

NEW VALIDATED RP-HPLC METHOD FOR THE DETERMINATION OF RITONAVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Received: 03 Aug 2013, Revised and Accepted: 04 Sep 2013

ABSTRACT

A simple validated RP HPLC method for the estimation of Ritonavir in pharmaceutical dosage form and bulk was developed for routine analysis. This method was developed by selecting Agilent TC C18 (250 x 4.6 mm, 5 μ) column as stationary phase and Water: Acetonitrile (20:80 v/v) pH adjusted to 3 as mobile phase. Flow rate of mobile phase was maintained at 1 ml/min at ambient temperature throughout the experiment. Quantification was achieved with ultraviolet (DAD) detection at 239 nm. The retention time obtained for Ritonavir was at 4.3 min. The detector response was linear in the concentration range of 10 – 80 μ g/ml. This method has been validated and shown to be Specific, Sensitive, Precise, Linear, Accurate, Rugged and Robust. Hence, this method can be applied for routine quality control of Ritonavir in dosage forms as well as in bulk drug.

Keywords: Ritonavir, Reverse Phase High Performance Liquid Chromatography, Ritovir capsules.

INTRODUCTION

Ritonavir [1] is an antiretroviral drug belongs to 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.

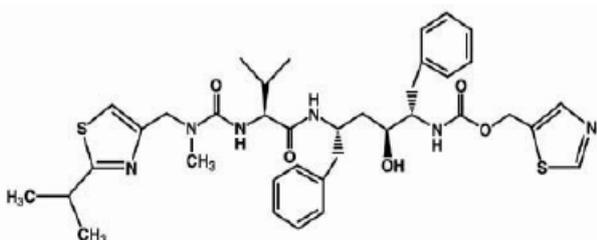


Fig. 1: Chemical Structure of Ritonavir

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]. From the literature survey, we found that Ritonavir was estimated by analytical methods such as Reversed Phase High Performance Liquid Chromatography (RP-HPLC) method [4-11], LC-MS [12] and HPTLC method [13]. The availability of an HPLC method with high sensitivity and rapid quantification will be very much useful for the determination of Ritonavir in pharmaceutical formulations. The aim of the study is to develop a simple, precise, accurate and validated Reversed-Phase HPLC method for the estimation of Ritonavir in pharmaceutical dosage form as per ICH guidelines. The statistical analysis proved that method is reproducible and selective for the analysis of Ritonavir in bulk drug and formulations.

MATERIALS AND METHODS

Pharmaceutical grade Ritonavir was supplied by Hetro Drugs Ltd., Hyderabad, India. The Methanol (HPLC grade), Acetonitrile (HPLC grade) were purchased from MERK and the triple distilled water was collected from in house production. The commercially available RITOVIR capsules (one equivalent to 100 mg of Ritonavir) which are manufactured by Hetro drugs Ltd. was purchased from market for analysis.

Instruments

Agilent technologies 1200 LC system with gradient pump connected to DAD UV detector, LC-GC AGN204PO balance was used for all weighing.

METHOD DEVELOPMENT

Chromatographic conditions

Chromatographic separation was achieved by using Agilent TC C18 (250 x 4.6 mm, 5 μ) column as stationary phase and composition of Water: Acetonitrile (20:80 v/v) pH adjusted to 3 as mobile phase. Flow rate was maintained at 1 ml/min at ambient temperature and the injection volume used as 20 μ l. The detection was carried out at 239 nm. The retention time obtained for Ritonavir was at 4.3 min. Diluent was prepared by mixing 800 ml of Acetonitrile with 200 ml of triple distilled water, filtered through Whatman filter paper (0.45 μ m) and degassed before use. A typical chromatogram of blank and Ritonavir standard drug was shown in Fig. 2 and 3.

Preparation of stock solution

Accurately weighed quantity of Ritonavir (10 mg) was transferred to 10.0 ml volumetric flask. Then small amount of methanol was added and ultrasonicated for 5 min and diluted upto the mark with methanol (Concentration: 1000 μ g/ml).

