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RP-HPLC method development and validation for simultaneous estimation of Lopinavir and Ritonavir in tablet dosage form

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ABSTRACT

Simultaneous Estimation of Lopinavir and Ritonavir were carried out by RP-HPLC using Acetate buffer (PH 6.5): Acetonitrile (55:45) and column Phenomenex Luna C-18 (250*4.6 mm, 5um) as a stationary phase and peak was observed at 255 nm which was selected as a wavelength for quantitative estimation. After the development of the method, it was validated for specificity, linearity, precision, accuracy, robustness and ruggedness studies.

1. INTRODUCTION

Chemical name of Lopinavir is (2S)-N-[(2S,4S,5S)-5-[2-(2,6-dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]3-methyl-2-(2-oxo-1,3-diazinan-1-yl)butanamide. It is freely soluble in methanol and ethanol, soluble in isopropanol and practically insoluble in water.

Lopinavir inhibits HIV protease, causing the enzyme incapable of processing the polyprotein precursor. This leads to the production of non-infectious and immature HIV particles.

Chemical name of 1,3-thiazol-5-ylmethylN-[(2S,3S,5S)-3-hydroxy-5-[(2S)-3-methyl-2-{[methyl({[2-(propan-2-yl)-1,3-thiazol-4yl]methyl})carbamoyl]amino}butanamido]-1,6-diphenylhexan-2-yl]carbamate. It is freely soluble in methanol and ethanol, soluble in isopropanol and practically insoluble in water.

Ritonavir, a selectively competitive reversible inhibitor of HIV protease, interferes with the formation of essential proteins and enzymes. After which, the formation of immature and non-infectious viruses follows. It also interferes with the production of infectious HIV and limits further infectious spread of the virus.

2. MATERIALS AND METHODS

Drug samples: Lopinavir and Ritonavir were generously given by Lyka laboratories, Mumbai. Lopinavir (Assay: 99.80%) and Ritonavir (Assay: 99.65%) were used as standards.

Tablets used: Brand: **EMELTRA**; Lopinavir 200mg and Ritonavir 50mg

Chemicals and solvents used: All the solvents and reagents used were HPLC grade. Water, Acetonitrile, Ammonium acetate, Acetic acid, Methanol and Distilled water.

Instruments used: Shimadzu Isocratic HPLC system with following configurations, LC-10AT Vp series, Isocratic solvent delivery system (pump). Rheodyne 7725i injector with 20 µl loop. Spinchrome data station. Analytical column: Phenomenex – Luna, C_{18} (250 x 4.6 mm i.d., 5µ). UV-Visible – SPD 10AVp series detector.

Preparation of mobile phase:

Preparation of buffer solution: 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: Mobile phase is prepared by mixing 550 ml of buffer and 450 ml of acetonitrile

Mobile-Phase Ratio: Buffer: Acetonitrile (55: 45 % V/V).

3. RESULTS AND DISCUSSION

Optimized chromatographic conditions:

Stationary Phase: Phenomenex Luna C18 (4.6x250 mm 5µ)

Pump	: LC-10AT Vp series
Injector	: Rheodyne
Flow rate	: 1.5 ml/min

Operating temperature : Room temperature
Detection wave length : 255 nm
Mobile phase ratio :Buffer:Acetonitrile (55:45 %V/V)
Diluent : Mobile Phase
Injection Volume : 20µl
Run Time : 20 min

