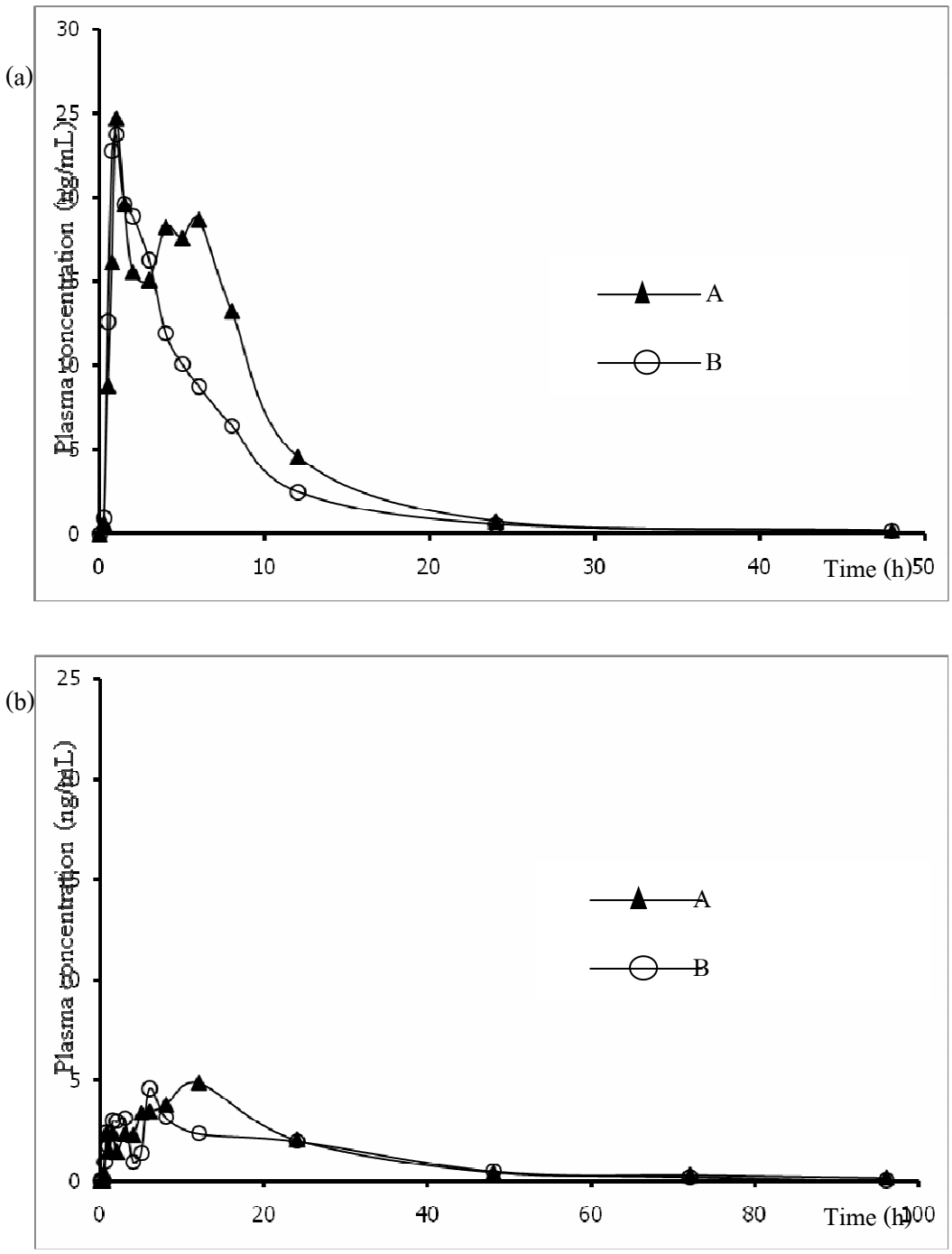


Figure 4.24 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 16

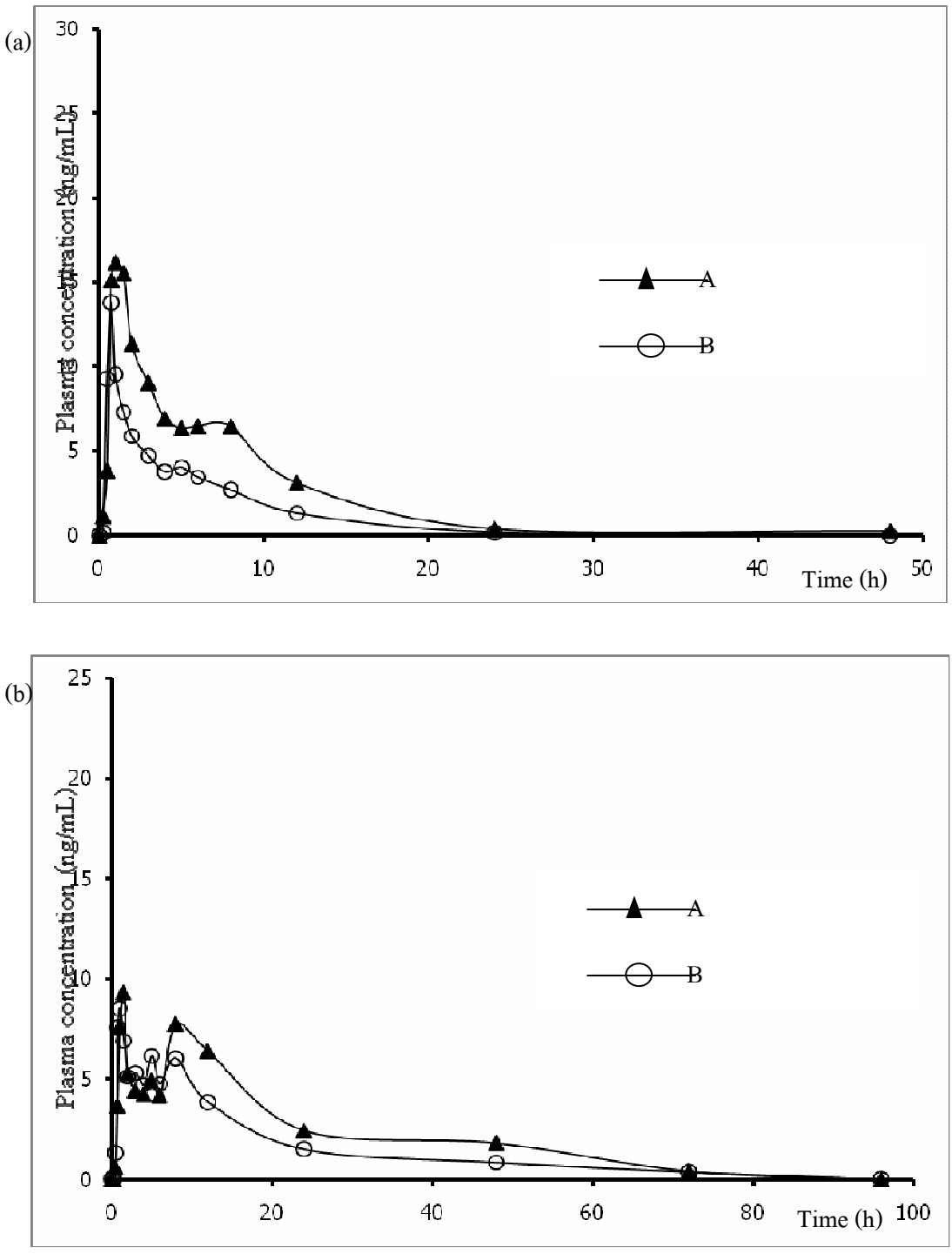
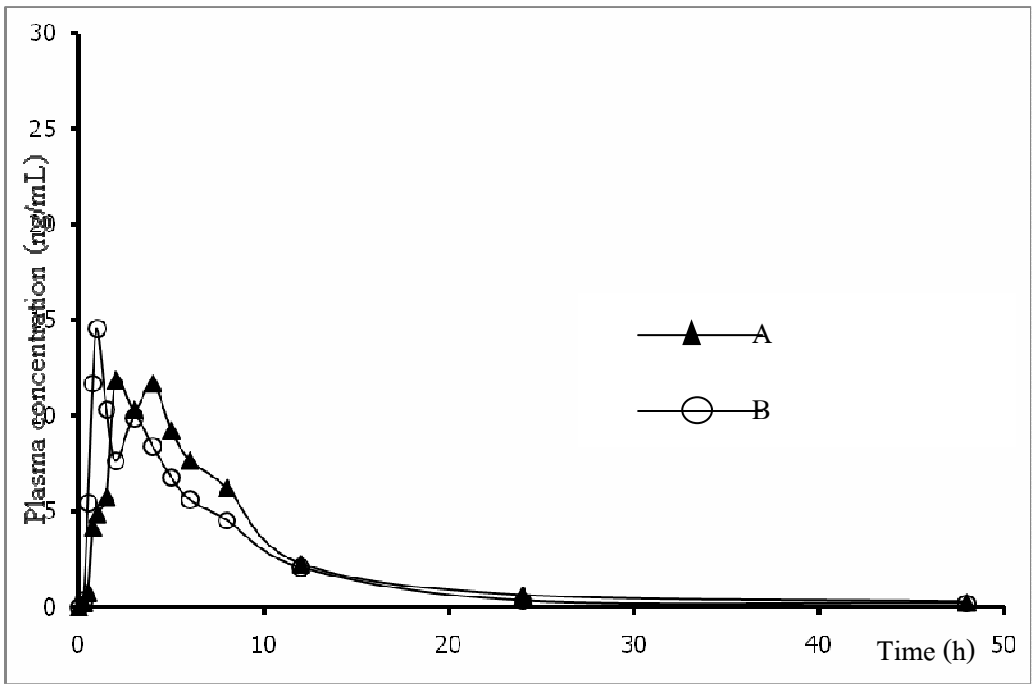


