*Available online at* www.scholarsresearchlibrary.com

Scholars Research Library

**Der Pharmacia Lettre, 2010: 2 (1) 196-200**
*(http://scholarsresearchlibrary.com/archive.html)*

ISSN 0975-5071
USA CODEN: DPLEB4

# Simultaneous Estimation of Ritonavir and Lopinavir by Absorption ratio (Q-analysis) UV Spectrophotometric Method in Combined Tablet Dosage Form

**Vaishali P. Nagulwar* and Kishore P. Bhusari**

*Sharad Pawar College of Pharmacy, Wanadongri, Hingna road, Nagpur, India*

---

## Abstract

The simple, accurate and precise absorption ratio method has been developed for the simultaneous estimation of ritonavir and lopinavir in combined tablet dosage form. The λmax of ritonavir and lopinavir were found to be 239nm and 259nm respectively. For the estimation by Q-analysis method, the λmax those selected were 239nm and an isoabsorptive point for both the drugs as 257.4nm. The linearity range lies between 5-30µg/mL for ritonavir and 20-120 µg/mL for lopinavir at their respective wavelengths. Both the drugs were found in good agreement with the label claimed in the marketed formulation. Ritonavir and lopinavir in standard mixture were determined as 98.6% and 101.3% respectively. In the tablets both the drugs were estimated as 98.3% and 99.9%. Statistical validation of the data has been carried out and it reveals that the proposed method is sensitive and economical too for the routine analysis of the drugs in combined dosage form.

**Keywords:** Ritonavir, Lopinavir and absorption ratio method.

---

## Introduction

Ritonavir and lopinavir both are antiretroviral drugs specifically belongs to protease inhibitors class**.** Literature survey reveals some methods for their determination by HPLC or in combination with other drugs [1-4], but no method was found for the selected combined dosage form by spectrophotometry. For the routine quantitative estimation of these drugs, a simple and economical method was required. Thus the proposed method has been developed which doesn't requires any sophisticated instruments and expensive reagents. Hence, an economical UV spectrophotometric absorption ratio (Q-analysis) method [5] was developed for the estimation of ritonavir and lopinavir in tablets.

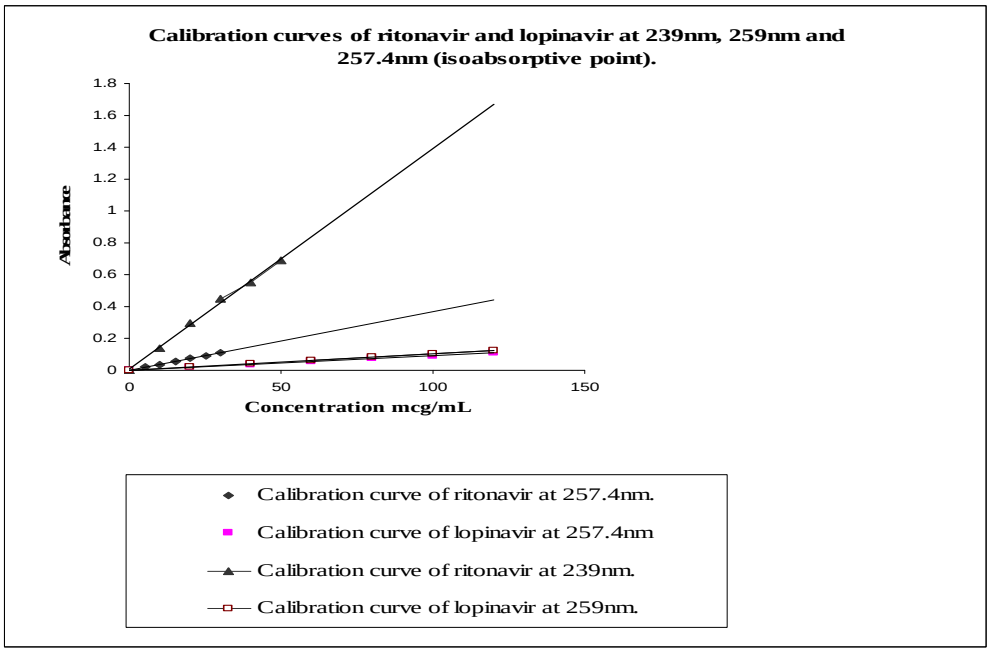
## Materials and Methods

Pure drugs of ritonavir and lopinavir (Cipla Ltd. Kurkumbh, Pune, INDIA), Acetonitrile (Qualigens Fine Chemicals Ltd. Mumbai) were used for the present study. Tablets were procured from a local pharmacy. Spectral absorbance measurements were made on Shimadzu UV-1601 with 10mm matched quartz cells.

