Int. J. Pharm. Investigation, 2026; 16(1):357-370.
https://www.jpionline.org

Original Article

Stability-Indicating RP-HPLC Method Development and Validation of Darunavir Using a Quality by Design (QbD) Approach, with Estimation in Prepared Solid Supersaturated Self-Emulsifying Drug Delivery Systems (SEDDS)

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ABSTRACT

Background: A new stability-indicating Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed to quantify Darunavir (DRV), an HIV-1 protease inhibitor, despite its limited water solubility and susceptibility to light. **Materials and Methods:** The method utilized the central composite design, aiming to minimize variations in regression coefficients. The primary objective of this study was to establish an RP-HPLC method that ensures accuracy, precision, and ease of automation. To date, there have been no recorded instances of utilizing methanol in RP-HPLC development. The focus was on demonstrating the cost-effectiveness of methanol in RP-HPLC method development, highlighting its economic advantages and good performance. The development and validation of HPLC methods are critical for various applications, including drug discovery, development, and manufacturing. DRV was analyzed at a wavelength of 267 nm utilizing a photodiode array detector, and separation of chromatographs was achieved using a Phenomenex Luna C-18 column. **Results:** The mobile phase comprised methanol and HPLC-grade water (with 0.1% formic acid). The flow rate of the mobile phase was set at 1 mL/min, with an injection volume of 10 µL. The retention time for DRV was found to be 8.85 min. The force degradation data indicate decreases in basic, oxidation, and photolytic conditions; nevertheless, in thermal and acidic settings, a significant degree of stability was shown after 2 hr. **Conclusion:** The study aimed to develop a simple, reliable reversed-phase HPLC method for quantifying DRV in pharmaceutical dosage forms and bulk drug samples.

Keywords: Darunavir, RP-HPLC, Central composite design, Analytical method validation.

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Received: 09-05-2025;
Revised: 28-07-2025;
Accepted: 16-09-2025.

INTRODUCTION

Darunavir (DRV) is an antiretroviral medication from the peptidic protease inhibitor class, commonly prescribed alongside other drugs to manage Human immunodeficiency viruses (HIV) infection and prevent the progression to Acquired Immunodeficiency Syndrome (AIDS) (Chavan RB *et al.*, 2015). Considered the latest HIV-1 protease inhibitor, DRV (Prezista (TM)) demonstrates efficacy against both drug-resistant HIV strains and wild-type viruses (Deeks ED., 2014). Its chemical structure, [(1S,2R) darunavir (DRV), 3-[Amino [2-methylpropyl]]Sulfonyl(4-aminophenyl)]1-propyl-2-hydroxy (phenylmethyl)- potassium hydroxide (3R, 3aS, 6aR). [2,3-b]-hexahydrofuro HIV type 1 treated with furan-3-yl ester monoethanolate, is a novel protease inhibitor (PI) as illustrated in Figure 1 (Guillarme D *et al.*, 2013). With a molecular weight of 547.6 and a chemical formula of $C_{27}H_{37}N_3O_7S$, DRV stood as an effective tool in combating HIV-1 infections. Developing an HPLC method with enhanced sensitivity and selectivity would have greatly aided in identifying DRV within pharmaceutical formulations. This protease inhibitor, DRV, worked by interacting with the HIV protease enzyme, effectively targeting a wide range of virus strains, including those that had developed resistance

DOI: 10.5530/ijpi.20261680

to earlier protease inhibitors. In contrast, an existing RP-HPLC method was discovered to be rapid, accurate, cost-effective, and user-friendly for quantifying DRV in dosage forms (Satyanarayana L *et al.*, 2011). High-Performance Liquid Chromatography (HPLC) stood as the second most utilized analytical technique within the pharmaceutical sector, just after RP-HPLC. DRV had been analyzed using various methods and equipment such as LC/MS, HPLC, HPLC-MS, and UV spectrophotometry, either individually or in combination (Lokhande DP. 2019). These spectrophotometry and chromatography techniques yielded reliable and precise data crucial for research and quality control across industries like pharmaceuticals, environmental monitoring, and food analysis (Tran GH *et al.*, 2024). Several experiments, including stress or forced degradation tests, were conducted to ensure the robustness of the proposed methodology. These experiments involved various conditions such as exposure to sunlight, acidic and alkaline environments, thermal stress, and oxidative conditions.

