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Research Article

QUALITY BY DESIGN (QBD) – BASED RP – HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF DARUNAVIR AND RITONAVIR

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ABSTRACT:
The Quality by Design (QbD) approach was employed to achieve method optimization by systematically evaluating the influence of critical method parameters (CMPs) such as mobile phase composition, flow rate, and detection wavelength using Design of Experiments (DoE). The optimized chromatographic conditions ensured sharp, well-resolved peaks with minimal tailing and reduced run time, confirming method efficiency and economy. The method was developed by using a Sunfire C18 column with a flow rate of 1ml/min the mobile phase was selected was Methanol: Ammonium Acetate (40:60 v/v) and the results had exhibited excellent linearity over the concentration range of 5–30 µg/ml for Ritonavir and 30–180 µg/ml for Darunavir, with correlation coefficients (R^2 = 0.999) for both analytes. The regression equations were y = 6626.1x + 340.57 for Ritonavir and y = 9239.1x + 991.54 for Darunavir. The mean assay results demonstrated high accuracy—99.95% for Ritonavir and 100.24% for Darunavir. The method was found to be specific, showing no interference from excipients or other components. System suitability and precision results yielded %RSD values within acceptable limits (≤2%), confirming repeatability and reproducibility. Accuracy studies showed recoveries of 99.75% (Ritonavir) and 99.87% (Darunavir), while LOD and LOQ were 0.02 µg/ml and 0.05 µg/ml for Ritonavir, and 0.59 µg/ml and 1.78 µg/ml for Darunavir, respectively, indicating the high sensitivity of the method. Robustness testing under minor deliberate variations further confirmed the reliability of the method within the defined design space. The proposed QbD-optimized RP-HPLC method is simple, accurate, robust, and economical, and can be effectively adopted for routine quality control analysis of Ritonavir and Darunavir in pharmaceutical formulations

Key Words: Darunavir, Ritonavir, RP-HPLC, QbD, DoE, Method Validation, Quality Control

INTRODUCTION:

CENTRAL COMPOSITE DESIGN

A 3^2 full factorial design was utilized to optimize the RP-HPLC method for the simultaneous detection of Darunavir and Ritonavir, emphasizing on two independent variables: the flow rate and the methanol content in the mobile phase. The flow rate was evaluated at 0.9, 1.0, and 1.10 mL/min, and the methanol concentration was altered to three different levels: 35%, 40%, and 45% (v/v with Ammonium Acetate). This setup resulted in twenty experimental runs, which allowed for a thorough evaluation of how variations in methanol concentration and flow rate affected significant chromatographic responses, including tailing factor, theoretical plate count, and retention duration. The factorial design technique assured a procedure that is resilient and trustworthy in conformity with QbD principles by making it easier to find the appropriate chromatographic parameters and providing insights into the interaction between the two variables. The experimental data was analyzed using surface plots and ANOVA, which made it easier to see how flow rate and methanol concentration interacted while determining the statistical significance of each element in connection to the chromatographic responses.

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Darunavir and Ritonavir are potent antiretroviral agents belonging to the protease inhibitor (PI) class, widely used in the management of human immunodeficiency virus type-1 (HIV-1) infections. Darunavir is a second-generation non-peptidic protease inhibitor that selectively binds to the active site of HIV-1 protease with high affinity, blocking the cleavage of viral Gag-Pol polyproteins and preventing the maturation of infectious virions.1,2 Darunavir is being studied as a possible treatment for SARS-CoV-2, the coronavirus responsible for COVID-19, due to in vitro evidence supporting its ability to combat this infection.3 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.4 Darunavir chemically written as (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-ylN-[(2S,3R)-3-hydroxy-4-[N-(2-methylpropyl)4-aminobenzenesulfonamido]-1-phenylbutan-2-yl]carbamate. When administered with ritonavir in combination antiretroviral therapy, darunavir significantly decreases viral load and increases CD4 cell counts, decreasing the morbidity and mortality of HIV infection.5 Darunavir is known to bind to different sites on the enzyme: the active site cavity and the surface of one of the flexible flaps in the protease dimer. Darunavir can adapt to changes in the shape of a protease enzyme due to its molecular flexibility.6,7 Ritonavir Chemically written as (1,3-thiazol-5-yl)methylN-[(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.4 it inhibits the HIV viral proteinase enzyme that normally cleaves the structural and replicative proteins that arise from major HIV genes, such as gag and pol. Gag encodes proteins involved in the core and the nucleocapsid, while pol encodes the the HIV reverse transcriptase, ribonuclease H, integrase, and protease.8 Combination therapy with Darunavir and Ritonavir forms a central component of highly active antiretroviral therapy (HAART) and has shown significant success in achieving durable viral suppression and immunologic recovery.9.10 The Darunavir/Ritonavir regimen has demonstrated clinical efficacy not only in adults but also in pediatric and elderly HIV patients due to its optimized pharmacokinetic profile and robust resistance profile 11. Additionally, recent research has explored its potential repurposing for emerging viral infections due to broad antiviral properties, although further clinical validation is necessary.12

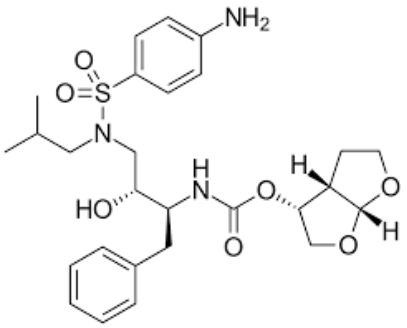


Figure 1: Structure of Darunavir

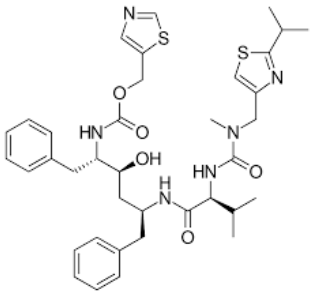


Figure 2: Structure of Ritonavir

A QbD is defined as "A systemic approach to the method development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management5." The QbD approach emphasizes product and process understanding with quality risk management and controls, resulting in higher assurance of product quality, regulatory flexibility, and continual improvement. The QbD method was based on the understanding and implementation of guidelines ICH Q8 Pharmaceutical Development, ICH Q9 Quality Risk Management, and ICH Q10 Pharmaceutical Quality System[13,14,15]. Analytical science is considered to be an integral part of pharmaceutical product development and hence go simultaneously during the entire product life cycle. Analytical QbD defined as a science and risk-based paradigm for analytical method development, endeavouring for understanding the predefined objectives to control the critical method variables affecting the critical method attributes to achieve enhanced method performance, high robustness, ruggedness, and flexibility for continual improvement[16,17]. Extensive literature research has unearthed a multitude of recorded analytical procedures[18-21], including the discovery of more economically efficient ways. Nevertheless, there is currently no documented approach for UPLC approach. Hence, a reliable and cost-effective approach is suggested for assessing the QbD and stability of Darunavir and Ritonavir, and their medicinal dose form using RP-UPLC must be validated and developed as per ICH guidelines.

