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Research Article

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## Analytical Method Development and Validation by RP-HPLC for Simultaneous Estimation of Lopinavir and Ritonavir in Combined Tablet Dosage form

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### ABSTRACT

A simple, sensitive and precise reverse phase high performance liquid chromatographic method has been developed for the simultaneous estimation of Lopinavir and Ritonavir in pharmaceutical dosage form. The mobile phase consisted of Methanol : phosphate buffer (70:30) 0.8ml /10 min and wavelength of detection at 260 nm. The retention times of were Lopinavir and Ritonavir 3.527 min and 3.003 min respectively. Chromatograms was run through Inertsil ODS C<sub>18</sub> (4.6, 150mm, 5µm). The % RSD of the Lopinavir and Ritonavir were found to be 2%. The % Recovery was obtained 98-102% of Lopinavir and Ritonavir. The linearity range of Lopinavir and Ritonavir 100-500µg/ml. The method was validated in terms of linearity, precision, accuracy, limit of detection, limit of quantification.. The coefficient of variance for both the drug was more than 0.999. The proposed method can be used for determination of these drugs in combined dosage forms.

**Keywords:** Lopinavir, Ritonavir, RP-HPLC

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## 1. Introduction

Lopinavir is a chemically named as (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 [1]. Lopinavir (ABT-378) is an antiretroviral of the protease inhibitor class. It is marketed by Abbott as Kaletra, a co-formulation with a sub-therapeutic dose of ritonavir, as a component of combination therapy to treat HIV/AIDS [2, 3]. Lopinavir inhibits the HIV viral protease enzyme. This prevents cleavage of the gag-pol polyprotein and, therefore, improper viral assembly results. This subsequently results in non-infectious, immature viral particles [4].

Ritonavir is a chemically named as 1,3-thiazol-5-ylmethyl N-[(2S,3S,5S)-3-hydroxy-5-[(2S)-3-methyl-2-[[methyl (2-(propan-2-yl)-1,3-thiazol-4-yl)methyl]]carbamoyl] amino]butanamide]-1,6-diphenylhexan-2-yl]carbamate[5]. An HIV protease inhibitor that works by interfering with the reproductive cycle of HIV. Ritonavir inhibits the HIV viral proteinase enzyme which prevents cleavage of the gag-pol polyprotein, resulting in noninfectious, immature viral particles [6].

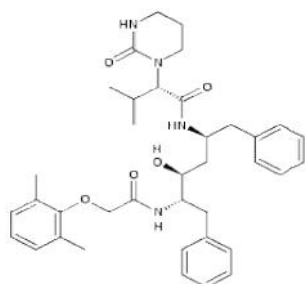


Figure 1: Structure of Lopinavir

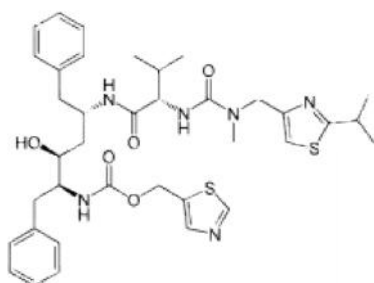


Figure 2: Structure of Ritonavir

## 2. Experimental

### HPLC Method Development:

#### Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions[7]. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 3.0), Methanol in proportion 30: 70 v/v respectively.

#### Wave length selection:

UV spectrum of 10 µg / ml Lopinavir and Ritonavir diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV

spectrum wavelength selected as 260. At this wavelength both the drugs show good absorbance [8].

#### Optimization of Column:

The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. Inertsil ODS (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 0.8ml/min flow[9].

#### Optimized chromatographic conditions:

Instrument used : Waters HPLC with auto sampler and PAD or detector.

Temperature : Ambient

Column : Inertsil ODS (4.6 x 150mm, 5µm)

Buffer : 6.8 grams of potassium dihydrogen ortho phosphate in 1000 ml water pH adjusted with ortho phasparic acid.

pH : 3.0

Mobile phase : 30% buffer 70% Methanol

Flow rate : 0.8 ml per min

Wavelength : 260 nm

Injection volume : 10 µl

Run time : 10min.

#### Preparation of buffer and mobile phase:

##### Preparation of Phosphate buffer:

Accurately weighed 6.8 grams of  $\text{KH}_2\text{PO}_4$  was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid[10].