Preparation of standard working solution

From the stock solution pipette out 1ml into 10 ml volumetric flask and make up the final volume with methanol (100 μ g/ml).

Preparation of mobile phase

The mobile phase was prepared by mixing Acetonitrile: water (80:20) the mobile phase was filtered through Whatman filter paper (0.45 μ m) and degassed before use.

Preparation of working sample solution

Select 20 capsules of RITOVIR (containing 100mg of Ritonavir). Weigh accurately each capsule and calculate the average content of 20 capsules. Weigh accurately powder equivalent to 10mg of Ritonavir and transferred to 10ml standard volumetric flask. Then add small amount methanol into the volumetric flask, sonicate for about 15min, and the final volume was made with same to obtain solution having the concentration of 1000 μ g/ml. The mixture was then filtered through Whatman filter paper (0.45 μ m). The above solution was suitably diluted with mobile phase to obtain the solution having the final concentration of 30 μ g/ml. A typical chromatogram of Ritonavir formulation (sample) drug was shown in Fig. 4. The assay results are shown in table 1.

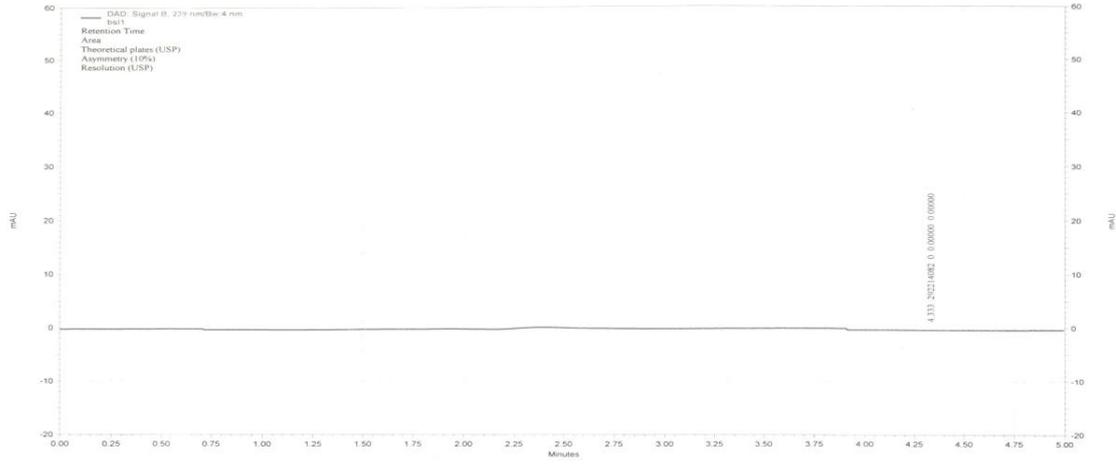


Fig. 2: Typical Chromatogram of Blank

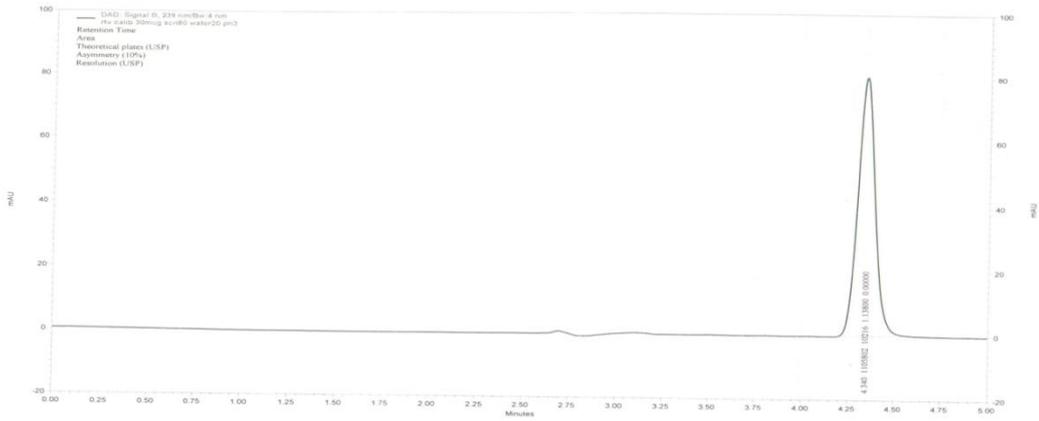


Fig. 3: Typical Chromatogram of Ritonavir standard drug

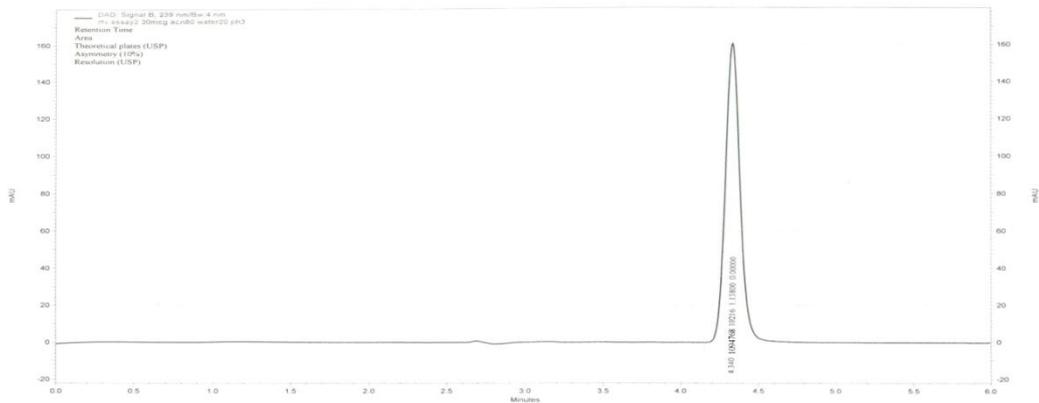


Fig. 4: Typical Chromatogram of Ritonavir (Ritovir) formulation

Table 1: Analysis of Formulation

Drug	Label claim (mg/Tablet)	Amount* found (mg/ Tablet)	% Amount found	%RSD
