

## Research Article



## REVERSE PHASE HPLC METHOD FOR DETERMINATION OF RITONAVIR IN PHARMACEUTICAL PREPARATIONS

Nagaraju P.T.\*, Sreekanh reddy K.P, Chinna ganganna P, Venugopal N, Ravindranath S, Bhanu Priya B, Dr. Satyanandam Sade  
Department of Pharmaceutical Analysis, Srinivasa Institute of Pharmaceutical Sciences, Proddatur, Andhra Pradesh, India.

\*Corresponding author's E-mail: [nraju04@gmail.com](mailto:nraju04@gmail.com)

Accepted on: 30-01-2012; Finalized on: 20-03-2012.

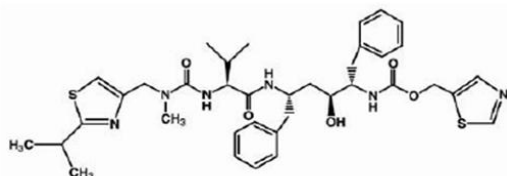
### ABSTRACT

A simple and reliable high-performance liquid chromatography (HPLC) method was developed and validated for Ritonavir in pharmaceutical preparations. The method was developed on Thermo Hypersil RP C-18 column (150 mm x 4.6 mm, 5 $\mu$ m) using a mobile phase of Acetonitrile : Potassium Dihydrogen Phosphate & DiPotassium Hydrogen Ortho Phosphate (45 : 55, v/v). The effluent was monitored by PDA detector at 239 nm. The total run time was 7 min with a flow rate of 1.0 ml/min. Calibration curve was linear over the concentration range of 20 – 120  $\mu$ g/ml. For Intra-day and inter-day precision % RSD values were found to be 0.38% and 0.41% respectively. Recovery of Ritonavir was found to be in the range of 99.90-100.50%. The limits of detection (LOD) and quantification (LOQ) were 0.09 and 0.027  $\mu$ g/ml respectively. The developed RP-HPLC method was successfully applied for the quantitative determination of Ritonavir in pharmaceutical dosage forms.

**Keywords:** Ritonavir, HPLC, Pharmaceutical preparation, Validation.

### INTRODUCTION

Ritonavir<sup>1</sup> is an Antiretroviral drug from the protease inhibitor class used to treat HIV infection and AIDS. Nomenclature : 10-Hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-Thiazolyl]-3, 6-dioxo-8,11-bis(phenyl methyl) 2,4,7,12- Tetraazatridecan-13-oicacid, 5-thiazolylmethyl ester, [5S- (5R, 8R, 10R, 11R)]. Molecular formula: C<sub>37</sub>H<sub>48</sub>N<sub>6</sub>O<sub>5</sub>S<sub>2</sub>. Solubility: Freely soluble in methanol and ethanol, soluble in isopropanol.



**Figure 1:** Structure of Ritonavir

Literature survey reveals that several analytical methods have been reported for the estimation of Ritonavir by UV<sup>2-5</sup> Spectroscopic, LC<sup>6</sup> HPLC<sup>7-15</sup>, LC-MS<sup>16,17</sup> and HPTLC<sup>18</sup> method was developed and applied in the determination of Ritonavir in biological fluids. HPLC methods for quantitative determination of Ritonavir in bulk drug samples and formulations were reported till date. The aim of this study was to develop a RP-HPLC method, which could be employed for the routine analysis of the drug in pharmaceutical dosage forms using simple mobile phase composition

### MATERIALS AND METHODS

#### Chemicals and Reagents

An analytically pure sample of Ritonavir was procured as gift sample from HETERO DRUGS. (Hyderabad, India). HPLC grade Acetonitrile and Potassium Dihydrogen Phosphate & DiPotassium Hydrogen Ortho Phosphate

was procured from E. Tablet formulation RITOVIR were procured from a local pharmacy with labelled amount 100 mg per tablet.

