RESEARCH ARTICLE

QbD-Driven Approach to Cleaning Method Development and Validation for Darunavir Analysis in Oral Films

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ABSTRACT

The current investigations involve developing a high-performance liquid chromatographic method that's simple to operate, fast to establish, accurate, precise, and economical through design-enabled methods. A positive experimental design is offered to utilize the central composite design of pH and the mobile phase, two crucial components of the RP-HPLC technology. The chromatographic conditions were optimized with the release of Design Expert software version 13.0. Symmetry C18 column (4.6×150mm, 5µm) was used in the procedure, along with acetonitrile (20:80 v/v) and potassium dihydrogen orthophosphate buffer pH 4.0 as the mobile phase. There was a 0.70 ml/min flow rate. 263 nm is the wavelength of detection. Darunavir's sharp, resolved peak was seen 2.3 minutes after swabbed samples from the 10*10 cm SS plate were injected. A linear calibration curve with an R^2 of 0.9991 was observed in the concentration range of 0.25 to 25µg/ml. For precision %RSD is 1.03,1.48. Accuracy % concentration 50%,100%,150% mean recovery 99.43-100.53.LOD&LOQ 1.12µg/ml, 3.39µg/ml. Robustness was tested with modest modifications to the wavelength and flow rate. Forced degradation studies were carried out and found to be within the limits. the degradation parameters such as Acid, Base, Oxidative, Photolytic, and thermal degradation parameters are according to the ICH guidelines. % Assay was done for oral films and it was found to be within the limits. Thus, the outcomes unequivocally demonstrated that the QbD technique could be effectively used to enhance the HPLC method for the measurement of darunavir in production settings.

Keywords: Darunavir, RP-HPLC, Quality by design, Method development, Stability studies.

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INTRODUCTION

To properly control HIV-1 infection, protease inhibitor medications, such as darunavir, are administered in addition to ritonavir and other HIV protease inhibitor medications[1]. A second-generation protease inhibitor called darunavir is intended to combat HIV drug resistance. In 2006, the FDA first approved something[2]. The medication is being researched as a possible treatment due to findings obtained in vitro showing darunavir's effectiveness against the coronavirus responsible for COVID-19, SARS-CoV-2. Darunavir belongs to the class of amino benzene sulphonamides, which are naturally occurring mixtures[3]. As seen in Figure 1, these are natural mixtures with a benzene sulphonamide moiety and an amine molecule attached to the benzene ring[4]. To ensure quality, QbD focuses on the analytical methods used in method development[5,6].

In the current research, the QbD-assisted cleaning approach technique was utilized. And we strictly adhered to ICH guidelines for the selection and optimization of many parameters that affect the development and authenticity of the method among the approaches utilized were risk assessment, Design of Experiment, Analytical Target Profile, and Critical Quality Attributes[7].We opted for a central composite design, 13.0(software).There were runs for optimization in the central composite design. We chose the tailing factor, retention time, aqueous phase pH, and number of mobile phases for this investigation. Nobody pursued their observation and such a chromatographic variance[8,9].The current study's goals were to create regular analytical methods and validate them using a quality-by-design methodology. According to the previous study, no QbD-assisted cleaning method of Darunavir by HPLC has been published.

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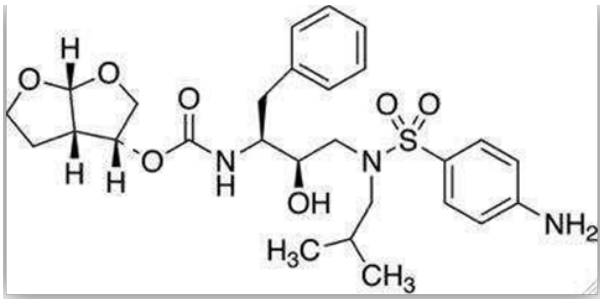


Figure 1: Structure of Darunavir

MATERIALS AND METHODS

Darunavir is obtained as a gift sample from Hetero Drugs Pvt.Ltd. All the reagents and solvents used in the study were of high quality and met the HPLC grades. Acetonitrile from Finar chemicals, high-quality deionized water was used which was obtained by double distillation process.

