เภสัชจลนศาสตร์ประชากรของเนวิราพีนในผู้ป่วยติดเชื้อเอชไอวี

นางสาวธนพร วัฒนกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชกรรมคลินิก ภาควิชาเภสัชกรรมปฏิบัติ
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2554

POPULATION PHARMACOKINETICS OF NEVIRAPINE IN HIV-INFECTED PATIENTS

Miss Thanaporn Wattanakul

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Pharmacy Program in Clinical Pharmacy

Department of Pharmacy Practice

Faculty of Pharmaceutical Sciences

Chulalongkorn University

Academic Year 2011

Thesis Title	POPULATION PHARMACOKINETICS OF NEVIRAPINE IN HIV-INFECTED PATIENTS
By	Miss Thanaporn Wattanakul
Field of Study	Clinical Pharmacy
Thesis Advisor	Baralee Punyawudho, Ph.D.
Thesis Co-advisor	Anchalee Avihingsanon, M.D.

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

..... Dean of the Faculty of
(Associate Professor Pintip Pongpech, Ph.D.) Pharmaceutical Sciences

THESIS COMMITTEE

..... Chairman
(Thitima Wattanavijitkul, Ph.D.)

..... Thesis Advisor
(Baralee Punyawudho, Ph.D.)

..... Thesis Co-advisor
(Anchalee Avihingsanon, M.D.)

..... Examiner
(Assistant Professor Wanchai Treyaprasert, Ph.D.)

..... External Examiner
(Weerawat Manosuthi, M.D.)

ธนพร วัฒนกุล : เภสัชจลนศาสตร์ประชากรของเนวิราพีนในผู้ป่วยติดเชื้อเอชไอวี. (POPULATION PHARMACOKINETICS OF NEVIRAPINE IN HIV-INFECTED PATIENTS) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: อ.ดร.บราลี ปัญญาวุธโธ, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: พญ.อัญชลี อวิหิงสานนท์ 79 หน้า.

ภูมิหลัง ระดับยาเนวิราพีนในพลาสมามีความสัมพันธ์กับการตอบสนองของไวรัส ความล้มเหลวในการรักษา และอาการไม่พึงประสงค์ ระดับยาเนวิราพีนมีความผันแปรระหว่างบุคคลสูง ดังนั้นการระบุที่มาของความผันแปรทางเภสัชจลนศาสตร์ของเนวิราพีนจึงมีความสำคัญต่อการปรับขนาดยา

วัตถุประสงค์ เพื่อสร้างแบบจำลองเภสัชจลนศาสตร์ประชากรของเนวิราพีน ประมาณค่าพารามิเตอร์เฉลี่ยเภสัชจลนศาสตร์ประชากร และระบุปัจจัยที่มีอิทธิพลต่อค่าพารามิเตอร์เภสัชจลนศาสตร์ประชากรของเนวิราพีนในผู้ป่วยติดเชื้อเอชไอวี

วิธีทำการศึกษา ทำการศึกษาเชิงพรรณนา แบบย้อนหลังในผู้ป่วยจำนวน 236 ราย โดยการรวบรวมข้อมูลจากการวิจัยทางคลินิกของเนวิราพีนในกลุ่มผู้ป่วยไทยที่ติดเชื้อเอชไอวี จากศูนย์ประสานความร่วมมือระหว่างไทยออสเตรเลีย เนเธอร์แลนด์ เพื่อการศึกษาวิจัยด้านโรคเอดส์ และสถาบันบำราศนราดูร สร้างแบบจำลองโดยใช้หลักการของ Nonlinear mixed-effects modeling โดยโปรแกรม NONMEM® การตรวจสอบแบบจำลองใช้การประมาณค่าแบบเบส์ (Bayesian estimation)

ผลการศึกษา แบบจำลองทางเภสัชจลนศาสตร์แบบหนึ่งห้อง ที่มีการดูดซึมยาและการขจัดยาแปรผันตรงกับความเข้มข้นของยา เป็นแบบจำลองที่เหมาะสมในการอธิบายเภสัชจลนศาสตร์ของเนวิราพีน อายุที่มากกว่า 40 ปี และระดับเอนไซม์แอสพาร์เตต อะมิโนทรานสเฟอเรส (AST) ที่สูงกว่า 60 ยูนิต/ลิตร ทำให้ค่าการชำระยาของเนวิราพีนลดลงร้อยละ 18 และร้อยละ 16 ตามลำดับ การใช้ไรแฟมพิซินร่วมระหว่างการรักษาเพิ่มค่าการชำระยาของเนวิราพีนร้อยละ 22 ผลการตรวจสอบแบบจำลองพบว่าค่าเฉลี่ยความผิดพลาดในการทำนาย (mean prediction error) เท่ากับ -0.10 มิลลิกรัม/ลิตร และไม่แตกต่างจากศูนย์อย่างมีนัยสำคัญทางสถิติ (p=0.49) กราฟแบรนด์-อัลท์แมนไม่แสดงอคติอย่างเป็นระบบ

สรุปผล การชำระยาของเนวิราพีนในประชากรกลุ่มนี้ต่ำกว่าประชากรกลุ่มอื่นที่เคยมีการรายงาน คุณลักษณะของผู้ป่วยที่มีอิทธิพลต่อเภสัชจลนศาสตร์ของเนวิราพีน ได้แก่ อายุแบบแบ่งกลุ่ม (≤40 ปี, >40 ปี), ระดับเอนไซม์ AST แบบแบ่งกลุ่ม (≤60 ยูนิต/ลิตร, >60 ยูนิต/ลิตร) และการใช้ไรแฟมพิซินร่วมระหว่างการรักษา แบบจำลองที่ได้จากการศึกษานี้สามารถนำไปใช้ปรับขนาดยาเนวิราพีนสำหรับผู้ป่วยแต่ละรายได้

ภาควิชา....เภสัชกรรมปฏิบัติ.... ลายมือชื่อนิสิต.....

สาขาวิชา...เภสัชกรรมคลินิก.... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา.....2554..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

5376556933 : MAJOR CLINICAL PHARMACY

KEYWORDS : POPULATION PHARMACOKINETICS / NEVIRAPINE / NONMEM

THANAPORN WATTANAKUL : POPULATION PHARMACOKINETICS OF NEVIRAPINE IN HIV-INFECTED PATIENTS. ADVISOR: BARALEE PUNYAWUDTHO, Ph.D., CO-ADVISOR : ANCHALEE AVIHINGSANON,M.D., 79 pp.

Background Nevirapine plasma concentration has been shown to be associated with virological response, treatment failure and adverse drug reactions. Nevirapine has a high interindividual variability, therefore identifying sources of the variability of nevirapine pharmacokinetics is important for dose optimization.

Objectives To develop a population pharmacokinetic model of nevirapine, to determine population mean pharmacokinetic parameters, and to identify factors influencing the pharmacokinetic parameters of nevirapine in HIV-infected patients.

Methods A retrospective descriptive study in 236 patients, data were extracted from clinical studies from The HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT) and Bamrasnaradura Infectious Disease Institute. The model was developed by a nonlinear mixed-effects modeling approach using NONMEM®. Model validation was performed using Bayesian estimation.

Results A one-compartment model with first-order absorption and elimination found to be the best model for describing nevirapine pharmacokinetics. Age more than 40 years and aspartate aminotransferase (AST) level more than 60 U/L decreased nevirapine apparent oral clearance (CL/F) by 18% and 16%, respectively. The concomitant use of rifampicin increased nevirapine CL/F by 22%. The results from model validation showed that the mean prediction error of the final model was -0.10 mg/L and was not significantly different from zero (p=0.49). The Bland-Altman plot showed no systematic bias.

Conclusions The nevirapine CL/F in this population was slightly lower than previously reported in other populations. The patient characteristics which influence nevirapine CL/F were age as categorical variable (≤40 years, >40 years), AST level as categorical variable (≤60 U/L, >60 U/L) and rifampicin use. The model obtained from this study can be used for nevirapine dosage optimization for individual patient.

Department : Pharmacy Practice Student's Signature .....

Field of Study : Clinical Pharmacy Advisor's Signature .....

Academic Year : 2011 Co-advisor's Signature .....

ACKNOWLEDGEMENTS

For successfully accomplishment of this thesis, I would like to thank you for contributions in this statement.

Firstly, I would like to express that I am thankful to my thesis advisor, Dr. Baralee Punyawudho, from the Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for expert advice in non-linear mixed effects modeling approach which I have never learned, closely attention to my work, proving my English writing and the encouragement throughout my study.

I am also grateful to Dr. Anchalee Avihingsanon, from The HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT), The Thai Red Cross AIDS Research Centre, Bangkok, my thesis co-advisor and Dr. Weerawat Manosuthi, from Bamrasnaradura Infectious Disease Institute, Nonthaburi, my thesis external committee for providing me the entire data and guidance. Without their contributions, this thesis cannot be successful.

My appreciation is also extended to my thesis committee; Dr. Thitima Wattanavijitkul, Assistant Professor Dr. Wanchai Treyaprasert, for their helpful discussion and suggestion for my thesis.

Thanks are also due to all physicians, nurses, and staffs at HIV-NAT and Bamrasnaradura Infectious Disease Institute for their co-operation and support in providing help for data collection and other facilities.

Finally, I would like to say thank you to my beloved family, my father, my mother, my aunt, and my brother for their love and encouragement throughout my study. Thanks for always staying by my side and supporting everything in my life. Thanks to all my friends who always keep me smile and help me go forward. Besides, I would like to express my thanks to all of those whose name have not been mentioned for their help in anyway. Thank you.

CONTENTS

Page

CHAPTER

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVEATIONS

AIDS	=	Acquired Immune Deficiency Syndrome
ALT	=	Alanine aminotransferase
AST	=	Aspartate aminotransferase
AUC	=	Area under the curve
CL/F	=	Apparent oral clearance
C_{max}	=	The maximum concentration
C_{min}	=	The minimum concentration
DV	=	Observed concentrations
exp	=	Exponential
HBV	=	Hepatitis B virus
HCV	=	Hepatitis C virus
HIV	=	Human immunodeficiency virus
HPLC	=	High Performance Liquid Chromatography
hr	=	Hour
IIV	=	Interindividual variability
IPRED	=	Individual predicted concentrations
k_a	=	Absorption rate constant
kg	=	Kilogram
L	=	Liter
mg	=	Milligram
MPE	=	Mean prediction error
NNRTI	=	Non-nucleoside reverse transcriptase inhibitor
NRTI	=	Nucleoside reverse transcriptase inhibitor
OFV	=	Objective function value
PI	=	Protease inhibitor
PRED	=	Population predicted concentrations
PXR	=	Pregnane X receptor

RMSE	=	Root mean square error
RUV	=	Residual unexplained variability
SD	=	Standard deviation
TB	=	Tuberculosis
TVCL	=	Typical value of the apparent oral clearance
TVk_a	=	Typical value of absorption rate constant
TVV	=	Typical value of the apparent volume of distribution
ULN	=	Upper limit of normal
V/F	=	Apparent volume of distribution
WRES	=	Weighted residual

CHAPTER I
INTRODUCTION

Background and significance of the problem

Human immunodeficiency virus (HIV) infection is one of the most important health problems in the world. Based on The World Health Organization [1] report, the number of people living with HIV in 2010 were 34.0 millions, while the number of newly infected patients were 2.7 millions. Moreover, it was found that 1.8 millions of people were dead because of Acquired Immune Deficiency Syndrome (AIDS). The two regions which had the most number of HIV-infected patients were Sub-Saharan Africa and South & South East Asia [1]. HIV situation in Thailand is also of concern. In 2011,The Bureau of Epidemiology Thailand [2] reported that there were 376,690 HIV-infected patients in Thailand, and the most common opportunistic infectious disease found in HIV-infected patients was tuberculosis (TB).

The use of antiretroviral therapy for the treatment of HIV-infection can reduce morbidity and mortality rate and also improve quality of life of the patients. Nevertheless, the use of antiretroviral agents still faces with adverse effects and drug resistance problems. Thailand national guideline on HIV/AIDS diagnosis and treatment 2010 recommended the use of the non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen in combination with 2 nucleoside reverse transcriptase inhibitors (NRTI) as a first-line therapy. In case of patients who cannot tolerate the adverse effects of NNRTIs, protease inhibitor (PI) was recommended [3, 4]. The efficacy of antiretroviral therapy in HIV-infected patients was found to be associated with antiretroviral plasma concentration [5]. When the trough plasma concentrations of antiretroviral agents decrease, the chance of drug resistance is higher. On the other hand, an increased of plasma concentrations of antiretroviral agents can lead to toxicity [5]. Due to these problems and a high variability of plasma drug concentrations of antiretroviral drugs among the patients, therapeutic drug monitoring of antiretroviral agents, especially for

NNRTI and PI was recommended to optimize dosage regimens among HIV-infected patients [5, 6].

Among TB-HIV co-infected patients, rifampicin-based regimen is recommended. However, rifampicin is the potent inducer of several metabolizing enzymes, the concomitant use of rifampicin with NNRTIs or PIs may lead to the decrease of NNRTIs or PIs plasma concentrations [3, 7, 8]. Moreover, the use of rifampicin causes skin and hepatic adverse events; therefore, there are overlapping toxicities from rifampicin and antiretroviral agents. The combination use of antiretroviral and rifampicin encounters the problem of drug-drug interaction and cause more complexity [3].

According to Thailand national guidelines on HIV/AIDS diagnosis and treatment 2010, nevirapine and efavirenz are the preferred first-line NNRTIs [3]. The efficacy of nevirapine combined in generic fixed-dose combination with lamivudine and stavudine or zidovudine (GPO-vir®) has been confirmed [7, 9-11]. Along with its low cost and convenience, nevirapine is the most commonly used NNRTI in Thailand [3]. However, skin rash and hepatotoxicity are still being important adverse events. These adverse events are found to be related to plasma drug concentration of nevirapine [5, 12, 13]. The higher nevirapine plasma drug concentration, the higher the risk of having adverse events has been observed. On the other hand, the low plasma drug concentration of nevirapine is related to drug resistance [5]. Previous studies reported the high interindividual variability (IIV) of nevirapine [14-20]. Several factors influencing the IIV of nevirapine pharmacokinetics including weight [14, 15, 18, 20], age [17, 18], hepatitis C virus (HCV) co-infection [15], elevated aspartate aminotransferase (AST) level [15], concomitant medications [17, 18] and *CYP2B6* genetic polymorphisms [19, 20]. The high degree of IIV of the drug's pharmacokinetics causes high variability of drug concentrations which related to efficacy and adverse events [21]. Thus, using standard dosage regimens of nevirapine suggested by the dosing guidelines may not suitable and individualization of drug's dosage regimens should be more appropriate.

Population pharmacokinetics is the study aiming to estimate the population mean pharmacokinetic parameters, IIV and residual unexplained variability (RUV) in drug absorption, distribution, metabolism and excretion in a target population [22-24]. The information gains from the population pharmacokinetic study can directly apply to help dosage optimization for individual patient [24].

Population pharmacokinetics of nevirapine in HIV-infected patients has been reported in European [14-17, 20] and South African populations [18]. However, there are a few studies in Asian populations [19, 25].

Based on the results from previous studies, it was shown that the factors influencing population mean pharmacokinetic parameters were different among the studies. Therefore, it is possible that nevirapine pharmacokinetics and factors influencing its pharmacokinetics may be different among the populations. Due to these reasons, the use of previous population pharmacokinetic model from other population to optimize nevirapine dosage regimen in Thai patients may not applicable.

Objectives

1. To develop population pharmacokinetic model of nevirapine in Thai HIV-infected patients

2. To estimate the population mean pharmacokinetic parameters, IIV and RUV of nevirapine in Thai HIV-infected patients

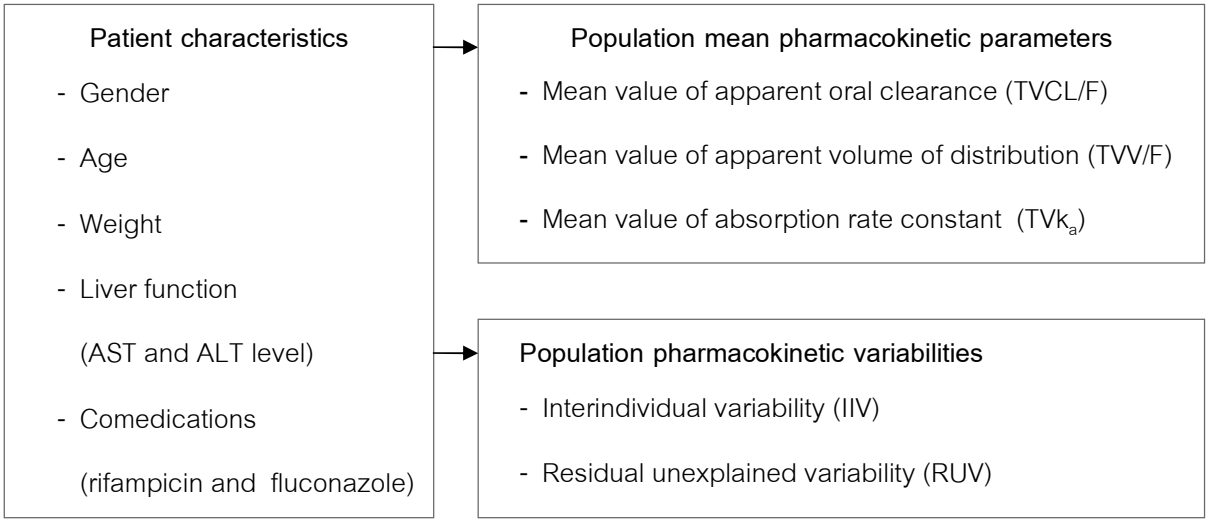
3. To investigate the relationship between patient characteristics and population mean pharmacokinetic parameters

Research hypotheses

1. The population mean pharmacokinetic parameters of nevirapine in Thai HIV-infected patients are different from other populations.

2. Gender, age, weight, liver function (AST and alanine aminotransferase (ALT) level) and comedications (rifampicin and fluconazole) influence nevirapine pharmacokinetic parameters and their variabilities.

Conceptual framework



Methodology

This study is a retrospective descriptive study. Data were extracted from clinical studies from The HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT) and Bamrasnaradura Infectious Disease Institute. Data were analyzed by nonlinear mixed-effects modeling approach using NONMEM® (version VI, GloboMax LLC, Hanover, MD).

Operation definitions

1. The population pharmacokinetics was defined as an approach aiming to estimate population mean pharmacokinetic parameters, and the IIV and the RUV in drug absorption, distribution, metabolism, and excretion [22-24].

2. The population mean pharmacokinetic parameters were defined as the mean value of apparent oral clearance, mean value of apparent volume of distribution and mean value of absorption rate constant of the population.

3. The interindividual variability was defined as a measure of unexplained random difference among individuals [26].

4. The residual unexplained variability was defined as a measure of remaining unexplained variability when all other sources of variability have been accounted for. The residual unexplained variability includes intraindividual variability, inter-occasion variability, measurement error, and model misspecification errors [24, 26].