#### Instrumentation

The HPLC system consisted of a Waters Alliance (Waters Corporation, MA, USA) equipped with a Waters 2695 solvent delivery module in a quaternary gradient mode and a Waters 2487 PDA detector. Data acquisition was performed by the EM-power 2 software.

#### Chromatographic Conditions

Chromatographic analysis was performed on a Thermo Hypersil reversed phase C-18 column with 150 mm x 4.6 mm i.d. and 5  $\mu$ m particle size. The mobile phase consists Acetonitrile: Potassium Dihydrogen Phosphate & DiPotassium Hydrogen Ortho Phosphate (45 : 55 v/v) and was set at a flow rate of 1.0 ml/min. The mobile phase was degassed and filtered through 0.2  $\mu$ m membrane filter before pumping into HPLC system. The effluent was monitored by PDA detector at 239 nm.

#### Preparation of Solutions

##### Preparation of Standard Solutions

The stock standard solution of Ritonavir was prepared with methanol to a concentration 50  $\mu$ g/ml. Five standard solutions ranging from 20 to 120  $\mu$ g/ml (20, 40, 60, 80, 100, 120 mcg/ml) were prepared in methanol by a serial dilution. Three quality control (QC) samples at the concentrations of 90%, 110% and 130% were prepared from the stock standard solution.

##### Procedure for pharmaceutical preparations

The average tablet mass calculated from mass of tablets of Ritovir (100mg Ritonavir tablet which was composed of Ritonavir and some excipients). They were then finely



ground, homogenized and portion of the powder was weighed accurately, transferred into a 10 ml volumetric flask and diluted up to mark with methanol. The mixture was sonicated for at least 30 min to aid dissolution and then filtered through a whatman no 41 paper. An appropriate volume of filtrate was diluted further with methanol so that the concentration of Ritonavir in the final solution was within the working range. The sample solution was then analyzed by HPLC (figure 2).

## RESULTS AND DISCUSSION

### Method development and optimization

The development of the RP-HPLC method for determination of drugs has received considerable attention in recent years because of its importance in routine quality control analysis. A RP-HPLC method was proposed as a suitable method for the estimation of Ritonavir in pharmaceutical dosage form. A good separation was achieved using a Thermo Hypersil RP C-18 column (150 mm x 4.6 mm, 5  $\mu$ m). The chromatographic conditions were adjusted in order to provide a good performance of the assay. The method involved a mobile phase consisting of Acetonitrile: Potassium Dihydrogen Phosphate & DiPotassium (45:55 v/v) accomplished at 239 nm. The retention time was 3.11 min at a flow-rate of 1.0 ml/min and the injection volume was 20  $\mu$ l. The total run time for an assay was approximately 7 min.

### Validation of the method

#### System suitability

A system suitability test of the chromatographic system was performed. Five replicate injections for a system suitability test were injected into the chromatographic system. Relative standard deviation and column efficiency for the five suitability injections were determined. For all sample analyses, the efficiency and %RSD were found  $\geq 2000$  and  $\leq 1.84\%$  respectively.

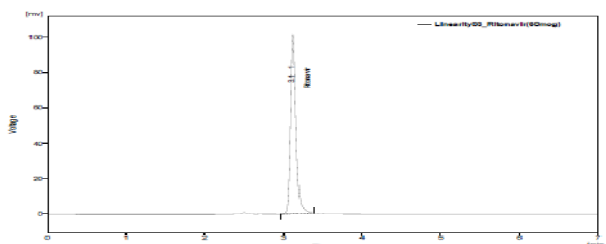


Figure 2: Chromatogram of Ritonavir at 239 nm

#### Linearity

Calibration curve was constructed for Ritonavir standard by plotting the concentration of compound versus peak area response. Standard solutions containing 20, 40, 60, 80, 100 and 120  $\mu$ g/ml of Ritonavir were injected into the HPLC column (figure 3). The linearity was calculated by the least square regression method. The regression equations were calculated from the calibration graphs (table 1).