Materials and Methods:

Darunavir and Ritonavir (API), and Darunavir/Ritonavir Combination tablet (Daruvir 600 R), Acetonitrile, Methanol, Ortho Phosphoric Acid, Distilled water. All of the solvents and chemicals were of HPLC quality and obtained from Rankem Chemicals Pvt Ltd.

Instrumentation:

The Method Development and Validation was performed by Waters HPLC Model 2695 equipped with PDA Detector and Empower 3 Software. For QbD Design Expert 13 Software, Analytical weighing Balance, Ultrasonicator, pH Meter, Hot air oven.

Chromatographic Condition:

An Isocratic Elution carried out by using **Methanol and Ammonium Acetate 40:60 v/v** as the Mobile Phase, Diluent used was Combination of Acetonitrile and Water in 1:1 ratio. **Sunfire C18** (4.6 x 250mm, 5µm) column was used to determine the Method at a flow rate of 1ml/min, by maintaining the column Temperature at 30^0C. In addition, with an injection volume of 10µL and the wavelength detected at 238nm.

API Formulation

Preparation of Standard stock solutions and Working Solution: Accurately weighed 60 mg of Darunavir, 10 mg of Ritonavir transferred to 50ml volumetric flask and 3/4 th of diluents was added to these flasks and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (1200µg/ml of Darunavir and 200µg/ml of Ritonavir) from this sol 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (120µg/ml of Darunavir and 20µg/ml of Ritonavir).

Sample Formulation

Preparation of Sample stock solutions and Working Solution: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to tablet was transferred into a 100ml volumetric flask and until the Tablets are completely dispersed. Equilibrate to room temperature, 25ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (6000µg/ml of Darunavir and 1000µg/ml of Ritonavir). From this 0.2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent to make 40µg/ml of Favipiravir.

Method Validation

The established technique is validated in accordance with ICH criteria for the purpose of validating analytical methods. The validation metrics were: system appropriateness, accuracy, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, robustness, specificity, and degradation studies.

System suitability parameters:

The chromatographic analysis was done in accordance with the designed and optimized parameters after the working standard solution was introduced into the HPLC system six times. By computing the % RSD of retention times, theoretical plates, and peak areas from six duplicate injections, the system appropriateness parameters were established.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision:

Repeatability (intraday) and intermediate precision (inter-day) were used to assess the developed analytical method's precision. The usage of an analytical process in a laboratory over a brief period of time that was evaluated by assaying the samples on the same day is known as repeatability. Intermediate precision was assessed via comparison of the assays on several days. SD and %RSD were determined.

Linearity:

Standard calibration curves were created with six distinct concentrations including the LOD and LOQ by making repeated volume to volume dilution of stock solution I over the range of 30-180 µg/ml for Darunavir and 5-30 µg/ml for Ritonavir. Linear calibration curves were produced between peak area and medication concentration. The linearity was tested using linear regression, which was calculated by the least square regression method

- 25 µg/mL: Take 0.25 mL of stock solution and dilute to 10 mL
- 50 µg/mL: Take 0.5 mL of stock solution and dilute to 10 mL
- 75 µg/mL: Take 0.75 mL of stock solution and dilute to 10 mL
- 100 µg/mL: Take 1.0 mL of stock solution and dilute to 10 mL

- 125 µg/mL: Take 1.25 mL of stock solution and dilute to 10 mL
- 150 µg/mL: Take 1.5 mL of stock solution and dilute to 10 mL

Accuracy:

Accuracy was performed in triplicate for various concentrations of Darunavir and Ritonavir equivalent to 50%, 100% and 150% of the standard amount were injected into the HPLC system per the test procedure. Dilution were as follows.

- 50 µg/mL: Take 0.1 mL of stock solution and dilute to 10 mL
- 100 µg/mL: Take 0.2 mL of stock solution and dilute to 10 mL
- 150 µg/mL: Take 0.3 mL of stock solution and dilute to 10 mL

Sensitivity:

Limit of detection and Limit of Quantification

Limits of detection (LOD) and limit of quantitation (LOQ) were established using the signal-to-noise ratio. The detection limit was stated to as the lowest concentration level resulting in a peak area of three times the baseline noise. The lowest concentration level that produced a peak area with a signal-to-noise ratio greater than ten was referred to as the quantitation limit.

Based on the response's standard deviation and calibration curve's slope, the LOD and LOQ can be estimated. The formulae given below can be used to calculate LOD and LOQ:

$$LOD = 3.3\sigma/S$$

$$LOQ = 10\sigma/S$$

where S is calibration curve of the slope and σ is the response of the standard deviation.

Sensitivity Stock solution: Take 0.25 mL of stock solution and dilute to 10 mL

- **LOD:** From above take 0.3 ml solution and dilute to 10 mL
- **LOQ:** From above take 0.9 ml solution and dilute to 10 mL

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guidelines.

Assay:

For the brand (Daruvir 600 R) with label claims of Ritonavir 100 mg and Darunavir 600 mg, the assay and percentage purity were computed. The value that was determined was compared against that of standard value without interference from the excipients used in the tablet dosage form

Degradation Studies

These investigations are carried out under various stress situations to describe the stability of the pure pharmacological material and are useful in establishing the best storage settings. These research cover base, peroxide, acid, neutral hydrolysis, photo, and heat degradation.

Oxidation:

After adding 1ml of a stock solution to 10ml of a 20% volume fraction of H2O2 and allowing it to sit in an oven at 60°C for 30 minutes, a chromatogram was produced by injecting a 20µg/ml & 120µg/ml solution at 10 µl into HPLC

Acid Degradation Studies:

1 ml of hydrochloric acid was added to 10 ml of vf with 1 ml stock and refluxed for 30 minutes at 60 ^{o}C. A 20µg/ml & 120µg/ml solution was injected at 10 µl into the system, resulting in the formation of a chromatogram.

