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QUALITY BY DESIGN (QbD) APPROACH TO DEVELOP HPLC METHOD FOR RITONAVIR: APPLICATION TO HYDROLYTIC, THERMAL, OXIDATIVE AND PHOTOLYTIC DEGRADATION KINETICS

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ABSTRACT

Stability of Ritonavir (RITO) was investigated using a stability indicating reverse phase high-performance liquid chromatography (RP-HPLC) method. Experimental designs were applied for multivariate optimization of the experimental conditions of (RP-HPLC) method. Three independent factors: methanol content in the mobile phase composition, buffer pH and flow rate were used to design mathematical models. Quality by Design (QbD) approach was used to facilitate method development. RITO was exposed to different stress conditions, including hydrolytic (acid, base, neutral), oxidative, thermal and photolytic. Relevant degradation was found to take place in all the conditions. The degradation of RITO followed (pseudo) first-order kinetics under experimental conditions. Using this optimum condition, baseline separation of both drugs with good resolution and a run time of less than 7 min were achieved. The kinetic parameters (rate constant, $t_{1/2}$, and t_{90}) of the degradation of RITO were calculated. The optimized assay condition was validated according to ICH guidelines to confirm specificity, linearity, accuracy and precision.

KEYWORDS: Ritonavir; QbD approach; Stability indicating HPLC; Degradation kinetics

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INTRODUCTION

Ritonavir (RITO, Figure 1) chemically known as [5S,8S, 10S,11S]-10-Hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1- methyl ethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis(phenyl methyl)- 2,4,7,12-tetraazatridecan-13-oic acid 5-thiazolylmethyl ester[1] is an antiretroviral drug from the protease inhibitor class used to treat HIV infection and AIDS. RITO was originally developed as an inhibitor of HIV protease. It is one of the most complex inhibitors and is now rarely used for its own antiviral activity, but remains widely used as a booster of other protease inhibitors [2] with HPLC as the predominant method, and dealt with the determination of RITO in biological fluids. Most of them are applied for the quantitation of RITO in plasma.[3]Literature survey revealed several analytical methods for the determination of ritoavir and lopinavir in tablets, capsules, and syrups which employ techniques such as high-performance liquid chromatography (HPLC)[4-5] Ultra performance liquid chromatography (UPLC)[6] and high performance thin layer chromatography (HPTLC).[7] In biological fluids, the active principles as well as their metabolites have been quantitatively determined by HPLC with UV detection, LC/MS/MS.[8-9] Micellarelectrokinetic chromatography methods.[10] and Tandem mass spectrometry.[11]The guidelines demonstrated require conduct of forced decomposition studies under a variety of conditions, such as oxidation, pH, light and dry heat followed by separation of the drug from its degradation products. The method is expected to allow the analysis of individual degradation products. An ideal stability-indicating method is one that quantifies the standard drug alone and also resolves its degradation products.Literature review reveals that there is no HPLC with QbD method reported for analysis of RITO, but there are several methods available for the estimation of other antiviral namely Ritonavir, valacyclovir, Azatanavir sulphate In the modern analytical laboratory, there is always a need for significant stability-indicating methods (SIMs) of analysis. Environmental factors, such as temperature, pH, buffer species, ionic strength, light, oxygen, moisture, additives and excipients, can play an important role in the stability of drug substances. Stress testing can help in identifying degradation products and provide important information about the intrinsic stability of drug substances. With the advent of the International Conference on Harmonization (ICH) guidelines.[12] requirements for the establishment of SIMs have become more clearly mandated. The guidelines explicitly require the conduct of forced decomposition studies under a variety of conditions, like pH, light, oxidation, dry heat, etc. and separation of drug from degradation products. The method is expected to allow analysis of individual degradation products.Moreover, kinetic studies on the decomposition of drugs using stability testing techniques are essential for their quality control and to predict the expiry date of pharmaceutical products. The scientific novelty of the present work is that the suggested method represents the first stability indicating HPLC method for the analysis of RITO. Besides, it is the first kinetic study for RITO degradation to calculate the strength of this azole molecule. A response surface methodology (RSM) approach was used to identify the optimum conditions for analysis during method development. The iterative procedure used in these studies included performing experiments in the region of the best known solution, fitting a response model to the experimental data and then optimizing the estimated response model. The conventional practice of modification of a single factor at a time may result in poor optimization as other factors are maintained at constant levels that do not depict the combined effect of all the factors involved in a separation. This approach is also time consuming and requires a vast number of experiments to establish optimum levels. These limitations can be eliminated by collectively optimizing all parameters using RSM. Furthermore RSM was used to evaluate the relative significance of several other factors in the presence of complex interactions. Compared with the traditional optimization method, RSM has distinct advantages such as the use of minimum number of experiments, shorter time of operation and feasibility of generating data that may be analysed statistically to provide valuable information on the interactions among experimental parameters. These designs are rotatable (or near rotatable) and require three levels for each factor. Diagrams of global optimum, which are more direct, were made.[13] Experimental design approach has been applied to optimize HPLC experimental conditions, such as the resolution and time of analysis for pramipexole in tablets.[14] and for the determination and optimization of voriconazole in pharmaceutical formulations Experimental design was used to optimize a liquid chromatographic method for the separation of six compounds and for the separation of the components of a cough syrup[15]. HPLC–ECD method was developed for the analysis of captopril using experimental design. HPLC method was developed using experimental design for the determination of tetranortriterpenoids in Carapa guianensis seed oil [16].Literature survey also revealed HPLC methods have been reported for estimation of Ritonavir in pharmaceutical formulations. The objective of this work was to develop a simple, sensitive, precise and accurate HPLC method for RITO that could be applied to the stress degradation kinetics.