Table 1: Linearity results for Ritonavir

Conc ( $\mu$ g / ml)	20	40	60	80	100	120
Peak Area	161.042	302.553	413.807	559.421	693.764	840.055
Correlation Coefficient	0.998					

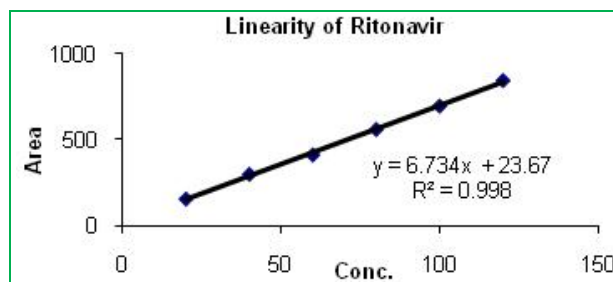


Figure 3: Calibration curve of Ritonavir at 239 nm

#### Accuracy

Accuracy was performed in triplicate after spiking pure drug equivalent to 90, 110, and 130% of the standard concentration of Ritonavir (20  $\mu$ g/ml). The results obtained (table 2) indicate that recovery was excellent, not less than  $100\% \pm 2$ .

Table 2: Accuracy results for Ritonavir

Sample	Percentage recovery	Mean
90%	100.73	100.01
90%	98.75	
90%	99.76	
110%	100.41	100.44
110%	100.59	
110%	99.12	
130%	100.78	100.06
130%	99.34	
130%	99.98	

Table 3: Precision results for Ritonavir

S. No.	Concentration ( $\mu$ g / mL)	Intraday precision (Area)	Interday precision (Area)
1	100	631.466	631.653
2	100	637.242	635.521
3	100	631.196	632.899
4	100	633.304	633.431
5	100	632.199	632.145
6	100	632.786	633.638
Mean		633.181	633.483
SD		2.43005	2.67517
%RSD		0.38	0.41

#### Sensitivity

Limit of detection (LOD) and quantification (LOQ) were estimated from the signal-to-noise ratio. LOD and LOQ values were found to be 0.09 and 0.28  $\mu$ g/ml, respectively.



### Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six injections of standard solution were injected into the chromatographic system in different time interval within a day. In the inter-day variation studies, six injections of standard solution were injected at different days. % RSD was calculated presented in table 3.

### Reproducibility (Ruggedness)

In addition to intra and inter day precision reproducibility study was also carried out and it was checked by determining precision on the same instrument, but by a different analyst. Results of reproducibility are shown in table 4.

**Table 4:** Ruggedness studies of Ritonavir by RP-HPLC method

Label claim (mg)	Analyst I		Analyst II	
	Amount found (mg)	Recovery $\pm$ SD* (%)	Amount found (mg)	Recovery $\pm$ SD* (%)
100	99.98	99.90 $\pm$ 0.96	100.06	100.50 $\pm$ 1.08

### CONCLUSION

A rapid and simple RP-HPLC method for determination of Ritonavir has been developed and validated. This chromatographic assay fulfilled all the requirements to be identified as a reliable and feasible method, including linearity, accuracy, sensitivity, precision, ruggedness and robustness. The chromatographic run time of 7 min allow a lot of samples in a short period of time. Therefore, the method is suitable for analysis of large samples during routine analysis of formulations and raw materials.

