Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(7):901-904

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

A RP-HPLC Method Development and Validation for the Estimation of Ritonavir in Bulk and Pharmaceutical Dosage Form

Santosh V. Gandhi* and Rasika R. Korhale

Department of Quality Assurance, AISSMS College of Pharmacy, Kennedy Road, Near R. T. O., Pune - 411001, Maharashtra, India

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ABSTRACT

The present study was to develop and validate reverse phase highperformance liquid chromatography (RP-HPLC) method for the estimation of Ritonavir. The RP-HPLC separation was achieved on JASCO HPLC system at 1ml/min flow rate on HiQSilC18 (250 x 4.6 mm, 5 µm) columnby using acetonitrile: 10 mM ammonium acetate buffer (pH 4) (85:15%V/V) as mobile phase at wavelength 239 nm. The data of linear regression analysis indicated a good linear relationship over the range of 5–30 µg/ml concentration with correlation coefficient value of 0.997. The accuracy of the method is indicated by good recovery in the range of 99.43%-100.53%and precision with RSD less than 2%.

Key words: Ritonavir, RP-HPLC, Validation, Crystallo co agglomerates

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INTRODUCTION

Retrovirus is the etiologic causative agent of acquired immunodeficiency syndrome (AIDS). Protease is an enzyme which is essential for the viral growth. These enzyme actions can be inhibited by the protease inhibitors, mainly in this class Indinavir, Ritonavir, Amprenavir, Nelfinavir, Atazanzvir, Saquinavir drugs are used in the treatment of HAART (Highly Active Anti-Retro viral Therapy). Ritonavir has the following structural formula [1].

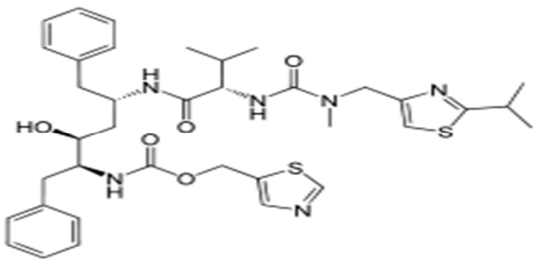

Fig.1 – Structure of Ritonavir

Literature survey revealed that, HPLC[2-4], stability indicating HPLC method[5-6] has been reported for ritonavir estimation alone in pharmaceutical formulation. Also HPLC method has been reported for determination of ritonavir in human serum[7]. Few HPLC methods are published for simultaneous estimation of ritonavir along with lopinavir[8-10]. The present studydescribes the development and validation of a simple, specific, accurate and precise RP-HPLC method for determination of ritonavir in tablet dosage formsprepared by usingcrytallo co agglomerates of the drug.The method was validated according to the International Conference on harmonization (ICH) guidelines[11].

EXPERIMENTAL SECTION

Instrumentation and chromatogrhaphical conditions

Separation of Ritonavir was carried on HPLC(Make-JASCO) equipped with HiQSiL C18 column (250×4.6 mm;5µm particle size), Rheodyne injector (50 µg/mL) and Jasco UV 2075 plus detector. The data acquisition was performed by Borwin chromatography software(version 1.5). Digital Balance Shimadzu make (Model AY-120) was used for weighing chemicals.

Separation was carried out at a flow rate of 1 mL/min using acetonitritle:10mM ammonium acetate buffer(85:15 v/v) as mobile phase and detection at 239 nm.

Materials

Pure Ritonavir is used as working standard, was received as gift from Lupin Laboratories Ltd., India. All chemicals and reagents i.e. Acetonitrile (HPLC), Glacial acetic acid (AR) and ammonium acetate (AR) employed were purchased from LobaChemie, Mumbai.

Preparation of optimized crystallo co agglomerates

In a crystallization vessel, ritonavir was dissolved in required amount of acetone (good solvent) to make saturated solution. This was added to aqueous solution of PEG 6000(bad solvent), with stirring using a mechanical stirrer (Remi motors, Mumbai) for 15 min, following which dichloromethane (DCM) was added slowly which acted as bridging liquid. The temperature of the crystallization system was maintained below 5°C. The stirring was continued to obtain agglomerates, which were then filtered and dried overnight at room temperature.

