PHARMACOKINETIC PARAMETERS OF ATAZANAVIR IN RITONAVIR-BOOSTED COMBINATION THERAPY IN THAI HEALTHY VOLUNTEERS: COMPARISON BETWEEN REDUCED DOSES OF RITONAVIR OR ATAZANAVIR

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พารามิเตอร์ทางเภสัชจลนศาสตร์ของอะทาซานาเวียร์เมื่อใช้ร่วมกับริโทนาเวียร์ในอาสาสมัครไทยสุขภาพดี: เปรียบเทียบระหว่างการลดขนาดยาริโทนาเวียร์หรืออะทาซานาเวียร์

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรดุษฎีบัณฑิต
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Thesis Title	PHARMACOKINETIC PARAMETERS OF ATAZANAVIR IN RITONAVIR-BOOSTED COMBINATION THERAPY IN THAI HEALTHY VOLUNTEERS: COMPARISON BETWEEN REDUCED DOSES OF RITONAVIR OR ATAZANAVIR
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Field of Study	Pharmaceutical Care
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ชาญกิจ พุฒิเลอพงศ์: พารามิเตอร์ทางเภสัชจลนศาสตร์ของอะทาซานาเวียร์เมื่อใช้ร่วมกับริโทนาเวียร์ในอาสาสมัครไทยสุขภาพดี: เปรียบเทียบระหว่างการลดขนาดยาริโทนาเวียร์หรืออะทาซานาเวียร์. (PHARMACOKINETIC PARAMETERS OF ATAZANAVIR IN RITONAVIR-BOOSTED COMBINATION THERAPY IN THAI HEALTHY VOLUNTEERS: COMPARISON BETWEEN REDUCED DOSES OF RITONAVIR OR ATAZANAVIR) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.ดร.ดวงจิตต์ พนมวัน ณ อยุธยา, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ.นพ.เกียรติ รักษ์รุ่งธรรม, 104 หน้า.

ขนาดยามาตรฐานของอะทาซานาเวียร์ร่วมกับริโทนาเวียร์ 300/100 มิลลิกรัม วันละ 1 ครั้ง มีระดับยาในเลือดที่สูงสำหรับผู้ป่วยไทยติดเชื้อเอชไอวี การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบเทียบค่าเภสัชจลนศาสตร์และภาวะบิลิรูบินในเลือดสูงของอะทาซานาเวียร์เมื่อใช้ร่วมกับริโทนาเวียร์ ระหว่างขนาดยามาตรฐานกับขนาดยาที่ลดลงของริโทนาเวียร์หรือทั้งอะทาซานาเวียร์และริโทนาเวียร์ในอาสาสมัครไทยผู้ใหญ่สุขภาพดีที่อยู่ในการศึกษาจนครบ 31 คน โดยอาสาสมัครจะถูกแบ่งออกเป็น 2 กลุ่มให้ได้รับอะทาซานาเวียร์และริโทนาเวียร์ในขนาดที่แตกต่างกัน (กลุ่มที่ 1:300/100 เทียบกับ 300/50, กลุ่มที่ 2: 300/100 เทียบกับ 200/50) พบว่า ลักษณะทั่วไปของอาสาสมัครทั้ง 2 กลุ่มไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ ค่าเฉลี่ยเรขาคณิตของอะทาซานาเวียร์ในกลุ่มที่ได้รับยา 300/100, 300/50 และ 200/50 มิลลิกรัม มีค่าดังนี้ คือ AUC $_{0\text{-}24}$ (มิลลิกรัม-ชั่วโมง/ลิตร) 40.66, 25.50, 24.97; C_{max} (มิลลิกรัม/ลิตร) 4.12, 2.83, 2.58; C_{min} (มิลลิกรัม/ลิตร) 0.53, 0.27, 0.24 ตามลำดับ AUC $_{0\text{-}24}$, C_{max} และ C_{min} ในกลุ่มที่ได้รับยา 300/50 และ 200/50 มีค่าต่ำกว่ากลุ่มที่ได้รับยา 300/100 มิลลิกรัม อย่างมีนัยสำคัญทางสถิติ แต่ไม่มีความแตกต่างกันอย่างมีนัยสำคัญระหว่างกลุ่มที่ได้รับยา 300/50 และ 200/50 ระดับยาต่ำสุดของอะทาซานาเวียร์ ที่จำแนกเป็นร้อยละในกลุ่มที่ได้รับยา 300/100, 300/50 และ 200/50 มีดังนี้ คือ อยู่ในระดับที่ต่ำกว่าที่ให้ผลการรักษา 0, 31, 33 อยู่ในระดับที่ให้ผลการรักษา 74, 56, 67 และเหนือระดับที่ให้ผลการรักษา 26, 13, 0 ตามลำดับ ระดับบิลิรูบินในเลือดลดลงอย่างมีนัยสำคัญทางสถิติภายหลังจากลดขนาดยาลง ดังนั้น การลดขนาดยาอะทาซานาเวียร์/ริโทนาเวียร์ มีผลลดระดับยาต่ำสุดของอะทาซานาเวียร์และการเกิดภาวะบิลิรูบินในเลือดสูง แต่ควรมีการศึกษาเพิ่มเติมในผู้ป่วยไทยที่ติดเชื้อเอชไอวี

ภาควิชา เภสัชกรรมปฏิบัติ ลายมือชื่อนิสิต.....

สาขาวิชา การบริบาลทางเภสัชกรรม ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา 2554 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

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KEYWORDS : PHARMACOKINETIC/ HYPERBILIRUBINEMIA/ ATAZANAVIR/ RITONAVIR/ THAI HELATHY VOLUNTEER

CHANKIT PUTTILERPONG: PHARMACOKINETIC PARAMETERS OF ATAZANAVIR IN RITONAVIR-BOOSTED COMBINATION THERAPY IN THAI HEALTHY VOLUNTEERS: COMPARISON BETWEEN REDUCED DOSES OF RITONAVIR OR ATAZANAVIR. ADVISOR: ASSOC.PROF.DUANCHIT PANOMVANA AYUDHYA, Ph.D., CO-ADVISOR: PROF.KIAT RUXRUNGTHAM, M.D., 104 pp.

The standard dose of atazanavir (ATV)/ritonavir (RTV) 300/100 mg once daily results in higher ATV exposure in Thai populations. This study was to compare the pharmacokinetic (PK) parameters and hyperbilirubinemia when lower than standard dose of ATV/RTV was given in 31 adults Thai healthy volunteers. Subjects were randomly assigned into 2 groups who were administered with the different doses of ATV/RTV (group I: 300/100 vs. 300/50, group II: 300/100 vs. 200/50). There were no statistically significant differences between the two groups in demographic data. The geometric means (GM) of ATV for 300/100, 300/50 and 200/50 mg once daily doses, respectively, were as follows: AUC_{0-24}(mg-h/L) 40.66, 25.50, 24.97; C_{max}(mg/L) 4.12, 2.83, 2.58; C_{min}(mg/L) 0.53, 0.27, 0.24, respectively. ATV AUC_{0-24}, C_{max} and C_{min} for 300/50 and 200/50 mg were statistically significant lower than 300/100 mg but did not show significantly different between 300/50 and 200/50 mg. ATV C_{min} for 300/100, 300/50 and 200/50 mg, respectively, were as follow: in subtherapeutic (%) 0, 31, 33; within therapeutic range (%) 74, 56,67; overtherapeutic (%) 26, 13, 0, respectively. There was significantly decrease in bilirubin concentration after dose reduction. Therefore, the reduced dose of ATV/RTV resulted in decreasing in both ATV C_{min} and hyperbilirubinemia. However, further investigation in Thai HIV-infected patients is required.

Department : Pharmacy Practice Student's Signature .....
Field of Study : Pharmaceutical Care Advisor's Signature .....
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LIST OF ABBREVIATIONS

AAG	=	alpha-1-acid glycoprotein
AEs	=	adverse events
ALT	=	alanine aminotransferase
ART	=	antiretroviral therapy
ARV	=	antiretroviral
AST	=	aspartate aminotranferase
ATV	=	atazanavir
AV	=	atrioventricular
ATV/RTV	=	ritonavir-boosted atazanavir
C_{avg}	=	average concentration
C_{max}	=	maximum concentration
C_{min}	=	minimum concentration
C_{trough}	=	trough concentrations
CL/F	=	clearance expressed as a function of bioavailability
CSF	=	cerebrospinal fluid
CTC	=	common toxicity criteria
CTCAE	=	common terminology criteria for adverse events
HAART	=	highly active antiretroviral therapy
LPV	=	lopinavir
NRTIs	=	nucleoside/nucleotide reverse transcriptase inhibitors
NNRTI	=	nucleoside reverse transcriptase inhibitor
OD	=	once daily
P-gp	=	P-glycoprotein
PI	=	protease inhibitor
PK	=	pharmacokinetic
RTV	=	ritonavir
TDM	=	therapeutic drug monitoring
UGT1A1	=	UDP-glycosyl transferase 1A1

CHAPTER I

INTRODUCTION

1.1 Rational and Background

Highly active antiretroviral therapy (HAART) is a combination of at least three drugs from two different classes of antiretroviral drugs and generally composed of two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) plus either one non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI).[1-3] HAART regimens reduces viral load, improves immune function and increases life expectancy leading to a dramatically improved outcome and turning HIV infection into a manageable chronic disease. The efficacy of HAART is well established and has provided benefits to many patients with HIV infection.[4] The morbidity and mortality related to HIV infection have dramatically decreased when HAART has been available. However, HAART regimens have also shown some limitations, such as poor adherence to the complex regimen, serious adverse events associated with long term use of antiretroviral and drug resistance. [1,5]

Protease inhibitors (PIs) are one of the main classes of antiretroviral drugs recommended for the initial treatment of HIV infection which have been shown to increase CD4 cell counts and dramatic reductions in the incidence of opportunistic events and have a high threshold for the development of resistance.[1,3,6-8] Certain PIs are often boosted with a low dose of ritonavir which can increase systemic exposure, prolong its half-life, reduce the risk of resistance and decrease the dose administration frequency of the main PI.[3,6] However, they can be associated with a number of adverse effects, such as gastrointestinal disturbances, liver toxicity and metabolic disorders, including alteration of lipid levels, insulin resistance and lipodystrophy.[8]

Atazanavir (ATV) is a potent and safe azapeptide protease nhibitor approved in adult HIV- infected patients and pediatric patients in some countries in combination with other antiretroviral drugs with once daily administration.[6,9] It has been established as the preferred initial regimen in published guidelines.[1-4] It is approved in Europe and the USA at a dose of 300 mg boosted with ritonavir (RTV) 100 mg once daily (atazanavir/ritonavir 300/100 mg once daily) to be taken with food but is also approved in the USA unboosted at 400 mg once daily for treatment-naive patients. Atazanavir benefits from once-daily dosing, low pill burden and also a favorable safety profile with less lipid abnormalities than other protease inhibitors. The optimal range for ATV trough concentration was determined between 0.15 and 0.85 mg/l which associated with the highest probability of virological response and the lowest probability of increase in serum bilirubin.[6,7,9-12]

Many clinical trials in antiretroviral therapy (ART)-naive patients who received dual-NRTI background therapy, boosted atazanavir was noninferior to unboosted ATV or lopinavir/ritonavir or fosamprenavir plus ritonavir.[13-5] In other study, Ritonavir-boosted atazanavir is associated with greater virologic control and immune response compared to non-boosted atazanavir without greater risk of adverse events except elevated bilirubin.[16] Higher atazanavir plasma concentration have been correlated with hyperbilirubinemia which is a frequent side effect of treatment with an ATV. Previous studies have reported cases of severe hyperbilirubinemia with a prevalence ranging from < 20% to 47%.[17-23] Altogether, these studies support the use of once-daily boosted ATV as a good first-line treatment option in ART-naive patients. Similarly, in ART-experienced patients with a history of virological failure who received dual-NRTI background therapy,boosted ATV was noninferior to lopinavir/ritonavir.[18,24] Furthermore, the use of unboosted ATV in treatment-experienced patients is not recommended. In addition, several switch studies in ART-experienced patients who achieved virological suppression on a PI-based regimen, switching to unboosted ATV was associated with

better maintenance of virological suppression and improvement in lipid profile than remaining on their previous regimen.[25-27]

The standard boosting doses typically 100 or 200 mg of ritonavir can cause side effects including gastrointestinal symptoms and blood lipid abnormalities. Minimizing the boosting dose could potentially improve tolerability and lower the cost of therapy. The adverse event profile of boosted PIs was affected by both ritonavir dose and the specific side effects associated with the various PIs. Asians are significantly higher exposure to PIs compared to the Caucasians.[28] Several dose-finding studies of boosted protease inhibitors have demonstrated that doses lower than those recommended in Caucasian populations exhibit in the Thai population similar pharmacokinetic properties with sustained virological suppression but reduced toxicity. A significant decrease of bilirubin was achieved after reducing the dose from 300/100 to 200/100 mg of ATV/RTV.[29] ATV/RTV at a dose of 200/100 mg once daily was effective in HIV-infected Thai patients.[30] Saquinavir, fosamprenavir and darunavir were boosted equally well by lower (50-100 mg) versus higher doses of ritonavir. Indinavir, tipranavir and lopinavir were boosted more by higher ritonavir doses but data on atazanavir were inconclusive.[31]

The combination reduced dose of ATV or RTV for daily dose could lower costs, decrease hyperbilirubinemia, improve patient adherence (a low pill burden) and complete virological suppression compared with other PIs. Therefore, reduced dose of RTV-boosted ATV is one of the best options plus appropriate backbone medications for HIV-1 infected Thai and other Asian ethnicities patients. However, no studies have been demonstrated the pharmacokinetic of reduced dose of RTV-boosted ATV in Thai population. Therefore, we will conduct this study to investigate the pharmacokinetics of reduced dose compared with standard dose of RTV-boosted ATV in Thai healthy volunteers.

1.2 Hypothesis

The reduced doses of RTV and/or ATV in Thai healthy volunteers provide the levels of ATV within the optimal therapeutic range comparable to standard dose of RTV-boosted ATV.

1.3 Objectives

1.3.1 To compare the pharmacokinetics of standard dose and reduced doses of ritonavir or both ritonavir and atazanavir.

1.3.2 To evaluate short term safety and tolerability standard dose and reduced doses of ritonavir or both ritonavir and atazanavir.

1.4 Expected Outcomes

1.4.1 This study will provide the information on the pharmacokinetic parameters of atazanavir between standard dose and reduced dose of ritonavir or both ritonavir and atazanavir in combination therapy in Thai healthy volunteers.

1.4.2 Reduced doses of ritonavir or both ritonavir and atazanavir may be benefit for Thai patients in clinical practice. It could be lower costs, decrease hyperbilirubinemia, improve patient adherence and complete virological suppression for Thai and other Asian ethnicities.

CHAPTER II

LITERATUERE REVIEWS

2.1 Introduction of atazanavir

Highly active antiretroviral therapy (HAART) regimens usually contain at least three drugs from two different classes of antiretroviral drugs. Current recommendation for the first preferred regimen for HIV-infected patients compose of a protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI) together with a dual nucleoside reverse transcriptase inhibitor (NRTI) backbone.[1-4,6]

Protease inhibitors are one of the main classes of antiretroviral drugs recommended for the initial treatment of HIV-infected patients. Certain PIs require the use of low-dose ritonavir to boost pharmacokinetic exposure through inhibition of metabolism by the cytochrome P450 3A4 enzyme pathway, reduce the risk of resistance and decrease the dose administration frequency of the major PI. However, boosted PIs can be associated with common adverse events such as gastrointestinal disturbances, metabolic abnormality including dyslipidemia, insulin resistance and potentially cardiovascular patients.[6-7]

Atazanavir (ATV) is a well established protease inhibitor developed by Bristol-Myers Squibb and approved in 2003 by the FDA in adult HIV-infected patients in combination with other antiretroviral drugs. Atazanavir is given orally as atazanavir sulfate. It was indicated for both antiretroviral naïve and experienced patients. The advantages of atazanavir over other PIs are its excellent oral bioavailability, once-daily dosing, low capsule burden, favorable effect on lipid profiles and a relatively favorable resistance profile.[7,32]

2.2 Chemistry

ATV, known as BMS-232632, is the first azapeptide PI. ATV sulfate has the following structural formula (Figure 2.1). The chemical name for atazanavir sulfate is (3S, 8S, 9S, 12S)-3,12-bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[[4-(2-pyridinyl) phenyl]methyl]-2,5,6,10,13-pentaazatetradecanedioic acid dimethyl ester, sulfate (1:1), with a molecular formula of $C_{38}H_{52}N_6O_7H_2SO_4$ and molecular weight of 802.9 (sulfuric acid salt). The free base molecular weight is 704.9. It is a white to pale yellow crystalline powder and slightly soluble in water. The pH of a saturated solution in water is about 1.9 at 24 ±3 °C.[32]

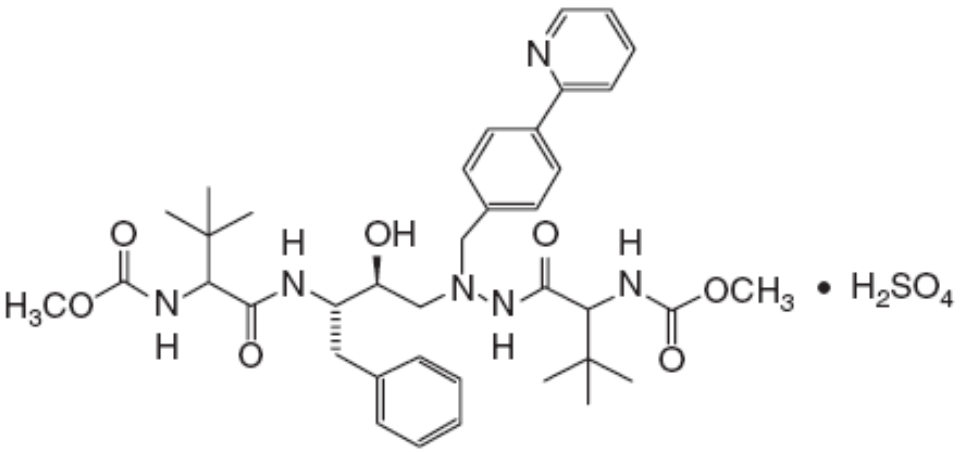

Figure 2.1: Chemical structure of atazanavir sulfate[9]

2.3 Mechanism of action

ATV is an azapeptide inhibitor of the HIV-1 protease. It selectively inhibits the virus-specific processing of viral Gag and Gag-Pol poliproteins of HIV-infected cells, thus preventing formation of mature virions.

2.4 Indication and usage

Atazanavir sulfate is indicated in combination with other antiretroviral drugs for treatment of HIV-infected patients for both antiretroviral-naïve and experienced adult and pediatric patients at least 6 years of age.[6,7,9,32,33] From many clinical trials, ritonavir-boosted atazanavir is associated with greater virological control and immune response.[34-36]

2.5 Dosage and administration

Atazanavir sulfate is developed by Bristol-Myer Squibb and approved in 2003 by the FDA. Atazanavir capsules are available for oral administration in strengths containing the equivalent of 100 mg, 150 mg, 200 mg and 300 mg as atazanavir sulfate.

For treatment-naïve patients, the recommended dosage of atazanavir is 300 mg with ritonavir 100 mg once daily or 400 mg without ritonavir once daily taken with food. For treatment-experienced patients, the recommended dosage of atazanavir is 300 mg with ritonavir 100 mg once daily taken with food. The recommended dosage of atazanavir for pediatric patients (6 to less than 18 years of age) is based on body weight and should not exceed the recommended adult dosage.[6,32,33]

2.6 Pharmacokinetics

Absorption

Atazanavir is rapidly absorbed with a peak plasma concentration (C_{max}) occurring after 2-3 hours. Steady state is achieved between days 4 and 8 with an accumulation of approximately 2.3 fold. The pharmacokinetics of multiple doses demonstrates nonlinear pharmacokinetics with greater than dose proportional increases in AUC and C_{max} values over the dose range of 200-800 mg once daily. In healthy

volunteers, the relative bioavailability of atazanavir capsule are approximately 60% in a single-dose study and about 68% in a multiple-dose study. The extent of absorption is highly dependent on gastric pH. Administration of ATV with light meal enhances bioavailability and reduces pharmacokinetic variability. Administration of a single 400 mg dose of ATV, AUC was increased from fasting levels by 70% and 35% in patients who had also received a light- or high-fat meal and interpatient variability in ATV levels decreased from 69% in fasting state to 37% and 43%, respectively.[37] Atazanavir should be taken with light meal to improve bioavailability and reduce variability.

Distribution

Atazanavir is ≥ 86% bound to human serum protein binding is independent of concentration. ATV binds to both alpha-1-acid glycoprotein (AAG) and albumin to a similar extent (89% and 86%, respectively).[6] The binding is a concentration independent. Population pharmacokinetic studies of ritonavir-boosted atazanavir in HIV-infected patients estimated the steady-state volume of distribution of atazanavir to be 80.8 L[38], 88.3 L[20] or 103 L (study in HIV-infected patients and healthy volunteers).[39] Atazanavir rarely enters the cerebrospinal fluid (CSF) and semen, with a CSF: plasma ratio ranging from 0.0021 to 0.0226 and a seminal fluid: plasma ratio ranging between 0.11 and 4.42. Atazanavir is substrate for P-glycoprotein (P-gp), an efflux transporter that will act to limit tissue compartment distribution.[7]

Metabolism

Atazanavir is predominately metabolized by hepatic cytochrome P450, primarily the CYP3A4/CYP3A5 isoenzymes. Atazanavir is a weak CYP3A inhibitor. Ritonavir-boosted with atazanavir has not been shown to induce its own metabolism or to increase the biotransformation of other drugs metabolized by CYP3A. It is also a direct inhibitor for UDP-glycosyl transferase 1A1 (UGT1A1). The major biotransformation pathways consisted of monooxygenation and dioxygenation. Other minor biotransformation

pathways for atazanavir or its metabolites consisted of glucuronidation, N-deakylation, hydrolysis and oxygenation with dehydrogenation. Two minor, pharmacologically inactive metabolites of atazanavir in plasma have been characterized. In HIV-infected patients with end-stage liver disease, unboostd ATV showed a pharmacokinetic profile which was similar to that of patients with boosted ATV and was able to maintain efficacy.[7,32] Atazanavir plus ritonavir once daily were achieved steady-state after administration for 10 days.[37,40-44] Population pharmacokinetic studies of atazanavir in HIV-infected patients estimated the steady-state oral clearance of atazanavir to be 7.6 L/hr[38], 12.9 L/hr[20] or 7.7 L/hr (study in HIV-infected patients and healthy volunteers).[39]

Elimination

Atazanavir and its metabolites undergo bilirubin and urinary elimination for 79% and 13% of an administrated dose, respectively. Unchanged drug accounted for approximately 20% and 7% of the administered dose in the feces and urine, respectively.[6,7,9,32] The mean steady-state elimination half-life of atazanavir in healthy subjects and HIV-infected patients were 18.1 hours and 8.6 hours, respectively for atazanavir 300 mg boosted with ritonavir 100 mg.[33] In population pharmacokinetic analyses of boosted atazanavir in HIV-infected patients estimated the elimination half-life of atazanavir to be 7.5 hours[38] and 8.8 hours.[20]

Special populations

Effect of age and sex

In Clinical trials, age and sex had no clinically significant effect on the pharmacokinetics of atazanavir in adults.[6,9,45]

Pregnant women

Although there are no adequate and well controlled on pharmacokinetics studies of atazanavir use during pregnancy, data from other PIs suggest a decrease in plasma exposure during the third trimester.

