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SPECTROPHOTOMETRIC DETERMINATION OF RITONAVIR IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, robust and selective and sensitive spectrophotometric method has been developed for the determination of Ritonavir in pharmaceutical formulations. The method was based on the scanning of methanolic solution of the drug and methanolic solution of formulation. The method showed high sensitivity with linearity range from 10 to 20 µg/mL. The lower limit of detection (LOD) was found to be 1.1 µg/mL and the limit of quantization (LOQ) was determined as the lowest concentration was found to be 3.3 µg/mL. The variables that affected the reaction were carefully studied and optimized. The proposed method was applied successfully for the determination of Ritonavir in pharmaceutical formulations. The percentage recovery was found to be 99.426 ± 0.59 (n = 9) for pharmaceutical formulation.

Key words: Ritonavir, Spectrophotometry, Formulation, Estimation.

INTRODUCTION

Ritonavir[1] 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. This inhibition leads to higher plasma concentrations of these latter drugs, allowing the clinician to lower their dose and frequency and improving their clinical efficacy. 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[2-4]. When lopinavir is administered with ritonavir as kaletra®, ritonavir inhibits the CYP 3A- mediated metabolism of lopinavir, thereby providing increased plasma levels of lopinavir.

It has the structural formula which was presented in Fig. 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 is official in Indian

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Pharmacopoeia[5] and United States Pharmacopoeia[6]. From the literature survey, it was found that ritonavir estimated by analytical methods such as spectrophotometric methods, reversed phase high performance liquid chromatographic (RP-HPLC) method[7-13], LC-MS[14] and HPTLC[15] method. 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.

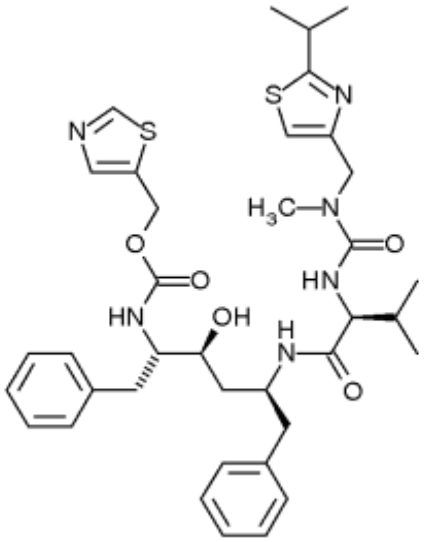

Fig. 1: Chemical structure of Ritonavir

EXPERIMENTAL

Materials and methods

Materials

For the development process, we used UV-visible spectrophotometer (Perkin-Elmer Lambda 25), Sonicater (Branson 2510), Electronic balance (precise 92 sm-202 A). Methanol (HPLC grade), water-double distilled water, pure ritonavir were obtained as gift sample and the drug was used as such for further analysis. Formulations were purchased from the local pharmacies and used for analysis.

Preparation of standard solution

An accurately weighed amount 100 mg of ritonavir was quantitatively transferred into a 100 mL calibrated flask, dissolved in methanol and made up the volume. Then 1 mL of the above solution was pipetted out and using micro-pipette transferred into a 100 mL standard flask diluted with methanol. The concentration of the working standard solution was 10 μg/mL.

Preparation of sample solution

Equivalent to 100 mg of ritonavir was weighed accurately, from the crushed tablet powder and transferred into a clean 100 mL standard flask. About 35 mL of methanol was added and solicited for 5 minutes and then made up to the volume with methanol.

The above solution was filtered through Whatman filter paper and the filtrate was collected. From the above filtrate 1 mL was pipetted out and transferred into a 100 mL standard flask, which then was

made up the volume with methanol and mixed well. Further dilutions were carried out to get 10 µg/mL concentration.

Validation

Method validation was performed in terms of specificity and selectivity, precision and accuracy, linearity and stability.

