International Journal for Pharmaceutical Research Scholars (IJPRS)
ISSN (Online): 2277 – 7873
RESEARCH ARTICLE **V-1, I-1, 2012**

Development and Validation of HPTLC Method for Simultaneous Analysis of Lopinavir and Ritonavir in their Combined Tablet Dosage Form

Mardia RB*[1], Suhagia BN[2], Pasha TY[3], Chauhan SP[4] and Solanki SD[5]
*[1,4]*Faculty of Pharmacy, Dharmsinh Desai University, Nadiad, India. [2]L. M. College of Pharmacy, Ahmedabad, Gujarat , India. [3]Parul Institute of Pharmacy, Baroda, Gujarat, India. [5]K. B. Raval College of Pharmacy, Ahmedabad, India.*

Manuscript No: IJPRS/V1/I1/00017, Received On: 16/03/2012, Accepted On: 26/03/2012

ABSTRACT

Simultaneous quantification of Lopinavir and Ritonavir in tablet by HPTLC method was developed and validated. The chromatograms were developed using a mobile phase of Chloroform: 1, 4 - Dioxane (7:3 %v/v) on pre-coated plate of silica gel GF aluminum TLC plate and quantified by densitometric absorbance mode at 210 nm. The R_f value for lopinavir and ritonavir was 0.74 and 0.58 respectively. The linearity of the method was found to be within the concentration range of 160-960 ng/spot for Lopinavir and for Ritonavir, it was 40-240 ng/spot. The lower limits of detection and quantification were 9.56 ng/spot and 28.96 ng/spot for Lopinavir and 6.82 ng/spot and 20.66 ng/spot for Ritonavir. The method was also validated for precision, specificity and recovery. This developed method was used to analyze fixed-dose tablet (Lopimune, Cipla Ltd) sample of Lopinavir and Ritonavir.

KEYWORDS

HPTLC, Lopinavir, Ritonavir

INTRODUCTION

Lopinavir as [1S-[1R*, (R*), 3R*, 4R*]]-N-[4-[[(2, 6-dimethylphenoxy) acetyl] amino]-3-hydroxy-5-phenyl-1-phenylmethyl) pentyl] tetrahydro - alpha-(1-methylethyl)-2-oxo-1(2H)-pyrimidineacetamide. (Fig. 1) and Ritonavir 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, [5S-5R*,8R*,10R*,11R*)] (Fig. 2) are anti-HIV drugs (HIV protease inhibitors). Lopinavir and Ritonavir have been reported to be quantified individually or in combination by spectrophotometric methods[1–3] and HPLC[4-7].

The literature survey reveals that, there are analytical methods available for determination of Lopinavir and Ritonavir from biological

***Address for Correspondence:**
Mr. Rajnikant B. Mardia
Faculty of Pharmacy,
Dharmsinh Desai University,
College road, Nadiad-387001,
Gujarat. India.
E-Mail Id: rajnikantmardia28@gmail.com

Figure: 1 Lopinavir

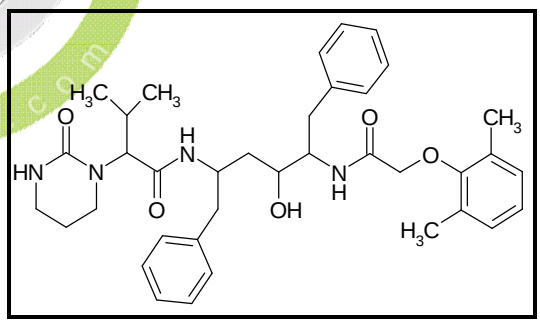
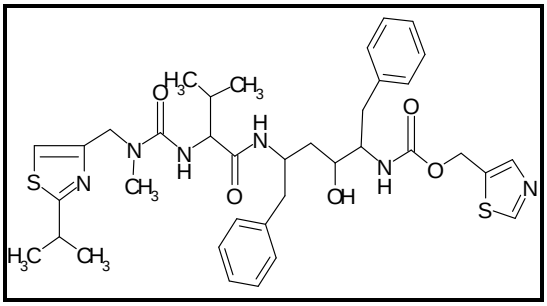


Figure: 2 Ritonavir



© Copyright reserved by IJPRS

matrices, bulk drug and dosage forms, and for determination of Lopinavir and Ritonavir with combination of other antiviral drugs by RP-HPLC/MS[8–12]. Patel D. et al.[13] had reported HPTLC method for the analysis of Lopinavir and Ritonavir using Merck TLC aluminium sheets of silica gel 60F-254 as a stationary phase and ethyl acetate: ethanol:toluene : diethylamine (7:2.0:0.5:0.5 %v/v) as mobile phase with detection at 266 nm. The linear range reported in the method is 8-20 mg/mL. In this study, we report the HPTLC method for the analysis of Lopinavir and Ritonavir using a solvent system of Chloroform: 1, 4 - Dioxane (7:3 %v/v).