Figure 4.25 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 17

(a)



(b)

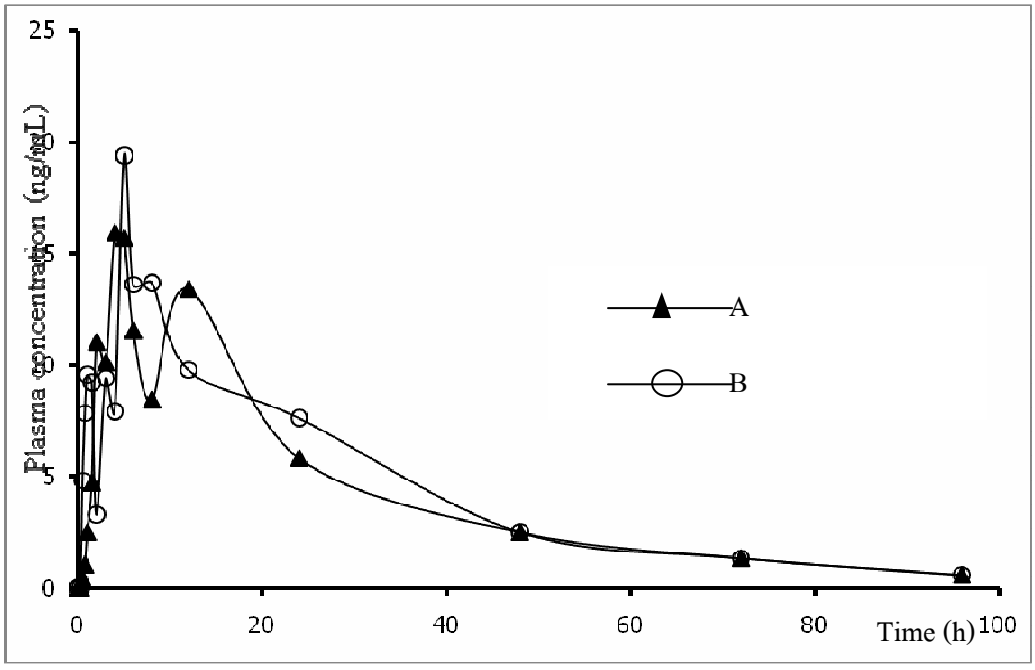


Figure 4.26 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 18

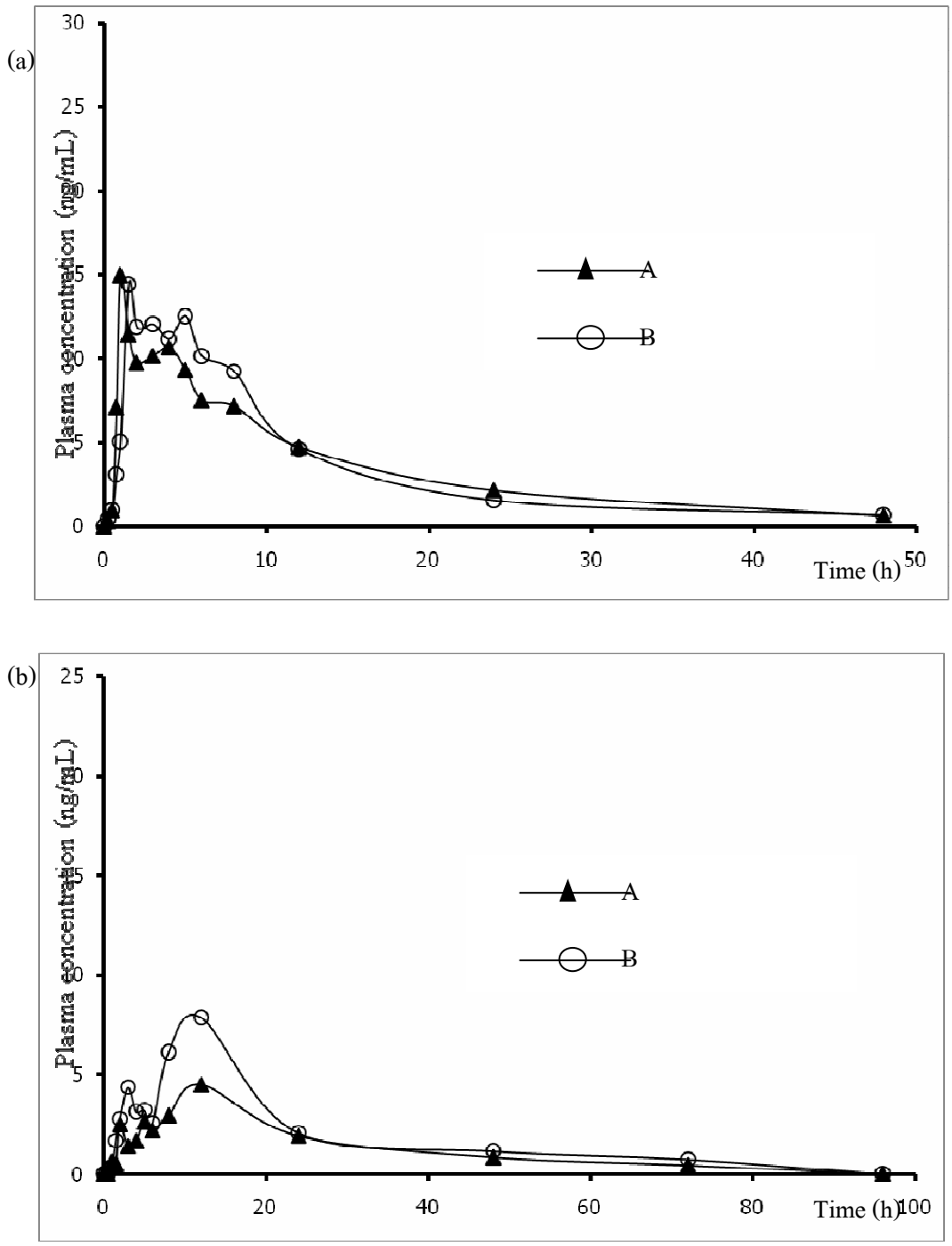


Figure 4.27 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 19

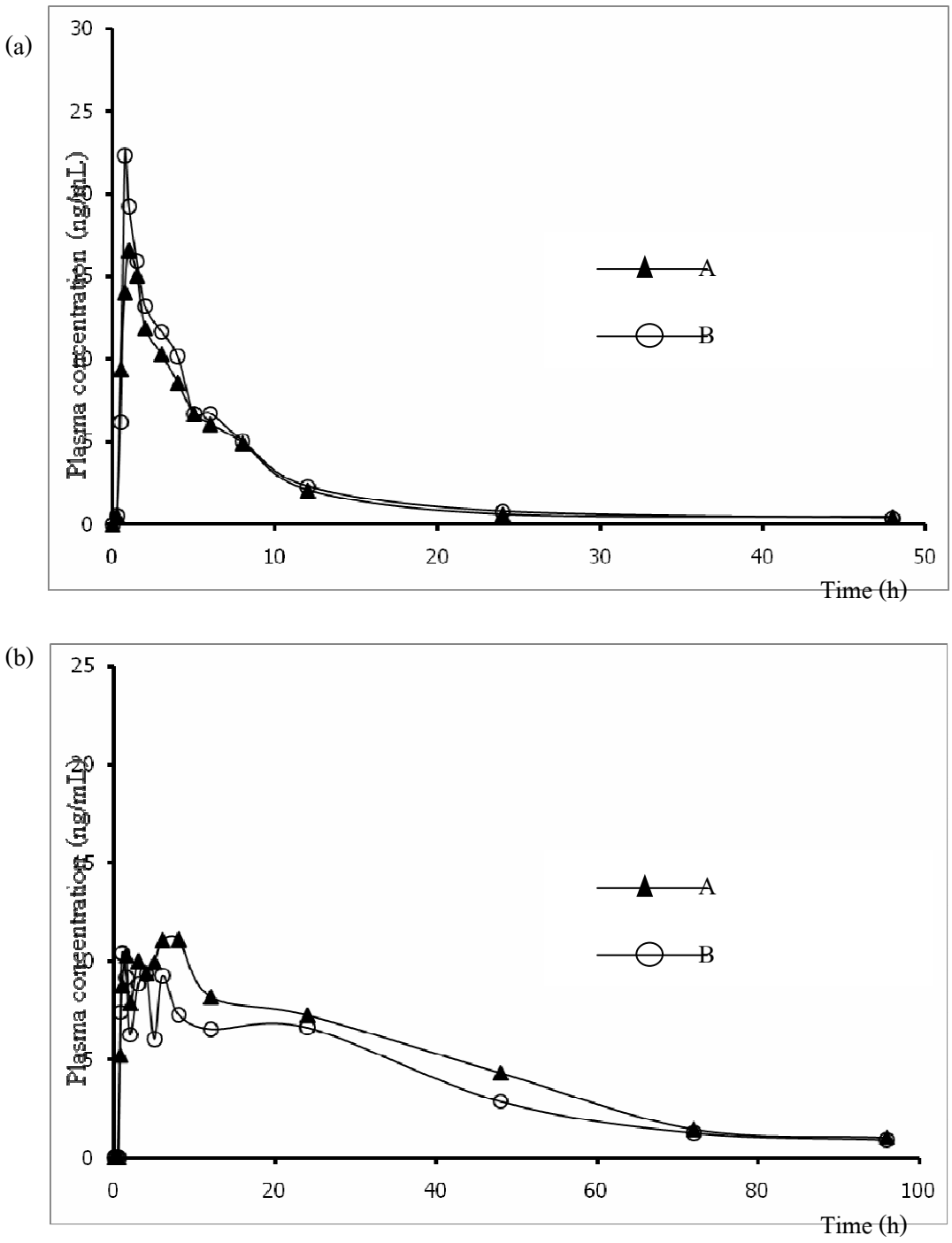


Figure 4.28 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 20

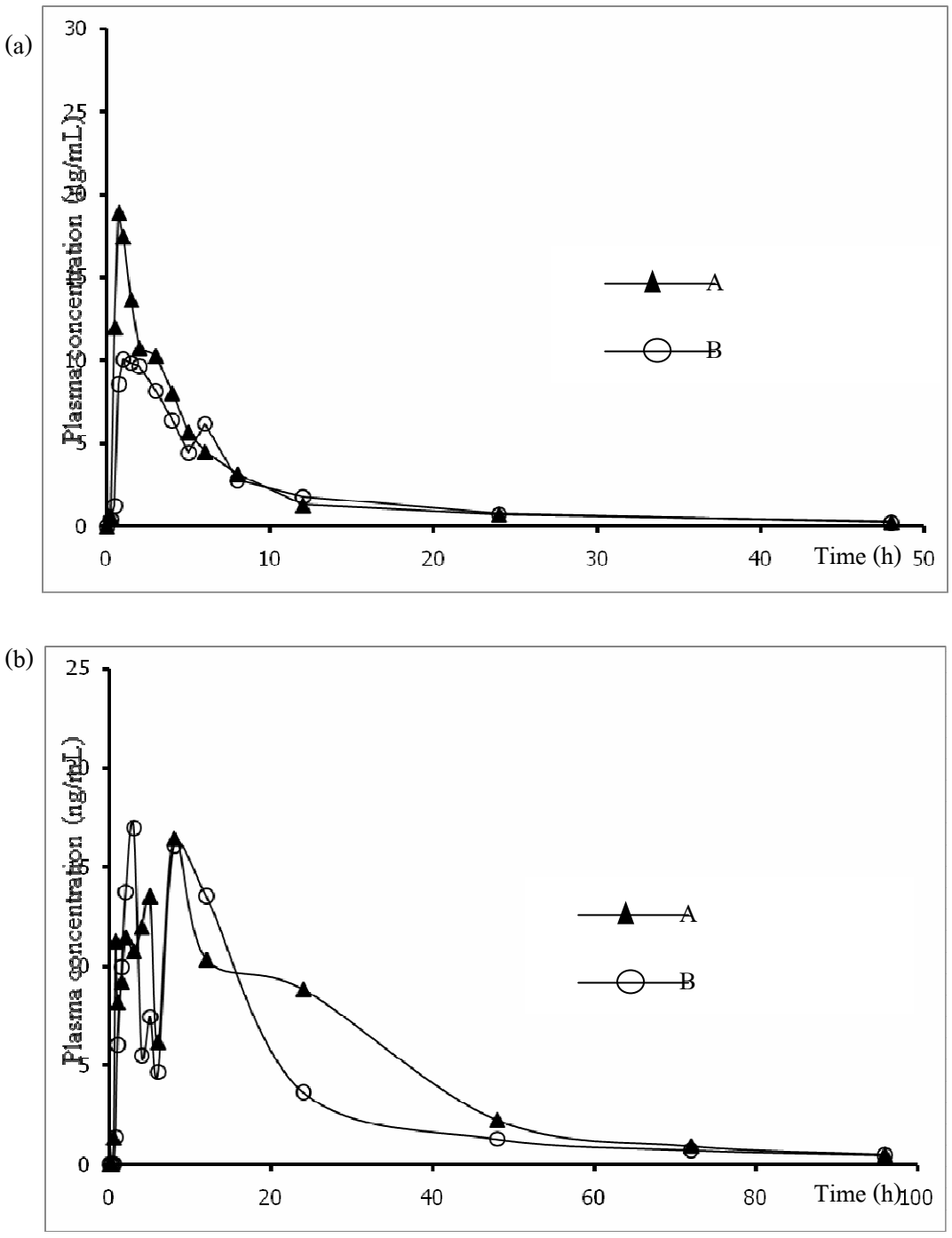


Figure 4.29 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 21

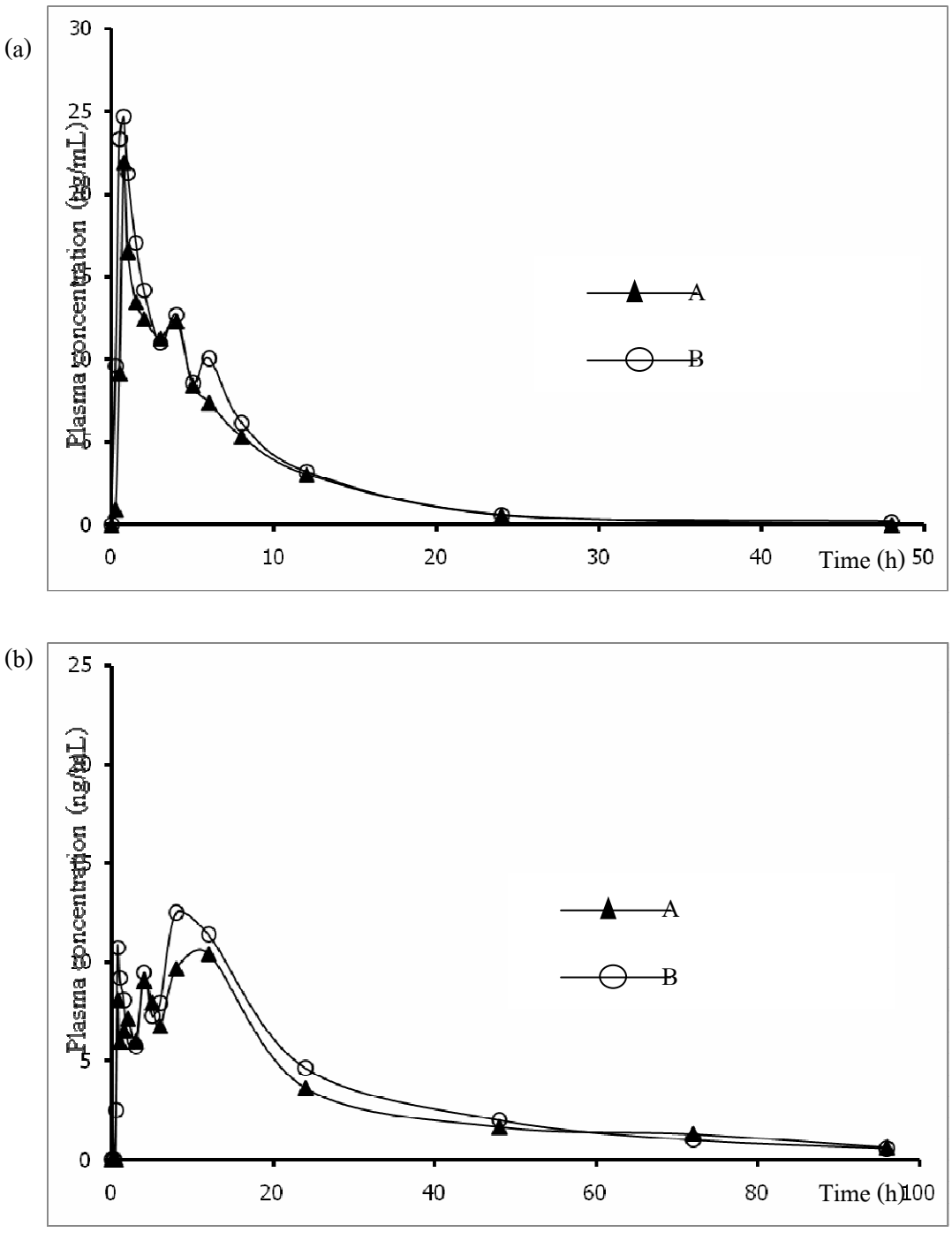


Figure 4.30 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 22