Hepatic impairment

Although there are no data in patients with hepatic impairment with HIV infection. Atatzanavir is metabolized in the liver by CYP3A, increased exposure to the drug in such patients is anticipated. Atazanavir should be used with caution in patients with mild-to-moderate hepatic impairment. For patients with moderate hepatic impairment (Child-Pugh Class B) who have not experienced prior virological failure, a dose reduction to 300 mg once daily should be considered. Atazanavir should not be used in patients with severe hepatic impairment (Child-Pugh Class C).[6,9,33]

Renal impairment

Atazanavir is eliminated primarily by the liver then impairment of atazanavir pharmacokinetic in patients with renal impairment is unlikely. Patients with severe renal impairment who are not managed with hemodialysis, no dose adjustment is required for atazanavir. Treatment-naïve patients with end stage renal disease managed with hemodialysis should received atazanavir 300 mg boosted with ritonavir 100 mg. Atazanavir should not be used to HIV-treatment -experienced patients with end stage renal disease managed with hemodialysis.[33]

2.7 Drug interactions

Atazanavir is a substrate and inhibitor of CYP3A with K_i value of 0.84 to 1.0 μM. Atazanavir is also a direct inhibitor for UGT1A1 (K_i = 1.9 μM) and CYP2C8 (K_i =2.1 μM).[7,33] It is also substrate and inhibitor of P-gp (P-gp inhibitor). Therefore, co-administration of atazanavir and drugs primarily metabolized by CYP3A, CYP2C8 or UGT1A1 and/or substrate of P-gp may result in increased plasma concentrations of the concomitant drug, which potentially could enhance or prolong both their therapeutic and adverse effects. Drugs affecting the gastric pH may alter atazanavir solubility and consequently its bioavailability.[7] Atazanavir and atazanavir boosted with ritonavir drug

interaction are summarized in Table 2.1 and drugs should not be used with atazanavir or atazanavir boosted with ritonavir are shown in Table 2.2.

2.8 Drug monitoring

Monitoring plasma drug concentrations has proven to be useful for PIs, and may allow tailoring of antiretroviral therapy. ATV plasma concentrations demonstrate high inter-individual variability, even in the presence of r, along with a low intra-individual variability, supporting therapeutic drug monitoring (TDM).[7] The recommended atazanavir trough concentrations (C_{trough}) has been defined between 0.15 - 0.85 mg/L to ensure maximal viral suppression with minimal side effects on hyperbilirubinemia especially when used ritonavir boosted with atazanavir.[32] Many studies have evaluated the relationship between atazanavir plasma concentrations and virological response. A threshold of 0.15 mg/L has been proposed for antiretroviral-naïve patients based on results of a retrospective study performed on 51 patients. In that study, virological responses according to atazanavir C_{trough} were as follows: 58.3% when < 0.15 mg/L, 75% when values between 0.15 and 0.85 mg/L, and 100% when > 0.85 mg/L. The occurrence of hyperbilirubinemia has been reported frequently side effects of atatzanavir with the prevalence ranging from < 20% to 47%.[17,46] Study of Colombo and colleagues reported a significant correlation between serum bilirubin concentration and atazanavir plasma trough concentration. This study showed that patients with an atazanavir C_{trough} higher than 0.85 mg/L presented a threefold-higher risk of bilirubin elevation than patients with a C_{trough} lower than that level. Half of the patients received atazanavir 400 mg without ritonavir once daily would lead to subtherapeuic levels. For these results strongly support the preferable use of atazanavir with ritonavir boosting, but in the absence of ritonavir, a twice daily schedule would be recommended for atazanavir.[20]

Table 2.1: Drug interaction between atazanavir and other drugs[1,7]

Concomitant drug	Effect on ATV or Concomitant Drug Concentrations	Dosing Recommendations and Clinical Comments
Antacids	↓ ATV	Give ATV at least 2 hours before or 1 hour after antacids
Amiodarone, bepridil, lidocaine, quinidine	↑ antiarrhythmic	Caution is warranted, TDM recommended.
Carbamazepine, lamotrigine, phenytoin, phenobarbital	expected ↓ ATV	Use with caution.
Diltiazem	↑ diltiazem and desacetyl-diltiazem	Caution is warranted. 50% dose reduction of diltiazem should be considered.
Tricyclic antidepressants (TCAs)	↑ TCAs	Use with caution, TDM is recommended.
H_2 receptor antagonists	↓ ATV	- H_2 receptor antagonist dose should not exceed a dose equivalent to famotidine 40 mg BID in ART-naïve patients or 20 mg BID in ART-experienced patients. - Give ATV 300 mg + RTV 100 mg simultaneously with and/or >10 hours after the H_2 receptor antagonist. - If using TDF and H2 receptor antagonist in ART-experienced patients, use ATV 400 mg + RTV 100 mg.
Trazodone	↑ trazodone	TDM is recommended and lower trazodone doses should be used.
Fluticason	↑ fluticasone	Caution is warranted.
Itraconazole, ketoconazole	↑ itraconazole, ketoconazole (ATV 400)	If ATV is used with RTV, itraconazole or ketoconazole doses of 200 mg/day should be used with caution.

Table 2.1: Drug interaction between atazanavir and other drugs[1,7] (continue)

Concomitant drug	Effect on ATV or Concomitant Drug Concentrations	Dosing Recommendations and Clinical Comments
Felodipine, nifedipine, nicardipine, verapamil	↑ felodipine, nifedipine, nicardipine, verapamil	Caution is warranted and ECG monitoring is recommended. Dose titration should be considered.
Atrovastatin, rosuvastatin	↑ atorvastatin, rosuvastatin	Use the lowest possible dose of atorvastatin or rosuvastatin with careful monitoring or consider other HMG-CoA reductase inhibitor such as pravastatin or fl uvastatin.
Proton pump inhibitors (PPIs)	↓ ATV	- PPIs are not recommended in patients receiving unboosted ATV. - PPIs should not exceed a dose equivalent to omeprazole 20 mg daily in PI-naïve patients. PPIs should be administered at least 12 hours prior to ATV/r. - PPIs are not recommended in PI-experienced patients.
Warfarin	↑ warfarin	- Monitor INR closely when stopping or starting PI and adjust warfarin dose accordingly.
Ethinyl estradiol, norethindrone	↓↑ oral contraceptives	Due to possible alteration of oral contraceptives concentrations, alternative/additional contraceptive measures should be used when co-administered with ATV or ATV/r.
Sildenafil, tadalafil, vardenafil	↑ sildenafil, tadalafil, vardenafil	Do not exceed 25 mg of sildenafil in 48 h, 10 mg of taldalafil in 72 hours or 2.5 mg of vardenafil in 72 h.

Table 2.1: Drug interaction between atazanavir and other drugs [1,7](continue)

Concomitant drug	Effect on ATV or Concomitant Drug Concentrations	Dosing Recommendations and Clinical Comments
Posaconazole	ATV AUC ↑ 146% (ATV/r) ATV AUC ↑ 268% (ATV)	Monitor for adverse effects of ATV.
Voriconazole	RTV 100 mg BID ↓ voriconazole AUC 39%	Do not coadminister voriconazole and RTV unless benefit outweighs risk. If administered, consider monitoring voriconazole level.
Clarithromycin	↑ clarithromycin ↑ 14-OH-clarithromycin ↑ ATV concentrations	May cause QTc prolongation. Dose reductions by 50% should be considered. Consider alternative therapy. Combination with boosted ATV has not yet been studied.
Didanosine (ddI)	↓ ATV, ↓ddI	Didanosine should be administered (with food) 1 h before or 2 h after ATV/r intake.
Tenofovir	↓ ATV, ↑ tenofovir	Avoid combination of tenofovir with ritonavir unboosted ATV.
Efavirenz	↓ ATV	The recommended dose in treatment-naïve patients is ATV/r 300/100 mg/day. No recommendation has been established in treatment-experienced patients.
Nevirapine	expected ↓ ATV	Co-administration is not recommended.
Etravirine	↑ etravirine, ↓ ATV	ATV should be boosted with ritonavir
Raltegravir	↑ raltegravir	The clinical relevance of these data is unknown. No changes in raltegravr dosign are recommended.

Table 2.2: Drugs not be used with atazanavir or atazanavir boosted with ritonavir[1,7]

Concomitant drug	Effect on ATV or Concomitant Drug Concentrations	Dosing Recommendations and Clinical Comments
Irinotecan	↑ irinotecan	Contraindicated
Rifampicin	severe ↓ ATV	Contraindicated
Indinavir	-	Contraindicated due to synergistic effect on hyperbilirubinemia.
Midazolam, triazolam	↑ midazolam, triazolam	Contraindicated
Dihydroergotamine, ergotamine, ergonovine, methylergonovine	↑ ergot derivatives	Contraindicated
Lovastatin, simvastatin	↑ lovastatin, simvastatin	Contraindicated
Cisapride	↑ cisapride	Contraindicated

2.9 Contraindication

Atazanavir is contraindicated in patients with previously demonstrated clinically significant hypersensitivity (e.g. Stevens-Johnson syndrome, erythema multiforme) to any of the components of this product and when coadministered with drugs that are highly dependent on CYP3A or UGT1A1 for metabolize, and for which elevated ATV plasma concentrations are associated with serious and/or life-threatening events.[32]

2.10 Adverse events

Adverse events during patients treated with highly active antiretroviral therapy (HAART) may lead to decrease in adherence and virological failure or therapy discontinuation. Most common adverse reactions are nausea, jaundice/scleral icterus,

rash, headache, abdominal pain, vomiting, insomnia, peripheral neurological, symptoms, dizziness, myalgia, diarrhea, depression and fever.

Gastrointestinal system

Gastrointestinal disturbances are common among patients taking PIs. Nausea was the most common adverse event, reported by 25-35% of the patients receiving atazanavir, followed by abdominal pain, headache and diarrhea, which occurred in approximately 20% of patients.[7,9,31,37]

Hyperbilirubinemia

Hyperbilirubinemia and clinical jaundice occurred in patients treated with atazanavir. Jaundice (6-12%) and scleral icterus (2-6%) occurred only in atazanavir-treated patients and was dose-related. Unconjugated revesible hyperbilirubinemia was the most common grade 3-4 laboratory abnormality that was dose-related, appearing within several days to 1 week, but was not clinically significant and rarely led to discontinuations. However, 7-8% of patients developed scleral icterus or clinical jaundice, which may limit their atazanavir acceptability. Atazanavir competitively inhibits UGT that reponsible for bilirubin glucuronidation (UGT1A1).[9,32,33,37] The risk of hyperbilirubinemia seems to be associated to atazanavir plasma concentration and is more frequent when high doses of atazanavir are used or when atazanavir is boosted with ritonavir. Higher atazanavir trough concentrations have been associated with increased indirect bilirubin levels. Hyperbilirubinemia is completely reversible after stopping atazanavir.[7]

Hepatotoxicity

Grade 3 - 4 elevations of aspartate transaminase (AST) or alanine transaminase (ALT) occurred in 4-14% of patients receiving ATV but these were not correlated with elevations of serum bilirubin and are more frequently seen in HIV-infected patients with

underlying chronic hepatitis B or C. Patients on atazanavir with underlying chronic liver disease should periodically be monitored in liver function tests.[7,33,37]

Cardiac conduction abnormalities

Atazanavir has been shown to prolong the PR interval of the electrocardiograms performed on healthy volunteers as well as in HIV-infected patients. Abnormalities in atrioventricular (AV) conduction were asymptomatic, concentration-dependent and generally limited to first-degree AV block. There have been rare reports of second-degree AV block and other conduction abnormalities. Atazanavir should be used with caution in these patients. The periodic electrocardiogram should be monitored in patients treated with atazanavir particularly when boosted with ritonavir.[7,9,33,37]

Rash

In controlled clinical trial, all grades of rash occurred in approximately 20% of patients treated with ATV. Rash were generally mild-to-moderate maculopapular skin eruptions. Atazanavir was often continued without stopping in patients who developed rash. The discontinuation rate for rash was less than 1% in clinical trials. Cases of Stevens-Johnson syndrome, erythema multiforme and toxic skin eruptions have been reported in patients receiving ATV and should be discontinued if severe rash develops.[6,33]

Nephrolithiasis

Cases of nephrolithiasis in HIV-infected patients receiving atazanavir have been reported after postmarketing surveillance. However, this complication is very rare. In one retrospective study, the prevalence of atazanavir-associated urolithiasis was 0.97%. Patients with low water intake, high urinary pH and prior history of urinary stones are at higher risk for atazanavir-associated urine crystallization.[7,33]

Others

Another concern of HAART is the occurrence of lipid abnormalities. Atazanavir was generally well tolerated in all clinical trials. Unlike to others PIs, ATV does not seem to negatively effects on lipid profiles and less impact on insulin and overall on glucose metabolism than all other PIs. From the BMS-034 study in a subgroup of patients receiving ATV for 48 weeks, no abnormal fat redistribution was noticed. The favorable effects of ATV on lipid profiles compared with the most other PIs was supported by many studies in which patients with viral suppression on another PI-containing regimen were switched to an ATV-based regimen.[6,7,32,33] Compared to other PIs, ATV has a unique profile as it was least associated with adverse effects. This favourable adverse effect profile was also seen in patients who received the ATV and low-dose ritonavir combination.

2.11 Conclusion

Atazanavir is anew generation of PIs and may have an advantage over other PIs because of its favorable effect on lipid profiles, less marked metabolic effects, generally well tolerated, once-daily dosing, low capsule burden and a relatively favorable resistance profile. However, highly frequent hyperbilirubinemia is observed some cases in clinical jaundice. Antiretroviral therapy (ART) regimens containing boosted ATV improved virological and immunological markers in adult HIV-infected with treatment-naïve and treatment-experienced patients. This combination of boosted ATV with two nucleoside or nucleotide reverse transcriptase inhibitors is one of the ART regimens recommended as preferred first-line therapy by current Department of Health and Human Services (DHHS) guideline of the United State of America and alternative first-line therapy by Thai national guidelines. This regimen of a similar efficacy, better tolerance and low pill burden which should improved adherence as compared with other PIs makes boosted ATV one of the best options for treatment HIV-infected patients.[1,6,9,32,37,47]

CHAPTER III

RESEARCH METHADOLOGY

3.1 Subjects

The study was conducted from October 2010 to May 2011 at Chula Clinical Research Center of The King Chulalongkorn Memorial Hospital.

Study Population

The subjects of this study were selected from Thai healthy volunteers. The study protocol was reviewed and approved by the institutional review board at the Faculty of Medicine, Chulalongkorn University. Written inform consent had to be obtained from each individual who was participate in this study after adequate explanation of the aims, methods, objectives and potential hazards of the study and before undertaking and study-related procedures. Thirty-one subjects fulfilled the following criteria were recruited in this study. The criteria for enrollment included:

Inclusion criteria:

1. Subjects were competent and willing to sign informed consent form after being given all the detailed information about the study and must be willing to comply with all study requirements. Informed consent form signed voluntarily.
2. Healthy male or non-pregnant, non-lactating females.
3. An age between 18 to 60 years old.

4. Women of childbearing potential must be using an adequate method of contraception to avoid pregnancy throughout the study and for a period of at least 1 month after the study.

Exclusion criteria:

1. Any significant acute or chronic medical illness that might interfere with drug absorption, distribution, metabolism or excretion.
2. Evidence of organ dysfunction or underlying with cardiovascular diseases or any clinically significant deviation from normal in physical examination, vital signs or clinical laboratory determinations.
3. Positive blood screen for HIV-1 and/or 2 antibodies.
4. ALT/AST more than 5x upper limit. (grade 3)
5. Total bilirubin more than 3x upper limit. (grade 3)
6. Current or recent (within 3 months) gastrointestinal disease.
7. Clinically relevant alcohol or history of alcohol or drug use considered by the Investigator to be sufficient to hinder compliance with treatment, follow-up procedures or evaluation of adverse events. Smoking is permitted, but tobacco intake should remain consistent throughout the study.
8. Exposure to any investigational drug or placebo within 3 months of first dose of study drug.
9. Use of any other drugs, including over-the-counter medications and herbal preparations, within two weeks prior to first dose of study drug that may interfere with the pharmacokinetics of ATV/RTV, unless approved/prescribed by the principal investigator as known not to interact with study drugs.
10. Females of childbearing potential without the use of effective non-hormonal birth control methods, or not willing to continue practicing these birth control methods for at least 30 days after the end of the treatment period.

11. Previous allergy to any of the constituents of the pharmaceuticals administered in this trial.

Sample size determination

We will demonstrate non equivalence in the mean ATV C_{trough} from different doses of RTV-boosted ATV in Thai healthy volunteers. From previous study about a low dose of RTV-boosted ATV in HIV-infected Thai adults shown the mean ATV C_{trough} and standard deviation of ATV/RTV 300/100 mg once daily was 1.36 and 0.87 mg/l, respectively. The mean ATV C_{trough} was higher than its therapeutic range. Therefore we will expect to decrease the mean ATV C_{trough} more than or equal 0.6 mg/l that led the C_{trough} level into the therapeutic range.[29] The minimum sample size needs to show that mean C_{trough} of ATV in ritonavir-boosted combination therapy between reduced dose ritonavir or atazanavir is lower than standard dose of RTV-boosted ATV will be determined as follows. The calculation will be based on the following assumptions:

1. The null hypothesis is that the mean ATV C_{trough} for standard dose of ATV/RTV the mean ATV C_{trough} for reduced dose of ATV/RTV is less than or equal to 0.6 mg/l.
2. The alternative hypothesis is that the difference mean of ATV C_{trough} is more than 0.6 mg/l.
3. The sample size is calculated which the standard deviation ATV C_{trough} is 0.87 in the standard dose of ATV/RTV.[29]
4. The test is conducted at the (α = 0.05, one sided; 1-β = 0.80).

Sample size calculation

$$N = \frac{(Z_{\alpha} + Z_{\beta})^2 S^2}{D^2}$$

α = 0.05, one sided, Z_{α} = 1.645; β = 0.20, one sided, Z_{β} = 0.842

S = 0.87 (standard deviation from ATV/RTV 300/100 mg)

D = 0.6 mg/l

$$N = \frac{(1.645 + 0.842)^2 (0.87)^2}{(0.6)^2} = 13$$

Under these assumptions, the required number of evaluate participants is approximately 13. However, we will calculate approximately 3 subjects on an expected lost to follow up rate of 20%. We will need each 16 subjects per group to compare the pharmacokinetic parameters of reduced dose and standard dose of ritonavir-boosted atazanavir. Therefore we initially consider the total sample size of 32 subjects in this study.

3.2 Methods

Study design and procedures

This study was designed as a prospective, open-label, randomized, parallel assignment, and pharmacokinetics study in Thai healthy volunteers at King Chulalongkorn Memorial Hospital. The study was conducted in four steps. Demographic data, clinical findings, laboratory results, treatment regimen and adverse events were recorded for every subject until to complete this study. Information obtained from the subjects and laboratories were recorded in case report form as shown in Appendix A.

In step one, at the screening or baseline visit, Healthy subjects as determined by their medical history, physical examination and laboratory screening were eligible to participate in the study. The participants were tested for HIV infection. HIV-positive subjects were not be recruited because subjects were not received HAART and it was not yet clear if an experimentally reduced dose of RTV-boosted ATV would be successfully treated HIV infection. If they had a positive test, participants would be offered full counseling and further supported to receiving the standard of treatment. After the successful screening or baseline visit and upon confirmation that the subjects were met all the inclusion criteria and none of the exclusion criteria. The investigator was

assigned subject numbers sequentially to the subjects as they checked-in for the study. Eligible subjects were randomly assigned by block size of four to two groups.

In step two, at period 1, sixteen subjects in both groups were received ATV/RTV 300/100 mg once daily for 11 days. In step three, at period 2, subjects in group 1 were received ATV/RTV 300/50 mg while subjects in group 2 were received ATV/RTV 200/50 mg on day 12 through 22. Each eligible subject was administered ATV/RTV over the course of a 22-day treatment phase and followed in the last step by an 8-day follow-up phase.

Atazanavir plus ritonavir were administered once daily for 10 days within each period for treatment group to achieved steady-state.[37,40-44] Study medication was administered with 240 ml of water following a light meal or snack. Because of a liquid formulation of RTV was not available yet. Subjects were received Aluvia® tablet that containing with 100 mg lopinavir and 25 mg ritonavir instead of liquid dosage form in this study. The use of caffeine or xanthine-containing foods or drinks (which may affect bilirubin levels) was prohibited from 24 hours prior to dosing on days 9 to 10 and days 21 to 22. Subjects were instructed to record all prescribed and over-the-counter medications taken in a medication diary. The flow chart of study was shown in Figure 3.1.

Study evaluation

Each participant was evaluated at study entry. Baseline measurements of complete blood count, renal function test (serum creatinine) and liver function tests (ALT and total bilirubin) were done prior to starting the study. Subjects in the both groups were assessed at follow-up visits on days 10, 21 and 30. Clinical responses, drug adherence, occurrence of adverse events related to study medications and use of concomitant medications were recorded at follow-up visits. Subjects were evaluated

adherence to treatment by pharmacist interview, review of the medication diary and pill count at each follow-up visits.

Subjects were visited the clinic on up to 5 occasions during the 4 weeks study period (at baseline, days 10, 11, 21 and 22. They were followed up at 8 days after the last dose of study medication by telephone. The subjects were return to the research unit on days 10, 11 in period 1 and days 21, 22 in period 2 for pharmacokinetic (PK) sampling to determine C_{trough} (pre-dose or 0 hour) and C_2 (post-dose or 2 hour after administered study drugs on day 11 and 22) of ATV and RTV for assuring the steady state and the full pharmacokinetic (PK) study. The full PK properties of ATV and RTV will be assessed on days 10 and 21. Subjects were confined to stay in the unit on days of intensive pharmacokinetic study.

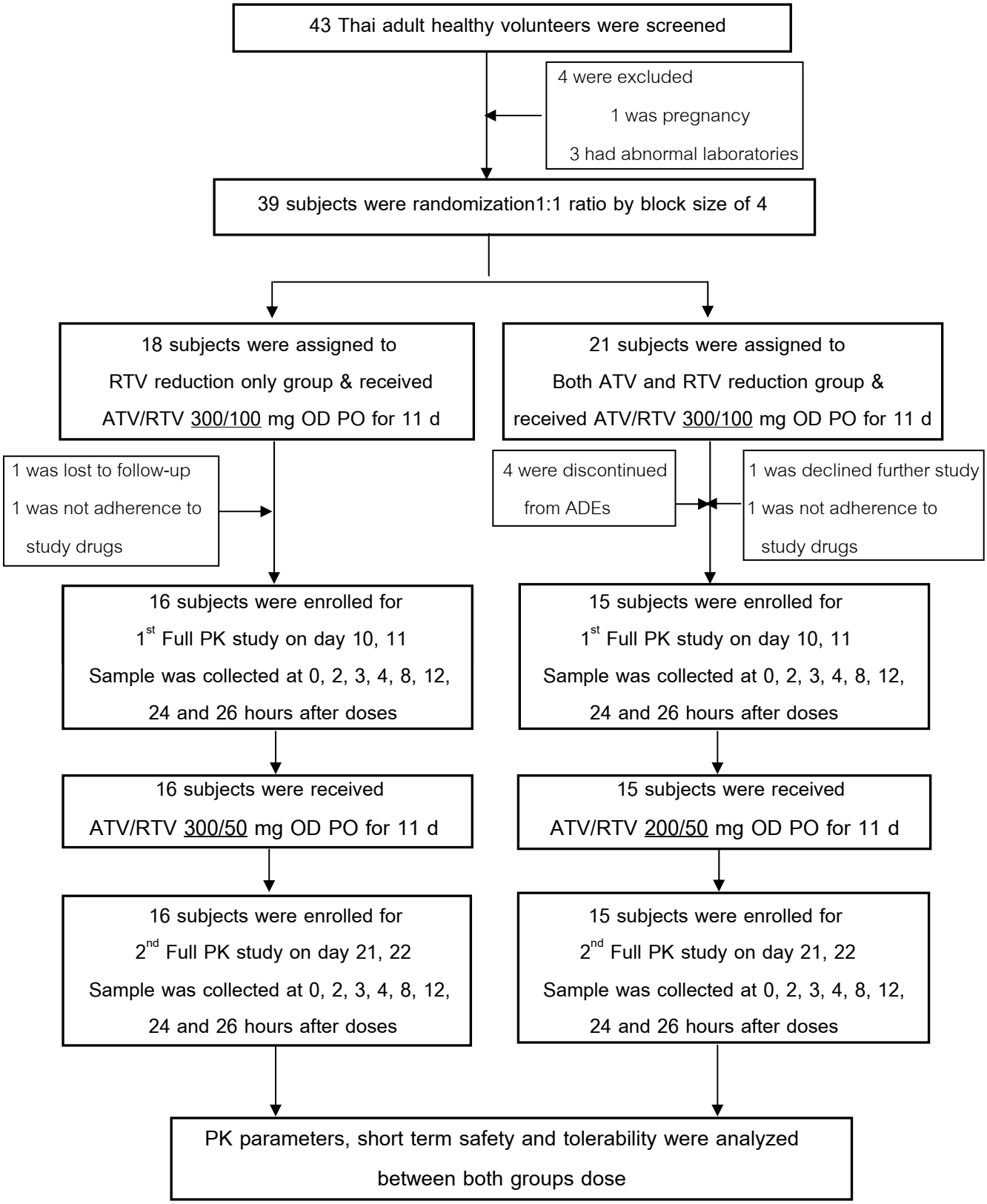

Figure 3.1: Flow chart of the study

On days 10, the first 24 hour PK study was done for ATV/RTV 300/100 mg once daily in all subjects. At days 21, the second 24 hour PK study was performed for ATV/RTV 300/50 and 200/50 mg once daily in group 1 and group 2, respectively. Drugs intake were directly observed and timed on the both day of pharmacokinetic study and administered following by a standardized breakfast of 2,500 kJ (25% from fat).