Significance of the study

The information about population mean pharmacokinetic parameters and factors influencing population mean pharmacokinetic parameters obtained from this study can be used as a tool for nevirapine dosage optimization in individual Thai HIV-infected patients to obtain a more appropriate, effective, and safer doses in clinical practice.

CHAPTER II
LITERATURE REVIEW

Review of Human immunodeficiency virus infection

Human immunodeficiency viruses (HIV) are lentiviruses, a family of mammalian retroviruses. HIV cause persistent chronic infection with gradual onset of clinical symptoms [27]. There are 2 major families of HIV, HIV-1 and HIV-2, but the most commonly involved epidemic is HIV-1 [27]. HIV can be transmitted via three primary ways; sexual, parenteral and perinatal [28]. Sexual intercourse is the most common cause of infection [28]. The clinical presentations of HIV infection vary among patients but often have viral syndrome such as fever, adenopathy, sore throat, and rash [28] which can be summarized in Table 1. Besides the clinical presentations, the detection of the high level of viral load and persistent decrease in CD4+ T-cell can be found [28].

Table 1 Clinical presentations of HIV infection [4, 28]

Sign and symptom	Percent
Fever	96
Adenopathy	74
Pharyngitis	70
Erythematous maculopapular rash	70
Myalgia	54
Diarrhea	32
Headache	32
Nausea, vomiting	27
Hepatomegaly, spleenomegaly	14
Weight loss	13
Oral trush	12
Neurologic symptoms	12

Antiretroviral agents were used for the treatment of HIV infection. The goals of therapy are mainly focused on a maximal suppression of viral load, an increase of CD4+ T-cell counts which indicates a strengthening of immune system. Additionally, a decrease of adverse drug events, a promotion of adherence, an improvement in quality of life, and a decrease of morbidity and mortality from opportunistic infection are also of concern. [28, 29]. A recommendation of starting antiretroviral therapy by Thailand national guidelines on HIV/AIDS diagnosis and treatment 2010 is based on patient symptoms and CD4+ T-cell counts as shown in Table 2 [3, 4].

The recommended antiretroviral therapy in Thailand is the NNRTI-based regimens in combination with 2 NRTIs. However, in the case that patients cannot tolerate to NNRTIs, PI is recommended [3, 4]. The preferred first-line and alternative antiretroviral regimens are summarized in Table 3.

Table 2 Indications for antiretroviral therapy initiation [3, 4]

Clinical Presentation	CD4+ T-cell counts (cells/mm^3)	Recommendations
AIDS-defining illness*	Any value	Treat
HIV-related Symptomatic**	Any value	Treat
Asymptomatic	<350	Treat
Asymptomatic	>350	Defer treatment; follow up clinical status and monitor CD4+ T-cell count every 6 months
Pregnancy	Any value	Treat, discontinue ART after delivery if pre-treatment CD4+ T-cell count is >350 cells/mm^3

*as described in the 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults [30] and with penicilliosis, which is considered AIDS-defining illness in Thailand [31]. **oral candidiasis, pruritic papular eruptions (PPE), unexplained fever or diarrhea > two weeks, >10% unexplained weight loss in 3 months, or herpes zoster involved > two dermatomes.

Table 3 The preferred first-line and alternative antiretroviral regimens recommendation [3, 4]

NRTIs		NNRTIs		PIs
Preferred				**Preferred**
AZT+3TC			If patients	LPV/r
TDF+3TC/FTC			cannot	
Alternative	Plus	EFV	tolerate	**Alternative**
ABC+3TC		NVP	→	ATV/r
d4T+3TC			NNRTI	DRV/r
ddI+3TC				SQV/r

AZT=zidovudine, 3TC=lamivudine, TDF=tenofovir, FTC=emtricitabine, ABC=abacavir, d4T=stavudine, ddI=didanosine, EFV=efavirenz, NVP=nevirapine, LPV/r=lopinavir/ritonavir, ATV/r =atazanavir/ritonavir, DRV/r=darunavir/ritonavir, SQV/r=saquinavir/ritonavir

Nevirapine

1. Chemistry

Nevirapine is presented as a white to off-white crystalline powder with the melting point of approximately 245 ˚C. The chemical name of nevirapine is 11-cyclopropyl-4-methyl-5, 11-dihydro-6*H*-dipyrido [3, 2-*b*: 2', 3'-*e*] [1, 4] diazepin-6-one, with the molecular weight of 266.3 [32]. Its structural formula is present in Figure 1.

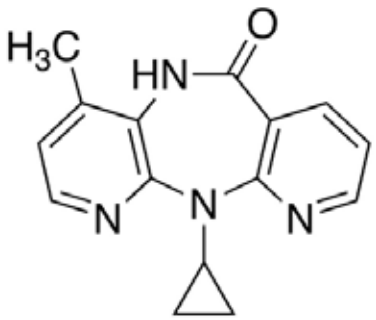


Figure 1 The structural formula of nevirapine

2. Indication

Nevirapine is indicated to be used as a treatment of HIV-1 infection in combination with other antiretroviral agents [32, 33]. Based on the clinical studies, nevirapine should not be used in adult females having CD4+ T-cell counts more than 250 cells/mm^3 or in adult males having CD4+ T-cell counts more than 400 cells/ mm^3 except those who considered to get the benefit outweighs the risk [32].

3. Dosage and administration

Nevirapine tablet contains 200 mg of nevirapine anhydrate as an active ingredient [32]. The most commonly used generic fixed-dose combination tablet in Thailand are GPO-vir S30 (containing nevirapine 200 mg, lamivudine 150 mg, and stavudine 30 mg) and GPO-vir Z250 (containing nevirapine 200 mg, lamivudine 150 mg, and zidovudine 250 mg).Dosage recommendation of nevirapine in HIV-infected adults and adolescents older than 16 years is 200 mg once daily for the first 14 days and then increases the dose to 200 mg twice daily as a maintenance dose [32, 34]. The lead in dose must always be used to reduce the developing of skin rash [32].

4. Mechanism of action

Nevirapine is a non-nucleoside reverse transcriptase inhibitor. It has an activity against HIV-1 by directly binding to reverse transcriptase and inhibits RNA-dependent and DNA-dependent DNA polymerase activities by disrupting the enzyme's catalytic site. Unlike other NRTIs which have to be intracellular phosphorelated to exhibit antiretroviral activity, nevirapine does not required intracellular mechanism to exert its activity [32, 34].

5. Pharmacokinetics of nevirapine

5.1 Absorption

Nevirapine is rapidly absorbed after oral administration in healthy volunteers and HIV-infected adults. A mean absolute bioavailability of nevirapine is approximately 90% [32]. The administration with food or antacid does not affect the extent of absorption [32, 33]. The oral absorption of nevirapine is completed in approximately 4 hours after drug administration [32, 34].

5.2 Distribution

Nevirapine is highly lipophilic and un-ionized at physiologic pH. Therefore, it is widely distributed in human body [32-34]. The apparent volume of distribution of nevirapine is 1.2-1.4 L/kg. Approximately 60% of nevirapine binds to plasma protein [32-34].

5.3 Metabolism and excretion

Nevirapine is mainly biotransformed by cytochrome P450 isozymes, CYP3A4 and CYP2B6 [32, 35]. It is metabolized by oxidative reaction to obtain several hydroxylated metabolites, 2-hydroxynevirapine, 3-hydroxynevirapine, 8-hydroxynevirapine, and 12-hydroxynevirapine [35]. These hydroxylated metabolites were then glucuronidated to 2-hydroxynevirapine glucuronide, 3-hydroxynevirapine glucuronide, 8-hydroxynevirapine glucuronide, and 12-hydroxynevirapine glucuronide, respectively [35]. More than 80% of glucuronidated conjugates were found in the urine and only 2.7% was excreted in the urine as parent compound .The biotransformation pathway of nevirapine is shown in Figure 2.

Nevirapine is an enzyme inducer of both CYP3A4 and CYP2B6 [32, 35]. As these enzymes are responsible for nevirapine metabolism, autoinduction is exhibited. There is evidence that autoinduction of nevirapine occurs during the first two weeks after initiation of the therapy [35]. After a completion of autoinduction, the apparent oral

clearance of nevirapine is found to be increased by 1.5-2 folds and half-life is decreased from approximately 45 hours to 25-30 hours [32, 35].

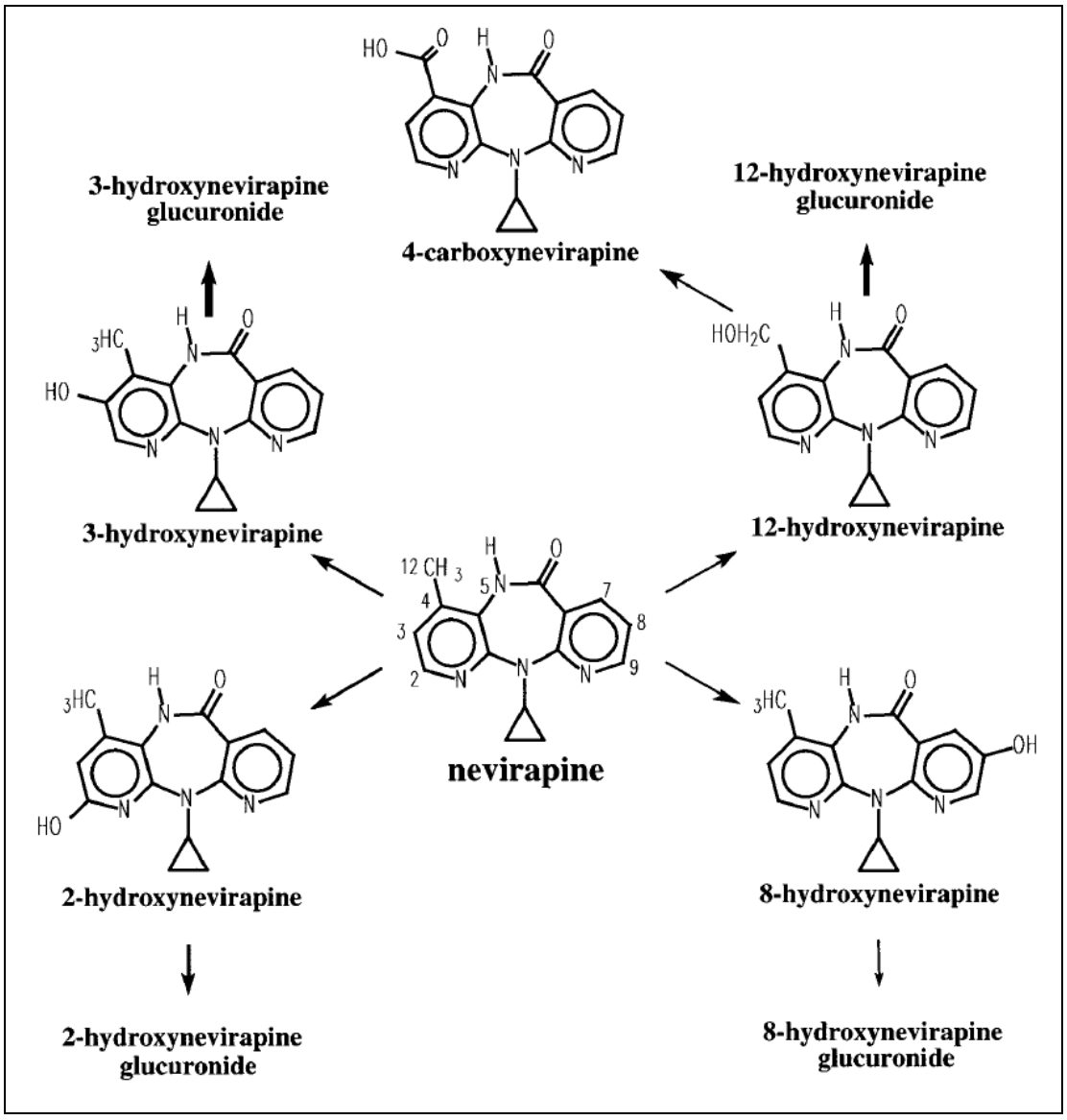


Figure 2 The biotransformation pathway of nevirapine [35]

6. Drug interaction

Nevirapine is mainly metabolized by CYP3A4 and CYP2B6. Drugs that are inducers or inhibitors of these enzymes can alter the metabolism of nevirapine. Rifampicin, a potent inducer of several CYP enzymes, can increase nevirapine metabolism leading to a decrease of nevirapine exposure. The use of nevirapine and drugs that are enzyme inhibitors of CYP3A4 and CYP2B6 including darunavir, fosamprenavir, clarithomycin, and fluconazole resulting in an increase of nevirapine concentrations [32].

As nevirapine is an enzyme inducer of both CYP3A4 and CYP2B6, the concomitant use of nevirapine with drugs that are metabolized by these enzymes may result in potentially drug-drug interaction. The plasma concentrations of these following drugs have been reported to be decreased when used concomitantly with nevirapine; zidovudine, efavirenze, indinavir, lopinavir, nelfinavir, saquinavir, tipranavir, clarithromycin, itraconazole, ketoconazole, warfarin, ethinyl estradiol, norethindrone, and methadone [32].

On the other hand, the drugs which are enzyme inducers or enzyme inhibitors of CYP3A4 and CYP2B6 can alter the pharmacokinetic parameters and plasma concentration of nevirapine . The changes in pharmacokinetic parameters of nevirapine in the presence of concomitant drugs are summarized in Table 4.

Table 4 Summary of changes in pharmacokinetic parameters of nevirapine in the presence of common concomitant drugs [32]

Concomitant drugs	Changes in pharmacokinetic parameters of nevirapine		
	AUC	C_{max}	C_{min}
Darunavir/ritonavir*	Increased	Increased	Increased
Fosamprenavir/ritonavir*	Increased	Increased	Increased
Clarithromycin	Increased	Increased	Increased
Itraconazole	Increased	Increased	Increased
Fluconazole	Increased	Increased	Increased
Rifampicin	Decreased	Decreased	Decreased

* In combination with ritonavir

7. Adverse drug reaction

The most serious adverse drug reactions of nevirapine are hepatitis/hepatic failure, Stevens-Johnson syndrome, toxic epidermal necrolysis, and hypersensitivity reaction [32]. The first 18 weeks of the treatment is a critical period and therefore monitoring of these adverse reactions should be performed. Even though, it was shown that the risk of hepatic events is high during the first 6 weeks of the therapy [32], monitoring should be continued at all time of the treatment.

The most common adverse drug reaction found during nevirapine use is the occurrence of rash [32, 34]. Rash was usually mild to moderate, maculopapular erythematous cutaneous eruption, with or without pruritus, and normally located on the trunk, face, and extremities. Severe or life-threatening rash was found about 2% among patients treated with nevirapine [32]. The summary of adverse drug reactions of nevirapine is presented in Table 5.

Table 5 Adverse drug reactions of nevirapine [32, 34]

Percentage of adverse drug reaction	Adverse drug reactions
> 10%	Rash (grade1/2: 13%, grade3/4: 1.5%)
	ALT > 250 U/L
	Symptomatic hepatic events
1-10%	Headache
	Fatigue
	Nausea
	Abdominal pain
	Diarrhea
	AST > 250 U/L

Review of population pharmacokinetics

The population pharmacokinetics was introduced by Sheiner, Beal, and colleagues in the early 1980s [36, 37]. They published a series of articles describing a new approach for analyzing the pharmacokinetic data and also introduced a new software called NONMEM (distributed by GloboMax LLC, Hanover, MD) [36]. To date, population pharmacokinetics has gained much interest and is integrated to the regulatory affair and many phases of drug development [37]. Moreover, it can be applied to be used as a tool for dosage optimization in therapeutic drug monitoring in clinical practice [24].

Population pharmacokinetics is the study of pharmacokinetics and describes the variability of plasma drug concentrations between individual patients in the population of interest [23, 37, 38]. The purpose of population pharmacokinetics is to obtain population mean pharmacokinetic parameters, their IIV and the RUV in drug absorption, distribution, metabolism, and excretion [22-24, 37]. Moreover, the population pharmacokinetics can be used to demonstrate a relationship between the pharmacokinetic parameters and patient's specific covariates. The nonlinear mixed-effects modeling approach is used to analyze the population data.

The nonlinear mixed-effects model consisted of 3 components; the base model or the structural model, the covariate model, and the variance or statistical model [37].

1. **The base (structural) model**

To develop population pharmacokinetic model, the first step is to identify the appropriate base model that best describe the data. The various base models should be tested and the appropriate model is chosen based on statistical criteria including likelihood ratio test, and graphical examination [37].

2. **The covariate model**

After the base model was identified, the covariate model is then developed using stepwise strategies. The specific patient characteristics that may influence the pharmacokinetics of a drug are used as covariates. Each covariate is added to the base model one at a time during stepwise forward inclusion. All significant covariates are included and are called the full model. During stepwise backward deletion each of the covariate in the full model is removed one at a time. The likelihood ratio test (LRT) is used for model discrimination which is computed by following equation;

$$LRT = 2(LL_f - LL_r) \text{ [37]}$$

Where LL_f and LL_r are the log-likelihoods for the full model and reduced model, respectively. The LRT is approximately χ^2-distributed with f-r degrees of freedom [37]. The objective function value (OFV) obtained from NONMEM is approximately equal to -2LL [39]. Therefore, the LRT can also be calculated by using the OFV. Based on this equation, the improvement in model fit can be assigned a significance level. For one degree of freedom, the decrease of 3.84 in the OFV of the full model is significant at $p \leq 0.05$ and the decrease of 6.63 in the OFV of the full model is significant at $p \leq 0.01$ [37]. The factors that may influence the pharmacokinetic parameters and have been used as covariates in population pharmacokinetic studies are summarized in Table 6.

Table 6 Factors that may influence the pharmacokinetic parameters [24, 37]

Factors that may influence the pharmacokinetic parameters
Weight
Age
Gender
Ethnicity
Concomitant medications
Environmental factors such as smoking, diet, and polluted exposure
Laboratory test such as AST, ALT, bilirubin, etc.
Renal impairment
Liver impairment
Disease states
Genetic variation

3. **The variance model (statistical model)**

The variance model includes two components, the model for IIV and the model for RUV.

3.1 The interindividual variability

The interindividual variability can be modeled by three different models;

3.1.1 Additive error model

$$P_i = TVP + \eta_i$$

3.1.2 Proportional error model

$$P_i = TVP \times (1+ \eta_i)$$

3.1.3 Exponential error model

$$P_i = TVP \times \exp (\eta_i)$$

Where P_i is the individual pharmacokinetic parameter, TVP is the typical value of the pharmacokinetic parameter in the population and η_i is the IIV, which is assumed to be normally distributed with a mean of zero and variance of ω^2 [37].

3.2 The residual unexplained variability

The residual unexplained variability is commonly modeled by the following equations;

3.2.1 Additive error model

$$C_{obs,ij} = C_{pred,\ ij} + \varepsilon_{ij}$$

3.2.2 Proportional error model

$$C_{obs,ij} = C_{pred,\ ij} \times (1+\varepsilon_{ij})$$

3.2.3 Exponential error model

$$C_{obs,ij} = C_{pred,\ ij} \times \exp(\varepsilon_{ij})$$

3.2.4 Combined additive and proportional error model

$$C_{obs,ij} = C_{pred,\ ij} \times (1+ \varepsilon_{1ij}) + \varepsilon_{2ij}$$

Where $C_{obs,ij}$ and $C_{pred,\ ij}$ represent the i^{th} observed and predicted concentration in the j^{th} individual and ε_{ij} represents the RUV which is assumed to be normally distributed with mean of zero and variance of σ^2[37].

