



AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: <http://www.ajptr.com/>

Quantitative Estimation of Lopinavir and Ritonavir in Tablet Dosage forms by RP-HPLC method

M. Jagadeeswaran^{1*}, N. Gopal², K. Pavan Kumar¹, T. Sivakumar¹

1. Nandha College of Pharmacy and Research Institute, Department of Pharmaceutical Analysis, Erode-638 052, Tamil Nadu, India.

2. Balaji Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, Narsampet, Warangal-506 331, Andhra Pradesh, India.

ABSTRACT

A reversed phase high-performance liquid chromatographic method was developed and validated for the quantitative determination of two antiviral drugs viz. lopinavir and ritonavir. Chromatography was carried out by gradient technique on a reversed-phase C₁₈ Column, Phenomenex (250 x 4.6 mm, 5 μ) with mobile phase mixture of Buffer: Acetonitrile (45:55 v/v) was used as a mobile phase and the pH was adjusted into 4.5 by using with O-phosphoric acid, at a flow rate of 1.2 ml/min. The UV range was detected at 240nm for lopinavir and ritonavir respectively. The different analytical performance parameters such as linearity, precision, accuracy, and specificity, limit of detection (LOD) and limit of quantification (LOQ) were determined according to International Conference on Harmonization ICH Q2B guidelines. The linearity of the calibration curves for each analyte in the desired concentration range is good ($r^2 > 0.9$). The recovery of the method was between 102.1% and 100.1% for lopinavir and ritonavir respectively. Hence the proposed method is highly sensitive, precise and accurate and it successfully applied for the reliable quantification of API content in the commercial formulations of lopinavir and ritonavir.

Key words: Lopinavir, Ritonavir, UV spectrophotometry, RP-HPLC.

*Corresponding Author Email: jaganmurugesh@gmail.com

Received 2 March 2012, Accepted 15 March 2012

Please cite this article in press as: Jagadeeswaran M *et al.*, Quantitative Estimation of Lopinavir and Ritonavir in Tablet Dosage forms by RP-HPLC method . American Journal of PharmTech Research 2012.

INTRODUCTION

One of the deadliest and unmanageable chronic health catastrophes is HIV/AIDS. It requires lifelong treatment with potent life saving essential drugs that include nucleoside reverse transcriptase inhibitors, non nucleoside reverse transcriptase inhibitors and protease inhibitors. Amongst these lopinavir and ritonavir drug combination is a protease inhibitor used as a second line regimen to treat patients with HIV¹.

Lopinavir (the active ingredient) (Figure 1.A) is chemically designated as [1*S*-[1*R**, (*R**) 3*R**, 4*R**]]-*N*-[4-[[[(2, 6-dimethylphenoxy) acetyl] amino]-3 -hydroxy-5-phenyl-1-(phenylmethyl) pentyl] tetrahydro- α -(1-methylethyl)-2-oxo-1 (2*H*)-pyrimidineacetamide. Ritonavir (Figure 1.B) is chemically designated as 10-Hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]- 3,6-dioxo- 8,11- bis(phenylmethyl)-2,4,7,12 -tetraazatridecan -13-oic acid, 5-thiazolylmethyl ester, [5*S*-(5*R**,8*R**,10*R**,11*R**)].

