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# DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTITATIVE ESTIMATION OF RITONAVIR IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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## ABSTRACT

A simple, precise, specific and accurate reverse phase HPLC method has been developed for the determination of Ritonavir in bulk and pharmaceutical dosage forms. The chromatographic separation was achieved on Symmetry C18 (4.6 x 100mm, 3.5 µm) column using a mixture of Buffer: Acetonitrile (50:50) as the mobile phase at a flow rate 1.0 ml/min. Linearity was observed in concentration range of 50-150µg/ml. The retention time of Ritonavir was 5.1 min. The analyte was monitored using UV detector at 239 nm. Results of analysis were validated statistically and by recovery studies. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness.

**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. It has the structural formula and shown 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 Pharmacopoeia [2] and United States Pharmacopoeia [3].

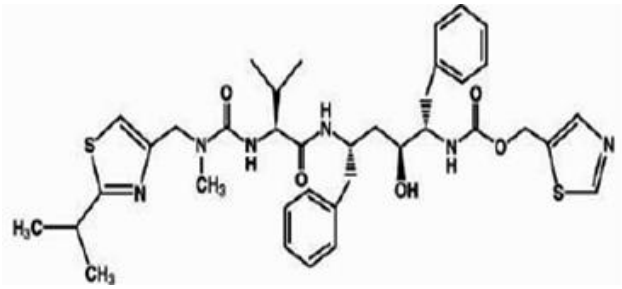

**FIG. 1: CHEMICAL STRUCTURE OF RITONAVIR**

From the literature survey, it was found that Ritonavir estimated by analytical methods such as reversed-phase high-performance liquid chromatographic (RP-HPLC) method [4, 5, 6, 7, 8, 9, 10], LC-MS [11] and HPTLC method [12]. 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.

**MATERIALS AND METHODS:** A Shimadzu HPLC model containing LC-20 AT pump, variable wavelength programmable UV/VIS detector and Rheodyne injector was employed for the investigation. All the chemicals used in the investigation were of HPLC grade. The chromatographic analysis was performed on a Symmetry C18 (4.6 x 100mm, 3.5 µm) column. The mobile phase consisted of buffer (Phosphate $pH_4$) and acetonitrile in the ratio of 50:50 (v/v). The optimized chromatographic conditions are summarized in **Table 1**.

**TABLE 1: OPTIMIZED CHROMATOGRAPHIC CONDITIONS FOR THE PROPOSED METHOD**

| Parameters | Optimized condition |
|---|---|
| Column | Symmetry C18 (4.6 x 100mm, 3.5 µm) |
| Mobile phase | Buffer (Phosphate pH4) and Acetonitrile (50:50) |
| Flow Rate | 1.0 ml / min |
| Injection volume | 20 µl |
| Detection | 239 nm in UV detector |
| Temperature | Ambient |
| Retention Time | 5.10min |
| Run time | 8min |

**Preparation of mobile phase:** Potassium dihydrogen orthophosphate was weighted (3.5 g) and dissolved in 500 ml of HPLC water. This solution was mixed with 500 mL of acetonitrile and mixed well and finally adjusted pH 4.0 using ortho phosphoric acid. The solution was sonicated for 10 min and filtered using Whatman filter paper.

**Preparation of standard stock solution of Ritonavir:** The standard solution of Ritonavir was prepared by dissolving 25mg of Ritonavir 100 ml of Mobile phase to give the concentration 250 µg / ml. The mobile phase and the solution were sonicated for 10 min and filtered through 0.45µm filter.

**Preparation of Calibration Curve:** From the standard stock solution, the various dilutions of Ritonavir in the concentration of 50, 75, 100, 125 and 150 µg / ml were prepared. The solutions were injected using a 20 µl fixed loop in to the chromatographic system at the flow rate of 1.0

ml/min and the effluents were monitored at 239 nm, chromatograms were recorded. The Ritonavir was eluted at 5.1 min as shown in (**Fig. 2**). The calibration curve was constructed by plotting average peak area versus concentration and was presented in (**Fig. 3**). The method was extended for determination of Ritonavir in pharmaceutical dosage form containing 100 mg.