Preparation of tablets of optimized crystallo co agglomerates

All the materials as shown in the formula (Table 1) were mixed by geometric mixing technique. Mixing was continued for about 30 minutes until a homogenous powder blend was obtained. Lactose was used as diluent, PVP K-30 was used as dry binder, SLS was used as dispersing agent, talc was used as lubricant and starch as disintegrant. Tablets were prepared by direct compression method using standard 10.5 mm concave punches on rotary tablet compression machine (Rimek Mini Press II MT). All the product and process variables like mixing time and hardness, were kept constant and within permissible limits.

Table 1: Formula for preparation of tablets

Sr.No	Ingredient	Amount (mg)
1	Ritonavircrytallo co agglomerates	100
2	Starch	18
3	SLS	12
4	PVP K-30	48
5	Talc	12
6	Lactose	QS

#Total weight of the tablets was kept 200 mg

Selection of Wavelength-

From standard stock solution further dilutions were made using acetonitrile and it was scanned over the range 200-400 nm. It was observed that drug showed considerable absorbance at 239 nm.
(Fig. 2)

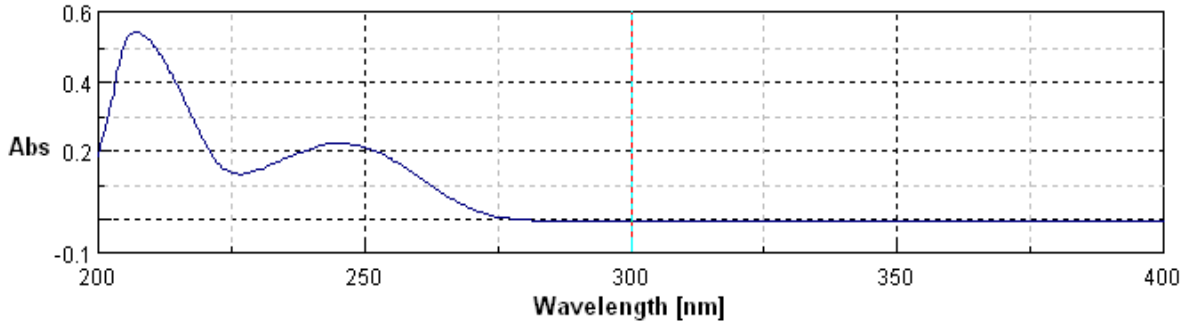

Fig.2 - UV Spectrum of Ritonavir (10µg/mL)

Preparation of standard stock solution-

Stock solution of Ritonavir was prepared by transferring accurately weighed 10 mg of Ritonavir into a 10mL volumetric flask and making up volume with Acetonitrile(1000µg/mL).

RESULTS AND DISCUSSION

VALIDATION OF ANALYTICAL METHOD:

Specificity

The specificity of the method was ascertained by peak purity profile studies. The peak purity values were found to be more than 995, indicating the no interference of any other peak of degradation product, impurity or matrix.

Linearity and Range

From the standard stock solution (1000 µg/mL) of Ritonavir, solution was prepared containing 100 µg/mL of Ritonavir in acetonitrile. This solution was further used to prepare range of solution containing six different concentrations. The linearity (relationship between peak area and concentration) was determined by analyzing six solutions over the concentration range of 5-30 µg/mL.

