High Performance Liquid Chromatographic Assay for the Determination of Protease Inhibitors (PIs) and Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs) in Human Plasma

Kantima Sangsiriwut, M.Sc., Thanomsak Anekthananon, M.D., Winai Ratanasuwan, M.D., Surapol Suwanagool, M.D., Teera Kolladarungkri, M.D.

Department of Preventive and Social Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

ABSTRACT

Objective: To develop and validate a high performance liquid chromatography (HPLC) method for simultaneous quantitative determination of five HIV protease inhibitors (PIs): indinavir (IDV), lopinavir (LPV), nelfinavir (NFV), ritonavir (RTV), saquinavir (SQV), and two non-nucleoside reverse transcriptase inhibitors (NNRTIs): nevirapine (NVP), and efavirenz (EFV) in human plasma.
Methods: A sample of 200 µL of plasma and an internal standard were extracted with tert-butyl methyl ether. The compounds were separated on a reversed-phase C18 column with gradient phase of 25 mM phosphate buffer (pH 4.9) and acetonitrile. The limit of quantation, accuracy, precision, specificity, stability and recovery were tested.
Results: The lower limit of quantitation for all drugs was 75 ng/mL. The standard curve was linear in the range of 75 ng/mL to 20,000 ng/mL. Intra-day and inter-day variability ranged from 0.1% to 2.4% and 0.3% to 4.1%, respectively. Accuracy ranged from 98.4%-102.4% for three quality controls (75, 100, and 1,000 ng/mL) for all drugs measured. The extraction recovery ranged from 98.7%-101.3%.
Conclusion: This method provides a simple, accurate, and precise method for monitoring of plasma concentrations of five PIs and two NNRTIs in the case of weak economy and out of date instrumental limitations.

Keywords: HPLC, HIV, non-nucleoside reverse transcriptase inhibitors, protease inhibitors

Siriraj Med J 2012;64: 1-6
E-journal: http://www.sirirajmedj.com

INTRODUCTION

The use of highly active antiretroviral therapy (HAART) has shown efficacy in controlling viral replication in HIV-1 infected patients since the 2000's. HAART reduces plasma HIV-RNA to below detectable limits in most cases. However, some patients do not have a sustainable antiviral response due to the development of drug resistance. Several studies have demonstrated a relationship between protease inhibitors (PIs) and the non-nucleoside reverse transcriptase inhibitors (NNRTIs) plasma concentrations and antiviral efficacy. However, ninety percent of HIV positive patients use the combination of these drugs. In the recent experiences of treatment with the NNRTIs and the PIs around the world, these drugs are metabolized by the cytochrome P450 system, mainly by CYP3A4 and CYP2D6. It was found that there was a high interaction when used in combination with other agents. For example, rifampicin and rifabutin reduce the blood levels of the protease inhibitors and reduce their antiviral efficacy.[1-2] Therefore, the measurement of plasma drug concentration is necessary for this situation. Therapeutic drug monitoring (TDM) of antiretroviral drugs has been highly used in experimental dosage evaluation during the last 10 years.[3-4] It is used to determine the best dosage regimen to reduce viral resistance with low plasma drug concentration and to limit the toxicity of the high plasma drug concentration. Quantification of antiretroviral drugs in human plasma is important to manage drug interactions and to evaluate the relationship between plasma concentrations and treatment response in future clinical research.

Correspondence to: Teera Kolladarungkri
E-mail: sitkd@mahidol.ac.th
Received 26 April 2011
Revised 11 August 2011
Accepted 22 August 2011

In recent years, several HPLC methods for simultaneous determination of antiretroviral drugs in plasma have been published.[5-8] However, a simplified technique is necessary for weak economy and out of date instrumental countries, because the general reported techniques suggest a solid-phase extraction and an ultraviolet detection at multi-wavelengths, all of which are actually not available in many routine laboratories. In this study, we describe a novel HPLC method with ultraviolet (UV) detection for quantifying the PIs and NNRTIs at the same time. The process of the plasma preparation uses a rapid liquid-liquid ether extraction and chromatography conditions which are more simple for detection and only require a single wavelength, so they are cheaper. Excellently, this method also requires less volume of plasma samples than the methods in recent literature.[5-8] The results will mainly be applied for clinical dosage evaluation research in both adult and pediatric populations in the near future.