Outcome evaluation

Primary outcome measure:

- Pharmacokinetic parameters of standard dose and reduced doses of ritonavir or both ritonavir and atazanavir.

Secondary outcome measure:

- Short term safety and tolerability of standard dose and reduced doses of ritonavir or both ritonavir and atazanavir.

Safety evaluations

All patients who received at least one dose of the study medications were included in the primary safety analysis, which were evaluated events that occurred from the initiation of the assigned study drugs to 7 days after discontinuation of the regimen. All patients will question regarding possible adverse events during the course of the study. All adverse events (AEs) were recorded. Safety and tolerability were assessed by monitoring adverse events using the subjects interview, physical examinations and laboratory tests performed during the course of the study (days 10 and 20), then were compared with normal data or values at baseline. The severity of adverse events and laboratory abnormalities were graded according to Common Toxicity Criteria (CTC), Common Terminology Criteria for Adverse Events (CTCAE) or a modified CTC of the National Institutes of Allergy and Infectious Diseases.[48-50] Reported adverse events were assessed by the investigator as mild (symptoms causing no or minimal interference with

usual social and functional activities), moderate (symptoms causing greater than minimal interference with usual social and functional activities), severe (symptoms causing inability to perform usual social and functional activities) or potentially life-threatening (symptoms causing inability to perform basic self-care functions or medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death). The relationship of adverse events to study drugs was rated as doubtful, possible, probable and definite by the investigator.

Discontinuation criteria:

Discontinuation criteria are a serious or life-threatening adverse event that precluded the continuation of the study drug such as hyperbilirubinemia more than 3x upper limit (grade 3) with ALT more than 5x upper limit (grade 3) with clinical sign and symptom of jaundice. Subjects were been withdrawn from this study and were received with appropriate treatment.

3.3 Blood sampling and drug analysis

On intensive PK day (Period 1), blood samples were obtained at pre-dose (0 hr) and at 2, 3, 4, 8, 12, 24 and 26 hours post-dose. Blood samples were centrifuged at 3,000 rpm for 10 minutes at 20ºC, and were kept at -20ºC until analysis. Plasma concentrations of atazanavir (ATV) and ritonavir (RTV) were measured by validated high-performance liquid chromatography (HPLC) methods with ultra-violet detection.

On intensive PK day (Period 2), blood samples were obtained at pre-dose (0 hr) and 2, 3, 4, 8, 12, 24 and 26 hours after drug intake. Blood samples were centrifuged at 3,000 rpm for 10 minutes at 20ºC, and were kept at -20ºC until analysis. Plasma concentrations of atazanavir (ATV), lopinavir (LPV) and ritonavir (RTV) were measured by a validated HPLC method with ultra-violet detection.

Sample preparation

A 500 µL aliquot of plasma (standard, QC, patient) was spiked with IS and mixed with 500 µL 0.1 mol/L NH_4OH and 5 mL methyl tert-butyl ether. After vortexing for 1 minute and centrifuging for 5 minutes, the organic layer was transferred and evaporated to dryness with nitrogen at 37°C. The residue was dissolved in 300 µL eluent and washed with 3 mL hexane and vortexed for 5 minutes. After centrifuging for 5 minutes, 50 µL of the eluent was injected into the chromatography system.

Chromatography conditions and equipment

The HPLC Spectra system consisted of a P4000 solvent pump, an AS3000 autosampler, and a UV1000 programmable wavelength UV detector. All these instruments were from Thermo Finnigan. The analytical column was an OmniSpher 3 C18 column (100 × 4.6 mm ID; particle size, 3 µm) protected by a Chromguard RP column; both were from Varian. Analytical runs were processed by Chromquest software. The chromatographic separation was performed at ambient temperature with gradient elution. The mobile phase components were acetonitrile and 50 mmol/L potassium phosphate adjusted to pH 5.60 with 50 mmol/L sodium phosphate. The acetonitrile concentration was increased linearly from 35% to 54% during a 28-minute period. The mobile phase flow rate was 1.5 mL/min. Protease Inhibitors and IS were detected at 215 nm. The injection volume was 50 µL.

Method validation of atazanavir, ritonavir and lopinavir

The quantitative determination of protease inhibitors (PIs), including atazanavir (ATV), lopinavir (LPV) and ritonavir (RTV) in human plasma was carried out by means of a validated reversed-phase high performance liquid chromatography method (HPLC) with Ultra-Violet Detection.

Specificity and Selectivity

Selectivity was done by analyzing 6 independent sources of the drug-free plasma. The drug-free plasma (blank plasma) was obtained from National Blood Centre, Thai Red Cross Society. Chromatogram of drug free plasma was shown in Appendix B (Figure 1-6).

Specificity of the samples were shown a clear separation between the active ingredients (atazanavir, lopinavir and ritonavir), other anti-retroviral drugs and unknown impurities. No interferences with endogenous substances were observed, in both the chromatogram of plasma free drug for atazanavir, lopinavir and ritonavir, spiked plasma of internal standard [IS] and in patient samples. Chromatogram of test mixtures and patient samples were shown in Appendix B (Figure 7-9).

Internal Standard [IS = A86093.0 (5S, 8S, 10S, 11S)-9-hydroxy-2-cyclopropyl-5 -(1-methylethy)-1-(2-(1-methylethyl)-4-thiazolyl)-3, 6-dioxo-8, 11-bis(phenylmethyl)-2, 4, 7, 12-tetraazatridecan -13-oic acid, 5-thiazolylmethyl-ester) from Abbott] at 0.05 mg/ml. The retention time of Antiretroviral Drugs (ARV) are as follows:

Nevirapine	NVP	1.28	
Indinavir	IDV	4.64	6.22*8
Darunavir	DRV	6.17	9.2*8
Nelfinavir metabolite	NFV-M8	11.55	
Atazanavir	**ATV**	**12.37**	
Saquinavir	SQV	13.25	
Ritonavir	**RTV**	**14.42**	
Lopinavir	**LPV**	**15.77**	
Internal Standard	IS	17.40	
Nelfinavir	NFV	20.99	

*8 Pollution of the I.S.

Calibration Curve and Linearity

A multipoint (n ≥ 6) calibration curve were generated for each analytical run and were used to calculate the concentration of atazanavir, lopinavir and ritonavir in the samples. This include one zero sample and six (lopinavir) and seven (atazanavir and ritonavir) non-zero sample covering the target range, including LLOQ. The target concentration range for the calibration curves were 0.045 – 30.000 mg/L for atazanavir and ritonavir, and 0.105 – 30.000 mg/L for lopinavir. Calibration curves and linearity curves are shown in Table 3.1 and Appendix B (Figure 10-12).

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

Table3.1: Linearity Data

ARV	Validation – Linearity Data		
	Day 1	Day 2	Day 3
Atazanavir	0.9997	0.9999	0.9998
Ritonavir	0.9998	0.9999	0.9995
Lopinavir	0.9998	1.0000	0.9995

Lower Limit of Quantification (LLOQ)

The target lower limit of quantification (LLOQ) was 0.045 mg/L for atazanavir and ritonavir, and 0.105 mg/L for lopinavir, as shown in Appendix B (Figure 13-14).

Quality Control (QC)

A multipoint (n ≥ 6) calibration curve were generated for each analytical run and were used to calculate the concentration of ATV, LPV and RTV in the samples. The QCs were prepared in bulk, aliquot and stored at ≤ -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 are performed using 3 levels, QC Low (QC L), QC Medium (QC M) and QC High (QC H), and the concentration were 0.15 mg/L, 1.50 mg/L, and 7.50 mg/L, respectively as shown in Appendix B (Figure 15-17).

Accuracy and Precision from Biological fluid

Accuracy of atazanavir, lopinavir and ritonavir 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 with in 80-120 %. As shown in Table 3.2, the % accuracy was in acceptable range.

With-in day and between-day precision was done to express the precision under the same operating conditions over a certain period of time. This was carried out on 3 separate days. The precision of atazanavir, lopinavir and ritonavir samples was done by using at least 5 samples for 3 concentration levels, i.e. low, medium, and high. The calculated coefficient of variation (%CV) did not deviate more than 20%, as shown in Table 3.2.

The therapeutic target of ATV C_{trough} is above 0.15 mg/L. The ATV level was linear over the range of 0.045 - 30.0 mg/L. The lower limit of quantification for ATV was 0.045 mg/L. The % accuracy was 101% at 0.15 mg/l, 102% at 1.5 mg/l, 103% at 7.5 mg/l. The with-in day %CV of precision was 2.06% at 0.15 mg/l, 2.68% at 1.5 mg/l, 5.07% at 7.5

mg/l. The between day %CV of precision was 2.25% at 0.15 mg/l, 0% at 1.5 mg/l, 0.74% at 7.50 mg/l.

The therapeutic target of LPV C_{trough} is above 1.0 mg/L. The LPV level was linear over the range of 0.105–30.0 mg/L. The lower limit of quantification for LPV is 0.105 mg/L. The % accuracy was 104% at 0.15 mg/l, 102% at 1.5 mg/l, 101% at 7.5 mg/l. The with-in day %CV of precision was 3.34% at 0.15 mg/l, 1.37% at 1.5 mg/l, 1.42% at 7.5 mg/l. The between day %CV of precision was 1.22% at 0.15 mg/l, 1.30% at 1.5 mg/l, 1.10% at 7.50 mg/l.

The ritonavir level was linear over the range of 0.045 - 30.0 mg/L. The lower limit of quantification for ritonavir was 0.045 mg/L. The % accuracy was 101% at 0.15 mg/l, 104% at 1.5 mg/l, 103% at 7.5 mg/l. The with-in day %CV of precision was 3.22% at 0.15 mg/l, 1.70% at 1.5 mg/l, 0.89% at 7.5 mg/l. The between day %CV of precision was 3.64% at 0.15 mg/l, 1.17% at 1.5 mg/l, 1.10% at 7.50 mg/l.

Table 3.2: The percentage coefficient of variation (CV) of precision and accuracy of atazanavir, lopinavir and ritonavir

ARV	QC	Concentration	Precision		% Accuracy
			% Between day	% Within day	
ATV	QC H	7.5	2.2356	5.7549	98
	QC M	1.5	0.6024	2.7707	96
	QC L	0.15	0.0000	8.5660	86
RTV	QC H	7.5	1.2858	3.0631	99
	QC M	1.5	2.4097	2.4499	97
	QC L	0.15	2.0604	8.4520	84
LPV	QC H	7.5	0.0000	2.9933	100
	QC M	1.5	0.6131	2.2931	100
	QC L	0.15	0.4924	8.2289	91

Stability

The stability of atazanavir, lopinavir and ritonavir are shown in Table 3.3.

Table 3.3: Stability of Atazanavir, Lopinavir and Ritonavir

Stability	Matrix	Conditions	Conditions		
			Atazanavir	Lopinavir	Ritonavir
Freeze/Thaw cycle	Plasma	-20 °C	3 cycle	3 cycle	3 cycle
Short Term	Whole blood	Room Temperature	5 days	5 days	5 days
	Plasma	Room Temperature	8 days	8 days	8 days
	Plasma	-20° C	4 months	4 months	4 months
Long Term	Plasma	-20° C	18 months	18 months	18 months
Stock solution	Methanol	-20° C	24 months	24 months	24 months
Post-Preparative	Extracted samples	Room Temperature	4 Weeks	4 Weeks	4 Weeks

The HIV Netherlands-Australia-Thailand (HIV-NAT) Clinical Research Laboratory participates in an international quality control and quality assessment program developed by the department of Clinical Pharmacology at the University Medical Centre Nijmegen (Nijmegen, the Netherlands).[51] and has been cross-validated with other PK laboratories.[52]

3.4 Pharmacokinetic parameters calculation

Pharmacokinetic parameters of ATV, LPV and RTV were calculated by using noncompartmental techniques with WinNonlin® Professional version 6.2 (Pharsight Corporation, Mountain View, California) using the linear up/log down trapezoidal rule.

AUC from time zero to the end of the dosing interval (AUC_{0-24}), C_{trough}, C_{max}, T_{max}, terminal phase rate constant, $t_{1/2}$ and apparent oral clearance parameters will be determined.

3.5 Statistical analysis

All data were performed by using the SPSS for windows version 17.0 and analyzed by descriptive statistics and inferential statistics. Paired t-test, Independent t-test or Wilcoxon signed-rank test will be used for comparison of pharmacokinetic parameters between the groups. A p-value of less than 0.05 was considered to be statistically significant for all analyses.

3.6 Ethical consideration

This study was complied with the standard for gathering subjects' information for confidential in every process since data collection, analysis, conclusion and publication. All data collected from patients were coded in order to protect their confidentially. There had no record any details that led to identify to the subjects. Only principal investigator and co-investigators could access directly to the patients' information. Subjects included in this study may be possible got risk from adverse event of the drugs used in this study. However, these patients were received close monitoring by clinical and laboratory assessment to ensure the subjects' safety with supportive treatment to alleviate that adverse event. Results from this study may be published in scientific journals or presented at medical meetings but subjects were not been personally identify.

CHAPTER IV

RESULTS

4.1 Study populations

The study was conducted from October 2010 to May 2011 at Chula Clinical Research Center of The King Chulalongkorn Memorial Hospital. All of the patients gave their consent to participate in this study. Forty-three Thai adult healthy volunteers were screened and 3 volunteers were excluded from this study (1 was pregnancy and 2 had abnormal laboratories). Thirty-nine volunteers were enrolled and randomly assigned 1:1 ratio into one of the two groups by block size of 4. A total of 31 healthy volunteers (22 men and 9 women) were completed the study and included in the further analysis; 4 discontinued because of adverse events, 2 did not adhere to the study protocol, 1 withdrew consent or declined to further study and 1 lost to follow up because of influenza during study. Thirty-one volunteers were divided into two groups; 16 volunteers in Group 1 (Reduced dose RTV) and 15 volunteers in Group 2 (Reduced dose ATV and RTV). Subjects in both groups were received ritonavir-boosted atazanavir in the different dose divided into 2 periods. At period 1, all subjects in both groups were received ATV/RTV 300/100 mg once daily for 11 days. At period 2, 16 subjects in group 1 were received ATV/RTV 300/50 mg while 15 subjects in group 2 were received ATV/RTV 200/50 mg on day 12 through 22.

Demographic data

Of the 39 healthy volunteers, 24 subjects (61.5%) were male. Mean (standard deviation) age, body weight and body mass index were 28.13 (5.53) years, 60.55 (10.20) kg and 21.60 (2.04) kg/m^2. The mean standard dose of atazanavir per body weight was 5.09 (0.82) mg/kg. The mean (standard deviation) baseline of total bilirubirin

was 0.83 (0.20) mg/dL. Baseline demographic data of the enrolled subjects in both groups had no statistically significant difference (P>0.05) and was summarized in Table 4.1

Table 4.1: Baseline demographic data of the enrolled subjects in this study

Characteristics	Mean ± Standard deviation (Median)		Total	P-value
	Group 1 RTV reduction only (N=18)	Group 2 Both ATV and RTV reduction (N=21)		
Gender; No. [%]				
- Male	11 [61.1]	13 [61.9]	24 [61.5]	1.000
- Female	7 [38.9]	8 [38.1]	15 [38.5]	
Age (yr)	29.17 ± 6.00 (28.5)	27.24 ± 5.07 (27.0)	28.13 ± 5.53 (27.0)	0.284
Height (cm)	165.56 ± 9.70 (166.0)	168.10 ± 8.75 (170.0)	166.92 ± 9.17 (166.0)	0.943
Weight (kg)	60.92 ± 10.68 (58.75)	60.65 ± 10.04 (61.10)	60.55 ± 10.20 (58.8)	0.898
Body mass index (kg/m^2)	21.90 ± 2.10 (21.53)	21.34 ± 2.00 (20.58)	21.60 ± 2.04 (20.93)	0.396
Standard ATV dose/weight (mg/kg)	5.11 ± 0.86 (5.11)	5.07 ± 0.81 (4.91)	5.09 ± 0.82 (5.10)	0.403
Total bilirubin (mg/dL)	0.81 ± 0.23 (0.80)	0.86 ± 0.18 (0.90)	0.83 ± 0.20 (0.90)	0.514

4.2 Pharmacokinetic (PK) parameters of atazanavir and ritonavir

Comparisons of the PK parameters obtained after ATV/RTV 300/100 and ATV/RTV 300/50

The mean plasma concentration-time profiles of atazanavir (ATV) and ritonavir (RTV) after subjects receiving ATV/RTV 300/100 and 300/50 daily were illustrated in Figure 4.1. The pharmacokinetic parameters of ATV and RTV and the results of the t-test for the difference of the values obtained after receiving two different dosage regimens were summarized in Table 4.2 and 4.3, respectively.

Pharmacokinetic parameters of atazanavir between ATV/RTV 300/100 and ATV/RTV 300/50 (G1P1 versus G1P2)

Comparisons of the geometric mean (90% CI) pharmacokinetic parameters of atazanavir between subjects in group 1 (RTV reduction only) at period 1; G1P1 (ATV/RTV 300/100) and subjects group 1 at period 2; G1P2 (ATV/RTV 300/50) were shown in Table 4.2. The reduction of the ritonavir dose was associated with significant decreasing in the area under the plasma concentration-time curve from time 0 to 24 hour (AUC_{0-24}), peak concentration (C_{max}), trough concentration (C_{min}) and average concentration (C_{avg}) of ATV while the clearance (CL/F) of ATV was significantly increase. Elimination rates constant (K_{el}) was slightly increased and in turn half-life ($T_{1/2}$) was decreased but did not reach the statistically significant level at α =0.05 (P=0.09). Time to peak concentration (T_{max}) was approximately no change. There were approximately 36%, 30%, 53% and 26% decrease in the geometric mean of AUC_{0-24}, C_{max}, C_{min} and C_{avg} of ATV, respectively when the dose of ritonavir was reduced from 100 mg to 50 mg daily even though the dose of ATV was remained at 300 mg daily.

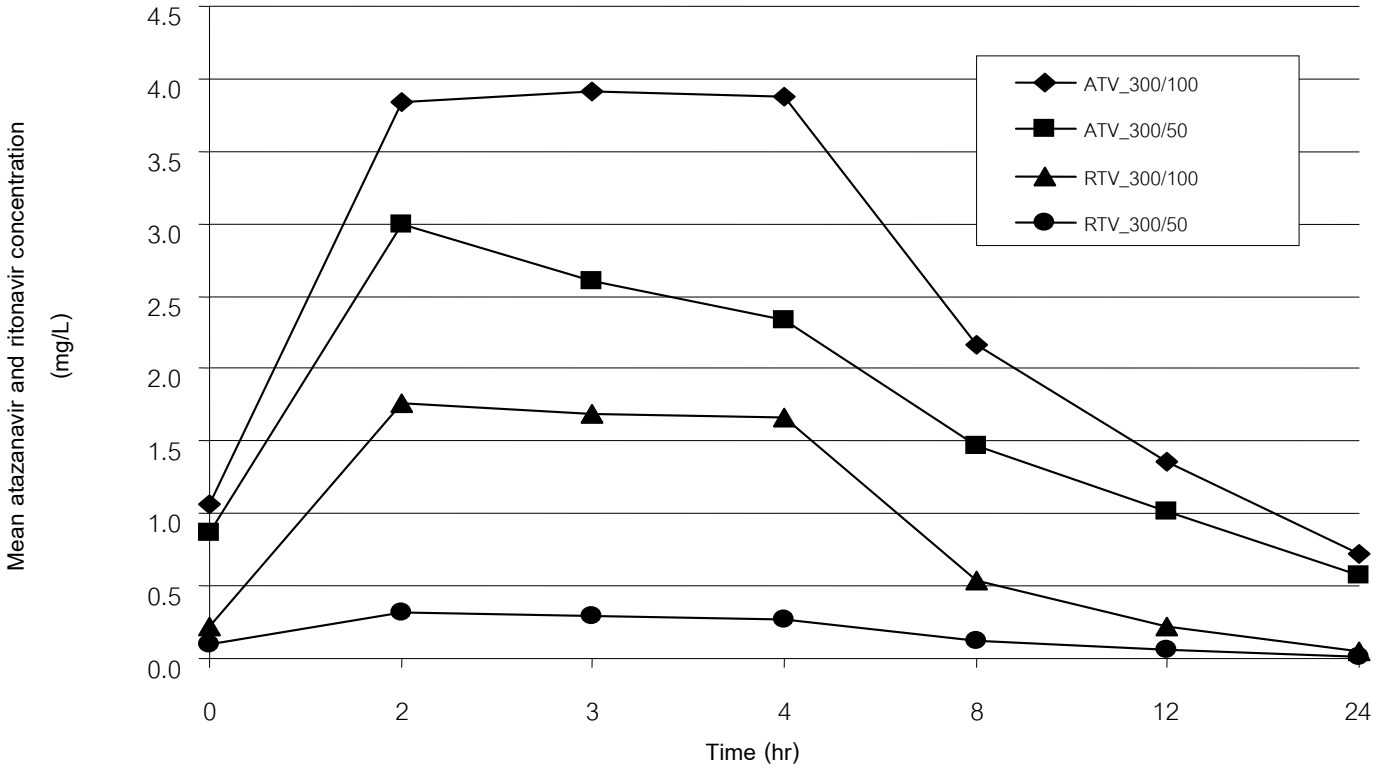

Figure 4.1: The mean atazanavir and ritonavir concentration-time curves in subjects receiving atazanavir/ritonavir 300/100 mg/d for 11 days and 300/50 mg/d for the next 11 days.

