



Research Article
**Journal of Pharmaceutical and Biomedical
 Analysis Letters**

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Analytical Method Development and Validation for the Estimation of Ritonavir in Bulk and Pharmaceutical Dosage Form

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Received: 30 April 2014, Accepted: 6 June 2014, Published Online: 18 June 2014

Abstract

A simple, robust, selective and sensitive spectrophotometric method has been developed for the determination of Ritonavir in pharmaceutical formulations. The method was based on the scanning of the drug in 0.1 N HCl and the same conditions for formulation. The method showed high sensitivity with linearity range from 10 to 60 µg/ml. The 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.

Keywords: UV spectroscopic, Ritonavir, linearity, precision, ICH guidelines

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 Manuscript ID: JPBMAL2130



PAPER-QR CODE

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1. 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- tetraazatridecan-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 [5-9], reversed-phase high-performance liquid chromatographic (RP-HPLC) method [10] and HPTLC method [11]. 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.

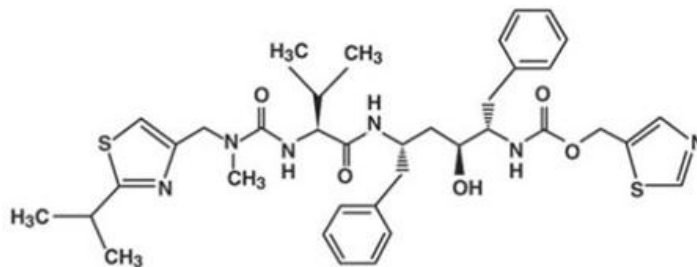


Figure 1. Chemical structure of Ritonavir

2. Materials and Methods

Materials

Ritonavir was obtained as a gift sample from MSN laboratories, Hyderabad. Distilled water was prepared in-house. Hydrochloric acid (HCl) was purchased from SD Fine chem ltd, Mumbai. PG Instruments T60 UV-Vis Spectrophotometer with a fixed slit width (2 nm) and 10 millimeter quartz cell was used to obtain spectrum and absorbance measurement. All the chemicals and reagents used were analytical reagent grade.

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 $\mu\text{g/ml}$ and the resulting solution was used as working standard solution.

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 $\mu\text{g/ml}$. From this stock solution, various dilutions of the sample solution were prepared and analyzed.

Method Validation

Method validation was performed in terms of specificity and selectivity, precision and accuracy, Linearity and stability as per ICH guidelines [12 and 13]

Linearity and range

Calibration standards of ritonavir, covering the range 10-20 $\mu\text{g/ml}$ 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.

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 three 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%, 100%, 150% of drug as average along with the tablet powder and further dilution was made to get 10 $\mu\text{g/ml}$ concentration. These solutions were used for further analysis to perform recovery studies

3. Results and Discussion

Calibration standards for ritonavir covering the range of 10-20 $\mu\text{g/ml}$ 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 absorbance 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 absorbance 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. 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 $y = 0.0115x - 0.0056$.

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 $y = 0.0115x - 0.0056$. 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.

4. Conclusion

From the validation study of the developed method for Ritonavir was found to be simple accurate, precise, and repeatable. Hence it can be applied for routine analysis of Ritonavir in its tablet dosage form for quality control.

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