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# Simultaneous UV spectrophotometric method for estimation of ritonavir and lopinavir in bulk and tablet dosage form

**Jyoti M. Salunke*, Dnyaneshwar S. Pawar, Vinit D. Chavhan and Minal R. Ghante**

*Department of Pharmaceutical Chemistry, STES's Smt Kashibai Navale College of Pharmacy, Kondhwa, Pune, India*

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**ABSTRACT**

*Two simple, precise and economical UV methods have been developed for the simultaneous estimation of Lopinavir and Ritonavir in bulk and pharmaceutical dosage form. Method A is Absorbance maxima method, which is based on measurement of absorption at maximum wavelength of 238 nm and 260 nm for Ritonavir and Lopinavir respectively. Method B is area under curve (AUC), in the wavelength range of 228-248 nm for Ritonavir and 250-270 nm for Lopinavir. Linearity for detector response was observed in the concentration range of 10-35µg/ml for Ritonavir and 100-500 µg/ml for Lopinavir. The accuracy of the methods was assessed by recovery studies and was found to be 99.64 % and 98.97% for Ritonavir and Lopinavir. The developed method was validated with respect to linearity, accuracy (recovery), precision and specificity. The results were validated statistically as per ICH Q2 R1 guideline and were found to be satisfactory. The proposed methods were successfully applied for the determination of for Ritonavir and Lopinavir in commercial pharmaceutical dosage form.*

**Keywords**: Ritonavir, Lopinavir, Simultaneous estimation, Absorbance maxima method, Area under curve.

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## INTRODUCTION

Ritonavir (RITO) is (5S, 8S, 10S, 11S)-10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3, 6-dioxo-8, 11-bis (phenyl methyl)-2, 4, 7, 12-etraazatridecan-13-oic acid 5-thiazolyl methyl ester. (Fig. 1). It is official in Indian Pharmacopoeia[1] and United States Pharmacopoeia[2]. Ritonavir[3] is an antiretroviral drug from the protease inhibitor class used to treat HIV infection and AIDS. Ritonavir is frequently prescribed with Highly Active Anti-Retroviral Therapy, not for its antiretroviral action, but as it inhibits the same host enzyme that metabolizes other protease inhibitors. The lower than therapeutic doses of ritonavir are commonly given in combination with agents such as Lopinavir, Indinavir, or Amprenavir to reduce the risk of resistance by increasing the time of drug exposure. Combination therapy with the HIV protease inhibitors lopinavir and ritonavir (Sustained release capsule with combination of lopinavir 133.3 mg and ritonavir 33.3 mg is available in market by brand name kaletra®) has been shown to be effective against drug-resistant HIV-13. These agents are metabolized by cytochrome P-450 (CYP) 3A in the liver. When lopinavir is administered with ritonavir as kaletra®, ritonavir inhibits the CYP 3A-mediated metabolism of lopinavir, thereby providing increased plasma levels of lopinavir.

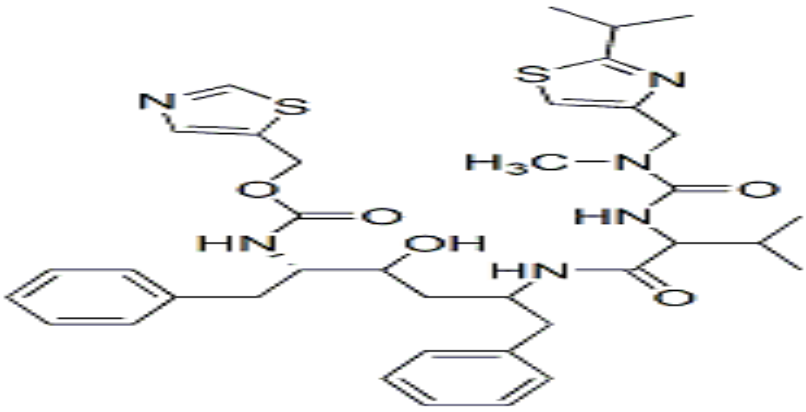

**Fig.1 Ritonavir**

Lopinavir[4] is chemically known as (2*S*)-*N*- [(2*S*, 4S, 5S)-5-[2-(2,6dimethylphenoxy) acetamido]-4-hydroxy-1, 6-diphenylhexan-2-yl] - 3-methyl-2-(2-oxo-1, 3-diazinan-1-yl) butanamide and its empirical formula is $C_{37}H_{48}N_4O_5$ with a molecular weight of 628.80. Lopinavir inhibits the HIV viral protease enzyme. This prevents cleavage of the gagpolpolyprotein and, therefore, improper viral assembly results. This subsequently results in non-infectious, immature viral particles. The chemical structure was shown in fig 2.

