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ORIGINAL ARTICLE **OPEN ACCESS**

Advanced, Simple, Sensitive, Selective Reverse Phase Liquid Chromatography Technique for The Quantitative Determination of Ritonavir and Lopinavir in In-House Dosage Form

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ABSTRACT

*For the estimation of Ritonavir (RITO) and Lopinavir (LOPI) in tablets, an accurate, simple, novel, specific, robust, and stability indicating approach was created. The developed method was rapid and economic. On a Kromasil C_{18} 250*4.6 mm, 5 column, orthophosphoric acid (OPA): acetonitrile (ACN) (48:52) was used to obtain the desired chromatographic separation. Flow rate of 1 mL/min with a dual wavelength UV detection was used (260 nm for LOPI and 238 nm for RITO). The retention time (Rt) of RITO and LOPI are 3.170 min and 2.214 min respectively. The developed method was specific and well separated from the impurities of both LOPI and RITO. The method is linear in a range of 50% to 150% for both LOPI and RITO. The r^2 values are 0.999 & 0.999 for LOPI and RITO respectively observed as correlation coefficient. Both the standard solutions and the test solutions confirmed 48-hour stability. The strategy indicates stability after a forced degradation inquiry. The developed approach can be applied to routinely analyse LOPI and RITO in fixed dose combinations.*

Keywords: *Lopinavir, Ritonavir, Fixed dose, Developed method.*

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INTRODUCTION

Lopinavir (LOPI) is a white to off-white, crystalline power, soluble in Dimethylformamide (DMF), Methanol, Dimethyl sulfoxide (DMSO), water, and ethanol. This comes under the classification of the protease inhibitor antiretroviral drug. This antiretroviral drug is used either alone or in concomitant with other antiretroviral drugs (such as ritonavir) for HIV treatment. Its molecular formula is $C_{37}H_{48}N_4O_5$ and molecular weight is 628.8008 g/mol. [1] Ritonavir (RITO) is a white to off-white crystalline compound, soluble in methanol, water, ethanol, DMF, and DMSO. The molecular formula is $C_{37}H_{48}N_6O_5S_2$ & Molecular weight is720.944 g/mol.

MATERIAL AND METHODS

Reagents and Chemicals:

LOPI and RITO – Pharmaceutically active ingredients (API) – MSN Laboratories Limited Hyderabad. Methanol (HPLC grade), ACN, Ortho Phosphoric acid and water were applied as solvent.

Instruments Used:

Analysis was conducted by analytical balance Sartorius, HPLC used is Shimadzu LC-2010 with photo diode array detector. Column used in HPLC is Kromasil C_{18} 250*4.6 mm with 5µ column µm. Other tools were employed, including a sonicator, a water bath, and a hot air oven made by thermo.

Preparation of Buffer: Preparation of Ortho Phosphoric Acid buffer (0.1%): Take one ml of Ortho phosphoric acid solution in a thousand ml of volumetric flask, make up to final volume with water.

Preparation of Mobile Phase: Ortho phosphoric acid: Acetonitrile (48:52) Filter this through 0.45 µm PVDF membrane filter.
Diluents: Acetonitrile:Water (50:50).
Standard stock solutions preparation: 10 mg of LOPI, 2.5 mg of RITO were weighed accurately and transferred to 10 ml volumetric flask. Then diluents were placed to 3/4th of the flask and for 10 minutes it was sonicated. The volume was made up with the diluents and represented as a stock solution. [2]
Standard working solutions preparation: One ml from every stock solution was transferred out into a ten ml volumetric flask, where it was adjusted with the diluents.
Sample stock solutions preparation: One tablet was weighed, powdered and then was taken in a 100 mL volumetric flask, 50mL of diluents mixed and for 25 mins it was sonicated, followed by makeup the volume with diluent and filtered.
Preparation of sample working solutions: From the filtered solution 1 ml was pipette out into a 10 ml volumetric flask and made upto 10ml with diluent.