### Method

The stock solutions having 1mg/ml solutions of both the drugs were freshly prepared in acetonitrile. Aliquots of both the stock solutions were diluted further again using acetonitrile to get the concentration of ritonavir as 5,10,15,20,25,30,40 and 50µg/ml at 239nm and that of lopinavir as 20,40,60,80, 100 and 120 µg/ml at 259nm, respectively to study the verification of Beer's law.(Fig 1).

**Fig.1: Calibration curves of ritonavir and lopinavir at 239nm, 259nm and 257.4nm (Isoabsorptive point)**



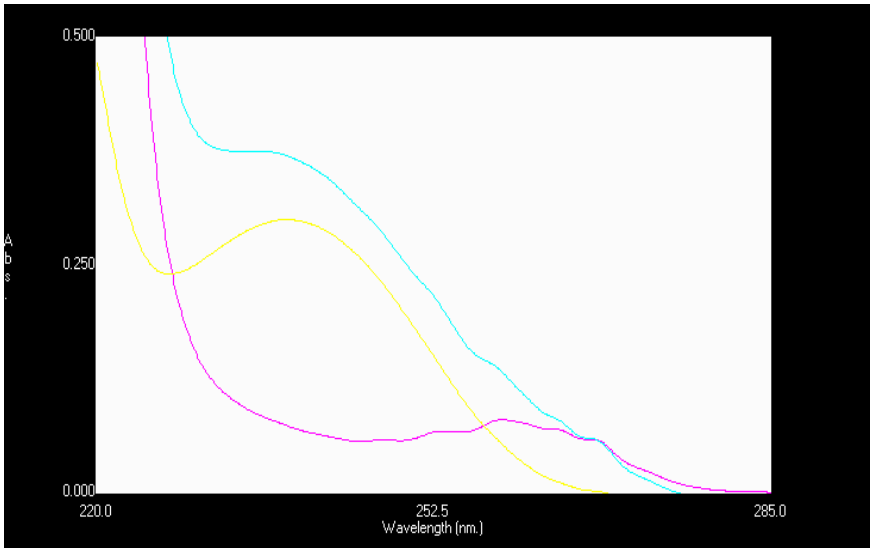

The isoabsorptive point of both the drugs was found at 257.4nm and the absorbance values at 257.4nm were recorded throughout the selected concentration range of both the drugs. (Fig2).

Drug concentrations of 20 µg/ml (ritonavir), 80µg/ml (lopinavir) and a mixture containing the same concentration of both the drugs were analyzed for the proposed method.

Tablets containing 50mg ritonavir and 200mg lopinavir (**Emeltra)** were weighed and finely powdered. A quantity of powder equivalent to 25mg ritonavir and 100mg lopinavir was accurately weighed and transferred to 25ml volumetric flask, dissolved in acetonitrile, filtered

through whatman filter paper No.1 and the volume was made up to 25ml with the same solvent. Aliquots of this solution were diluted with acetonitrile to get the working standards of 20 µg/ml ritonavir (~ 80 µg/ml lopinavir).

**Fig 2. Overlain spectrum of ritonavir (yellow) and lopinavir (pink) in proportion of 20:80 µg/mL along with spectra of their mixture (green)**




The sample solutions were scanned over the range of 190-400nm and the absorbance of the sample solutions at 239nm and 257.4 nm were measured. For determining the concentration of ritonavir and lopinavir by the proposed method, the absorbance and the absorptivity values at the particular wavelengths were calculated (Table 1) and substituted in the following equation:

$$cx = (Q_0-Q_2) \times A_1/(Q_1-Q_2) \times a_1,$$

$$cy = (Q_0-Q_1) \times A_1/(Q_2-Q_1) \times a_2,$$

where, cx and cy are the concentration of ritonavir and lopinavir respectively. $A_1$ is the absorbance of sample at 257.4nm, $a_1$ and $a_2$ are the absorptivity values of ritonavir and lopinavir at 257.4nm respectively.

**Table 1: The absorptivity values of ritonavir and lopinavir in the proposed method**

| Absorptivity values | Wavelengths (nm) | |
|---|---|---|
| | **239** | **257.4** |
| $ax_1$ | 14.97 | - |
| $ax_2$ | - | 3.65 |
| $ay_1$ | 0.89 | - |
| $ay_2$ | - | 0.9125 |

* $ax_1$ and $ax_2$ are the absorptivity values of ritonavir at the respective wavelengths
** $ay_1$ and $ay_2$ are the absorptivity values of lopinavir at the respective wavelengths.