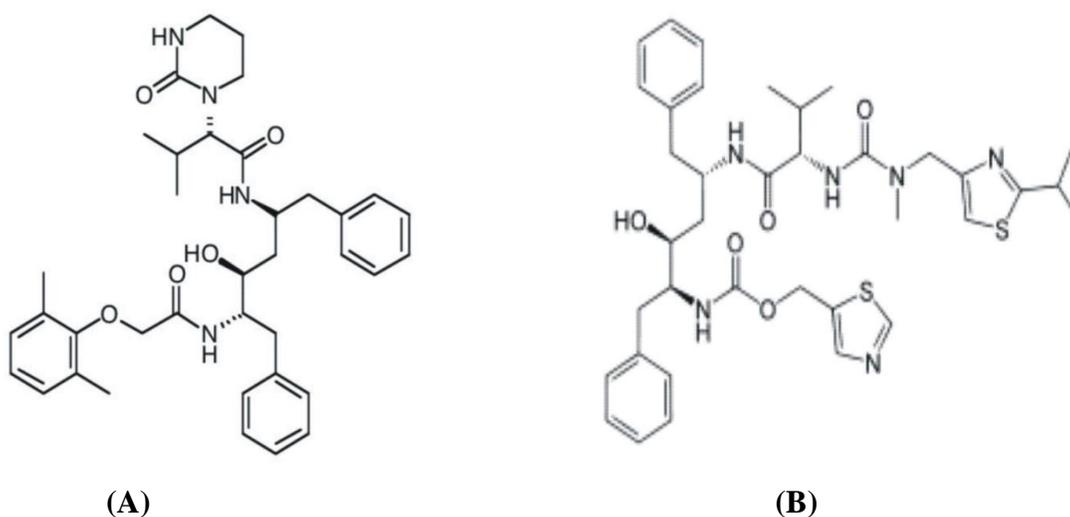


Figure 1: Chemical structures of Lopinavir (A) and Ritonavir (B)

Literature survey revealed several analytical methods for the determination of ritoavir and lopinavir in tablets, capsules, and syrups which employ techniques such as high-performance liquid chromatography (HPLC)²⁻⁴, Ultra performance liquid chromatography (UPLC)⁵, and high performance thin layer chromatography (HPTLC)⁶. In biological fluids, the active principles as well as their metabolites have been quantitatively determined by HPLC with UV detection, LC/MS/MS^{7, 8}, Spectroscopic method⁹, Micellar electrokinetic chromatography method¹⁰ and Tandem mass spectrometry¹¹.

The proposed method was optimized and validated in accordance with International Conference on Harmonization (ICH) guidelines¹². The aim of present work is to develop a simple, rapid,

precise, accurate and selective reversed phase chromatographic method and to estimate the lopinavir and ritonavir in bulk and its solid dosage forms.

MATERIALS AND METHODS

The bulk drugs of lopinavir and ritonavir were obtained as gift samples from abbot Laboratories Ltd, Gawhati, India. HPLC grade acetonitrile and ammonia were obtained from Sigma Aldrich (Switzerland). Combination tablets of Lopinavir 200 mg and Ritonavir 50 mg from abbot were purchased from local market. Milli-Q-Water was used in all experiments. All the solutions for analysis were prepared and analyzed freshly.

Instrumentation and analytical conditions

Chromatography was performed using a shimadzu LC-10ATvp series, (Kyoto, Japan) equipped with SPD-10A UV-Vis detector. Data acquisition and processing was performed using chemistry station software (LC solution). The methods were conducted using a gradient reverse phase technique. The analytical conditions (mobile phase composition, flow rate and analytical wavelengths) for the two drugs have been summarized in Table 1. The mobile phases were prepared freshly, filtered through 0.45 μ membrane filter (Millipore, USA) and sonicated (Branson sonicator 3210, Germany) for 10 min before use in order to deaerate.

Preparation of standard and quality control solutions

Primary stock solutions of lopinavir (100 mg) and ritonavir (100 mg) were prepared in ultra pure water and further diluted with water to obtain working standards in the concentration range of 40–200 μ g/ml and 10-50 μ g/ml for lopinavir and ritonavir respectively. Quality control (QC) samples were run with each batch of working standards in order to calculate the validation parameters. QC samples were prepared in ultra pure water spiked with analytes at different concentrations following the same procedure as for calibration standards, using a different primary stock. The samples were analyzed with reagent blanks. The prepared standard solutions were validated with suitable parameters according to International Conference on Harmonization.