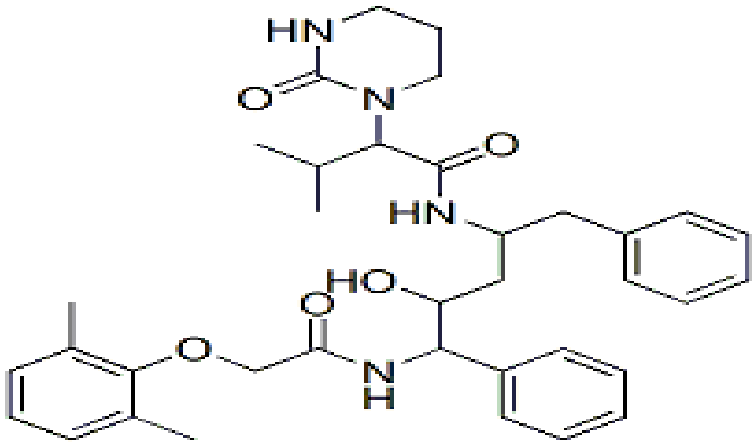

**Fig.2 Lopinavir**

Different methods were reported in estimation of individual [5, 6, 7] as well as combination of Lopinavir and ritonavir. Simultaneous determinations of Lopinavir and ritonavir dosage form were also reported like HPLC[8], LC/MS[9], RP-HPLC[10], and UV-Spectroscopy[11]. Therefore an attempt was made to develop a new rapid and sensitive UV Spectrophotometric method and to validate as per ICH-guidelines[12]. A comprehensive literature research reveals the lack of a Spectrophotometric analytical method for simultaneous estimation of Ritonavir and Lopinavir in pharmaceutical formulations. A successful attempt was made to develop accurate, precise and simple method of analysis for estimation of both the drugs in combined dosage form.

## MATERIALS AND METHODS

**Materials:**

Lopinavir and Ritonavir were generous gift samples from Ranbaxy Laboratories Limited, (Gurgaon, India). Commercial Lopimune tablets containing 50 mg of Ritonavir and 200 mg of Lopinavir were purchased from local market and used within their shelf-life period. All other chemicals used were of analytical grade.

**Instrumentation:**

A Jasco double beam UV–visible spectrophotometer, Model: V-630, with a fixed bandwidth (2nm) and 1-cm quartz cell was used for Spectral and absorbance measurements. In addition, electronic balance, micropipette and sonicator were used in this study.

**Procedure:**
Preparation of standard stock solution-Standard stock solutions of each LOPI and RITO was prepared by dissolving 10 mg of standard RITO and 40 mg of Standard LOPI separately in 10 ml distilled water with vigorous shaking. Aliquot in the range of 10-35 µg/ml for RITO and 100-500 µg/ml for LOPI was prepared using this stock solution.

**Method A: Absorption Maxima Method**
For the selection of analytical wavelength, standard solution of RITO and LOPI were scanned in the spectrum mode from 400 nm to 200 nm separately. From the spectra of drug λmax of RITO, 238 nm [Fig.3], and λmax of LOPI, 260 nm [Fig.4], were selected for the analysis. Aliquots of standard stock solution were made and calibration curve was plotted .