##### Preparation of mobile phase:

Accurately measured 300 ml (30%) of above buffer and 700 ml of Methanol HPLC (70%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

**Diluent Preparation:** The Mobile phase was used as the diluents.

#### Preparation of the Lopinavir & Ritonavir Standard & Sample Solution:

##### Standard Solution Preparation:

Accurately weigh and transfer 10 mg of Lopinavir and Ritonavir 10mg of working standard into a 10mL & 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent [11]. Further pipette 3ml & 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

##### Sample Solution Preparation:

Accurately weigh 10 tablets crush in mortar and pestle and transfer equivalent to 10 mg of Lopinavir and Ritonavir (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 3 ml of Lopinavir e and Ritonavir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents [12].

**Procedure:** Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Lopinavir and Ritonavir peaks and calculate the % Assay by using the formulae.

**System Suitability:**

Tailing factor for the peaks due to Lopinavir and Ritonavir in Standard solution should not be more than 2.0. Theoretical plates for the Lopinavir and Ritonavir peaks in Standard solution should not be less than 2000.

**Calculation: (for Lopinavir)**

$$\text{Assay \%} = \frac{\text{AT} \times \text{WS} \times \text{DT} \times \text{P} \times \text{Avg. wt}}{\text{AS} \times \text{DS} \times \text{WT} \times 100 \times \text{Label Claim}} \times 100$$

Where:

AT = average area counts of sample preparation.

As= average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = LABEL CLAIM OF Lopinavir e mg/ml.

**Calculation: (for Ritonavir)**

$$\text{Assay \%} = \frac{\text{AT} \times \text{WS} \times \text{DT} \times \text{P} \times \text{Avg. wt.}}{\text{AS} \times \text{DS} \times \text{WT} \times 100 \times \text{Label Claim}} \times 100$$

Where:

AT = average area counts of sample preparation.

As= average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = Label Claim of Ritonavir mg/ml.

**Table 1:** Sample and Standard Details

S. No.	Samples
1	Lopinavir and Ritonavir Tablets 50mg & 0.5mg
2	Lopinavir & Ritonavir working standards

**Method Validation Summary:**

**Precision:**

**Preparation of stock solution:** Accurately weigh and transfer 25 mg of Lopinavir and Ritonavir working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent [13]. Further pipette 3 ml of Lopinavir & Ritonavir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Procedure:**

The standard solution was injected for five times and measured the area for all five Injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

**Acceptance Criteria:**

The % RSD for the area of five standard injections results should not be more than 2%.

**Intermediate Precision/Ruggedness:**

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

**Preparation of stock solution:** Accurately weigh and transfer 25 mg of Lopinavir and 10mg of Ritonavir working

standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent [14]. Further pipette 3ml of Lopinavir & Ritonavir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Procedure:**

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

**Acceptance Criteria:** The % RSD for the area of five standard injections results should not be more than 2%.

**Accuracy:**

**Preparation of Standard stock solution:**

Accurately weigh and transfer 10 mg of Lopinavir and Ritonavir 10mg of working standard into a 10mL & 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent [15]. Further pipette 3ml & 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

**Preparation Sample solutions:**

**For preparation of 50% solution (With respect to target Assay concentration):** Accurately weigh and transfer 5mg of Lopinavir and 5.3mg of Ritonavir working standard into a 10mL and 100 ml of clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 3 ml of Lopinavir e & 0.3 ml of Ritonavir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents [16].

**For preparation of 100% solution (With respect to target Assay concentration):**

Accurately weigh and transfer 10 mg of Lopinavir and 10 mg of Ritonavir working standard into a 10mL and 100 ml of clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 3 ml of Lopinavir & 0.3 ml of Ritonavir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**For preparation of 150% solution (With respect to target Assay concentration):**

Accurately weigh and transfer 14.4mg of Lopinavir and 14.5mg of Ritonavir working standards into a 10mL and 100ml of clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 3 ml of Lopinavir & 0.3 ml of Ritonavir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents [17].

**Procedure:**

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Lopinavir & Ritonavir and calculate the individual recovery and mean recovery values.

**Acceptance Criteria:**

- The % Recovery for each level should be between 98.0 to 102.0%.

**Linearity:**

**Preparation of stock solution:** Accurately weigh 10 tablets crush in mortar and pestle and transfer equivalent to 10 mg of Lopinavir and Ritonavir (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent [18].

**Preparation of Level – I (100ppm of Lopinavir & 10ppm of Ritonavir):** 1ml and 0.1 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

**Preparation of Level –II (200ppm of Lopinavir & 20ppm of Ritonavir):** 2ml and 0.2 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

**Preparation of Level–III (300ppm of Lopinavir & 30ppm of Ritonavir):** 3ml and 0.3 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluents [19].

