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Forced Degradation and Stability Indicating Method Development and Validation of Ratinovir by RP-HPLC In Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A stable, simple, accurate, precise, robust and highly selective Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method was developed and validated using ritonavir. Chromatographic separation was achieved using cyber labs, LC 100 separation module, Agilent C18 column at temperature 30°C. Flow rate selected was 1ml/min. Both drugs are identified with UV detector at 256nm. Mobile phase employed was Methanol: Water (50:50), which resulted best sensitivity. Developed method was validated in terms of linearity, range (25-150µg/ml), precession (correlation coefficient is less than 0.999), robustness, accuracy(recovery was 101.96%) and under stress conditions drug degradation was less than 10%.The validation of proposed stability indicating method was verified by recovery studies and can be applicable in routine pharmaceutical analysis.

Keywords: RP-HPLC, methanol, HPLC grade water, stress studies, stability, method development, validation

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INTRODUCTION

Ritonavir 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. It is now more commonly used as a booster of other protease inhibitors and is available in both liquid formulation and as capsules. Structure was given in fig no. 1. Ritonavir,1,3-thiazol-5-ylmethyl N-[(2S,3S,5S) - 3-hydroxy – 5 - [(2S) – 3 – methyl – 2 - { [methyl ({[2- (propan-2-yl)-1,3-thiazol-4-yl] methyl}) carbamoyl] amino}butanamido]-1,6-diphenylhexan-2-yl] carbamate.[(1)]

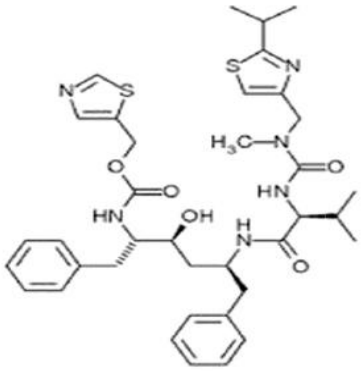

Figure 1: Structure of Ritonavir

A Very few analytical methods have been reported for the assay of ritonavir individually and various methods have been reported for combination with other drugs in formulations. Literature survey reveals that no analytical method was there for determination of ritonavir individually in dosage forms. So it was felt worthwhile to develop a stable, simple, rapid, accurate, precise and more economical high performance liquid chromatographic method for estimation of ritonavir in bulk and its dosage form.[(2-6)]

MATERIALS AND METHOD

Instruments

Reference standards of Ritonavir was obtained as gift samples from Aurobindo Pharmaceuticals, Hyderabad. Market formulation of this norvir was procured from the local market. HPLC grade water and methanol were obtained from Merck (India). The LC system cyber labs with UV detector. The output signal was monitored and integrated using LC solutions Software.

Preparation of Mobile Phase:

Mix a mixture of Methanol 500 ml (50%) and 500 ml of Water HPLC (50%) and degas in ultrasonic water bath for 5 minutes.

Diluent: Acetonitrile

Primary Standard Solution Preparation:

Accurately weigh and transfer 25 mg of Ritonavir working standard into a 25ml clean dry volumetric flask add about 10ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Primary Sample Solution Preparation:

Weigh and finely powder not fewer than 10 tablets. Transfer 25 mg of Ritonavir Tablet powder into a 25ml clean dry volumetric flask add about 10ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Filter the solution through 0.45µm nylon filter. Further dilute 25ml with diluent.

RESULTS AND DISCUSSION

The purpose of the present study was to develop a stable, rapid and sensitive RP-HPLC method for the estimation of ritonavir in bulk and pharmaceutical dosage form.