The use of population pharmacokinetic approach in pharmacokinetic data analysis shows some advantages compared with the traditional approach. However, there are some disadvantages of population pharmacokinetic approach. Table 7 shows the advantages and disadvantages of population pharmacokinetic approach.

Table 7 Advantages and disadvantages of the population pharmacokinetic approach [24, 37]

Advantages
1. Data are normally collected from patients of interest
2. Both intensive and sparse samples can be used for analysis
3. Study can be done in special populations such as neonates, elderly, HIV-infected patients, critical care patients, and patients with cancer as a sparse sampling design can be used
4. Data can be combined from different sources
5. Covariates that explain IIV can be identified
6. Information obtained can be used for individualized prediction of dose
Disadvantages
1. Perceived as complicated and difficult to implement
2. Few expert pharmacometricians available for consultation and education
3. Methods are difficult to understand
4. Less power than Phase I studies
5. Time consuming
6. Different analysts may develop different models

The population pharmacokinetics of nevirapine

The population pharmacokinetics of nevirapine has been investigated in various populations such as European [14-17, 20, 25] South African [18, 25], and South American patients [25]. However, there are a few data in Asian population [19, 25]. Previous population pharmacokinetic studies of nevirapine found that a one-compartment model with first-order absorption and elimination was best described the pharmacokinetics of nevirapine [14-20, 25]. The mean pharmacokinetic parameters were different among the populations. The mean apparent oral clearance was estimated to be 2.81-3.84 L/hr, the mean apparent volume of distribution was 77-223 L, and the mean absorption rate constant was 0.49-3.57 hr^{-1}. The interindividual variability for CL/F

was approximately 25-34% [14-20, 25]. The covariates that found to be significant for nevirapine CL/F were weight [14-16, 20], age [17, 18], HCV co-infection [15], elevated AST level [15], albumin level [18], creatinine clearance [19], and *CYP2B6* polymorphisms [19, 20]. The significant covariates for V/F were weight [14, 18] and darunavir coadministration [17].

Factors influencing pharmacokinetics of nevirapine

The previous pharmacokinetic studies of nevirapine reported that several factors can influence pharmacokinetic parameters of nevirapine such as age, weight, liver function, comedications, and genetic variation [14-20, 25, 40].

1. **Race**

Race can be a factor that influences pharmacokinetic parameters of nevirapine [37]. The results from the 2NN pharmacokinetic substudy which is a large-scale, international, multicenter study, showed that HIV-infected patients from Thailand had nevirapine CL/F lower than patients from South America and Western countries by 11% and 28%, respectively [25]. However, a previous population pharmacokinetic study of nevirapine showed that race is not associated with pharmacokinetics of nevirapine and dose optimization among different races is not required [41].

2. **Gender**

A pharmacokinetic study by Marubbi et al showed that females had a higher C_{trough} , C_{max}, and $AUC_{(0-24)}$ than males. However, the difference does not reach a statistical significance. [42]. The 2NN pharmacokinetic substudy reported that gender is one of the significant covariates for nevirapine CL/F. The results showed that females had nevirapine CL/F 13.8% lower than males [25]. However, several population pharmacokinetic studies of nevirapine showed no significant effect of gender on nevirapine pharmacokinetic parameters [14-20, 40, 41].

3. Age

Age was found to be a significant covariate for nevirapine CL/F in previous population pharmacokinetic studies of nevirapine [17, 18]. Age-related changes in pharmacokinetics are common, since aging people has impairment of many organ functions. Additionally, there is evidence that the IIV is increased in this population [43].

4. Weight

Many physiological parameters are associated with body weight [37, 44]. Previous population pharmacokinetic studies of nevirapine reported that weight is a significant covariate for both nevirapine CL/F [14, 15, 18, 20] and V/F [14, 18].

5. Liver function

Nevirapine is extensively metabolized by CYP3A4 and CYP2B6 in the liver, therefore liver impairment may alter pharmacokinetics of nevirapine. A study by de Maat et al found that nevirapine CL/F was decreased by 13.2% in HIV-infected patients having AST level > 1.5 x upper limit of normal (ULN) compared with patients having normal AST level.

6. Renal function

The influence of renal function on nevirapine pharmacokinetic parameters is unexpected, because nevirapine is mainly metabolized in the liver. A previous study reported that creatinine clearance affected nevirapine CL/F [19]. In this study, creatinine clearance explained 0.3% of the interindividaul variability. It was suggested that the uremic toxins in renal insufficiency patients may affect the hepatic transporters and metabolizing enzymes and may explain the influence of renal insufficiency on nevirapine CL/F [19, 45].

7. Disease

Previous population pharmacokinetic studies of nevirapine showed that hepatitis B virus (HBV) co-infection [25] and HCV co-infection [15] could affect nevirapine CL/F. A study by Kappellhof et al [25] found that nevirapine CL/F was 19.5% lower in patients with HBV co-infection, while, a study by de Maat et al [15] found that chronic HCV co-infection decreased nevirapine CL/F by 27.4%. However, a study by Vogel et al showed that HCV co-infection did not affect the pharmacokinetics of nevirapine in patients with preserved liver function [46].

8. Comedications

As nevirapine is extensively metabolized by CYP3A4 and CYP2B6, concomitant use of drugs that are inducers or inhibitors of these enzymes may alter nevirapine pharmacokinetic parameters. Several population pharmacokinetic studies of nevirapine reported the effect of comedications on nevirapine pharmacokinetic parameters [16-18]. Rifampicin, a potent enzyme inducer of both CYP3A4 and CYP2B6, was reported to be an important factor that can increase nevirapine CL/F [18, 32, 47-49]. A previous population pharmacokinetic study showed that the use of rifampicin increased nevirapine CL/F by 37.4% in South African patients [18].

A previous study found that CL/F and V/F of nevirapine were decreased significantly when nevirapine was used in combination with darunavir [17]. Moreover, it was reported that a concomitant use of fluvoxamine decreased nevirapine CL/F by 33.7% and the decrease of nevirapine CL/F appeared to be dose dependent [16].

Fluconazole is recommended to be used in HIV-infected patients having cryptococcal meningitis as opportunistic infection. A retrospective study by Manosuthi et al [50] found that patients who received fluconazole had nevirapine trough plasma concentration higher than those who did not receive fluconazole ($p < 0.001$).

9. Genetic variation

CYP3A4 and CYP2B6 isoenzymes show high IIV in expression and activities [20, 51, 52]. Previous population pharmacokinetic studies of nevirapine reported the impact of *CYP2B6* polymorphisms on nevirapine CL/F [19, 20].

Chou et al found that nevirapine CL/F was decreased among patients with different *CYP2B6* polymorphisms. Nevirapine CL/F was decreased by 12% in patients with *CYP2B6 516GT* genotype and 46% in patients with *CYP2B6 516TT* genotype. Schipani et al [20] reported similar results, this study found that *CYP2B6 516TT* and *CYP2B6 983TC* was associated with 37% and 40% lower of nevirapine CL/F.

Uttayamakul et al [53] studied the effects of *CYP2B6 G516T* polymorphisms on nevirapine plasma concentrations when co-administered with rifampicin in HIV/TB coinfected Thai patients. The results showed that the mean plasma drug concentrations of nevirapine in patients with TT genotype were higher than those with GG and GT genotypes.

CHAPTER III
METHODOLOGY

Methods and data collection

This study is a retrospective descriptive study. The data used in this study were part of the data collected for clinical studies investigating the plasma concentration of nevirapine, efficacy, and adverse events in Thai HIV-infected patients receiving nevirapine-based antiretroviral therapy from HIV-NAT and Bamrasnaradura Infectious Diseases Institute [48-50]. Time of blood draw, dosing history including nevirapine dosage regimen and amount of the last dose before blood sampling time, laboratory data i.e. AST level, ALT level, patient characteristic data, and comedications were extracted.

Sample size

The formula used to calculate sample size in this study was

$$N \geq 15p \text{ [54]}$$

Where

N = sample size

p = number of patient characteristics which were tested in the study, including gender, age, weight, liver function (AST and ALT level) and concomitant drugs (rifampicin and fluconazole). Therefore, p equals to 7.

$$N \geq 15(7)$$

$$N \geq 105$$

Thus, sample size required in this study should be at least 105 patients.

Inclusion and exclusion criteria

1. Inclusion criteria

1.1 Patients who were HIV-infected and were being treated with nevirapine as part of antiretroviral agent for at least 2 weeks.

1.2 Patients were at least 18 years old.

1.3 Patients who had nevirapine plasma concentrations recorded in the database.

2. Exclusion criteria

2.1 Patients who were pregnant or breastfeeding women.

2.2 Patients who had incomplete characteristic data.

Nevirapine concentration determination

Nevirapine plasma concentrations were determined by High Performance Liquid Chromatography (HPLC) in the laboratory center of HIV-NAT. All the concentrations were analyzed by the same technician. The information about HPLC determination [55] is shown in Appendix A. Because nevirapine exhibits autoinduction property, all patients were received nevirapine for at least 2 weeks before blood sampling to ensure a completion of the induction phase.

Data analysis methods

1. Demographic data were analyzed by the Statistical Package for Social Sciences; SPSS (version 17.0, SPSS Co., Ltd., Bangkok Thailand). The continuous data were summarized by mean and standard deviation. Frequency and percent were used for describing categorical data.

2. The population pharmacokinetic model of nevirapine was developed by a nonlinear mixed-effects modeling approach using NONMEM software (version VI, Globomax LLC, Hanover, MD).

Population pharmacokinetic model building

1. **Data cleaning and data splitting**

The original dataset obtained from HIV-NAT and Bamrasnaradura Infectious Diseases Institute were pooled. The data that did not meet the inclusion and exclusion criteria were excluded from the dataset. The cleaned data were then randomly divided into 2 datasets. Two-third of the patients were assigned to be in an index dataset which was used in model building process. One-third of the patients were assigned to be in a validation dataset. The randomization was performed by the Statistical Package for Social Sciences (version 17.0, SPSS Co., Ltd., Bangkok Thailand).

2. **Population pharmacokinetic model building**

2.1 Structural pharmacokinetic model

NONMEM was used to develop the structural pharmacokinetic model that best described the data. One- and two-compartment models with first-order absorption and elimination were tested. The best structural model was chosen based on the goodness-of-fit plot using S-PLUS (Insightful Corp, Seattle, Washington), Xpose [56], and the decrease of the OFV. The first-order conditional estimation method with interaction (FOCE-I) was used throughout the model building process. The allometric scaling was used for clearance and volume of distribution using these following equations;

$$TVCL = \theta_1 \times (WT_i/WT_{std})^{0.75}$$

$$TVV = \theta_2 \times (WT_i/WT_{std})$$

Where TVCL and TVV represent the typical (mean) value of the apparent oral clearance (CL/F) and apparent volume of distribution (V/F) in the population, θ_1 and θ_2 is the CL/F and V/F for population with a standard weight, WT_i represent the individual weight, WT_{std} represent the standard weight which is the mean value of weight in the population.

2.2 Random effect model

The interindividual variability was modeled in three different ways;

2.2.1 Additive error model

$$P_i = TVP + \eta_i$$

2.2.2 Proportional error model

$$P_i = TVP \times (1+ \eta_i)$$

2.2.3 Exponential error model

$$P_i = TVP \times \exp (\eta_i)$$

Where P_i is the individual pharmacokinetic parameter, TVP is the typical value of the pharmacokinetic parameter in the population and η_i is the IIV, which is assumed to be normally distributed with mean of zero and variance of ω^2.

The residual unexplained variability was modeled using the following models;

2.2.4 Additive error model

$$C_{obs,ij} = C_{pred,\ ij} + \varepsilon_{ij}$$

2.2.5 Proportional error model

$$C_{obs,ij} = C_{pred,\ ij} \times (1+\varepsilon_{ij})$$

2.2.6 Exponential error model

$$C_{obs,ij} = C_{pred,\ ij} \times \exp(\varepsilon_{ij})$$

2.2.7 Combined additive and proportional error model

$$C_{obs,ij} = C_{pred,\ ij} \times (1+ \varepsilon_{1ij}) + \varepsilon_{2ij}$$

Where $C_{obs,ij}$ and $C_{pred,ij}$ represent the i^{th} observed and predicted concentration in the j^{th} individual and ε_{ij} represents the RUV which is assumed to be normally distributed with mean of zero and variance of σ^2.

The following criteria were used for selecting the random effect model; the goodness-of-fit plot using S-PLUS (Insightful Corp, Seattle, Washington), Xpose, and the decrease of the OFV.

3. Covariate model building

When the appropriate base pharmacokinetic model and random effect model were obtained, the patient characteristics including gender, age, weight, AST level, ALT level and concomitant drugs (rifampicin and fluconazole) were tested as a covariate. The likelihood ratio test and graphical analysis were used to choose the most suitable model. Each covariate was added to the structural model one by one during forward inclusion. The decrease of the OFV of at least 3.84 ($p \leq 0.05$, χ^2, df=1) was used as a criteria for the addition of the covariate. All significant covariates during forward inclusion were included in the full model. During backward deletion, each of the covariate in the full model was removed one at a time. An increase of the OFV of at least 6.63 ($p \leq 0.01$, χ^2, df=1) was used as a criteria to retain the covariate in the final model.

4. Model validation

The final model was further validated in the validation dataset. The final model was used to predict individual nevirapine plasma concentration by Bayesian estimation [57]. The Bayesian predicted concentrations and the observed concentrations were then compared. The individual predicted concentrations (IPRED) were obtained from the final model using “post hoc” option in NONMEM without evaluation (MAXVAL=0) setting the mean parameter values, IIV, and RUV to be equal to the values obtained from the final model. The IPRED were then compared with the individual observed concentrations (DV). The bias and precision between IPRED and DV were described by mean prediction error (MPE) and root mean square error (RMSE), respectively. The MPE and RMSE were calculated by the following equations;

4.1 Calculation of MPE

$$\text{MPE} = \frac{1}{N} \times \sum (\text{predicted concentration} - \text{observed concentration})$$

4.2 Calculation of RMSE

$$RMSE = \sqrt{\frac{1}{N} \times (\text{predicted concentration} - \text{observed concentration})^2}$$

The agreement between IPRED and DV were described by Bland-Altman plot which is a plot of the the difference between IPRED and DV against their mean [58]. Therefore, the X-axis is the mean of IPRED and DV and the Y-axis is the difference of IPRED and DV.

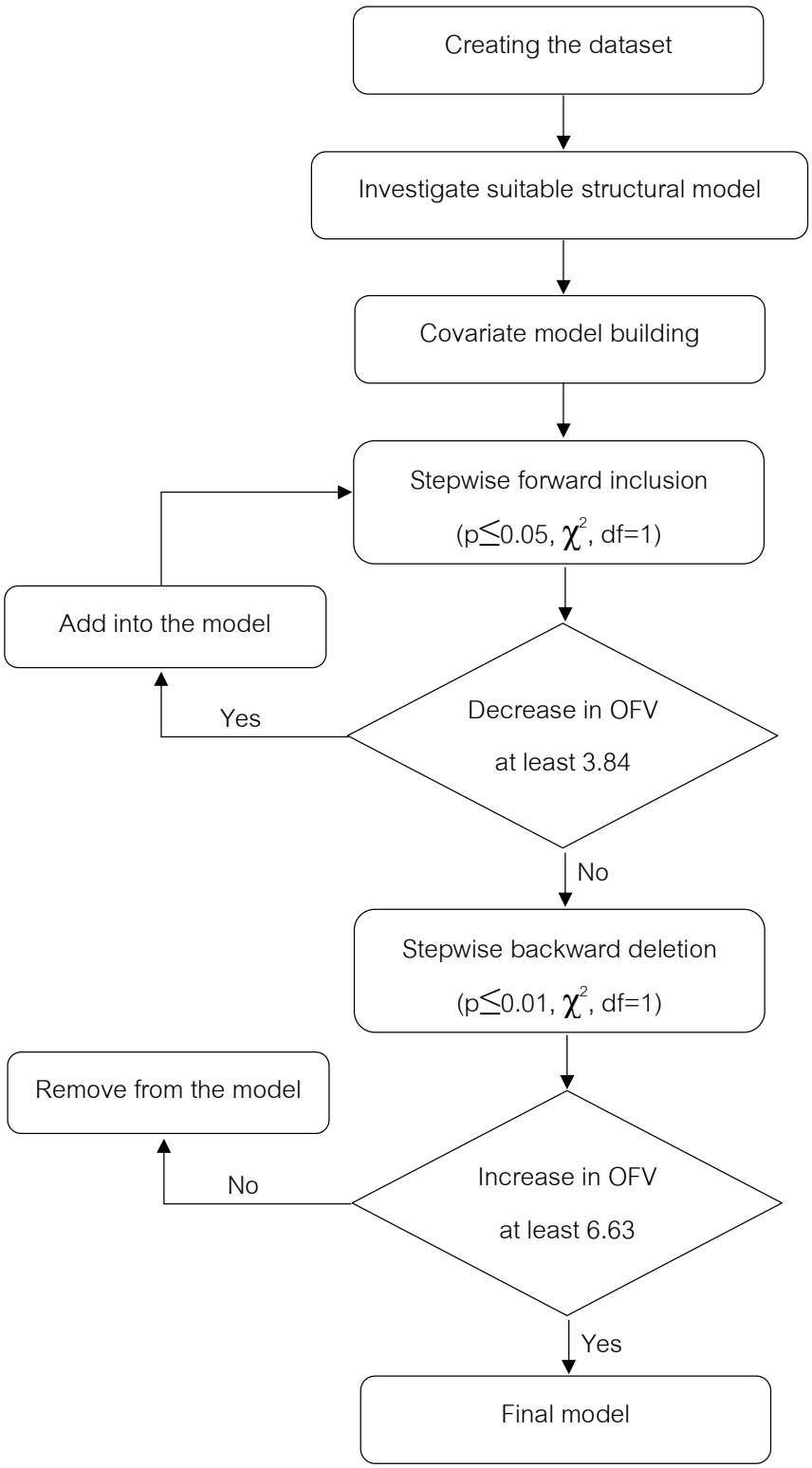


Figure 3 Process of population pharmacokinetic model building

CHAPTER IV
RESEARCH RESULT

A total of 236 patients with 399 concentrations were included in this study. Patients were randomly divided into 2 datasets, an index dataset consisted of 176 patients with 294 concentrations and a validation dataset consisted of 60 patients with 105 concentrations. Most of patients received nevirapine combining in a fixed dose combination of nevirapine 200 mg, lamivudine 150 mg, and stavudine 30 mg (GPO-vir S30). The most common dosage regimen is 200 mg twice daily. However, some patients were given different dosage regimen, 1 patient received nevirapine 600 mg once daily, and 3 patients received nevirapine 400 mg once daily.