RESULTS AND DISCUSSION

RP-HPLC method

A RP-HPLC method was developed for two anti-retroviral drugs, which can be conveniently employed for routine quality control in pharmaceutical dosage forms. The chromatographic conditions were optimized in order to provide a good performance of the assay. The mobile phase for each drug was selected based on its polarity. Different ratios of Buffer: ACN

combinations were tried for lopinavir and ritonavir and the fixed mobile phase are listed in Table 1. The optimization of flow rate is critical since the extent of longitudinal broadening is inversely related to flow rate of mobile phase. In either case of high or low flow rates, an ideal Gaussian curve of the peak is not obtained as the peak symmetry parameters are affected, i.e. asymmetry factor deviates from unity (Figure 2, 3 & 4). The retention times of Ritonavir and Lopinavir were 4.323 and 5.650 min respectively. The chromatograms have been showed (Figure 5). The methods were specific as none of the excipients interfered with the analytes of interest. Hence, the methods were suitably employed for assaying the commercial anti-retroviral individual formulations.

Table 1: The optimized chromatographic conditions for analysis of Lopinavir and Ritonavir

| | | |
|---|----------------------|---|
| 1 | Stationary phase | Phenomenex C ₁₈ analytical column, (250×4.6 mm, 5 μm) |
| 2 | Mobile phase | Solvent A: Ammonia solution adjusted the pH to 4.5 with O-Phosphoric acid Solvent B: Buffer: ACN (45:55 v/v) |
| 3 | Flow rate | 1.2 ml/min |
| 4 | Detection wavelength | 240 nm |
| 5 | Injection volume | 20 μl |
| 6 | Temperature | Ambient temperature |