METHOD VALIDATION

Specificity: After determining the suitability of system, standard, placebo, and blank solutions were injected into the HPLC. At the Rts of LOPI and RITO, no disruptions from blank, standard, or placebo solutions was noticed.
Linearity: To check the linearity plotted a graph by comparing the test solution's concentration on the X-axis and the corresponding solution's reaction on the Y-axis, from 50% to the 150% against standard concentrations for each analytes. [3]
Precision: The outcomes were as previously said after six injections were given from a single volumetric flask of working standard solution. For two medications, average area, standard deviation, and percent RSD were computed. The obtained %RSD for LOPI and RITO was 0.8% and 1.1%, respectively. This method was used to adopt the system precision because the precision limitation was less than "2".
Each injection from each working sample solution was given on day two after the sample production, and the results were as they are shown in the above tabulation. Repeated sampling using a sample stock solution was performed, and six functioning sample solutions of the same quantities were generated. Average area, standard deviation and % RSD were estimated for two drugs and obtained as 0.7% and 0.6% respectively for LOPI and RITO. As the Precision limit was less than “2” the system precision was adopted in this method. [4]
Accuracy: The accuracy of test method was carried out using RITO and LOPI, API and placebo at 80 %, 100 %, 120 % spike levels in triplicate. Calculated the percentage recovery and recorded the results.

BENCH TOP STABILITY STUDY FOR TEST AND STANDARD SOLUTION

Preparation: The assay was performed for LOPI, RITO tablets according to the test method for 50-200 mg and kept on bench top for 48 hr after analyzing the initial amount. Injected the samples at initial, 24 hr and 48 hr. Calculated the assay against the newly processed standard solution and checked the difference in assay of the samples between the initial and bench top stability samples. [5-6]
Robustness: Performed the robustness by altering the flow rate by ± 0.1mL/min from 1.0 mL/min, column oven temperature by ± 5 ºC from 35 ºC and buffer pH by ± 0.2 from 5.0. By changing the aforementioned parameters, the standard solution was created and the system appropriateness criteria were assessed. The system's suitability standards were within each adjusted parameter's bounds.
Filter Integrity Test: Performed the filter validation studies on RITO- LOPI Tablets 50-850 mg by preparing the test sample. A portion of the test sample was centrifuged and the remaining portion of test solution was filtered with PVDF and PTFE filters. Calculated the % assay and calculated the difference in assay from the assay obtained by centrifuging. [7-8]
Forced Degradation Study: Performed the forced degradation of test method to demonstrate the non-interference of impurities, degradation products in quantification of analyte by different types of stress conditions like acid degradation, base degradation, degradation peroxide and thermal degradation. [9-10]

Table 1: Optimized Chromatographic Conditions

Column	Kromasil C_{18} 250*4.6 mm, 5µ
Detector	Dual wavelength UV detector
Wavelength	260 nm for LOPI& 238 nm for RITO
Flow rate	1.0 mL/min
Injection Volume	20 µL
Column oven Temp.	35^0C
Sample tray	Ambient (25^0C)
Run time	10.0 Min

Table 2: Specificity

S.No.	Sample Solution Name	Retention Time
1	Blank	No Peaks
2	Placebo	No Peaks
3	Standard	LOPI- 2.230 RITO- 3.187

TABLE 3: Linear regression analysis of RITO and LOPI

Parameters	RITO	LOPI
Linearity range (µg/mL)	10-60	10-60
Correlation Coefficient (R2)	0.9999	0.9999
Slope	25913	31824

Table 4: Precision Studies Of RITO And LOPI

Drug	Actual Concentration	Precision Data	% RSD
RITO	10 µg	9.99	0.0786773
LIPO	10µg	10.02	0.117498

Table 5: Percentage Recovery Study Results Of RITO And LOPI

Amount Taken (µg/ml)		Amount Added (µg/ml)		% Recovery		% RSD	
RITO	LIPO	RITO	LIPO	RITO	LIPO	RITO	LIPO
32	32	40	40	100.2	100.5	0.000262	0.000141643
40	40	40	40	99.8	99.9	0.000197	0.000128214
48	48	40	40	99.7	100	0.000108	0.00017469