$Q_0$ was obtained by using the equation, (absorbance of sample at 237nm)/ (absorbance of sample at 257.4nm), $Q_1$ was obtained from (absorptivity of ritonavir at 237nm)/ (absorptivity of ritonavir

at 257.4nm), $Q_2$ was obtained from (absorptivity of lopinavir at 237nm)/ (absorptivity of lopinavir at 257.4nm).

The amount and % claim calculated for both the drugs. To study the linearity, accuracy and precision of the proposed method, the recovery studies were carried out by adding a known amount of standard drug to the preanalyzed sample and the % recovery was calculated. The results are depicted in Table 2.

**Table 2: The results of the analysis of commercial formulations and the recovery studies**

| **Standard mixture** | **Concentration (µg/mL)** | **Content estimated (µg/mL)** | **% amount estimated** | **Standard deviation (± )** | - |
|---|---|---|---|---|---|
| Ritonavir<br>Lopinavir | 20<br>80 | 19.72<br>81.04 | 98.6<br>101.3 | 0.99<br>0.92 | -<br>- |
| **Tablet** | **Label claim (mg/tab)** | **Amount found (mg/tab)** | **% label claim** | **Standard deviation (±)** | **% recovery ± SD** |
| **Emeltra**<br>Ritonavir<br>Lopinavir | <br>50<br>200 | <br>49.15<br>199.80 | <br>98.3<br>99.9 | <br>1.20<br>0.07 | <br>98.5 ± 1.06<br>99.2 ± 0.56 |

## Results and Discussion

The findings of the present technique of analysis for ritonavir and lopinavir in standard mixture were 98.6% and 101.3% respectively. The marketed tablet formulation has also been analyzed and the percent of the label claimed were ritonavir- 98.3% and lopinavir - 99.9%. The recovery studies done by standard addition method has given satisfactory results as ritonavir- 98.5% and lopinavir- 99.2%. The regression analysis of the calibration curves and the optical characteristics such as Beer's law limits, detection limit, molar absorptivities and sandell's sensitivities were also determined (Table 3).

**Table 3: Regression analysis of calibration curves and summary of validation parameters**

| **Parameters** | **Ritonavir** | **Lopinavir** | **Ritonavir** | **Lopinavir** |
|---|---|---|---|---|
| Wavelength(nm) | 239 | 257.4 | 257.4 | 259 |
| Beer's law limit(µg/ml) | 10-30 | 20-120 | 5-30 | 20-120 |
| ε-Molar absorptivity(l/mol/cm) | $1.0793 \times 10^3$ | $0.05737 \times 10^3$ | $0.26315 \times 10^3$ | $0.06311 \times 10^3$ |
| Limit of detection (µg/ml) | 0.1 | 0.5 | 0.1 | 0.5 |
| Limit of quantitation (µg/ml) | 0.5 | 10 | 0.5 | 10 |
| Sandell's sensitivity (µg/cm$^2$) | 0.66800 | 10.9589 | 2.73972 | 9.96264 |
| **Regression equation*** | | | | |
| Intercept(α) | 0.0081286 | 8.92857E-05 | 8.92857E-05 | 0.00028215 |
| Slope(β) | 0.0138689 | 0.0009131 | 0.0036522 | 0.0010084 |
| Correlation coefficient(r) | 0.9986329 | 0.9999598 | 0.9999598 | 0.99982358 |

Where,***y= α+ β x**, x is the concentration of the analyte and y is the absorbance value.

## Conclusion

The proposed absorption ratio method is simple, accurate and economical for routine analysis of two drugs without prior separation. The amount found was in good agreement with the label claim of the formulation. The value of standard deviation was satisfactorily low indicating the reproducibility and accuracy of the method.

## Acknowledgements

The authors are thankful to Cipla Ltd., Kurkumbh, Pune, for providing the pure drugs. They are also grateful to the Director of the institute for providing the necessary facilities for the completion of the work.

## References

[1] Y Usami; T Oki; M Nakai; M Sagisaka; T Kaneda; *Chem Pharm Bull*, **2003**; 51(6), 715-718.
[2] R Naser; T Richard; K Angela*; J. Chromatogr B*, **2004**; 805(2), 241-247.
[3] C Sun; R Naser; K Angela; *J. Chromatogr B*, **2007**; 848(2), 374-378.
[4] A Antonio; S Marco; S Mauro; L Baietto; B Stefano; et.al; *J Chromatogr B*, **2007**; 859(2), 234-240.
[5] AG Davidson, AH Beckett, JB Stenlake. Practical Pharmaceutical Chemistry, 4th ed, CBS publishers and distributors, New Delhi, **2002**; 284-286.