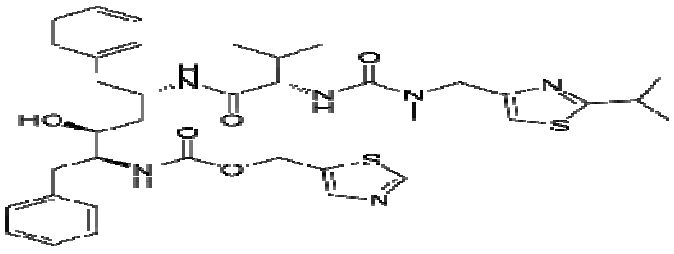

Figure 1
Chemical Structure of Ritonavir

Chemicals, reagents and solutions

All the reagents as HPLC grade Water and Methanol (HPLC grade), were purchased from Merck Chemicals, India. Reference standard Ritonavir was procured Lupin Limited, Pune(India), as gift samples. Potassium dihydrogen orthophosphate, tetra butyl ammonium hydroxide (TBAH) and o-phosphoric acid, hydrochloric acid, sodium hydroxide, and 30% hydrogen peroxide used were of analytical grade and purchased from S D Fine Chem. Ltd. (Mumbai, India). Buffer was prepared by dissolving 1360 mg (10 mM) of potassium dihydrogen orthophosphate and 3330 mg of TBAH (10 mM) in 1 L of HPLC grade water.

HPLC instrumentation and chromatographic conditions

The HPLC system consisted of two pumps (Analytical Technologies P2230 HPLC pump), a manual injector with 20 µL capacity per injection, and a temperature-controlled column oven. The UV–VIS detector (Analytical Technologies UV 2230) was operated at a wavelength of 254 nm. The software used was chromatography workstation A-2000, version 1.6. Columns used were Lichrospher C 18, 250 mm · 4.6 mm, 5.0ml (Merck, Germany), Atlantis C 18, 250 mm · 4.6 mm, 5.0 lm (Waters Corporation, USA) and Alltima C-8, 250 mm · 4.6 mm, 5.0 lm (Grace, USA). Chromatographic separation of RITO was achieved at ambient temperature using a Lichrospher RP C18 (250 mm · 4.6 mm, 5 lm) analytical column; the mobile phase consisted of methanol–potassium dihydrogen orthophosphate (pH 3.8; 10 mM, tetra butyl ammonium hydroxide; 10 mM) (40:60, v/v) at a flow rate of 1.0 mL/min. pH of buffer was adjusted with o-phosphoric acid. Before use, the mobile phase was filtered through a 0.22 µm nylon membrane filter and sonicated for 15 min. Injection volume was 20 µL, and the optimum wavelength selected for quantification was 254 nm. Photo stability Chamber Model: CS-90 (GMP) internal diameter 50x40x85 cm. Design Expert Software Experimental using Statistical (Version 10.0.4.0) Design Wizard- Optimization- factorial/RSM-HTC study type-response surface, design type-central composite Model –Quadratic Subtype Randomized.

Construction of the calibration curve

Standard stock solution of RITO was prepared in methanol at a concentration of 10 mg ml/min and further diluted with the mobile phase to furnish the working standard stock solution of 100 ml/min. The working standard stock solution was diluted with the mobile phase to prepare calibration samples in the concentration range of 0.5–100µg/ml. Triplicate injections of 20 µL were made for each calibration sample and chromatographed under the specified HPLC conditions decayed previously. Peak areas were plotted against the corresponding concentration to obtain the calibration curve.

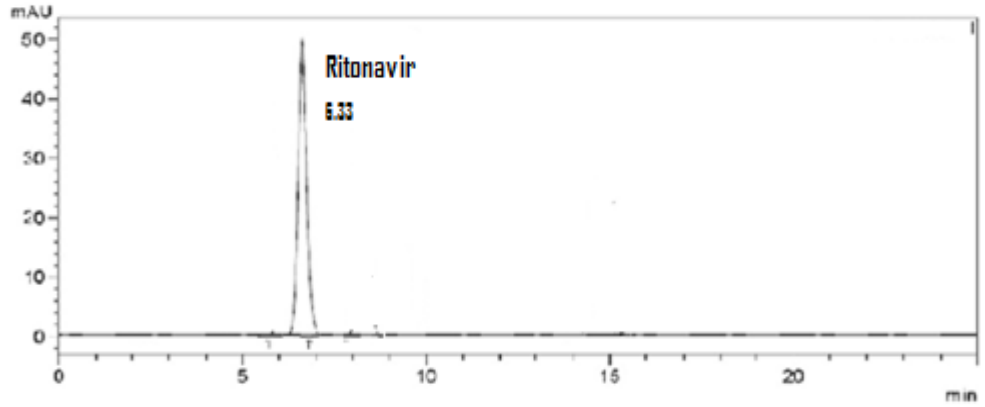

Figure 2
Typical chromatogram of (RT 6.33 min)

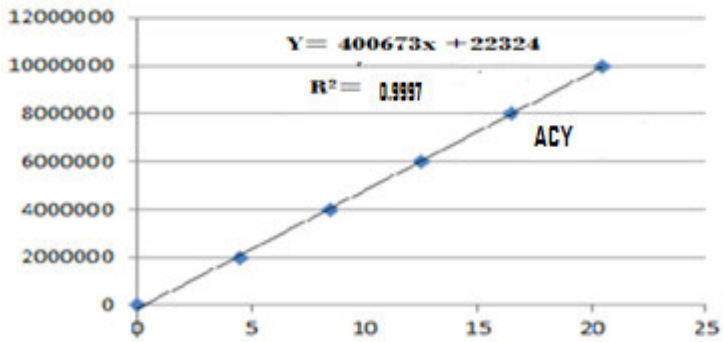

Figure 3
Standard Calibration Curve for RITO

Forced degradation of RITO

Hydrolytic conditions: acid, alkali and water induced degradation

Standard stock solution (1 mL) was transferred to each of five 10 mL volumetric flasks and the volume was made up to the mark with 2 N HCl, 5 N HCl, 2 N NaOH, 5 N NaOH and water separately. These were subjected to the conditions specified in Table 1.

