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HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF RITONAVIR IN PURE FORM AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, selective, rapid and precise HPLC method was developed for the estimation of ritonavir in pure form and in pharmaceutical dosage forms. The drug was separated on a Luna C18 (4.6×250mm, 5µm) column with mobile phase comprising of acetonitrile: water in the ratio of 80:20%v/v. Retention time of the drug was found to be4.09 min. Linearity of the method was found to be 10-50µg/mL. Assay of the formulation was found to be 100.27%. The method was validated according to the ICH guidelines. The method was found to be suitable for the routine quality control analysis of ritonavir.

INTRODUCTION

Ritonavir is an antiretroviral drug from the protease inhibitor class used to treat HIV infection and AIDS [1]. It is an HIV protease inhibitor that interferes with the reproductive cycle of HIV. Although it was initially developed as an independent antiviral agent, it has been shown to possess advantageous properties in combination regimens with low-dose ritonavir and other protease inhibitors. Ritonavir inhibits the HIV viral proteinase enzyme that normally cleaves the structural and replicative proteins that arise from major HIV genes, such as gag and pol.

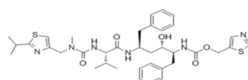

Fig. 1: Structure of Ritonavir

Chemically ritonavir is 10-Hydroxy -2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4thiazolyl]-3,6-dioxo-8,11-bis(phenylmethyl)-2,4,7,12tetraazatridecan-13-oic acid,5-thiazolyl methyl ester, [5S-(5R*,8R*,10R*,11R*)]. Structure of ritonavir is given in figure 1. It is white to light tan powder. Practically insoluble in water, freely soluble in methanol and ethanol, and soluble in isopropanol. Molecular weight of ritonavir is 720.944. Literature survey revealed that few spectrophotometric [2, 3], HPLC [4-9] and LC-MS/MS [10, 11] methods have been reported for estimation of ritonavir. In the present investigation we have developed a simple, rapid and precise HPLC method for the estimation of ritonavir in pure form and pharmaceutical dosage forms.

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EXPERIMENTAL:

Chemicals, Reagents, and Solutions: Pharmaceutical grade of ritonavir was kindly supplied by Sura Labs (Hyderabad, India).Water; methanol and acetonitrile (HPLC grade) were purchased from Merck, India. Mobile phase was prepared by mixing 200 ml of HPLC grade water with 800 ml of acetonitrile and degassed by sonication for 15 minutes and then filtered through 0.2 µ membrane filter. Mobile phase was also used as diluent. Standard stock solution of ritonavir was prepared in diluent at a concentration of 1.0 mg/mL and further diluted with diluent to furnish working standard stock solution of 100 µg/mL. The working standard stock solution was used to prepare calibration samples.

HPLC Instrumentation and Chromatographic Conditions: HPLC system used for the investigation was Shimadzu HPLC with manual injector and PDA Detector. Software used was the Lab solution and column used was Luna C18 (4.6×250mm, 5 µ). Mobile phase used was acetonitrile: water in the ratio of 80:20 v/v and flow rate was 1 mL/min. Run time used for the chromatography was 10 min and analyte was monitored at 254 nm. Injection volume was 20 µL.

Standard Solutions and Calibration Curve: The standard stock solution was diluted with mobile phase to prepare working standard solution and calibration samples. Calibration samples were prepared in the concentration range of 10 to 50 µg/mL. Triplicate injections were made for each calibration sample and chromatographed under the specified HPLC conditions described previously. The peak area of each concentration was plotted against the corresponding concentrations to obtain the calibration graph. Regression equation was calculated.

Analysis of the Pharmaceutical Dosage Forms: Tablet powder equivalent to 10 mg of ritonavir was taken in 10 mL volumetric flask, and volume was made up with diluent, vortexed for 10 min, and sonicated for 15 min. Further pipette out 0.4 mL of above stock solution into a 10mL volumetric flask and dilute up to the mark with diluent. The resultant solution was injected into the HPLC system and amount of drug present in the formulation was calculated.