Linearity and range

Calibration standards of ritonavir, covering the range 10-20 µg/mL were prepared with the suitable dilution made from ritonavir stock solution. The calibration curves were obtained by plotting the intensity of absorbance against of concentration of ritonavir. The slope and intercept of the calibration line were determined by linear regression using the least squares method.

Specificity and selectivity

The interference from endogenous compounds was investigated by the analysis of six different blank matrices.

Precision and accuracy

Method validation regarding reproducibility was achieved by replicate injection of extracted standard solution at low, medium and high concentration levels, where intensity of absorbance was measured in comparison to the intensity of absorbance of the standard.

Intermediate precision study was conducted during routine operation of the system over a period of six consecutive days. Statistical evaluation revealed relative standard deviations at different values of six replicates. Within-day repeatability was studied by six replicate at three concentration levels.

Accuracy was estimated as the deviation to the observed mean concentration from actual concentration and found to be less than 2% for all the concentration. The procedure which was stated was done in addition of 50%, 75%, 100% of drug as average along with the tablet powder and further dilution was made to get 10 µg/mL concentration. These solutions were used for further analysis to perform recovery studies.

Stability

Problems of stability are usually encountered with these compounds, mainly at ambient temperature. The stability of ritonavir was verified concentrations were measured for 6 h in the interval of 1 h and found that the differences are within the limit.

RESULTS AND DISCUSSION

Calibration standards for ritonavir covering the range of 10-20 µg/mL were prepared by the method mentioned above and the serial dilutions were made with methanol. The spectrum was presented in Fig 2. The calibration curve was obtained by plotting the intensity by absorbence of the ritonavir versus analyte concentration. The slope and intercept of the calibration like was determined by linear regression using the least square method. The data are presented in Table 1 and the calibration curve is presented in Fig 3. Regression analysis of the calibration curve showed a linear relationship between the intensity of absorbence of ritonavir and the concentration with co-relation co-efficient higher than in all the curves assayed in pure form. The values are presented in Table 1.

Table 1: Regression data of the calibration lines for quantitative determination of Ritonavir by UV method

Parameters	Ritonavir
Measured wavelength (λ_{max})	242
Linearity range, µg/mL	10 – 20
Slope	0.0115
Intercept	-0.0056
Correlation coefficient (r)	0.9989
LOD, µg/mL	1.1
LOQ, µg/mL	3.3
Repeatability of absorbance, RSD %	0.16
Repeatability of wavelength, RSD %	0.14
Reproducibility of absorbance, RSD %	0.31
Reproducibility of wavelength, RSD %	0.03

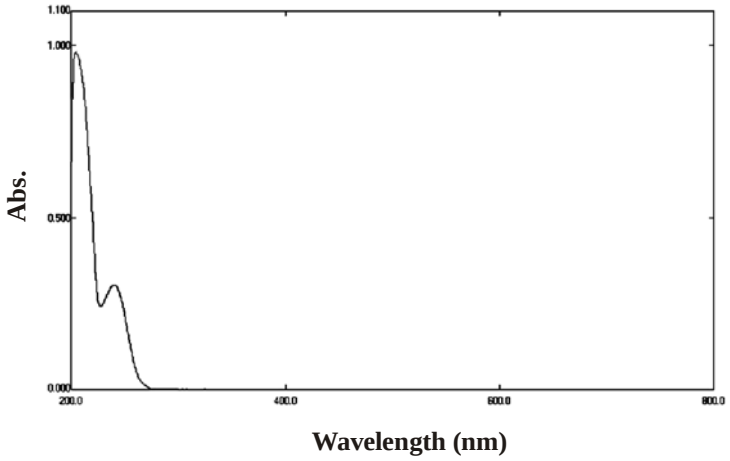

Fig. 2: Absorption spectrum of Ritonavir

The precision was carried out as described in method and the results were presented in Table 2. The values obtained in the repeatability (precision) shows that there is no significant difference in the precision values hence; the developed method can be used to analyze the ritonavir in tablet formulation. The mean of the precision value is 99.86. The regression equation was found to be y = 0.0115 x - 0.0056