Patient demographic data

An index dataset consisted of 123 males and 53 females. Among them, there were 74 patients received rifampicin (42.0%), 14 patients received fluconazole (8.0%) and 8 patients received both rifampicin and fluconazole (4.5%) as comedications. Most of patients in an index dataset had normal hepatic function with the mean AST and ALT of 42.09 and 43.61 U/L, respectively. In a validation dataset, there were 37 males and 23 females. Similarly to patients in an index dataset, there were 26 patients received rifampicin (43.3%) and 3 patients received fluconazole (5.0%) and 1 patient received both rifampicin and fluconazole (1.7%) as comedications. Patients in a validation dataset had normal hepatic function but the mean value of AST and ALT were slightly lower than patients in an index dataset (37.72 and 38.02 U/L, respectively). The difference of the patient characteristics between the index and validation dataset were tested. Continuous variables were tested using independent sample t-test and χ^2 test was used for categorical variables. No differences among the two groups were found. Patient demographic data of two datasets are summarized in Table 8. The distributions of the continuous covariates are presented in Figure 4.

Table 8 Summary of patient demographic data

Patient demographic	Index dataset n (%)	Validation dataset n (%)	p-value
Number of patients	176	60	
Number of concentrations	294	105	
Gender (SEX)			0.239[a]
Males (SEX=1)	123 (69.9)	37 (61.7)	
Females (SEX=0)	53 (30.1)	23 (38.3)	
Dosage regimen			
200 mg twice daily	173 (98.3)	59 (98.3)	0.984 [a]
400 mg once daily	3 (1.7)	0	0.309 [a]
600 mg once daily	0	1 (1.7)	0.086 [a]
Concomitant medication			
Rifampicin (RFP)	74 (42.0)	26 (43.3)	0.862 [a]
Fluconazole (FLU)	14 (8.0)	3 (5.0)	0.445 [a]
Rifampicin+Fluconazole	8 (4.5)	1 (1.7)	0.315 [a]
Patient demographic	**Index dataset** Mean±SD (Range)	**Validation dataset** Mean±SD (Range)	**p-value**
Age (AGE, years)	36.84±8.46 (18-65)	38.83±8.37 (26-66)	0.115[b]
Height (HT, cm)	164.56±8.25 (143-187)	164.23±8.21 (141-182)	0.786 [b]
Weight (WT,kg)	56.37±10.65 (38-95)	56.18±12.02 (35.50-105)	0.911 [b]
BMI (kg/m^2)	20.77±3.34 (13.29-32.02)	20.79±3.99 (13.22-38.57)	0.979 [b]
IBW (kg)	59.91±8.39 (45.14-81.33)	59.26±8.56 (45.14-76.80)	0.612 [b]
BSA (m^2)	1.60±0.17 (1.25-2.15)	1.59±0.19 (1.24-2.19)	0.830 [b]
AST (U/L)	42.09±33.36 (9-252)	37.72±25.63 (18-159)	0.519 [c]
ALT (U/L)	43.61±41.92 (8-253)	38.02±23.90 (8-132)	0.899 [c]

[a] Comparison of the frequency between an index dataset and a validation dataset by χ^2 test

[b] Comparison of the mean values between an index dataset and a validation dataset by independent sample t-test,

[c] Comparison of the mean values between an index dataset and a validation dataset by Mann-Whitney U Test.

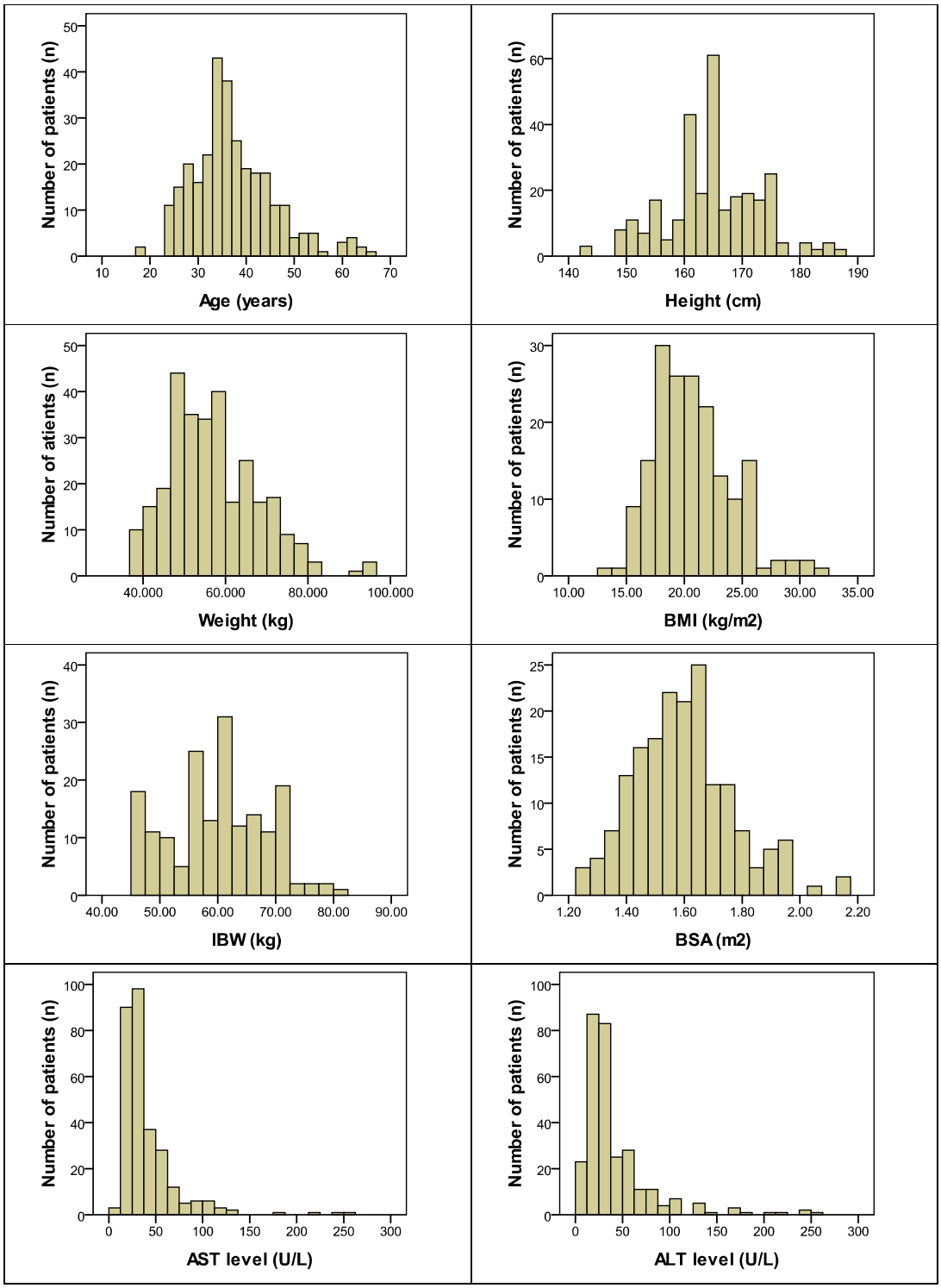


Figure 4 The distribution of the continuous covariates of the patients in the index data set

Population pharmacokinetic analysis

1. **Structural pharmacokinetic model building**

For the structural model building step, an allometric scaling using the power model [44] was used, since many physiological parameters are related to body weight [37, 44]. Moreover, the previous population pharmacokinetic studies found that weight was a significant covariate influencing pharmacokinetic parameters of nevirapine [14, 15, 18, 20]. Based on the allometric scaling, the exponent of the power model for CL/F and V/F of 0.75 and 1 was used, respectively [37, 44]. The allometric scaling of CL/F and V/F are presented in following equation;

$$TVCL = \theta_1 \times (WT/56)^{0.75}$$

$$TVV = \theta_2 \times (WT/56)$$

One-compartment model and two-compartment model with first-order absorption and elimination were tested. For one-compartment model (ADVAN2 TRANS2), only the model using an exponential error model for IIV was minimized successfully, while two-compartment model (ADVAN 4 TRAN 4) failed to achieve a successful minimization. The parameter estimates from a one- and two-compartment model are shown in Table 9 and Table 10, respectively.

Table 9 Results from one-compartment model with different IIV and RUV models

Model for IIV	Model for RUV	OFV	Parameter estimate (SE %)						
			CL (L/hr)	V (L)	k_a (hr^{-1})	ω_{CL}	ω_V	ω_{ka}	σ
Additive	Additive	-[a]	-	-	-	-	-	-	-
	Proportional	897.892[b]	2.62	124	0.309	0.9159	0.5477	0.4669	0.2917
	Combined	882.819[c]	2.64	386	1.27e+007	1.0099	0.5504	4.9193	Proportional: 0.1612 Additive: 1.1489
	Exponential	897.892[b]	2.62	124	0.309	0.9159	0.5477	0.4669	0.2917
Proportional	Additive	-[a]	-	-	-	-	-	-	-
	Proportional	892.709[b]	2.47	33.6	0.203	0.3271	0.4098	0.1992	0.2723
	Combined	873.395[b]	2.47	76.4	0.241	0.3507	0.4505	0.1646	Proportional: 0.1791 Additive: 1.0488
	Exponential	892.709[b]	2.47	33.6	0.203	0.3271	0.4098	0.1992	0.2723
Exponential	Additive	903.435[b]	2.6	1370	0.0166	0.4460	9.7979	11.3137	1.9799
	Proportional	875.928[d]	2.41 (4.6)	17.9 (59.8)	0.0789 (66.9)	0.3886 (15.2)	0.7113 (71.9)	0.9338 (48.7)	0.2366 (20.9)
	Combined	862.322[d]	2.47 (4.5)	74.5 (58.9)	0.186 (43.7)	0.4135 (14.1)	1.3266 (41.6)	0.0206 (6854.5)	Proportional: 0.1865 (63.2) Additive: 0.7823(86.4)
	Exponential	875.928[d]	2.41 (4.6)	17.9 (59.8)	0.0789 (66.9)	0.3885 (15.2)	0.7113 (71.9)	0.9338 (48.7)	0.2366 (20.9)

[a] Estimation omitted

[b] Minimization terminated

[c] Minimization successful but covariance step aborted

[d] Minimization successful

Table 10 Results from two-compartment model with different IIV and RUV models

Model for IIV	Model for RUV	OFV	Parameter estimate (SE %)										
			CL (L/hr)	V1 (L)	Q (L/hr)	V2 (L)	k_a (hr^{-1})	ω_{CL}	ω_{V1}	ω_{Q}	ω_{V2}	ω_{ka}	σ
Additive	Additive	-[a]	-	-	-	-	-	-	-	-	-	-	-
	Proportional	900.524[b]	2.68	182	0.366	82.8	0.268	0.9176	0.5477	0.5477	0.5477	0.5576	0.3159
	Combined	895.023[b]	2.67	192	0.1	79.6	0.225	0.9528	0.5477	0.5477	0.5477	0.5338	Proportional: 0.2844 Additive: 0.8865
	Exponential	900.524[b]	2.68	182	0.366	82.8	0.268	0.9176	0.5477	0.5477	0.5477	0.5576	0.3159
Proportional	Additive	-[a]	-	-	-	-	-	-	-	-	-	-	-
	Proportional	-[a]	-	-	-	-	-	-	-	-	-	-	-
	Combined	-[a]	-	-	-	-	-	-	-	-	-	-	-
	Exponential	-[a]	-	-	-	-	-	-	-	-	-	-	-
Exponential	Additive	872.336[b]	2.44	6800	53	16.3	0.0556	0.3949	38.7298	4.8682	0.5147	0.5856	1.4212
	Proportional	888.335[c]	2.63	442	0.1	1	8.16e+009	0.4277	0.0054	114.018	0.5648	0.0054	0.2662
	Combined	862.386[b]	2.47	77.4	0.1	2620	0.189	0.4147	1.3416	0.1058	0.5495	0.0054	Proportional: 0.1862 Additive: 0.7854
	Exponential	888.335[c]	2.63	442	0.1	1	8.16e+009	0.4277	0.0054	114.018	0.5648	0.0054	0.2662

[a] Estimation omitted

[b] Minimization terminated

[c] Minimization successful, however parameter estimate is near its boundary

However, k_a and V/F estimated from the models that minimized successfully had a high standard error (greater than 50%). Moreover, the estimates of k_a and V/F cannot precisely estimate. This could be due to the fact that most of blood samples were collected at through concentrations, as presented in Figure 5. There are 2 observed concentrations measured during the absorption phase (time after last dose < 4 hours). Therefore, k_a and V/F were fixed at the literature values of 1.66 hr^{-1} and 77 L, respectively [25].

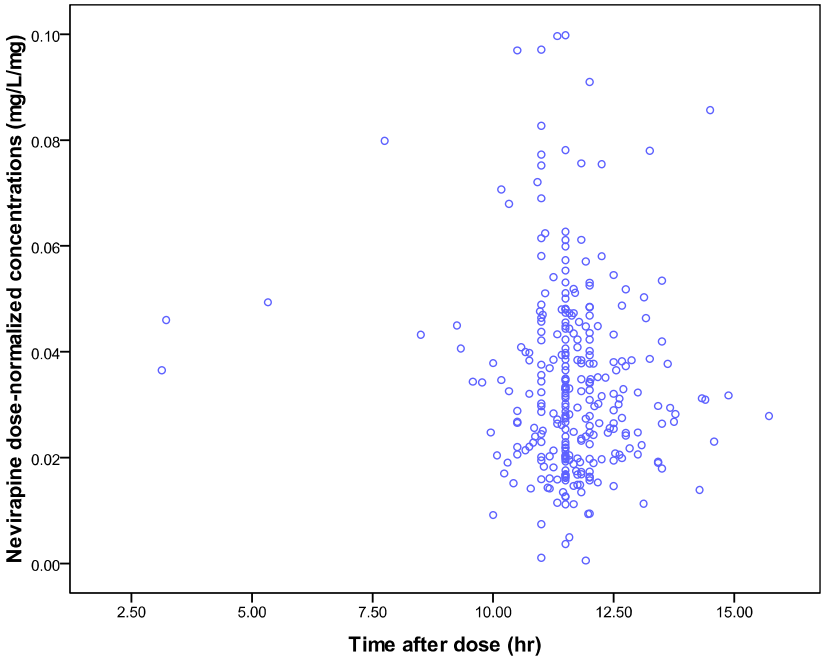


Figure 5 The plot of nevirapine dose-normalized concentrations (mg/L/mg) vs. time after last dose (hr)

The parameter estimates from the rerun of the models that minimized successfully when V/F and k_a were fixed are presented in Table 11.

Table 11 Parameter estimates from different error models for RUV after fixed k_a and V/F

Model for IIV	Model for RUV	OFV	Parameter estimate (SE %)		
			CL (L/hr)	ω_{CL}	σ
Exponential	Proportional	901.333	2.42 (3.7)	0.4159 (16.2)	0.2634 (19.3)
	Combined	882.602	2.38 (3.6)	0.4231 (13.2)	Proportional: 0.2035 (27.1)
					Additive: 0.7823 (43.1)
	Exponential	901.333	2.42 (3.7)	0.4159 (16.2)	0.2634 (19.3)

As the combined proportional and additive error model for RUV gave the smallest OFV and the better goodness of fit than other models. Therefore, the model with exponential error model for IIV and combined proportional and additive error model for RUV was chosen as the final structural model. The goodness of fit plot of the chosen structural model is presented in Figure 6-8. From these plots, a consistency between the observed and predicted concentration is shown. Moreover, significant deviation is not observed in the weighted residual (WRES) plot. Therefore, the final structural model is adequately explained the pharmacokinetics and variability of the data.

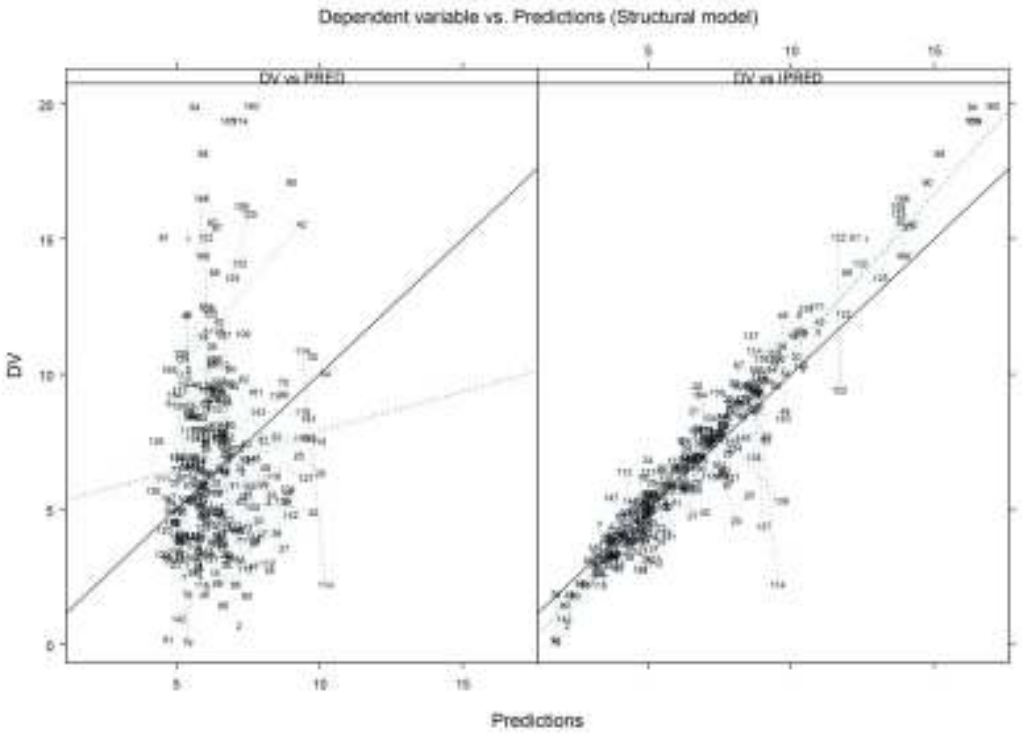


Figure 6 Goodness of fit plot of the structural model; observed concentrations (DV) vs. population predicted concentrations (PRED) and observed concentrations (DV) vs. individual predicted concentrations (IPRED)