RESULTS AND DISCUSSION

HPLC method was developed by performing different runs with different mobile phase combinations like acetonitrile: water and methanol: water at different proportions. In methanol: water mobile phase, the peak shape was not good. The peak shape was good by using the mobile phase acetonitrile: water in the ratio of 80:20. The column used was C18 column. The optimized chromatogram was shown in figure2. The retention time of the drug was found to be 4.09 min. The method was validated in accordance with International Conference on Harmonization (ICH) guidelines [12], for system suitability, linearity, specificity, precision, accuracy, limit of detection, limit of quantification, and robustness. System-suitability test was an integral part of method development and has been used to ensure adequate performance of the chromatographic system. % RSD of retention time, number of theoretical plates (N), and tailing factor (T) were evaluated for five replicate injections of the drug at a concentration of 30 µg/mL. The % RSD of retention time was found to be 0.1%, number of theoretical plates was found to be 3224 and tailing factor observed was 0.89. The method was found to be specific as there is no interference from the excipients present in the formulation. Model chromatogram of ritonavir formulation was shown in Figure 3. Linearity of the proposed method was evaluated according to ICH guidelines. Ritonavir showed linearity in the concentration range of 10-50 µg/mL. The regression equation obtained was Y= 6468X+4516, where Y is peak area and X is concentration of ritonavir (µg/mL). The correlation coefficient was found to be 0.9982. Intra-day and inter-day precision was evaluated by injecting three different concentrations (10, 30 and 50 µg/mL) of ritonavir. For intra-day variation, sets of three replicate of the three concentrations were analyzed on the same day; for inter-day variation, three replicates were analyzed on three different days. The intra-day and inter-day precision (% RSD) was found to be 1.2%, within the acceptable limit of not more than 2%.

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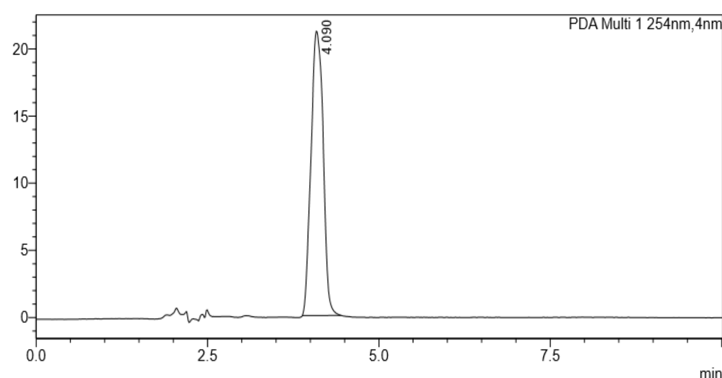

Figure 2: Model chromatogram of ritonavir at a concentration of 40 µg/mL

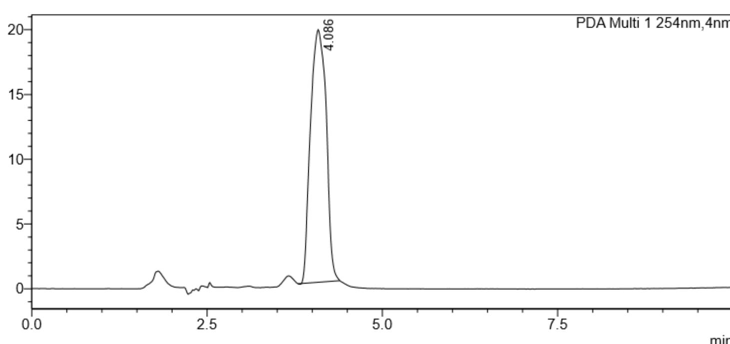

Figure 3: Model chromatogram of Ritonavir formulation.

Limit of detection (LOD) was defined as the lowest concentration of ritonavir resulting in a signal-to-noise ratio of 3:1, and limit of quantification (LOQ) was expressed as a signal-to-noise ratio of 10:1. Due to the difference in detector response, different concentrations ranging from 1to 10 µg/ mL were prepared and analyzed. The LOD and LOQ obtained were of 2.0 and 6.0 µg /mL, respectively. Accuracy of the method was determined by performing the recovery experiments. Known amount of the standards at 80%, 100%, and 120% levels was fortified to aliquot of the formulation sample containing 20 µg of ritonavir. Peak areas of the standards were calculated by the difference of peak areas between fortified and unfortified samples. Three replicate samples of each concentration level were prepared and the % recovery at each level (n = 3) was determined. The percentage recovery obtained was within the acceptable limit of 98-102%. A method is robust if it is unaffected by small changes in operating conditions. To evaluate HPLC method robustness, a few parameters were deliberately varied. The parameters included variation of flow rate and variation of mobile phase composition. Flow rate varied was ±0.1 mL and acetonitrile content of mobile phase was varied by 80 ±2 (v/v). There was no significant variation in the retention time, peak area and tailing factor. So the method was found to be robust. The assay of the tablets was found to be 100.27%.

Conclusions:

A simple, selective, rapid and precise HPLC method was developed for the estimation of ritonavir in bulk drugs and in pharmaceutical formulations. The method was validated in terms of system suitability, specificity, linearity, accuracy, precision, LOD&LOQ and robustness. The method was found to be suitable for the routine quality control analysis of ritonavir in bulk drugs and in pharmaceutical formulations.

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