Instruments

A Waters HPLC system (1525), with binary HPLC pump, UV detector (2487), column C18 with internal diameter 100×4.6× 5µm. UV spectrophotometer (UV 3200) from Lab India, polypropylene swab sticks from Himedia, 10*10*2mm thickness stainless steel plate, sonicator (2200MH) by Soltec, Analytical balance by Shimadzu.

HPLC Conditions

The mobile phase consisted of acetonitrile and potassium dihydrogen orthophosphate buffer pH 4.0 in a 20:80v/v ratio. The solvents are pushed from reservoirs to the column at a flow rate of 0.7 milliliters per minute after being filtered with a 0.45-micrometer membrane filter. A 10-minute run time was specified. At 263 nm, the eluents were observed.

Sample Preparation

Preparation of Mobile Phase A

Buffer Solution pH 4.0 was used to create mobile phase A.

Preparation of Mobile Phase B

Acetonitrile was used to create Mobile Phase B.

Getting the Buffer Solution Ready 4.0 pH

Precisely weigh 1.36 g of potassium dihydrogen phosphate in 1000 ml of water. Use orthophosphoric acid to adjust the pH to 4.0 ± 0.05 and filter through a 0.45µ nylon membrane filter.

Making of Standard Stock Solution

Darunavir stock solution was made by weighing about 50 mg of the pure medication and transferring it to a 50 ml volumetric flask. Acetonitrile was added to raise the concentration of the drug solution to the necessary 1000 µg/ml.

Working Standard Solution

A 10 milliliter volumetric flask is filled with one milliliter of the stock solution via pipetting. The reactor's capacity was increased with ACN to bring the concentration to 100 µg/ml. Multiple dilutions were prepared by using the 100 µg/ml concentration solution. From the above solution, further serial dilutions were made to obtain concentrations of 0.25,0.5,1.2,3,4,5,10,15,25 µg/ml.

Cleaning Method

Stainless steel plates and swabs were utilized in the cleaning procedure. A tiny amount of the chosen solvent (acetonitrile) was pipetted out and applied dropwise to the stainless-steel plate after the plate had been gently cleaned with the solvent. A hairdryer was then utilized to dry the plate. Subsequently, residues were gathered by applying a swab stick in all directions to the surface. Lastly, the swab was put in a test tube holding a solvent, and acetonitrile was added to the solution to make it up to 10 ml. After measuring the solution's RT and absorbance and using that result as a blank, repeat the process with varying concentrations and apply it to the manufacturing machinery to find any traces of the previous batch's production.

METHODOLOGY

Optimization with QbD

QbD was used to design trials that varied in the interaction of factors on dependent variables. A CCD study was planned with two independent components (mobile phase composition and buffer pH) and two dependent response factors (retention time, and tailing factor). Eleven runs were performed to optimize the independent variables and evaluate the dependent variables.

RESULTS AND DISCUSSION

Retention Time

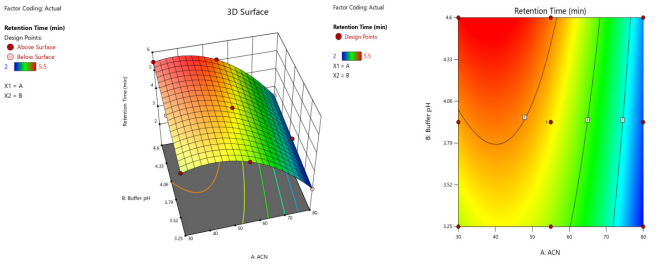
The retention time was optimized using CCD and ANOVA for the Quadratic model (p-values < 0.0500). With a signal-to-noise ratio of 218.152, there is a strong enough signal. You can use this layout to guide the design space.