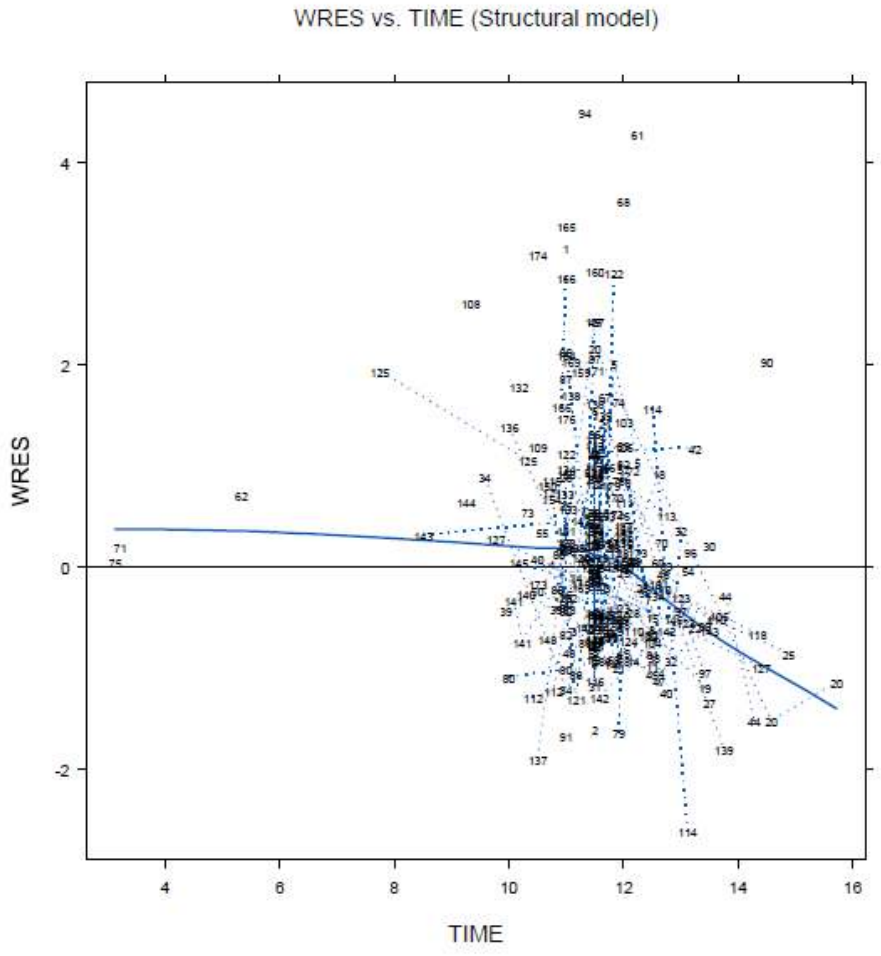


Figure 7 Goodness of fit plot of the structural model; Weighted residual (WRES) vs. Time

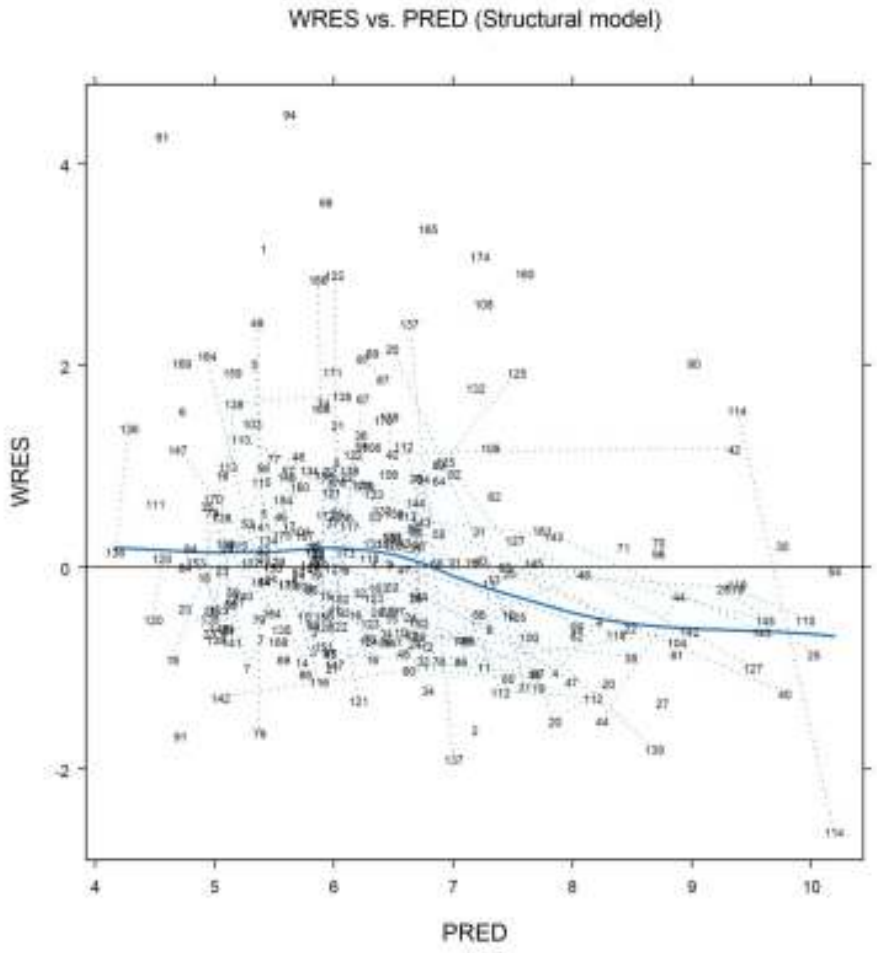


Figure 8 Goodness of fit plot of the structural model; Weighted residual (WRES) vs. population predicted concentration (PRED)

2. Covariate model building

Initially, the relationship between each of the patient characteristics and pharmacokinetic parameters was investigated by graphical displays. The plots between each patient characteristic and individual CL/F are presented in Figure 9. The graphical displays of the relationship between nevirapine observed concentrations and each covariate was shown in Figure 10.

According to Figure 9, the individual plots of continuous variables show the trend of a linear relationship between clearance and most of the covariates, thus a linear covariate model was used in the covariate model building. The continuous variables were centered by their mean when they were used as covariates. Besides, the graphical displays also show the possible point of change of clearance at about AGE=40 years, AST=60 U/L and ALT=60 U/L. Thus, these variables were categorized into two groups and were tested as categorical variables. A summary of the categorical variables of index and validation datasets are shown in Table 12.

Table 12 Summary of categories of age, AST and ALT

Category	Index dataset	Validation dataset	p-value
	n (%)	n (%)	
Categories of age (CAGE)			0.060[a]
0= Age ≤ 40	128 (71.5)	35 (58.3)	
1= Age > 40	51 (28.5)	25 (41.7)	
Categories of AST (CAST)			0.171[a]
0= AST ≤ 60	152 (84.9)	55 (91.7)	
1= AST > 60	27 (15.1)	5 (8.3)	
Categories of ALT (CALT)			0.788[a]
0= ALT ≤ 60	149 (83.2)	51 (85.0)	
1= ALT > 60	30 (16.8)	9 (15.0)	

[a] Comparison of the frequency between an index dataset and a validation dataset by χ^2 test

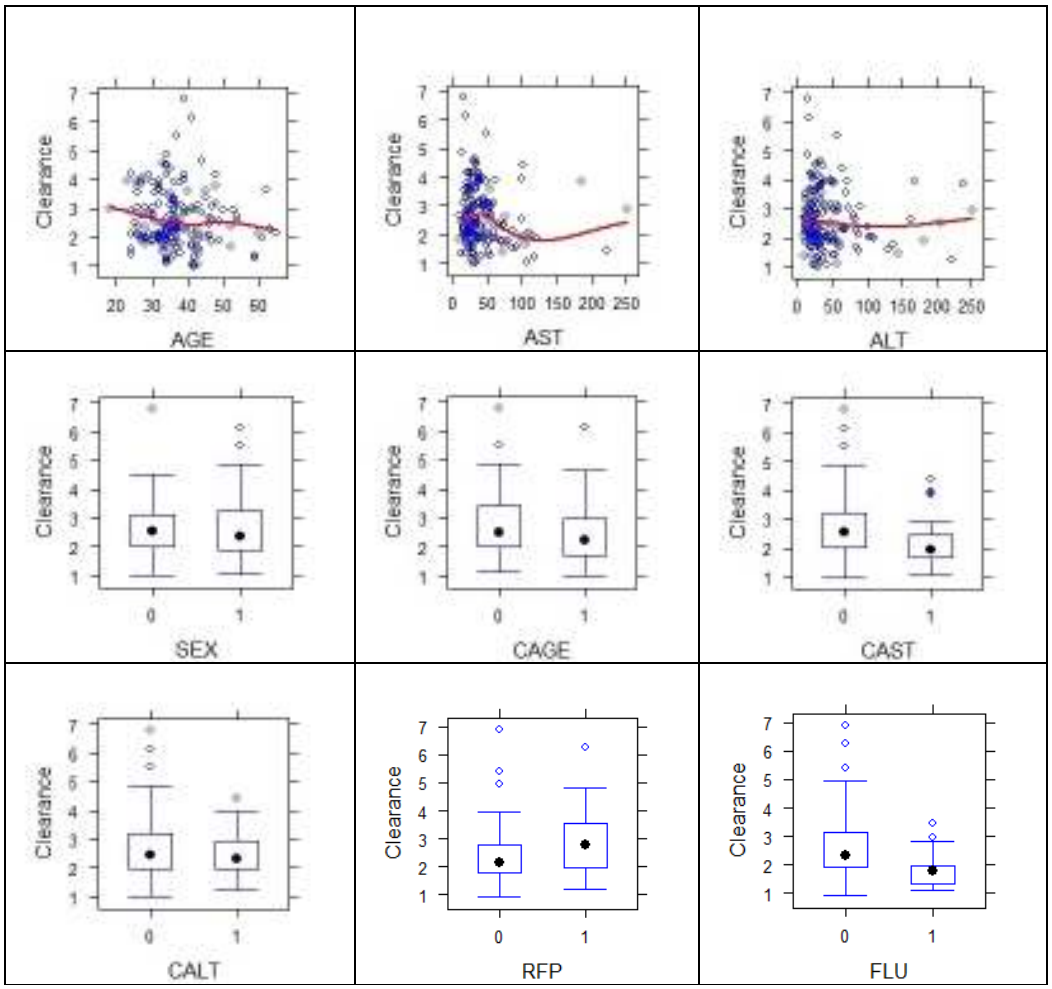


Figure 9 The graphical displays of the relationship between clearance and covariates

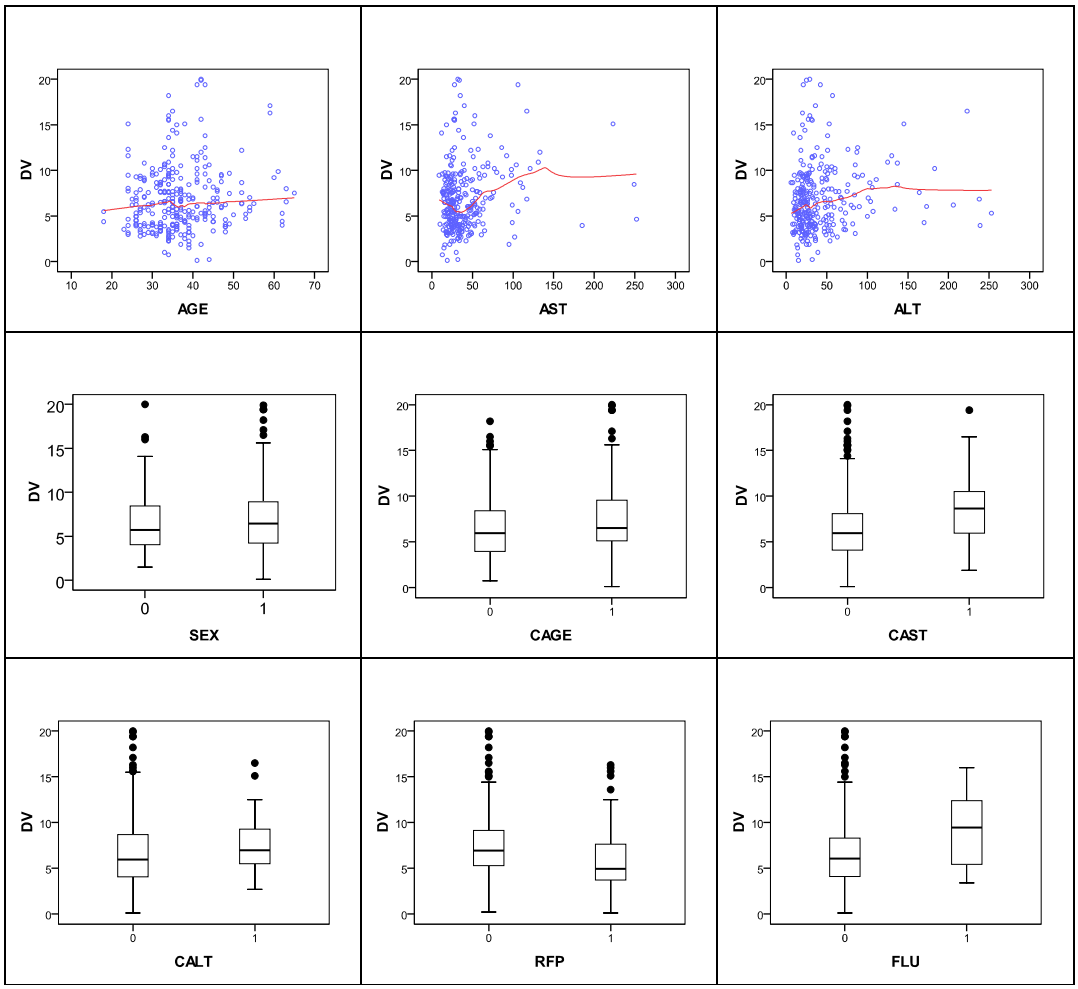


Figure 10 The graphical displays of the relationship between observed concentrations (DV) and covariates

During stepwise forward inclusion, each covariate was introduced to the structural model one at a time. A covariate which decreased the OFV of at least 3.84 ($p \leq 0.05$, χ^2, df=1) was added into the model for further analysis. The significant covariates in the process of stepwise forward inclusion were FLU, CAGE, CAST and RFP. Then, the model which included FLU, CAGE, CAST and RFP as covariates was used as a full model for further backward deletion process.

During backward deletion, the covariate was removed one at a time. If the OFV increased at least 6.63 ($p \leq 0.01$, χ^2, df=1), the covariate was retained in the final model. After backward deletion, the final model included CAST, CAGE and RFP as significant covariates. The results from stepwise forward inclusion and backward deletion were shown in Table 13-19.

The goodness of fit plots of the final model obtained from NONMEM was performed. The plots of observed concentrations (DV) vs. population predicted concentrations (PRED), DV vs. individual predicted concentrations (IPRED) and weighted residual (WRES) and PRED of the final model compared with the structural model can better predict the higher concentration than the structural model (Figure 11-13).

The final population pharmacokinetic model of nevirapine obtained from NONMEM is presented as follow;

$$TVCL = (\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 CAGE) \times (1+\theta_3 CAST) \times (1+\theta_4 RFP)$$

Table 13 The results of stepwise forward inclusion (univariate analysis)

Model	OFV	dOFV
Structural model $TVCL=\theta_1 \times ((WT/56)^{0.75})$ Fixed V=77 x (WT/56),Ka=1.66	882.602	
Add age into the model $TVCL=\theta_1 \times ((WT/56)^{0.75})+\theta_2(AGE-37)$	879.070	3.532
Add AST into the model $TVCL=\theta_1 \times ((WT/56)^{0.75})+\theta_2(AST-42)$	880.259	2.343
Add ALT into the model $TVCL=\theta_1 \times ((WT/56)^{0.75})+\theta_2(ALT-44)$	879.090	3.512
Add sex into the model $TVCL=\theta_1 \times ((WT/56)^{0.75}) \times (1+\theta_2 SEX)$	879.374	3.228
Add age as categorical variable into the model $TVCL=\theta_1 \times ((WT/56)^{0.75}) \times (1+\theta_2 CAGE)$	877.695	4.907*
Add AST as categorical variable into the model $TVCL=\theta_1 \times ((WT/56)^{0.75}) \times (1+\theta_2 CAST)$	875.230	7.372*
Add ALT as categorical variable into the model $TVCL=\theta_1 \times ((WT/56)^{0.75}) \times (1+\theta_2 CALT)$	879.950	2.652
Add RFP into the model $TVCL=\theta_1 \times ((WT/56)^{0.75}) \times (1+\theta_2 RFP)$	863.533	19.069*
Add FLU into the model $TVCL=\theta_1 \times ((WT/56)^{0.75}) \times (1+\theta_2 FLU)$	879.988	2.614

* OFV decreased at least 3.84 ($p \leq 0.05$, χ^2, df=1)

Table 14 The results of stepwise forward inclusion (RFP was added)

Model	OFV	dOFV
Structural model $TVCL=\theta_1 \times ((WT/56)^{0.75}) \times (1+\theta_2 RFP)$ Fixed V=77 x (WT/56),Ka=1.66	863.533	
Add age into the model $TVCL=(\theta_1 \times ((WT/56)^{0.75}))+\theta_2(AGE-37)) \times (1+\theta_3 RFP)$	859.457	4.076*
Add AST into the model $TVCL=((\theta_1 \times ((WT/56)^{0.75}))+\theta_2(AST-42)) \times (1+\theta_3 RFP)$	861.106	2.427
Add ALT into the model $TVCL=((\theta_1 \times ((WT/56)^{0.75}))+\theta_2(ALT-44)) \times (1+\theta_3 RFP)$	861.015	2.518
Add sex into the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 SEX) \times (1+\theta_3 RFP)$	859.975	3.558*
Add age as categorical variable into the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 CAGE) \times (1+\theta_3 RFP)$	857.011	6.522*
Add AST as categorical variable into the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 CAST) \times (1+\theta_3 RFP)$	855.454	8.079*
Add ALT as categorical variable into the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 CALT) \times (1+\theta_3 RFP)$	861.440	2.093
Add FLU into the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 FLU) \times (1+\theta_3 RFP)$	858.810	4.723*

* OFV decreased at least 3.84 ($p \leq 0.05$, χ^2, df=1)

Table 15 The results of stepwise forward inclusion (CAST and RFP were added)

Model	OFV	dOFV
Structural model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 CAST) \times (1+\theta_3 RFP)$ Fixed V=77 x (WT/56),Ka=1.66	855.454	
Add age into the model $TVCL=(\theta_1 \times ((WT/56)^{0.75}))+\theta_2(AGE-37)) \times (1+\theta_3 CAST) \times (1+\theta_4 RFP)$	849.857	5.597*
Add ALT into the model $TVCL=((\theta_1 \times ((WT/56)^{0.75}))+\theta_2(ALT-44)) \times (1+\theta_3 CAST) \times (1+\theta_4 RFP)$	855.436	0.018
Add sex into the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 SEX) \times (1+\theta_3 CAST) \times (1+\theta_4 RFP)$	852.280	3.174
Add age as categorical variable into the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 CAGE) \times (1+\theta_3 CAST) \times (1+\theta_4 RFP)$	847.704	7.750*
Add ALT as categorical variable into the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 CALT) \times (1+\theta_3 CAST) \times (1+\theta_4 RFP)$	855.319	0.135
Add FLU into the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 FLU) \times (1+\theta_3 CAST) \times (1+\theta_4 RFP)$	851.819	3.635

* OFV decreased at least 3.84 ($p \leq 0.05$, χ^2, df=1)

Table 16 The results of stepwise forward inclusion (CAGE CAST, and RFP were added)