Alkali Degradation Studies:

A mixture of 1 ml of stock and 1 ml of NaOH in 10 ml of vf was refluxed for 30 minutes at 60°C. A 40 µg/ml solution was injected at 10 µl into HPLC, resulting in the production of a chromatogram.

Dry Heat Degradation Studies:

The stock solution was allowed to undergo thermal deterioration in an oven set at 105oC for 6 hours. Subsequently, a chromatogram was prepared by injecting a 20µg/ml & 120µg/ml solution at 10 µl into HPLC.

Photo Stability studies:

The stock underwent degradation by exposure to UV radiation in the laboratory for a duration of 7 days. Upon injecting a 20µg/ml & 120µg/ml solution at a volume of 10 µl into HPLC, a chromatogram was developed.

Neutral Degradation Studies:

After refluxing the stock for 6 hours at 60 degrees Celsius, a chromatogram was prepared by injecting a 20µg/ml & 120µg/ml solution at 10 µl into HPLC

RESULT AND DISCUSSION

Parameter Selection

Various preliminary HPLC trials were carried out for selection of Column and organic modifier. The choice of C18 column based on the preliminary investigation was done using **Sunfire C18 (250×4.6 mm, 5.0µm)**, Selection of a suitable organic modifier is also important to get better selectivity with adequate separation of all analytes. Commonly used organic solvents for the reversed phase HPLC include Acetonitrile and Methanol, from that trials Methanol showed to be an ideal and suitable organic modifier compared to acetonitrile, because Ritonavir and Darunavir was solubilized in methanol compare to acetonitrile. Therefore, **methanol was selected and finalized as the organic modifier for further optimization study**.

Optimization of method

The method was optimized via Central composite design. The earliest trials are needed to optimize the final approach. It was necessary to tune the organic concentration, flow rate, and column temperature. In order to maximize these characteristics, which were adjusted over three levels (high, mid, and low), central composite design was employed. different ranges of parameters ranging from **35-45% Aqueous Phase**, **temperature 27 ^{0}C - 33 ^{0}C** and **0.9-1.10ml/min flow rate** respectively were taken and counter and 3D surface plot showing the effect of each parameter on Retention Time, Theoretical plates and Resolution (CQA) were generated. A desirability function used to the optimal settings to estimate retention period, asymmetry, theoretical plates

Table 1 Optimized Condition

Parameter	*Condition*
Mobile phase	*Methanol: Ammonium Acetate (40:60 v/v)*
Flow rate	*1 ml/min*
Column	*Sunfire C18 (4.6 x 250mm, 5µm)*
Detector wave length	*238nm*
Column temperature	*30°C*
Injection volume	*10µL*
Diluent	*Water and Acetonitrile in the ratio 50:50*

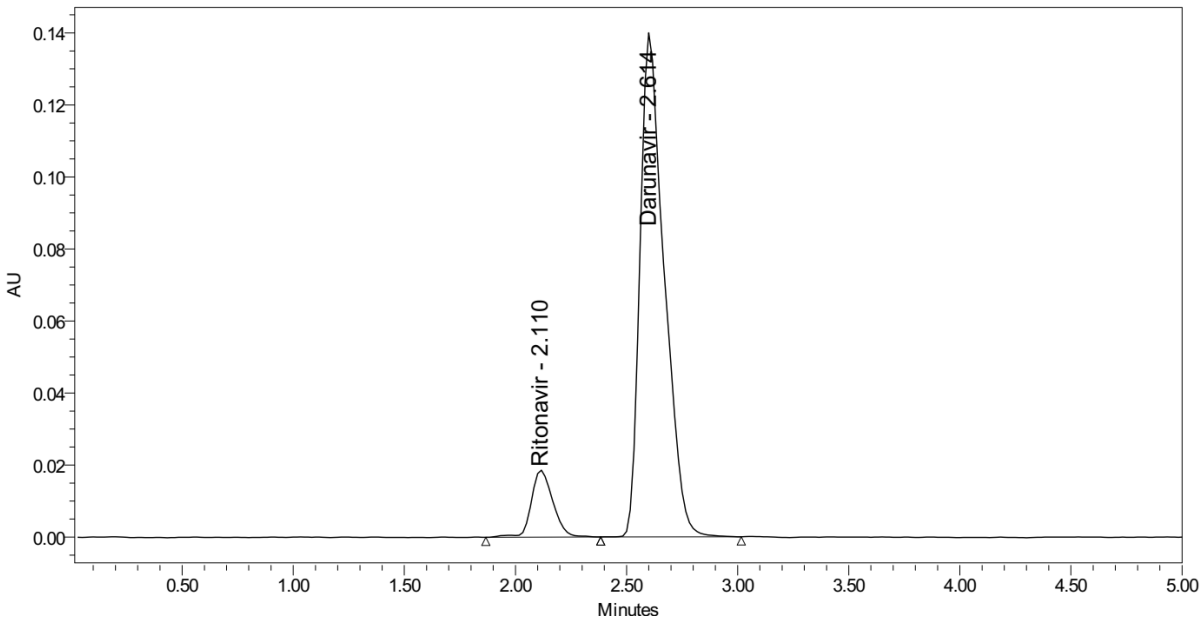


Figure 2: Optimized Chromatogram

Design summary of CCD

Design Summary					
File version: DX 13.0.0 Study Type: Response surface Design Type: central composite design Design Model: Quadratic			***ATP: Robustness CQA: Retention time, Theoretical plates and Tailing factor Runs: 20***		
CMPs	***Unit***	*Type*	*Subtype*	***Min.***	***Max.***
Aqueous Phase	***%***	*Numeric*	*Continuous*	***35 %***	***45 %***
Flow rate	***ml/min***	*Numeric*	*Continuous*	***0.9 ml/min***	***1.10 ml/min***
Temp	***^{0}C***	*Numeric*	*Continuous*	***27 ^{0}C***	***33 ^{0}C***

Factors

Factor	*Name*	*Level*	*Low Level*	*High Level*	*Std. Dev.*	*Coding*
A	*FR*	*1.00*	*0.90*	*1.10*	*0.0000*	*Actual*
B	*MP*	*40.00*	*35.00*	*45.00*	*0.0000*	*Actual*
C	*Temp*	*30.00*	*27.00*	*33.00*	*0.0000*	*Actual*