EXPERIMENTAL

CHEMICALS AND REAGENTS

Pure Lopinavir and Ritonavir powders were kind gifts from Emcure Pharmaceuticals Ltd., Pune, India. Commercial tablets (Lopimune, CiplaLtd) containing Lopinavir (200mg) and Ritonavir (50mg) were used for the study. Chloroform, 1, 4-dioxane and methanol used were of analytical grade (E.Merck, Mumbai, India). All the other chemicals used were also of analytical grade (E.Merck,India).

INSTRUMENTATION AND CONDITIONS

HPTLC plates pre-coated with silicagel GF aluminum TLC plate, (10cm X 10cm) were from Merck. Densitometry was carried out with a CAMAG TLC Scanner3, fitted with a win-CATS 1.4.0 planar chromatography manage software. Samples were applied to the HPTLC plates using the spray-on technique of CAMAG LINOMAT V under nitrogen gas flow, and developed in a CAMAG 10cm X 10cm twin trough chambers.

STANDARD PREPARATION

Lopinavir (200mg) and Ritonavir (50mg) were accurately weighed and transferred into100 mL volumetric flask, and dissolved in methanol. The volume was made upto the mark with methanol. The resulting stock solution was further 10 times diluted with methanol to get the final concentration of 200 mcg/mL Lopinavir and 50 mcg/mL Ritonavir which was used for calibration purpose of both the drugs.

PREPARATION OF SAMPLE SOLUTION

For analysis of tablet dosage form, twenty tablets, each containing 200mg of Lopinavir and 50mg ritonavir, were weighed and their average weight was calculated. The tablets were finely powdered and powder equivalent to 200mg Lopinavir and 50mg Ritonavir was accurately weighed and transferred in to 100mL volumetric flask. 60mL methanol was added to it and shaken for 30 minutes. The volume was made upto the mark with methanol. The solution was sonicated for 30min, filtered through the Whattman No.41 filterpaper. This solution was further diluted with methanol to get the same concentration as that of the final standard solution.

CHROMATOGRAPHIC CONDITIONS

Lopinavir and Ritonavir reference standard solution was prepared using methanol as solvent. From the prepared standard solution, 0.8, 1.6, 2.4, 3.2, 4.0 and 4.8 µL aliquots were applied to the HPTLC plates as spot bands of 6mm using LINOMAT V. Application positions were at least 15mm from the sides and 10mm from the bottom of the plates. Mobile phase components were mixed prior to use and the development chamber was left for saturation with mobile phase vapors for 10min before each run. Development of the plate was carried out by the ascending technique to a migration distance of 7cm. Then the plates were dried on a hot plate. All the analysis was carried out in a laboratory with temperature control (20–24^{0}C). Densitometry scanning was done in absorbance mode at 210 nm using a deuterium lamp. The slit dimensions were set at 6mm x 0.30 mm, the scanning speed of 10mm/s, and the data resolution at 100 mm/step. Single wavelength detection was performed since the main components were only analyzed.

METHOD VALIDATION

The developed method was validated as per the International Conference on Harmonization (ICH)[14,15] guidelines with respect to linearity

© Copyright reserved by IJPRS

and range, specificity, precision, accuracy, limit of detection and limit of quantification.

RESULTS AND DISCUSSION

LINEARITY AND RANGE

A stock standard solution containing Lopinavir and Ritonavir was prepared in methanol and 10 times diluted. 0.8, 1.6, 2.4, 3.2, 4.0 and 4.8 µL aliquots of the diluted standard solution was applied to the HPTLC plate to deliver 160, 320, 480, 640, 800 and 960 ng of Lopinavir per spot and 40, 80, 120, 160, 200 and 240 ng of Ritonavir per spot. This was done in triplicate and repeated for three days. For each concentration, the applied spot bands were evenly distributed across the plate to minimize possible variation along the silica layer. The results are indicated in Table 1.