In a Quality by Design (QbD) framework, the quality of HPLC methodologies had gained significant importance. During the development phase of a method, it was crucial to ascertain the robustness and ruggedness of HPLC procedures early on, ensuring consistent performance throughout the product's lifecycle (Bhatt DA *et al.*, 2011). The principles of QbD involved a systematic understanding of key process variables and method attributes, along with their impact on the quality of results (Koli R *et al.*,). Through the utilization of Design of Experiments (DoE), parameters such as column selection, mobile phase composition, and detection wavelength were optimized to formulate a method that delivered heightened sensitivity, accuracy, and precision. The aim of this research was to create a dependable, accurate, and cost-effective approach for the simultaneous determination of both medications, integrating the principles of QbD (D'Avolio A *et al.*, 2007).

RP-HPLC method using water-acetonitrile (40-60 v/v) with formic acid was developed before (Patel BN *et al.*, 2012). A method development employing an HPLC column operated in constant mobile phase composition, utilizing 0.01M ammonium format (pH 3.0) buffer along with acetonitrile in a proportion of 55:45 (v/v), with a flow rate of 1.0 mL/min has developed before but no methanol has been used (Reddy BR *et al.*, 2013). RP-HPLC method has been developed using an eluent phase comprising 0.02M dipotassium hydrogen orthophosphate with water and acetonitrile in the ratio of 40:60 v/v (Fukuda IM *et al.*, 2018). "To date, no documented instances have been found regarding the utilization of methanol in Reversed Phase High-performance Liquid Chromatography (RP-HPLC) method development."

Advantages

Lower cost: Methanol is often more economical compared to acetonitrile, making it a preferred choice for budget-conscious laboratories.

Good solvent properties: Methanol exhibits excellent solubility for a wide range of compounds, making it versatile for different types of samples.

Environmental consideration

Methanol is considered less hazardous and has a lower environmental impact compared to some other solvents, aligning with sustainable laboratory practices. When methanol is compared with acetonitrile, the efficiency of methanol in terms of its effectiveness in achieving desired chromatographic separations potentially requires less solvent per analysis compared to acetonitrile. Consider the long-term cost implications of using methanol, including reduced overall consumption and potential savings in method optimization and troubleshooting.

The goal of the study is to develop a cost-effective, precise, and dependable method for calculating Darunavir (DRV) that integrates Quality by Design (QbD) concepts. This will be achieved by verifying the precision and dependability of the suggested methodology. This objective provides a fresh approach to DRV estimation and adds to the method's greater resilience. This study's reasoning emphasizes the need of ensuring accuracy, dependability, and efficiency in pharmaceutical analysis while also aligning with current concept of methodological improvement and quality assurance (Paul K *et al.*, 2021; D'Avolio A *et al.*, 2007; Godambe RD *et al.*, 2018; Swartz ME *et al.*, 2018; Ettaboina SK *et al.*, 2022; Sarkar *et al.*, 2006; Truong DH *et al.*, 2016; Chavan RB *et al.*, 2015).

MATERIALS AND METHODS

Chemicals and reagents

HPLC-grade methanol is supplied by Merck, an Indian company located in Mumbai. Formic acid, orthophosphoric acid, concentrated HCl, NaOH, sodium hydroxide pellets, and water (Milli-Q) were gathered from the pharmacy school at KLE College in Belagavi.