Oxidizing conditions

Hydrogen peroxide-induced Degradation Standard stock solution (1 mL) was transferred to each of two 10 mL volumetric flasks and the volume was made up to the mark with 3% H2O2 and 10% H2O2 separately. These were subjected to the conditions specified in Table 1.

Thermal conditions

Dry heat and moist heat induced degradation Standard stock solution (1 mL) was transferred to each of two 10 mL volumetric flasks and the volume was made up to the mark with methanol. These were subjected to the conditions indicated in Table 1.

Photolytic degradation

Exposure to sun light Standard stock solution (1 mL) was transferred to a 10 mL volumetric flask and the volume was made up to the mark with methanol. This was subjected to the conditions mentioned in Table 1.

Sample collection

Storage and preparation before collecting samples, the volume was made up to the mark with respective solvent. Sample (200µL) was collected at specified sampling points as indicated in Table 1. The samples from acid and base induced degradation were neutralized by adding 200 ll of appropriate strength of NaOH and HCl. All samples were stored at 2–8 0C in the refrigerator. On the day of analysis samples were diluted with the mobile phase up to 10 mL, filtered with a 0.22 lm membrane syringe filter and injected three times for each sample into HPLC.

Table 1
Hydrolytic, oxidative, thermal and photolytic stress testing conditions for drugs

Stress condition	solvents	Temperature 0 C	Time (days)	Sampling time (days)
Hydrolytic	H_2O	60	25	1,2,6,8,10,15,20
Neutral	2 N HCl	60	30	1,2,4,6, 8,10,15,20
Acidic	5 N HCl	60	30	1,2,4,8,10,15,20
Basic	2N NaOH 5N NaOH	60	30	1,2,4,8,10,15,20
Oxidizing	3% H_2O_2	60	30	1,2,4,8,10,15,20
thermal	10% H_2O_2	Room temp.	7	1,4,6,8,20
Moist heat	Methanol	Room temp.	7	1,4,8,20
Dry heat	Methanol	60	14	1,2,4,6, 8,10,15,20
Photolytic	Methanol	60	14	1,2,4,8,10,15,20
Direct sunlight	Methanol	-	25	1,2,4,6,8,10,15,20