Model	OFV	dOFV
Structural model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 CAGE) \times (1+\theta_3 CAST) \times (1+\theta_4 RFP)$ Fixed V=77 x (WT/56),Ka=1.66	847.704	
Add ALT into the model $TVCL=((\theta_1 \times ((WT/56)^{0.75}))+\theta_2(ALT-42)) \times (1+\theta_3 CAGE) \times$ $(1+\theta_4 CAST) \times (1+\theta_5 RFP)$	847.681	0.023
Add sex into the model $TVCL=((\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 SEX) \times (1+\theta_3 CAGE) \times$ $(1+\theta_4 CAST) \times (1+\theta_5 RFP)$	845.426	2.278
Add ALT as categorical variable into the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 CALT) \times (1+\theta_3 CAGE) \times$ $(1+\theta_4 CAST) \times (1+\theta_5 RFP)$	847.638	0.066
Add FLU into the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 FLU) \times (1+\theta_3 CAGE) \times$ $(1+\theta_4 CAST) \times (1+\theta_5 RFP)$	843.673	4.031*

* OFV decreased at least 3.84 ($p \leq 0.05$, χ^2, df=1)

Table 17 The results of stepwise forward inclusion (FLU, CAGE, CAST and RFP were added)

Model	OFV	dOFV
Structural model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 FLU) \times (1+\theta_3 CAGE) \times (1+\theta_4 CAST) \times (1+\theta_5 RFP)$ Fixed $V=77 \times (WT/56)$, $Ka=1.66$	843.673	
Add ALT into the model $TVCL=((\theta_1 \times ((WT/56)^{0.75}))+\theta_2(ALT-44)) \times (1+\theta_3 FLU) \times (1+\theta_4 CAGE) \times (1+\theta_5 CAST) \times (1+\theta_6 RFP)$	843.605	0.068
Add sex into the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 SEX) \times (1+\theta_3 FLU) \times (1+\theta_4 CAGE) \times (1+\theta_5 CAST) \times (1+\theta_6 RFP)$	840.836	2.837
Add ALT as categorical variable into the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 CALT) \times (1+\theta_3 FLU) \times (1+\theta_4 CAGE) \times (1+\theta_5 CAST) \times (1+\theta_6 RFP)$	843.579	0.094

Table 18 The results of stepwise backward deletion

Model	OFV	dOFV
Structural model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 FLU) \times (1+\theta_3 CAGE) \times (1+\theta_4 CAST) \times (1+\theta_5 RFP)$ Fixed V=77 x (WT/56),Ka=1.66	843.673	
Remove FLU from the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 CAGE) \times (1+\theta_3 CAST) \times (1+\theta_4 RFP)$	847.704	4.031
Remove age as categorical variable from the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 FLU) \times (1+\theta_3 CAST) \times (1+\theta_4 RFP)$	851.819	8.146*
Remove AST as categorical variable from the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 FLU) \times (1+\theta_3 CAGE) \times (1+\theta_4 RFP)$	851.825	8.152*
Remove RFP from the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 FLU) \times (1+\theta_3 CAGE) \times (1+\theta_4 CAST)$	867.169	23.496*

* OFV increased at least 6.63 ($p \leq 0.01$, χ^2, df=1) and retained in the model

Table 19 The results of stepwise backward deletion (FLU was removed)

Model	OFV	dOFV
Structural model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 CAGE) \times (1+\theta_3 CAST) \times (1+\theta_4 RFP)$ Fixed V=77 x (WT/56),Ka=1.66	847.704	
Remove age as categorical variable from the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 CAST) \times (1+\theta_3 RFP)$	855.454	7.750*
Remove AST as categorical variable from the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 CAGE) \times (1+\theta_3 RFP)$	857.011	9.307*
Remove RFP from the model $TVCL=(\theta_1 \times ((WT/56)^{0.75})) \times (1+\theta_2 CAGE) \times (1+\theta_3 CAST)$	869.292	21.588*

* OFV increased at least 6.63 ($p \leq 0.01$, χ^2, df=1) and retained in the model

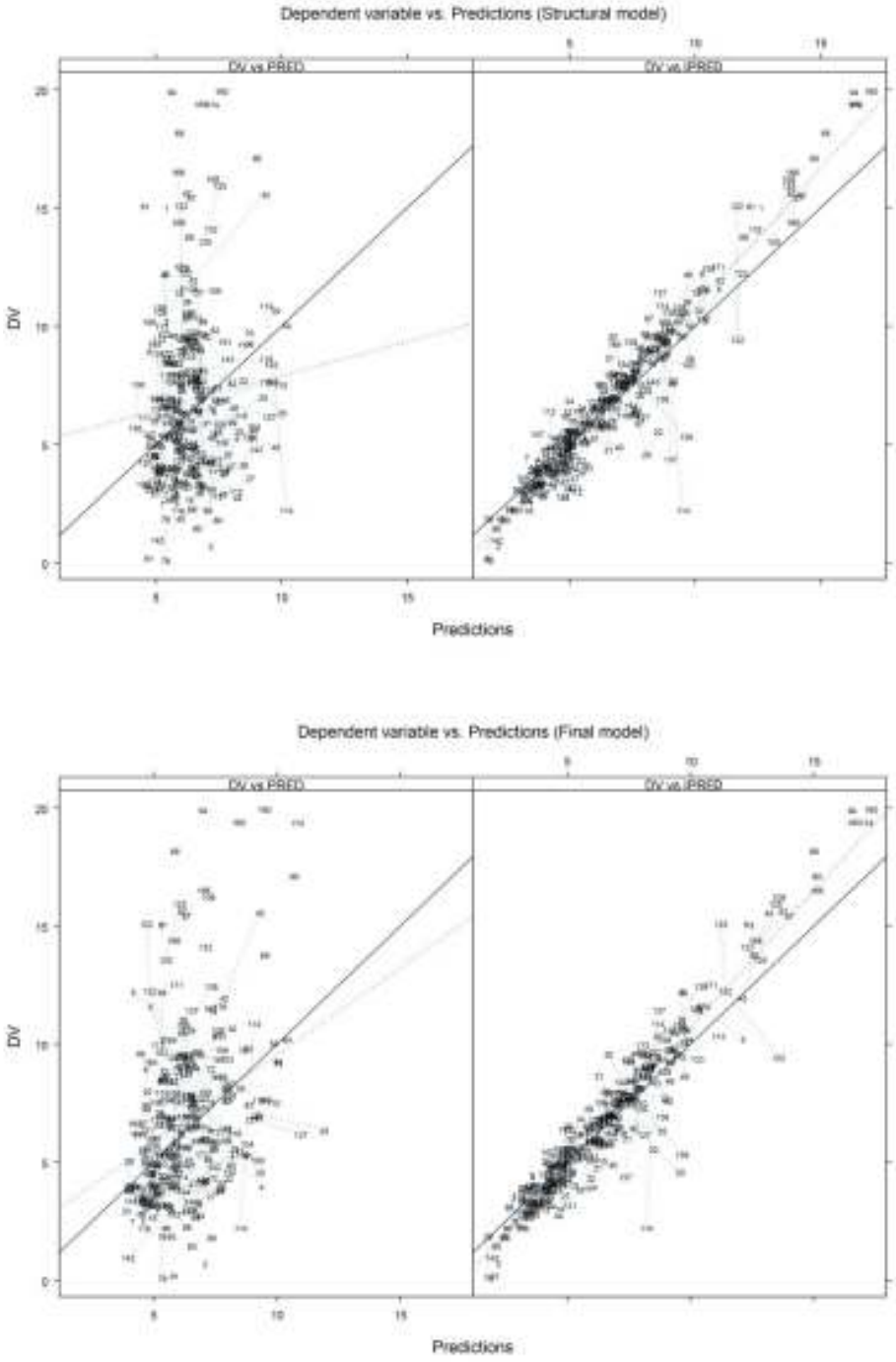
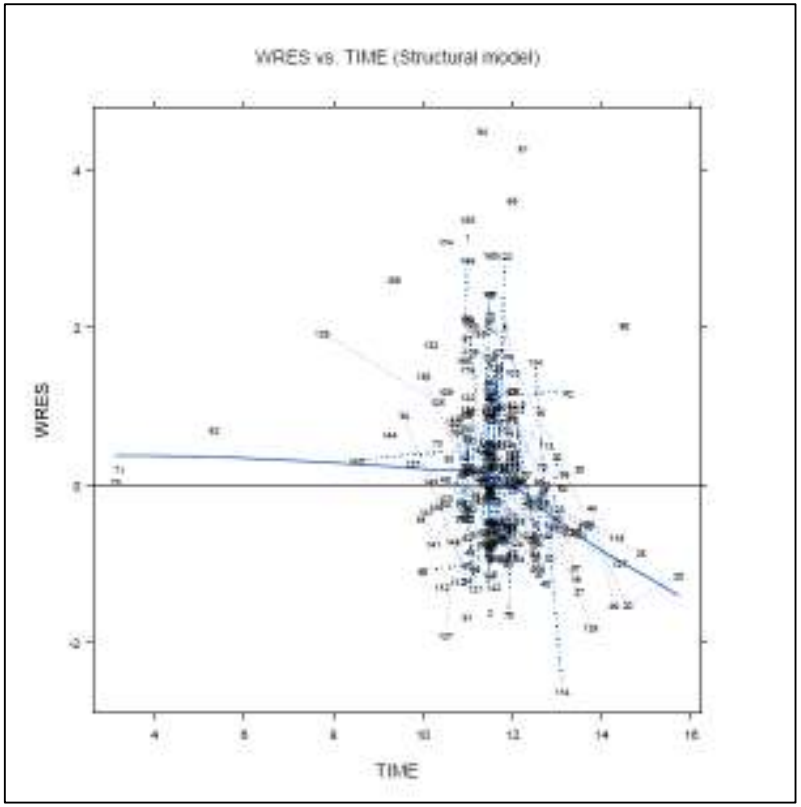


Figure 11 The goodness of fit plots of structural model and final model; Observed concentrations (DV) vs. population predicted concentrations (PRED) and observed concentrations (DV) vs. individual predicted concentrations (IPRED)



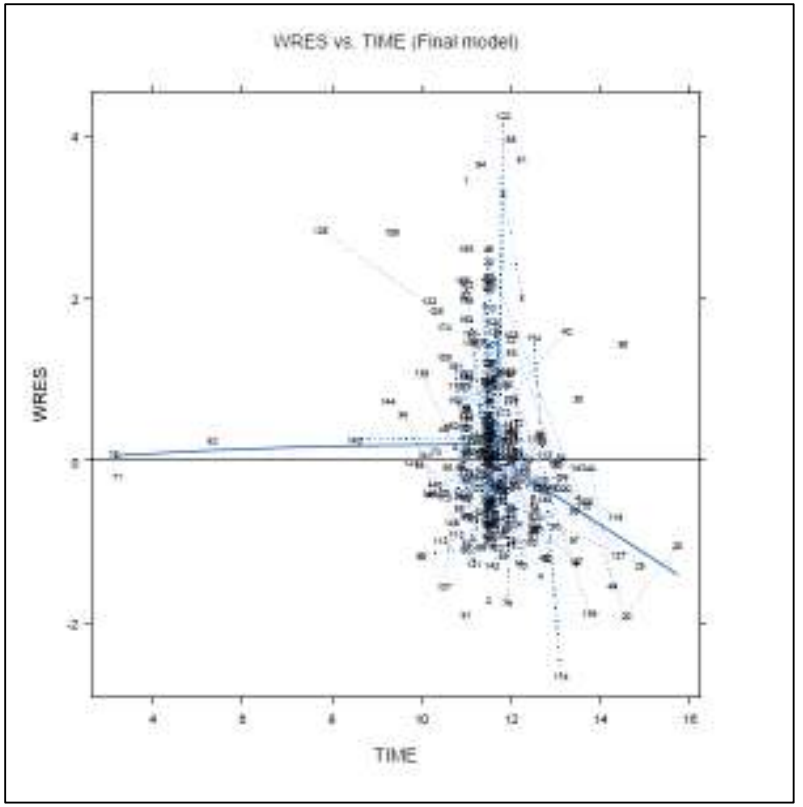
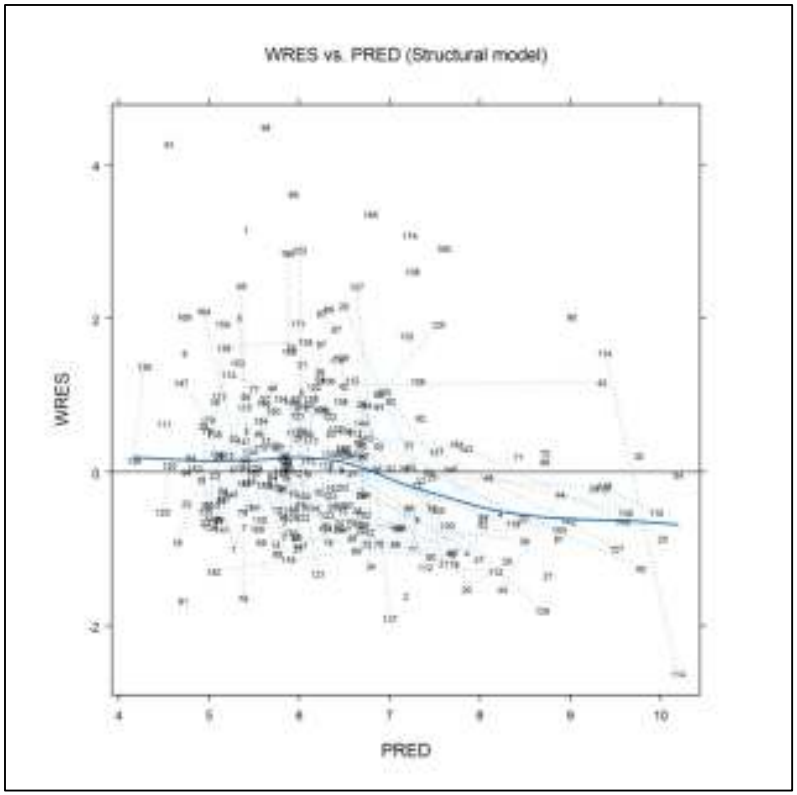


Figure 12 The goodness of fit plots of structural model and final model; Weighted residual (WRES) vs. Time



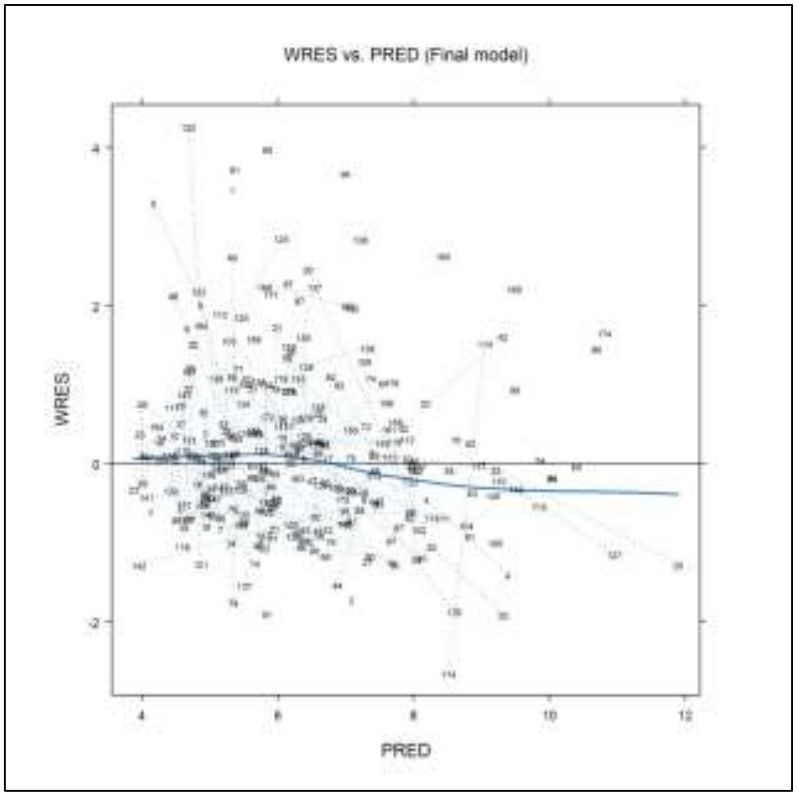


Figure 13 The goodness of fit plots of structural model and final model; Weighted residual (WRES) vs. population predicted concentrations (PRED)

The percent of coefficient of variation (%CV) of CL/F of the final model was estimated to be 38.99% which was lower than the values obtained from the structural model (42.31%). The proportional and additive RUV were 19.87% and 0.73 mg/L, respectively. The parameter estimates and their 95% confidence interval are summarized in Table 20.

Table 20 Final parameter estimates and 95% confidence intervals from NONMEM

Parameter	NONMEM		
	Estimate	95% confidence intervals*	
		Lower	Upper
θ_1	2.41	2.21	2.61
θ_2	-0.18	-0.30	-0.07
θ_3	-0.16	-0.25	-0.06
θ_4	0.22	0.11	0.33
IIV (CL/F), %CV	38.99	32.97	44.19
RUV (proportional), %CV	19.87	14.58	24.03
RUV (additive), mg/L	0.73	0.39	0.96

* Estimate ± 1.96 x standard error of the parameter estimates

Therefore, the equation of CL/F can be rewritten as follow;

$$CL/F = (2.41 \times ((WT/56)^{0.75})) \times (1-0.18 \times CAGE) \times (1-0.16 \times CAST) \times (1+0.22 \times RFP)$$

From the equation, weight was related to CL/F by a power function with an exponent of 0.75. Age more than 40 years and AST level more than 60 U/L decreased CL/F by 18% and 16%, respectively. Concomitant use of rifampicin increased CL/F by 22%.

3. Model validation

A validation of the final population pharmacokinetic model of nevirapine was performed by Bayesian estimation [57]. A validation dataset was used in this process. The individual predicted concentrations from Bayesian estimation were compared with the observed concentrations. The bias and precision of the model prediction was calculated as MPE and RMSE, respectively. The individual predicted concentrations (IPRED) and observed concentrations (DV) of each patient in validation dataset are shown in Table 21.