Chromatography instrument and conditions

The analysis was conducted using a 10 µL injection volume on the Shimadzu Agilent 1220 Infinity II instrument (LC-20AD, Japan). The instrument, equipped with a quaternary pump with degasser (G7111A), auto-injector (G7129A), and PDA detector (G7115A), was utilized. The data were interpreted and analyzed utilizing the OpenLab CDS software. Chromatographic separation and analysis were performed using a Phenomenex Luna C18 analytical column with the following specifications: C-18(2) 100, internal diameter of 250 mm, length of 4.60 mm, and particle size

of 5 µm. This column is manufactured by Phenomenex Inc., a US-based company headquartered in Canada. The mobile phase ratio for DRV was optimized to MeOH: 0.1% formic acid in water (60:40), flowing through the column at a rate of 1 mL/min. Before use, ultrasonic degassing was applied to the mobile phase using a PVDF filter membrane (0.45 µm; Millex HV®, Millipore, USA). Sample analysis was performed with an injection volume of 10 µL, and DRV was detected at a wavelength of 267 nm.

Preparation of primary stock and standard samples

The DRV stock solution was prepared by utilizing separate 10 mL volumetric flasks to achieve a concentration of 1 mg/mL. Accurate measurements of the drug's firm standard weight were taken and then dissolved in methanol. The quantities of these solutions were adjusted using the mobile phase. Following this, both solutions underwent a sonication process for 6 min. 1 mL of the stock solution was then taken and diluted with the mobile phase to a concentration of 100 µg/mL to create the working solution. The calibration standards were prepared by serially diluting the Darunavir working solution to obtain concentrations ranging from 10 to 50 µg/mL.

Preliminary method development studies

The relative maximum solubility profiles of both medications (methanol and acetonitrile) were determined through an evaluation of their solubility, considering available literature and their individual physicochemical properties. However, during the method development process, the aqueous phase was adjusted using orthophosphoric acid and formic acid, and equal amounts of the organic and aqueous phases were blended. In order to examine the solubility profile and improve chromatographic estimations, various mobile phase ratios were explored across different pH levels.

Method development

The mobile phase was pumped for 30 min to perform baseline correction and ensure column saturation. A standard calibration curve was established for each drug. The previously prepared stock solutions were divided into multiple aliquots using suitable diluents. Each concentration was administered six times into the liquid chromatography system. The exact peak and retention times of each medication were noted. Unique calibration curves were generated for each drug by plotting the mean peak area on the Y-axis against the corresponding concentration values on the X-axis. Regression equations were then derived from these calibration curves and applied to determine the accurate composition of the product.

Quality Target Product Profile (QTPP) and Critical Quality Attributes (CQAs)

The first step in implementing a QbD approach is defining the Quality Target Product Profile (QTPP), which considers the desired outcome of the product. This involves identifying the aspects of a product's features and performance that were relevant to patient care improvement. A framework for selecting QTPPs is provided by the ICH Q8 guideline on dosage. Factors such as pharmacokinetics, stability, route of administration, dosage form, strength, and various quality attributes (such as particle size, zeta potential, PDI, EE, etc.) also play a role in determining the QTPP. CQA are characteristics of a product that depend on the results of an experiment. Examples include excipients, components in drugs, and process variables. To achieve the desired outcome of creating a high-quality product, these variables can be carefully considered and controlled. Therefore, it is crucial to identify CQA during the screening procedure by utilizing current, dependable data, and literature (Satyanarayana L *et al*., 2011).

Preparation of Solid SEDDS

DRV's initial solubility in a range of oils, co-surfactants, and surfactants was established. The SEDDS formulations were prepared using clove oil, Tween 80, and Transcutol P as the oil, surfactant, and co-surfactant, respectively (Truong DH *et al*., 2016). The self-emulsifying region was displayed on the ternary phase diagram by evaluation. The Polydispersity Index (PDI) and droplet size of the final emulsions were used to optimize the formulations. A solid self-emulsifying drug delivery system was created utilizing solid inert carriers Aerosil 200, using an adsorption method to increase solubility (Satyanarayana L *et al*., 2011; Gurumukhi VC *et al*., 2021).

Evaluation of the analytical method employing the Quality by Design (QbD) approach

It consists of several essential steps that are required to ensure reliable and stable analytical processes. Firstly, the objective of the method, which includes target analytes and desired performance parameters, is defined. Secondly, Critical Method Parameters (CMPs) that have a significant impact on the method's outcome are identified. These CMPs are then categorized as either CQAs or Critical Process Parameters (CPPs). Thirdly, a risk assessment is conducted to prioritize and assess potential risks related to the method. DoE is then utilized to methodically investigate the effects of CMPs on CQAs. Finally, the data is collected, and statistical tools are used to analyze the data obtained, resulting in the creation of a design space and ideal chromatographic conditions (Gurumukhi VC *et al*., 2021; Fukuda IM *et al*., 2018; Perumal DD *et al*., 2022).