The Responses of Trial

		Factor 1	**Factor 2**	**Factor 3**	**Response 1**	**Response 2**	**Response 3**	**Response 4**	**Response 5**
Std	Run	A:FR	B:MP	C:Temp	RT1	RT2	RS	NTP1	NTP2
		ml/min	%	0 C	min	num	num	num	num
1	9	0.9	35	27	2.594	3.193	2.6	1.19	3185
2	5	1.1	35	27	2.116	2.604	2.4	1.19	2488
3	15	0.9	45	27	2.406	2.98	3	1.09	2801
4	13	1.1	45	27	1.964	2.425	2.7	0.99	2364
5	8	0.9	35	33	2.323	2.87	2.5	1.19	2783
6	6	1.1	35	33	1.935	2.384	2.3	1.19	2098
7	14	0.9	45	33	2.157	2.667	2.8	1.09	2578
8	12	1.1	45	33	1.796	2.217	2.5	0.99	2084
9	4	0.831821	40	30	2.523	3.137	2.8	1.29	2958
10	3	1.16818	40	30	1.814	2.242	2.4	1.19	2021
11	20	1	31.591	30	2.338	2.837	2.3	1.19	2801
12	7	1	48.409	30	2.039	2.511	2.8	0.94	2370
13	19	1	40	24.9546	2.348	2.889	2.8	1.06	2899
14	2	1	40	35.0454	1.935	2.391	2.5	1.11	2262
15	18	1	40	30	2.101	2.608	2.6	1.24	2435
16	11	1	40	30	2.102	2.608	2.6	1.26	2441
17	16	1	40	30	2.102	2.608	2.6	1.26	2467
18	17	1	40	30	2.106	2.615	2.6	1.27	2459
19	10	1	40	30	2.108	2.617	2.6	1.24	2496
20	1	1	40	30	2.119	2.628	2.7	1.24	2490

Final Responses

Response	Name	Units	Observations	Minimum	Maximum	Mean	Std. Dev.	Ratio
R1	RT1	min	20.00	1.796	2.594	2.15	0.2172	1.44
R2	RT2	min	20.00	2.217	3.193	2.65	0.2669	1.44
R3	RS	num	20.00	2.3	3	2.61	0.1820	1.30
R4	NTP1	num	20.00	0.94	1.29	1.16	0.1031	1.37
R5	NTP2	num	20.00	2021	3185	2524.00	304.43	1.58

Factor Coding: Actual

All Responses

Design Points

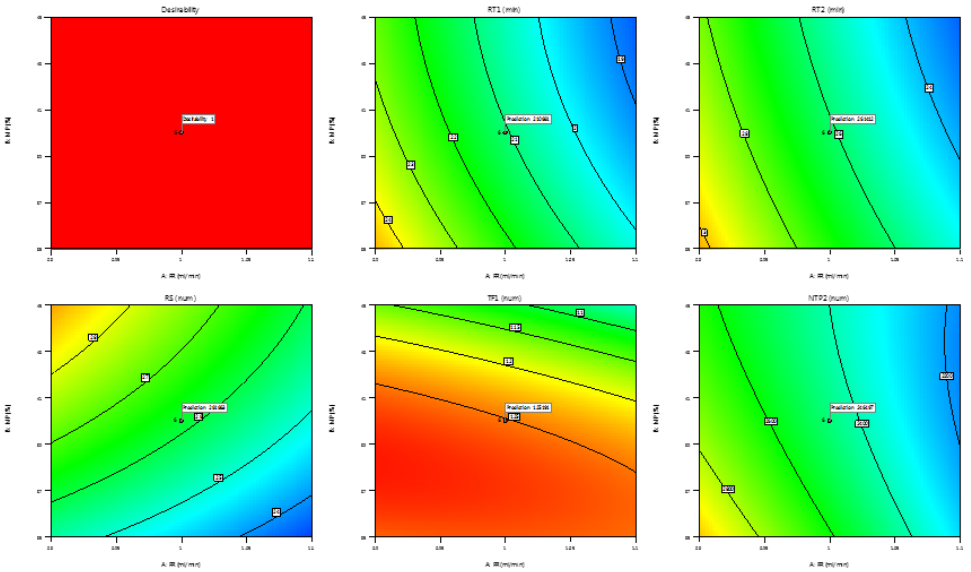
0 1

X1 = A

X2 = B

Actual Factor

C = 30



Factor Coding: Actual

All Responses

Design Points

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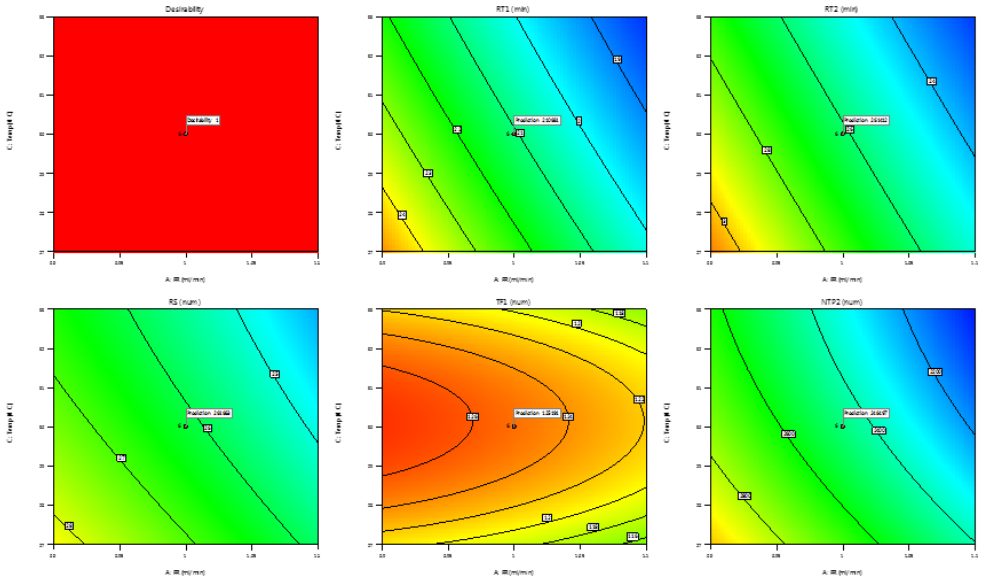
X1 = A