**Preparation of Level – IV (400ppm of Lopinavir & 40ppm of Ritonavir):** 4ml and 0.4 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

**Preparation of Level –V (500ppm of Lopinavir & 50ppm of Ritonavir):** 5ml and 0.5 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent

**Procedure:**

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient [20].

**Acceptance Criteria:** Correlation coefficient should be not less than 0.999.

**Limit of Detection:****Limit of Detection: (For Lopinavir):****Preparation of 300µg/ml solution:**

Accurately weigh and transfer 10 mg of Lopinavir working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent [21]. Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Preparation of 0.12µg/ml solution):**

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipette 0.4mL of 1µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluents [22].

**Calculation of S/N Ratio:**

Average Baseline Noise obtained from Blank

Signal Obtained from LOD solution

$$S/N = 152/52 = 2.9$$

**Acceptance Criteria:** S/N Ratio value shall be 3 for LOD solution.

**Limit of Detection: (For Ritonavir)****Preparation of 3µg/ml solution:**

Accurately weigh and transfer 10mg of Ritonavir working standard into a 100ml clean dry volumetric flask add about

7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent [23]. Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Preparation of 0.015µg/ml solution):**

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents [24]. Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Calculation of S/N Ratio:**

Average Baseline Noise obtained from Blank

Signal Obtained from LOD solution

$$S/N = 156/52 = 3.0$$

**Acceptance Criteria:**

S/N Ratio value shall be 3 for LOD solution.

**Limit of Quantification:****Limit of Quantification (for Lopinavir HCL)****Preparation of 300µg/ml solution:**

Accurately weigh and transfer 10 mg of Lopinavir working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent [25]. Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Preparation of 0.42µg/ml solution):**

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Pipette 1.0mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluents [26].

Pipette 1.4 mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

**Calculation of S/N Ratio:**

Average Baseline Noise obtained from Blank

Signal Obtained from LOQ solution

$$S/N = 522/52 = 10.03$$

**Acceptance Criteria:** S/N Ratio value shall be 10 for LOQ solution.

**Limit of Quantification : (For Ritonavir)****Preparation of 3µg/ml solution:**

Accurately weigh and transfer 10mg of Ritonavir working standard into a 100mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent [27]. Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Preparation of 0.05µg/ml solution):** Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents [28]. Pipette 1.7mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. 152 µV

**Calculation of S/N Ratio:**

Average Baseline Noise obtained from Blank

Signal Obtained from LOQ solution

$$S/N = 524/52 = 10.$$

**Robustness:** As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

- The flow rate was varied at 0.8 ml/min to 1.2ml/min Standard solution 300ppm of Lopinavir & 3ppm of Ritonavir was prepared and analysed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly.
- Hence it indicates that the method is robust even by change in the flow rate  $\pm 10\%$ .
- \*Results for actual flow (1.0ml/min) have been considered from Assay standard [29].
- The Organic composition in the Mobile phase was varied from 50% to 50%. Standard solution 300  $\mu\text{g/ml}$  of Setraline & 3 $\mu\text{g/ml}$  of Ritonavir was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method. On evaluation of the above results, it can be concluded that the variation in 10% [30].
- Organic composition in the mobile phase affected the method significantly [31]. Hence it indicates that the method is robust even by change in the Mobile phase  $\pm 10$
- Results for actual Mobile phase composition (55:45 Methanol: Buffer (ph-2.8) has been considered from Accuracy stand [32].

### 3. Results and Discussion

#### Validation Parameters:

##### 3.1 Precision:

Precision of the method was carried out for standard solutions as described under experimental work. The corresponding chromatograms and results are shown below

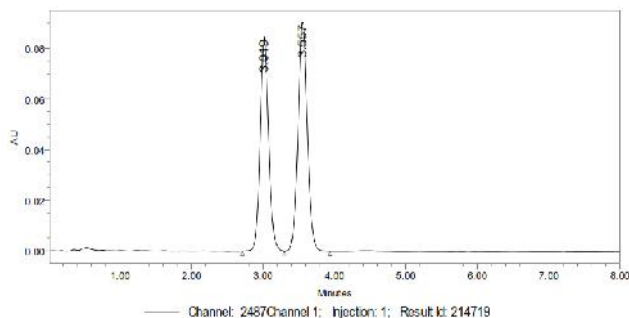


Figure 3: Chromatogram for standard injection-1

Table 2: Results of method precession for Lopinavir

Name : Lopinavir			
	Name	RT	Area
1	Lopinavir	3.557	819305
2	Lopinavir	3.547	807157
3	Lopinavir	3.544	804070
4	Lopinavir	3.537	808474
5	Lopinavir	3.534	804505
Mean			808702
Std. Dev.			6203.7
% RSD			0.77