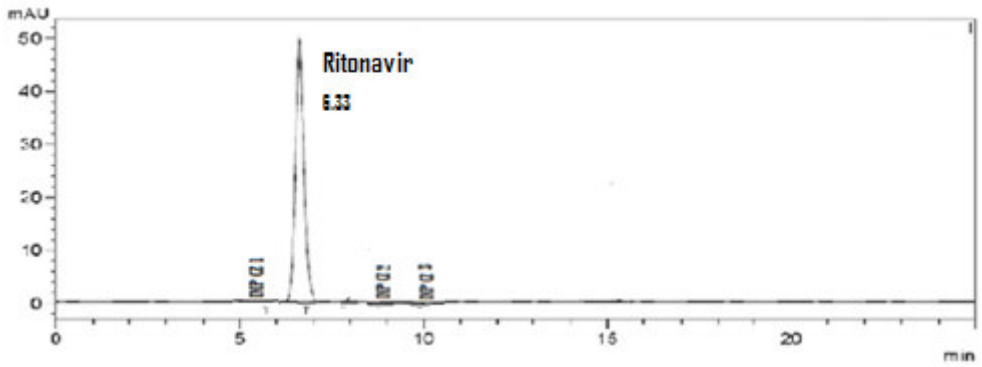

Figure4
Chromatogram of 2N HCl Treated Tablet Sample

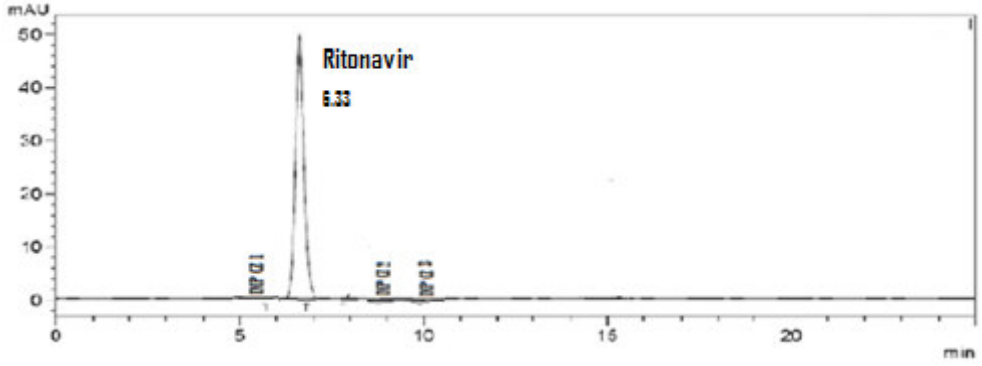

Figure 5
Chromatogram of 2N NaOH Treated Tablet Sample

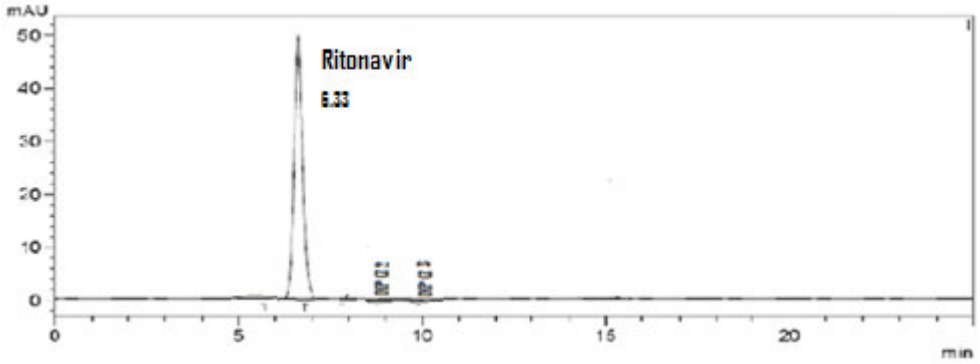

Figure6
Chromatogram of H_2O_2(3 %) Treated Tablet Sample

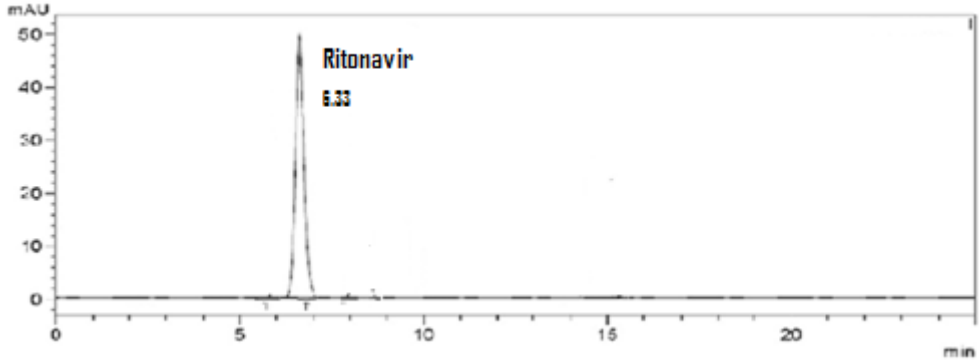

Figure 7
Chromatogram of Dry Heat Treated Tablet Sample

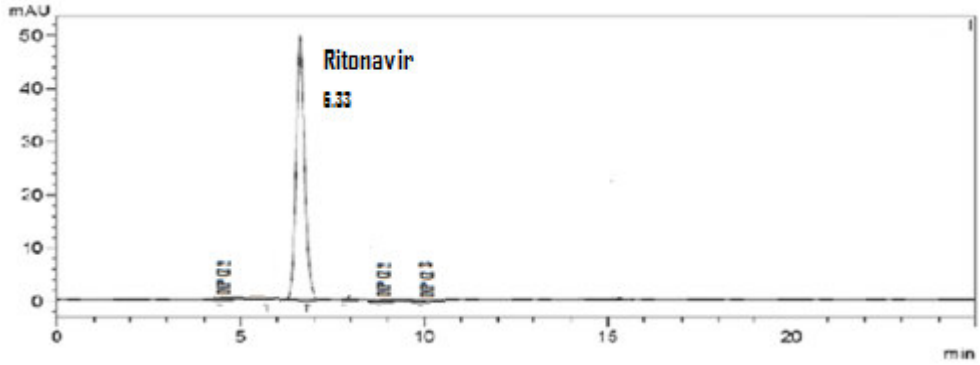

Figure 8
Chromatogram of UV Radiation Treated Tablet Sample

RESULTS AND DISCUSSION

Method development and optimization

Optimum wavelength of 254 nm was selected to reduce the base line noise at absorption maximum (208 nm) of RITO. Based on RITO solubility, methanol was selected as organic phase. Initially, reversed-phase analytical columns (C18 and C8) were tested with mobile phase composed of variable composition of methanol (80–20% v/v) and water. Then water was replaced with buffer (10 mM potassium dihydrogen orthophosphate) at different pH levels ranging from 2.8 to 6.9 with a flow rate of 1 ml/min. In the above employed conditions; RITO did not get any capacity factor (k). RITO was eluted along with the mobile phase, i.e. the retention volume was equal to void volume. Seeing this elution behavior of RITO, which is a basic drug having pKa of 5.52, we employed ion pair methodology, using TBAH as an ion pair agent at the concentration of10 mM.pH of the buffer (10 mM potassium dihydrogen orthophosphate) was adjusted to 3.8 with o-phosphoric acid, i.e., more than two units below the pKa (5.5) to ionize RITO by 100%. Based on the above conditions, the mobile phase composed of methanol: buffer at the 40:60 ratio eluted RITO through the C18 stationary phase (Lichrospher, RP C18, 250 mm · 4.6 mm, 5 l) having a k of 1.44. In order to get a satisfactory k between 2 and 3, pH of buffer was varied between 3.6 and 4.2, level of methanol was varied between 20% and 30% v/v and TBAH concentration was varied between 5 and 10 mM. Twenty-seven experiments were conducted using the full factorial design (3 factors, 3 levels, 27 runs), in order to rationally examine the effects of TBAH concentration, buffer pH and organic phase concentration on the capacity factor of RITO. Experimental factors and levels used in the experimental design are shown in Table 2. The factors and ranges selected for consideration were based on previous univariate studies and chromatographic intuition. The data generated were analyzed using Statistica (Version 6.0).Figure.16 shows the influence of