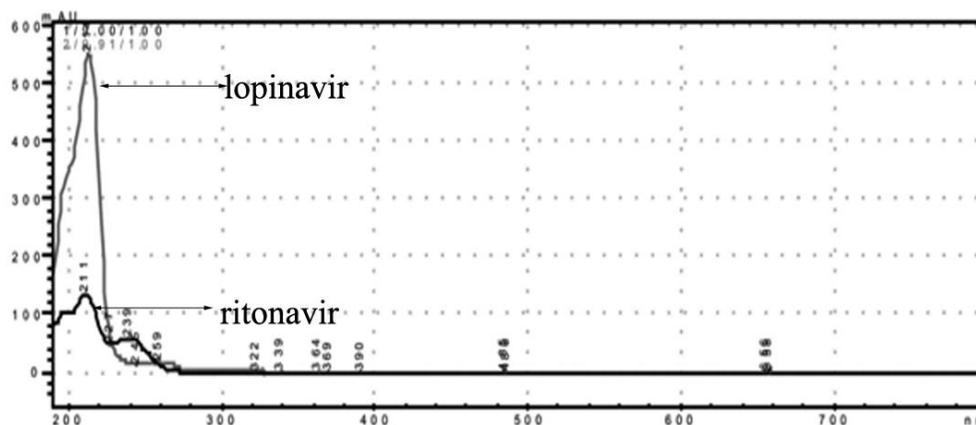


Figure 2: Overlay spectrum of Lopinavir and Ritonavir

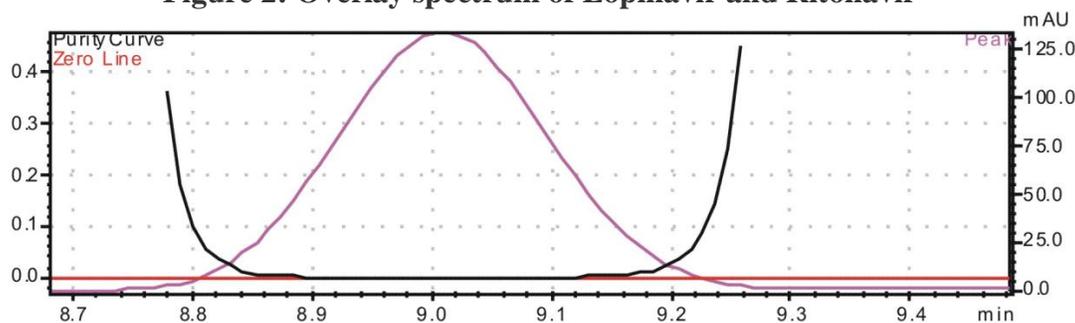


Figure 3: Peak purity curve of Ritonavir

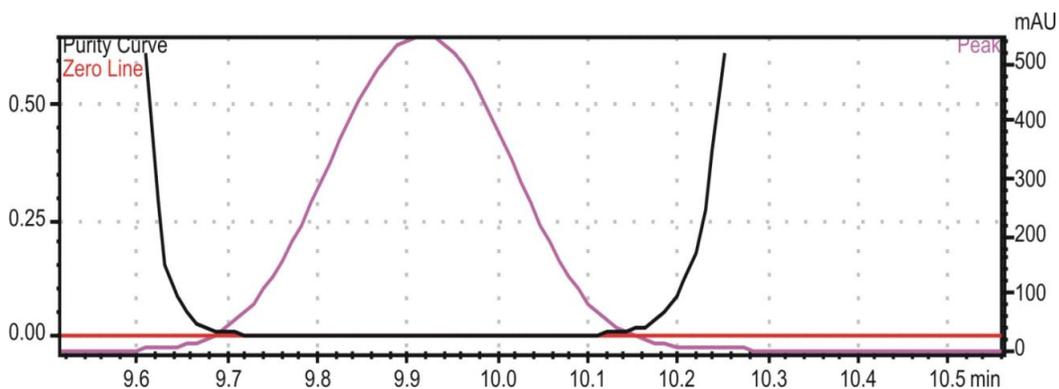


Figure 4: Peak purity curve of Lopinavir

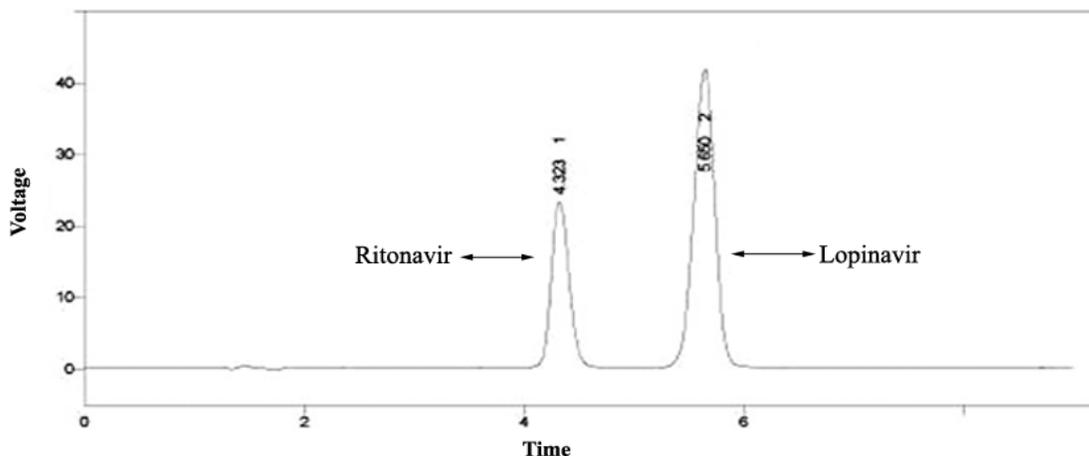


Figure 5: Typical chromatogram showing the elution of Ritonavir and Lopinavir at their respective retention times

Linearity

Calibration curves were obtained from the peak area and concentration of the drug were subjected to regression analysis and correlation coefficients. Table 2 represents the mean RP-HPLC area responses for ritonavir and lopinavir at different concentrations. As shown, the responses for the drug was strictly linear ($r^2 > 0.999$) in the concentration range of 10-50 $\mu\text{g/ml}$ for ritonavir and 40-200 $\mu\text{g/ml}$ for lopinavir respectively. The slope and intercept for lopinavir was found to be 21201 and 10566 where as for ritonavir was found to be 15278 and 26980 respectively.