RITOVIR	100	100.18	100.18	1.3

*Mean of three readings

Method Validation

The method was validated for its linearity range, accuracy, precision, sensitivity and specificity. Method validation is carried out as per ICH guidelines [14-16].

Linearity

Calibration curve was constructed by plotting peak area Vs concentration of Ritonavir solutions, and the regression equation was calculated. The calibration curve was plotted over the concentration range 10-80µg/ml. accurately measured Ritonavir standard working solution of 1,2,3,4,5,6,7 and 8ml were transferred to a series of 10ml volumetric flasks and diluted up to the mark with mobile phase. 20 µl aliquots of each solution were injected into the HPLC system which is operated according to chromatographic condition as described above. The plot of calibration curve was shown in Fig. 5.

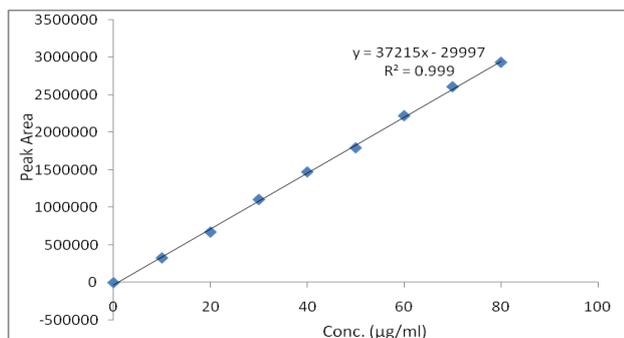


Fig. 5: Calibration curve of Ritonavir

Accuracy

The accuracy of the methods was determined by calculating recoveries of Ritonavir by the standard addition methods. The accuracy of the method was determined by preparing solutions of different concentrations in which the amount of marketed formulation (RITOVIR-100mg) was kept constant (30mg) and the amount of pure drug was varied that is 24mg, 30mg and 36mg for 80%, 100% and 120% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery was shown in table 3.

Method precision

The precision of the instruments was checked by repeatedly injecting (n=3) solutions of Ritonavir (20µg/ml).