Table 1: Optimising parameters for Darunavir analysis by CCD

Run	*Run order*	*Coded factors levels*	
		Factor A	*Factor B*
1	11	55	3.925
2	6	80	3.925
3	4	80	4.6
4	3	30	4.6
5	8	55	4.6
6	7	55	3.25
7	1	30	3.25
8	10	55	3.925
9	9	55	3.925
10	2	80	3.25
11	5	30	3.925
Level of factor			
Parameter	*Low(-1)*	*Intermediate(0)*	*High(+1)*
A: ACN	30	55	80
B: Buffer pH	3.25	3.92	4.6

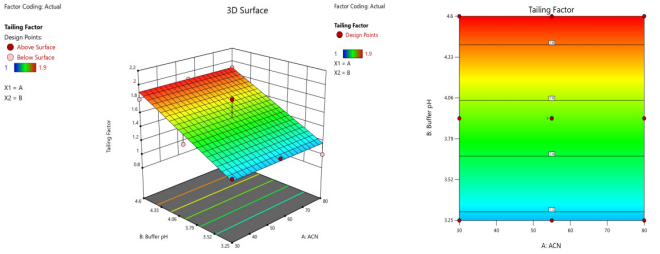
Table 2: CCD experimental design with levels and their measured responses

Std	*Run*	*F1*	*F2*	*R1*	*R2*
		A: ACN	*B: Buffer pH*	*Retention Time*	*Tailing Factor*
11	1	55	3.925	4.7	1.8
6	2	80	3.925	2.3	1.09
4	3	80	4.6	2.5	1.9
3	4	30	4.6	5.5	1.8
8	5	55	4.6	5.1	1.9
7	6	55	3.25	4.3	1.2
1	7	30	3.25	4.4	1.2
10	8	55	3.925	4.7	1.8
9	9	55	3.925	4.7	1.8
2	10	80	3.25	2	1
5	11	30	3.925	4.9	1.4



2(a) 2(b)

Figure 2: (a) 3D response surface showing the effects of an independent element on the Retention time; (b) A contour plot showing how the independent component affects the retention time



(a) (b)

Figure 3: (a) 3D response surface showing the effects of an independent element on the Tailing factor; (b) A contour plot showing how the independent component affects the Tailing factor

Tailing Factor

The tailing factor was optimized by utilizing CCD and ANOVA for the Quadratic model (p-values < 0.0500). The signal-to-noise ratio is 7.545 which indicates a sufficient signal. This layout may be utilized to guide the design space.