MATERIALS AND METHODS

1. Chemicals

Powders of nevirapine (NVP), indinavir (IDV), lopinavir (LPV), nelfinavir (NFV), saquinavir (SQV), and ritonavir (RTV) were obtained from Thai Governmental Pharmaceutical Organization. Efavirenz powder was obtained from MERCK (Rahway, NJ, USA). Internal standard, carbamazepine (IS) was purchased from Sigma (St. Louis, MO, USA). HPLC grade super gradient acetonitrile, methanol and tert-butyl methyl ether were purchased from LABSCAN (Bangkok, Thailand). NH_4OH and hexane were purchased from Merck.

2. Instruments and HPLC conditions

The HPLC system consisted of a Waters (Milford, MA, USA) Alliance liquid chromatography system, which included a Model 2695 Separate Module and a Model 2487 Dual Wavelength UV detector. The analytical column was a Symmetry C18 analytical column, 5 µm (150 x 4.6 mm I.D.) and protected with a sentry guard column C18, which were both from Waters (Milford, MA, USA). Separation was performed at 35°C using a column heater (temperature control system, Waters). The mobile phase consisted of 25 mM potassium phosphate buffer (pH 4.9) and acetonitrile. The composition of mobile phase was 53% 25 mM potassium phosphate buffer (pH 4.9) and 48% acetonitrile (vol:vol) from 0 to 10 minutes followed by switch to 48% 25 mM potassium phosphate buffer (pH 4.9) and 53% acetonitrile in a minute and this composition was constant from 11 to 36 min. (48% + 53% = 101%.)Then the column was re-equilibrated for 5 min. with the initial condition before the next injection. The mobile phase was filtered through a 0.2 µM membrane prior to use. All drugs were detected at 212 nM. The flow-rate was 0.8 mL/min. The analysis time was set at 42 minutes per sample. The injection volume was 10 µL.

3. Standard, quality control and internal standard preparation

The stock solution of indinavir, lopinavir, nelfinavir, saquinavir, ritonavir, nevirapine and efavirenz were prepared at concentrations of 1 mg/mL in methanol. Ten milligrams of each analyzed drug was dissolved in a 10 mL volumetric flask to give a 1 mg/mL drug concentration.

For preparation of a standard curve, each standard stock solution was serially 10 fold diluted in 50% methanol to a final concentration of 10 µg/mL. The standard curve covering the concentration ranges between 75 and 20,000 ng/mL (75, 150, 300, 1,000, 4,000, and 20,000 ng/mL) were prepared by adding appropriate volumes of these diluted solutions to 200 µL of drug free human plasma. The quality control (QC) samples in the concentration of 75, 150, and 1,000 ng/mL were prepared in the same way with standard curve preparation.

A 1 mg/mL stock solution of carbamazepine (internal standard) was prepared in methanol and further diluted 1:10 in 50% methanol to give a 100 µg/mL working solution. Stocks and working solutions of all drugs and internal standards were aliquots and stored at -20°C until used.

4. Sample extraction

A 200 µL of plasma (blank plasma, standard and QC standard) were added to 10 ml glass tubes as well as one tube of drug free plasma (blank). Ten µL of 100 µg/mL internal standard solution was added to all tubes. Five mL of tert-butyl methyl ether was added to all tubes and stacked horizontally for 10 min. followed by centrifuging at 5,000 rpm for 10 min. The organic layer was separated in a new tube and evaporated to dryness under a gentle stream of nitrogen at 42°C. The residue was diluted in 300 µL of mobile phase followed by adding 3 mL of hexane. The samples were shaken horizontally for 10 min. follow by centrifuging at 5,000 rpm for 10 minutes and 10 µL of the solution was injected for analysis.

5. Extraction recovery

Extraction recoveries were measured in triplicate at the 75, 150, and 1,000 ng/mL by comparing the amount of all drugs of interest from extracted standard sample with non-extracted standard sample at these same levels.

6. Accuracy and precision

Accuracy, intraday and interday precision for each drug were determined by analyzing 5 replicate QC samples at three different concentrations (75, 150, and 1,000 ng/mL) for 3 separate days.

7. Selectivity

Selectivity was determined by comparing the chromatogram of spiked plasma with blank plasma to ensure that no interfering peaks were found in the chromatogram of the drugs of interest and IS.

8. Stability

Stability testing was determined by analyzing QC samples under various conditions. The QC samples at 75, 150 and 1,000 ng/mL of the drugs of interest were separated into 3 sets. The first set was heated at 56°C for 30 min. to inactivate the HIV. The second set was subjected to 3 freeze-thaw cycles and the third set was stored at room temperature for 24 hrs. Each QC sample was analyzed by comparing with the same concentration of freshly thawed QC samples.