Table: 1 Linearity results

Components	Concentration range (ng/spot)	Equation for regression line	R^2
Lopinavir	160-960	y = 10.46x + 2162	0.993
Ritonavir	40-240	y = 24.68x + 289.0	0.999

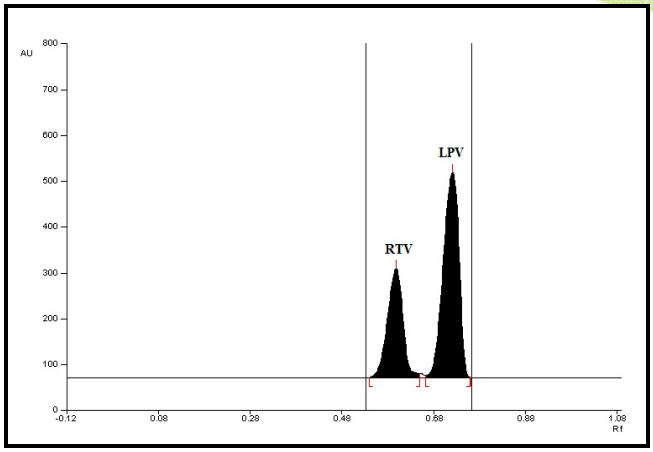


Figure: 3 HPTLC chromatogram of Ritonavir (240 ng/spot) and Lopinavir (960 ng/spot) standard with corresponding Rf at 210 nm

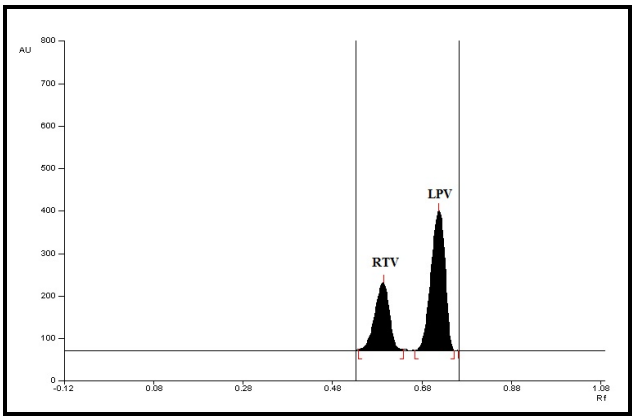


Figure: 4 HPTLC chromatogram of Ritonavir (150 ng/spot) and Lopinavir (600 ng/spot) tablet sample with corresponding Rf at 210 nm

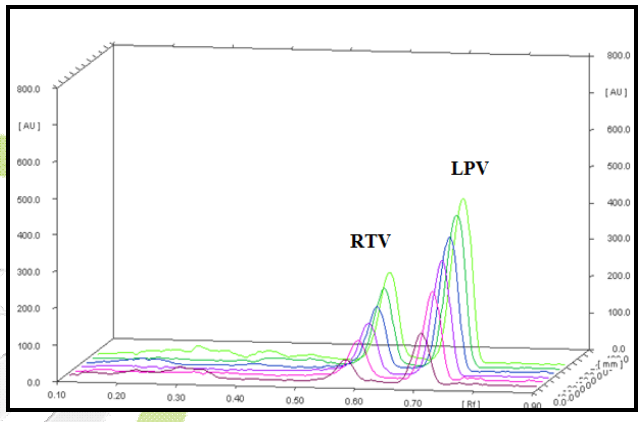


Figure: 5 3D Chromatogram showing peaks of Ritonavir and Lopinavir standards in different concentrations