TABLE 6: FORCED DEGRADATION LOPI AND RITO

Sl. No.	Stress Condition	LOPI	RITO	Acceptance criteria
1	Acid Degradation	Passes	Passes	Peak purity shall pass
2	Base Degradation	Passes	Passes	
3	Degradation Peroxide	Passes	Passes	
4	Thermal Degradation	Passes	Passes	

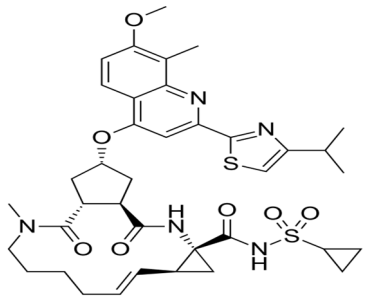

Fig. 1: Lopinavir

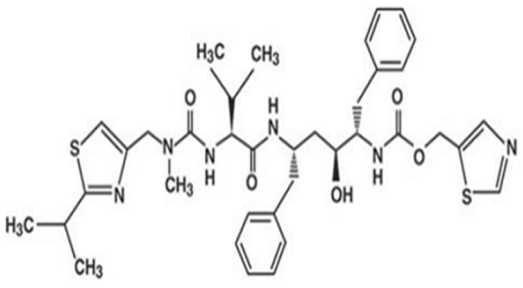

Fig. 2: Ritonavir

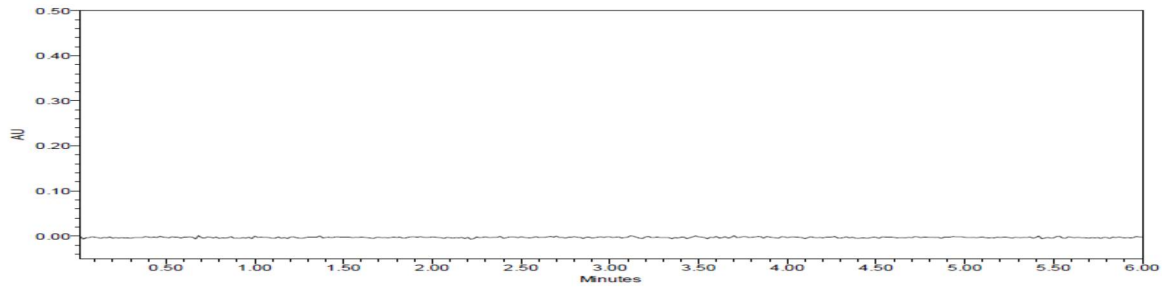

Fig 3: Interpretation of Chromatogram - Blank

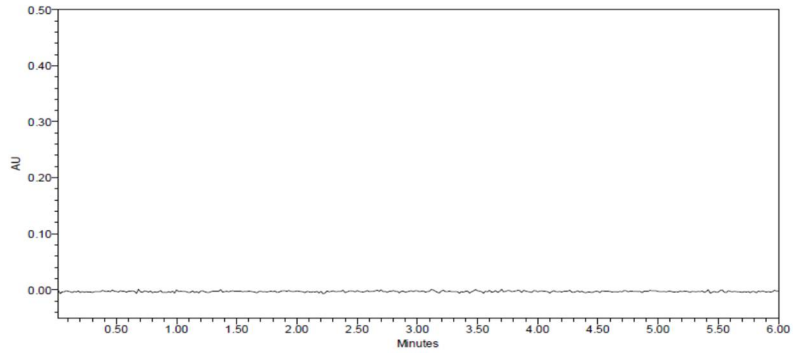

Fig 4: Interpretation of Chromatogram – Placebo

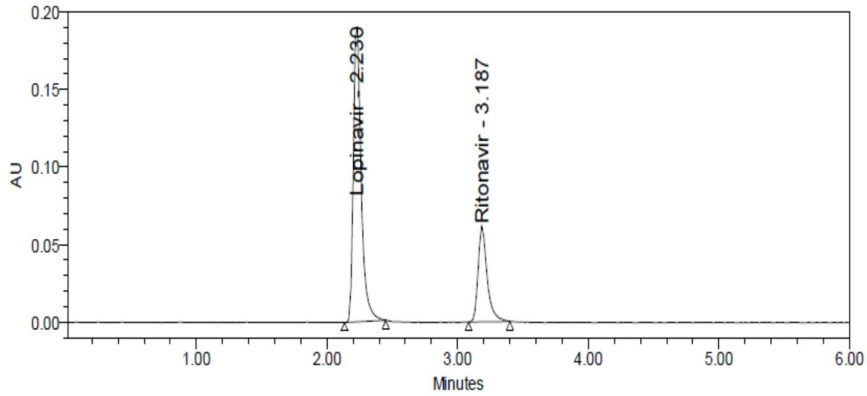

Fig 5: Interpretation of Typical Chromatogram of LOPI and RITO