X2 = C

Actual Factor

B = 40



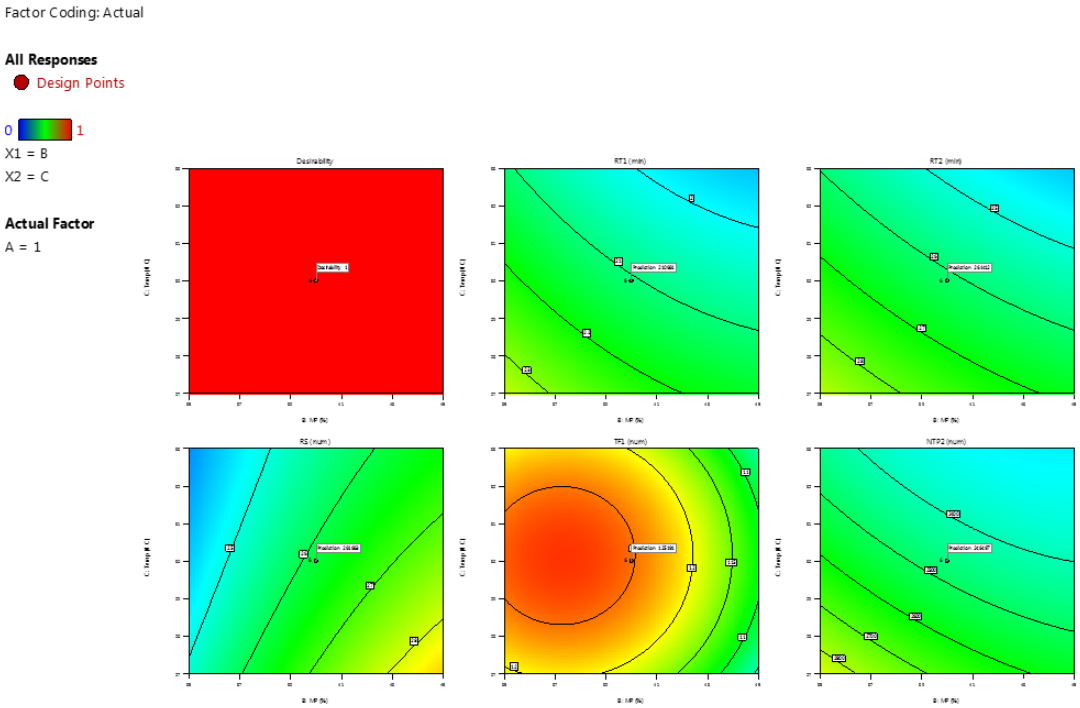


Figure 3 Multi Contour plots of all Response

Validation

System Suitability:

Table 2: System Suitability

Ritonavir				
Injection	***RT***	***area***	***Plate Count***	***Tailing***
Injection-1	*2.337*	133407	2987	1.17
Injection-2	*2.338*	132089	2927	1.20
Injection-3	*2.340*	133183	2901	1.18
Injection-4	*2.342*	132823	2960	1.18
Injection-5	*2.353*	133812	3052	1.14
Injection-6	*2.354*	133870	2978	1.16
Mean		*133197*		
Std ev		*669.8*		
RSD		*0.5*		

Darunavir					
Injection	***RT***	***area***	***Plate Count***	***Tailing***	***Resolution***
Injection-1	2.890	1112499	2837	1.30	2.8
Injection-2	2.890	1109160	2914	1.28	2.9
Injection-3	2.893	1104169	2930	1.29	2.9
Injection-4	2.893	1115552	2822	1.32	2.8
Injection-5	2.903	1117065	2848	1.28	2.8
Injection-6	2.904	1111628	2830	1.30	2.8
Mean		*1111679*			
Std ev		*4635.3*			
RSD		*0.4*			

Six injections of the standard Favipiravir solution were performed, and the chromatograms that corresponded to each injection were acquired. Observations showed that the percent RSD was less than 2%, the USP tailing was less than 2, and the theoretical plate count surpassed 2,000. Every condition for system appropriateness was satisfied and falls within permissible bounds.

Linearity:

Six concentrations ranging from 10 to 60 µg/ml were prepared and linearity was estimated in a duplicate manner. The linearity equation for Favipiravir was y = 63434x + 13743. For the calibration curve over the concentration range, the data have shown a good correlation.

Table 3: Linearity Data

	Ritonavir		***Darunavir***
Concentration (ppm)	****Peak area***	***Concentration (ppm)***	****Peak area***
0	*0*	***0***	*0*
5	33846	30	272403
10	66139	60	555473
15	99520	90	838584
20	133784	120	1113290
25	166809	150	1395665
30	198028	180	1652175
y :	*6626.1x + 340.57*	***y :***	*9239.1x + 991.54*
R^2	*0.999*	***R^2***	*0.999*
Slope	*6626.1*	***Slope***	*9239.1*
Intercept	*340.57*	***Intercept***	*991.54*
LOD	*0.02µg/ml*	***LOD***	*0.59µg/ml*
LOQ	*0.05 µg/ml*	***LOQ***	*1.78 µg/ml*

