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Development and Validation of Spectrophotometric Method for Quantitative estimation of Ritonavir in Bulk and Pharmaceutical Dosage Forms

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Abstract: Two Simple, precise and economical UV methods have been developed and validated for the quantitative for the estimation of Ritonavir in bulk and pharmaceutical dosage forms. Ritonavir has the absorbance maxima at 239 nm (Method A), and in the first order derivative spectra, showed sharp peak at 232 nm (Method B). Beer's law was found to be obeyed in the concentration range of 10-50 µg/mL for the Method A and B. The developed method was validated according to ICH guidelines and was found to be accurate and precise. The proposed method can be successfully applied for the estimation of Ritonavir in bulk and pharmaceutical dosage forms. Results of the analysis were validated statistically and by recovery studies.
Keywords: Ritonavir, UV Spectrophotometry, Derivative Spectroscopy.

INTRODUCTION:

Ritonavir was the first protease inhibitor for which clinical efficacy was demonstrated [1]. The chemical name of Ritonavir is (5S,8S,10S,11S)-10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis(phenylmethyl)-2,4,7,12-etraazatridecan-13-oic acid 5-thiazolyl methyl ester. It has the structural formula and shown in (Figure 1).

It is official in Indian Pharmacopoeia [2] and United States Pharmacopoeia [3]. 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 [4]. From the literature survey, it was found that Ritonavir estimated by analytical methods such as spectrophotometric methods [5-6], reversed-phase high-performance liquid chromatographic (RP-HPLC) method [7] and HPTLC method [8]. Apart from the above no other methods such as zero and first order derivative spectrophotometric method was reported for the quantitative determination of Ritonavir in pharmaceutical dosage forms. The developed method was simple, precise, specific and accurate. The statistical analysis proved that method is reproducible and selective for the analysis of Ritonavir in bulk drug and tablet formulations.

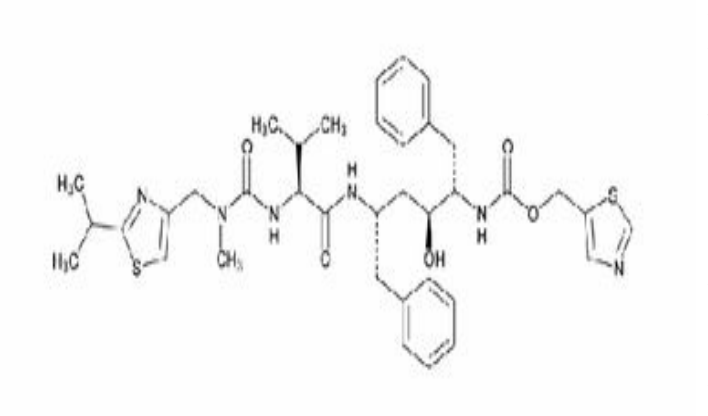

Figure 1: Chemical Structure of Ritonavir

2. EXPERIMENTAL

2.1 Instruments and reagents

An analytically pure sample of Ritonavir was procured as gift sample from Matrix laboratories (Hyderabad, India). Analytical grade methanol was used as solvent for dilution. A Shimadzu UV-1800 UV/VIS spectrophotometer was used with 1 cm matched quartz cell. Tablet formulation [RITOVIR (Brand I), Hetero Drugs Limited, Hyderabad and EMPETUS (BrandII), Emcure Pharmaceuticals Ltd, Pune, India] were procured from a local pharmacy with labelled amount 100 mg per tablet.

2.2 Preparation of working standard drug solution

The standard Ritonavir (100 mg) was weighed accurately and transferred to volumetric flask (100 ml). It was dissolved properly and diluted up to the mark with methanol to obtain final concentration of 1000 µg/ml and the resulting solution was used as working standard solution.

2.3 Analysis of marketed formulations

For the estimation of Ritonavir in tablets formulations, 20 tablets of two different brands were weighed and triturate to fine powder. Tablet powder equivalent to 100 mg of Ritonavir for each was weighed and transfer into 100 ml volumetric flask than dissolved with methanol and further diluted with methanol. It was kept for ultra-sonication for 30 min; this was filtered through Whatman filter paper No. 41 and then final dilution was made with methanol to get the final stock solution of 1000 µg/ml. From this stock solution, various dilutions of the sample solution were prepared and analysed.