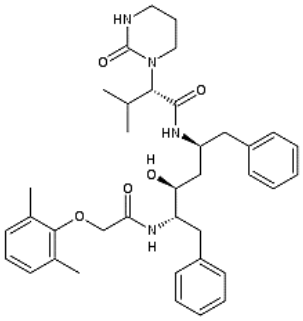

Figure.1.Molecular structure of Lopinavir

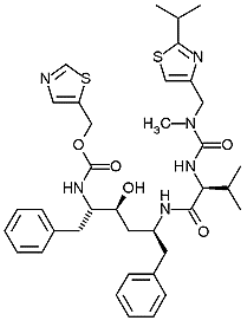

Figure.2.Molecular structure of Ritonavir

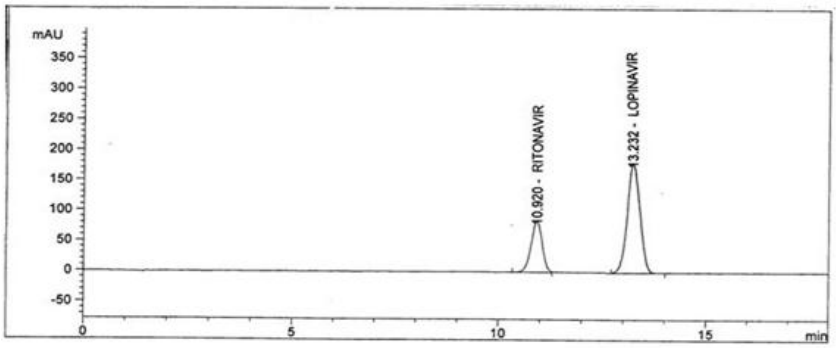

Figure.3.Chromatogram at optimized conditions

Estimation: Estimation of Lopinavir and Ritonavir in dosage forms by the developed RP-HPLC method was carried out. The standard and sample solutions were prepared and the chromatograms were recorded. The assay procedure was performed and the assay percentage was calculated. The assay percentage of individual drugs and amount present per tablet were calculated.

Validation of the method: The suitability of the system was studied by the values obtained for Theoretical plate, Resolution and tailing factor of the chromatogram of standard drugs. The selectivity of the method was revealed by the repeated injection of mobile phase and no interference was found. The accuracy of the method was determined by recovery experiments. The recovery studies were carried out by preparing 6 individual samples with same procedure from the formulation and injecting. The percentage recovery was calculated. From the data obtained, added recoveries of standard drugs were found to be accurate.

The system and method precision of the method were demonstrated by inter day, intraday and repeatability of injection studies. All the solutions were injected into the chromatographic system. The peak area and percentage relative standard deviation were calculated. From the data obtained, the developed HPLC method was found to be precise.

The standard drug solutions of varying concentrations ranging from 20% to 120% of the targeted level of the assay concentration (ie) 5 µg/ml to 30 µg/ml of Ritonavir and 20µg/ml to 120µg/ml of Lopinavir, were examined by the proposed method. The response factor, slope, intercept, correlation co-efficient and Residual sum of squares values were calculated. The slope, intercept and correlation coefficient(r) were found to be 49.77, 134.3 and 0.999 respectively for Ritonavir and 6.004, 66.80 and 0.999 respectively for Lopinavir. The calibration curves were plotted using response factor Vs concentration of the standard solutions. The calibration graph shows that linear response was obtained over the range of concentrations used in the assay procedure. These data demonstrates that the methods have adequate sensitivity to the concentrations of the analytes.

The robustness of the method was studied by carrying out experiments by changing conditions discussed earlier. The response factors for these changed chromatographic parameters were almost same as that of the fixed chromatographic parameters and hence developed method is said to be robust.

Table.1.Parameters of simultaneous estimation of Lopinavir and Ritonavir by RP-HPLC

Parameter	Limits	Observation	
		Lopinavir	Ritonavir
Specificity	No interference	No interference	No interference
System precision Method precision	RSD NMT 2.0%	0.06 0.062	0.20 0 .53
Linearity range	Correlation coefficient NMT –0.999	0.999	0.999
Accuracy	% Recovery range 98 – 102 %	99.88-99.90	99.86-99.99
Limit of Detection	Signal noise ratio should be more than 3:1	0.153	0.043
Limit of Quantitation	Signal noise ratio should be more than 10:1	0.462	0.132
Asymmetry factor	NMT 2%	1.012	1.001
Number of Theoretical Plates	NLT 2000	7599	6782
Robustness, Change in column temperature, Change in buffer pH		No effect on system suitability parameters	No effect on system suitability parameters

4. CONCLUSION

Based on the results observed, it was concluded that proposed method can be used for routine analysis of Lopinavir and Ritonavir.

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