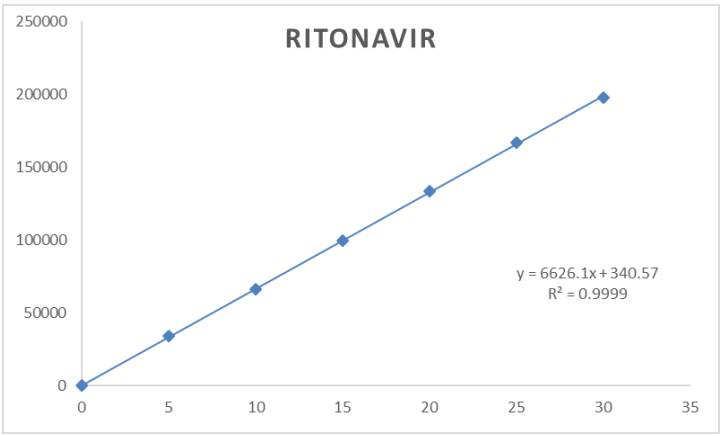


Figure 3: Calibration Curve of Ritonavir

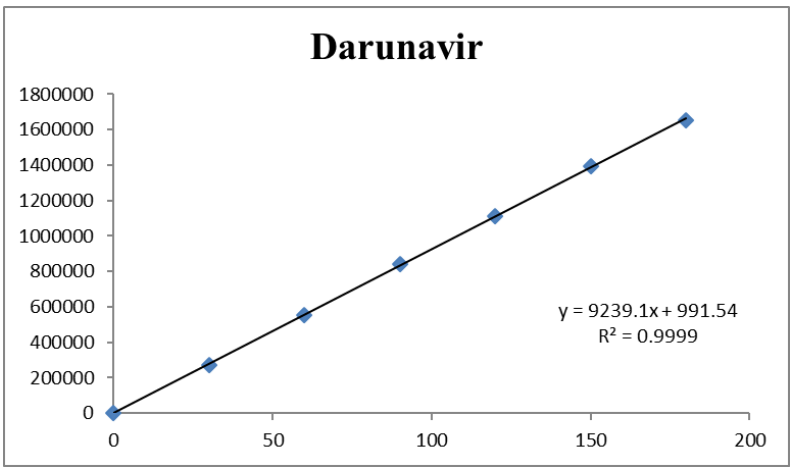


Figure 3: Calibration Curve of Darunavir

Accuracy:

Three doses were given at each level, and the mean % recovery was calculated. Favipiravir's recovery rate was observed to be between 99% and 100.%, which is within the acceptable ranges

Table 4: Accuracy Data

	Ritonavir				Darunavir			
% Level	Added	recovered	%Recovery	Avg %	Added	recovered	%Recovery	Avg %
50%	10	9.96	99.62	99.84	60	60.00	100.00	100.50
		10.03	100.27			60.26	100.43	
		9.96	99.62			60.64	101.07	
100%	20	19.93	99.63	100.02	120	119.21	99.34	99.66
		20.10	100.52			119.63	99.70	
		19.98	99.90			119.95	99.96	
150%	30	29.82	99.41	99.40	180	178.95	99.42	99.45
		29.78	99.28			179.05	99.47	
		29.86	99.52			179.05	99.47	
% recovery		99.75				99.87		

Precision:

Table 5: Precision Data

	Ritonavir		***Darunavir***	
S. No	***Day 1***	***Day 2***	***Day 1***	***Day 2***
Injection-1	133002	133147	1119932	1115461
Injection-2	132674	133065	1113107	1101221
Injection-3	133474	132767	1113423	1114330
Injection-4	133237	132103	1116869	1114935
Injection-5	133561	132841	1119996	1116965
Injection-6	134468	132735	1116332	1103335
Mean	133403	132776	1116610	1111041
S.D	613.8	368.8	3002.4	6876.4
%RSD	0.5	0.3	0.3	0.6

Robustness:

Table 6: Robustness data for Ritonavir

Ritonavir							
conditio	***Optimized condition***	***Condition applied***	***area***	***RT***	***Plate Count***	***Tailing factor***	***RSD***
Flow rate (-0.1ml/min)	**1ml/min**	0.9ml/min	125588	2.347	2494	1.2	1.0
Mobile phase (-5%v/v)	**40:60**	35: 65	154331	2.147	2940	1.3	0.4
Column temp (-3^{0}c)	**30^{0}c**	27^{0}c	121527	2.101	2186	1.2	1.3
Optimized			**126598**	**2.110**	**2318.4**	**1.2**	**-**
Flow rate (+0.1ml/min)		1.1ml/min	111024	1.921	2014	1.23	0.4
Mobile phase (+5%v/v)		45: 55	122082	2.096	2283	1.22	0.8
Column temp (+3^{0}c)		33^{0}c	121546	2.120	2048	1.22	1.0

Table 6: Robustness data for Darunavir

Darunavir								
condition	Optimized condition	Condition applied	area	RT	Plate Count	Tailing factor	RS	RSD
Flow rate (-0.1ml/min)	1ml/min 40:60 30^0c	0.9ml/min	1098664	2.904	2523	1.31	2.7	0.1
Mobile phase (-5%v/v)		35: 65	1252685	2.653	2121	1.42	2.3	0.1
Column temp (-3^0c)		27^0c	1023669	2.600	2246	1.33	2.5	0.4
Optimized			1157823	2.614	2327.4	1.35	2.4	-
Flow rate (+0.1ml/min)		1.1ml/min	937527	2.378	2030	1.34	2.3	0.2
Mobile phase (+5%v/v)		45: 55	1001171	2.589	2363	1.31	2.5	0.2
Column temp (+3^0c)		33^0c	1019010	2.624	2072	1.39	2.4	0.1

Assay

Table 7: % Assay Purity Data

Formulation	***Label claim(mg)***	***% Assay****
Daruvir 600 R	***Ritonavir 100 mg***	***99.95 %w/w***
	Darunavir 600mg	***100.24 %w/w***

Degradation studies:

Table 8: Force Degradation Studies of Ritonavir

Ritonavir					
S.No	Stress Conditions	Optimized area	Peak area	% Degradation	Peak Purity
1	Acid	126598	124309	6.86	Passes
2	Base		125905	5.66	Passes
3	Oxidation		123748	7.28	Passes
4	Thermal		129795	2.75	Passes
5	Photolytic		130136	2.49	Passes
6	Hydrolytic		132600	0.65	Passes

Table 8: Force Degradation Studies of Darunavir

Darunavir					
S.No	Stress Conditions	Optimized area	Peak area	% Degradation	Peak Purity
1	Acid	1157823	1038107	6.80	Passes
2	Base		1035413	7.05	Passes
3	Oxidation		1039748	6.66	Passes
4	Thermal		1078644	3.17	Passes
5	Photolytic		1080818	2.97	Passes
6	Hydrolytic		1110244	0.33	Passes