Intermediate Precision (Reproducibility)

The intra-day and inter-day precision of the proposed methods were determined by the corresponding responses three times on the same day and on three different days over a period of one week for three different concentration of Ritonavir (10,20 and 50µg/ml)

Table 3: Accuracy studies of Ritonavir

Amount of sample taken (µg/ml)	Amount of standard added (µg/ml)	% of Std added	Amount recovered (µg/ml)*	% amount recovered*	% RSD
30	24	80	24.06	100.27	0.432
30	30	100	29.99	99.86	
30	36	120	36.23	100.45	

*Average of three determinations (n=3)

Table 4: Precision studies of Ritonavir

Amount of std taken (µg/ml)	Intra-day precision Mean*±% RSD	Inter-day precision Mean*±% SD
10	99.91±0.580	100.03±0.618
20	101.51±0.498	99.65±0.260
50	100.23±0.970	99.34±0.686

*Average of three determinations (n=3)

Robustness

Robustness of the method was determined by carrying out the analysis at three different wavelengths (i.e. 239±2 nm) and three different flow rates (i.e. 1±0.1 ml/min).

Ruggedness

Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective peak areas were noted. The result was indicated by % RSD.

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were calculated using the following equations as per International Conference of Harmonization (ICH) guidelines.

$$\text{LOD} = 3.3 X \alpha / S$$

$$\text{LOQ} = 10 X \alpha / S$$

RESULTS AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phases of different compositions were tried. A satisfactory separation and good peak symmetry for Ritonavir were obtained with a mobile phase consisting of Acetonitrile: water (80: 20 v/v) pH adjusted to 3. Quantification was achieved with UV detection at 239nm based on peak area. Complete resolution of the peaks with clear baseline was obtained. System suitability parameters was calculated and compared with the standard limit as per ICH. The following tables shows the results obtained which are related different analytical method validation parameters.

Table 2: Linearity of Ritonavir

Parameter	Result
Linearity range	10-80µg/ml
Slope	37215
Intercept	-29997
Correlation coefficient	0.999

The Linearity and correlation coefficient of Ritonavir was found to be 10-80µg/ml, and 0.999 respectively.

The accuracy experiments were carried out by the standard addition method. The recoveries obtained by 99.86 to 100.45% for Ritonavir. The values indicate that method is highly accurate (Tab-3).

The low %RSD values of intra-day and inter-day precision studies (0.682 and 0.521) for Ritonavir reveal that the proposed method is precise (Tab-4).

The low %RSD values of Robustness and Ruggedness (0.658 and 0.691) for Ritonavir reveal that the proposed method is robust and rugged (Tab-5).

LOD for Ritonavir was found to be 2.65 and LOQ for Ritonavir was found to be 8.06. This data concludes that the method is sensitive for the determination of Ritonavir (Table-6).

Table 5: Robustness and Ruggedness studies of Ritonavir

Parameter		Mean ^a ±% SD
Robustness	Change in flow rate (±0.1ml/min)	99.92±0.337
	Change in λ _{max} (± 2nm)	100.65 ±0.98
Ruggedness	1 st analyst	99.65±0.517
	2 nd analyst	99.34±0.865

*Average of three determinations (n=3)

Table 6: LOD and LOQ of Ritonavir

STD	LOD (µg/ml)	LOQ (µg/ml)
Ritonavir	2.65	8.06

CONCLUSIONS

A simple, accurate, precise, selective and sensitive RP- HPLC assay method with DAD detection for Ritonavir in pharmaceutical dosage form has been developed and validated. The method will be extensively used for the estimation of Ritonavir in bulk and pharmaceutical formulation.

ACKNOWLEDGEMENTS

The authors are very much thankful to Prof. A. Prameela Rani, Principal, University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Guntur, for providing the necessary chemicals and instrumentation for our work. K. Sujana, Asst. Prof., Department of Pharmaceutical Analysis, University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Guntur for giving valuable guidance to us and also thankful to their classmates and friends for their co-operation to them in this work.

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