Table 3: Results of method precession for Ritonavir

Name : Ritonavir			
	Name	RT	Area
1	Ritonavir	3.019	691143
2	Ritonavir	3.011	685431
3	Ritonavir	3.004	683543
4	Ritonavir	2.997	683564
5	Ritonavir	2.994	683532
Mean			685443
Std. Dev.			3289.7
% RSD			0.48

**Acceptance criteria:** %RSD for sample should be NMT 2  
The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

#### Intermediate Precession (Ruggedness):

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

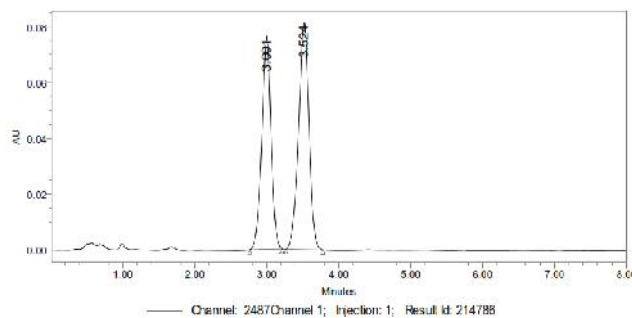


Figure 4: Chromatogram for sample injectioncn-1

Table 4: Results of Intermediate precision for Lopinavir and Ritonavir

Name : Lopinavir			
	Name	RT	Area
1	Lopinavir	3.524	813507
2	Lopinavir	3.533	817673
3	Lopinavir	3.533	815189
4	Lopinavir	3.517	815816
5	Lopinavir	3.530	815356
Mean			815508
Std. Dev.			1492.7
% RSD			0.18

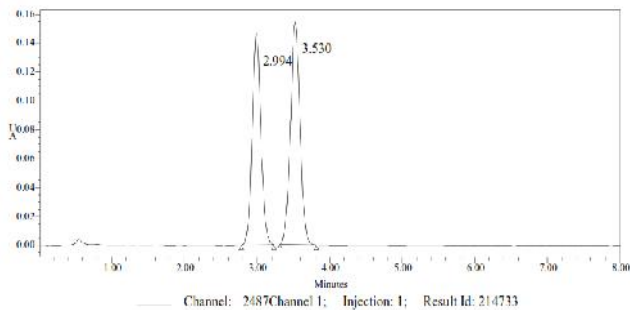
Name : Ritonavir			
	Name	RT	Area
1	Ritonavir	3.001	673725
2	Ritonavir	3.009	672535
3	Ritonavir	3.010	676216
4	Ritonavir	2.997	679037
5	Ritonavir	3.007	677101
Mean			675723
Std. Dev.			2611.5
% RSD			0.39

**Acceptance criteria:**

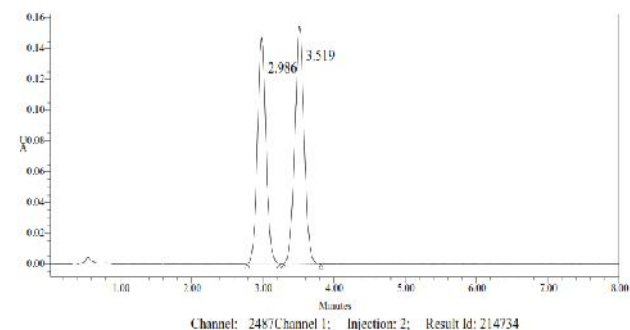
- a. %RSD of five different sample solutions should not more than 2
- b. The %RSD obtained is within the limit, hence the method is rugged.

**3.2 Accuracy:**

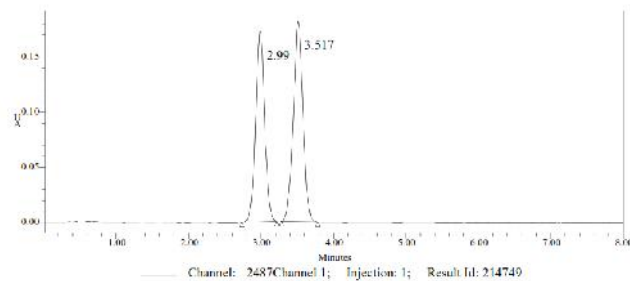
Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.