9. Standard curve and statistical analysis

In this study, EmpowerPro software (Water, Milford, MA, USA) was used to generate the standard curve by plotting the areas under the curve ratio of the drugs of interest/internal standard of extracted spiked plasma versus various concentrations of the drugs of interest. The correlation coefficients and coefficient variations (CV%) in percent were evaluated in this study. Finally, the values from the linear regression were used for calculation of the drugs of interest concentrations in the samples from their areas under curve ratios.

RESULTS

The chromatogram of drug free plasma and spiked plasma with internal standard have been shown in Fig 1. The spike of other chromatograms are different in height, which are related to their different concentrations. Then, we used 150 ng/ml for clear demonstration in Fig 1. The assay run time was 42 minutes. The mean retention times were 4.56, 6.43, 8.09, 17.28, 22.55, 25.18, 26.93 and 35.81 minutes for nevirapine (NVP), internal standard (IS), indinavir (IDV), saquinavir (SQV), ritonavir (RTV), lopinavir (LPV), efavirenz (EFV) and nelfinavir (NFV), respectively. At a detection wavelength of 212 nm, assays performed on drug-free human plasma have no presence of any interfering peaks at the retention times of the interes-ted drugs and internal standard (Fig 1A). These results indicated that the established conditions were valid when measuring these drugs simultaneously.

The standard curve in human plasma ranging from 75 to 20,000 ng/mL for all drugs were analyzed for each run. A least-squares linear regression was used to calculate the equation relating the peak-area ratio between the drugs of interest/IS and the concentration of the drugs of interest. The standard curves were linear in the range of 75 to 20,000 ng/mL for all drugs. The correlation coefficients were higher than 0.99 (CV <10%). The limit of detection (LOD) of this assay was the same as the lower limit of quantitation (LOQ) (75 ng/mL).

Intra-day and inter-day precision data for plasma analysis of the drugs of interest were evaluated over the range of 75-1,000 ng/mL. Precision, accuracy, and extraction recovery of our HPLC method have been shown in Table 1. All variability has been expressed as CV%. The CVs calculated for NVP in the intra-day and inter-day assays ranged from 0.18% to 0.53% and 0.50% to 2.47%, respectively. In IDV, CVs ranged from 0.26% to 0.83% and 0.54% to 3.64%, and in SQV, CVs ranged from 0.11% to 1.23% and 0.46% to 3.67%. For RTV, CVs ranged from 0.12% to 2.44% and 0.46% to 3.55%, In LPV, CVs ranged from 0.18% to 1.72% and 0.54% to 3.42%. In EFV, CVs ranged from 0.09% to 0.7% and 0.45% to 3.69%, while in NFV, CVs ranged from 0.15% to 1.50% and 0.32% to 4.12%.

Accuracies ranged from 99.9% to 100.8%, 99.1% to 100.4%, 99.8% to 100.2%, 100.2% to 102.4%, 100.3% to 102.2%, 99.8% to 100.5%, and 98.4% to 100.1% for NVP, IDV, SQV, RTV, LPV, EFV and NFV, respectively.

Drug recoveries from plasma ranged from 99.5% to 100.3%, 99.8% to 100.1%, 99.7% to 100%, 100% to 101.3%, 100.2% to 100.8%, 99.8% to 100.3%, 98.7% to 100.7% and 99.5% to 101% for NVP, IDV, SQV, RTV, LPV, EFV, NFV and IS respectively.

The stability of all drugs under various conditions at three concentrations of QC standard has been shown in Table 2. All the assayed antiretroviral drugs were stable for 24 hours at room temperature and for heating at 56°C for 30 min. No degradation was observed at -20°C after three freeze-thaw cycles. At least 96% of the initial concentrations were recovered. Therefore, all of these analyses were considered to be stable in the tested conditions.