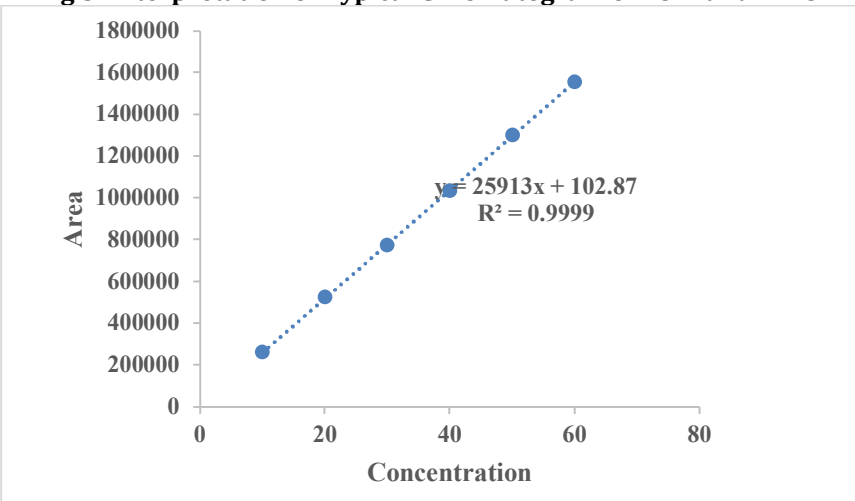

Fig 6: Linearity Graph of RITO

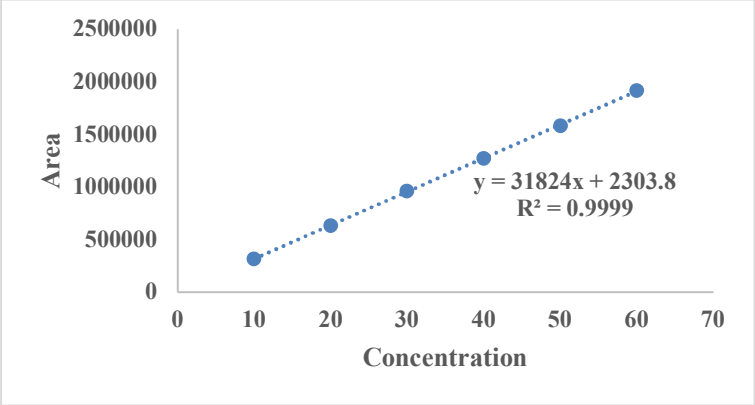

Fig 7: Linearity Graph of LOPI

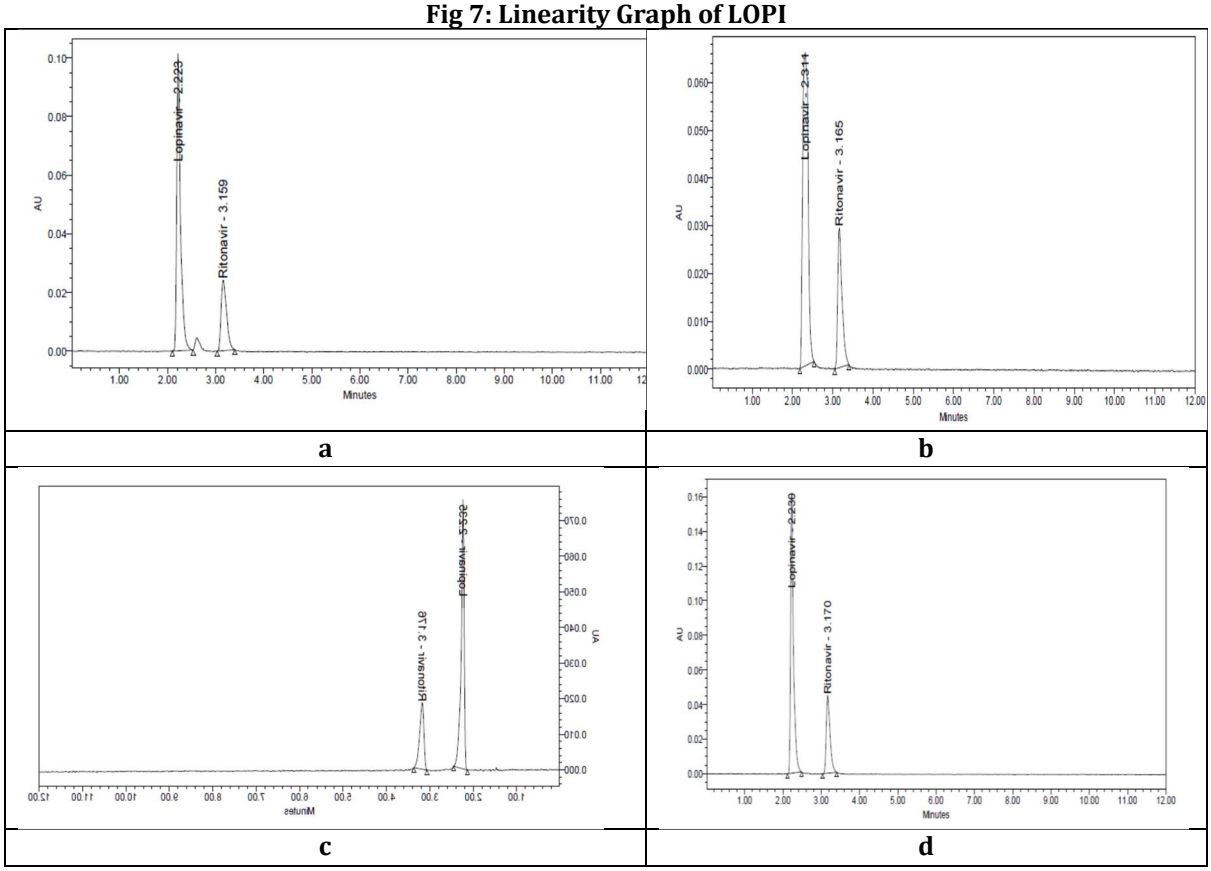

Fig 8: Degradation Chromatograms: (a) Acid Chromatogram, (b) Base Chromatogram, (c) Peroxide Chromatogram, (d) Thermal Chromatogram

CONCLUSION

A new RP-HPLC method has been developed for the simultaneous estimation of RITO and LOPI in marketed formulation. The method showed good satisfactory outcome (Resolution) between the above discussed two drugs and also with degradants in forced degradation study. The two analyte peaks were well separated from the impurities of LOPI and RITO. The developed technique was validated for linearity, specificity, robustness, precision, accuracy, and system stability. It has proven to be economical, distinctive simple, accurate, and suggestive of stability. As a result, the proposed RP-HPLC method is suitable for routine RITO and LOPI analysing in pharmaceutical dosage forms in quality control labs.

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CONFLICT OF INTEREST

According to the authors, they are free of any conflicts of interest. There are no studies by any of the authors in the article that involved using either human or animal subjects.

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