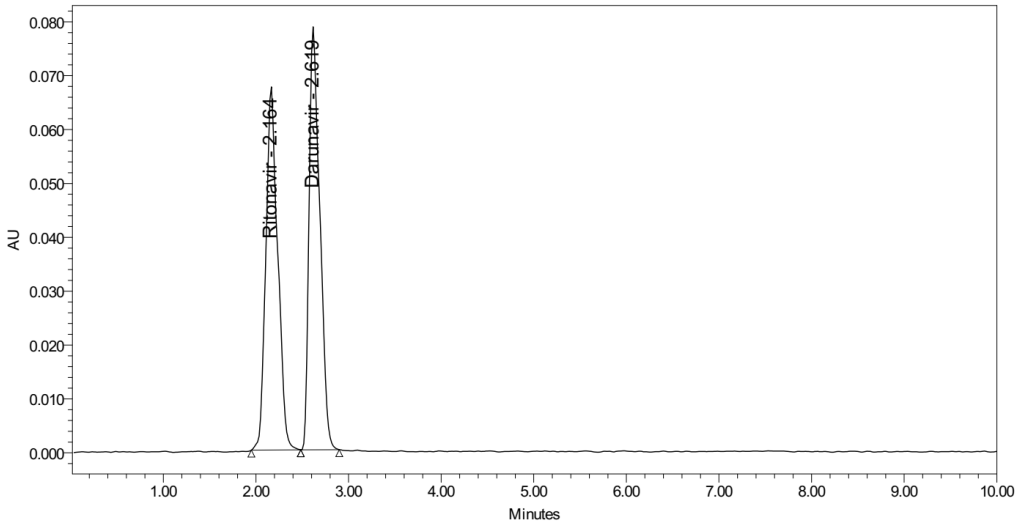


Figure 4: Acid Chromatogram

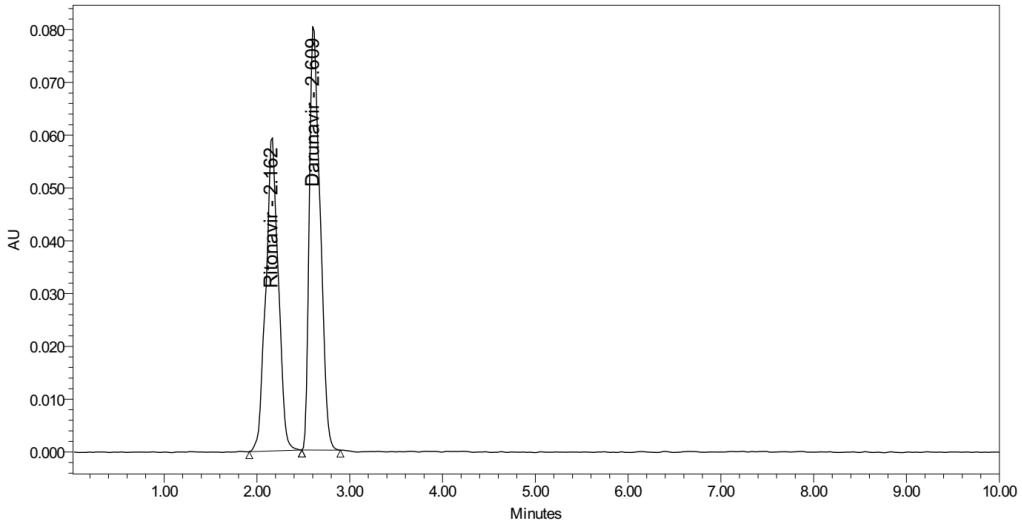


Figure 5: Base Chromatogram

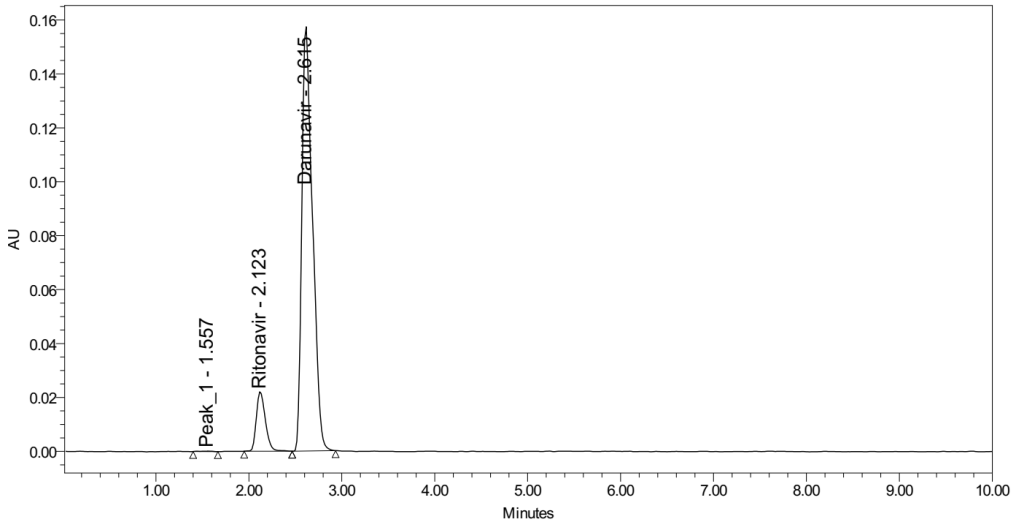


Figure 5: Oxidative Chromatogram

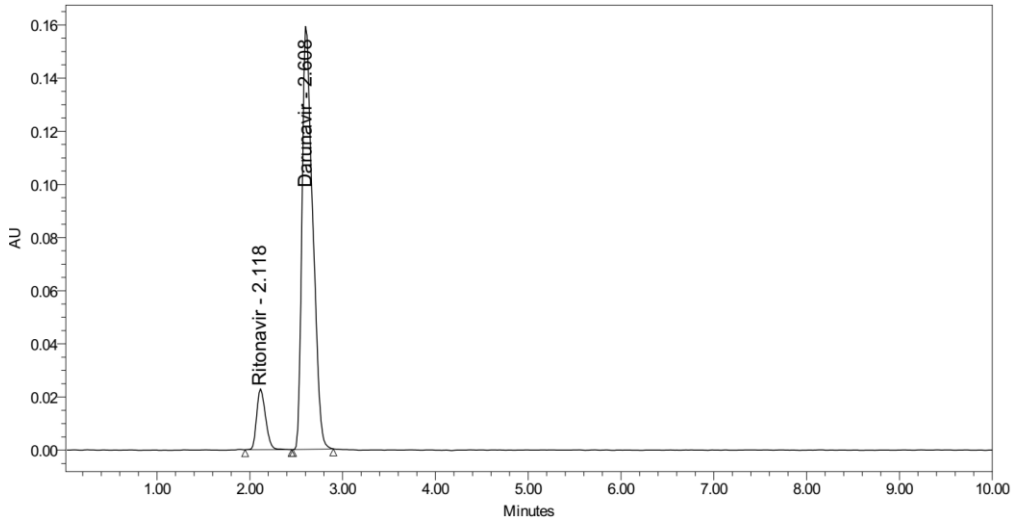


Figure 5: Thermal Chromatogram

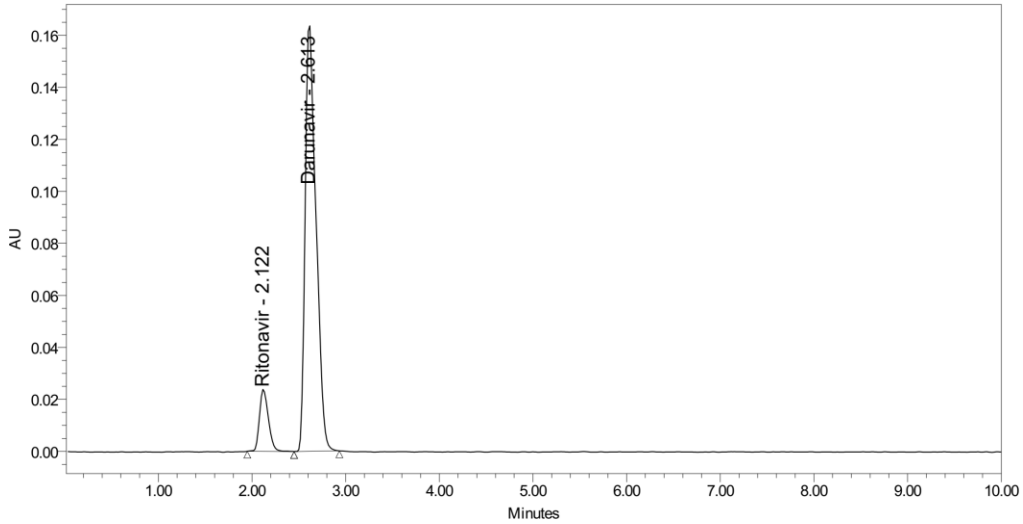


Figure 5: Photolytic Chromatogram

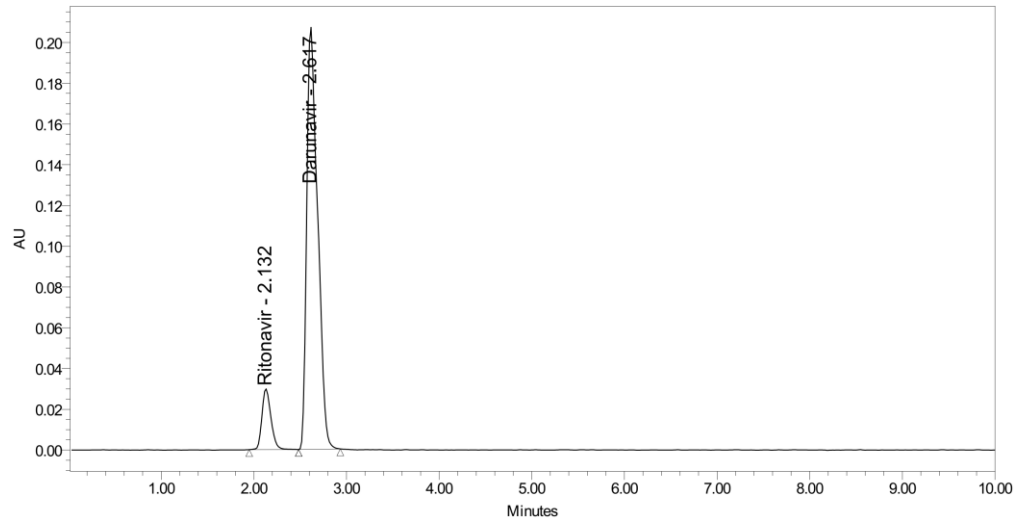


Figure 5: Water Chromatogram

CONCLUSION:

The present study was aimed at developing a sensitive, precise and accurate stability indicating HPLC method for the analysis of Darunavir and Ritonavir in bulk drug and pharmaceutical dosage forms by using QbD approach using Design Expert® software. The Central composite design experimental design describes the interrelationships of mobile phase and pH at three different level and responses to be observed were retention time, theoretical plates, and peak asymmetry with the help of the Design Expert 13.0.5.0 version. Here, a better understanding of the factors that influence chromatographic separation with greater confidence in the ability of the developed HPLC method to meet their intended purposes is done. The QbD approach to analytical method development was used for better understanding of method variables with different levels. it is a faster way of developing method, which helps choosing much better method condition during the development process through design space. The validation results confirmed the usefulness of the method. The method was found to be precise, accurate and linear, whereas the stress degradation studies identified the potential degradation products which can form during the shelf life of the