2.4 Calibration curve

Method A: Absorption Maxima Method

For the selection of analytical wavelength, 30 µg/mL solution of Ritonavir was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. From the spectra of drugs (Figure 2), λ max of Ritonavir, 239 nm was selected for the analysis. The calibration curve was prepared in the concentration range of 10-50 µg/mL at 239 nm. By using the calibration curve, different concentrations of the sample solution were calculated.

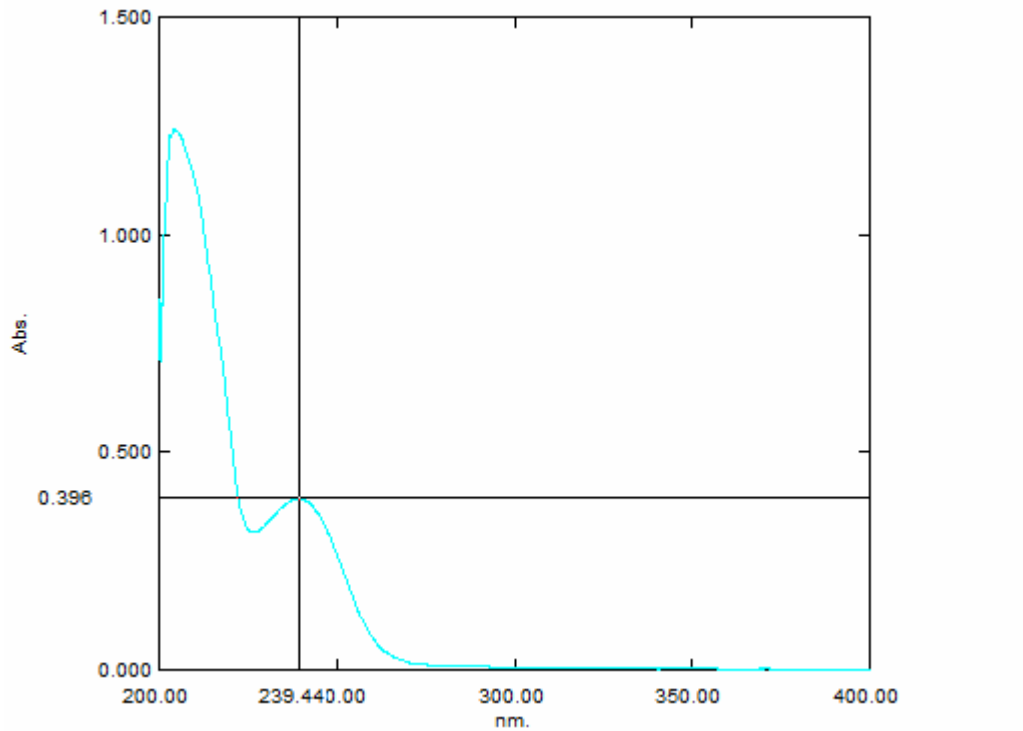

Figure 2: Zero order spectra of Ritonavir

Method B: First Order Derivative Spectroscopic method

In this method, 30 µg/mL solution of Ritonavir was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. The Absorption spectra thus obtained were derivatized from zero to first order. The first order derivative spectra showed sharp peak at 232 nm when n=1 and linearity was measured at 232 nm (Figure 3). The calibration curve for Ritonavir was plotted in the concentration range of 10-50 µg/mL at wavelength 232 nm. Similarly absorbances of samples solution were measured and amount of Ritonavir was determined from standard calibration curve.