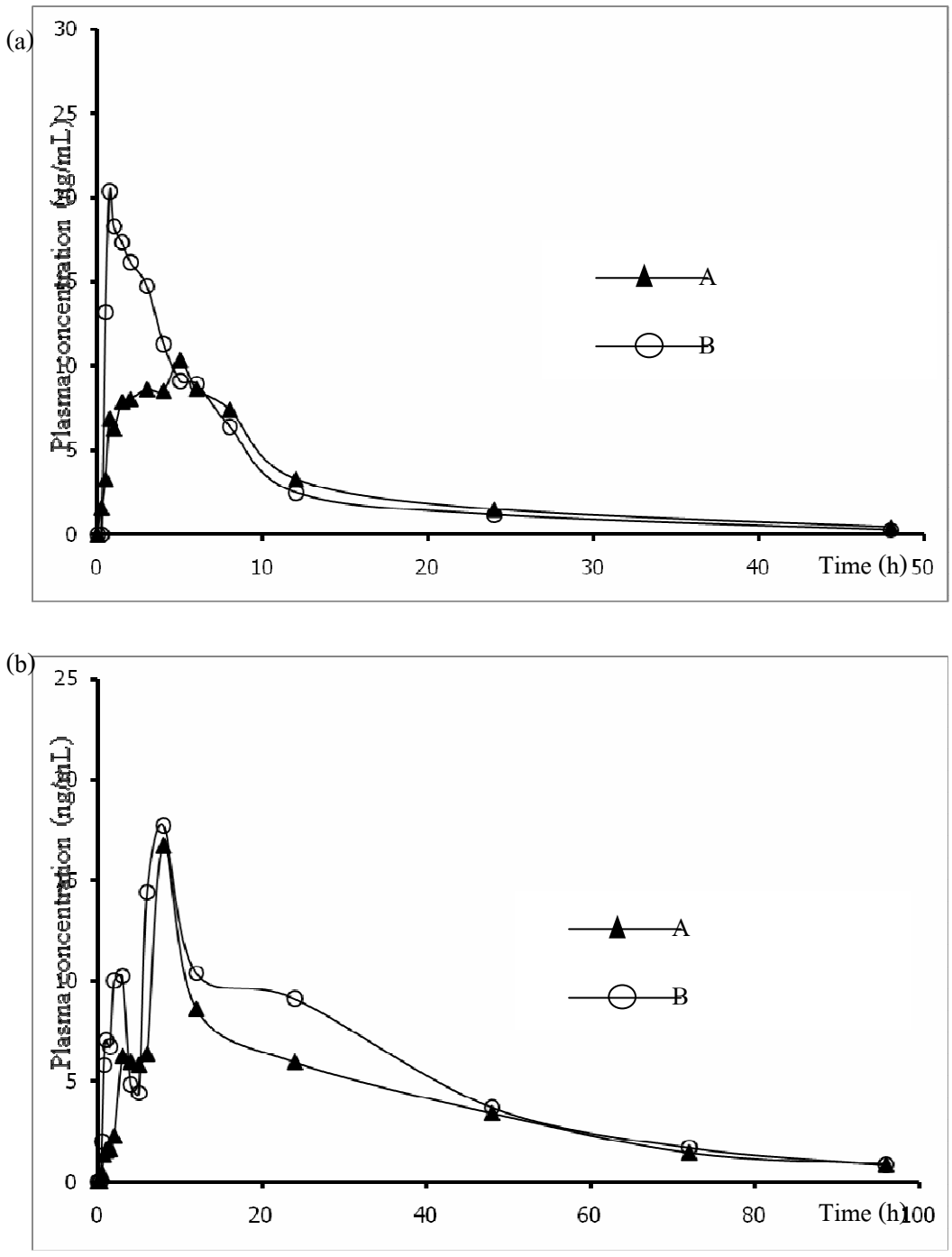
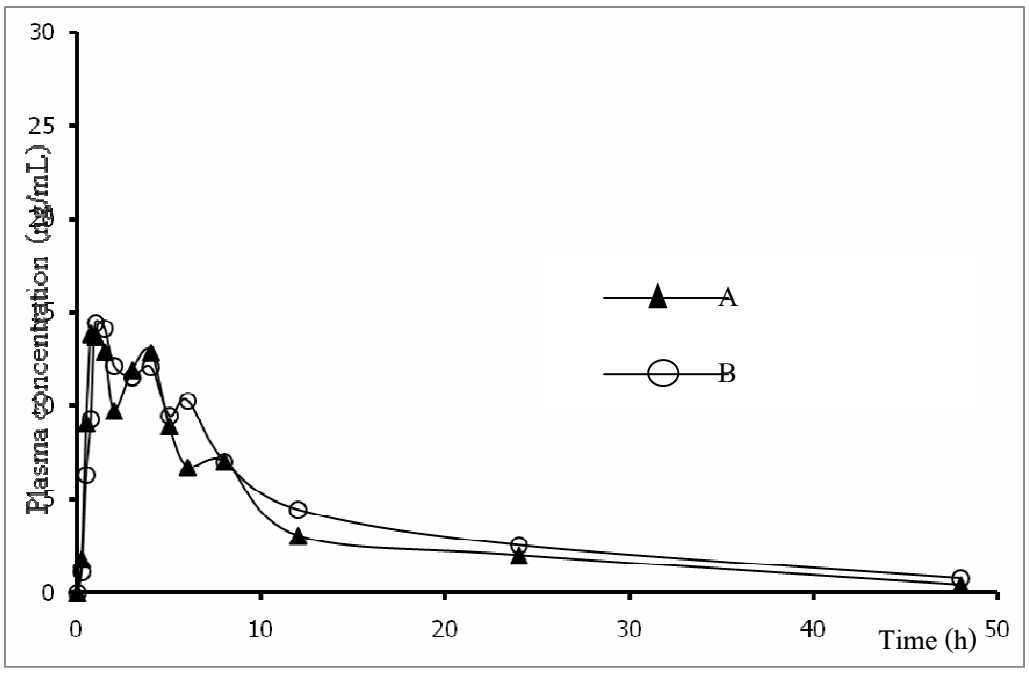


Figure 4.31 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 23

(a)



(b)

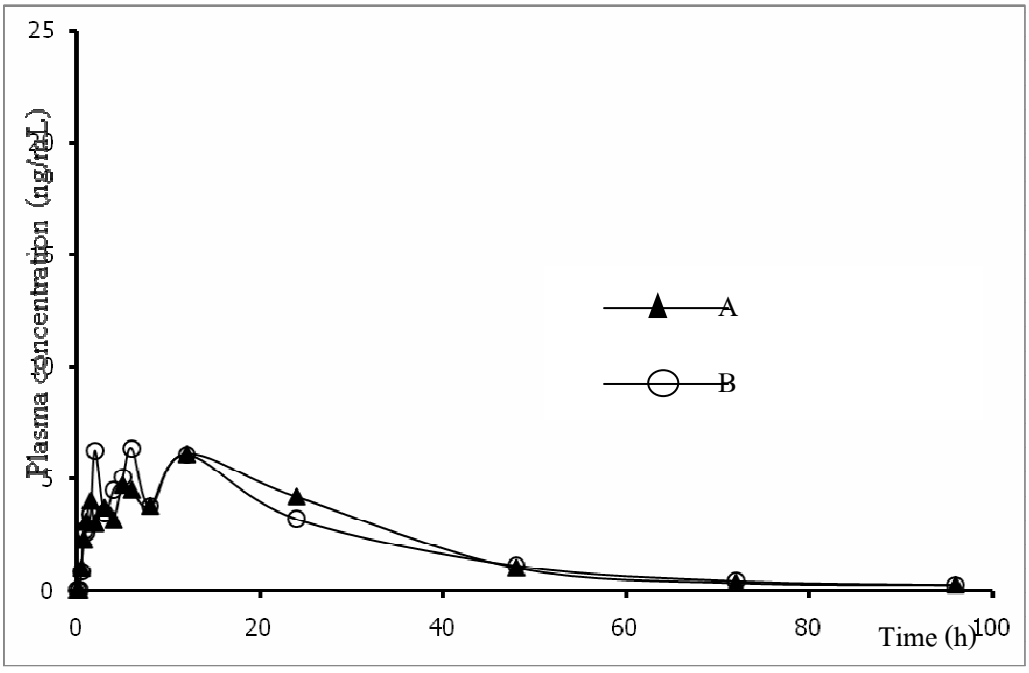


Figure 4.32 Plasma concentration-time profiles of RIS (a) and 9OHRIS (b) after single oral administration of 2 mg of Test (A) and reference (B) risperidone tablet to subject No. 24

Table 4.25 Adverse events of test and reference 2 mg risperidone tablet in the 24 subjects

Side effect	Reference		Test	
	Number	%	Number	%
Sedation	24	100	24	100
Orthostatic hypotension	3	12.5	3	12.5
Headache	1	4.2	2	8.3
Syncope	1	4.2	0	0

CHAPTER 5

CONCLUSION

5.1 Bioanalytical Method

Plasma concentration of RIS and 9OHRIS were simultaneously determined by liquid chromatography, electrospray ion trap, tandem mass spectrometric method. The method employs a simple liquid-liquid extraction for sample preparation with quantitative recovery for all analytes of interest. The LOQ is low enough to determine RIS and 9OHRIS with good intra- and inter-assay accuracy and precision. The validation results indicated that the method can be used for pharmacokinetic studies with desired precision and accuracy.

5.2 Bioequivalence study

The relative bioavailability study of a Thailand locally made 2 mg risperidone film coated tablet to the innovator's product was completed in 22 healthy Thai male volunteers. The pharmacokinetic parameters ($AUC_{0->t}$ and C_{max}) are as follows:-

Risperidone:

The $AUC_{0->48}$ were 100.67±48.89 ng.h/mL (test) and 97.74±45.78 ng.h/mL (reference). The C_{max} of test and reference products was 15.70±4.98 ng/mL and 17.00±5.81 ng/mL, respectively. There were no statistically significant difference of the pharmacokinetic parameters between the studied products (p>0.05) and the 90% CIs of the pharmacokinetic parameters based on ln transformed data were within the acceptable value of 80 – 125%.