**Figure 5:** Chromatograms for Sample Concentration-50%



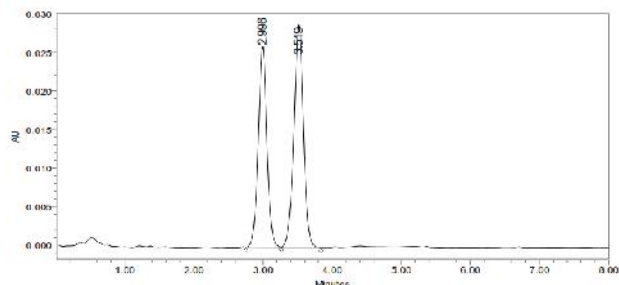
**Figure 6:** Chromatogram for sample concentration-100%



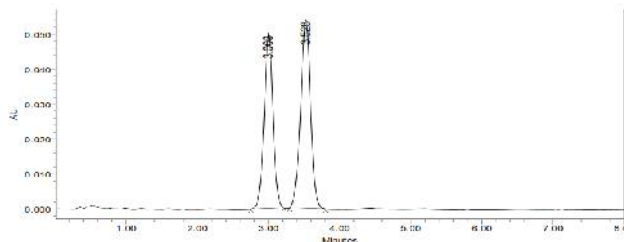
**Figure 7:** Chromatogram for sample concentration-150%

**3.3 Linearity:**

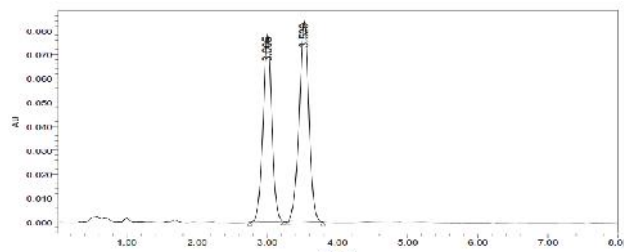
The linearity range was found to lie from 100µg/ml to 500µg/ml of Lopinavir, 10µg/ml to 50µg/ml Of Ritonavir and chromatograms are shown below.



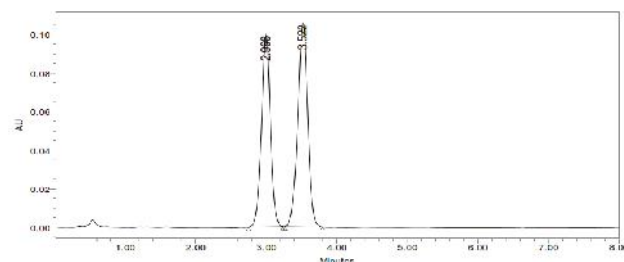
**Figure 8:** Chromatogram for linearity concentration-100µg/ml of Lopinavir & 10 µg/ml of Ritonavir



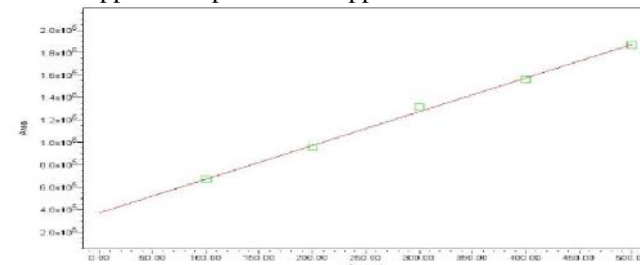
**Figure 9:** Chromatogram for linearity concentration-200 µg/ml of Lopinavir & 20 µg/ml of Ritonavir



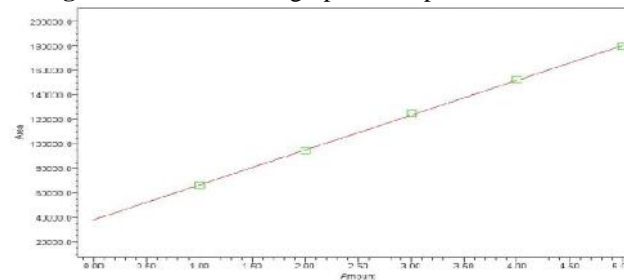
**Figure 10:** Chromatogram for linearity concentration--300 µg/ml of Lopinavir & 30 µg/ml of Ritonavir