Table 4.2: Comparisons of the pharmacokinetic parameters of atazanavir in group 1 after subjects receiving standard dose in period 1 (ATV/RTV 300/100 OD; G1P1) to those obtained after subjects reduced dose of ritonavir in period 2 (ATV/RTV 300/50 OD; G1P2)

Pharmacokinetic parameters	Geometric Mean (90% CI) Arithmetic Mean		Ratio of Geometric Mean (G1P2/G1P1)	P-value
	G1P1 ATV/RTV 300/100 (N=16)	G1P2 ATV/RTV 300/50* (N=16)		
AUC_{0-24} (mg.h/L)	39.80 (33.21 - 47.71) 43.26	25.50 (19.85 - 32.76) 30.40	0.64	0.000
C_{max} (mg/L)	4.03 (3.43 - 4.73) 4.30	2.83 (2.29 - 3.50) 3.18	0.70	0.001
C_{min} (mg/L)	0.58 (0.44 - 0.76) 0.72	0.27 (0.17 - 0.43) 0.53	0.47	0.000
C_{avg} (mg/L)	1.66 (1.38 -1.99) 1.80	1.06 (0.83 - 1.36) 1.27	0.64	0.000
$T_{1/2}$ (hr)	12.33 (10.85 - 14.02) 12.88	11.10 (9.29 - 13.26) 12.21	0.90	0.090
T_{max} (hr)	2.40 (2.11 - 2.72) 2.50	2.25 (2.04 - 2.48) 2.31	0.94	0.480
K_{el} (1/hr)	0.06 (0.05 - 0.06) 0.06	0.06 (0.05 - 0.07) 0.07	1.00	0.090
CL/F (L/hr)	7.54 (6.29 - 9.03) 8.13	11.76 (9.16 - 15.11) 13.42	1.56	0.000

* (+ LPV 200 mg)

Pharmacokinetic parameters of ritonavir between ATV/RTV 300/100 and ATV/RTV 300/50 (G1P1 versus G1P2)

Table 4.3 presented comparison between of the geometric mean (90% CI) of ritonavir pharmacokinetic parameters in subjects with G1P1 (ATV/RTV 300/100) and those observed in subjects with G1P2 (ATV/RTV 300/50). The reduction of ritonavir dose was associated with significantly decrease in the AUC_{0-24}, C_{max} and C_{avg} of RTV while C_{min} was not compared because most of subjects had very low level of RTV at C_{min} that might have C_{min} which were lower than the lower limit of quantification (LLOQ). The geometric mean ratios of CL/F were significantly higher 2.89 (P=0.000). K_{el} and $T_{1/2}$ were not significantly different and T_{max} remained the same. The administration of 300/50 of ATV/RTV once daily showed a RTV AUC_{0-24} that was 83% lower than that obtained after administration of the standard 300/100 mg once daily dose of ATV/RTV. At the same time, the ritonavir C_{max} and C_{avg} were 84% and 83% lower, respectively.

Table 4.3: Comparisons of the pharmacokinetic parameters of ritonavir in group 1 after subjects receiving standard dose in period 1 (ATV/RTV 300/100 OD; G1P1) to those obtained after subjects receiving reduced dose of ritonavir in period 2 (ATV/RTV 300/50 OD; G1P2)

Pharmacokinetic parameters	Geometric Mean (90% CI) Arithmetic Mean		Ratio of Geometric Mean (G1P2/G1P1)	P-value
	G1P1 ATV/RTV 300/100 (N=16)	G1P2 ATV/RTV 300/50* (N=16)		
AUC_{0-24} (mg.h/L)	9.15 (6.77 - 12.39) 12.07	1.58 (1.11 - 2.25) 2.46	0.17	0.000
C_{max} (mg/L)	1.53 (1.15 - 2.04) 1.92	0.25 (0.19 - 0.34) 0.33	0.16	0.000
C_{min} (mg/L)**	0.09 (NA) 0.10	0.116 (NA) NA	NA	NA
C_{avg} (mg/L)	0.38 (0.28 - 0.52) 0.50	0.07 (0.05 - 0.09) 0.10	0.17	0.000
$T_{1/2}$ (hr)	5.78 (4.95 - 6.71) 6.13	6.24 (5.24 - 7.43) 6.81	1.08	0.416
T_{max} (hr)	2.48 (2.15 - 2.87) 2.63	2.48 (2.20 - 2.78) 2.56	1.00	0.972
K_{el} (1/hr)	0.12 (0.10 - 0.14) 0.13	0.11 (0.09 - 0.13) 0.12	0.92	0.416
CL/F (L/hr)	10.92 (8.07 - 14.77) 13.07	31.56 (22.20 - 44.86) 39.67	2.89	0.000

* (+ LPV 200 mg)

** Data available from only 3 and 1 subjects in G1P1 and G1P2, respectively. The others had very low RTV concentration at C_{min} (lower than LLOQ; < 0.045 mg/L)

Comparisons of the PK parameters obtained after ATV/RTV 300/100 and ATV/RTV 200/50

The mean plasma concentration-time profiles of atazanavir and ritonavir, after subjects receiving ATV/RTV 300/100 and 200/50 daily are illustrated in Figure 4.2. The pharmacokinetic parameters of two drugs and the results of the t-test for the difference of the values obtained after receiving two different dosage regimens were reported in Table 4.4 and 4.5, respectively.

Pharmacokinetic parameters of atazanavir between ATV/RTV 300/100 and ATV/RTV 200/50 (G2P1 versus G2P2)

Comparisons of the geometric mean (90% CI) pharmacokinetic parameters of atazanavir between subjects administered ATV/RTV 300/100 (G2P1) versus ATV/RTV 200/50 (G2P2) in group 2 (Both ATV and RTV reduction) were shown in Table 4.4. The decrease of both atazanavir and ritonavir doses were associated with significantly decrease in the AUC_{0-24}, C_{max}, C_{min} and C_{avg}. In contrast, CL/F was higher but did not reach statistically significant level at P=0.05. K_{el} and $T_{1/2}$ were not significantly different while T_{max} was nearly the same. There were approximately 40%, 39%, 51% and 40% decrease in the geometric mean of AUC_{0-24}, C_{max}, C_{min} and C_{avg}, respectively when the dose of atazanavir was reduced from 300 mg to 200 mg along with the dose of ritonavir which was reduced from 100 mg to 50 mg.

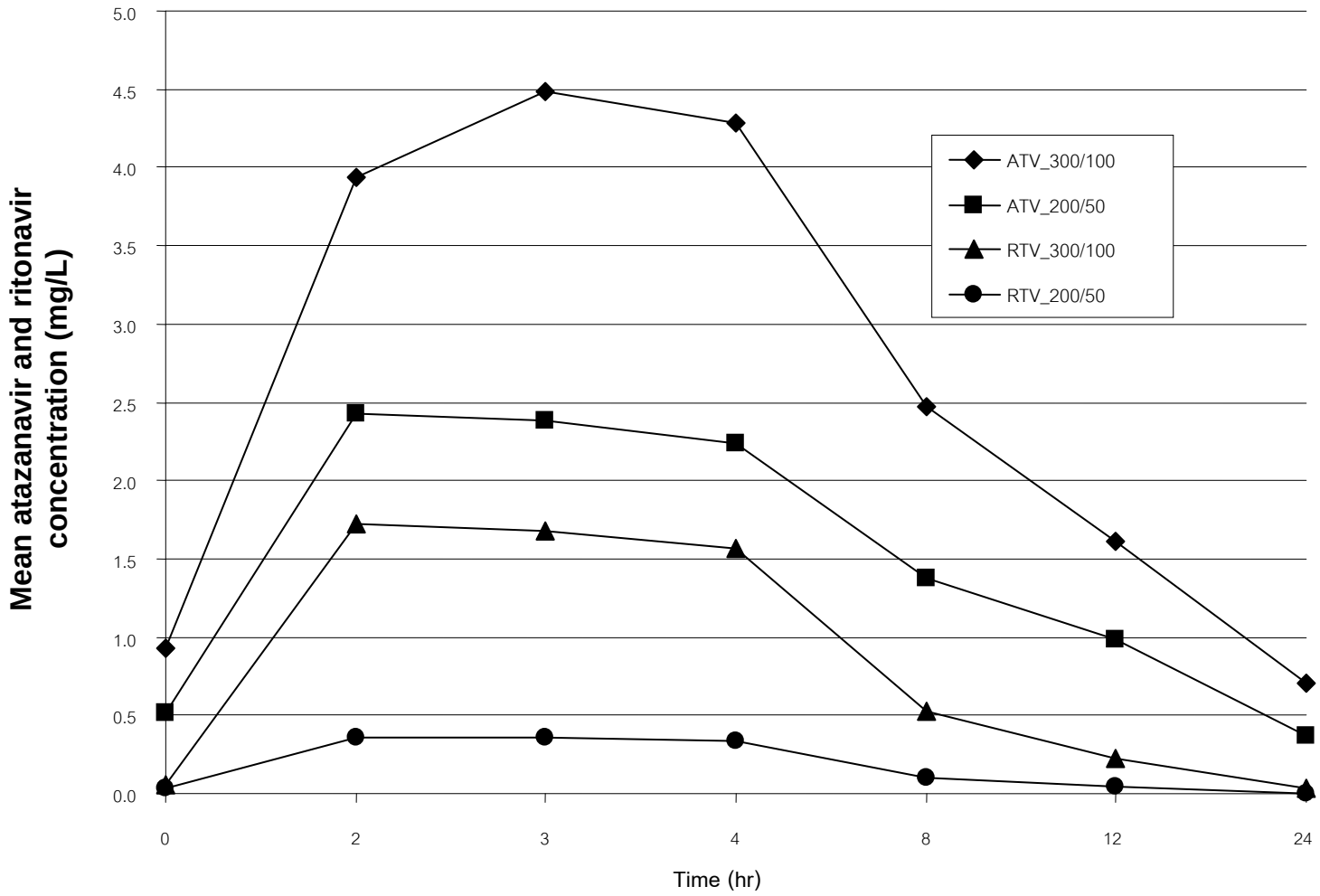

Figure 4.2: The mean atazanavir and ritonavir concentration-time curves in subjects receiving with atazanavir/ritonavir 300/100 mg/d for 11 days and 200/50 mg/d for the next 11 days.

Table 4.4: Comparisons of the pharmacokinetic parameters of atazanavir after subjects receiving standard dose (ATV/RTV 300/100 OD; G2P1) and to those obtained after subjects receiving reduced dose of atazanavir and ritonavir (ATV/RTV 200/50 OD; G2P2)

Pharmacokinetic parameters	Geometric Mean (90% CI) Arithmetic Mean		Ratio of Geometric Mean (G2P2/G2P1)	P-value
	G2P1 ATV/RTV 300/100 (N=15)	G2P2 ATV/RTV 200/50* (N=15)		
AUC_{0-24} (mg.h/L)	41.60 (32.37 - 53.45) 47.76	24.97 (20.92 - 29.81) 26.81	0.60	0.000
C_{max} (mg/L)	4.23 (3.36 - 5.32) 4.77	2.58 (2.23 - 2.98) 2.71	0.61	0.001
C_{min} (mg/L)	0.49 (0.35 - 0.67) 0.61	0.24 (0.15 - 0.37) 0.33	0.49	0.004
C_{avg} (mg/L)	1.73 (1.35 - 2.23) 1.98	1.04 (0.87 - 1.24) 1.12	0.60	0.000
$T_{1/2}$ (hr)	12.78 (11.25 - 14.51) 13.26	11.18 (9.78 - 12.78) 11.63	0.88	0.155
T_{max} (hr)	2.56 (2.24 - 2.92) 2.67	2.32 (2.05 - 2.61) 2.40	0.90	0.295
K_{el} (1/hr)	0.05 (0.05 - 0.06) 0.06	0.06 (0.05 - 0.07) 0.06	1.20	0.154
CL/F (L/hr)	7.21 (5.61 - 9.27) 8.28	8.01 (6.71 - 9.56) 8.58	1.11	0.295

* (+ LPV 200 mg)

Pharmacokinetic parameters of ritonavir between ATV/RTV 300/100 and ATV/RTV 200/50 (G2P1 versus G2P2)

Table 4.5 summarized comparisons of the geometric mean (90% CI) pharmacokinetic parameters of ritonavir between subjects in G2P1 (ATV/RTV 300/100) and G2P2 (ATV/RTV 200/50). The reduction of atazanavir and ritonavir dose was associated significantly decrease in the AUC_{0-24}, C_{max} and C_{avg}. In contrast, the geometric mean CL/F was significantly higher. K_{el} and $T_{1/2}$ were not significantly changed and T_{max} remained unchanged. The administration of 200/50 of ATV/RTV once daily showed a ritonavir AUC_{0-24} that was 81% lower than that received after the standard 300/100 mg once daily. The ritonavir C_{max}, C_{min} and C_{avg} were 80%, 92% and 80% lower, respectively.

Comparisons of the PK parameters obtained after ATV/RTV 300/50 and ATV/RTV 200/50

The pharmacokinetic parameters of two drugs and results of the t-test for the difference between both groups were reported in Table 4.6 and 4.7, respectively.

Table 4.5: Comparison of the pharmacokinetic parameters of ritonavir after subjects receiving standard dose (ATV/RTV 300/100 OD; G2P1) and to those obtained after subjects receiving reduced dose of atazanavir and ritonavir (ATV/RTV 200/50 OD; G2P2)

Pharmacokinetic parameters	Geometric Mean (90% CI) Arithmetic Mean G2P1 ATV/RTV 300/100 (N=15)	G2P2 ATV/RTV 200/50* (N=15)	Ratio of Geometric Mean (G2P2/G2P1)	P-value
AUC_{0-24} (mg.h/L)	9.98 (7.75 - 12.87) 11.43	1.94 (1.47 - 2.55) 2.32	0.19	0.000
C_{max} (mg/L)	1.59 (1.26 - 2.00) 1.79	0.32 (0.24 - 0.42) 0.39	0.20	0.000
C_{min} (mg/L)**	0.07 (NA) 0.08	NA (NA) NA	NA	NA
C_{avg} (mg/L)	0.42 (0.32 - 0.54) 0.48	0.08 (0.06 - 0.11) 0.10	0.20	0.000
$T_{1/2}$ (hr)	5.77 (5.01 - 6.65) 6.04	7.11 (4.87 - 10.39) 11.30	1.23	0.355
T_{max} (hr)	2.38 (2.11 - 2.69) 2.47	2.56 (2.24 - 2.92) 2.67	1.08	0.520
K_{el} (1/hr)	0.12 (0.10 - 0.14) 0.13	0.10 (0.07 - 0.14) 0.12	0.83	0.356
CL/F (L/hr)	10.02 (7.77 - 12.91) 11.62	25.31 (19.36 - 33.08) 29.34	2.53	0.000

* (+ LPV 200 mg)

** Data available from only 3 subjects in G2P1 and others had very low RTV concentration at C_{min} (lower than LLOQ; < 0.045 mg/L) but all subjects in G2P2 had RTV concentration lower than LLOQ.

Pharmacokinetic parameters of atazanavir between ATV/RTV 300/500 and ATV/RTV 200/50 (G1P2 versus G2P2)

Comparisons of the geometric mean (90% CI) pharmacokinetic parameters of atazanavir between subjects who received ATV/RTV 300/50 (G1P2) and subjects who received ATV/RTV 200/50 (G2P2) were shown in Table 4.6. Neither pharmacokinetic parameters (AUC_{0-24}, C_{max}, C_{min} C_{avg}, $T_{1/2}$, K_{el} and T_{max}) showed statistically significant different between the two groups of subjects receiving different dosage regimens except for CL/F. The subjects who were administered with ATV/RTV 300/50 showed significantly higher CL/F than the subjects who were administered with ATV/RTV 200/50. There were approximately 2%, 10%, 13% and 3% decrease in the geometric mean of AUC_{0-24}, C_{max}, C_{min} and C_{avg}, respectively when the dose of atazanavir was reduced from 300 mg to 200 mg combined with boosted ritonavir 50 mg.

Table 4.6: Comparisons of the pharmacokinetic parameters of atazanavir between subjects receiving reduced dose of ritonavir alone (ATV/RTV 300/50 OD; G1P2) and reduced doses of both atazanavir and ritonavir (ATV/RTV 200/50 OD; G2P2)

Pharmacokinetic parameters	Geometric Mean (90% CI) Arithmetic Mean		Ratio of Geometric Mean (G1P2/G2P2)	P-value
	G1P2 ATV/RTV 300/50* (N=16)	G2P2 ATV/RTV 200/50* (N=15)		
AUC_{0-24} (mg.h/L)	25.50 (19.85 - 32.76) 30.40	24.97 (20.92 - 29.81) 26.81	1.02	0.907
C_{max} (mg/L)	2.83 (2.29 - 3.50) 3.18	2.58 (2.23 - 2.98) 2.71	1.10	0.533
C_{min} (mg/L)	0.27 (0.17 - 0.43) 0.53	0.24 (0.15 - 0.37) 0.33	1.13	0.734
C_{avg} (mg/L)	1.06 (0.83 - 1.36) 1.27	1.04 (0.87 - 1.24) 1.12	1.03	0.907
$T_{1/2}$ (hr)	11.10 (9.29 - 13.26) 12.21	11.18 (9.78 - 12.78) 11.63	0.99	0.956
T_{max} (hr)	2.25 (2.04 -2.48) 2.31	2.32 (2.05 - 2.61) 2.40	0.97	0.758
K_{el} (1/hr)	0.06 (0.05 - 0.07) 0.07	0.06 (0.05 - 0.07) 0.06	1.00	0.955
CL/F (L/hr)	11.76 (9.16 - 15.11) 13.42	8.01 (6.71 - 9.56) 8.58	1.47	0.038

* (+ LPV 200 mg)

Pharmacokinetic parameters of ritonavir between ATV/RTV 300/50 and ATV/RTV 200/50 (G1P2 versus G2P2)

Table 4.7 summarized comparisons of the geometric mean (90% CI) pharmacokinetic parameters of ritonavir between subjects in G1P2 (ATV/RTV 300/50) and G2P2 (ATV/RTV 200/50) groups. None of the pharmacokinetic parameters (AUC_{0-24}, C_{max}, C_{avg}, $T_{1/2}$, T_{max}, K_{el} and CL/F) showed statistically significant different between the two groups. Most of the ritonavir concentrations at C_{min} in both groups were very lower than LLOQ that couldn't be compared between both groups. The administration of boosted ritonavir dose 50 mg once daily along with different dose of atazanavir, either 300 mg or 200 mg, demonstrated the resemblance pharmacokinetic parameters of ritonavir between the two groups.

Table 4.7: Comparisons of the pharmacokinetic parameters of ritonavir between subjects receiving reduced dose of ritonavir alone (ATV/RTV 300/50 OD; G1P2) and reduced doses of both atazanavir and ritonavir (ATV/RTV 200/50 OD; G2P2)

Pharmacokinetic parameters	Geometric Mean (90% CI) Arithmetic Mean		Ratio of Geometric Mean (G1P2/G2P2)	P-value
	G1P2 ATV/RTV 300/50* (N=16)	G2P2 ATV/RTV 200/50* (N=15)		
AUC_{0-24} (mg.h/L)	1.58 (1.11 - 2.25) 2.46	1.94 (1.47 - 2.55) 2.32	0.82	0.438
C_{max} (mg/L)	0.25 (0.19 - 0.34) 0.33	0.32 (0.24 - 0.42) 0.39	0.78	0.294
C_{min} (mg/L)	0.116 (NA) NA	NA (NA) NA	NA	NA
C_{avg} (mg/L)	0.07 (0.05 - 0.09) 0.10	0.08 (0.06 - 0.11) 0.10	0.80	0.393
$T_{1/2}$ (hr)	6.24 (5.24 - 7.43) 6.81	7.11 (4.87 - 10.39) 11.30	0.88	0.578
T_{max} (hr)	2.48 (2.20 - 2.78) 2.56	2.56 (2.24 - 2.92) 2.67	0.97	0.740
K_{el} (1/hr)	0.11 (0.09 - 0.13) 0.12	0.10 (0.07 - 0.14) 0.12	0.91	0.578
CL/F (L/hr)	31.56 (22.20 - 44.86) 39.67	25.31 (19.36 - 33.08) 29.34	1.25	0.393

* (+ LPV 200 mg)

** Data available from only 1 subjects in G1P2 and others had very low RTV concentration at C_{min} (lower than LLOQ; < 0.045 mg/L) but all subjects in G2P2 had RTV concentration lower than LLOQ.

4.3 Pharmacokinetic parameters of lopinavir

Comparisons of the PK parameters obtained after ATV/RTV 300/50 and ATV/RTV 200/50

In this study, subjects received 2 tablets Aluvia® which each tablet containing 100 mg lopinavir (LPV) and 25 mg ritonavir instead of taking tablet containing only 50 mg of ritonavir. The pharmacokinetic parameters of lopinavir and the results of the t-test for the difference between the two groups (ATV/RTV 300/100 versus ATV/RTV 200/50) were summarized in Table 4.8.

Pharmacokinetic parameters of lopinavir between ATV/RTV 300/50 and ATV/RTV 200/50 (G1P2 versus G2P2)

Comparisons of the geometric mean (90% CI) pharmacokinetic parameters of lopinavir between subjects received equally dose of lopinavir along with ATV/RTV 300/50 mg or ATV/RTV 200/50 mg were shown in Table 4.8. Different doses of atazanavir when combined with 50 mg boosted ritonavir and 200 mg lopinavir did not cause statistically significant difference in any pharmacokinetic parameter of lopinavir including AUC_{0-24}, C_{max}, C_{min}, C_{avg}, $T_{1/2}$, T_{max}, K_{el} and CL/F.

Table 4.8: Comparisons of the pharmacokinetic parameters of lopinavir between subjects receiving reduced dose of ritonavir alone (ATV/RTV 300/50 OD; G1P2) and reduced doses of both atazanavir and ritonavir (ATV/RTV 200/50 OD; G2P2)

Pharmacokinetic parameters	Geometric Mean (90% CI) Arithmetic Mean		Ratio of Geometric mean (G1P2/G2P2)	P-value
	G1P2 ATV/RTV 300/50* (N=16)	G2P2 ATV/RTV 200/50* (N=15)		
AUC_{0-24} (mg.h/L)	41.33 (31.53 - 54.19) 50.19	49.90 (40.88 - 60.90) 54.68	0.83	0.339
C_{max} (mg/L)	5.15 (4.39 - 6.06) 5.47	5.99 (5.16 - 6.95) 6.30	0.86	0.241
C_{min} (mg/L)	0.05 (0.03 - 0.11) 0.37	0.05 (0.03 - 0.09) 0.11	1.08	0.894
C_{avg} (mg/L)	1.72 (1.31 - 2.26) 2.09	2.08 (1.70 - 2.54) 2.28	0.83	0.339
$T_{1/2}$ (hr)	6.29 (5.20 - 7.60) 7.03	5.95 (5.21 - 6.78) 6.19	1.06	0.677
T_{max} (hr)	2.43 (2.19 - 2.70) 2.50	2.27 (2.05 - 2.52) 2.33	1.07	0.425
K_{el} (1/hr)	0.11 (0.09 - 0.13) 0.12	0.12 (0.10 - 0.13) 0.12	1.10	0.676
CL/F (L/hr)	4.84 (3.69 - 6.34) 5.77	4.01 (3.28 - 4.89) 4.36	1.21	0.339

* (+ LPV 200 mg)

4.4 Therapeutic optimum trough plasma concentration of atazanavir

The suggested minimum target trough concentrations in patients with HIV-1 susceptible to atazanavir has been defined to be 0.15 mg/L. Therapeutic target optimum trough plasma concentration of atazanavir has been recommended to be between 0.15-0.85 mg/L. In this study, subjects received different dose of atazanavir and/or boosted with ritonavir. The percentages of subjects whose atazanavir C_{min} (ATV C_{min}) were within, above or below the therapeutic range were showed in Table 4.9.