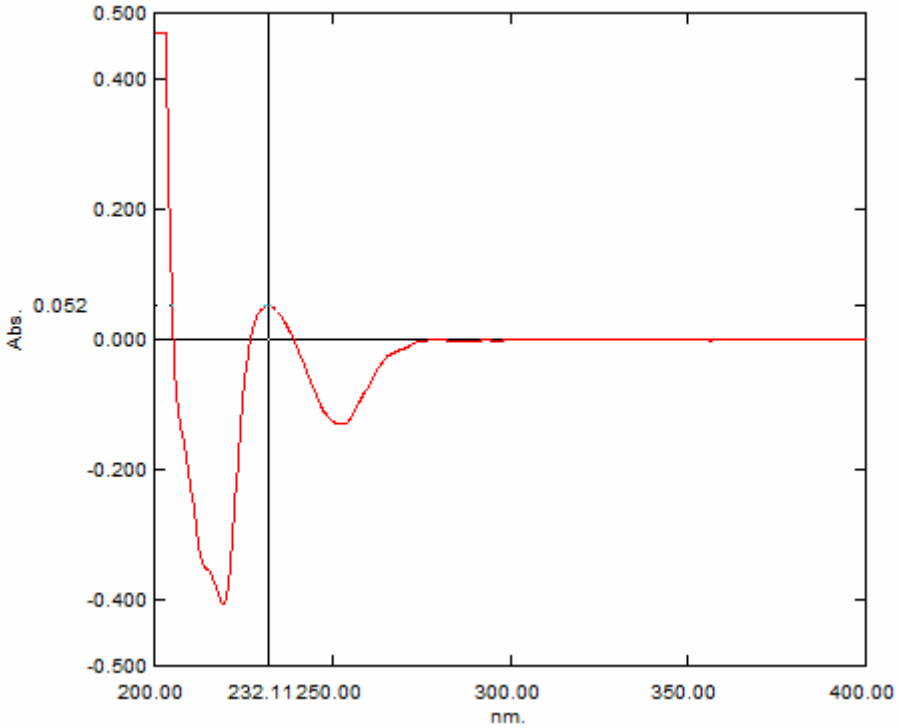

Figure 3: First order derivative spectrum of Ritonavir with n=1

Table 1: Optical characteristics and Other Parameters

PARAMETERS	RESULTS	
	METHOD A	METHOD B
Absorption Maxima (nm)	239	232
Beer's-Lambert's range (µg/ml)	10-50	10-50
Regression equation (y)* Slope (b) Intercept (a)	 0.013 0.048	 0.0017 0.0007
Correlation coefficient	0.9998	0.9996
Sandell's sensitivity (mcg / cm^2-0.001 absorbance units)	0.0755667	0.5760
Precision (% RSD) Intraday precision Interday precision	 0.29 0.78	 1.58 1.75
Accuracy	98.14	98.83
Limit of detection (µg / ml)	0.39	1.09
Limit of quantification (µg / ml)	1.2	3.30

*y = a + bx; when x is the concentration in mg/ml and y is absorbance unit.

Table 2: Analysis of tablet formulation

METHOD	Tablet	Label claimed (mg)	Amount found (mg)	%Recovery ± SD**
A	BRAND I	100	99.50	100.92 ± 0.17
	BRAND II	100	99.84	101.36 ± 0.09
B	BRAND I	100	100.34	100.47 ± 0.19
	BRAND II	100	99.76	99.91 ± 0.46

**Average of six determinations

3. RESULT AND DISCUSSION

Ritonavir showed a broad spectrum, the derivative spectroscopy method applied has the advantage that it locates hidden peak in the normal spectrum. It eliminates the interference caused by the excipients and the degradation products present, if any, in the formulation. The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures [9-11].

Ritonavir has the absorbance maxima at 239 nm (Method A), and in the first order derivative spectra, showed sharp peak at 232 nm (Method B). The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 10-50 µg/ml and given in Table 1. Recovery studies were carried out at three different levels i.e. 50 %, 100 %, and 150 % by adding the pure drug to the previously analysed tablet powder sample. Percentage recovery for Ritonavir was determined by all the methods and they were found to be under acceptance criteria which are 98% to 102 % according to ICH guidelines [9-11]. The results are in Table 1. The percentage recovery value indicates non interferon from excipients used in formulation. The result of analysis of marketed formulation is shown in Table 2. The reproducibility and accuracy of the method was found to be good, which was evidenced by low standard deviation.

4. CONCLUSION

The most striking features of two methods are its simplicity and rapidity, non- requiring consuming sample preparations such as extraction of solvents, heating, degassing which are needed for HPLC procedure. It can be concluded that the proposed methods are fully validated and found to be simple, sensitive, accurate, precise, reproducible, rugged and robust and relatively inexpensive. So, the developed methods can be easily applied for the routine Quality Control analysis of Ritonavir in pharmaceutical preparations.

5. ACKNOWLEDGEMENT

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