each factor on the capacity factor. Organic phase and TBAH were significant by linear regression; by quadratic regression only organic phase was significant and TBAH effect was smaller. Effect of pH was non-significant by both linear and quadratic regression. Two dimensional contour plots are presented in Figure.15 and are very useful for studying the interaction effects of the factors on the capacity factor. The model that has been developed can be used to predict the capacity factor of RITO within the limits of the experiments.The optimized chromatographic conditions obtained from the design were mixture of 10 mM potassium dihydrogen orthophosphate (pH 3.8) containing 10 mM TBAH and methanol (40:60, v/v), at a flow rate of 1.0 mL/min. These chromatographic conditions achieved reasonable retention (k= 2.01) and symmetric peak shape for RITO with a retention time of 6.31 min (Figure. 16). No interference from the blank and cream formulation excipients was observed at the retention time of RITO (Figure. 16). Percentage of recovery (n= 6) obtained from the formulation was 100.3 ±1.2.

Table 2
Factors and level used in the experimental design

Factor	Level (-1)	Level (0)	Level (+1)
TBAH (mM)	3.4	3.5	3.7
pH	3.5	3.8	4.1
Organic phase	20	25	30

Solution stability

The stability of RITO in the mobile phase was investigated by analyzing the standard of RITO (60µgml/min) at 0, 3, 6, 9, 12 and 24 h. No significant variation in the peak area of standard shown in Table 3.

Table 3
Stability of drug in the mobile phase

Time	Peak area (50 ug/ml of drugs)
0	627823
3	641243
6	684745
9	713246
12	743176
24	802197

Mean± SD. RSD (%), n = 6

Method validation

To confirm the suitability of the method for its intended purpose, the method was validated in accordance with the ICH guidelines [17]for system suitability, linearity, limits of detection and quantification, Accuracy, intra- day and inter-day precision, specificity and robustness.

System suitability

System-suitability test was an integral part of method development and has been used to ensure adequate performance of the chromatographic system. Retention time (RT), capacity factor (k), number of theoretical plates (N) and tailing factor (T), were evaluated for six replicate injections of the drug at a concentration of 60µgml/min. The results presented in Table 4 are within the acceptable limits.

Table 4
System suitability data

Property	Mean ± SD, n=6	RSD (%)	Required limits
Retention time (RT)	6.33 ± 0.03	0.13	RSD≤ 2
Capacity factor (k)	2.04 ± 0.002	0.19	-
Theoretical plates (N)	33126 ±144	0.63	N ≥ 2000
Tailing factor (T)	1.06 ± 0.01	1.32	T ≤ 2

Linearity

Linearity of the proposed method was evaluated according to the ICH guidelines. RITO showed linearity in the concentration range of 10–60µgml/min, (r2 = 0.9979). The regression equation obtained was Y= 30.321X+13.247, where Y is peak area and X is concentration of RITO (µg/ml). This equation was used to determine the amount of RITO present in the stability samples.

Limits of detection and quantification

The limit of detection (LOD) was defined as the lowest concentration of RITO resulting in a signal-to-noise ratio of 3:1 and limit of quantification (LOQ) was expressed as a signal-to noise ratio of 10:1. Due to the difference in detector response, different concentrations ranging from 0.01 to 2 µg/ml were prepared and analyzed. The LOD and LOQ obtained were 0.3 and 0.9 µg/ml, respectively.

Table 5

Level of standard added (%)	Amount of sample standard added (µg)	Sample	Mean peak area ± SD, RSD (%) n=3		Amount of standard found (µg)	Recovery for standard (%)
			Standard + sample	Standard		
80	40	6212.19 ±15.31,1.07	8227.29 ±23.31,1.83	691.09 ±23.31,1.03	40.10	100.09
100	50	6423.21 ±15.31,1.27	8461.26 ±25.31,1.23	819.81 ±25.31,1.16	50.07	100.3
120	60	6652.29 ±15.31,1.10	8832.39 ±27.31,1.08	938.85 ±27.31,1.24	60.02	100.03

Recovery of the standard from stress degraded samples by standard addition method

Accuracy

Accuracy of the method was determined by performing the recovery experiments. Known amount of the standard at 80%, 100% and 120% levels was fortified to the degradation sample. Peak area of the standard was calculated by the difference of peak area between fortified and unfortified samples. Three replicate samples of each concentration level were prepared and the percentage recovery at each level (n = 3) was determined (Table 5). For RITO, the results obtained are in good agreement with the added amounts.

Intra-day and inter-day precision

Intra-day and inter-day precision was evaluated by injecting four different concentrations (10, 20, 40, and 60µgml/min) of RITO. For intra-day variation, sets of six replicates of the four concentrationswere analyzed on the same day; for inter-day variation, six replicates were analyzed on six different days. The intra-day and inter-day precision (%RSD) was found to be less than 2% (Table 6), indicating that the method was precise.

Table 6
Results of intra-day and inter-day precision

Concentration µg/ml	Intra- day precision		Inter-day precision	
	Peak area Mean ± SD (n=6)	RSD (%)	Peak area Mean ± SD (n=6)	RSD (%)
10	64619.29 ±13.29,1.62	1.89	6401.19 ±12.64,1.79	1.39
20	76928.23 ±14.42,1.22	1.56	7201.10 ±12.18,1.11	1.17
40	85293.12±15.29,1.67	1.64	8602.11±12.19,1.33	1.29
60	107718.09 ±16.83,1.21	1.32	10296.09 ±12.23,1.72	1.11

Specificity

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. The specificity of the HPLC method was illustrated in Figure. 10 and 11, where complete separation of RITO was noticed in the presence of degradants. The average RT ± standard deviation for RITO was found to be 5.08 ±0.01 min, for six replicates. The peaks obtained were sharp and had clear baseline separation.