About 70%-80% of subjects receiving ATV/RTV 300/100 mg had their ATV C_{min} within the recommended therapeutic range. While approximately 20%-30% of subjects administered with ATV/RTV 300/100 mg had ATV C_{min} which were higher than the therapeutic range. None of the subjects taking ATV/RTV 300/100 mg once daily had their ATV C_{min} levels which were lower than the suggested minimum target trough concentration of 0.15 mg/L. Fifty-six percentage of subjects receiving atazanavir 300 mg combined with reduced dose 50 mg of ritonavir had their ATV C_{min} within the therapeutic range while 31% of their ATV C_{min} was subtherapeutic and 13% was above therapeutic range. The reduction of both atazanavir and ritonavir doses in G2P2 (ATV/RTV 200/50), majority of subjects (66.67%) had their ATV C_{min} levels in the recommended therapeutic range (0.15-0.85 mg/L) while about 33% of subjects had their ATV C_{min} levels lower than the minimum recommend trough concentration of 0.15 mg/L. None of the subjects taking ATV/RTV 200/50 mg once daily had their ATV C_{min} levels higher than the suggested target trough concentration of 0.85 mg/L. About 67% of subjects in both groups had their ATV C_{min} levels within therapeutic ranges, 33% in G2P1 (ATV/RTV 300/100) had their ATV C_{min} levels above 0.85 mg/L while 33% in G2P2 (ATV/RTV 200/50) had their ATV C_{min} levels lower than the minimum trough concentration of 0.15 mg/L.

Table 4.9: Percentage of subjects receiving different doses of atazanavir and/or ritonavir whose atazanavir C_{min} were within, above or below the recommended therapeutic range

Atazanavir Cmin (mg/L)	Number (%) of subjects			
	RTV reduction only (N=16)		Both ATV and RTV reduction (N=15)	
	ATV/RTV 300/100	ATV/RTV* 300/50	ATV/RTV 300/100	ATV/ RTV* 200/50
< 0.15	0 (0)	5 (31.25)	0 (0)	5 (33.33)
0.15 - 0.85	13 (81.25)	9 (56.25)	10 (66.67)	10 (66.67)
>0.85	3 (18.75)	2 (12.50)	5 (33.33)	0 (0)
P-value	0.127		1.00	

* (+ LPV 200 mg)

4.5 Safety and tolerability

Most commonly reported treatment-related adverse events were shown in Table 4.10. All regimens were generally well tolerated. None of the subjects included in this study discontinued the study drugs due to adverse drug reaction. Grade 1, 2, 3 and 4 of blood bilirubin alterations according to the CTCAE classification were defined as >Upper limit normal (ULN)-1.5 ULN, >1.5-3 ULN, >3-10 ULN and >10 ULN, respectively.[50]

Hyperbilirubinemia was the most common adverse events found in both groups approximately 90-100% which related to the receiving of atazanavir. All of the subjects receiving ATV/RTV 300/100 mg had hyperbilirubinemia which about 88% had severity in grade 2 or 3. While subjects taking the reduced dose of atazanavir and/or ritonavir, hyperbilirubinemia did not found in 3 subjects (16.67%) in G1P2 (ATV/RTV 300/50)

group and 5 subjects (23.81%) in G2P2 (ATV/RTV 200/50) group. Majority of the subjects while administering 300 mg or 200 mg of atazanavir along with 50 mg of ritonavir had grade 1 of hyperbilirubinemia (29-50%).

Jaundice also occurred higher in subjects receiving the standard dose of atazanavir boosted with ritonavir. Thirteen subjects (33%) in the ATV/RTV 300/100 mg group had jaundice which was more frequent than 9 subjects (23%) found in the ATV/RTV 300/50 mg or 200/50 mg group.

Loose stool or diarrhea was reported to be the most common adverse reactions caused by lopinavir in the combination with ritonavir in Aluvia® tablet. In this study, there were 4 subjects (25%) and 6 subjects (40%) in the ATV/RTV 300/50 mg or 200/50 mg group, respectively.

Rash was generally side effects found in subjects receiving atazanavir. Rash was reported in 2 subjects in group 1 (RTV reduction only) that were the same subject in the standard dose (ATV/RTV 300/100) and reduced dose 50 mg of RTV (ATV/RTV 300/50). Four subjects in group 2 (Both ATV and RTV reduction) were reported rash and discontinued to further study.

Dizziness was only found in 2 subjects in the ATV/RTV 300/100 mg once daily. This might be the side effect caused by atazanavir and/or ritonavir. All adverse events and laboratory abnormalities were reversible.

Table 4.10: Adverse events recorded while subjects receiving different doses of atazanavir and/or ritonavir

Adverse events	Number (%) of subjects			
	RTV reduction only (N=18)		Both ATV and RTV reduction (N=21)	
	ATV/RTV 300/100	ATV/RTV* 300/50	ATV/RTV 300/100	ATV/ RTV* 200/50
Hyperbilirubinemia				
- Grade 1	2 (11.11)	9 (50.00)	1 (4.76)	6 (28.57)
- Grade 2	10 (55.56)	3 (16.67)	14 (66.67)	2 (9.52)
- Grade 3	5 (27.78)	1 (5.56)	6 (28.57)	2 (9.52)
Jaundice	5 (27.78)	5 (27.78)	10 (53.33)	4 (19.05)
Loose stool	0 (0)	4 (22.22)	2 (9.52)	6 (28.57)
Rash	2 (11.11)	2 (11.11)	4 (19.05)	0 (0)
Dizziness	0 (0)	0 (0)	2 (9.52)	0 (0)

* (+ LPV 200 mg)

Figure 4.3 illustrated the distribution of total bilirubin concentrations on day 11 of each dosage regimen study, divided into 4 groups based on the different doses of atazanavir and/or ritonavir which the subjects consumed. Total bilirubin concentrations were higher in subjects taking standard dose of ATV/RTV (300/100 mg) than subjects taking the reduced dose of atazanavir and/or ritonavir (300/50 or 200/50 mg).

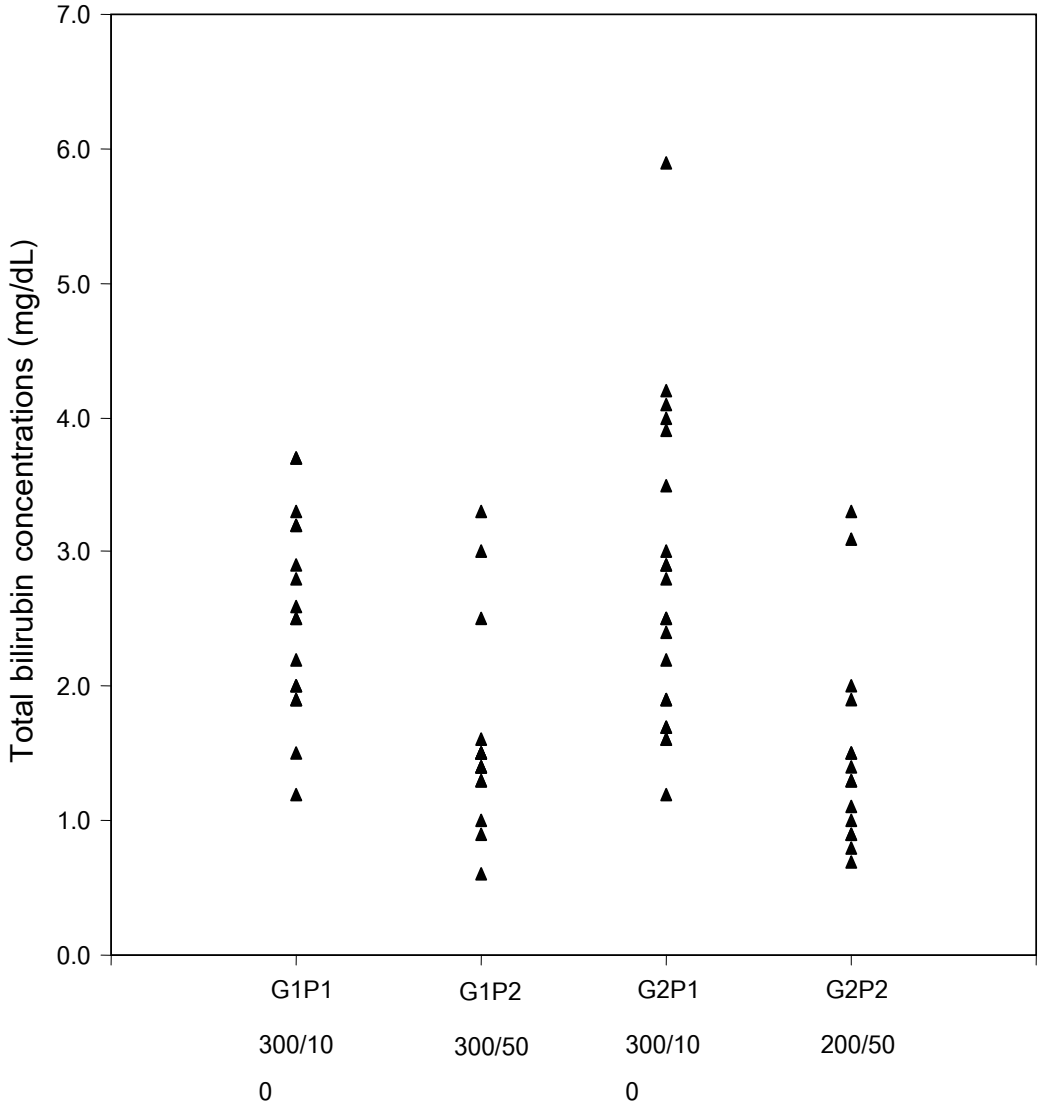

Figure 4.3: The distribution of total bilirubin concentrations in subjects receiving different doses of atazanavir and/or ritonavir

Comparison of the mean total bilirubin concentrations classified based on the therapeutic level of atazanavir C_{min} for subjects receiving different doses of atazanavir and/or ritonavir were shown in Table 4.11 and 4.12. Atazanavir and ritonavir concentrations were completely obtained from 16 and 15 subjects in group 1 (Reduction RTV only) and group 2 (Both ATV and RTV reduction), respectively. Subjects whose ATV C_{min} levels were above the therapeutic range (>0.85 mg/L) had the mean total bilirubin concentrations which were significantly higher than subjects whose ATV C_{min} levels were within the therapeutic range (0.15-0.85 mg/L) and/or subtherapeutic range (<0.15 mg/L). Significantly higher mean total bilirubin concentration was observed in patient with higher ATV C_{min}.

The reduction of atazanavir and/or ritonavir dose resulted in the lower of the mean total bilirubin level. Comparison between ATV/RTV 300/100 mg and 300/50 mg in group 1 showed statistically significant difference in the mean of total bilirubin (P=0.002). There was approximately 38% decrease in the mean total bilirubin when the dose of ritonavir was reduced from 100 mg to 50 mg combined with 300 mg of atazanavir. Similarly, the mean total bilirubin also showed significant difference between subjects receiving ATV/RTV 300/100 mg and 200/50 mg in group 2 (P=0.000). There was approximately 46% decrease in the mean total bilirubin when the dose of atazanavir was reduced from 300 mg to 200 mg and the dose of ritonavir was reduced from 100 mg to 50 mg.

Table 4.11: Comparison of the mean total bilirubin concentrations classified according to the ATV C_{min} therapeutic level of subject receiving the standard dose and reduced dose of atazanavir and/or ritonavir

Atazanavir C_{min} (mg/L)	Mean ± SD (range) of total bilirubin concentrations (mg/dL)			
	RTV reduction only (N=16)		Both ATV and RTV reduction (N=15)	
	ATV/RTV 300/100	ATV/RTV* 300/50	ATV/RTV 300/100	ATV/ RTV* 200/50
< 0.15	-	1.06 ± 0.35 (0.60 - 1.50)		1.06 ± 0.29 (0.70 - 1.40)
0.15 - 0.85	2.42 ± 0.72 (1.20 - 3.70)	1.66 ± 0.62 (1.30 - 3.30)	2.38 ± 0.88 (1.20 - 4.20)	1.74 ± 0.86 (0.80 - 3.30)
>0.85	3.27 ± 0.45 (2.80 -3.70)	2.75 ± 0.35 (2.50 - 3.00)	3.58 ± 0.70 (2.40 - 4.10)	-
Average	2.58 ± 0.74 (1.20 - 3.70)	1.61 ± 0.72 (0.60 - 3.30)	2.78 ± 0.99 (1.20 - 4.20)	1.51 ± 0.78 (0.70 - 3.30)
P-value	0.002		0.000	

* (+ LPV 200 mg)

As shown in Table 4.12, subjects administered with either ATV/RTV 300/50 or ATV/RTV 200/50, the total bilirubin on day 11 after drugs administration showed no statistically significant difference.

Table 4.12: Comparison of the mean total bilirubin concentrations classified according to the ATV C_{min} therapeutic level of subject receiving different doses of atazanavir along with 50 mg of ritonavir

Atazanavir C_{min} (mg/L)	Mean ± SD (range) of total bilirubin concentrations (mg/dL)			
	RTV reduction only (N=16)	Both ATV and RTV reduction (N=15)	RTV reduction only (N=16)	Both ATV and RTV reduction (N=15)
	ATV/RTV 300/100 (N=16)	ATV/RTV 300/100 (N=15)	ATV/RTV* 300/50 (N=16)	ATV/ RTV* 200/50 (N=15)
< 0.15	-		1.06 ± 0.35 (0.60 - 1.50)	1.06 ± 0.29 (0.70 - 1.40)
0.15 - 0.85	2.42 ± 0.72 (1.20 - 3.70)	2.38 ± 0.88 (1.20 - 4.20)	1.66 ± 0.62 (1.30 - 3.30)	1.74 ± 0.86 (0.80 - 3.30)
>0.85	3.27 ± 0.45 (2.80 -3.70)	3.58 ± 0.70 (2.40 - 4.10)	2.75 ± 0.35 (2.50 - 3.00)	-
Average	2.58 ± 0.74 (1.20 - 3.70)	2.78 ± 0.99 (1.20 - 4.20)	1.61 ± 0.72 (0.60 - 3.30)	1.51 ± 0.78 (0.70 - 3.30)
P-value	0.518		0.734	

* (+ LPV 200 mg)

CHAPTER V

DISCUSSION

Atazanavir is the first once daily protease inhibitor approved in 2003 by the US FDA to be used in adult HIV-infected patients in combination with other antiretroviral drugs. Atazanavir has shown high efficacy and safety and indicated for both treatment-naïve and treatment-experienced patients compared with other protease inhibitors. The advantages of atazanavir over other PIs are its excellent oral bioavailability, once-daily dosing, low capsule burden, favorable effect on lipid profiles and a relatively favorable resistance profile.[7,32] The recommended dosage of atazanavir is 300 mg coadministred with 100 mg of ritonavir once daily or 400 mg without ritonavir once daily taken with food. Several clinical trials in antiretroviral therapy (ART)-naive patients who received dual-NRTI background therapy indicated that boosted atazanavir was noninferior to unboosted atazanavir or lopinavir/ritonavir or fosamprenavir plus ritonavir.[13-5] In other study, ritonavir-boosted ATV is associated with greater virologic response and fewer virological failure compared to non-boosted atazanavir without greater risk of adverse events except elevated bilirubin.[16,34] The use of unboosted atazanavir in treatment-experienced patients is not recommended.

Ritonavir is formulated in 100 mg capsule and has been used for boosting at dose of 100 mg either once daily or twice daily with protease inhibitors such as fosamprenavir, atazanavir, darunavir, indinavir, lopinavir, and saquinavir. Ritonavir is known to elevate lipid profiles even at doses of 100-200 mg daily. Reducing the boosted dose of ritonavir could improve tolerability, lower pill burden and lower costs. Many protease inhibitors were boosted equally well with lower (50-100 mg) versus higher doses of ritonavir.[44,53] Two clinical trials have evaluated 50 mg doses of ritonavir in liquid formulation and reported that 50 mg doses were sufficient to boost either

saquinavir or fosamprenavir.[44,54] Recently in 2011, two clinical trials have assessed the pharmacokinetics of atazanavir and darunavir boosted with 50 mg and 100 mg ritonavir in healthy volunteers. These studies presented that the 50 mg dose and 100 mg dose of ritonavir led to bioequivalent AUC and C_{max} levels of atazanavir and darunavir while there were slightly lower C_{min} levels of these 2 protease inhibitors when boosted with the 50 mg dose of ritonavir.[55] However, data on atazanavir boosted with ritonavir at dose of 50 mg in Thai were not conclusive.

5.1 Demographic data

This study is the first to investigate the pharmacokinetic parameters of standard dose and reduced doses of ritonavir or both ritonavir and atazanavir in Thai healthy volunteers. We compared the pharmacokinetics of atazanavir and bilirubin levels of after taking standard dose and reduced dose of ritonavir or reduced doses of both atazanavir and ritonavir in Thai healthy volunteers. There were no statistically significant differences in baseline demographic data of the enrolled subjects in both groups.

5.2 Pharmacokinetic (PK) parameters of atazanavir

The AUC_{0-24}, C_{max}, C_{min} of atazanavir were lower than those previously reported for Thai HIV-infected patients.[29] This result was contrast to the comparison studies between Agarwala S and colleagues[33,41] and Taburet A and colleagues[6,7,33,56] which demonstrated higher AUC_{0-24}, C_{max} and C_{min} of atazanavir in Caucasian healthy volunteers than in Caucasian HIV-infected patients. However, the result from our study was in consistent with comparison of the results obtained from Agarwala S and colleagues[33,41] and Hill A and colleagues[55] which demonstrated the higher atazanavir of AUC_{0-24}, C_{max} and C_{min} in Caucasian HIV-infected patients than Caucasian healthy volunteers. As the results from several studies summarized atazanavir of AUC_{0-24}, C_{max}

and C_{min} which has the highest value in Thai HIV-infected patient, Caucasian HIV-infected patient or Caucasian healthy volunteers and Thai healthy volunteer, respectively. These results of analysis agreed with numerous studies about pharmacokinetics of indinavir[57-60] and saquinavir[54,61-63] boosted with ritonavir, which demonstrated the indinavir and saquinavir pharmacokinetic parameters in Thai HIV-infected patients higher than Caucasian HIV-infected patients. The reason for this difference pharmacokinetics between Thai and Caucasian populations may be body weight, food intake, racial, body composition, and genetic factor. In this study, the mean (median) of body weight of Thais was 61 (59) kg as compared to 65-85 kg for Caucasians in several studies.[30,39,64] Stage of HIV infection may alter Phase I and Phase II drug metabolizing enzyme activity that a consequence of immune activation and cytokine exposure.[65] When atazanavir was used in HIV-infected patients, the blood levels were reported to be higher than those obtained in healthy volunteers which might due in part to the overall lower CYP3A activity (50%) and P-glycoprotein (22-30%) in HIV-infected patients.[66]

Comparison PK parameters between ATV/RTV 300/100 and 300/50

When ritonavir dose alone was reduced from 100 mg to 50 mg (ATV/RTV 300/50), the AUC_{0-24}, C_{max}, C_{min} of atazanavir were statistically significantly lower than those obtained after administered with standard dose (ATV/RTV 300/100) while the CL/F of atazanavir was significant increased. This might indicate that the reduced dosage of ritonavir (50 mg) was not enough to inhibit the enzyme activity which took part in metabolizing atazanavir (300 mg) to its maximum inhibitory effect as compared to that obtained after the standard dose of ritonavir (100 mg). Therefore, the boosting effect was reduced and in turns the AUC_{0-24}, C_{max}, C_{min} of atazanavir were decreased 36%, 30% and 53%, respectively when compared with the standard dose.

Comparison PK parameters between ATV/RTV 300/100 and 200/50

When both atazanavir and ritonavir doses were reduced (ATV/RTV 200/50), the AUC_{0-24}, C_{max}, C_{min} of atazanavir were significantly lower than those obtained after administered with standard dose (ATV/RTV 300/100) while the CL/F of atazanavir was not significantly increased. This might indicate that the 50 mg dosage of ritonavir could inhibit the enzyme activity which metabolized 200 mg of atazanavir at the same level that 100 mg dosage of ritonavir could inhibit the enzyme activity for metabolizing 300 mg of atazanavir. The AUC_{0-24}, C_{max}, C_{min} of atazanavir was decreased 40%, 39% and 51%, respectively when compared with the standard dose simply due to the reduced dosage of atazanavir from 300 to 200 mg.

Comparison PK parameters between ATV/RTV 300/50 and 200/50

The AUC_{0-24}, C_{max}, C_{min} of the subjects taking ATV/RTV 300/50 or 200/50 was not significantly different. Since the CL/F of the subjects who were taking ATV/RTV 300/50 was increased by approximately 50% even though the dosage of atazanavir was remained at 300 mg as the standard dose of atazanavir. While the subjects who were taking ATV/RTV 200/50, even though the CL/F of atazanavir was nearly the same as that obtained after administered with standard dose of atazanavir. The dose of atazanavir was decreased from 300 mg to 200 mg, the AUC_{0-24}, C_{max}, C_{min} was reduced accordingly.

5.3 Therapeutic optimum trough plasma concentration of atazanavir

The results of atazanavir C_{min} level were classified into therapeutic target ranges that has been recommended between 0.15-0.85 mg/L.[1,7,20,67] The study of Gonzalez de Requena D and colleagues, virological responses according to atazanavir C_{min} concentrations were as follows: 58.3% when < 0.15 mg/L, 75% when values between

0.15 and 0.85 mg/L, and 100% when > 0.85 mg/L.[67] Higher atazanavir C_{min} than therapeutic ranges associated with hyperbilirubinemia. The percentage of C_{min} which were above therapeutic range, within therapeutic range and below therapeutic range for subjects while taking ATV/RTV 300/100, ATV/RTV 300/50 and ATV/RTV 200/50 were 0%, 74% and 26%; 31%,56% and 13%; 33%, 67% and 0%, respectively. None of the subjects taking ATV/RTV 300/100 had their C_{min} below the therapeutic range. At the same time, none of the subjects taking 200/50 had their C_{min} above the therapeutic range. The result from different doses of ATV/RTV might be related the vary combination ratio of atazanavir and ritonavir. The ratio of atazanavir and ritonavir dose in subjects received ATV/RTV 300/50 and ATV/RTV 200/50 were 6:1 and 4:1 but different from the ATV/RTV 300/100 that was 3:1. The difference of these ratios might be related to its maximum inhibitory effect of ritonavir which affected to atazanavir concentration. The results from this study indicated that 50 mg of ritonavir might not be enough to boosted 300 mg of atazanavir to its maximum level. Previous study in HIV-infected patients by Avihingsanon A and colleagues[29] reported the AUC_{0-24}, C_{max} and C_{min} after administered ATV/RTV 200/100 to be 41.04 mg.h/L, 4.60 mg/L and 0.70 mg/L, respectively while the CL/F was significantly increased. This ratio of atazanavir and ritonavir was 2:1 that might be appropriate to inhibit enzyme activity and had the proper AUC_{0-24}, C_{max} and C_{min}. The appropriate ratio of atazanavir and ritonavir would give the best pharmacokinetic parameters of atazanavir for Thai populations. However, other protease inhibitors boosted with ritonavir might be the difference ratio of them for providing the appropriate pharmacokinetic parameters. Study from van der Lugt J and colleagues[54] presented 50 mg boosting dose of ritonavir did not affect saquinavir plasma concentrations in Thai HIV-1-infected patients and recommended to use saquinavir 1,500 mg boosting with ritonavir 50 mg instead of ritonavir 100 mg. Unlike the prior study by Boyd M and colleagues[59] presented that adequate exposure to indinavir with a reduced doseindinavir from 800 mg to 400 mg with 100 mg ritonavir twice daily. Hill A and colleagues[31] reviewed data from 17 phamacokinetic trials using different ritonavir doses

with protease inhibitors that found saquinavir was boosted well by lower ritonavir dose but indinavir and lopinavir were boosted with higher ritonavir doses.

