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A NOVEL ISOCRATIC RP-HPLC METHOD DEVELOPMENT & VALIDATION OF LOPINAVIR AND RITONAVIR

A. Kiran Kumar*, K. Krishna Chaitanya, N. Sanni Babu

A. U .College of pharmaceutical sciences, Andhra University, Visakhapatnam, Andhra Pradesh-530 003, INDIA.

**Corresponding author E-mail: kiranankalla@gmail.com*

ABSTRACT

A Novel, Selective and Rapid, Isocratic Reversed Phase High Performance Liquid Chromatographic (RP-HPLC) method for the analysis of Lopinavir and Ritonavir in binary mixture has been developed and validated. The Liquid Chromatographic system consisting of LC -10AT VP series model chromatograph, the separation was achieved from Zorbax C18 column (150 x 4.6mm, 5 µm) using mobile phase containing a mixture of buffer and acetonitrile was prepared in the ratio of 55:45%v/v. The samples were monitored at 210 nm for detection at a flow rate of 1.5 mL/min and the retention time of Lopinavir and Ritonavir was found to be 4.323 and 5.656mins. The calibration curve was linear over the concentration range 50-300µg/mL for Lopinovir and of 12-76µg/mL for Retinovir respectively. The proposed method is accurate in the range of 98.6% - 101.00% recovery and precise (%RSD of intraday variation and %RSD of inter day variation were found to be within the acceptable criteria). Therefore, this method can be used as a more convenient and efficient option for the analysis of Lopinavir and Ritonavir in Quality control laboratory.

Key words: Lopinovir, Retinovir, Novel RP-HPLC and Isocratic.

INTRODUCTION

Lopinavir is chemically known as [1 S-[1 R*,(R*), 3 R*, 4 R*]]- N-[4-[[(2,6 dimethyl phenoxy)acetyl]amino]-3-hydroxy-5-phenyl-1-(phenylmethyl)pentyl]tetrahydro-alpha-(1-methyl ethyl) -2-oxo-1(2H)-pyrimidin eacetamide. Ritonavir is chemically known as 10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methyl ethyl) - 4-thiazolyl]-3,6-dioxo-8,11 bis (phenyl methyl)-2,4,7,12-tetraazatridecan-13-oic acid, 5-thiazolylmethyl ester, [5 S- (5 R*,8 R*,10 R*,11 R*)]. On literature[4-9] survey it was found that Lopinavir and Ritonavir are estimated by several methods including HPLC, LC-MS in plasma, also determined its intermediates and metabolites. However only one HPLC method was found for estimation of Lopinavir and Ritonavir and no method is available for such estimation in the pharmacopoeia, in view of the need for a suitable method for routine analysis of Lopinavir and Ritonavir in formulations, attempts are being made to develop simple, precise and accurate analytical method and extend it for their determination in formulation. The proposed method is suitable for the analysis of pharmaceutical formulation in analytical laboratories.

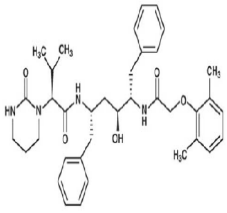

Figure: 1 Structure of Lopinavir

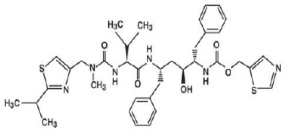

Figure: 2 Structure of Ritonavir

MATERIALS AND METHODS:

Instrumentation:

The liquid chromatographic system consisting of the following components was used for analysis. LC -10AT VP series model chromatograph equipped with symmetry C8 (4.6 x 150mm, 5 µm) was employed for the study. Sample injection was done and the output signal was monitored and integrated by Spinchrome software.

Drugs and chemicals : The reference standard Lopinavir & Ritonavir were obtained from Hetero Pharma Ltd. Methanol, Acetonitrile and Glacial Acetic acid is of HPLC grade and Ammonium acetate was of GR Grade (Merck Ltd. Mumbai, India) Milli-Q water was used throughout the analysis.

Preparation of buffer: Weighed & transferred about 0.77g of Ammonium acetate into a beaker containing 1000ml of Water and dissolved completely. The pH of the Solution was adjusted to 6.5±0.05 with glacial acetic acid and then filtered through 0.45µm membrane filter.

Preparation of Mobile phase: A mixture of buffer and acetonitrile was prepared in the ratio of 55:45%v/v.

Preparation of Ritonavir Standard stock solution: Accurately weighed and transferred about 50 mg of Ritonavir working standard into a 100 ml volumetric flask and then added 70ml of diluent and sonicated to dissolve and volume was made up with diluent.

Preparation of Lopinavir Standard stock solution: Accurately weighed and transferred about 100 mg of Lopinavir working standard into a 100 ml volumetric flask and then added 70ml of diluent and sonicated to dissolve and volume was made up with diluent.

Standard preparation: Transferred 5.0 ml of Ritonavir standard stock solution and 10 ml of Lopinavir standard stock solution into a 50 ml volumetric flask, diluted to volume with diluent and mixed and then filtered the solution through 0.4µm membrane filter.

Optimization of method (or) Procedure: Equilibrate the column for at least 30minutes with mobile phase flowing through the system with flow rate of 1.5mL/minute. Detection was set at a wavelength of 210 nm, with the optimized chromatographic conditions a steady base line was recorded. Separately inject appropriate aliquots (20µL) of diluent standard preparations and sample preparations into the chromatograph, record the chromatograms and measure the peak area responses for the major peak. The chromatogram of Lamivudine and Tenofovir blank, placebo and standard were shown in Figure: 3 and 4.