product. In order to affect proper elution of the component peaks, mixtures of Acetonitrile with phosphate buffer, Orthophosphoric acid and Disodium hydrogen phosphate buffer in different combinations and were tested as mobile phase. Finally, **Sunfire C18 (4.6 x 250mm, 5µm)and mixture of 0.1N Ammonium Acetate (pH 4.0) (60.0 v/v) and Methanol (40.0 v/v)** was proved to be the most suitable of the combination since the chromatographic peaks were better defined and resolved and almost free from tailing. The retention time for Ritonavir and Darunavir was at **2.110 ± 10% min 2.614 ± 10% min** and respectively injected at a flow rate **of 1.0 mL/min**. The proposed HPLC method was also applied for the forced degradation studies on Darunavir and Ritonavir under a variety of conditions like acid and base hydrolysis, oxidation, and heat and photo stability. The drugs were found to be stable except in acidic, Basic, Peroxide stress conditions. The drug peaks in these degradations were found to be homogenous No major degradants were found in Neutral stress, photo stability and Thermal degradation studies. As the developed method could effectively separate Darunavir and Ritonavir from the degradants, it can be employed as a stability indicating assay. System suitability testing was performed prior to the study of each validation parameter and the verified parameters like tailing factor (< 2.0), resolution (> 2.0), column efficiency (> 2000) and repeatability (% RSD < 2) ensured that the equipment, electronics, and analytical operations for the samples analysed could be constituted as an integral system that can be evaluated as a whole.

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