Table 2: Assay results from Ritonavir tablets and mean recoveries in spiked tablets

Parameters	Ritonavir
Labelled claim, mg	100
Amount found, mg*	99.86
RSD %	2.87
Added, %	50, 75, 100

Cont…

Parameters	Ritonavir
Found, %**	98.89, 99.52, 98.87
Recovery, %	100.98
RSD, % of recovery	0.25

*Mean of six determinations,
** three determinations

The stability studies of solution were carried out as described in method and found that there was no significant difference while the final sample solution was kept for 6 h at ambient temperature.

The mean of the three different recovery studies are presented in Table 2. In that overall mean of the recovery studies was to be 100.98 %. The drug ritonavir in formulation was well identified under this condition. There is no interference is observed in different blank samples of ritonavir.

Figure 3 shows the regression analysis of calibration curve for ritonavir in pure form showed a linear relationship between the intensity of absorbance and the concentration with correlation. Correlation co-efficient was found to be higher than 0.998 in all the curves assayed.

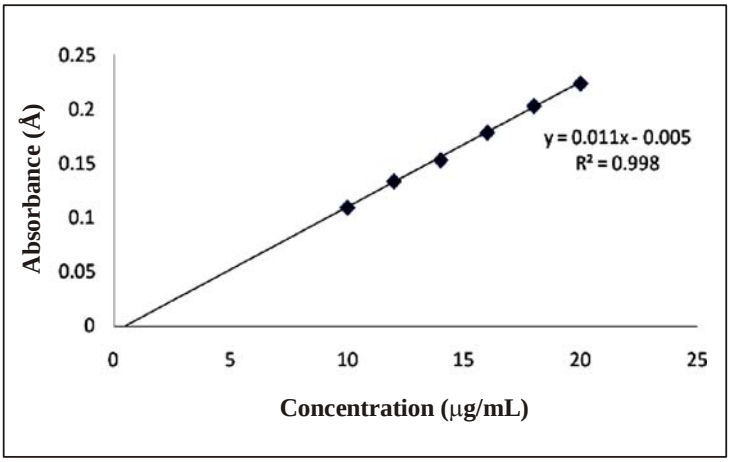

Fig. 3: Regression analysis of the calibration curve for ritonavir showed a linear relationship between the intensity of absorbance and the concentration, with correlation coefficients higher than 0.9989 in all the curves assayed

The LOD determined as the amount drug was found to be 1.1 μg/mL and the LOQ was determined as the lowest concentration was found to be 3.3 μg/mL in formulation.

The result of the interferences study showed that no interference of any component with the drug has been proved and was found from the recovery of ritonavir was 100.98%. This indicates the absence of interferences of any component with drug.

Ruggedness was performed as described in method by two different analysts, the method could not be repeated in a different laboratory or using different equipment and their results were shown that there is no significant changes found in the assay. Robustness was performed as described in method and the results has been proved that there are no significant changes when the drug analyzed indifferent wavelength.

CONCLUSION

A spectrophotometreic method for quantifying ritonavir in formulation has been developed and validated. The linear range of the proposed spectrophotometric method was 10-20 µg/mL. The assay is selective, precise, accurate and linear over the concentration range from 10-0 µg/mL, the concentration of ritonavir used for the precision study is 99.86 µg/mL in formulations could be precisely quantified and detected was approximated 1.1 µg/mL and 3.3 µg/mL respectively. Also, the proposed method involved spectrophotometric measurements with comparable analytical performance devoid from any potential interference. This gives the advantage of flexibility in performing the analysis on any available instrument. Furthermore, all the analytical reagents are inexpensive, have excellent shelf life, and are available in any analytical laboratory. Therefore, these methods can be recommended for the routine analysis of ritonavir in quality control and clinical laboratories.

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