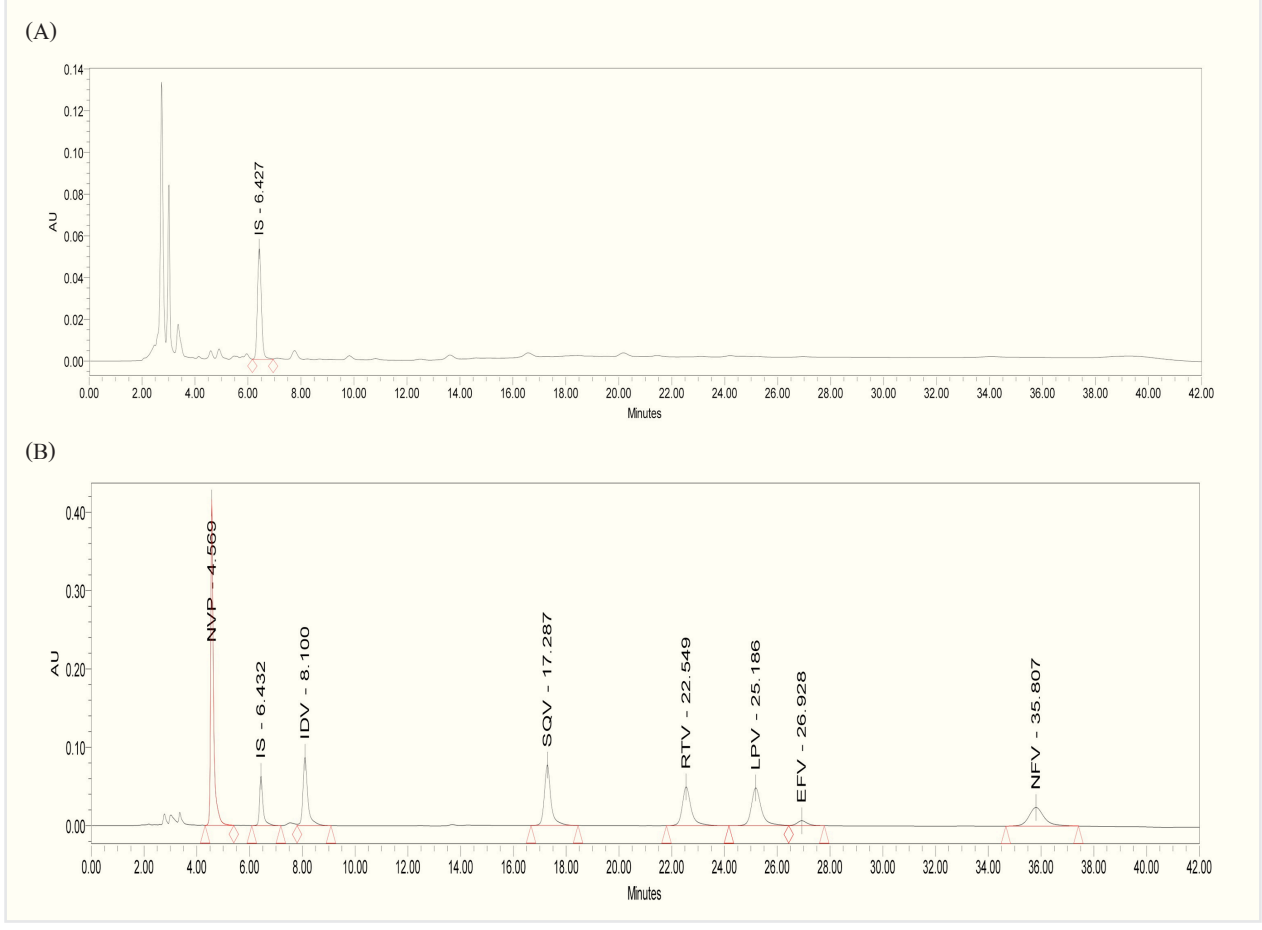

Fig 1. Representative Chromatograms of (A) blank plasma; (B) a 150 ng/mL of all study drugs. NVP: nevirapine; IS: internal standard; IDV: indinavir; SQV: saquinavir; RTV: ritonavir; LPV: lopinavir; EFV: efavirenz; NFV: nelfinavir.

TABLE 1. Intra-day and Inter-day Precision and Accuracy for Five PIs and Two NNRTIs.