Robustness

A method is robust if it is unaffected by small changes in operating conditions. To evaluate HPLC method's robustness few parameters were deliberately varied. The parameters included variation of C18 columns from different manufacturers, pH of the buffer, flow rate and percentage of methanol in the mobile phase. Two analytical columns were used during the experiment, one from Germany (Lichrospher C 18 column) and the other from USA (Atlantis C 18 column). Each of the three examined factors (pH, flow rate, and methanol percentage) selected was changed one at a time to estimate the effect. Replicate injections (n = 6) of standard solution (60 µg/ml) were performed under small changes of chromatographic parameters (factors). Flow rate was varied by 1± 0.1 mL min^{-1}; level of methanol in the mobile phase was varied by 25 ±2% (v/v), while pH was varied by 2.8 ±0.1. Results obtained are presented in Table 7, indicating that the results remained unaffected by small variations of these parameters. The results from the two columns indicated that there is no significant difference between the results from the two columns.

Table 7
Results for the analysis of robustness

Factors	**level**	**Retention Mean ± SD (n=6)**	**Time (min) Mean ± SD (n=6)**	**Peak area Mean ± SD (n=6)**
A Flow rate (ml/min)				
0.9	-1	6.33 ± 0.02	1.31 ± 0.03	64921.28 ±,1.27
1.0	0	6.31 ± 0.01	1.32 ± 0.01	64222.41 ±1.27
1.1	+1	6.32 ± 0.02	1.12 ± 0.01	68417.28 ±1.27
mean		6.32 ± 0.02	1.56 ± 0.02	65449.39 ±1.27
B percentage of methanol in the mobile phase (v/v)				
23	-1	6.32 ± 0.01	1.22 ± 0.03	63631.28 ±1.24
25	0	6.31 ± 0.02	1.30 ± 0.01	64948.20 ±,1.19

27	+1	6.33 ± 0.03	1.31 ± 0.01	64754.89 ±,1.57
mean		6.32 ± 0.02	1.33 ± 0.02	65128.79 ±,1.39
C pH of buffer				
2.7	-1	6.30 ± 0.02	1.47 ± 0.03	61641.53 ±,1.27
2.8	0	6.33 ± 0.02	1.28 ± 0.02	64641.69 ±,1.27
2.9	+1	6.32 ± 0.03	1.26 ± 0.01	64671.59 ±,1.27
Mean		6.32 ± 0.02	1.25 ± 0.02	64620.39 ±,1.17
D: columns from different manufactures				
I Lichrospher C 18 column		6.33 ± 0.02	1.22 ± 0.03	64281.45 ±1.37
II Atlantis C18 column		6.30 ± 0.02	1.28 ± 0.02	64754.21 ±1.32
Mean		6.32 ± 0.03	1.24 ± 0.01	64157.73 ±1.27,1.24

Stability-indicating property

An analytical method is stability-indicating if this method can separate all the process-related impurities and all the degradation.Quality by Design (QbD) approach to develop HPLC method for Ritonavir: Application S319 degradation products from the major peak of the sample. The model chromatograms of RITO under acidic and oxidative stress conditions are presented in Figure.4 and 6. RITO under acidic and basic stress conditions showed same degradant peaks at the retention time of 5.4, 5.8, 9.1 and 10.0 and 10.2 min .Stress samples under dry heat, moist heat, water hydrolysis and photolysis showed two degradant peaks at 4.5, 9.1 and 10.3 min. Under oxidative stress conditions, RITO showed two degradant peaks at 9.0, 10.0 min; the peak observed at corresponds to the blank. This indicates that the drug is susceptible to hydrolytic (acid, base and water), oxidative, thermal and photolytic degradation. In all the above cases the degradant peaks did not interfere with the RITO peak, suggesting that the method enabled specific analysis of RITO in the presence of its degradation products. Scheme 1 presents the proposed degradation mechanism of RITO in different stress conditions. 2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6*H*-purin-6-one monosodium (I) and pyrimidine (III) might be the major degradation products in hydrolytic/thermal/photolytic stress conditions; 2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)-5*H*-purin-4-one monosodium might be the degradation products in oxidative stress conditions.2 + log Ct/Co Time (days).

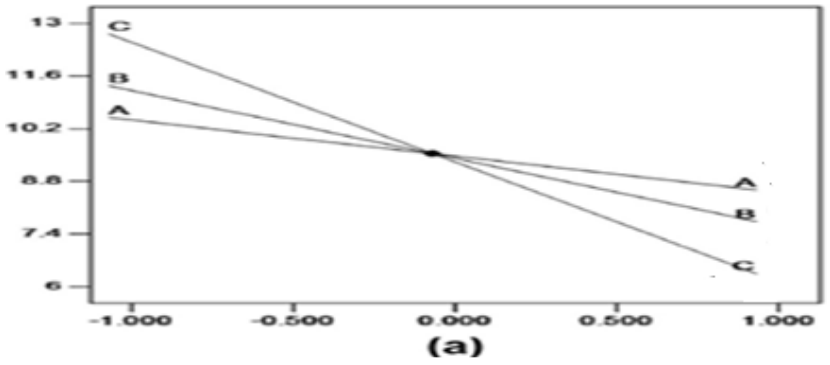

Figure 9

9 perturbation (a) the effect of each of the independent factors on RT of RITO, while keeping other factors at their respective mid-point levels (A; pH = 3.8; B: flow rate =1.0ml/min C % MeOH = 50 % v/v)