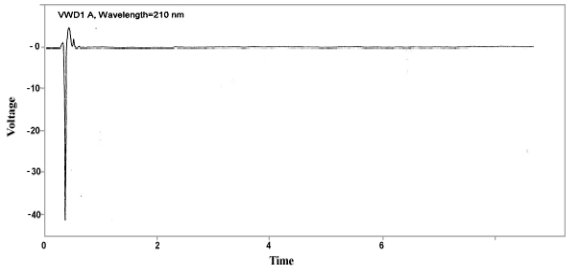

Figure: 2 A Typical Chromatogram of Lopinavir and Ritonavir of Blank

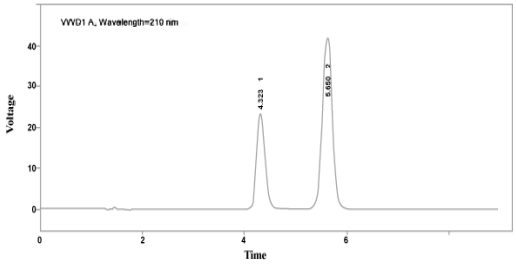

Figure: 4 A Typical Chromatogram of Lopinavir and Ritonavir Standard

ANALYTICAL METHOD VALIDATION

Precision: Precision is the measure of how close the data values to each other for a number of measurements under the same analytical conditions. Six replicate measurements/injections of standard preparation (System Precision), six replicate analysis of the samples through the complete analytical procedure from sample preparation (Method Precision) was performed. The results were found to be for Ritonavir 101.2 % and Lopinavir 98.5 %.

Accuracy: The accuracy of the HPLC method was confirmed by recovery studies by spiking 50,100 & 150% of pure drugs for Lopinavir and Ritonavir to the pre analyzed samples and the samples after dilution injected into the system (n=3).

Table: 2 Accuracy of Ritonavir

Amount added(mg)	Amount found(mg)	% Recovery	Statistical Analysis of % Recovery	
25.25	25.06	99.2	MEAN	99.5
25.12	25.13	100	SD	0.44
25.25	25.08	99.3	%RSD	0.44
50.25	49.72	98.9	MEAN	99.3
50.12	49.99	99.7	SD	0.4
50.12	49.72	99.2	%RSD	0.4
75.25	73.87	98.2	MEAN	98.6
75.2	74.63	99.2	SD	0.53
75.25	74.06	98.4	%RSD	0.54

Table: 3 Accuracy of Lopinavir

Amount added(mg)	Amount found(mg)	% Recovery	Statistical Analysis of % Recovery	
	101.03	100.5	MEAN	100.8
100.25	101.15	100.9	SD	0.26
100.2	101.18	101.0	%RSD	0.26
200.25	197.43	98.6	MEAN	98.8
200.12	198.61	99.2	SD	0.32
200.2	197.59	98.7	%RSD	0.32
300.2	299.60	99.8	MEAN	100.1
300.2	302.09	100.6	SD	0.42
300.25	300.33	100.0	%RSD	0.42

Linearity & Range: A linear response of peak area was observed over the concentration range 12.7- 76.2 µg/mL and 50-300µg/mL for Ritonavir and Lopinavir respectively. 20 µL of each samples solution was injected (n=3) under above chromatographic conditions and average peak area for Ritonavir and Lopinavir was measured. The linearity data and calibration curves of Ritonavir and Lopinavir are presented in Table: 4& 5.

Table: 4 Linearity of Ritonavir

Concentration ppm	Average Area
12.710	321303
25.420	639970
38.130	963132
50.840	1276473
63.550	1614124
76.260	1925699

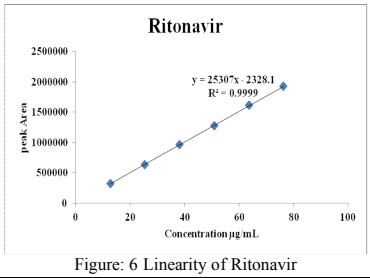

Figure: 6 Linearity of Ritonavir

Table: 5 Linearity of Lopinavir

Concentration ppm	Average Area
50.125	1595307
100.250	3102538
150.375	4583171
200.500	6138331
250.65	7666791
300.750	9024778

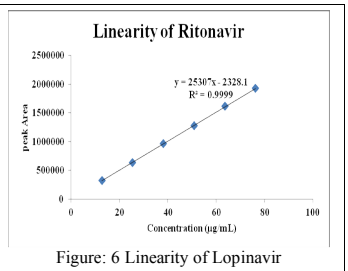

Figure: 6 Linearity of Lopinavir

Robustness: The robustness of the method was determined as per USP guidelines under a variety of conditions including changing the in the flow rate by ± 20% or ±0.2 mL, composition of organic phase ± 2%, change, pH of buffer by ± 0.2 and using different columns. No marked changes were observed in the system suitability parameters and peak area.

Results and Discussion: By applying the proposed method, the retention times of Ritonavir and Lopinavir were found to be 4.323 and 5.656mins. The number of theoretical plates obtained was 7591 and 6776 for Ritonavir and Lopinavir respectively, which indicates the efficiency of the column. The regression equations of concentration of Ritonavir and Lopinavir over their peak areas were found to be y = 25307x - 2328.1(R^2 = 0.9999) and y = 29864x + 112385(R^2 = 0.9997) respectively where Y is the peak area and X is the concentrations of Ritonavir and Lopinavir (µg/mL). The high percentage recovery indicates that the proposed method is highly accurate.

Conclusion: A simple, specific, accurate, precise, RP-HPLC method has been developed which can be used for accurately quantitative estimation of Lamivudine and Tenofovir for routine analysis of individual and combination of drugs. Method was validated as per ICH Q2 (R1) so it can be used by pharmaceutical industries.

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