5.4 Safety and tolerability

Hyperbilirubinemia was the most common adverse events which related to the subjects receiving atazanavir. The percentage of subjects showed hyperbilirubinemia grade 2 or 3 while taking ATV/RTV 300/100, ATV/RTV 300/50 and ATV/RTV 200/50 were 90%, 22% and 19%, respectively. Several studies had found high correlation between atazanavir C_{min} and serum bilirubin level.[17,19,46,67-70] Subjects with high atazanavir C_{min} showed hyperbilirubinemia. Comparison of the hyperbilirubinemia grade 3 in subjects received ATV/RTV 300/100 between the study of Avihingsanon A and colleagues[29] and this study, found 36% in Thai HIV-infected patients and 28% in Thai healthy volunteers. Because of higher ATV C_{min} in Thai HIV-infected patients than Thai healthy volunteers that related hyperbilirubinemia. Our study showed that subjects with ATV C_{min} higher than 0.85 mg/dL had a threefold higher risk of bilirubin elevation than subjects with ATV C_{min} lower than this level. There was significantly decrease in the mean total bilirubin concentrations after reduction atazanavir with ritonavir once daily from standard dose (300/100 mg) to reduced dose (300/50 or 200/50 mg). This results is consistent with previous study of Colombo S, et al.[20] and Gonzalez de Requena D, et al.[67] However, we observed that 6% 65% and 16% of subjects presented with bilirubin elevation when their ATV C_{min} levels were in lower, within and higher therapeutic ranges. As a result, it might be the weak direct correlation between ATV C_{min} and bilirubin concentration that is in agreement with previous observations.[67] Because of the bilirubin elevation were influenced of the genetic polymorphisms that recently reported by Rodriguez-Novoa S et al.[69-70] In studies of Rodriguez-Novoa S et al., polymorphisms at MDR 1-3435 significantly influence ATV levels, involving Caucasian patients with CT/TT genotypes, was associated with lower ATV levels. In the same study, researcher found that

atazanavir concentrations directly correlate with bilirubin levels, the severe hyperbilirubinemia was associated with the presence of the UGT1A1-TA7 allele.

Because of a liquid formulation of ritonavir or ritonavir tablet 50 mg was not available yet in Thailand. We used Aluvia® 2 tablets that one tablet containing with 100 mg lopinavir and 25 mg ritonavir instead of 50 mg of ritonavir in this study. Difference dose of ritonavir boosted with atazanavir affected to atazanavir and ritonavir pharmacokinetic parameters. Comparison the pharmacokinetic parameters between subjects receiving ATV/RTV 300/100 mg versus 300/50 mg and 300/100 versus 200/50, the reduction of the ritonavir dose was associated with significant decrease in ATV AUC_{0-24}, C_{max}, C_{min} and C_{avg}. The equal dose of ritonavir combined with atazanavir did not affect to atazanavir, ritonavir and lopinavir pharmacokinetic parameters. Comparison of the pharmacokinetic parameters between subjects received ATV/RTV 300/50 mg and ATV/RTV 200/50 mg, the different dose of atazanavir was not associated significant differences in AUC_{0-24}, C_{max}, C_{min} and C_{avg} of atazanavir, ritonavir and lopinavir. These results are in agreement with several previous studies that atazanavir did not affect to lopinavir and ritonavir pharmacokinetic parameters.[71-73] But this finding disagreement with previous studies that showed decrease in lopinavir concentrations,[74] whereas in the study by Ribera E and colleagues found that atazanavir increased lopinavir and ritonavir.[75] Because of the patients received ATV 300 mg once daily combined with LPV/RTV 400/100 mg twice daily that was the different dose from our study. On the other hands, many studies reported that lopinavir was increased ATV C_{min} with or without reaching statistical significance.[71-75] Increasing of ATV C_{min} may be explained by a greater inhibition of atazanavir metabolism by higher dose of ritonavir 200 mg. In this study, subjects received 200 mg of lopinavir with 50 mg ritonavir in both groups that lopinavir may be increased ATV pharmacokinetic parameters. However, it can't be concluded this effect because the different regimen between standard dose and reduced dose.

CHAPTER VI

CONCLUSION

Conclusion

Of the 39 Thai healthy volunteers were enrolled in this study at Chula Clinical Research Center of The King Chulalongkorn Memorial Hospital and randomized into two groups; 18 subjects in Group 1 (Reduced dose RTV only) and 21 subjects in Group 2 (Both ATV and RTV reduction). Subjects in both groups were received ritonavir-boosted atazanavir in the different dose divided into 2 periods. At period 1, all subjects in both groups were received ATV/RTV 300/100 mg once daily for 11 days. At period 2, all subjects in group 1 were received ATV/RTV 300/50 mg while all subjects in group 2 were received ATV/RTV 200/50 mg on day 12 through 22. Baseline demographic data of the enrolled subjects in both groups had no statistically significant difference ($P>0.05$). Only 31 subjects were completed through this study and remained 16 subjects in group 1 and 15 subjects in group 2.

Reduced dose of ritonavir to 50 mg (ATV/RTV 300/50) might not be able to boost atazanavir to the same pharmacokinetic levels (AUC_{0-24}, C_{max}, C_{min}) as the standard dose (ATV/RTV 300/100) but significant decrease of hyperbilirubinemia. However, reduced dose of rionavir alone (ATV/RTV 300/50) or reduced doses of both atazanavir and ritonavir (ATV/RTV 200/50) resulted in none significant difference of AUC_{0-24}, C_{max}, C_{min}.

The optimum amount of ritonavir-boosted effect depends on the type and amount of the substrate and also depends on the conditions of the subjects (healthy volunteers or HIV-infected patients).

There are some limitations of this study. First, this study concentrated on the steady state pharmacokinetic parameters only and not long-term efficacy and safety of the reduced dose of ritonavir and/or atazanavir. Second, this study had not analyzed genetic polymorphisms which correlated the pharmacokinetic parameters and toxicity of atazanavir. Third, there was difference ritonavir dosage form using in regimen between standard dose and reduced dose. Only subjects in reduced dose of ATV and/or RTV received lopinavir which might be affected atazanavir pharmacokinetic parameters.

This is the first pilot study in Thai healthy volunteers to compare the pharmacokinetic parameters and safety of standard dose and reduced doses of ritonavir or both ritonavir and atazanavir. There was a difference in pharmacokinetic parameters of atazanavir between Thai and Caucasian populations. Several studies summarized Thai HIV-infected patients had higher pharmacokinetic parameters for atazanavir than Caucasian healthy volunteers or HIV-infected patients and Thai healthy volunteers. The coadministration reduced dose of atazanavir or ritonavir for daily dose could lower costs, reduce hyperbilirubinemia, improve patient adherence and complete virological suppression compared with other PIs. Therefore, reduced dose of RTV-boosted ATV is one of the best options plus appropriate backbone medications for HIV-infected Thai and other Asian ethnicities patients. Based on this study, we can not recommend the appropriate dose of ritonavir and/or atazanavir for Thai healthy volunteers. Further studies in Thai HIV-infected patients are required to find out the appropriate doses of ritonavir and/or atazanavir and evaluated long-term efficacy and safety.

REFERENCES

[1] Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. [Online]. 2011. Available from: http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf. [2011, 20th November]

[2] Clumeck, N., Pozniak, A., and Raffi, F. European AIDS Clinical Society (EACS) guidelines for the clinical management and treatment of HIV-infected adults. HIV Med 9,2 (2008): 65-71.

[3] Gazzard, B.G., et al. British HIV Association Guidelines for the treatment of HIV-1-infected adults with antiretroviral therapy 2008. HIV Med 9,8 (2008):563-608.

[4] Hammer, S.M., et al. Antiretroviral treatment of adult HIV-1 infection: 2008 recommendations of the International AIDS Society-USA panel. JAMA 300,5 (2008):555-70.

[5] Powderly, W.G. Limitations of current HIV-1 therapies: opportunities for improvement. JAIDS 33,1 (2003): S7-16.

[6] Croom, K.F., Dhillon, S., and Keam, S.J. Atazanavir: a review of its use in the management of HIV-1 infection. Drugs 69,8 (2009):1107-40.

[7] Rivas, P., Morello, J., Garrido, C., Rodriguez-Novoa, S., and Soriano, V. Role of atazanavir in the treatment of HIV infection. Ther Clin Risk Manag. 5,1 (2009):99-116.

[8] Murphy, R.L. Reviving protease inhibitors: new data and more options. J Acquir Immune Defic Syndr 33,1 (2003): S43-52.

[9] Le Tiec, C., Barrail, A., Goujard, C., and Taburet, A.M. Clinical pharmacokinetics and summary of efficacy and tolerability of atazanavir. Clin Pharmacokinet 44,10 (2005):1035-50.

[10] Gatell, J., et al. Efficacy and safety of atazanavir-based highly active antiretroviral therapy in patients with virologic suppression switched from a stable, boosted or unboosted protease inhibitor treatment regimen: the SWAN Study (AI424-097) 48-week results. Clin Infect Dis 44,11 (2007):1484-92.

[11] Soriano, V., et al. Efficacy and safety of replacing lopinavir with atazanavir in HIV-infected patients with undetectable plasma viraemia: final results of the SLOAT trial. J Antimicrob Chemother 61,1 (2008):200-5.

[12] Mallolas, J., et al. Efficacy and safety of switching from boosted lopinavir to boosted atazanavir in patients with virological suppression receiving a LPV/r-containing HAART: the ATAZIP study. J Acquir Immune Defic Syndr 51,1 (2009):29-36.

[13] Malan, D.R., Krantz, E., David, N., Wirtz, V., Hammond, J., and McGrath, D. Efficacy and safety of atazanavir, with or without ritonavir, as part of once-daily highly active antiretroviral therapy regimens in antiretroviral-naive patients. J Acquir Immune Defic Syndr 47,2 (2008):161-7.

[14] Smith, K.Y., et al. Fosamprenavir or atazanavir once daily boosted with ritonavir 100 mg, plus tenofovir/emtricitabine, for the initial treatment of HIV infection: 48-week results of ALERT. AIDS Res Ther 5 (2008):5.

[15] Molina, J.M., et al. Once-daily atazanavir/ritonavir versus twice-daily lopinavir/ritonavir, each in combination with tenofovir and emtricitabine, for management of antiretroviral-naive HIV-1-infected patients: 48 week efficacy and safety results of the CASTLE study. Lancet 372,9639 (2008):646-55.

[16] Horberg, M., et al. Efficacy and safety of ritonavir-boosted and unboosted atazanavir among antiretroviral-naive patients. HIV Clin Trials 9,6 (2008):367-74.

[17] Torti, C., et al. Hyperbilirubinemia during atazanavir treatment in 2,404 patients in the Italian atazanavir expanded access program and MASTER Cohorts. Infection 37,3 (2009):244-9.

[18] Johnson, M., et al. Atazanavir plus ritonavir or saquinavir and lopinavir/ritonavir in patients experiencing multiple virological failures. AIDS 19,7 (2005): 685-94.

[19] Smith, D.E., Jeganathan S., and Ray J. Atazanavir plasma concentrations vary significantly between patients and correlate with increased serum bilirubin concentrations. HIV Clin Trials 7,1 (2006):34-8.

[20] Colombo, S., et al. Population pharmacokinetics of atazanavir in patients with human immunodeficiency virus infection. Antimicrob Agents Chemother 50,11 (2006):3801-8.

[21] Sanne, I., Piliero P., Squires K., Thiry A., and Schnittman S. Results of a phase 2 clinical trial at 48 weeks (AI424-007): a dose-ranging, safety, and efficacy comparative trial of atazanavir at three doses in combination with didanosine and stavudine in antiretroviral-naive subjects. J Acquir Immune Defic Syndr 32,1 (2003):18-29.

[22] Squires, K., et al. Comparison of once-daily atazanavir with efavirenz, each in combination with fixed-dose zidovudine and lamivudine, as initial therapy for patients infected with HIV. J Acquir Immune Defic Syndr 36,5 (2004):1011-9.

[23] Wood, R., et al. Long-term efficacy and safety of atazanavir with stavudine and lamivudine in patients previously treated with nelfinavir or atazanavir. J Acquir Immune Defic Syndr 36,2 (2004):684-92.

[24] Cohen, C., et al. Comparison of atazanavir with lopinavir/ritonavir in patients with prior protease inhibitor failure: a randomized multinational trial. Curr Med Res Opin 21,10 (2005):1683-92.

[25] Gatell, J., et al. Efficacy and safety of atazanavir-based highly active antiretroviral therapy in patients with virologic suppression switched from a stable, boosted or unboosted protease inhibitor treatment regimen: the SWAN Study (AI424-097) 48-week results. Clin Infect Dis 4,11 (2007):1484-92.

[26] Soriano, V., et al. Efficacy and safety of replacing lopinavir with atazanavir in HIV-infected patients with undetectable plasma viraemia: final results of the SLOAT trial. J Antimicrob Chemother 61,1 (2008):200-5.

[27] Noor, M.A., Flint O.P., Maa J.F., and Parker R.A. Effects of atazanavir/ritonavir and lopinavir/ritonavir on glucose uptake and insulin sensitivity: demonstrable differences in vitro and clinically. AIDS 20,14 (2006):1813-21.

[28] van der Lugt, J., and Avihingsanona A. Clinical pharmacology and pharmacokinetics of antiretrovirals in Asia. Asian Biomedicine 3,1 (2009):53-62.

[29] Avihingsanon, A., et al. A low dose of ritonavir-boosted atazanavir provides adequate pharmacokinetic parameters in HIV-1-infected Thai adults. Clin Pharmacol Ther 85,4 (2009):402-8.

[30] Chetchotisakd, P., and Anunnatsiri S. Low-dose, once-daily atazanavir/ritonavir (200/100): an effective treatment for HIV-infected patients in Thailand. J Acquir Immune Defic Syndr 49,2 (2008):230-1.

[31] Hill, A., van der Lugt, J., Sawyer, W., and Boffito, M. How much ritonavir is needed to boost protease inhibitors? Systematic review of 17 dose-ranging pharmacokinetic trials. AIDS 23,17 (2009):2237-45.

[32] Bentue-Ferrer, D., Arvieux, C., Tribult, O., ruffault, A., and Bellissant, E. Clinical pharmacology, efficacy and safety of atazanavir: a review. Expert Opin Drug Metab Toxicol. 5,11 (2009):1455-68.

[33] Bristol-Myers Squibb Company. Reyataz® (atazanavir sulfate) capsules: US prescribing information [Online]. Available from: http://packageinserts. bms.com/pi/pi_reyataz.pdf. [2010, 29th January]

[34] Malan, D.R., et al. 96-week efficacy and safety of atazanavir, with and without ritonavir, in a HAART regimen in treatment-naive patients. J Int Assoc Physicians AIDS Care. 9,1 (2010):34-42.

[35] Wood, E., Hogg, R.S., Yip, B., Moore, D., Harrigan, P.R., and Montaner, J.S. Superior virological response to boosted protease inhibitor-based highly active antiretroviral therapy in an observational treatment programme. HIV Med 8,2 (2007):80-5.

[36] Horberg, M., et al. Efficacy and safety of ritonavir-boosted and unboosted atazanavir among antiretroviral-naïve patients. HIV Clin Trials 9,6 (2008):367-74.

[37] Goldsmith, D.R., and Perry, C.M. Atazanavir. Drugs 63,16 (2003):1679-93.

[38] Solas, C., et al. Population pharmacokinetics of atazanavir in human immunodeficiency virus-infected patients. Ther Drug Monit 30,6 (2008):670-3.

[39] Dickinson, L., et al. Population pharmacokinetics of ritonavir-boosted atazanavir in HIV-infected patients and healthy volunteers. J Antimicrob Chemother 63,6 (2009):1233-43.

[40] Mathias, A.A., West, S., Hui, J., and Kearney, B.P. Dose-response of ritonavir on hepatic CYP3A activity and elvitegravir oral exposure. Clin Pharmacol Ther 85,1 (January 2009):64-70.

[41] Agarwala, S., Russo, R., Mummaneni, V., Randall, D., Geraldes, M., and O'Mara, E. Steady-state pharmacokinetic (PK) interaction study of atazanavir (ATV) with ritonavir (RTV) in healthy subjects. 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy 2002 Sep 27-30; SanDiego (CA): Abstract No. H-1716.

[42] Hsu, A., et al. Multiple-dose pharmacokinetics of ritonavir in human immunodeficiency virus-infected subjects. Antimicrob Agents Chemother 41,5 (1997):898-905.

[43] O'Maram, E., et al. Steady-state pharmacokinetic interaction study between BMS-232632 and ritonavir in healthy subjects. 8th Conference on Retroviruses and Opportunistic Infections; 2001 Feb 4; Chicago (IL): Abstract No. 740.

[44] Hsu, A., Granneman, G.R., and Bertz, R.J. Ritonavir. Clinical pharmacokinetics and interactions with other anti-HIV agents. Clin Pharmacokinet 35,4 (1998):275-91.

[45] von Hentig, N., et al. The steady-state pharmacokinetics of atazanavir/ritonavir in HIV-1- infected adult outpatients is not affected by gender-related co-factors. J Antimicrob Chemother 62,3 (2008):579-82.

[46] Uglietti, A., Novati, S., Gulminetti, R., and Maserati, R. Correlations between atazanavir Ctrough and hyperbilirubinemia: a case report. J Med Case Reports 3 (2009):9307.

[47] Sungkanuparp, S., et al. Thai national guidelines for antiretroviral therapy in HIV-1 infected adults and adolescents 2010. Asian Biomedicine 4 (2010):515-28.

[48] National Institute of Allergy and Infectious Disease. Division of AIDS table for grading the severity of adult and pediatric adverse events. [Online]. 2004. Available from: http://www.ucdmc.ucdavis.edu/clinicaltrials/documents/DAIDS_AE_GradingTable_FinalDec2004.pdf. [2010, 20th January]

[49] DCTD, NCI, NIH, DHHS. Common Toxicity Criteria (CTC) version 2.0. [Online] 1999. Available from: http://ctep.info.nih.gov/protocolDevelopment/electronic_applications/docs/ctcv20_4-30-992.pdf. [2010, 20th January]

[50] DHHS, NCI, NIH. Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. [Online] Available from: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.02_2009-09-15_QuickReference 5x7.pdf. [2010, 20th January]

[51] Droste, J.A., Verweij-van Wissen, C. P., and Burger, D.M. Simultaneous Determination of the HIV Drugs indinavir, amprenavir, saquinavir, ritonavir, lopinavir, nelfinavir, the nelfinavir hydroxymetabolite M8, and nevirapine in human plasma by reversed-phase high-performance liquid chromatography. Ther Drug Monit 25 (2003): 393-9.

[52] Droste, J.A., Aarnoutse, R.E., Koopmans, P.P., Hekster, Y.A., and Burger D.M. Evaluation of antiretroviral drug measurements by an inter-laboratory quality control program. J Acquir Immune Defic Syndr 32 (2003):287-91.

[53] Cooper, C.L., van Heeswijk, R.P., Gallicano, K., Cameron, D.W. A review of low-dose ritonavir in protease inhibitor combination therapy. Clin Infect Dis 36,12 (2003):1585-92.

[54] van der Lugt, J., et al. Reducing the boosting dose of ritonavir does not affect saquinavir plasma concentrations in HIV-1-infected individuals. AIDS 23 (2009):1176-79.

[55] Hill, A., Khoo, S., Boffito, M., Back, D. Should we switch to a 50-mg boosting dose of ritonavir for selected proteases inhibitors? J Acquir Immune Defic Syndr 58 (2011):e137-8.

[56] Taburet, A., et al. Interactions between atazanavir-ritonavir and tenofovir in heavily pretreated HIV-infected patients. Antimicrob Agents Chemother 48,6 (2004):2091-6.

[57] Saah, A.J., Winchell, G.A., Nessly, M.L., Seniuk, M.A., Rhodes, R.R., and Deutsch P.J. Pharmacokinetic profile and tolerability of indinavir-ritonavir combinations in healthy volunteers. Antimicrob Agents Chemother 45,10 (2001):2710-5.

[58] Hsu, A., et al. Pharmacokinetic interaction between ritonavir and indinavir in healthy volunteers. Antimicrob Agents Chemother 42,11 (1998):2784-91.

[59] Boyd, M., et al. Pharmacokinetics of reduced-dose indinavir/ritonavir 400/100 mg twice daily in HIV-1-infected Thai patients. Antiviral Therapy 10 (2005):301-7.

[60] van Heeswijk, R., et al. The steady-state plasma pharmacokinetics of indinavir alone and in combination with a low dose of ritonavir in twice daily dosing regimens in HIV-1-infected individuals. AIDS 13 (1999):F95-9.

[61] Autar, R.S., et al. Interindividual variability of once-daily ritonavir boosted saquinavir pharmacokinetics in Thai and UK patients. Journal of Antimicrobial Chemotherapy 56 (2005):908-13.

[62] Kilby, J.M., et al. Safety and pharmacokinetics of once-daily regimens of soft-gel capsule saquinavir plus minidose ritonavir in human immunodeficiency virus-negative adults. Antimicrob Agents Chemother 44,10 (2000):2672-8.

[63] la Porte, C. Saquinavir, the pioneer antiretroviral protease inhibitor. Expert Opin Drug metab Toxicol 5,10 (2009):1313-22.

[64] von Hentig, N., et al. Tenofovir comedication does not impair the steady-state pharmacokinetics of ritonavir-boosted atazanavir in HIV-1-infected adults. Eur J Clin Pharmacol 63,10 (2007):935-40.

[65] Jones, A.E., et al. Variability in drug metabolizing enzyme activity in HIV-infected patients. Eur J Clin Pharmacol 66 (2010):475-85.

[66] Jetter, A., et al. Do activities of cytochrome P450 (CYP)3A, CYP2D6 and P-glycoproetin differ between healthy volunteers and HIV-infected patients? Antiviral Therapy 15 (2010): 975-83.

[67] Gonzalez de Requena, D., Bonora, S., Cavechia, I. Atazanavir Ctrough is associated with efficacy and safety at 24 weeks: Definition of therapeutic range. 6th International Workshop on Clinical Pharmacology of HIV Therapy. April 28–30 2005; Quebec City, Quebec, Canada: Abstract 60.

[68] Molto, J., et al. Monitoring atazanavir concentrations with boosted or unboosted regimens in HIV-infected patients in routine clinical practice. Ther Drug Monit 29,5 (2007):648-51.

[69] Rodriguez Novoa, S., et al. Plasma levels of atazanavir and the risk of hyperbilirubinemia are predicted by the 3435C–>T polymorphism at the multidrug resistance gene 1. Clin Infect Dis 42,2 (2006):291-5.

[70] Rodriguez-Novoa, S., et al. Genetic factors influencing atazanavir plasma concentrations and the risk of severe hyperbilirubinemia. AIDS 21,1 (2007):41-6.

[71] Pham, P.A., et al. Beneficial pharmacokinetic interaction between atazanavir and lopinavir/ritonavir. J AIDS 45,2 (2007):201-5.

[72] Giambenedetto, S., et al. Aiazanavir and lopinavir with ritonavir alone or in combination: analysis of pharmacokinetic interaction and predictors of drug exposure. HIV Medicine 9 (2008):239-45.

[73] von Hentig, N., et al. Decrease of atazanavir and lopinavir plasma concentrations in a boosted double human immunodeficiency virus protease inhibitor salvage regimen. Antimicrob Agents Chemother 52,6 (2008):2273-5.

[74] Colombo, S., et al. Ritonavir-boosted atazanavir-lopinavir combination: a pharmacokinetic interaction study of total, unbound plasma and cellular exposures. Antivir Ther 11,1 (2006):53-62.

[75] Ribera, E., et al. Atazanavir and lopinavir/ritonavir: pharmacokinetics, safety and efficacy of a promising double-boosted protease inhibitor regimen. AIDS 20 (2006):1131-9.