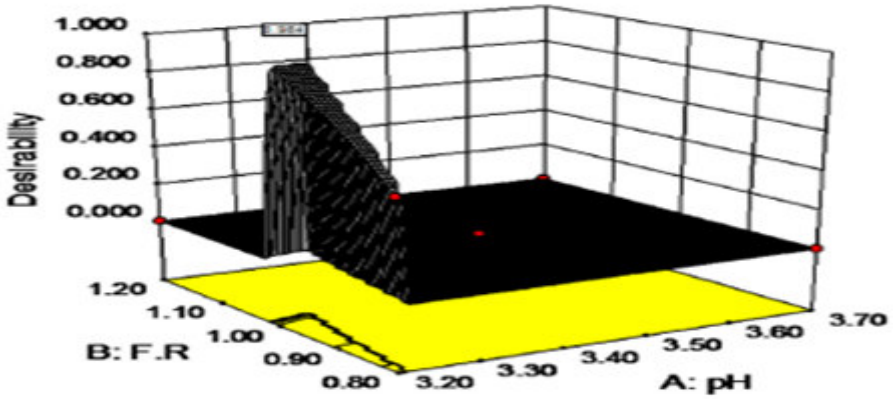

Figure10

Graphic representation of the overall desirability function D Flow Rate (B) is plotted against pH (A) withMeOH % v/v (C) held constant at 60 % v/v.

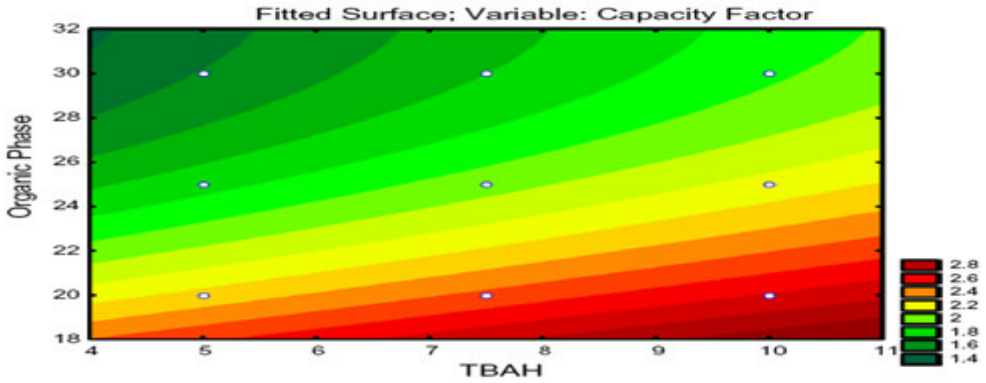

Figure 11
Contour plot for capacity factor as a function of TBAH concentration and organic phase for RITO

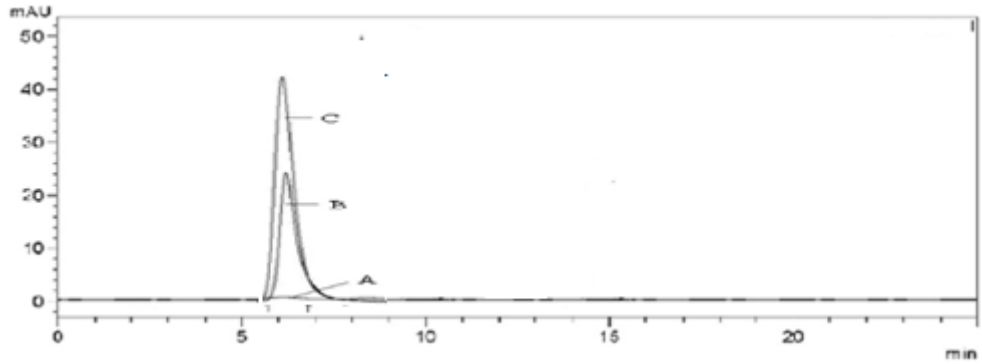

Figure 12
Representative chromatograms of RITO blank (A) standard (B) and formulation

Kinetic investigation

Treatment of RITO under specified stress conditions resulted in a gradual decomposition of RITO in all conditions. Since the degradation was performed with a large excess of solvent (9 mL) compared to drug solution (1 mL), the degradation of RITO followed pseudo-first-order kinetics [18] as a linear relationship between log percentage of RITO remaining and time was established, having good correlation coefficients (Figure. 29–32). Pseudo-first-order is the term used when two reactants are involved in the reaction but one of them is in such a large excess that any change in its concentration is negligible compared with the change in concentration of the other reactant (drug). The kinetic parameters are presented in Table 8. Rate constant (K), time left for 50% potency (t1/2) and time left for 90% potency (t90) for each stress condition were calculated using Eqs. (1)– (3), respectively [19]: where K is the rate constant, [C0] is the concentration of RITO at time t= 0 and [Ct] is its concentration at time t.The K values per day were found to be 4.73 ×10 -3,14.03 ×10 -3,6.65 ×10 -3,16.72 ×10 -3,19.13 ×10 -3,59.40 ×10 -3,25.62 ×10 -3,38.42 ×10 -3,19.47 ×10 -3,18.63 ×10 -3 for 2 N NaOH, 5 N NaOH, 2 N HCL, 5 N HCL, 3% H2O2, 10% H2O2, dry heat, moist heat, water hydrolysis and photolytic conditions, respectively. The rate constant values were increased as the strength of NaOH, HCl and H2O2 increased. K value was increased approx. six times as the strength of NaOH was increased from 2 N to 5 N, while the rate of degradation was increased up to approx. three times under the same conditions of acid (HCL) treatment, indicating more susceptibility of RITO under basic media compared to acidic. The K value for water induced degradation was found to be similar to the degradation by 5 N HCL and 5 N NaOH, specifying the importance of water toward RITO degradation. Between thermal treatments K value for dry heat was found to be higher than moist heat. Extensive degradation was observed in oxidative conditions, where K value was found to be highest among all the tested conditions. Hence the effect of oxygen needs to be considered for topical formulation of RITO. Suitable antioxidants need to be a part of the topical formulation of RITO. K value obtained for photolytic degradation was similar to water hydrolysis, 5 N NaOH and 5 N HCL. This illustrates the prominent effect of light toward the stability of RITO. $t_{1/2}$ and t_{90} values for all the tested stress conditions are shown in Table 8, both $t_{1/2}$ and t_{90} were found to be lowest Figure 15 First order plots for the degradation of RITO under hydrolytic and photolytic stress conditions (each point represents the mean ± SD, n=3). Quality by Design (QbD) approach to develop HPLC method for Ritonavir: Application S321 and highest (198.77and 30.87days) for alkaline hydrolysis with 2 N NaOH.