The MPE and RMSE were -0.10 mg/L and 1.50 mg/L, respectively. The bias of final population pharmacokinetic model prediction was not different from zero (MPE = -0.10 mg/L, p=0.49). The result of one-sample t-test of MPE compared to zero is shown in Table 22.

$$\text{MPE} = \frac{1}{N} \times \sum (\text{predicted concentration} - \text{observed concentration})$$

$$= \frac{1}{105} \times (-10.63)$$

$$= -0.10 \text{ mg/L}$$

$$\text{RMSE} = \sqrt{\frac{1}{N} \times (\text{predicted concentration} - \text{observed concentration})^2}$$

$$= \sqrt{\frac{1}{105} \times (-10.63)^2}$$

$$= 1.50 \text{ mg/L}$$

Table 21 Comparison of IPRED and DV obtained from Bayesian estimation

ID	IPRED	DV	Prediction error	ID	IPRED	DV	Prediction error
1	8.32	8.97	-0.65	13	5.98	5.19	0.79
2	8.68	9.44	-0.76	13	4.68	4.95	-0.27
3	9.70	11.05	-1.35	13	7.71	9.08	-1.37
4	7.30	7.27	0.03	14	2.32	0.03	2.29
5	4.96	6.56	-1.60	15	4.12	3.69	0.43
5	6.03	3.34	2.69	16	3.70	3.91	-0.21
5	6.81	7.69	-0.88	16	3.70	3.77	-0.07
6	3.95	3.94	0.01	16	4.48	4.13	0.35
6	6.08	1.85	4.23	17	6.47	7.30	-0.83
6	4.74	5.84	-1.10	17	5.44	5.23	0.21
7	6.65	6.98	-0.33	18	4.36	5.28	-0.92
7	7.77	8.10	-0.33	18	5.39	3.88	1.51
8	4.82	3.05	1.77	18	3.81	4.34	-0.53
8	2.44	3.28	-0.84	19	9.13	10.30	-1.17
8	3.08	2.35	0.73	19	11.46	11.66	-0.20
9	2.26	1.33	0.93	20	5.63	5.90	-0.27
9	2.29	2.66	-0.37	21	8.78	9.29	-0.51
9	3.89	3.61	0.28	22	4.42	4.33	0.09
10	6.34	2.98	3.36	23	5.05	4.39	0.66
10	5.06	5.06	0.00	24	3.44	3.97	-0.53
10	6.17	7.76	-1.59	24	6.36	5.39	0.97
11	6.99	9.30	-2.31	25	9.40	10.64	-1.24
11	6.90	5.91	0.99	26	4.92	4.85	0.07
11	8.32	7.65	0.67	27	7.13	6.65	0.48
12	5.84	4.60	1.24	28	9.80	11.27	-1.47
12	6.04	5.18	0.86	29	15.82	18.02	-2.20
12	7.17	9.61	-2.44	29	16.24	17.48	-1.24

Table 21 Comparison of IPRED and DV obtained from Bayesian estimation (continued)

ID	IPRED	DV	Prediction error	ID	IPRED	DV	Prediction error
32	5.83	6.04	-0.21	48	5.81	6.76	-0.95
32	7.53	7.22	0.31	48	7.70	6.63	1.07
33	4.57	4.50	0.07	49	4.74	4.42	0.32
34	6.81	6.83	-0.02	49	4.37	4.49	-0.12
35	4.53	4.35	0.18	50	6.68	7.98	-1.30
36	5.77	5.88	-0.11	50	6.70	7.41	-0.71
37	10.04	11.99	-1.95	50	9.14	6.06	3.08
38	6.74	7.14	-0.40	51	6.30	6.02	0.28
39	4.37	4.21	0.16	52	8.12	9.54	-1.42
40	4.50	3.88	0.62	52	7.98	6.27	1.71
40	3.60	4.23	-0.63	53	23.74	28.95	-5.21
41	3.43	3.68	-0.25	53	27.39	27.46	-0.07
41	3.40	3.05	0.35	54	7.13	1.49	5.64
41	3.95	3.88	0.07	54	8.43	4.23	4.20
42	10.22	11.41	-1.19	54	6.48	10.24	-3.76
42	13.47	13.92	-0.45	55	12.18	14.77	-2.59
43	5.26	5.83	-0.57	56	3.70	2.89	0.81
43	4.74	4.39	0.35	56	4.47	4.84	-0.37
44	2.12	2.06	0.06	57	11.66	12.85	-1.19
44	2.17	1.38	0.79	58	6.88	7.34	-0.46
45	4.67	4.62	0.05	58	6.70	7.11	-0.41
45	3.75	4.08	-0.33	58	6.64	6.71	-0.07
46	4.26	4.08	0.18	59	7.63	7.82	-0.19
47	6.20	4.98	1.22	60	7.87	8.82	-0.95
47	5.33	6.13	-0.80	Sum of prediction error			-10.63

Table 22 One-sample t-test of MPE compared to zero

One-Sample Test						
Test Value = 0						
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
MPE	-0.689	104	0.49	-0.10	-0.39	0.19

When the Bland-Altman was plotted to describe the agreement between IPRED and DV (Figure 14), the plots are equally distributed and most of them are within mean±SD. Therefore, the final model can be deemed adequate.

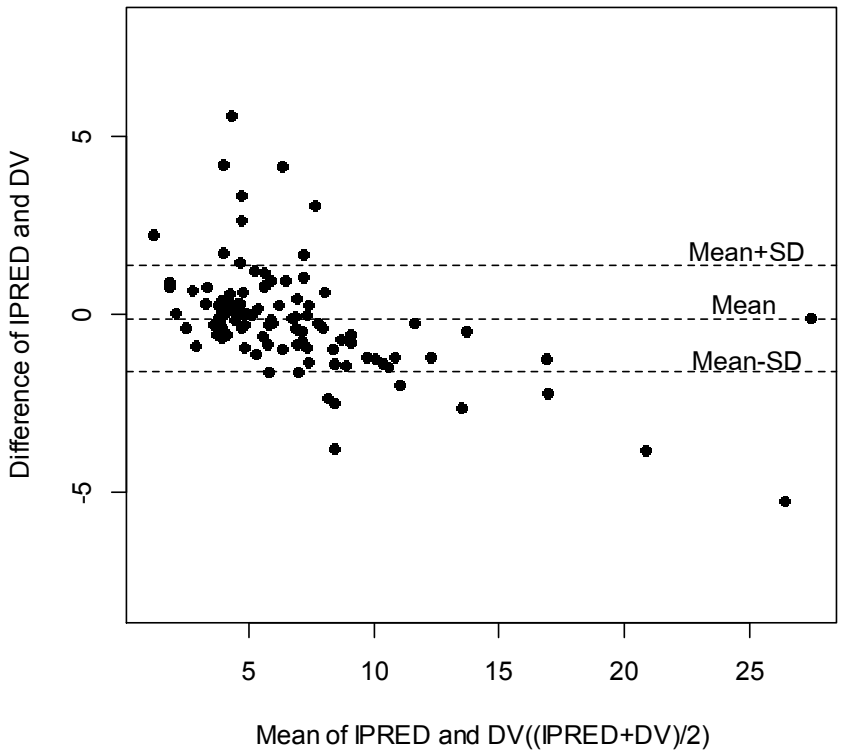


Figure 14 Bland-Altman plot described the agreement between individual predicted concentrations (IPRED) and observed concentrations (DV)

CHAPTER V
DISCUSSION AND CONCLUSION

Nevirapine is one of the preferred first-line NNRTIs for HIV-infected patients in Thailand [3] and is the most widely used as part of an antiretroviral therapy. Since nevirapine is generally combined in a generic fixed-dose combination tablet (GPO-vir®), it has low cost and convenience. The efficacy of nevirapine has been confirmed in several studies [7, 9-11]. However, the problematic situations of nevirapine use are adverse effects and drug resistance which relates to nevirapine plasma concentration. The complexity of comedication use is also an obstacle. Therefore, dosage optimization for individual patient is required to improve efficacy and safety of nevirapine use in HIV-infected patients.

This study used the population pharmacokinetic approach to build the population pharmacokinetic model of nevirapine which can be used as a tool for guiding dosage optimization in Thai HIV-infected patients. The population pharmacokinetics has an ability to estimate the population mean pharmacokinetic parameters, their variability and allows the investigation of the impact of patient's specific covariates on the pharmacokinetic parameters.

In this study, a one-compartment model with first-order absorption and elimination was found to be the best model for describing nevirapine pharmacokinetics which is consistent with previous population pharmacokinetic studies of nevirapine in HIV-infected patients [15-20, 25, 40]. During the structural model building, k_a and V/F were fixed at the literature values of 1.66 hr^{-1} and 77 L, respectively [25]. Because the concentration-time profiles available in the analysis did not contain enough information to estimate k_a and V/F.

In this analysis, weight was included into the structural model using an allometric scaling. Several previous population pharmacokinetic studies of nevirapine found that the pharmacokinetics of nevirapine were influenced by weight [14, 15, 18, 20] and there is a recommendation that pharmacokinetic parameters, such as clearance, volume of

distribution are normally related to body size [44, 59, 60], allometric scaling of CL/F and V/F was considered in this analysis.

In the covariate model building, covariates were introduced to the model in a stepwise fashion. The significant covariates for CL/F found in this analysis were age as categorical variable (≤40 years, >40 years), AST level as categorical variable (≤60 U/L, >60 U/L) and rifampicin use.

According to the parameter estimates obtained from the final model of this study, the patients having weight, age and AST level equal to 56 kg, 37 years and 42 U/L and did not use rifampicin as comedication, has a CL/F of 2.41 L/hr. The CL/F estimated from this study tends to be lower than the values previously reported (2.81-3.84 L/hr) [15-20, 25, 40]. The result is consistent with a study by Kappelhoff et al [25] who found that patients from Thailand have nevirapine CL/F lower than patients from South America and Western countries by 11% and 28%, respectively. Therefore, dose adjustment of nevirapine in Thai patients may be warrant.

Two previous population pharmacokinetic studies of nevirapine found that age was significant covariate for CL/F [17, 18] which is consistent with our study. This could be due to the influence of age on liver function. However, the effect of age on liver function was unclear. Some studies showed no significant difference of liver function test between adult and healthy elderly [61, 62]. Moreover, a study measuring CYP450 activity in human liver microsome found no significant difference in CYP450 activity among different age [62]. However, there is the study reported that the activity of CYP450 (1A2, 2C9, 2C19, 2E1 and 3A4) was decreased in elderly adult (>65 years) when compared with young/mature adult (20-64 years) [43]. Besides, advanced age is related to reduction of liver size and liver blood flow [43, 62] which could lead to reduction of liver clearance.

A previous population pharmacokinetic study found that AST > 1.5 ULN was one of significant covariates for CL/F [15]. Since nevirapine is extensively metabolized by CYP3A4 and CYP2B6 in the liver, abnormality of hepatic function indicated by an

increased level of hepatic enzyme such as AST may reduce capacity of nevirapine CL/F.

In this study, rifampicin increases nevirapine CL/F by 22%. Interestingly, a previous study found a larger extent of rifampicin induction effect. Elsherbiny et al [18] found that concomitant administration of rifampicin increased nevirapine oral clearance by 37.4% in South African patients. One possible explanation of the different degree of rifampicin increasing nevirapine CL/F is the variation of genetics involving in the induction effect of rifampicin.

Pregnane X receptor (PXR), a nuclear receptor plays an important role as xenosensors and regulators of CYP450 metabolizing enzymes [63]. The presence of PXR polymorphisms can alter the induction of CYP3A4 and CYP2B6 promoter activity [63]. Rifampicin is established as a PXR agonist and can activate the promoter activity of CYP3A4 and CYP2B6 [63, 64]. The single nucleotide polymorphisms (SNPs) of PXR that was found to increase rifampicin activation of CYP3A4 were *7635A>G* and *8055C>T* [63]. The prevalence of these SNPs is different among ethnicities [63, 65-71]. Svard et al [63] reported that the frequencies of *7635A>G* and *8055C>T* polymorphisms in Sub-Saharan African were 0.965 and 0.425, respectively. In Asian (Vietnamese), it was showed that the frequencies of *7635A>G* and *8055C>T* polymorphisms were 0.59 and 0.40, respectively [71]. The different frequency of these polymorphisms may reflect the difference of the induction of CYP3A4 and CYP2B6 promoter activity of rifampicin via PXR.

Fluconazole is known as a moderate CYP3A4 inhibitor, concomitant use with nevirapine can cause potential drug-drug interaction. Manosuthi et al [50] found that the nevirapine plasma concentrations in patients who received fluconazole as comedication were lower than those who did not receive fluconazole ($p<0.001$). In our study, the graphical display (Figure 6) showed that the patients who received fluconazole as comedication had lower nevirapine CL/F than the patients who did not receive fluconazole and fluconazole was added to the full model during stepwise forward inclusion. However, it failed to reach significant level in the process of stepwise

backward deletion. The possible reason could be that the number of patients who used fluconazole as comedication in this analysis were small (14 patients, 8%). Therefore, the effect of fluconazole on nevirapine pharmacokinetics cannot be detected. Moreover, among 14 patients receiving fluconazole, 8 patients received both fluconazole and rifampicin. Thus, it is possible that the induction effect of rifampicin could interfere with the inhibition effect of fluconazole in this study.

When the significant covariates were included into the final model, the IIV for CL/F was 38.99% which is reduced by 3.32% compared with the structural model. This IIV of CL/F is similar to previous population pharmacokinetic studies of nevirapine (24.0-33.8%). Other factors including genetics may partly explain IIV of nevirapine. Previous studies showed that *CYP2B6* polymorphisms significantly affected nevirapine CL/F. Two population pharmacokinetic and pharmacogenetic studies of nevirapine found that the *CYP2B6 516G>T* polymorphisms (*CYP2B6 516TT*) decreased nevirapine CL/F by 37% [19, 20]. This suggests that the effect of *CYP2B6* polymorphisms should be further incorporated in the population pharmacokinetic study of nevirapine.

The result from model validation in this study showed that MPE of our model was -0.10 mg/L and was not significantly different from zero (p=0.49). The RMSE was 1.50 mg/L. The Bland-Altman plot showed no systematic bias. Therefore, we can conclude that the final model seems to be adequate.

In conclusion, the population pharmacokinetic model of nevirapine is successfully obtained in this study. The nevirapine CL/F in this population was slightly lower than previously reported in other populations. The patient characteristics influencing nevirapine CL/F were age as categorical variable (≤40 years, >40 years), AST level as categorical variable (≤60 U/L, >60 U/L) and rifampicin use. The results from model validation show the accuracy of the model. Thus, this population pharmacokinetic model can use to guide a dosage optimization for individual HIV-infected Thai patients to improve efficacy and safety of the drug.

Limitations

1. The data used in this study were sparse data; therefore, the concentration-time profiles available were not enough to precisely estimate ka and V/F. Therefore, ka and V/F were fixed to values reported from the previous population pharmacokinetic study.

2. This study did not investigate the influence of genetic factors that may explain part of the IIV of nevirapine pharmacokinetics.

3. The influence of fluconazole were not be quantified in this study due to the small number of patients using fluconazole, even though previous study showed the effect of fluconazole on nevirapine pharmacokinetics.

Recommendation

Previous population pharmacokinetic studies of nevirapine found that *CYP2B6* polymorphisms (*516G>T* and *983T>C*) influenced nevirapine CL/F. Thus, an effect of the genetic variations of enzymes responsible for nevirapine metabolism should be further investigated.

REFERENCES

[1]World Health Organization. Global Health Observatory Data Repository. [Online]. 2011. Available from: http://apps.who.int/ghodata/?vid=22100# [2012, May 21].

[2]The Bureau of Epidemiology. AIDS situation in Thailand. [Online]. 2012. Available from: http://www.aidsthai.org/article/view/302690 [2012, May 21].

[3]Sungkanuparph, S.,Techasathit, W.,Utaipiboon, C.,Chasombat, S.,Bhakeecheep, S.,Leechawengwongs, M., et al. Thai national guidelines for antiretroviral therapy in HIV-1 infected adults and adolescents 2010. Asian Biomedicine. 4 (August 2010): 515-528.

[4]Department of disease control. National Guidelines on HIV/AIDS Diagnosis and Treatment: Thailand 2010. [Online]. 2010. Available from: http://dpc9.ddc.moph.go.th/crd/tranfers/guideline/hiv2010.html [2012, Apr 12].

[5]Kappelhoff, B.S.,Crommentuyn, K.M.,de Maat, M.M.,Mulder, J.W.,Huitema, A.D., Beijnen, J.H. Practical guidelines to interpret plasma concentrations of antiretroviral drugs. Clin Pharmacokinet. 43 (October 2004): 845-853.

[6]Aarnoutse, R.E.,Schapiro, J.M.,Boucher, C.A.B.,Hekster, Y.A., Burger, D.M. Therapeutic drug monitoring: an aid to optimising response to antiretroviral drugs? Drugs. 63 (2003): 741-753.

[7]Manosuthi, W.,Sungkanuparph, S.,Vibhagool, A.,Rattanasiri, S., Thakkinstian, A. Nevirapine- versus efavirenz-based highly active antiretroviral therapy regimens in antiretroviral-naive patients with advanced HIV infection. HIV Med. 5 (March 2004): 105-109.

[8]Manosuthi, W.,Sungkanuparph, S.,Thakkinstian, A.,Vibhagool, A.,Kiertiburanakul, S.,Rattanasiri, S., et al. Efavirenz levels and 24-week efficacy in HIV-infected patients with tuberculosis receiving highly active antiretroviral therapy and rifampicin. AIDS. 19 (September 2005): 1481-1486.

[9]Anekthananon, T.,Ratanasuwan, W.,Techasathit, W.,Sonjai, A., Suwanagool, S. Safety and efficacy of a simplified fixed-dose combination of stavudine, lamivudine and nevirapine (GPO-VIR) for the treatment of advanced HIV-infected patients: a 24-week study. J Med Assoc Thai. 87 (July 2004): 760-767.

[10]Tin, E.E.,Bowonwatanuwong, C.,Desakorn, V.,Wilairatana, P.,Krudsood, S., Pitisuttithum, P. The efficacy and adverse effects of GPO-VIR (stavudine+lamivudine+nevirapine) in treatment-naive adult HIV patients. Southeast Asian J Trop Med Public Health. 36 (March 2005): 362-369.

[11]Chasombat, S.,Lertpiriyasuwat, C.,Thanprasertsuk, S.,Suebsaeng, L., Lo, Y.R. The National Access to Antiretroviral Program for PHA (NAPHA) in Thailand. Southeast Asian J Trop Med Public Health. 37 (July 2006): 704-715.

[12]de Maat, M.M.,ter Heine, R.,Mulder, J.W.,Meenhorst, P.L.,Mairuhu, A.T.,van Gorp, E.C., et al. Incidence and risk factors for nevirapine-associated rash. Eur J Clin Pharmacol. 59 (September 2003): 457-462.

[13]Gonzalez de Requena, D.,Nunez, M.,Jimenez-Nacher, I., Soriano, V. Liver toxicity caused by nevirapine. AIDS. 16 (January 2002): 290-291.

[14]Molto, J.,Valle, M.,Miranda, C.,Cedeno, S.,Miranda, J.,Santos, J.R., et al. Once- or twice-daily dosing of nevirapine in HIV-infected adults: a population pharmacokinetics approach. J Antimicrob Chemother. 62 (October 2008): 784-792.

[15]de Maat, M.M.,Huitema, A.D.,Mulder, J.W.,Meenhorst, P.L.,van Gorp, E.C., Beijnen, J.H. Population pharmacokinetics of nevirapine in an unselected cohort of HIV-1-infected individuals. Br J Clin Pharmacol. 54 (October 2002): 378-385.

[16]de Maat, M.M.,Huitema, A.D.,Mulder, J.W.,Meenhorst, P.L.,van Gorp, E.C.,Mairuhu, A.T., et al. Drug Interaction of Fluvoxamine and Fluoxetine with Nevirapine in HIV-1-Infected Individuals. Clin Drug Investig. 23 (2003): 629-637.