APPENDICES

Appendix A

Screening Number ☐☐☐

Case Report Form

Pharmacokinetic parameters of atazanavir in ritonavir-boosted combination therapy in Thai healthy volunteers: comparison between reduced dose of ritonavir or atazanavir

Clinical Trial Site: Chula Clinical Research Center

Faculty of Medicine,

Chulalongkorn University

Visit 1: Screening Date …./..../…..

Screening ICF signed □ YES □ NO

1. Demographic data

1.1 Gender □ Male □ Female

1.2 Date of birth..……. (dd/mm/yyyy)

1.3 Age …… yrs

1.4 Race …….

1.5 Nationality……..…

1.6 Height …. m

1.7 Weight … kg

1.8 BMI …….(kg/m^2)

1.9 Religious □ Buddhism □ Muslim □ Chris train □ Other …….

1.10 Marital status □ single □ marriage □ divorce

1.11 Occupation ……

1.12 Food Habit □ Veg. □ Non Veg. □ Muslim

1.13 Smoking habit □ No □ Yes □ Frequency …..…….roll(s)/day

1.14 Alcohol consumption □ No □ Yes □ Frequency ……drink/day

1.15 Caffeine consumption □ No □ Yes □ Frequency ……..….cup(s)/day

1.16 Vital signs BP…..…mm Hg Pulse……./min Temp…… ..°C RR …./min

2. History of Present Illness

Medical history: Is there any relevant medical history in the following systems?

Code	System	*Yes	No	Code	System	*Yes	No
2.1	Allergy to any drugs or food			2.10	Cardiovascular		
2.2	Allergy disorder			2.11	Dermatological		
2.3	Drugs within last 1 month			2.12	Musculo-skeletal		
2.4	Asthma			2.13	Haematological		
2.5	Pulmonary tuberculosis			2.14	Neoplasia		
2.6	Diabetes mellitus			2.15	Neurological		
2.7	Hypo/ Hyperthyroidism			2.16	Psychological		
2.8	Gastro-intestinal			2.17	Eyes, ear, nose, throat		
2.9	Hepato-biliary			2.18	Other		

* If **Yes** for any of the above, enter the code for each condition in the boxes below (next page); give further details (including dates) and state if the condition is currently or potentially active. If giving details of surgery please specify the underlying cause. Use a separate line for each condition.

Visit 1: Screening

Code	Details	Currently Active?	
		Yes	No

Recorded by: …….. (Nurse)

3. Physical examination (to be carried out by medical staff only)			
Code	**System**	***Abnormal**	**Normal**
3.1	General Appearance	□	□
3.2	Head and Neck	□	□
3.3	Eyes, ear, nose, throat	□	□
3.4	Heart	□	□
3.5	Chest and Lungs	□	□
3.6	Breast	□	□
3.7	Abdomen	□	□
3.8	Pelvic	□	□
3.9	Neurologic	□	□
3.10	Mental status	□	□
3.11	Skin	□	□
3.12	Musculo-skeletal	□	□
3.13	Extremities and back	□	□

* If **Abnormal** enter the code for each condition in the boxes below and give brief details. Please use a separate line for each condition.

Visit 1: Screening

Code	Details

Recorded by: …….. (Physician)

4. Laboratory test	**Date** …./…../…...	**Result** **Abnormal**	**Normal**
CBC			
- RBC			
- WBC		□	□
- Hemoglobin			
- Hematocrit			
- Platelets			
Renal function			
BUN		□	□
Scr			
Liver function			
Albumin			
AST			
ALT		□	□
Alk. phosphatase			
TB			
DB			
Anti-HIV	□ Negative □ Positive	□	□
Urine Pregnancy Test	□ Negative □ Positive	□	□

Are all final results: □ Normal □ Abnormal not contradict

□ Abnormal contradict study*

*Descriptions:

……...

……..……

…….

Recorded by: …….. (Nurse/Physician)

Visit 1: Screening

5. Inclusion criteria	**Yes**	**No***
5.1 Subject is competent and willing to sign informed consent form.	☐	☐
5.2 Healthy male or non-pregnant, non-lactating females	☐	☐
5.3 Ages between 18 to 60 years old	☐	☐
5.4 Women of childbearing potential must be using an adequate method of contraception to avoid pregnancy throughout the study and for a period of at least 1 month after the study.	☐	☐
5.5 Normal laboratory values on the laboratory evaluations		

* If any inclusion criteria are ticked no then the patient is not eligible for the study.

6. Exclusion criteria	**Yes***	**No**
6.1 History of previous allergy to any of the constituents of the pharmaceuticals administered in this trial.	☐	☐
6.2 Positive blood screen for HIV-1 and/or 2 antibodies	☐	☐
6.3 History/evidence of gastrointestinal disorders, liver disorders, kidney disorder, cardiovascular diseases or other disorders that might interfere with drug absorption, distribution, metabolism or excretion.	☐	☐
6.4 History of exposure to any investigational drug or other drugs that may interfere with the pharmacokinetics of ATV/RTV, within 3 months or two weeks prior to first dose of study drug, respectively	☐	☐
6.5 History of alcoholism (more than 2 years), moderate drinkers (more than 3 drinks per day) [one drink is equal to one unit of alcohol (one glass wine, half pint beer, or one measure of spirit)]	☐	☐
6.6 History of usually smoking (more than 10 cigarettes per day), if moderate smokers (less than 10 cigarettes per day) and must be stop at least a day before the study and until the completion of the second phase	☐	☐

* If any exclusion criteria are ticked yes then the patient is not eligible for the study.

7. Subject is SUITABLE /UNSUITABLE to participate in the study.

☐ SUITABLE (ENROLLMENT TO STUDY PERIOD, SUBJECT ID □-□□)

☐ UNSUITABLE (Please specify

……..……
……..……
……..……

Recorded by: ……. Checked by:…….
(Investigator/ Physician)

Visit 2: Study period I **Subject ID** □-□□

2.1 Starting date on day 1 (ATV/RTV 300/100 mg OD): ……/……../……...

2.2 Visit date 1 (on days 10): ……/……../……...

2.3 Date of reporting to PK clinical facility

Visit	**Date**	**Time in** (hr:min)	**Time out** (hr:min)
Day 1			
Day 2			

2.4 Vital signs and well being status

Time	**SBP/DBP (mm Hg)**	**PR (beats/min)**	**RR (breaths/min)**	**Temperature (°C)**	**Well being**
At the time before Pre-dose					☐ Well ☐ Unwell*
At the time before check out					☐ Well ☐ Unwell*

* If unwell please specify:
……
……
……..…..……

Any Illness since last visit ☐ Yes ☐ No

Any Medication since last visit ☐ Yes ☐ No

If "Yes" please specify**:**
……
……
……
……

2.5 Laboratory tests (Date …….)
Lab Results: ☐ Normal ☐Abnormal

Recorded by: …….. (Nurse/Physician)

Visit 2: Study period I Subject ID □-□□

2.6 Physical examination before check-in (to be carried out by medical staff only)			
Code	**System**	***Abnormal**	**Normal**
2.6.1	General Appearance	□	□
2.6.2	Head and Neck	□	□
2.6.3	Eyes, ear, nose, throat	□	□
2.6.4	Cardiovascular	□	□
2.6.5	Gastrointestinal System	□	□
2.6.6	Skin	□	□
		YES	NO
2.6.7	Any other abnormal findings	□	□

* If **Abnormal** or **Yes** enter the code for each condition in the boxes below and give brief details. Please use a separate line for each condition.

Code	**Details**

Subject is fit for check-in: ☐ Yes ☐ No

Recorded by: ……
(Physician /Doctor /PI)

2.7 Dosing record

Treatment	**Dosing time**	**Pharmacist/Physician**
ATV/RTV 300/100 mg OD + water 250 ml		

Mouth check done ☐ Done ☐ Not done

Recorded by: ……..…….....
(Pharmacist/Physician)

Visit 2: Study period I **Subject ID □-□□**

2.8 1st Full PK study (on days 10): ……/……../……...

Sample Number	Sampling time	Theoretical time (hr:min)	Real time (hr:min)	Remark
1	0 hr	7.00	:	Day 1
Drug administration		8.00	:	
2	2 hr	10.00	:	
3	3 hr	11.00	:	
4	4 hr	12.00	:	
5	8 hr	16.00	:	
6	12 hr	20.00	:	
7	24 hr (Before drug administration)	8.00	:	Day 2
Drug administration		8.00	:	
8	26 hr	10.00	:	

Subject remained in upright posture for 2 hours after dosing ☐ Yes ☐ No

Recorded by: ……..
(Nurse)

Visit 2: Study period I **Subject ID □-□□**

2.9 Type of meal

Type of Meal, (Schedule time), Menu	**Consumption**	**Start Time** (hr: min)					**End Time** (hr: min)				
				:					:		:
				:					:		
				:					:		
				:					:		
				:					:		

Recorded by: ……..
(Nurse)

2.10 Subject is fit for check-in: ☐ Yes ☐ No

2.11 Did any serious adverse event/ adverse event occur during this period of the study? (If Yes fill serious adverse event/ adverse event record form) ☐ Yes ☐ No

* If occurred AE, SAE, ADR please recorded and report to PI

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Recorded by: ……..……..
(Physician/ PI/ Nurse)

Visit 3: Study period II Subject ID □-□□

3.1 Drug regimen on days 12 to 21: □ Group 1 (ATV/RTV 300/50 mg OD)

□ Group 2 (ATV/RTV 200/50 mg OD)

3.2 Visit date 1 (on days 21): ……/……../……...

3.3 Date of reporting to PK clinical facility

Visit	**Date**	**Time in** (hr:min)	**Time out** (hr:min)
Day 1			
Day 2			

3.4 Vital signs and well being status

Time	**SBP/DBP (mm Hg)**	**PR (beats/min)**	**RR (breaths/min)**	**Temperature (°C)**	**Well being**
At the time before Pre-dose					☐ Well ☐ Unwell*
At the time before check out					☐ Well ☐ Unwell*

* If unwell please specify:
……
……

Any Illness since last visit ☐ Yes ☐ No

Any Medication since last visit ☐ Yes ☐ No

If “Yes” please specify**:**
……
……
……

3.5 Laboratory tests (Date …….)

Lab Results: ☐ Normal ☐Abnormal

Recorded by: …….. (Nurse/Physician)

Visit 3: Study period II Subject ID □-□□

3.6 Physical examination before check-in (to be carried out by medical staff only)			
Code	**System**	***Abnormal**	**Normal**
3.6.1	General Appearance	□	□
3.6.2	Head and Neck	□	□
3.6.3	Eyes, ear, nose, throat	□	□
3.6.4	Cardiovascular	□	□
3.6.5	Gastrointestinal System	□	□
3.6.6	Skin	□	□
		YES	NO
3.6.7	Any other abnormal findings	□	□

* If **Abnormal** or **Yes** enter the code for each condition in the boxes below and give brief details. Please use a separate line for each condition.

Code	**Details**

Subject is fit for check-in: ☐ Yes ☐ No

Recorded by: ……..……..

(Physician /Doctor /PI)

3.7 Dosing record

Treatment	**Dosing time**	**Pharmacist/Physician**
□ Group 1 (ATV/RTV 300/50 mg OD) **OR** □ Group 2 (ATV/RTV 200/50 mg OD) + water 250 ml		

Mouth check done ☐ Done ☐ Not done

Recorded by: ……..

(Pharmacist/Physician)

Visit 3: Study period II Subject ID □-□□

3.8 2nd Full PK study (on days 21): ……/……../……...

Sample Number	Sampling time	Theoretical time (hr:min)	Real time (hr:min)	Remark
1	0 hr	7.00	:	Day 1
Drug administration		8.00	:	
2	2 hr	10.00	:	
3	3 hr	11.00	:	
4	4 hr	12.00	:	
5	8 hr	16.00	:	
6	12 hr	20.00	:	
7	24 hr (Before drug administration)	8.00	:	Day 2
Drug administration		8.00	:	
8	26 hr	10.00	:	

Subject remained in upright posture for 2 hours after dosing ☐ Yes ☐ No

Recorded by: ……..……..
(Nurse)

Visit 3: Study period II **Subject ID □-□□**

3.9 Type of meal

Type of Meal, (Schedule time), Menu	**Consumption**	**Start Time** (hr: min)					**End Time** (hr: min)				
				:					:		:
				:					:		
				:					:		
				:					:		
				:					:		

Recorded by: ……..
(Nurse)

3.10 Subject is fit for check-in: ☐ Yes ☐ No

3.11 Did any serious adverse event/ adverse event occur during this period of the study? (If Yes fill serious adverse event/ adverse event record form) ☐ Yes ☐ No

* If occurred AE, SAE, ADR please recorded and report to PI

……
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……
……
……

Recorded by: ……..
(Physician/ PI/ Nurse)

Adverse Events Record Form										**Subject ID □-□□**
Has the patient experienced any Adverse Events since signing the Informed Consent?								Yes, specify below		No
AE no.	**Adverse Event** (diagnosis (if known) or signs/symptoms)	**Start Date** dd/mm/yyyy **and Time** (24 hour clock)	**Stop Date** dd/mm/yyyy **and Time** (24 hour clock)	**Outcome** 1 = Recovered 2 = Recovered with sequelae 3 = Continuing 4 = Patient Died 5 = Change in AE 6 = Unknown	**Severity** 1 = Mild 2 = Moderate 3 = Severe 4 = Potentially life-threatening	**Plausible relationship to Study Drug**	**Action taken with Study Drug** 1 = None 2 = Dose Reduction Temporarily 3 = Dose Reduced 4 = Discontinued Temporarily 5 = Discontinued	**Withdrawn due to AE?**	**Serious AE (SAE)?**	**If SAE does it require immediate reporting? (see Protocol)?**
		/ / :	/ / :			☐ Yes ☐ No		☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
		/ / :	/ / :			☐ Yes ☐ No		☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
		/ / :	/ / :			☐ Yes ☐ No		☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
		/ / :	/ / :			☐ Yes ☐ No		☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No

Visit 4: Follow-up & Off Study

4.1 Follow-up date (on days 28): ……/……../……...
Subject's status: □ Normal
□ Abnormal ……

Date Off Study: ___ ___ / ___ ___ / ___ ___ ___ ___

Date Last Study Medication Taken: ___ ___ / ___ ___ / ___ ___ ___ ___

Reason Off Study

(Please mark only the primary reason. Reasons **other than Completed Study** require explanation next to the response)

☐ Completed study

☐ AE/SAE **(complete AE CRF & SAE form, if applicable)**

……

☐ Lost to follow-up

……

☐ Non-compliant participant

……

☐ Concomitant medication

……

☐ Medical contraindication

……

☐ Withdraw consent

……

☐ Death.

……

☐ Other

……

Signature …… **Date** ……

Appendix B

Validation of analytical method for ATV, RTV and LPV concentration

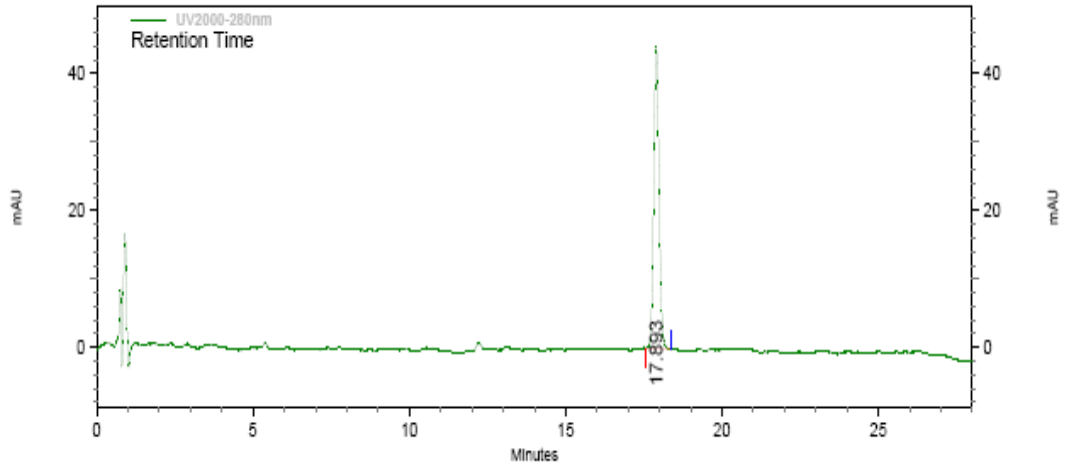

Figure 1: Chromatogram of drug-free-plasma. Retention time (RT) of Internal Standard (IS) = 17.89 minute

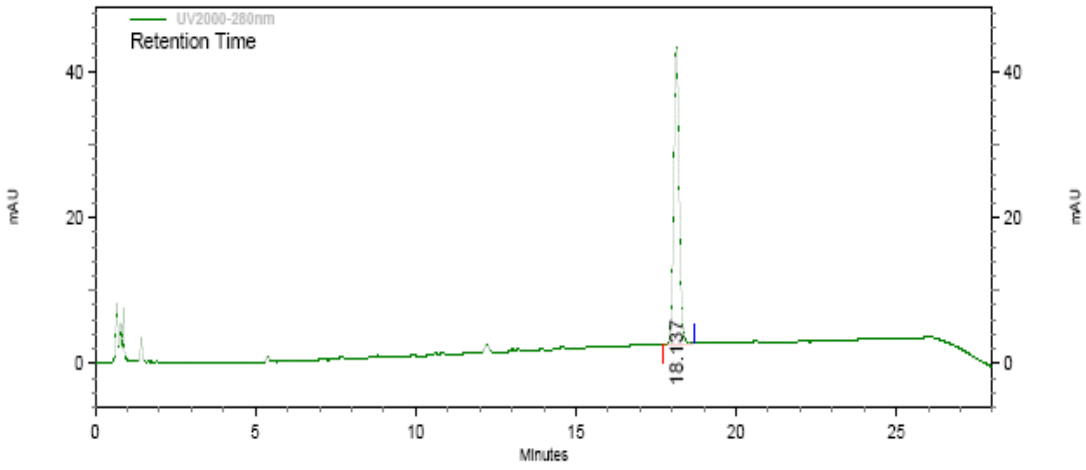

Figure 2: Chromatogram of drug-free-plasma. Retention time (RT) of Internal Standard (IS) = 18.13 minute

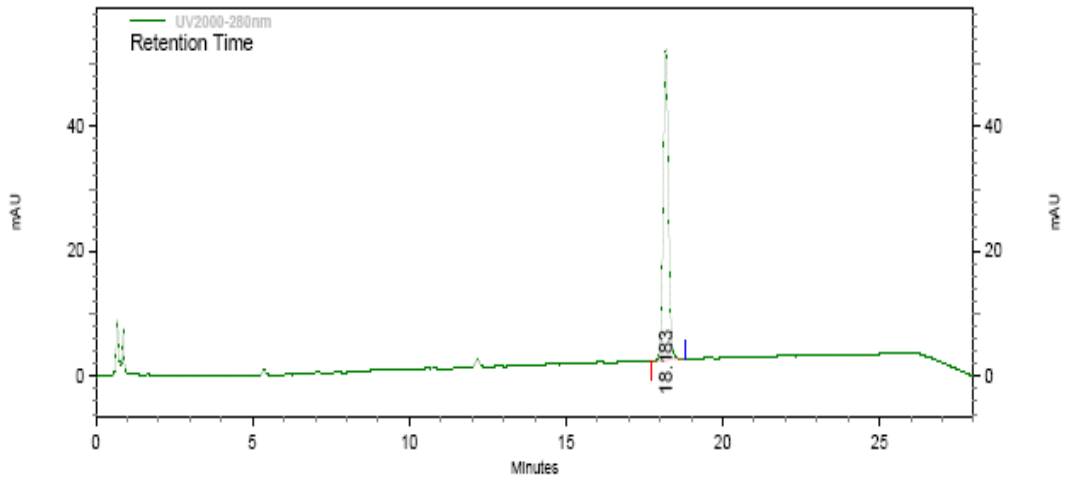

Figure 3: Chromatogram of drug-free-plasma. Retention time (RT) of Internal Standard (IS) = 18.18 minute

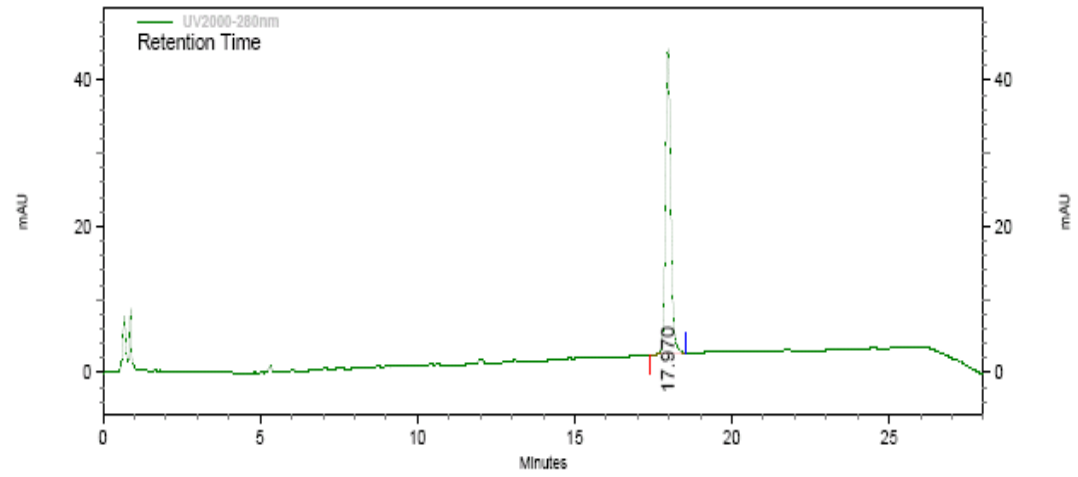

Figure 4: Chromatogram of drug-free-plasma. Retention time (RT) of Internal Standard (IS) = 17.97 minute

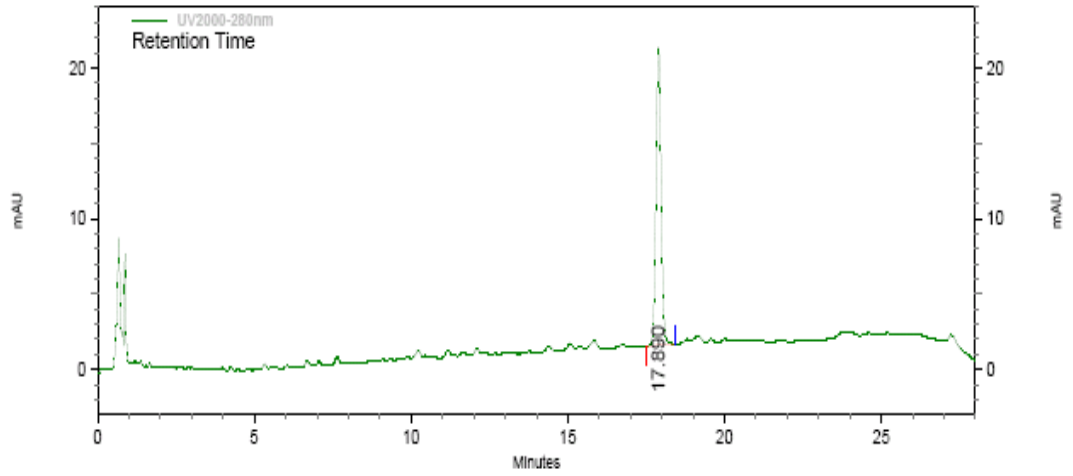

Figure 5: Chromatogram of drug-free-plasma. Retention time (RT) of Internal Standard (IS) = 17.89 minute