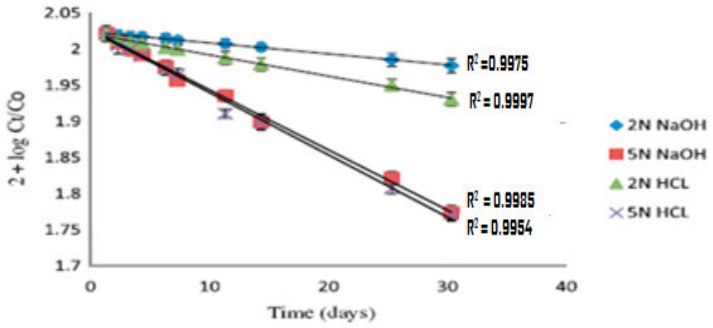

Figure 13
First order plots for the degradation of RITO under acidic and basic stress condition
(each point represents the mean ± SD, n=3)

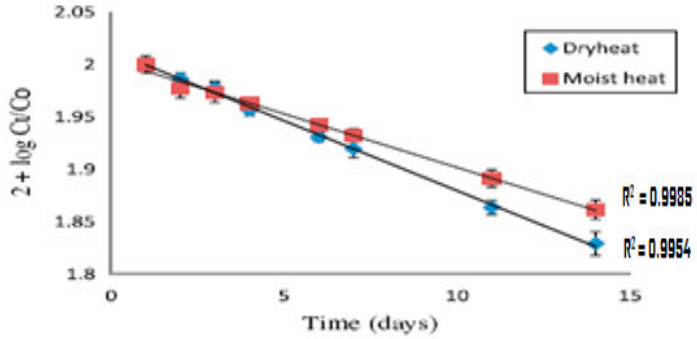

Figure14
First order plots for the deradation of RITO under thermal stress conditions
(each point represents the mean ± SD, n=3)

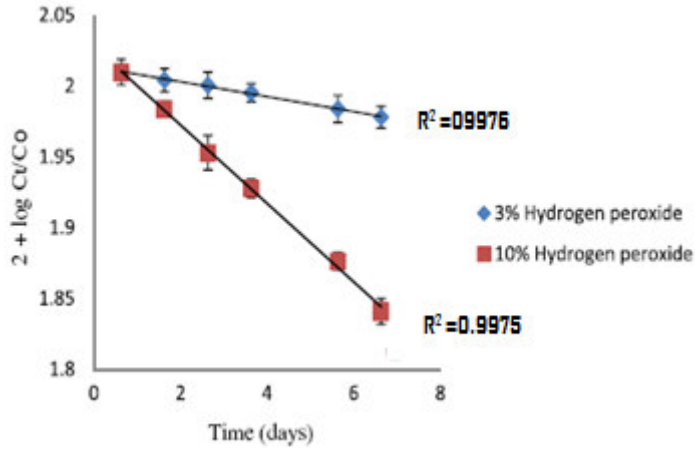

Figure15
First order plots for the degradation of RITO under oxidative stress condition
(each point represents the mean ± SD, n=3)

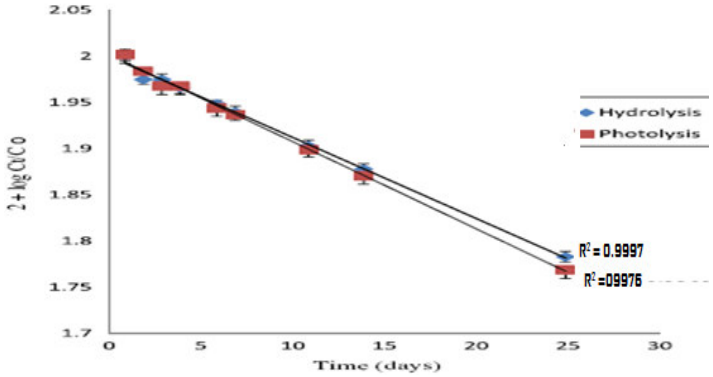

Figure16
First order plots for the degradation of RITO under hydrolysis and photolytic stress conditions
(each point represents the mean ± SD, n=3)

CONCLUSION

The proposed HPLC method provides simple, accurate and reproducible quantitative analysis for the determination of RITO in the presence of its degrades. It was found that RITO was rapidly degraded under oxidative, hydrolytic (acid and alkali) and photolytic conditions. The degradation of RITO was found to be of pseudo-first-order kinetics in analyte's concentration. The reaction rate increases with increase in strength of the acid/base/H_2O_2 solution. This study suggests that the formulation scientist needs to incorporate antioxidants in the topical formulation of RITO and also care should be taken to prevent photolysis upon its exposure to sun light.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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