Method Optimization: After conducting several trials one method was optimized. The optimized parameters were given in table no.1 and chromatogram was shown in figure.2

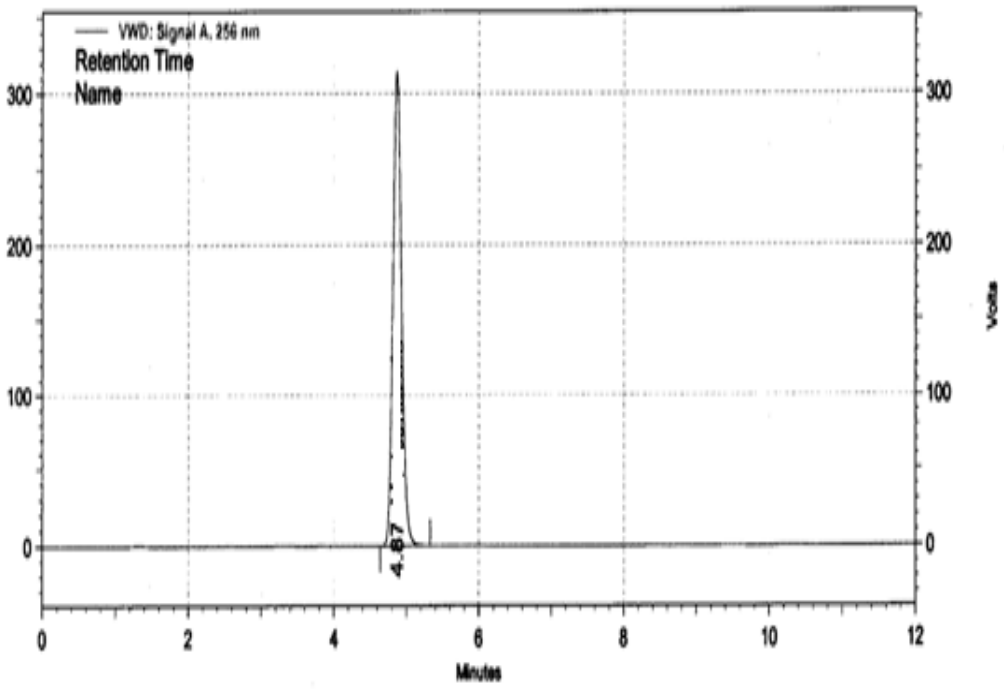

Figure 2: Chromatogram of Ritonavir

Table 1: Optimized Method Parameters

Parameters	Conditions
Column (Stationary Phase)	Aligent C18 column
Mobile Phase	Methanol: Water(50:50)
Flow rate (ml/min)	1ml/min
Run time (min)	12min
Column temperature (°C)	30°C
Volume of injection loop (µl)	10µl
Detection wavelength (nm)	256
Drug Rt (min)	4.87 min

Validation:

The described method has been validated for the estimation of ritonavir using following parameters as per ICH guidelines.[7]

Accuracy

Accuracy of the method was demonstrated at three different concentration levels (50-150%) by spiking a known quantity of standard drugs into analyzed sample in triplicate. The percentage recovery was found to be 101.9%. The results of accuracy (Table 2) revealed that the method was more accurate.

Table 2: Accuracy Results

%Concentration (at specification Level)	Area	Amount Added(µg)	Amount Found(µg)	% Recovery	Mean Recovery
50%	22443206	50	51.18	102.0	101.96%
100%	44677531	100	102.4	102.4	
150%	70699935	150	152.3	101.5	

Precision:

For the precision of the method, three replicate were injected into the system on same day and %RSD was calculated, i.e 0.17. Results of precision are given in Table 3, which indicated that the method is precise.

Table 3: Precision Results

Injection	Area
Injection-1	44371522
Injection-2	44462066
Injection-3	44477531
Injection-4	44513231
Injection-5	44467035
Injection-6	44286277
Avg	44429610.33
SD	77094.61836
%RSD	0.17

Intermediate Precision:

For the precision of the method, three replicate were injected into the system on two different non consecutive days, in each case %RSD was 0.53. Results of precision are given in Table 4, which indicated that the method is precise.

Table 4 Observation of Intermediate System Precision

Injection	Area
Injection-1	44171522
Injection-2	44462066
Injection-3	43977531
Injection-4	43713231
Injection-5	43867035
Injection-6	43986277
Average	44029610.33
Standard Deviation	237442.4425
%RSD	0.53

Linearity:

To establish linearity of the proposed method, five different sets of drug solution was prepared and analyzed. Standard curves were constructed in the concentration range of 25-150µg/ml of ritonavir (Figure 3). The correlation coefficient was determined as 0.9965 and the results were shown in Table 5.