Drugs	Expected (ng/mL)	Intra-day (n=5) Measured (ng/mL)	CV (%)	Inter-day (n=15) Measured (ng/mL)	CV (%)	Accuracy (%)	Recovery (%)
NVP	75	74.04 ± 0.39	0.53	74.04 ± 1.74	2.36	99.87	99.52
	150	150.44 ± 1.29	0.86	150.44 ± 3.72	2.47	100.77	100.29
	1000	1000.06 ± 1.80	0.18	1000.06 ± 4.97	0.50	100.06	100.01
IDV	75	74.82 ± 0.58	0.76	74.82 ± 2.73	3.64	99.13	99.76
	150	150.08 ± 1.24	0.83	150.08 ± 3.05	2.03	99.85	100.05
	1000	1000.79 ± 2.5 6	0.26	1000.79 ± 5.34	0.54	100.37	100.08
SQV	75	74.75 ± 0.92	1.23	74.75 ± 2.75	3.67	100.20	99.67
	150	149.88 ± 0.46	0.31	149.88 ± 2.22	1.48	99.79	99.92
	1000	999.72 ± 1.11	0.11	999.72 ± 4.56	0.46	99.96	99.97
RTV	75	75.03 ± 1.83	2.44	75.03 ± 2.66	3.55	102.37	100.04
	150	151.90 ± 1.16	0.76	151.90 ± 3.76	2.47	101.21	101.27
	1000	1000.94 ± 1.18	0.12	1000.94 ± 4.63	0.46	100.18	100.09
LPV	75	75.63 ± 1.30	1.72	75.63 ± 2.59	3.42	102.19	100.84
	150	151.17 ± 1.46	0.97	151.17 ± 3.16	2.09	101.22	100.78
	1000	1001.76 ± 1.76	0.18	1001.76 ± 5.44	0.54	100.29	100.18
EFV	75	74.82 ± 0.52	0.70	74.82 ± 2.76	3.69	99.8	99.76
	150	150.38 ± 0.13	0.09	150.38 ± 3.18	2.11	100.29	100.25
	1000	1002.10 ± 2.47	0.25	1002.10 ± 4.51	0.45	100.46	100.21
NFV	75	74.64 ± 1.12	1.50	74.64 ± 3.07	4.12	98.41	98.72
	150	151.11 ± 1.75	1.16	151.11 ± 3.54	2.34	99.43	100.74
	1000	999.48 ± 1.51	0.15	999.48 ± 3.20	0.32	100.11	99.95

NVP: Nevirapine, IDV: Indinavir, SQV: Saquinavir, RTV : Ritonavir, LPV: Lopinavir, EFV: Efavirenz, NFV: Nelfinavir

CONCLUSION

In conclusion, this HPLC method was prepared by the common and effective instruments that we can find in the low and middle economy countries. The process of HPLC has been developed by the conception of liquid-liquid extraction instead of solid-phase extraction which is more expensive. In this HPLC method, the column in this study was chosen by the multi-purpose quality. The limitation of the substance and internal standard were corrected by using the original substances which were contributed in Thailand by the certified companies and Thai Governmental Pharmaceutical Organization. This method has been completely validated with respect to precision, accuracy, stability, LOQ, recovery and linea-rity. The quality of the study was determined by inter-day and intra-day report. The stability and effectiveness of the method were reported by QC study and drug recoveries with more than 90 percent in every different condition. Comparison with the other published study in the HPLC method, our HPLC-UV method can determine the common dose of the NNRTIs and the PIs with recommended AIDS-regimens for the third world. Moreover, this method has more advantages than the previously reported methods[9-14] in cost of column, sample preparations, simple technique and very efficient outcome chromatograms. The reason for using the rapid liquid-liquid extraction was the more flexible technique and it was less expensive than the expensive and complicated solid-phase extraction. The less time running the process in the solid-phase method (15 minutes) is the only advantage over this method (45 minutes) in the same column. Mostly, the advantage in this method was the amount of blood sample which required only 200 µL of plasma for one analysis compared to more than 500 µL in other reports. This small amount of blood sample makes it applicable for pediatric populations as well. Finally, we have shown that our HPLC method is the sensitive, accurate, and precise method for monitoring the plasma concentrations of antiretroviral drugs in both pediatric and adult patients. From the data in the Handbook of Drug Monitoring Methods: Nevirapine's therapeutic level is between 3-20 mg/L, Indinavir is 0.1-10 mg/L, Saquinavir is 0.015-0.5 mg/L, Ritonavir is 1-14 mg/L, Lopinaivir is 5.5-15 mg/L, Efavirenz is 1-4 mg/L and Nelfinavir is 1-4 mg/L.[15] Since this method has detected that the antiretroviral drugs levels are effective as low as 75 ng/ml (0.075 mg/L), it can cover the most common plasma levels of the antiretroviral drugs. Hopefully, this method will be used for evaluating the relationship between plasma levels, dosage and clinical response in HIV patients in study in the near future.