Optimized QbD Parameters

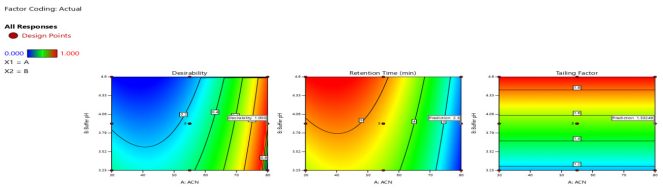


Figure 4: Final Optimum conditions

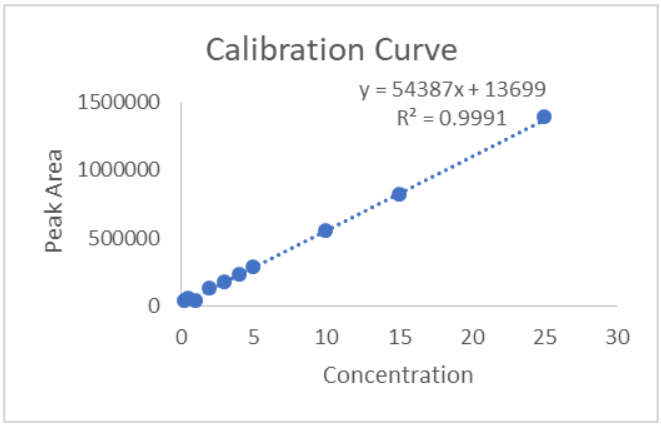


Figure 5: Calibration Curve of Darunavir

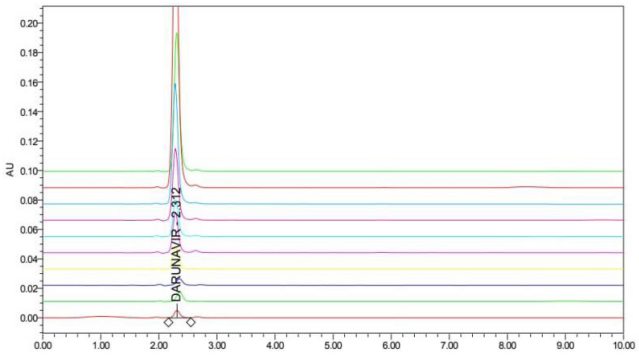


Figure 6: Linearity chromatogram Darunavir

Validation parameters

Linearity

The linearity curve was created throughout the ranges of 0.25, 0.5,1.2,3,4,5,10,15, and 25 µg/ml.It was discovered that darunavir had a 0.999 correlation coefficient, indicating linearity. Y= 54387x + 13699s was the equation for the linear regression. Table 3 presents the estimated regression properties, including slope, intercept, and percentage RSD.

Precision

The precision of the approach was assessed using variance assessments conducted both within and between days. Six of the same concentrations were examined on separate days for inter-day variation investigations and the percentage RSD was computed. This implies that the process is accurate. As Table 4 illustrates, the obtained results fell within the recognized parameters.

Table 3: Linearity study of Darunavir

S. No	*Concentration (µg/ml)*	*Area*
1	0.25	40898
2	0.5	57698
3	1	40858
4	2	129565
5	3	176447
6	4	229968
7	5	286532
8	10	548745
9	15	818737
10	25	1383490

Table 4: Precision study of Darunavir

	Inter day		*Intra day*	
Concentration (µg/ml)	*Retention time*	*Area*	*Retention time*	*Area*
25	2.33	2503163	2.32	2632109
25	2.33	2503298	2.32	2623012
25	2.33	2567361	2.32	2524471
25	2.33	2540654	2.32	2601164
25	2.33	2539557	2.32	2610153
25	2.33	2553489	2.32	2610863
Mean	2.33	2534587	2.32	2600295
SD	26303.61		38696.48	
%RSD	1.03		1.48	

Accuracy

Accuracy samples were prepared at three different levels. For each accuracy level, three injections were administered, and the percentage of recovery was computed and recorded. Since the figures in Table 5 are within the acceptable range of 98.0% to 102%, the method was determined to be accurate by the acceptance criteria.

Robustness

The effects of these adjustments are then observed by injecting the solutions. if the count of plates, tailing factors, or chromatogram did not change. It suggests that the approach is robust and adaptable to wavelength and flow rate changes. Table 6 presents a tabulation of the obtained data's overall standard deviation and percentage RSD.

Ruggedness

Six samples of the same batch of Darunavir were analyzed independently in two sets to determine ruggedness. The days of the experiment, the people carrying them out, and the tools/columns should all be distinct from one another. Table 7 lists the total standard deviation and percentage RSD for the collected data. The method's ruggedness was demonstrated when the mean of two RSD readings was determined to be within the acceptable range.

LOD & LOQ

The LOD was calculated using the formula LOD=3.3×(N/S), where N is the standard deviation of the peak area and (S)

Table 5: Accuracy study of Darunavir

Level	*Amount of pure drug*	*Amount of sample*	*Total concentration*	*% Recovery*	*Mean recovery*	*%RSD*
50%	1µg/ml	2µg/ml	170323	99.7		
50%	1µg/ml	2µg/ml	170433	100	100.36	0.904
50%	1µg/ml	2µg/ml	171022	101.4		
100%	2µg/ml	2µg/ml	258130	99.2		
100%	2µg/ml	2µg/ml	257120	98.4	99.43	1.174
100%	2µg/ml	2µg/ml	260110	100.7		
150%	3µg/ml	2µg/ml	307002	100.7		
150%	3µg/ml	2µg/ml	305186	99.36	100.53	1.093
150%	3µg/ml	2µg/ml	308012	101.54		