9-hydroxyrisperidone:

The $AUC_{0->96}$ of test and reference drug products was 277.32±120.86 ng.h/mL and 273.16±121.23 ng.h/mL, respectively. The C_{max} of test and reference drug products was 11.06±5.13 ng/mL, and 11.47±5.21 ng/mL, respectively. There were no statistically significant difference of the pharmacokinetic parameters between the studied products (p>0.05) and the 90% CI of the pharmacokinetic parameters based on ln transformed data were within the acceptable value of 80 – 125%.

It can be concluded that the Thailand locally made product was bioequivalent with the innovator's product in both rate and extent of absorption.

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Laboratory data of 24 healthy volunteers enrolled in the study.

	Normal range	Results
Hemoglobin	13.3 - 16.0 g/dL	13.72±0.81
Hematocrit	40 -48%	36.7±12.21
WBC	5,000 - 10,000 cells/cu.mm	6308.33±1385.93
Platelets	Adequate	Adequate
FBS	70 - 110 mg%	77.75±6.36
BUN	5 - 23 mg%	12.52±4.28
Cr	0.8 - 1.4 mg%	1.08±0.11
SGOT	Up to 37 U/L	21.58±8.12
SGPT	Up to 40 U/L	24.04±5.8
ALP	39 - 117 U/L	81.74±20.64
TB	0.2 - 1.0 mg%	0.89±0.2
ALB	39 - 117 U/L	5.11±0.32
TP	6.7 - 8.3 g%	7.26±0.43

ที่ ศธ 0521.1.07/ 0681

คณะเภสัชศาสตร์
มหาวิทยาลัยสงขลานครินทร์
ตู้ ปณ. 7 คอหงส์
อ.หาดใหญ่ จ.สงขลา 90112

หนังสือฉบับนี้ให้ไว้เพื่อรับรองว่า

โครงการวิจัยเรื่อง : การศึกษาชีวสมมูลของยาเม็ด Risperidone ขนาด 2 มิลลิกรัม ในอาสาสมัครชายไทยสุขภาพดี

คณะผู้ดำเนินการวิจัย : ภญ.ดร.จุติมา บุญเลี้ยง และคณะ

ได้ผ่านการพิจารณาและได้รับความเห็นชอบจากคณะกรรมการ Ethics Committee ซึ่งเป็นคณะกรรมการพิจารณาศึกษาวิจัยในคนของคณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ แล้ว

ให้ไว้ ณ วันที่ 29 มีนาคม 2549

..... ประธานกรรมการ
(ผู้ช่วยศาสตราจารย์ ดร.สิริรัศมิ์ ปิ่นสุวรรณ)
รองคณบดีฝ่ายวิจัยและบริการวิชาการ
ปฏิบัติราชการแทน คณบดีคณะเภสัชศาสตร์

รหัส | | | | |

อาสาสมัคร

หนังสือแสดงความยินยอมเข้าร่วมโครงการวิจัย

(Consent Form)

โครงการวิจัยเรื่อง การศึกษาชีวสมมูลของยาเม็ดริสเพอริโดนขนาด 2 มิลลิกรัม ในอาสาสมัครชายไทยสุขภาพดี

วันให้คำยินยอม วันที่…….เดือน……พ.ศ……..

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

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

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

ก่อนลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้อ่านเอกสารชี้แจงข้อมูลและคำแนะนำสำหรับอาสาสมัครของโครงการวิจัยนี้ จำนวน 3 หน้าแล้ว และได้รับการอธิบายด้วยวาจาจาก…….. (ผู้วิจัย) ให้ทราบวัตถุประสงค์ของการวิจัย วิธีวิจัย อันตรายหรืออาการข้างเคียงที่อาจเกิดขึ้นจากการวิจัยหรือจากยาที่ใช้ ประโยชน์ที่จะได้รับจากการวิจัย รวมทั้งความรับผิดชอบของผู้วิจัยที่มีต่อข้าพเจ้าและ สิทธิของข้าพเจ้าในฐานะเป็นอาสาสมัครอย่างละเอียด และข้าพเจ้าได้มีโอกาสซักถามผู้วิจัย จนเป็นที่พอใจและมีความเข้าใจดีแล้ว โดยมีพยานชื่อ……..…รับรู้

ผู้วิจัยได้ตอบคำถามต่างๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจ ไม่ปิดบัง ซ่อนเร้นจนข้าพเจ้าพอใจ

ข้าพเจ้าเข้าร่วมโครงการศึกษานี้โดยสมัครใจ และมีสิทธิ์ที่จะบอกเลิกการเข้าร่วมโครงการวิจัยนี้เมื่อใดก็ได้ โดยการบอกเลิก จะไม่มีผลกระทบต่อสิทธิและการปฏิบัติใดๆ ที่ข้าพเจ้าจะได้รับต่อไป

ข้าพเจ้าอนุญาตให้ผู้วิจัยเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าแก่หน่วยงานที่เกี่ยวข้องได้ตามที่ผู้วิจัยเห็นสมควร ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับ และจะเปิดเผยได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย

ในการวิจัยครั้งนี้จะมีการเจาะเลือดใน 3 ช่วงของการศึกษา คือ ช่วงที่ 1 การเจาะเลือดเพื่อคัดเลือกอาสาสมัครเป็นจำนวน 2 ครั้ง ครั้งละประมาณ 5 มิลลิลิตร ช่วงที่ 2 เจาะเลือดในการศึกษาคาบที่ 1 เป็นจำนวน 14 ครั้งๆ ละ 5 มิลลิลิตร และช่วงที่ 3 เจาะเลือดในการศึกษาคาบที่ 2 เป็นจำนวน 14 ครั้งๆ ละ 5 มิลลิลิตร รวมปริมาตรเลือดที่ถูกเจาะในการศึกษาทั้ง 3 ช่วง ประมาณ 150 มิลลิลิตร

ผู้วิจัยได้อธิบายให้ข้าพเจ้าทราบและเข้าใจเป็นอย่างดีแล้วว่า การเจาะเลือดเพียงเล็กน้อยโดยทั่วไปจะไม่เกิดอันตรายใดๆ แก่ข้าพเจ้าเลย นอกจากอาจมีรอยช้ำบริเวณเจาะเล็กน้อย ซึ่งอาจหายได้เองภายใน 7 วัน

ผู้วิจัยรับรองว่า หากเกิดอันตรายใดๆ จากการวิจัยดังกล่าว ข้าพเจ้าจะได้รับการรักษาพยาบาลโดยไม่คิดมูลค่า และจะได้รับการชดเชยรายได้ที่สูญเสียไประหว่างการรักษาพยาบาลดังกล่าว ตลอดจนเงินทดแทนความพิการที่อาจจะเกิดขึ้น และรายละเอียดเกี่ยวกับการรักษาพยาบาลหรือเงินชดเชยดังกล่าวข้าพเจ้าสามารถติดต่อได้ที่ ภาควิชาเภสัชเคมี คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ โดยบุคคลที่เป็นผู้ประสานงานเรื่องนี้คือ ภญ.ดร.จุติมา บุญเลี้ยง

หากการกระทำของผู้วิจัยไม่เป็นที่ชัดเจน ข้าพเจ้าสามารถแจ้งต่อประธานคณะกรรมการพิจารณาจริยธรรมการวิจัย คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ หมายเลขโทรศัพท์ 0-7428-8909-10 ในวันและเวลาราชการ