[17]Dailly, E.,Raffi, F.,Perre, P.,Martin, J.,Deslandes, G., Jolliet, P. Influence of darunavir coadministration on nevirapine pharmacokinetics in HIV-infected patients: a population approach. HIV Med. 10 (October 2009): 586-589.

[18]Elsherbiny, D.,Cohen, K.,Jansson, B.,Smith, P.,McIlleron, H., Simonsson, U.S. Population pharmacokinetics of nevirapine in combination with rifampicin-based short course chemotherapy in HIV- and tuberculosis-infected South African patients. Eur J Clin Pharmacol. 65 (January 2009): 71-80.

[19]Chou, M.,Bertrand, J.,Segeral, O.,Verstuyft, C.,Borand, L.,Comets, E., et al. Population pharmacokinetic-pharmacogenetic study of nevirapine in HIV-infected Cambodian patients. Antimicrob Agents Chemother. 54 (October 2010): 4432-4439.

[20]Schipani, A.,Wyen, C.,Mahungu, T.,Hendra, H.,Egan, D.,Siccardi, M., et al. Integration of population pharmacokinetics and pharmacogenetics: an aid to optimal nevirapine dose selection in HIV-infected individuals. J Antimicrob Chemother. 66 (June 2011): 1332-1339.

[21]Sheiner, L., Grasela, T. An introduction to mixed effect modeling: Concepts, definitions, and justification. Journal of Pharmacokinetics and Pharmacodynamics. 19 (1991): 11S-24S.

[22]Steimer, J. Population models and methods, with emphasis on pharmacokinetics. In Rowland M. and Aarons L. (eds), New strategies in drug development and clinical evaluation: the population approach, pp. 31-40. Luxembourg: Commission of European Communities; 1992.

[23]Aarons, L. Population pharmacokinetics: theory and practice. Br J Clin Pharmacol. 32 (December 1991): 669-670.

[24]Ette, E.I., Williams, P.J. Population pharmacokinetics I: background, concepts, and models. Ann Pharmacother. 38 (October 2004): 1702-1706.

[25]Kappelhoff, B.S.,van Leth, F.,MacGregor, T.R.,Lange, J.,Beijnen, J.H., Huitema, A.D. Nevirapine and efavirenz pharmacokinetics and covariate analysis in the 2NN study. Antivir Ther. 10 (2005): 145-155.

[26]American College of Clinical Pharmacology. Pharmacometrics. [Online]. 2011. Available from: http://www.accp1.org/pharmacometrics/theory.htm [2012, May 12].

[27]Laurence , L.B.,John , S.L., Keith , L.P. Goodman & Gilman's the pharmacological basis of therapeutics. New York: McGraw-Hill, 2006.

[28]Barbara , G.W.,Joseph , T.D.,Terry, L.S., Cindy, W.H. Pharmacotherapy handbook. New York McGraw-Hill, 2006.

[29]Mary , A.K.-K.,Lloyd, Y.Y.,Brain, K.A.,Robin, L.C.,B.Joseph, G.,Wayne, A.K., et al. Applied therapeutics : the clinical use of drugs. Philadelphia: Wolters Klumer/Lippincott Williams & Wilkins, 2009.

[30]MMWR Recomm Rep. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. [Online]. 1992. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=1361652 [2012, May 15].

[31]Supparatpinyo, K.,Chiewchanvit, S.,Hirunsri, P.,Uthammachai, C.,Nelson, K.E., Sirisanthana, T. Penicillium marneffei infection in patients infected with human immunodeficiency virus. Clin Infect Dis. 14 (April 1992): 871-874.

[32]Boehringer Ingelheim (Canada) Ltd. Product Information. Viramune®, nevirapine. [Online]. 2010. Available from: http://www.boehringer-ingelheim.ca/content/dam/internet/opu/ca_EN/documents/humanhealth/product_monograph/Viramune-pm.pdf [2012, May 12].

[33]Smith, P.F.,DiCenzo, R., Morse, G.D. Clinical pharmacokinetics of non-nucleoside reverse transcriptase inhibitors. Clin Pharmacokinet. 40 (2001): 893-905.

[34]Lacy, C.F.,Armstrong, L.L.,P.Goldman, M., Lane, L.L. Drug information handbook international : with Canadian and international drug monographs. Hudson, Ohio : Lexi-Comp, 2005.

[35]Riska, P.,Lamson, M.,MacGregor, T.,Sabo, J.,Hattox, S.,Pav, J., et al. Disposition and biotransformation of the antiretroviral drug nevirapine in humans. Drug Metab Dispos. 27 (August 1999): 895-901.

[36]Bonate, P.L. Recommended reading in population pharmacokinetic pharmacodynamics. AAPS J. 7 (2005): E363-373.

[37]Bonate, P.L. Pharmacokinetic-pharmacodynamic modeling and simulation. New York: Springer, 2005.

[38]Ette, E.I.,Williams, P.,Fadiran, E.,Ajayi, F.O., Onyiah, L.C. The process of knowledge discovery from large pharmacokinetic data sets. J Clin Pharmacol. 41 (January 2001): 25-34.

[39]Sheiner, L.B.,Rosenberg, B., Marathe, V.V. Estimation of population characteristics of pharmacokinetic parameters from routine clinical data. J Pharmacokinet Biopharm. 5 (October 1977): 445-479.

[40]Svensson, E.,van der Walt, J.S.,Barnes, K.I.,Cohen, K.,Kredo, T.,Huitema, A., et al. Integration of data from multiple sources for simultaneous modelling analysis: experience from nevirapine population pharmacokinetics. Br J Clin Pharmacol. (February 2012).

[41]de Maat, M.M.,Nellen, J.F.,Huitema, A.D.,Wit, F.W.,Mulder, J.W.,Prins, J.M., et al. Race is not associated with nevirapine pharmacokinetics. Ther Drug Monit. 26 (August 2004): 456-458.

[42]Marubbi, F.,Meneghetti, G.,Maserati, R.,Regazzi, M.,Villani, P.,Seminari, E., et al. Sex differences in nevirapine disposition in HIV-infected patients. AIDS. 17 (2003): 2399-2400.

[43]Mangoni, A.A., Jackson, S.H. Age-related changes in pharmacokinetics and pharmacodynamics: basic principles and practical applications. Br J Clin Pharmacol. 57 (January 2004): 6-14.

[44]Holford, N.H. A size standard for pharmacokinetics. Clin Pharmacokinet. 30 (May 1996): 329-332.

[45]Gandhi, M.,Benet, L.Z.,Bacchetti, P.,Kalinowski, A.,Anastos, K.,Wolfe, A.R., et al. Nonnucleoside reverse transcriptase inhibitor pharmacokinetics in a large unselected cohort of HIV-infected women. J Acquir Immune Defic Syndr. 50 (April 2009): 482-491.

[46]Vogel, M.,Bertram, N.,Wasmuth, J.C.,Wyen, C.,Voigt, E.,Schwarze-Zander, C., et al. Nevirapine pharmacokinetics in HIV-infected and HIV/HCV-coinfected individuals. J Antimicrob Chemother. 63 (May 2009): 988-991.

[47]Boehringer Ingelheim International. Product Monograph on use of VIRAMUNE® in the treatment of adults and children with HIV infection. [Online]. 2008. Available from: www.viramune.com/downloads/Vir_mono_apr06.pdf [2011, September 21].

[48]Avihingsanon, A.,Manosuthi, W.,Kantipong, P.,Chuchotaworn, C.,Moolphate, S.,Sakornjun, W., et al. Pharmacokinetics and 48-week efficacy of nevirapine: 400 mg versus 600 mg per day in HIV-tuberculosis coinfection receiving rifampicin. Antivir Ther. 13 (August 2008): 529-536.

[49]Manosuthi, W.,Sungkanuparph, S.,Thakkinstian, A.,Rattanasiri, S.,Chaovavanich, A.,Prasithsirikul, W., et al. Plasma Nevirapine Levels and 24-Week Efficacy in HIV-Infected Patients Receiving Nevirapine-Based Highly Active Antiretroviral Therapy with or without Rifampicin. Clinical Infectious Diseases. 43 (July 2006): 253-255.

[50]Manosuthi, W.,Athichathanabadi, C.,Uttayamakul, S.,Phoorisri, T., Sungkanuparph, S. Plasma nevirapine levels, adverse events and efficacy of antiretroviral therapy among HIV-infected patients concurrently receiving nevirapine-based antiretroviral therapy and fluconazole. BMC Infect Dis. 7 (2007): 14.

[51]Chang, T.K.,Bandiera, S.M., Chen, J. Constitutive androstane receptor and pregnane X receptor gene expression in human liver: interindividual variability and correlation with CYP2B6 mRNA levels. Drug Metab Dispos. 31 (January 2003): 7-10.

[52]Code, E.L.,Crespi, C.L.,Penman, B.W.,Gonzalez, F.J.,Chang, T.K., Waxman, D.J. Human cytochrome P4502B6: interindividual hepatic expression, substrate specificity, and role in procarcinogen activation. Drug Metab Dispos. 25 (August 1997): 985-993.

[53]Uttayamakul, S.,Likanonsakul, S.,Manosuthi, W.,Wichukchinda, N.,Kalambaheti, T.,Nakayama, E., et al. Effects of CYP2B6 G516T polymorphisms on plasma efavirenz and nevirapine levels when co-administered with rifampicin in HIV/TB co-infected Thai adults. AIDS Research and Therapy. 7 (2010): 8.

[54]Hair, J.F.,Black, W.C.,Babin, B.J., Anderson, R.E. Multivariate data analysis. New Jersey: Prentice-Hall, 2009.

[55]Hollanders, R.M.,van Ewijk-Beneken Kolmer, E.W.,Burger, D.M.,Wuis, E.W.,Koopmans, P.P., Hekster, Y.A. Determination of nevirapine, an HIV-1 non-nucleoside reverse transcriptase inhibitor, in human plasma by reversed-phase high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl. 744 (July 2000): 65-71.

[56]Jonsson, E.N., Karlsson, M.O. Xpose—an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. Computer methods and programs in biomedicine. 58 (1998): 51-64.

[57]Sheiner, L.B., Beal, S.L. Bayesian individualization of pharmacokinetics: simple implementation and comparison with non-Bayesian methods. J Pharm Sci. 71 (December 1982): 1344-1348.

[58]Bland, J.M., Altman, D.G. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1 (Feb 8 1986): 307-310.

[59]Peters, R. The Ecological Implications of Body Size (Cambridge Studies in Ecology). Cambridge University Press, 1986.

[60]Holford, N.H. Input from the deep south compartment. A personal viewpoint. Clin Pharmacokinet. 29 (September 1995): 139-141.

[61]Kampmann, J.P.,Sinding, J., Moller-Jorgensen, I. Effect of age on liver function. Geriatrics. 30 (August 1975): 91-95.

[62]Koff, R.S.,Garvey, A.J.,Burney, S.W., Bell, B. Absence of an age effect on sulfobromophthalein retention in healthy men. Gastroenterology. 65 (August 1973): 300-302.

[63]Svard, J.,Spiers, J.P.,Mulcahy, F., Hennessy, M. Nuclear receptor-mediated induction of CYP450 by antiretrovirals: functional consequences of NR1I2 (PXR) polymorphisms and differential prevalence in whites and sub-Saharan Africans. J Acquir Immune Defic Syndr. 55 (December 2010): 536-549.

[64]Navaratnarajah, P.,Steele, B.L.,Redinbo, M.R., Thompson, N.L. Rifampicin-independent interactions between the pregnane X receptor ligand binding domain and peptide fragments of coactivator and corepressor proteins. Biochemistry. 51 (January 2012): 19-31.

[65]Moreira, R.P.,Jorge, A.A.,Mendonca, B.B., Bachega, T.A. Frequency of genetic polymorphisms of PXR gene in the Brazilian population. Clinics (Sao Paulo). 66 (2011): 1041-1044.

[66]King, C.R.,Xiao, M.,Yu, J.,Minton, M.R.,Addleman, N.J.,Van Booven, D.J., et al. Identification of NR1I2 genetic variation using resequencing. Eur J Clin Pharmacol. 63 (June 2007): 547-554.

[67]Hustert, E.,Zibat, A.,Presecan-Siedel, E.,Eiselt, R.,Mueller, R.,Fuss, C., et al. Natural protein variants of pregnane X receptor with altered transactivation activity toward CYP3A4. Drug Metab Dispos. 29 (November 2001): 1454-1459.

[68]Wang, X.D.,Deng, X.Y.,Chen, J.,Li, J.L.,Chen, X.,Zhao, L.Z., et al. Single nucleotide polymorphisms of the pregnane x receptor gene in Han Chinese and a comparison with other ethnic populations. Pharmacology. 81 (2008): 350-354.

[69]Lamba, J.,Lamba, V.,Strom, S.,Venkataramanan, R., Schuetz, E. Novel single nucleotide polymorphisms in the promoter and intron 1 of human pregnane X receptor/NR1I2 and their association with CYP3A4 expression. Drug Metab Dispos. 36 (January 2008): 169-181.

[70]Zhang, B.,Xie, W., Krasowski, M.D. PXR: a xenobiotic receptor of diverse function implicated in pharmacogenetics. Pharmacogenomics. 9 (November 2008): 1695-1709.

[71]Piedade, R.,Schaeffeler, E.,Winter, S.,Asimus, S.,Schwab, M.,Ashton, M., et al. PXR variants and artemisinin use in Vietnamese subjects: frequency distribution and impact on the interindividual variability of CYP3A induction by artemisinin. Antimicrob Agents Chemother. 56 (April 2012): 2153-2157.

APPENDIX

APPENDIX A

Determination of nevirapine plasma concentration

1. Blood Collection

Six mL Lithium heparinized blood was collected for determination of plasma nevirapine levels. The plasma was separated by centrifugation at 3000 rpm for 10 minutes at 20 $^{\circ}$C on the same day as blood collection and stored at -20 $^{\circ}$C until analysis.

2. Bioanalysis and Validation

Determinations of nevirapine concentrations were done by reverse phase high performance liquid chromatography (HPLC) method, with Ultra-Violet Detection, at the HIV-Netherlands-Australia-Thailand (HIV-NAT) Research Laboratory, The Thai Red Cross AIDS research Centre, Bangkok, Thailand. This method was originally developed at the Clinical Pharmacy, Laboratory, Radboud University, Nijmegen, Netherlands. The nevirapine assay has been externally validated by the International Interlaboratoy Program for the Quality Control of Therapeutic Drug Monitoring in HIV Treatment.

2.1 Bioanalysis

A multipoint ($n \geq 5$) calibration curve was generated for each analytical run and was used to calculate the concentration of nevirapine in the samples. This include one zero sample and five non-zero samples covering the target range, including LLOQ. The target concentration range for the calibration curves were 0.15 – 15.00 mg/L. The following conditions apply for each calibration curves:

±20% deviation of the LLOQ standard from the nominal concentration

±15% deviation of other non-LLOQ standard from the nominal concentration

The QCs were prepared in bulk, aliquot, and stored at $\leq$-20°C with the study samples. A set of QC samples (low, medium, high) were analyzed at start and at completion of each analytical run. The results for the QC samples were used to accept or reject analytical runs containing study samples.

The acceptance rule was: At least 4 of the 6 QC samples should be within ± 20% of their respective nominal value; 2 of the 6 QC samples (not at the same concentration or at the same position in the run) may be outside ± 20% of their respective nominal value. The quality control is performed using 3 levels, QC Low (QC L), QC Medium (QC M) and QC High (QC H), and the concentration were 0.20 mg/L, 0.80 mg/L, and 5.0 mg/L.

2.1.1 Chemicals

2.1.1.1 Nevirapine (NVP, Boehringer)

2.1.1.2 Acetonitrile (HPLC Grade, Lab Scan, ACN)

2.1.1.3 Potassium-di-hydrogen-phosphate (Merck)

2.1.1.4 Water (HPLC Grade, JT Baker)

2.1.1.5 Perchloric acid 70-72% (AR Grade, Merck)

2.1.2 Reagents

2.1.2.1 0.55M perchloric acid

2.1.2.2 Nevirapine Mobile Phase:70% 0.06 M KH_2PO_4 + 30% Acetonitrile

2.1.3 Extraction

Nevirapine plasma concentrations were measured by means of protein precipitation followed by reversed-phase HPLC with ultraviolet detection. In brief, 150 μl of 0.55 M perchloric acid was added to 150 μl of the plasma sample. The sample was mixed on a vortex mixer for 20 seconds and centrifuged, and 50 μl aliquots of clear supernatant were injected in the chromatographic system.

2.1.3.1 HPLC Column

2.1.3.1.1 Analytical column: Hypersil ODS, 250 x 4.6 mm (Alltech, cat. no. 288216)

2.1.3.1.2 Guard column: Hypersil ODS C18, 7.5 x 4.6 mm ALL-GUARD (Alltech, cat. no. 96013).

2.1.3.2 Pump: Quaternary HPLC-pump (P4000)

2.1.3.2.1 Flow: 1.5 ml/min.

2.1.3.2.2 Eluent: 5.72 g KH_2PO_4 in 700 ml Water + 300 ml Acetonitrile

2.1.3.3 Autosampler: (AS3000)

2.1.3.3.1 Injection volume: 50 µl

2.1.3.4 Detector: UV-detector (Thermo Quest UV1000)

2.1.3.4.1 Detector Wavelength: 280 nm

2.1.3.4.2 Filter rise time: 1.0

2.1.3.4.3 Range: 1.0

2.1.3.5 Integrator: (SN4000)

2.1.3.5.1 Integrate chromatogram peak: using "*ChromQuest*" Software version 4.2

2.2 Validation

Accuracy of nevirapine from biological fluid is done by using at least 5 samples for 3 concentration levels, i.e. low, medium, and high. This was carried out on 3 separate days. The % accuracy is determined by comparing the concentration of the samples from the experiment with the amount used. The % accuracy should be within 80 – 120 %. As shown below.

2.2.1 Accuracy in plasma:

2.2.1.1 96.9% at 0.2 mg/l

2.2.1.2 91.5% at 0.8 mg/l

2.2.1.3 102.6% at 5.0 mg/l

2.2.2 Lower Limit of Quantification: 0.15 mg/L

2.2.3 Extraction recovery in plasma: 101.8 ± 4.6%

2.2.4 Range of calibration curve: 0.15-15.0 mg/l

2.2.5 Stability

The stability data of nevirapine is shown below

	Matrix	Conditions	Time Interval
Stock solution	DMSO	-20° C	27 months
Freeze & Thaw cycle 3 times	Plasma	-20° C	3 cycles
Short Term Stability	Plasma	Room Temperature	164 hours
	Plasma	4° C	164 hours
	Whole blood	Room Temperature	164 hours
	Whole blood	4° C	164 hours
	Extracted samples	Room Temperature	13 hours
	Plasma	-20° C	9 months
Long Term Stability	Plasma	-40° C	27 months

VITAE

Miss Thanaporn Wattanakul was born on 26th August 1985. She graduated bachelor degree in Pharmacy from Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand in 2009. She worked as pharmacist at Fascino's drugstore in Bangkok since 2009. She had been enrolled in Master degree program of Pharmacy Practice Department, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand since 2010.