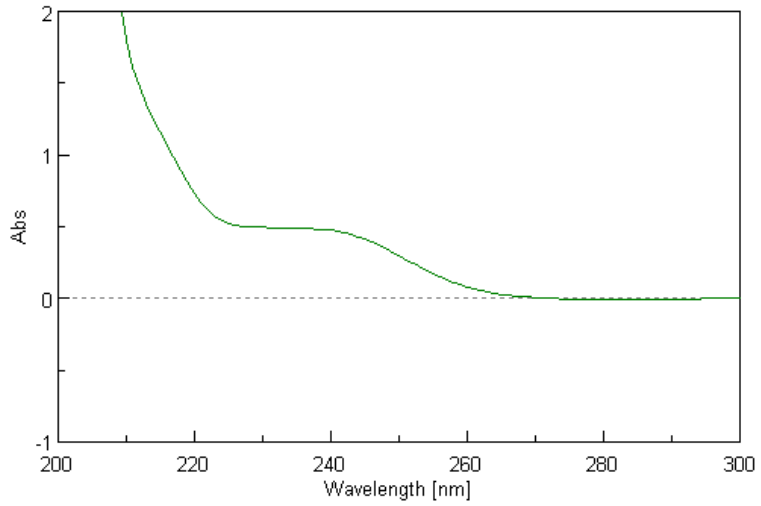


**Fig.3 It shows λmax of RITO**

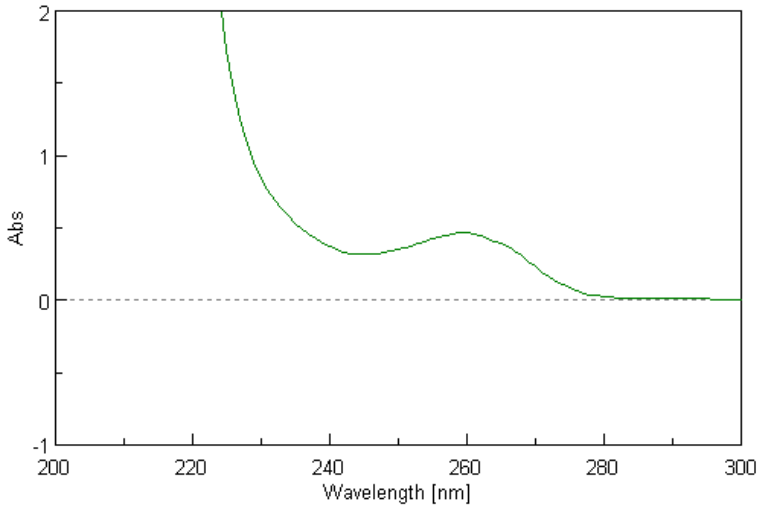


**Fig.4 It shows λmax of LOPI**

**Simultaneous estimation of Ritonavir and Lopinavir:**
The wavelength maxima of Ritonavir and Lopinavir were determined and found to be 238 nm (λ1) and 260nm (λ2) respectively where there was no interference among the drugs. The overlain spectrum is shown in Fig.5.

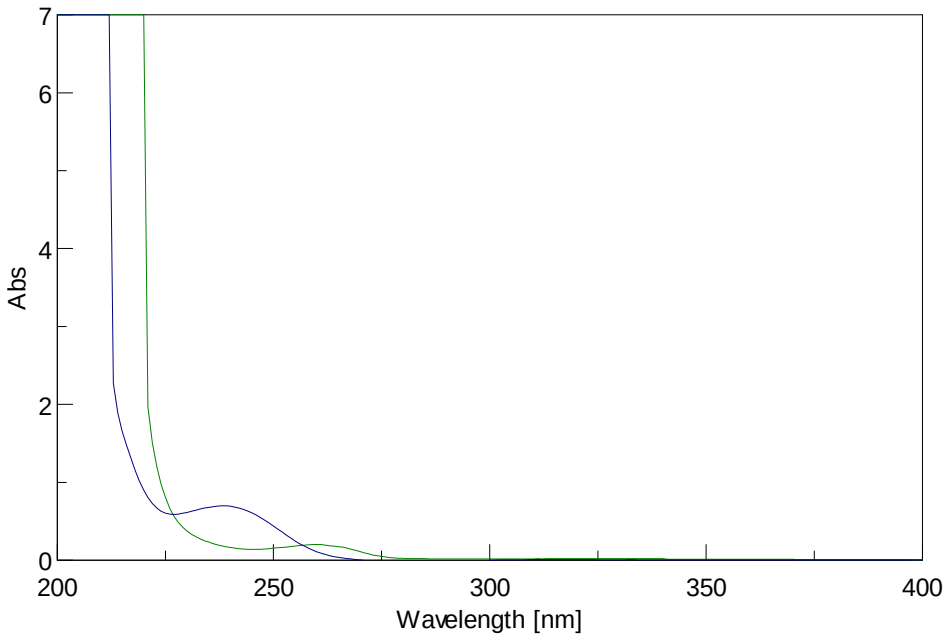


**Fig.5 Isobestic point of RITO and LOPI**

**Method B: Area under Curve Method**

From the spectra of drug obtained after scanning of standard solution of RITO and LOPI separately, area under the curve in the range of 228-248 nm and 250-270 nm was selected for the analysis. The calibration curve was prepared in the concentration range of 10-35 µg/ml for RITO and 100-500 µg/ml for LOPI at their respective AUC range. Both drugs followed the Beer-Lambert's law in the above mentioned concentration range. The calibration curves were plotted as absorbance against concentration of RITO and LOPI. The coefficient of correlation (r), slope and intercept values of this method are given in Table 1.