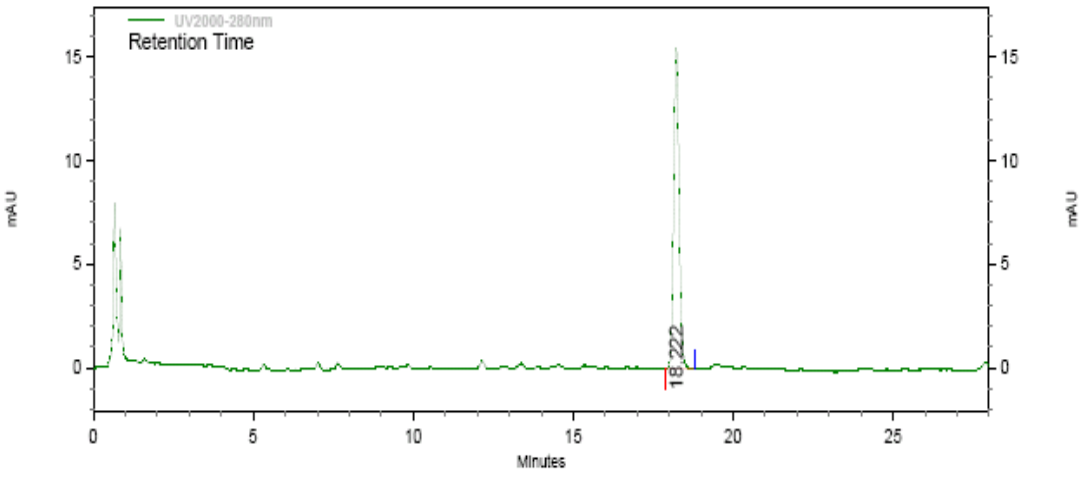

Figure 6: Chromatogram of drug-free-plasma. Retention time (RT) of Internal Standard (IS) = 18.22 minute

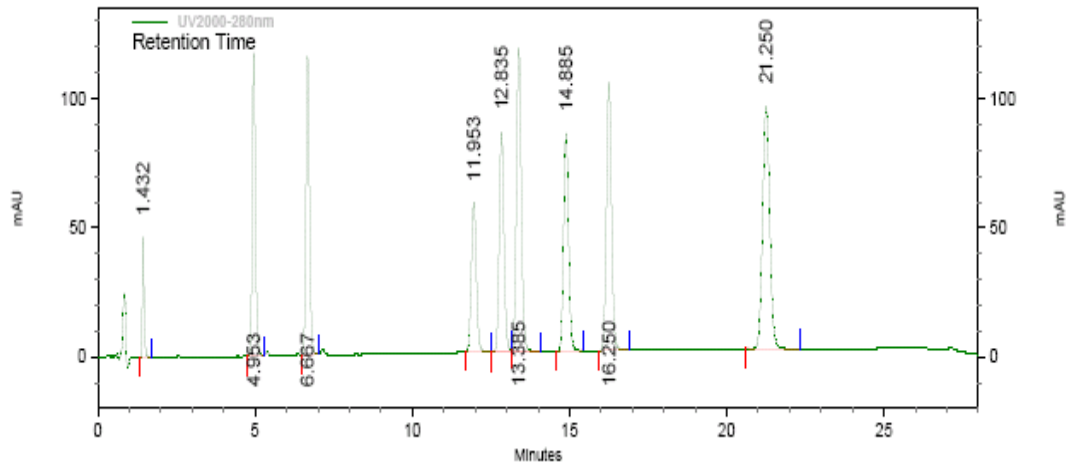

Figure 7: Chromatogram of test mixtures; spiked plasma of NVP, IDV, DRV , NFV-M8, ATV (12.83 minutes), SQV, RTV (14.88 minutes) , LPV (16.25 minutes) and NFV

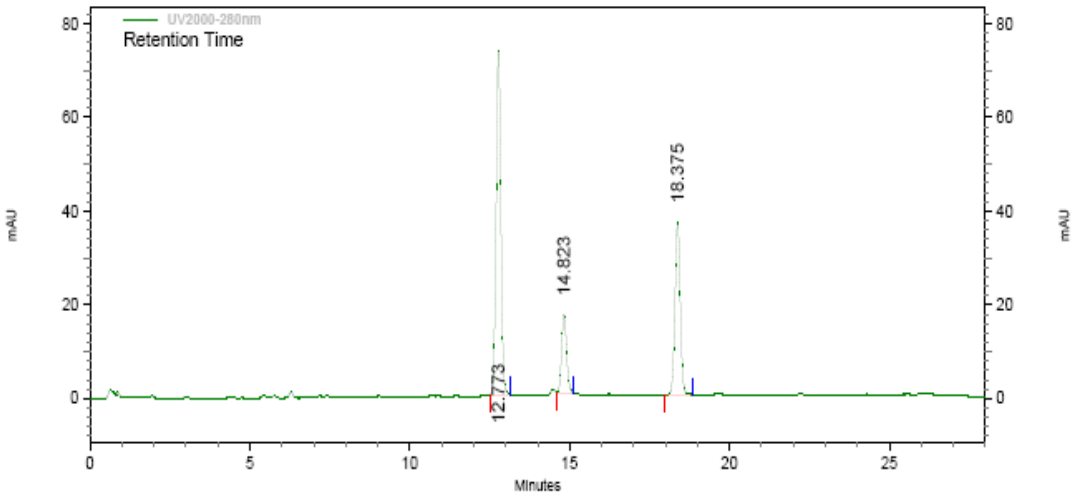

Figure 8: Chromatogram of patient samples, RT of ATV, RTV and IS 12.70, 14.82 and 18.37 minutes, respectively

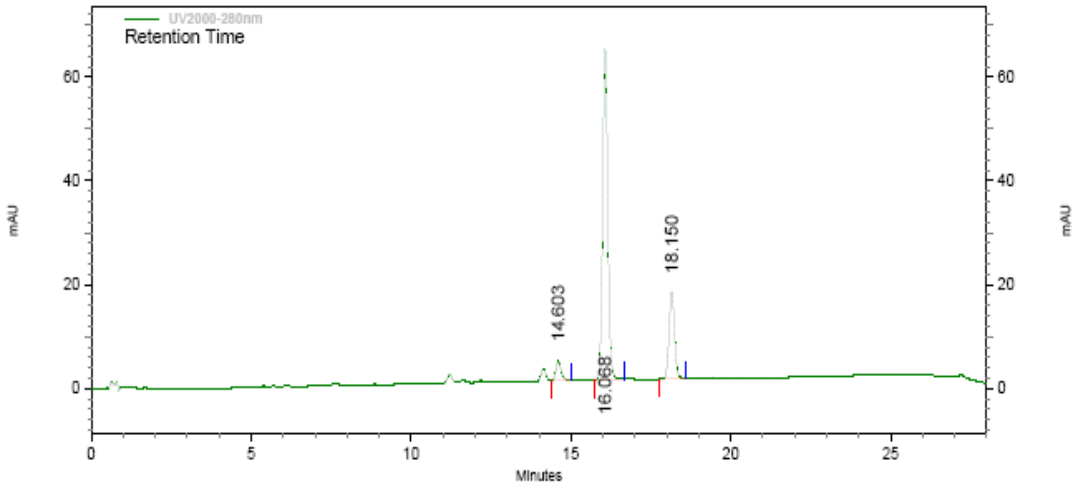

Figure 9: Chromatogram of patient samples, RT of RTV, LPV and IS 14.60, 16.06 and 18.15 minutes, respectively

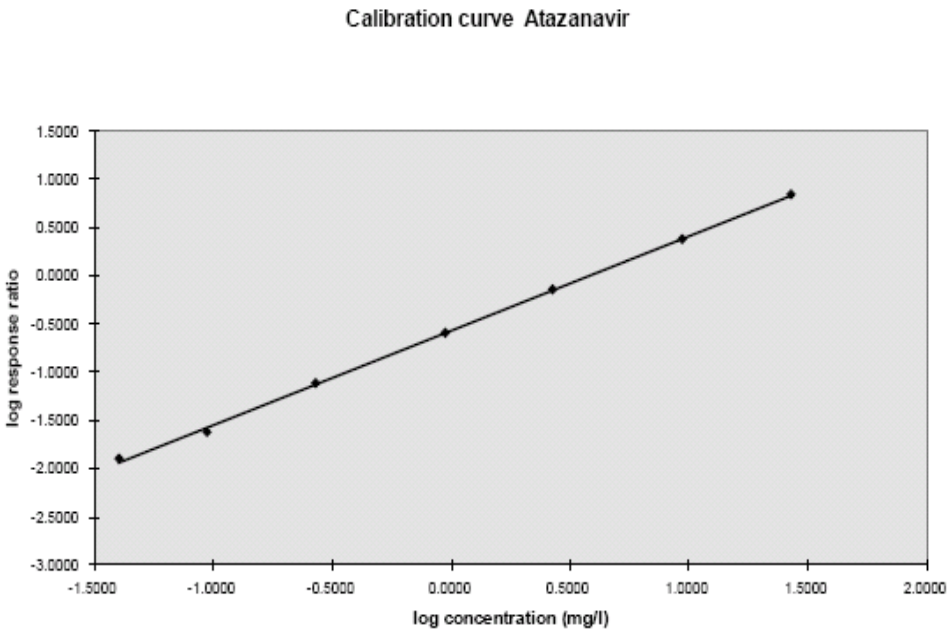

Figure 10: Calibration curve and linearity of atazanavir

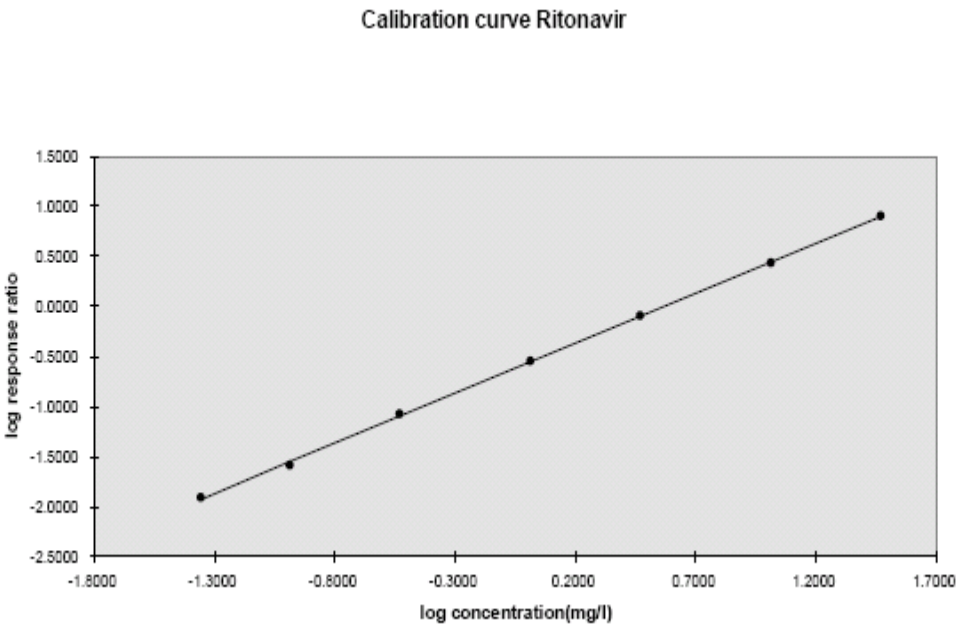

Figure 11: Calibration curve and linearity of ritonavir

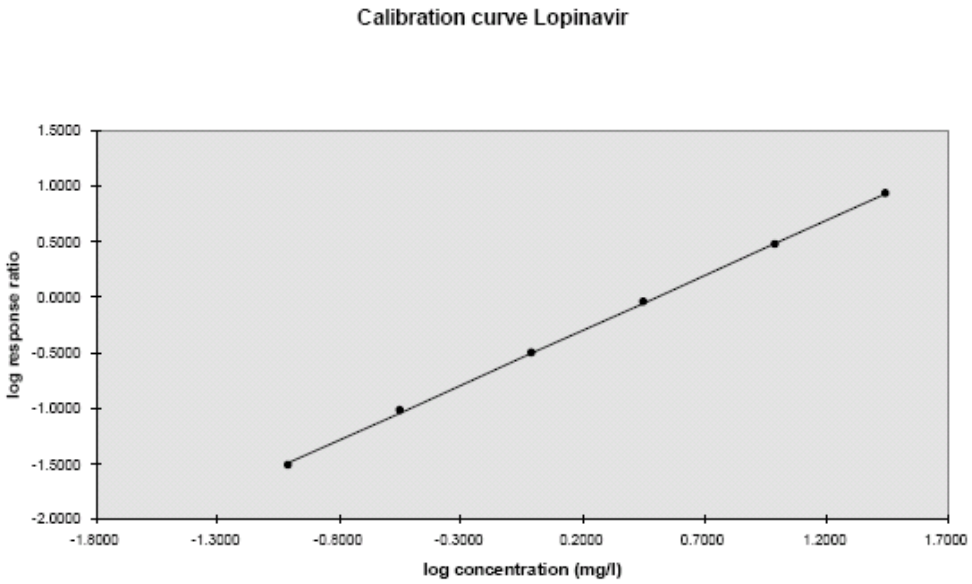

Figure 12: Calibration curve and linearity of lopinavirritonavir

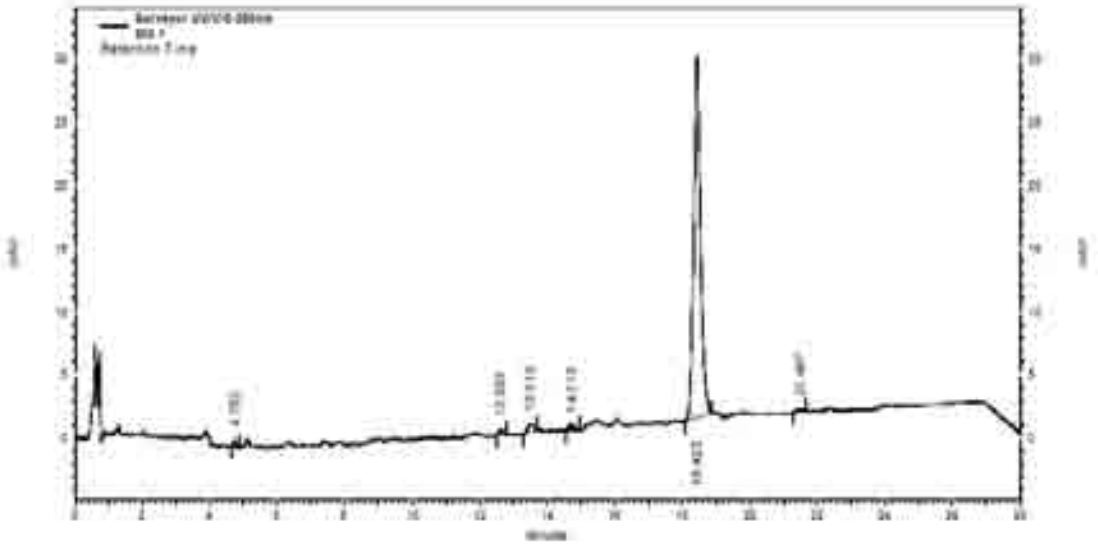

Figure 13: Chromatogram of spiked plasma of ATV, RTV (LLOQ) and IS [A86093.0 (Abbott)] at 0.045 mg/L (ATV and RTV) and 0.05 mg/ml (IS). RT of ATV, RTV and IS is 12.63, 14.71 and 18.42 minute, respectively.

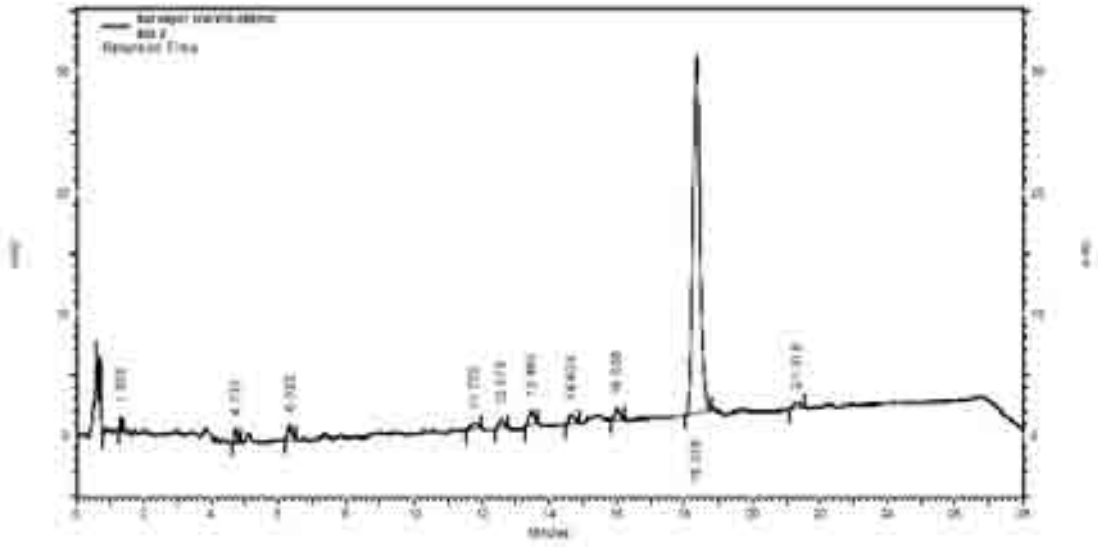

Figure 14: Chromatogram of spiked plasma of LPV (LLOQ) and IS [A86093.0 (Abbott)] at 0.105 mg/L (ATV and RTV) and 0.05 mg/ml (IS). RT of , LPV and IS 16.00 and 18.33 minute, respectively.

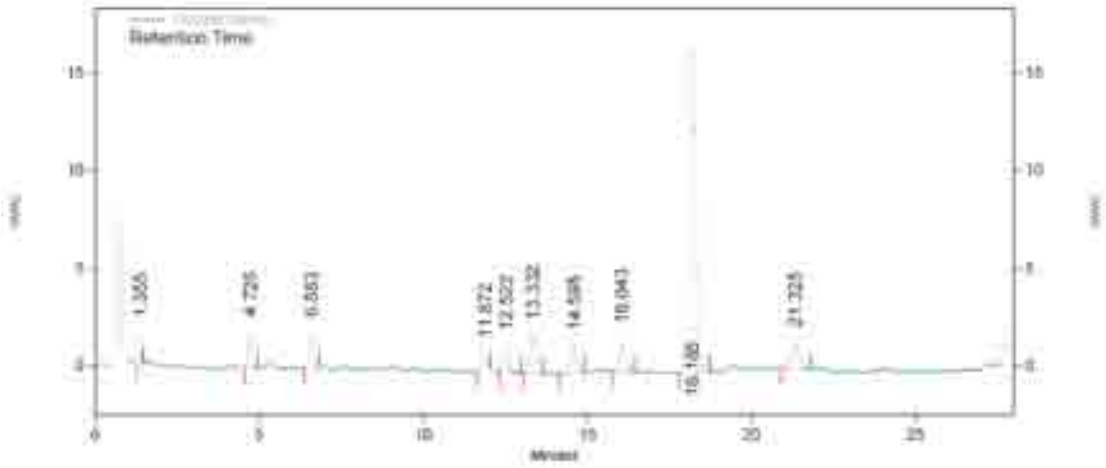

Figure 15: Chromatogram of spiked plasma ATV, RTV, LPV (QC L) and IS at 0.15 mg/L (ATV and RTV) and 0.05 mg/ml (IS). RT of ATV, RTV, LPV and IS are 12.52, 14.59, 16.04 and 18.18 minutes, respectively.

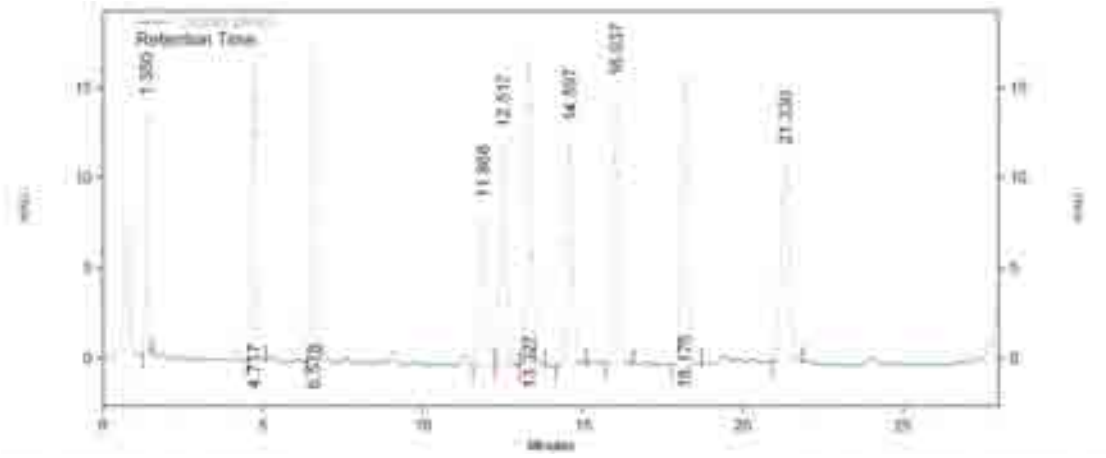

Figure 16: Chromatogram of spiked plasma ATV, RTV, LPV (QC M) and IS at 1.5 mg/L (ATV and RTV) and 0.05 mg/ml (IS). RT of ATV, RTV, LPV and IS are 12.51, 14.59, 16.03 and 18.17 minutes, respectively.

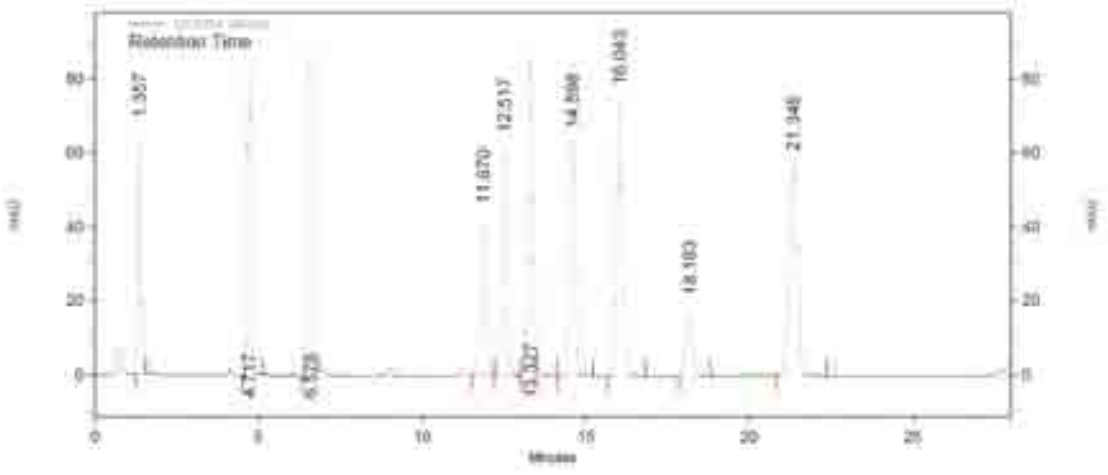

Figure 17: Chromatogram of spiked plasma ATV, RTV, LPV (QC H) and IS at 7.5 mg/L (ATV and RTV) and 0.05 mg/ml (IS). RT of ATV, RTV, LPV and IS are 612.51, 14.59, 16.04 and 18.18 minutes, respectively.

VITA

Mr. Chankit Puttilerpong was born on the ninth of March in 1975 at Hua Chiew Hospital, bangkok. He graduated with Bachelor degree in Pharmaceutical Sciences (first class honors) in 1996 and Master degree in Hospiatal and Clinical Pharmacy in 2000 from Faculty of Pharmaceutical Sciences, Chulalongkorn University. From 1996 to 2007, he worked a pharmacist at Prachuapkhirikhan Hospital, Prachuapkhirikhan, Thailand. His current position is a lecturer in Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, and Chulalongkorn University.