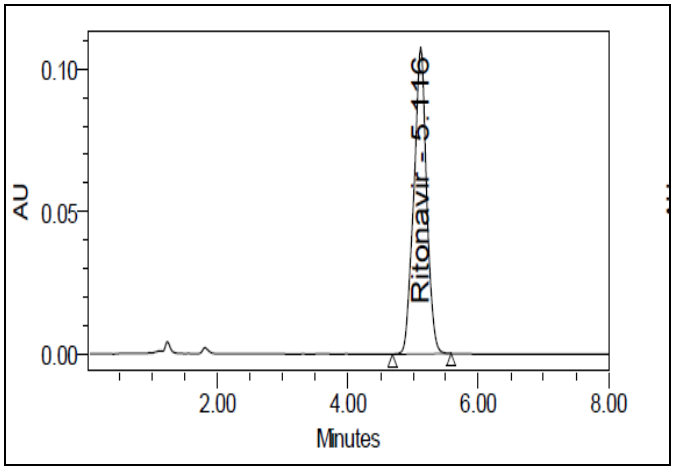


**FIG. 2: TYPICAL RP-HPLC CHROMATOGRAM OF RITONAVIR BY THE PROPOSED METHOD**

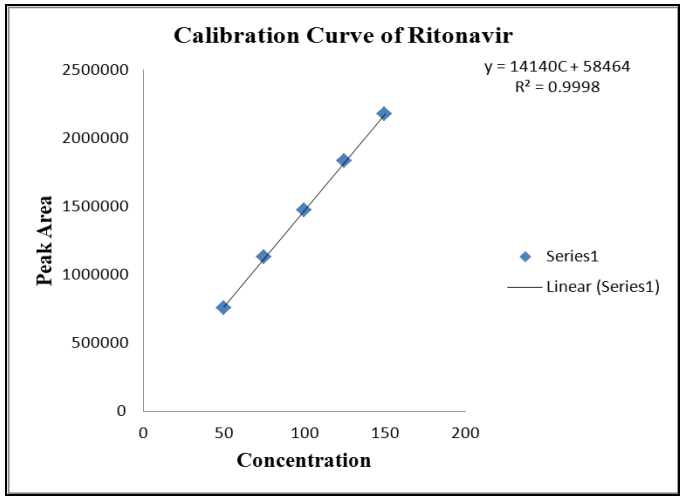


**FIG. 3: CALIBRATION CURVE OF RITONAVIR BY THE PROPOSED METHOD**

**Preparation for 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 analyzed. The proposed methods were validated as per the ICH guidelines [13-15].

**RESULTS AND DISCUSSION:** A system suitability test was applied to representative chromatograms for various parameters. The results obtained were within acceptable limits and are represented in **Table 2**.

**TABLE 2: SYSTEM SUITABILITY TEST PARAMETERS FOR THE PROPOSED METHOD**

| Parameters | Values |
|---|---|
| Theoretical plates | 2945.50 |
| Tailing factor | 0.98 |
| Retention Time | 5.01 |

Thus, the system meets suitable criteria. The calibration curve was obtained for a series of concentration in the range of 50-150 µg / ml and it was found to be linear. The data of regression analysis of the calibration curves are shown in **Table 3**.

**TABLE 3: REGRESSION ANALYSIS OF THE CALIBRATION CURVE FOR THE PROPOSED METHOD**

| Parameters | Values |
|---|---|
| Linearity Range(µg/ml) | 50-150 |
| Correlation coefficient($r^2$) | 0.9998 |
| Regression equation | Y= 14140C + 58464 |
| Slope | 14140 |
| Intercept | 58464 |

The precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample. The % RSD was found and lying with in 2. This showed that the precision of the method was satisfactory. The accuracy of the method was inferred by establishing the precision and linearity studies of the standard. The % RSD was less than 2.0. This showed that the recoveries of Ritonavir by the proposed methods are satisfactory. Ruggedness and Robustness were determined and the % RSD values were calculated from precision study was less than 2.0. Limit of detection (LOD) and Limit of quantitation (LOQ) were determined by the proposed methods. The results of validation parameters are summarized in **Table 4**.

**TABLE 4: SUMMARY OF VALIDATION PARAMETERS FOR THE PROPOSED METHOD**

| Parameters | Values |
|---|---|
| Limit of detection (µg/ml) | 0.013 |
| Limit of quantitation (µg/ml) | 0.43 |
| [a]Precision ( % RSD) | |
| Intra Day precision | 0.35 |
| Inter Day precision | 0.22 |
| [a]Ruggedness ( % RSD) | |
| Analyst I | 0.11 |
| Analyst II | 0.06 |
| [a]Robustness (% RSD) | |
| Changed condition I ( ratio of mobile phase) | |
| 55 : 45 (Buffer : Acetonitrile) | 0.20 |
| 45 : 55 (Buffer : Acetonitrile) | 0.29 |
| Changed condition II ( flow rate of mobile phase) | |
| 0.8 ml / min | 0.38 |
| 1.2 ml / min | 0.06 |

[a]Mean of six determinations, RSD indicates relative Standard deviation

The results of tablet analysis and recovery studies obtained by the proposed method were validated by statistical evaluation and are given in **Table 5**.

**TABLE 5: ASSAY RESULTS OF RITONAVIR USING PROPOSED METHOD**

| Brand used | Labeled amount (mg) | Amount found (mg) | %Recovery ± SD[**] |
|---|---|---|---|
| Tab-A | 100 | 99.78 | 99.65 ± 0.11 |
| Tab-B | 100 | 100.20 | 100.23 ± 0.06 |

[**]Mean of six determinations

**CONCLUSION:** Thus, it can be concluded that the method developed in the present investigation was simple, sensitive, accurate, rugged, robust, rapid and precise. Hence, the above said method can be successfully applied for the estimation of Ritonavir in pharmaceutical dosage forms..

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