Chemometrics-assisted RP-HPLC method development

In this study, a factorial design incorporating both independent and dependent variables was utilized for a comprehensive HPLC procedure. The optimization process was carried out using the Central Composite Design (CCD) in the DoE software version 13.0. Notably, CCD was chosen due to its simplicity compared to

the Box-Behnken Design (BBD) and its superior performance in terms of the responses of independent variables. CCD includes a higher number of experimental points at the center of the design space compared to BBD. The study mainly concentrated on two distinct independent variables: the organic phase percentage (A) and the flow rate (B). The dependent variables studied were the retention time (Y1) and tailing factor (Y2). The obtained results from 11 test runs are detailed in Table 1 and were analyzed using polynomial equations and ANOVA analysis. The experimental ranges for the variables were set as follows: the organic phase ratio ranged from 88% to 94%, the flow rate varied from 0.6 to 1.0 mL/min, and the column temperature spanned from 25 to 35°C. Variance analysis using the response surface methodology (ANOVA) was conducted to investigate the relationship between the variables and to validate the model's applicability, as shown in Table 2.

Validation of the method

According to the criteria of the ICH guidelines, the method validation was carried-out (Joyce P *et al.*, 2019; Marson BM *et al.*, 2020; El Himri M *et al.*, 2022).

Linearity

To assess the linearity of the method, DRV was prepared and utilized within a concentration range of 10-50 µg/mL. Each solution was prepared in triplicate. The calibration curve was constructed by plotting the peak area against the concentration. Results were then obtained after conducting the linearity tests for three consecutive days using the same concentration range.

Accuracy

The percentage mean recovery of the samples is considered as the outcome of the recovery experiment. To determine the recovery of DRV, triplicates of three different concentrations-50%, 100%, and 150%-were prepared. The % recoveries were then determined after analyzing the samples.

Sensitivity and precision

The Limits of Detection (LoD) and Quantification (LOQ) were determined using the calibration curve. Precision was assessed in two categories: intra-day (intermediate) and inter-day (repeatability).

Intra-day-Three replicates of standard solutions for each drug were prepared at concentrations of 10 µg/mL, 30 µg/mL, and 50 µg/mL. Analysis was carried out at three distinct time intervals within the same day.

Inter-day (Intermediate)-Three sets of standard drug solutions were prepared at levels of 10 µg/mL, 30 µg/mL, and 50 µg/mL, and analysis was performed over three consecutive days.