ACKNOWLEDGMENTS

This study was supported by a Siriraj Grant for Research Development and Medical Education, Faculty of Medicine Siriraj Hospital, Mahidol University.

TABLE 2. Stability for the 5 PIs and 2 NNRTIs in drug free plasma.

Drugs	Concentration (ng/mL)	Conditions		Recovery (%)	CV (%)
Nevirapine	75	Heat at 56ºC		99.51	0.85
		24 hours at room temperature		102.40	0.94
		3 freeze-thaw cycles		99.436	2.73
	150	Heat at 56ºC		102.67	1.30
		24 hours at room temperature		99.33	0.67
		3 freeze-thaw cycles		98.67	1.79
	1,000	Heat at 56ºC		99.74	0.46
		24 hours at room temperature		99.60	0.28
		3 freeze-thaw cycles		99.85	0.34
Indinavir	75	Heat at 56ºC		99.51	2.12
		24 hours at room temperature		99.91	1.34
		3 freeze-thaw cycles		96.79	2.69
	150	Heat at 56ºC		102.44	2.09
		24 hours at room temperature		100.89	1.53
		3 freeze-thaw cycles		99.11	2.06
	100	Heat at 56ºC		99.37	0.35
		24 hours at room temperature		100.13	0.42
		3 freeze-thaw cycles		99.67	0.37
Saquinavir	75	Heat at 56ºC		99.82	1.61
		24 hours at room temperature		100.84	2.09
		3 freeze-thaw cycles		98.16	0.73
	150	Heat at 56ºC		102.44	0.75
		24 hours at room temperature		101.56	2.01
		3 freeze-thaw cycles		97.56	2.20
	100	Heat at 56ºC		99.87	0.25
		24 hours at room temperature		100.08	0.24
		3 freeze-thaw cycles		99.58	0.33
Ritonavir	75	Heat at 56ºC		99.87	1.54
		24 hours at room temperature		97.47	3.72
		3 freeze-thaw cycles		100.51	1.18
	150	Heat at 56ºC		101.78	2.65
		24 hours at room temperature		100.89	1.38
		3 freeze-thaw cycles		96.29	1.48
	1000	Heat at 56ºC		99.87	0.38
		24 hours at room temperature		100.09	0.22
		3 freeze-thaw cycles		99.84	0.47
Lopinavir	75	Heat at 56ºC		99.24	4.86
		24 hours at room temperature		100.70	2.25
		3 freeze-thaw cycles		97.32	3.60
	150	Heat at 56ºC		101.78	2.00
		24 hours at room temperature		100.00	1.16
		3 freeze-thaw cycles		99.33	0.67
	1000	Heat at 56ºC		99.26	0.17
		24 hours at room temperature		99.96	0.32
		3 freeze-thaw cycles		99.84	0.47
Efavirenz	75	Heat at 56ºC		98.58	3.73
		24 hours at room temperature		98.57	1.50
		3 freeze-thaw cycles		97.73	1.53
	150	Heat at 56ºC		102.89	0.37
		24 hours at room temperature		99.56	1.39
		3 freeze-thaw cycles	96.44	1.44	
	1000	Heat at 56ºC		99.46	0.30
		24 hours at room temperature		99.96	0.41
		3 freeze-thaw cycles		99.52	0.25
Nelfinavir	75	Heat at 56ºC		99.47	2.64
		24 hours at room temperature		99.01	0.88
		3 freeze-thaw cycles		96.68	3.02
	150	Heat at 56ºC		102.44	0.99
		24 hours at room temperature		101.56	1.37
		3 freeze-thaw cycles		100.04	0.32
	1000	Heat at 56ºC		99.44	0.44
		24 hours at room temperature		100.04	0.32
		3 freeze-thaw cycles		99.70	0.03