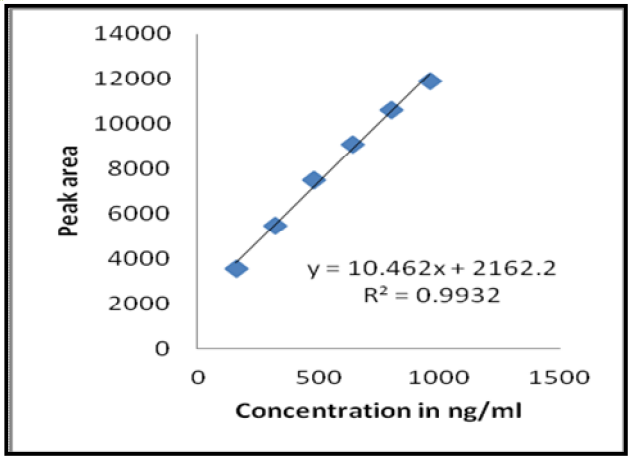


Figure: 6 Calibration curve of Lopinavir

© Copyright reserved by IJPRS

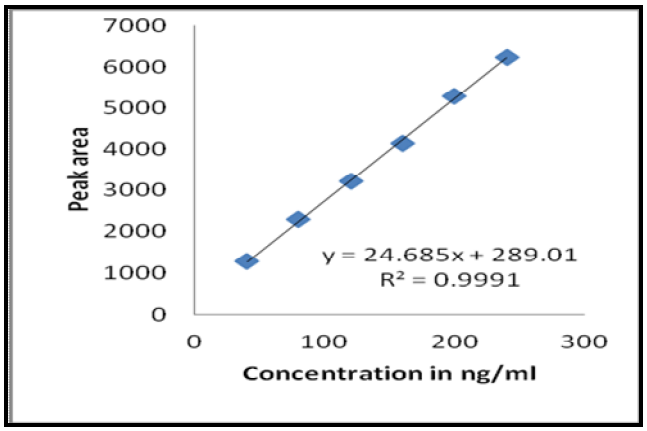


Figure: 7 Calibration curve of Ritonavir

PRECISION

The repeatability (intra-day precision) is expressed as percentage relative standard deviations (%RSD) for the Lopinavir at the concentrations of 320, 480 and 640 ng/spot, their % RSD values were 0.48, 1.52 and 0.86, respectively, and for the time-different intermediate precision (inter-day precision) the % RSD values were 1.38, 0.92 and 1.29,respectively. The % RSD values of intra-days precision for Ritonavir at the concentrations of 80, 120 and 160 ng/spot were 0.94, 0.45, 1.68, and for inter-days precision the %RSD levels were 1.43, 0.82 and 0.62, respectively. The pooled repeatability precisions were 0.95 and 1.02 for Lopinavir and Ritonavir concentrations, respectively, and the pooled time-different intermediate precisions were 1.19 and 0.96, respectively. The %RSD levels of intra-day and inter-day precision were less than 2.0 in all cases, which indicated that there were no significant variations in the analysis of Lopinavir and Ritonavir at the concentrations, which are shown in Table 2.

ACCURACY

The accuracy was assessed by the methodological recovery. The recovery of the method was calculated by comparing the determined concentration of spiked samples to the theoretical concentrations. The mean percentage recovery for each compound was calculated at each concentration level and reported with its standard deviation. The intra-day and inter-day percentages of accuracy obtained for Lopinavir at the concentrations of 320, 480 and 640 ng/spot, and for Ritonavir at the concentrations of 80, 120 and 160 ng/spot, are respectively shown in Table2. The% recoveries of intra-day for Lopinavir were 98.57 ± 1.37%, 97.83 ± 1.74% and 99.58 ± 1.32% respectively, the mean recovery for all the concentration levels was 98.66 ± 1.48 %. For Ritonavir, the %recoveries of intra-day were 99.26 ± 1.63%, 97.69 ± 1.82% and 99.34 ± 1.94%, respectively (Table2). The mean value covering all the concentration levels was 98.76 ± 1.80 %.

LIMITS OF DETECTION AND QUANTIFICATION

The limit of detection was found by the equation LOD = 3.3 x σ/s as well as limit of quantitation was found by the equation LOQ = 10 x σ/s. The limit of detection was found to be 9.56 ng/spot and 6.82 ng/spot for Lopinavir and Ritonavir, respectively. The limit of quantification was found to be 28.96 ng/spot and 20.66 ng/spot for Lopinavir and Ritonavir, respectively.

SPECIFICITY

The chromatogram of the solution, which was not spiked with Lopinavir and Ritonavir, did not

Table: 2 Accuracy and intermediate precision

Drugs	Concentration (ng/spot)	Intra-day		Inter-day	
		Accuracy (%)	Precision (% RSD)	Accuracy (%)	Precision (% RSD)
Lopinavir	320	98.57 ± 1.37	0.48	98.84 ± 1.84	1.38
	480	97.83 ± 1.74	1.52	99.74 ± 1.52	0.92
	640	99.58 ± 1.32	0.86	99.28 ± 1.77	1.29
Ritonavir	80	99.26 ± 1.63	0.94	100.05 ± 1.12	1.43
	120	97.69 ± 1.82	0.45	97.21 ± 1.36	0.82
	160	99.34 ± 1.94	1.68	101.28 ± 1.27	0.62

© Copyright reserved by IJPRS

show any spot, while the chromatogram of the solution of the tablet matrix spiked with Lopinavir and Ritonavir showed clear, compact and well- separated peaks of Lopinavir and Ritonavir (Fig. 4). Moreover, from Fig. 4, it can be seen that no other peaks were eluted besides the two active compounds. The method was therefore considered to be specific.

RESULTS OF ANALYSIS OF TABLET FORMULATION

Analysis of samples of marketed antiretroviral tablet containing Lopinavir 200mg and Ritonavir 50mg was carried out and the amounts recovered were expressed as a percentage amount of the label claims. The percentage recovery of Lopinavir and Ritonavir was 98.66 ± 1.48 %. and 98.76 ± 1.80 %.,respectively, and these values are complying with the assay specifications for active drugs in the United States of Pharmacopoeia (90.0–110.0%), which are required to be met by most drug formulations.