Table 6: Robustness study of Darunavir

	Wavelength				*Flow rate*			
S. No	*262 nm*		*264nm*		*0.60ml*		*0.80ml*	
	Retention Time	*Area*	*Retention Time*	*Area*	*Retention Time*	*Area*	*Retention Time*	*Area*
1	2.3	1202764	2.3	2644009	2.3	1001363	2.3	1495299
2	2.3	1219352	2.3	2712557	2.3	1034203	2.3	1490886
3	2.3	1189046	2.3	2670739	2.3	1013136	2.3	1509860
4	2.3	1201150	2.3	2657592	2.3	1035256	2.3	1522517
5	2.3	1213252	2.3	2717864	2.3	1012414	2.3	1540694
6	2.3	1222964	2.3	2720624	2.3	1012261	2.3	1505403
Average	1208088		2687231		1018106		1510777	
SD	12758.67		33803.78		13600.2		18427.13	
RSD	1.056105		1.257941		1.335834		1.219713	

Table 7: Ruggedness study of Darunavir

	Column 1		*Column 2*	
	Day-1		*Day-2*	
	Analyst-1		*Analyst-2*	
Concentration (µg/ml)	*Retention Time*	*Area*	*Retention Time*	*Area*
25	2.32	2632109	2.31	2613010
25	2.32	2623012	2.31	2623513
25	2.32	2524471	2.31	2602261
25	2.32	2601164	2.32	2601060
25	2.32	2610153	2.33	2510153
25	2.32	2610863	2.31	2614653
Mean	2600295		2594108	
SD	38696.48		41969.7	
%RSD	1.48		1.61	

Table 8: LOD & LOQ study of Darunavir

Drug Name	*LOD*	*LOQ*
Darunavir	1.59µg/ml	4.83 µg/ml

Table 9: Formulation of Oral Film

S. No.	*Ingredients*	*Formulation*
1	Darunavir	50(mg)
2	HPMC	400(mg)
3	Mannitol	25(mg)
4	Propylene glycol	0.15(ml)
5	Citric acid	5(mg)
6	Ethanol	10(ml)

Figure 7: Oral film

is the slope of the calibration curve.Based on the slope and the response's residual standard deviation, the limits of detection and quantification were assessed. Table 8 displays the Darunavir LOD and LOQ values.

Assay of oral film

The required quantity of Polymer HPMC E15 was taken, dissolved in the solvent ethanol in a beaker, and kept aside for soaking for ½ an hour. For 15 minutes, the mixture is stirred to achieve a uniform consistency. Within 10-15 mins the other excipients mentioned in Table- 9 were added in order. The solution is kept for stirring on a magnetic stirrer for 1-2hrs. To remove air bubbles the solution is kept aside for a few minutes. Once the mixture is well distributed throughout the petri dish, it can be dried in a hot air oven or at room temperature for 24 hours.The necessary volume of ACN was used to dissolve the oral film.

$$\frac{\text{Sample area}}{\text{STD area}} \times \frac{\text{wt. of STD}}{\text{dilution of STD}} \times \frac{\text{dilution of sample}}{\text{wt. of sample}} \times \frac{\text{purity}}{100} \times \frac{\text{wt. of tablet}}{\text{label claim}} \times 100$$

$$\frac{3070679}{2859052} \times \frac{50}{1000} \times \frac{1000}{50} \times \frac{99.5}{100} \times \frac{285}{300} \times 100$$

$$= 101.51\%$$

CONCLUSION

The systematic QbD-based methodology has effectively been used to build a quick, easy, sensitive, stability-indicating analytical strategy for evaluating darunavir and its degradation products in pharmaceutical formulations and bulk drugs. The experimental design identifies and describes the three crucial HPLC procedure components: the column, pH, and mobile phase. Preliminary optimised conditions are obtained for every column, pH, and mobile phase combination after the interrelationships are explored. Here, the factors impacting chromatographic separation are more known, and the techniques' ability to achieve their goals is more firmly believed. To define the design space and streamline the procedure, a central composite design was employed. To illustrate the relationship between the retention factor and mobile phase composition, 3-D response surface graphs and contour plots were made using software for design experts. All of the validated parameters were discovered to be within the acceptable range. The verified technique is robust, precise, accurate, linear, and durable. The HPLC technique for darunavir was developed in the lab using this strategy with success. It is even possible to build several techniques at once using the QbD methodology, such as the assay and cleaning procedures.

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