**Application of the proposed methods for the determination of RITO and LOPI in tablet dosage form:**

For the estimation of drugs in the tablet formulation, 20 tablets were weighed and weight equivalent to 50 mg of RITO and 200 mg of LOPI was transferred to 50 ml volumetric flask and ultrasonicated for 20 minutes and volume was made up to the mark with distilled water. The solution was then filtered through a Whatmann filter paper (No.42). The filtrate was appropriately diluted further. In Method-A, the concentration of RITO and LOPI was determined by measuring the absorbance of the sample at 238 nm and 260 nm respectively in zero order spectrum modes. By using the calibration curve, the concentration of the sample solution was determined.

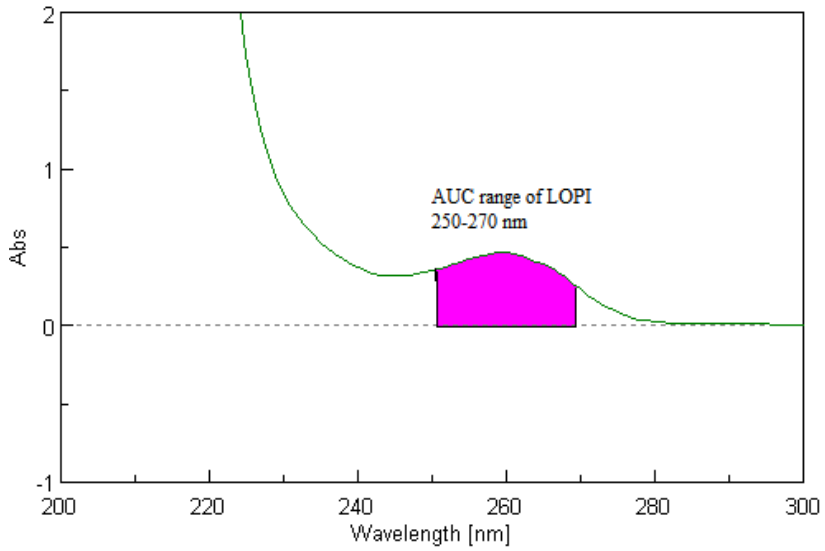


**Fig.6 It shows AUC of LOPI**

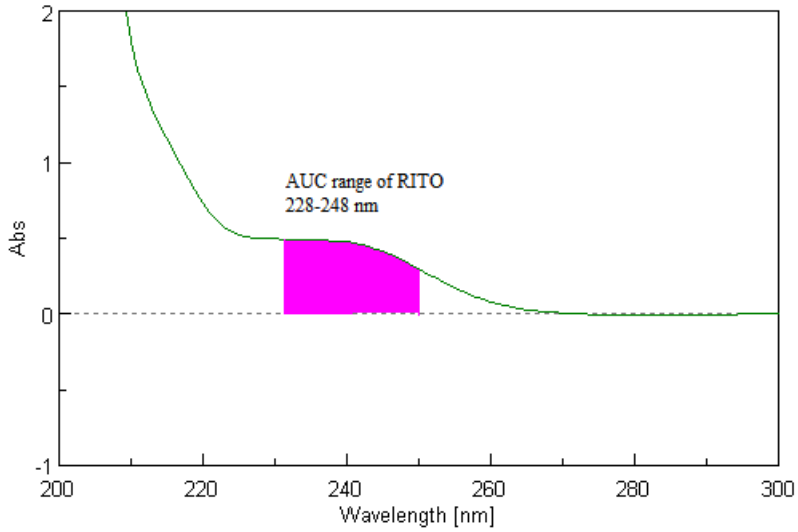


**Fig.7 It shows AUC of RITO**

In Method-B, the concentration of RITO and LOPI was determined by measuring area under curve in the range of 228-248(Fig.7)nm and 250-270(Fig.6) nm. By using the calibration curve, the concentration of the sample solution was determined.

**Validation of the developed methods**[12]**:**
The methods were validated with respect to accuracy, linearity, precision and selectivity.

**Accuracy:** Accuracy of an analysis was determined by systemic error involved. Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. Recovery studies carried out for both the methods by spiking standard drug in the powdered formulations 80%, 100%, 120% amount of each dosage content as per ICH guidelines**.**

**Linearity:** The linearity of measurement was evaluated by analyzing different concentration of the standard solution of RITO and LOPI. Result should be expressed in terms of correlation co-efficient.

**Precision:** The reproducibility of the proposed method was determined by performing tablet assay at different time intervals (morning, afternoon and evening) on same day (Intra-day assay precision) and on three different days (Inter-day precision). Result of intra-day and inter-day precision is expressed in % RSD.