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจทุกประการ จึงได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ โดยผู้วิจัยได้ให้เอกสารชี้แจงข้อมูลและคำแนะนำสำหรับอาสาสมัคร และสำเนาหนังสือแสดงความยินยอมเข้าร่วมโครงการวิจัย แก่ข้าพเจ้าอย่างละ 1 ฉบับ

ข้าพเจ้า □ ประสงค์ □ ไม่ประสงค์ ที่จะทราบผลการตรวจร่างกายและตรวจเลือดไม่ว่ากรณีใดๆ

ลงนาม…….... ผู้ยินยอมเข้าร่วมโครงการวิจัย ลงนาม……...…….....……ผู้วิจัย

ลงนาม……..... พยาน ลงนาม……..…….. พยาน

เอกสารชี้แจงข้อมูลและคำแนะนำสำหรับอาสาสมัคร

(Subject Information Sheet)

ชื่อโครงการวิจัย การศึกษาชีวสมมูลของยาเม็ดริสเพอริโดนขนาด 2 มิลลิกรัม ในอาสาสมัครชายไทยสุขภาพดี
(Bioequivalence Study of Risperidone 2 mg Tablets in Healthy Thai Male Volunteers)

สถาบันที่ทำการศึกษา คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ และศูนย์วิทยาศาสตร์การแพทย์สงขลา

เรียน ท่านผู้อ่านที่นับถือ

คณะผู้วิจัยขอเชิญท่านเป็นอาสาสมัครในโครงการวิจัยข้างต้น เพื่อประกอบการตัดสินใจของท่าน คณะผู้วิจัยขอให้ข้อมูลเกี่ยวกับโครงการวิจัยดังรายละเอียดข้างล่าง ขอให้ท่านใช้เวลาอ่านและทำความเข้าใจรายละเอียดตามเอกสารที่ท่านได้รับจำนวน 3 หน้า อย่างถี่ถ้วน ในกรณีที่ท่านไม่เข้าใจหรือสงสัยประการใด ท่านสามารถซักถามผู้วิจัยหรือผู้ที่ให้ข้อมูลแก่ท่านได้อย่างเต็มที่ ไม่ต้องเกรงใจ ถ้าท่านสมัครใจเข้าร่วมในโครงการวิจัยนี้ ขอให้ท่านลงชื่อในเอกสารแสดงความยินยอมเข้าร่วมโครงการวิจัย ท้ายเอกสารนี้

สรุปโครงการวิจัยโดยย่อ

รีสเพอริโดน (Risperidone) เป็นยาที่ใช้ในการรักษาโรคจิตเภทที่มีประสิทธิภาพดีในการควบคุมอาการของโรค โดยทำให้เกิดอาการข้างเคียงน้อย โดยทั่วไปผู้ป่วยสามารถทนต่อยาได้ดี การศึกษาชีวสมมูลของยามีวัตถุประสงค์เพื่อเปรียบเทียบอัตราและปริมาณการดูดซึมของยาเม็ด รีสเพอริโดน ระหว่างผลิตภัณฑ์ยาที่ผลิตโดยบริษัทยาในประเทศไทยกับยาต้นแบบ การศึกษาเป็นแบบข้ามเชิงสุ่ม ในอาสาสมัครผู้ชายจำนวน 24 คน โดยการศึกษามีสองรอบ การศึกษาในรอบที่สองจะเว้นระยะห่างหลังจากการเจาะเลือดในการศึกษารอบแรกสิ้นสุดอย่างน้อย 10 วัน ก่อนการศึกษาในแต่ละรอบ อาสาสมัครจะได้รับการตรวจร่างกายและตรวจเลือดที่โรงพยาบาลสงขลานครินทร์ ตามเกณฑ์ข้อกำหนดของการศึกษาวิจัย เพื่อประเมินสุขภาพ อาสาสมัครที่แพทย์ยืนยันว่ามีสุขภาพดี และเป็นไปตามเกณฑ์การคัดเลือกอาสาสมัครเข้าร่วมการศึกษาจะได้รับการพิจารณาให้เข้าร่วมการศึกษา ในการศึกษาแต่ละรอบอาสาสมัครจะได้รับประทานยาเม็ดรีสเพอริโดน ขนาด 2 มิลลิกรัม จำนวน 1 เม็ด เพียงครั้งเดียว พร้อมน้ำปริมาตร 240 มิลลิลิตร โดยอาจได้รับผลิตภัณฑ์ยาต้นแบบ หรือผลิตภัณฑ์ยาที่ผลิตในประเทศไทยสลับกันในแต่ละรอบ ก่อนการรับประทานยาอาสาสมัครต้องงดอาหารอย่างน้อย 10 ชั่วโมงโดยสามารถดื่มน้ำได้ตามต้องการยกเว้น 1 ชั่วโมงก่อนและหลังรับประทานยา โดยจะมีการเก็บตัวอย่างเลือดทั้งหมด 17 ครั้ง ครั้งละ 5 มิลลิลิตร คิดเป็นปริมาตรรวม 85 มิลลิลิตร ที่เวลา 0 (ก่อนรับประทานยา), 15, 30, 45, 60 และ 90 นาที และที่เวลา 2, 3, 4, 5, 6, 8, 12, 24, 48, 72 และ 96 ชั่วโมง หลังการรับประทานยา โดยจะมีการเจาะเลือดจากเส้นเลือดดำท้องแขน โดยการใส่ท่อค้างไว้ เพื่อลดความเจ็บปวดและเพื่อความสะดวกในการเก็บตัวอย่างเลือด โดยที่อาสาสมัครไม่ต้องเจ็บทุกครั้งที่มีการเก็บตัวอย่างเลือด หลังจากเก็บตัวอย่างเลือดที่เวลา 12 ชั่วโมงแล้วจะถอดท่อที่ท้องแขนออก และเจาะเลือดใหม่ที่เวลา 24, 48, 72 และ 96 ชั่วโมง เลือดที่เก็บได้จะนำไปวิเคราะห์หาระดับความเข้มข้นของยาและเมแทบอไลต์ที่ออกฤทธิ์ เพื่อหาค่าระดับความเข้มข้นสูงสุดของยา เวลาที่ระดับความเข้มข้นของยามีค่าสูงสุด พื้นที่ใต้กราฟทั้งหมด อัตราการขับยาออกจากร่างกาย และค่าครึ่งชีวิตของยาในร่ายกาย และเปรียบเทียบข้อมูลที่ได้จากผลิตภัณฑ์ยาที่ผลิตโดยบริษัทยาในประเทศไทยกับยาต้นแบบ ด้วยวิธีการทางสถิติเพื่อใช้ประกอบในการขึ้นทะเบียนยา

วัตถุประสงค์

เพื่อเปรียบเทียบอัตราและปริมาณการดูดซึมของผลิตภัณฑ์ยาเม็ด Risperidone ที่ผลิตโดยบริษัทยาในประเทศไทยกับยาต้นแบบ

ประโยชน์ที่คาดว่าได้รับ

อาสาสมัครอาจไม่ได้รับประโยชน์โดยตรงจากการศึกษานี้ แต่ผลจากการศึกษาจะเป็นข้อมูลที่สำคัญให้แก่บุคลากรทางการแพทย์ ในการพิจารณาเลือกใช้ผลิตภัณฑ์ยาที่มีคุณภาพเท่าเทียมกันโดยอาจไม่จำเป็นต้องใช้ผลิตภัณฑ์ยาต้นแบบจาก
ต่างประเทศ ซึ่งมีราคาแพง เป็นการประหยัดงบประมาณของชาติ และลดภาระค่าใช้จ่ายของผู้ป่วย