Accuracy and precision

Accuracy and precision were determined by elaboration of three standard calibration curves, two from the same day (intra-day) and third one from a different day (inter-day). The intra-day and inter-day precisions (% RSD) at different concentration levels were found to be less than 2 % (Table 3). Moreover the % RSD (less variation) showed good precision of the developed HPLC methods.

Table 2: Linearity of the developed RP-HPLC method

| S. No | Conc. of Ritonavir (µg/ml) | Conc. of Lopinavir (µg/ml) | Area of Ritonavir | Area of Lopinavir | Response factor of Ritonavir | Response factor of Lopinavir |
|-------|----------------------------|----------------------------|-------------------|-------------------|------------------------------|------------------------------|
| 1 | 10 | 40 | 180692 | 108837 | 0.255 | 0.182 |
| 2 | 20 | 80 | 333938 | 313309 | 0.472 | 0.499 |
| 3 | 30 | 120 | 484976 | 520991 | 0.686 | 0.582 |
| 4 | 40 | 160 | 630074 | 766285 | 0.891 | 1.013 |
| 5 | 50 | 200 | 796551 | 942405 | 1.092 | 1.092 |

Table 3: Precision data for the analysis of lopinavir and ritonavir

| S.No | Lopinavir | | | | Ritonavir | | | |
|------|---------------|-----------|---------------|-----------|---------------|-----------|---------------|-----------|
| | Intra-day | | Inter-day | | Intra-day | | Inter-day | |
| | Conc. (µg/ml) | % RSD |
| 1 | 40 | 1.31±0.56 | 40 | 1.58±0.43 | 10 | 0.85±0.49 | 10 | 0.94±0.32 |
| 2 | 80 | 1.48±0.68 | 80 | 1.54±0.36 | 20 | 1.32±0.81 | 20 | 1.54±0.76 |
| 3 | 120 | 0.71±0.09 | 120 | 1.98±0.23 | 30 | 0.92±0.62 | 30 | 0.56±0.23 |
| 4 | 160 | 1.84±0.48 | 160 | 1.62±0.33 | 40 | 0.87±0.45 | 40 | 0.86±0.43 |
| 5 | 200 | 1.23±0.54 | 200 | 1.41±0.36 | 50 | 0.95±0.36 | 50 | 0.96±0.45 |

The respective RP-HPLC area responses from the accuracy determination study are shown in Table 4. Recovery experiment was carried out by applying the standard addition method. Drug assay was performed in triplicate by spiking with equivalent amount of raw material into each volumetric flask for each spike level to get the concentrations of lopinavir and ritonavir equivalent to 80%, 100%, and 120% of the standard concentrations of lopinavir and ritonavir. The average percentage recovery of both the drugs was found to be within the limits and it is highly accurate.

Table 4: Analysis of formulations and recovery studies by RP-HPLC

| Level of addition (%) | Ingredient | Amount Added (mg) | % Recovered (mg) | Mean% recovery* |
|-----------------------|------------|-------------------|------------------|-----------------|
| 80 | Lopinavir | 80 | 79.12 | 100.12 |
| | Ritonavir | 40 | 40.66 | |
| 100 | Lopinavir | 100 | 99.86 | 99.74 |
| | Ritonavir | 50 | 49.71 | |
| 120 | Lopinavir | 120 | 120.11 | 100.26 |
| | Ritonavir | 60 | 60.79 | |

* An average of three determinations

LOQ and LOD

The LOD and LOQ were determined from the calculated standard deviations of each calibration standard and it was found to be 0.013 µg/ml and 0.465 µg/ml for lopinavir and ritonavir

respectively. The calculated LOQ and LOD concentrations confirmed that the method is sensitive.