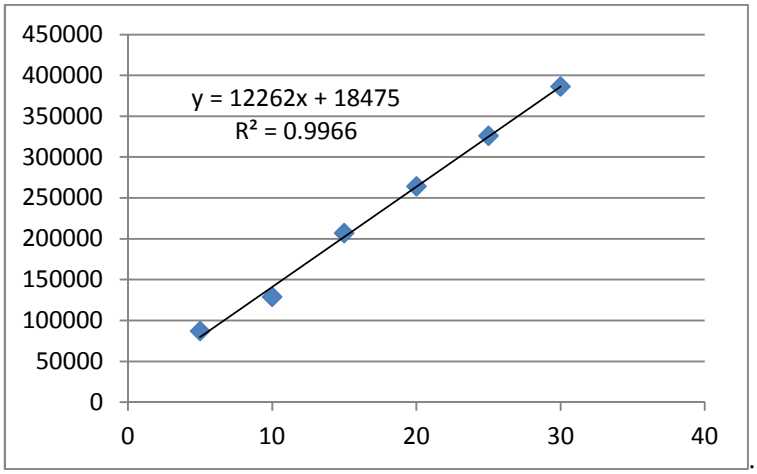

Fig.3 - Calibration curve of Ritonavir

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. For the intra-day studies, 3 replicates at 3 different concentrations (10, 20, 25µg/mL) were analyzed in a day and percentage relative standard deviation (%RSD) was calculated.

For the inter day variation studies, 3 different concentrations were analyzed on 3 consecutive days and % RSD was calculated. The results obtained for intraday and inter day variations were found to be within limits (less than 2% RSD). The results obtained are shown in Table 2.

Table 2- Results of intraday and interday precision

Concentration (µg/ml)	Intra-day Precision		Inter-day Precision	
	Average area	% R.S.D	Average area	% R.S.D
10	127007.13	1.45	125398.87	0.97
20	268344.76	1.86	260361.07	1.63
25	328947.00	1.37	322185.90	0.76

Limit of detection (LOD) and limit of quantitation (LOQ)

From the linearity data the LOD and LOQ was calculated, using the formula LOD = 3.3 σ/S and LOQ = 10 σ/S where, σ = standard deviation of the y intercept of linearity equations and S = slope of the calibration curve of the analyte. LOD and LOQ were found to be 0.591 µg/mL and1.792µg/mL respectively.

Assay

Ritonavir tablet formulation analysis was carried out as mentioned under section preparation of sample solution. Procedure was repeated for six times. The results obtained are shown in Table 3.

Table 3- Assay of tablet formulation

Drug	Peak Area	Amount Recovered (µg/mL)	% Recovery	% Recovery (Avg)	% RSD
Ritonavir	143739.87	10.21	102.15	98.53	1.73
	139211.32	9.84	98.46		
	138016.24	9.74	97.48		
	137863.77	9.73	97.36		
	136983.19	9.66	96.64		
	139961.32	9.90	99.07		

Accuracy

To check accuracy of the method, recovery studies were carried by spiking the standard drug to the Ritonavir tablet sample solution, at three different levels around 50, 100 and 150%. Basic concentration of sample solution chosen was 10 µg/ml. % recovery was determined from linearity equation. The results obtained are shown in Table 4.

Table 4- Accuracy study of Ritonavir

Level	Sample (µg/ml)	Standard (µg/ml)	Area	Amount recovered (µg/ml)	% recovery ± SD
50%	10	5	203154.29	15.06	100.53± 1.40
			206064.29	15.29	
			200924.29	14.87	
100%	10	10	264003.66	20.02	99.43± 0.92
			259972.26	19.69	
			263761.89	20.00	
150%	10	15	325957.37	25.07	99.57± 0.78
			321154.67	24.68	
			324012.55	24.91	

Robustness

Robustness of the method was checked by carrying out the analysis under conditions during which mobile phase composition (± 2% Composition), detection wavelength (± 2 nm), flow rate (± 0.05 ml/min) were altered and the effect on the area were noted. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters indicating that the method is robust.

CONCLUSION

The developed method was found to be simple, sensitive, selective, accurate and repeatable for analysis of Ritonavir in bulk and pharmaceutical dosage form without any interference from the excipients.

Acknowledgement

Authors are thankful to the Principal and Management, AISSMS College of Pharmacy, Pune for providing required facilities for research work.

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