ข้อปฏิบัติสำหรับอาสาสมัครก่อนและระหว่างเข้าร่วมการศึกษา

1. อาสาสมัครต้องงดอาหารก่อนการศึกษาอย่างน้อย 10 ชั่วโมง โดยสามารถดื่มน้ำได้ตามต้องการยกเว้น 1 ชั่วโมงก่อนและหลังการศึกษา
2. หลังรับประทานยาอย่างน้อย 4 ชั่วโมง อาสาสมัครจึงรับประทานอาหารได้ โดยอาหารที่อาสาสมัครรับประทานเป็นอาหารชนิดเดียวกันที่ผู้ทำการศึกษาจัดให้
3. ตลอดการศึกษา อาสาสมัครต้องไม่สูบบุหรี่ ไม่ดื่มเครื่องดื่มที่มีแอลกอฮอล์
4. ในระหว่างการเก็บตัวอย่างเลือดอาสาสมัครต้องไม่ออกกำลังกาย
5. อาสาสมัครต้องไม่รับประทานเครื่องดื่มที่มีส่วนผสมของคาเฟอีน เช่น ชา กาแฟ ชอคโกแลต เป็นต้น ก่อนการศึกษาอย่างน้อย 48 ชั่วโมงและหลังการรับประทานยาจนกระทั่งเสร็จสิ้นการเก็บตัวอย่างเลือดจุดสุดท้ายในแต่ละรอบของการศึกษา
6. อาสาสมัครต้องไม่ได้รับยาใดๆ ก่อนการศึกษาอย่างน้อย 2 สัปดาห์ และจนกระทั่งสิ้นสุดการศึกษา

ความเสี่ยงที่อาจเกิดขึ้นระหว่างการศึกษา

1. อาสาสมัครอาจเกิดอาการข้างเคียงจากการรับประทานยา อาการข้างเคียงที่พบบ่อยได้แก่ อาการง่วงนอน, วิงเวียน, ปวดศีรษะ และ เครียด ส่วนอาการข้างเคียงที่พบได้น้อยได้แก่ ความดันโลหิตต่ำ, คลื่นไส้, อาเจียน, ปวดท้อง ซึ่งเป็นอาการข้างเคียงที่พบเมื่อให้ยาในขนาดสูง (มากกว่า 4 มิลลิกรัมต่อวัน) สำหรับในการศึกษานี้อาสาสมัครจะได้รับยาในขนาดต่ำ (2 มิลลิกรัม) เพียงครั้งเดียว โอกาสเกิดอาการข้างเคียงจึงมีน้อยมาก นอกจากนี้อาการข้างเคียงที่เกิดขึ้นไม่รุนแรง และกลับสู่สภาพปกติได้
2. การใส่ท่อที่เส้นเลือดดำท้องแขนเพื่อเก็บตัวอย่างเลือด และการเจาะเลือด อาจทำให้เกิดความเจ็บปวดเล็กน้อยและเป็นรอยช้ำได้ แต่อาการนี้จะหายได้เองภายใน 7 วัน

การดูแลอาสาสมัครและดำเนินการเมื่อเกิดอันตรายจากการศึกษา

ในระหว่างการศึกษา อาสาสมัครจะอยู่ภายใต้การดูแลอย่างใกล้ชิดจากแพทย์ผู้ดูแลอาสาสมัคร พยาบาล และผู้ร่วมวิจัย โดยจะทำการตรวจวัดความดันเลือด และอัตราการเต้นของชีพจร ก่อนการรับประทานยา และทุกสองชั่วโมงหลังการรับประทานยา จนครบ 12 ชั่วโมง และที่เวลา 24, 48, 72 และ 96 ชั่วโมง ตลอดจนทำการซักถามเพื่อติดตามอาการไม่พึงประสงค์ตลอดระยะเวลาการศึกษา หากตรวจพบอาการผิดปกติอันเกี่ยวเนื่องจากการได้รับยาในโครงการวิจัยนี้ อาสาสมัครจะได้รับการดูแลวินิจฉัยและรักษาจากแพทย์ผู้ดูแลอาสาสมัคร และจะ

ได้รับการรักษาทางการแพทย์อย่างเต็มที่โดยคณะผู้ศึกษาได้เตรียมยาฉุกเฉิน และยาแก้แพ้ต่างๆ พร้อมทั้งยาสามัญที่จำเป็นไว้พร้อม ณ สถานที่ทำการศึกษา

การตอบแทน ชดเชยแก่อาสาสมัคร

อาสาสมัครไม่ต้องเสียค่าใช้จ่ายใดๆในการเข้าร่วมการศึกษา และจะได้รับค่าตอบแทนเป็นเงิน 2,000 บาท (สองพันบาทถ้วน) ต่อการได้รับยา 1 ครั้ง

หากอาสาสมัครเกิดอาการผิดปกติใดๆ อันมีสาเหตุเกี่ยวข้องกับการศึกษาวิจัย ทางคณะผู้ศึกษาวิจัยและบริษัทผู้สนับสนุนทุนวิจัย ซึ่งเป็นผู้ผลิตยาทดสอบที่ใช้ในการศึกษานี้ จะรับผิดชอบดูแลและจ่ายค่ารักษาพยาบาล ค่าชดเชยรายได้ที่สูญเสียไประหว่างการรักษาพยาบาล และเงินทดแทนความพิการ ที่อาจเกิดขึ้นแก่อาสาสมัครอย่างสมเหตุผล

สภาวการณ์ และ/หรือเหตุผลที่อาจเพิกถอนอาสาสมัครจากการวิจัย

1. อาสาสมัครเกิดอาการไม่พึงประสงค์ที่แพทย์เห็นควรให้ออกจากการศึกษา
2. อาสาสมัครไม่ปฏิบัติตนตามข้อกำหนดของการศึกษา
3. อาสาสมัครต้องการถอนตัวออกจากการศึกษา

การเก็บรักษาความลับ

ข้อมูลเกี่ยวกับการตรวจร่างกายและผลการทดลองของอาสาสมัครจะถูกเก็บเป็นความลับไม่แสดงชื่อและนามสกุลจริง โดยจะเปิดเผยเฉพาะในรูปของผลการวิจัยโดยรวม อย่างไรก็ตามท่านสามารถแจ้งความประสงค์ที่จะรับทราบผลการตรวจเลือดและการตรวจร่างกายของตนเองได้ และในกรณีที่ผลการตรวจมีความผิดปกติที่แพทย์ผู้ตรวจมีความเห็นว่ามีความสำคัญต่อสุขภาพ ซึ่งอาจทำให้ท่านไม่เข้าเกณฑ์การเข้าร่วมการศึกษา ท่านจะได้รับการแจ้งให้ทราบ เว้นแต่ท่านได้แจ้งความประสงค์ว่าไม่ต้องการทราบผลการตรวจร่างกาย ในหนังสือแสดงความยินยอมเข้าร่วมโครงการวิจัย

การติดต่อกับผู้ดำเนินการศึกษา

หากอาสาสมัครมีคำถามใดๆ เกี่ยวกับการศึกษานี้ หรือมีอาการผิดปกติเกิดขึ้นจากการได้รับยาในการศึกษา สามารถติดต่อกับคณะผู้วิจัยดังนี้

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Pipatrattanaseree, W. and Boonleang, J. Liquid chromatography electrospray ion trap tandem mass spectrometric method for the determination of risperidone and 9-hydroxyrisperidone in human plasma. *The 67th World Congress of Pharmacy and Pharmaceutical Sciences 2007*, 31 August – 6 September, 2007, China. (Abstract)