Specificity

The developed method is specific as none of the excipients interfered with the analytes of interest. Hence, this method is suitably employed for assaying the commercial anti-retroviral individual formulations.

CONCLUSION

The proposed RP-HPLC is simple, reliable and selective. It also provides satisfactory accuracy and precision with lower limits of detection and quantification. Moreover the shorter duration of analysis for lopinavir and ritonavir make these reported methods suitable for routine quantitative analysis in pharmaceutical dosage forms. The recoveries achieved are good by both the methods.

ACKNOWLEDGEMENTS

The authors are thankful to Abott Laboratories Ltd., Gawhati, India for procuring the gift samples and also thankful to the management, Nandha College of Pharmacy and Research Institute, Erode, Tamilnadu, India.

REFERENCES

1. Sean CS, Martindale. The complete drug reference, 34th ed., London, UK: The Pharmaceutical Press, 2005; 649-653
2. Usami Y, Oki T, Nakai M, Sagisaka M, Kaneda T. A simple HPLC method for simultaneous determination of lopinavir, ritonavir and efavirenz. *Chem Pharm Bull (Tokyo)* 2003; 51:715-718.
3. Donato EM, Dias CL, Rossi RC, Valente RS, Froelich PE et al. LC method for studies on the stability of lopinavir and ritonavir in soft gelatin capsules. *Chromatogr* 2006; 63: 437-443.
4. Phechkrajang CM, Thin EE, Sratthaphut L, Nacapricha D, Wilairat P. Quantitative Determination of Lopinavir and Ritonavir in Syrup Preparation by Liquid Chromatography. *Mahi Univ J Pharm Sci* 2009; 36: 1-12.
5. Marina VA, Jorge PR, Eduardo S, Julia P, Rafael L. Ultra-performance liquid chromatographic method for simultaneous quantification of HIV non-nucleoside reverse transcriptase inhibitors and protease inhibitors in human plasma. *J Braz Chem Soc* 2011; 22: 134-141.

6. Sulebhavikar AV, Pawar UD, Mangoankar KV, Prabhu NDN. HPTLC method for simultaneous determination of lopinavir and ritonavir in capsule dosage form. *E-J Chem* 2008; 5: 706-712.
7. Temghare GA, Shetye SS, Joshi SS. Rapid and sensitive method for quantitative determination of lopinavir and ritonavir in human plasma by liquid chromatography- Tandem Mass Spectrometry. *E-J Chem* 2009; 6: 223-230.
8. Myasein F, Kim E, Zhang J, Wu H, El-Shourbagy TA. Rapid, simultaneous determination of lopinavir and ritonavir in human plasma by stacking protein precipitations and salting-out assisted liquid/liquid extraction, and ultrafast LC–MS/MS. *Anal Chim Acta* 2009; 651: 112-116.
9. Vaishali P, Nagulwar, Kishore PB. Simultaneous estimation of ritonavir and lopinavir by absorption ratio (Q-analysis) UV spectrophotometric method in combined tablet dosage form. *Der Pharmacia Lettre* 2010; 2: 196-200.
10. Carvalho AZ, El-Attug MN, Zayed SE, Hove EV, Duppen JV, Hoogmartens J, Schepdael AV. Micellar electrokinetic chromatography method development for determination of impurities in ritonavir. *J Pharm Biomed Anal* 2010; 53: 1210-1216.
11. Estrela RC, Ribeiro FS, Seixas BV, Suarez-Kurtz G. Determination of lopinavir and ritonavir in blood plasma, seminal plasma, saliva and plasma ultra-filtrate by liquid chromatography/tandem mass spectrometry detection. *Rapid Comm Mass Spectro* 2008; 22: 657-664.
12. Anonymous, ICH Guidelines: Validation of Analytical Procedures: Methodology Q2 (B) Text and Methodology: 2003.