CONCLUSION

A quick, precise and accurate method based on HPTLC has been developed for routine analysis of Lopinavir and Ritonavir in fixed-dose combination tablets. The method was validated for linearity, precision, accuracy and specificity. It has the advantage over HPLC methods in general. It consumed less than 35mL of mobile phase per run (8 samples per plate), whereas HPLC methods would consume more than 50mL per runs of similar number of samples. If we consider the time from sample preparation to densitometric evolution for one plate, the new method took an average of 1h, whereas HPLC methods would generally take more than 2h for the same number of samples. It is cheap, quick and does not use chloroform, therefore suitable for routine analysis of Lopinavir and Ritonavir in fixed-dose combination tablets. When compared with the reported HPLC method, the developed HPTLC method is both time and cost effective for the determination of Lopinavir and Ritonavir mixtures like bulk and tablet dosage form. It can be applied for single component analysis of lopinavir and ritonavir separately in bulk and tablet also.

REFERENCES

1. Thakkar H, Patel K, “A first-derivative spectrophotometric method for the estimation of lopinavir in tablets”, Chronicles of Young Scientists, 2010, 1(3), 22-25.
2. Nagulwar V, Bhusari K, “Simultaneous estimation of ritonavir and lopinavir by absorption ratio (Q-analysis) UV spectrophotometric method in combined tablet dosage form”, Pharmacia Lettre, 2010, 2(1), 196-200.
3. Dias, Carolina L, Bergold, “UV-Derivative Spectrophotometric Determination of Ritonavir Capsules and Comparison with LC Method”, Analytical Letters, 2009, 42(12), 1900-1910.
4. Behera, Anindita, Moitra, “Simple validated isocratic RP-LC method for estimation of ritonavir in bulk and tablet dosage form”, Pharmacia Lettre, 2011, 3(1), 145-151.
5. Dias CL, Rossi RC, Donato, “LC determination of ritonavir, a HIV protease inhibitor, in soft gelatin capsules”, Chromatographia, 2005, 62(11-12), 589-593.
6. Donato EM, Dias CL, Rossi, “LC method for studies on the stability of lopinavir and ritonavir in soft gelatin capsules”, Chromatographia , 2006, 63(9-10), 437-443.
7. Suneetha A; Kathirvel S, Ramachandrika G, “A validated RP HPLC method for simultaneous estimation of lopinavir and ritonavir in combined dosage form” International Journal of Pharmacy and Pharmaceutical Sciences, 2011, 3(1), 49-51.
8. Marzolini C, Telenti A, Buclin T, Biollaz J, Decosterd LA, “Simultaneous determination of the HIV protease inhibitors indinavir, amprenavir, saquinavir, ritonavir, nelfinavir and the non-nucleoside reverse transcriptase inhibitor efavirenz by high-performance liquid chromatography after solid-phase extraction”, Journal of chromatography Biomedical sciences and applications, 2000, 740(1), 43-58.

© Copyright reserved by IJPRS

9. Damaramadugu R, Inamadugu J, Kanneti R, "Simultaneous Determination of Ritonavir and Lopinavir in Human Plasma after Protein Precipitation and LCMS-MS" Chromatographia, 2010, 71(9/10), 815-824.
10. D'Avolio A, Simiele M, Baietto L, "HPLC-MS method for the quantification of nine anti-HIV drugs from dry plasma spot on glass filter and their long term stability in different conditions" Journal of Pharmaceutical and Biomedical Analysis, 2010, 52(5), 774-780.
11. Myasein F, Kim E, Zhang J, "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" ,Analytica Chimica Acta, 2009, 651(1), 112-116.
12. Yadav M, Rao R, Kurani H, "Application of a rapid and selective method for the simultaneous determination of protease inhibitors, lopinavir and ritonavir in human plasma by UPLC-ESI-MS/MS for bioequivalence study in Indian subjects" Journal of Pharmaceutical and Biomedical Analysis, 2009, 49(4), 1115-1122.
13. Patel D, Desai S, Savaliya R, "Simultaneous HPTLC determination of lopinavir and ritonavir in combined dosage form" Asian Journal of Pharmaceutical and Clinical Research, 2011, 4(1), 59-61.
14. International Conference on Harmonization Guidance for Industry, In Q2A Text on Validation of Analytical Methods, Switzerland, IFPMIA, 1994, 1–4.
15. International Conference on Harmonization Guidance for Industry, In Q2B Text on Validation of Analytical Methods, Switzerlsis April 1999;41(2).

© Copyright reserved by IJPRS