**Figure 11:** Chromatogram for linearity concentration-400 ppm of Lopinavir & 40ppm of Ritonavir



**Figure 16:** Calibration graph for Lopinavir at 260 nm



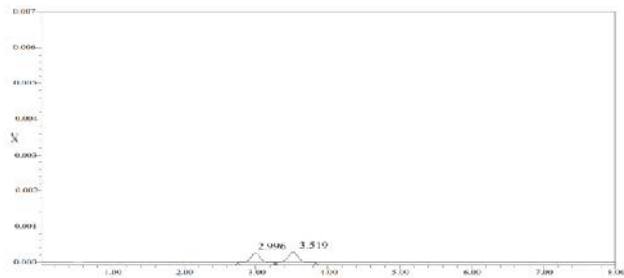
**Figure 17:** Calibration Graph for Ritonavir at 260 nm

**3.4 Limit of Detection (LOD):**

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

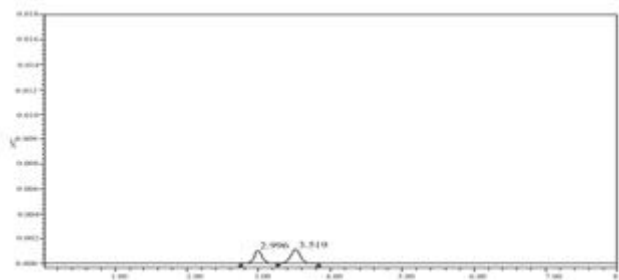
**3.5 Limit of Quantification (LOQ):**

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.



	Name	Retention Time (min)	Area (μV*sec)	Height (μV)
1	Ritonavir	2.996	1143	132
2	Lopinavir	3.519	1305	136

Figure 18: Chromatogram of Lopinavir & Ritonavir showing LOD



	Name	Retention Time (min)	Area (μV*sec)	Height (μV)
1	Ritonavir	2.996	4141	478
2	Lopinavir	3.519	4617	481

Figure 19: Chromatogram of Lopinavir & Ritonavir showing LOQ

Table 5: Instruments used

S.No	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module, PDA detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000 <sup>+</sup>
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 6: Chemicals and Reagents used

S.No	Chemical	Brand
1	Lopinavir	Mylon
2	Ritonavir	Cipla
3	KH <sub>2</sub> PO <sub>4</sub>	FINER chemical LTD
4	Water and Methanol for HPLC	LICHROSOLV (MERCK)
5	Acetonitrile for HPLC	MOLYCHEM
6	Ortho phosphoric Acid	MERCK

Table 7: Accuracy (recovery) data for Lopinavir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	644765	5.0	5.036	100.7%	99.84%
100%	803722	10.0	10.003	100.0%	
150%	962917	14.4	14.224	98.780%	

Table 8: Accuracy (recovery) data for Ritonavir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	544711	5.3	5.34	100.8%	100.51%
100%	675935	10	10.10	100.01%	
150%	812764	14.2	14.45	99.68%	

Table 9: Area of different concentration of Lopinavir

S.No	Linearity Level	Concentration	Area
1	I	100ppm	277182
2	II	200ppm	521695
3	III	300ppm	808274
4	IV	400ppm	1033875
5	V	500ppm	1285804
Correlation Coefficient			0.999

**Table 10:** Area of different concentration of Ritonavir

S.No	Linearity Level	Concentration	Area
1	I	10ppm	226418
2	II	20ppm	432920
3	III	30ppm	677256
4	IV	40ppm	869825
5	V	50ppm	1095759
Correlation Coefficient			0.999

**Table 11:** Results of LOD

Drug name	Baseline noise ( $\mu\text{V}$ )	Signal obtained ( $\mu\text{V}$ )	S/N ratio
Lopinavir	52	152	2.9
Ritonavir	52	156	3

**Table 12:** Results of LOQ

Drug name	Baseline noise ( $\mu\text{V}$ )	Signal obtained ( $\mu\text{V}$ )	S/N ratio
Lopinavir	52	522	10.03
Ritonavir	52	524	10.1

#### 4. Conclusion

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Lopinavir and Ritonavir was done by RP-HPLC. The Phosphate buffer was  $\text{p}^{\text{H}}$  3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/v. Inertsil C<sub>18</sub> column C18 (4.6 x 150mm, 5 $\mu\text{m}$ ) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Lopinavir and Ritonavir were found to be from 100-500  $\mu\text{g/ml}$  of Lopinavir and 10-50 $\mu\text{g/ml}$  of Ritonavir. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Lopinavir and Ritonavir. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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