**Table 1: Table shows Optical characteristics and precision**

| Sr.No. | Parameter | RITO | LOPI |
|---|---|---|---|
| 1. | λ max | 238 | 260 |
| 2. | Beer's law limit (µg/ml) | 10-35 | 100-500 |
| 3. | Correlation coefficient (r) | 0.999 | 0.999 |
| 4. | Slope(m) | 0.0148 | 0.0009522 |
| 5. | Intercept | -0.0196 | 0.00154 |

**Table 2: Table shows Results of Analysis of Tablet Formulation (N*=6)**

| Method | Drug | Label Claim mg | Amount of drug estimated (mg/tab) | % Label claim * ± | %Recovery |
|---|---|---|---|---|---|
| A | RITO | 50 | 49.53 | 99.06± 0.67 | 99.51 |
| B | | | 49.15 | 98.3± 1.20 | 98.5 |
| | | | | | |
| A | LOPI | 200 | 197.04 | 98.52± 1.05 | 98.92 |
| B | | | 199.80 | 99.9± 0.07 | 99.2 |

**Table 3: Table shows Result of Recovery studies**

| Excess drug added to the analyte (%) | Drug | % Recovery | | % RSD | | SE | |
|---|---|---|---|---|---|---|---|
| | | Method A | Method B | Method A | Method B | Method A | Method B |
| 80 | RITO | 99.64 | 100.01 | 0.408 | 0.287 | 0.670 | 0.154 |
| 100 | | 99.64 | 100.06 | 0.365 | 0.192 | 0.416 | 0.127 |
| 120 | | 100.27 | 100.78 | 0.094 | 0.226 | 0.767 | 0.328 |
| | | | | | | | |
| 80 | LOPI | 98.97 | 98.97 | 0.296 | 0.288 | 0.812 | 0.821 |
| 100 | | 99.56 | 100.02 | 0.436 | 0.126 | 0.673 | 0.987 |
| 120 | | 100.37 | 100.32 | 0.114 | 0.122 | 0.994 | 0.985 |

**a) RSD: Relative Standard deviation**
**b) SE: Standard error**

**Table 4: Table shows Result of Intra-day and Inter-day precision**

| Method | Drug | Intra-day Precision | | | Inter-day Precision | | |
|---|---|---|---|---|---|---|---|
| | | SD | %RSD | SE | SD | %RSD | SE |
| A | RITO | 0.616 | 0.417 | 0.205 | 0.587 | 0.377 | 0.156 |
| B | | 0.543 | 0.357 | 0.124 | 0.354 | 0.236 | 0.115 |
| | | | | | | | |
| A | LOPI | 0.272 | 0.836 | 0.112 | 0.197 | 0.758 | 0.106 |
| B | | 0.180 | 0.764 | 0.103 | 0.167 | 0.657 | 0.098 |

## RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient and accurate way for analysis of Ritonavir and Lopinavir in its bulk and pharmaceutical dosage form. Absorbance maxima of RITO at 238 nm and LOPI at 260 nm were selected for the analysis. Linearity for detector response was observed in the concentration range of 10-35 µg/ml for RITO and 100-500 µg/ml for LOPI. Percent label claim for RITO and LOPI in tablet analysis was found in the range of 99.51% and 98.92% [Table 2]. Standard deviation and coefficient of variance for six determinations of tablet formulation, was found to be less than ± 2.0 indicating the precision of the methods. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery. % recovery for RITO and LOPI was found in the range of 99.64% and 98.97% values of standard deviation and coefficient of variation was satisfactorily low indicating the accuracy of all the methods. % RSD for Intraday assay precision for RITO was found to be 0.616 and 0.543 for Method A and B, and for LOPI, 0.272 and 0.180 for Method A and B. Interday assay precision for RITO was found to be 0.587 and 0.354 for Method A and B and for LOPI 0.197 and 0.167 for Method A and B. Based on the results obtained, it is found that the proposed methods are accurate, precise, reproducible & economical and can be employed for routine quality control of Ritonavir and Lopinavir in bulk drug and its pharmaceutical dosage form.

## CONCLUSION

UV spectrophotometric methods for Ritonavir and Lopinavir were developed separately in bulk and tablet dosage form by, Absorbance maxima method and Area under curve method. Further, UV Spectrophotometric methods for the simultaneous estimation of Ritonavir and Lopinavir were in bulk and combined dosage form. The methods were validated as per ICH guidelines. The standard deviation and % RSD calculated for these methods are <2, indicating high degree of precision of the methods. The results of the recovery studies showed the high degree of accuracy of these methods. In conclusion, the developed methods are accurate, precise and selective and can be employed successfully for the estimation of RITO and LOPI in bulk and pharmaceutical dosage form.

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