### REFERENCES

1. <http://www.rxlist.com/norvir-drug.htm>.
2. K.Chiranjeevi, K.P.Channabasavaraj, P.Srinivas Reddy, P.T.Nagaraju. Development and Validation of Spectrophotometric Method for Quantitative estimation of Ritonavir in Bulk and Pharmaceutical Dosage Forms. Int J Chemtech Res 3(1):2011; 58-62.
3. K. Chiranjeevi, G. Vijaya Kumar, K.P. Channabasavaraj, Y. Manjunath. Third Order Derivative Spectrophotometric Estimation Of Ritonavir In Bulk And Pharmaceutical Dosage Forms. Int.J.Ph.Sci 3(1):2011; 1017-1020.
4. Dias CL, Bergold AM, Pedro EF. UV-Derivative Spectrophotometric Determination of Ritonavir Capsules and Comparison with LC Method. Anal Letrs 42(12):2009; 1900-1910.
5. Vaishali PN, Kishore PB. Simultaneous Estimation of Ritonavir and Lopinavir by Absorption ratio (Q-analysis) UV Spectrophotometric Method in Combined Tablet Dosage Form. Der Pharmacia Lettre 2(1):2010; 196-200.
6. Dias CL, Rossi RC, Donato EM, Bergold AM, Froehlich PE. LC Determination of Ritonavir, a HIV Protease Inhibitor, in Soft Gelatin Capsules. J chromatographia 62:2005; 589-593.
7. K.Chiranjeevi, K.P.Channabasavaraj. Development and Validation Of RP-HPLC Method For Quantitative Estimation Of Ritonavir In Bulk And Pharmaceutical Dosage Forms.Int J Ph Sci & Res 2(3):2011; 596-600.
8. Richard M, Hoetelmans W, High-Performance Liquid Chromatographic determination of Ritonavir in human plasma, cerebrospinal fluid and saliva. J Chromatogr B Biomed Appl 705(1):1998; 119-126.
9. Rebiere H, Mazel B, Civade C, Bonnet PA. Determination of 19 antiretroviral agents in pharmaceuticals or suspected products with two methods using High-Performance Liquid Chromatography. J chromatogr B 850:2007; 376-383.
10. Yekkala RS, Ashenafi D, Marien I, Xin H, Haghedooren E, Hoogmartens J et al. Evaluation of an International Pharmacopoeia method for the analysis of Ritonavir by Liquid Chromatography. J pharm Biomed Anal 48(3):2008; 1050-1054.
11. Veronica A, Pilar M, Cecilia FL, Eduardo LM. Determination of Saquinavir and Ritonavir in human plasma by RP-HPLC and the analytical error function. J pharm Biomed Anal 36(4):2004; 835-840.
12. Usami Y, Tsuyoshi OK, Masahiko N, Sagisaka M, Kaneda T. A simple HPLC method for simultaneous determination of Lopinavir, Ritonavir and Efavirenz. Chem pharm Bull 51:2003; 715-718.
13. Proust V, Toth K, Hulin A, Taburet AM, Gimenez F, Singlas E. Simultaneous High-Performance Liquid Chromatographic determination of the antiretroviral agents Amprenavir, Nelfinavir, Ritonavir Saquinavir, Delavirdine and Efavirenz in human plasma. J chromatogr B 742:2000; 453-458.
14. Marsh KC, Eiden E, McDonald E. Determination of Ritonavir, a new HIV protease inhibitor, in biological samples using Reversed-Phase High-Performance Liquid Chromatography. J Chromatogr B Biomed Sci Appl 704(1-2):1997; 307-13.
15. Dailly E, Raffi F, Jolliet P. Determination of Atazanavir and other antiretroviral drugs (Indinavir, Amprenavir, Nelfinavir and its active metabolite M8, Saquinavir, Ritonavir, Lopinavir, Nevirapine and Efavirenz) plasma levels by High Performance Liquid Chromatography with UV detection. J chromatogr B 813(1-2):2004; 353-358.
16. Jingdian C, Jayewardene AL, Stone JA, Motoya T, Aweeka FT. Simultaneous determination of five HIV protease inhibitors Nelfinavir, Indinavir, Ritonavir, Saquinavir and Amprenavir in human plasma by LC/MS/MS. J pharm Biomed Anal 30: 2002; 524.
17. Temghare GA, Shetye SS, Joshi SS. Quantitative Determination of Lopinavir and Ritonavir in Human Plasma by Liquid Chromatography-Tandem Mass Spectrometry. E J chem 6(1):2009; 223-230.
18. Sulebhavikar AV, Pawar UD, Mangoankar KV, Prabhunavelkar ND. HPTLC Method for Simultaneous Determination of Lopinavir and Ritonavir in Capsule Dosage Form. E J Chem 5(4):2008; 706-712.

\*\*\*\*\*