REFERENCES

1. Barry M, Gibbons S, Back D, Mulcahy F. Protease inhibitors in patients with HIV disease. Clinically important pharmacokinetic considerations. Clin Pharmacokinet. 1997 Mar;32(3):194-209.
2. Fichtenbaum CJ, Gerber JG. Interactions between antiretroviral drugs and drugs used for the therapy of the metabolic complications encountered during HIV infection. Clin Pharmacokinet. 2002;41(14):1195-211.
3. Masquelier B, Breilh D, Neau D, Lawson-Ayayi S, Lavignolle V, Ragnaud JM, et al. Human Immunodeficiency Virus Type 1 Genotypic and Pharmacokinetic Determinants of the Virological Response to Lopinavir-Ritonavir-Containing Therapy in Protease Inhibitor-Experienced Patients. Antimicrob Agents Chemother. 2002 Sep;46(9):2926-32.
4. Duval X, Mentré F, Lamotte C, Chêne G, Spire B, Dellamonica P, et al. Indinavir plasma concentration and adherence score are codeterminant of early virologic response in HIV-infected patients of the APROCO cohort. Ther Drug Monit. 2005 Feb;27(1):63-70.
5. Droste JAH, Verwey-van Wissen CPWGM, Burger DM. Simultaneous Determination of the HIV drugs indinavir, ampinavir, saquinavir, ritonavir, lopinavir, nelfinavir, the nelfinavir hydroxymetabolite M8 and nevirapine in human plasma by reversed-phase high-performance liquid chromatography. Ther Drug Monit. 2003 Jun;25(3):393-9.
6. Poirier JM, Robidou P, Jaillon P. Simple and simultaneous determination of the hiv-protease inhibitors amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir and saquinavir plus M8 nelfinavir metabolite and the nonnucleoside reverse transcriptase inhibitors efavirenz and ne virapine in human plasma by reversed-phase liquid chromatography. Ther Drug Monit. 2005 Apr;27(2):186-92.
7. Verbesselt R, Van Wijngaerden E, de Hoon J. Simultaneous determination of 8 HIV protease inhibitors in human plasma by isocratic high-performance liquid chromatography with combined use of UV and fluorescence detection: amprenavir, indinavir, atazanavir, ritonavir, lopinavir, saquinavir, nelfinavir and M8-nelfinavir metabolite. J Chromatogr B Analyt Technol Biomed Life Sci. 2007 Jan 1;845(1):51-60.
8. Notari S, Bocedi A, Ippolito G, Narciso P, Pucillo LP, Tossini G, et al. Simultaneous determination of 16 anti-HIV drugs in human plasma by high-performance liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci. 2006 Feb 2;831(1-2):258-66.
9. Elens L, Veriter S, Di Fazio V, Vanbinst R, Boesmans D, Wallemacq P, et al. Quantification of 8 HIV-protease inhibitors and 2 nonnucleoside reverse transcriptase inhibitors by ultra-performance liquid chromatography with diode array detection. Clin Chem. 2009 Jan;55(1):170-4.
10. Dailly E, Raffi F, Jolliet P. Determination of atazanavir and other anti-retroviral drugs (indinavir, amprenavir, nelfinavir and its active metabolite M8, saquinavir, ritonavir, lopinavir, nevirapine and efavirenz) plasma levels by high performance liquid chromatography with UV detection. J Chromatogr B Analyt Technol Biomed Life Sci. 2004 Dec 25;813(1-2):353-8.
11. Weller DR, Brundage RC, Balfour HH, Jr, Vezina HE. An isocratic liquid chromatography method for determining HIV non-nucleoside reverse transcriptase inhibitor and protease inhibitor concentrations in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci. 2007 Apr 1;848 (2):369-73.
12. Titier K, Lagrange F, Pehourcq F, Edno-Mcheik L, Moore N, Molimard M. High-performance liquid chromatographic method for the simultaneous determination of the six HIV-protease inhibitors and two non-nucleoside reverse transcriptase inhibitors in human plasma. Ther Drug Monit. 2002 Jun;24(3):417-24.
13. Rezk NL, Crutchley RD, Yeh RF, Kashuba AD. Full validation of an analytical method for the HIV-protease inhibitor atazanavir in combination with 8 other antiretroviral agents and its applicability to therapeutic drug monitoring. Ther Drug Monit. 2006 Aug;28(4):517-25.
14. Pelerin H, Compain S, Duval X, Gimenez F, Benech H, Mabondzo A. Development of an assay method for the detection and quantification of protease and non-nucleoside reverse transcriptase inhibitors in plasma and in peripheral blood mononuclear cells by liquid chromatography coupled with ultraviolet or tandem mass spectrometry detection. J Chromatogr B Analyt Technol Biomed Life Sci. 2005 May 5;819(1):47-57.
15. Dasgupta A. Handbook of Drug Monitoring Methods. Humana Press Inc., Totowa NJ. Chapter10, Therapeutic Drug Monitoring in Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome; 2008. p. 201-210.