Table 5: Linearity Observation

S. No.	Linearity Level	Concentration	Area
1	I	25ppm	12899451
2	II	50ppm	22443206
3	III	75ppm	34338670
4	IV	100ppm	44677531
5	V	125ppm	56407238
6	VI	150ppm	70699935
Correlation Coefficient			0.9965

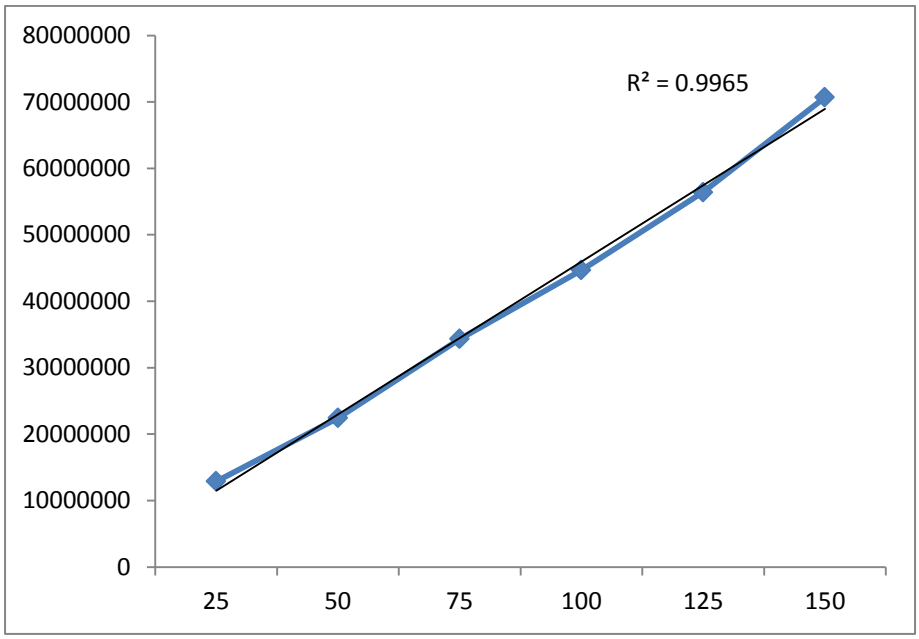

Figure 3: Linearity of Ritonavir

LOD and LOQ:

LOD AND LOQ were calculated and results were shown in table 6

Table 6: LOD & LOQ results

Drug Name	LOD	LOQ
Ritonavir	1.44µg/ml	4.39µg/ml

Robustness:

Experiment was conducted by changing the parameters like flow rate and Temperature. The system suitability parameters were within the limit. Results were shown in table 7.

Table 7: Results for robustness

Condition	%RSD	Theoretical Plates	Asymmetry
Column temperature 25^0c	0.17	8557	1.3
Column temperature 30^0c	0.17	8673	1.1
Column temperature 35^0c	0.32	8555	1.5
Flow 0.9ml	0.30	8412	1.4
Flow 1.0ml	0.30	8557	1.1
Flow 1.1 ml	0.65	8713	1.3

FORCED DEGRADATION STUDIES:

Samples were subjected to stress conditions like acid, alkaline, oxidative, thermal and photolytic and percentage of drug degradation was calculated. Impurity peaks were observed in stress conditions. Drug degradation was less than 10% in all stress conditions. The results were shown in table 8.

Table 8 Forced degradation results:

S.No	Stressed Condition	Area	Drug Recovery %	Degraded %
1	Acid hydrolysis	41550104	93	7
2	Alkaline hydrolysis	40254455	90.1	9.9
3	Oxidative hydrolysis	41013973	91.8	8.2
4	Thermal stress	43783980	98	2
5	Photolytic stress	41103328	92	8

CONCLUSION

The new stability indicating RP-HPLC method was developed and validated as per guidelines for the determination of Ritonavir in pharmaceutical dosage form. The proposed method was found to be stable, accurate, precise, simple, economic, rapid and having good specificity. The developed method can be applied for the assay of commercial tablets containing Ritonavir in routine quality control analysis.

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