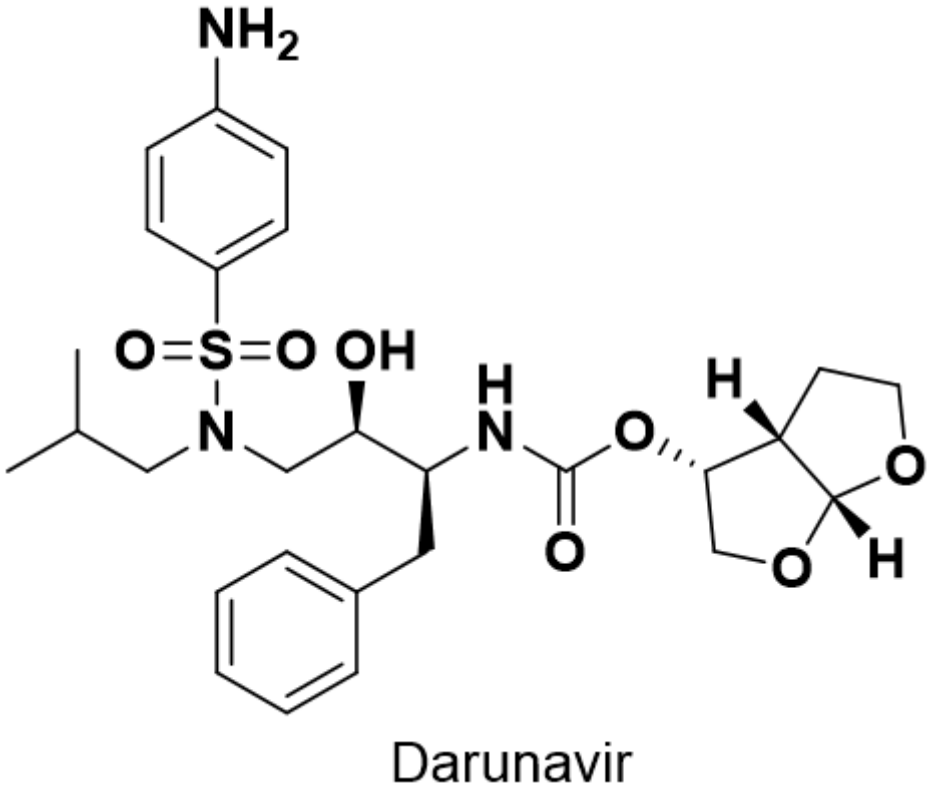

Figure 1: Structure of Darunavir.

Table 1: Experimental trials, variables, and measured responses were employed using a Central Composite Factorial Design for optimization study.

Run	Factor A	Factor B	Y1RT	Y2TF
1	50	0.8	7.35	0.64
2	50	0.8	7.45	0.78
3	50	0.8	7.59	0.86
4	60	1.0	8.69	1.1
5	60	1.0	8.73	1.25
6	60	1.0	8.85	1.36
7	60	1.0	8.98	1.59
8	70	1.2	9.52	2.63
9	70	1.2	9.68	2.87
10	70	1.2	9.84	3.1
11	60	1.0	8.98	1.59

Factor A: Organic phase, Factor B: Flow rate.

Table 2: Selected response surface method 2^2 Variables and responses.

Independent variables	Levels		
	Low (-1)	Medium (0)	High (+1)
A= organic Phase % (v/v)	50	60	70
B= flow rate (mL/min)	0.8	1	1.2
	Constraints	Importance++++++	
	In range		
	In range		
Dependent variable$Y_{1=}$RT of Darunavir	Maximum	+++	
Y_2= tailing factor of Darunavir	Minimum	+++	

System suitability

To assess the appropriateness of the chromatographic system, the peak area of DRV was analyzed by running the samples six times.

Forced degradation studies

Forced drug degradation experiments were performed to subject drug samples to stress conditions such as oxidation, photo degradation, heat degradation, acid and base hydrolysis, and the introduction of degraded products. These experiments assist in assessing the inherent stability of the product, as well as the rates of degradation for the active ingredients and their potential breakdown products (Bhardwaj SK *et al.*, 2015; Ghante M *et al.*, 2016).

Preparing of sample stock solution

An accurately weighed standard compound sample of 10 mg DRV was used for forced degradation testing. The sample was dissolved by transferring it to a 10 mL volumetric flask and adding 10 mL of diluent, followed by sonication for 10 min (Modini AK *et al.*, 2023).

Acid degradation (1 N HCl)

1 mL of the sample stock solution was mixed with 1 mL of 1 N HCl in a 10 mL volumetric flask, sealed with paraffin, and heated in a water bath at 80°C for 2 hr. Following removal from the water bath, the flask was allowed to reach ambient temperature. Neutralization was achieved by adding 1 N NaOH to the solution. The combination was then diluted to the desired volume using diluents and mixed thoroughly. Chromatograms were acquired by injecting the solution into the system to analyze the sample and its stability.

Alkali degradation (1 N NaOH)

1 mL of the sample stock solution was transferred to a 10 mL volumetric flask, sealed with paraffin, and then heated for 2 hr at 80 °C in a water bath. Following removal from the water bath, the flask was allowed to reach ambient temperature. Neutralization was achieved by adding 1 N HCl to the solution. The solution was then diluted to volume using diluents and stirred. Chromatograms were obtained by injecting the solution into the system to evaluate the sample stability.

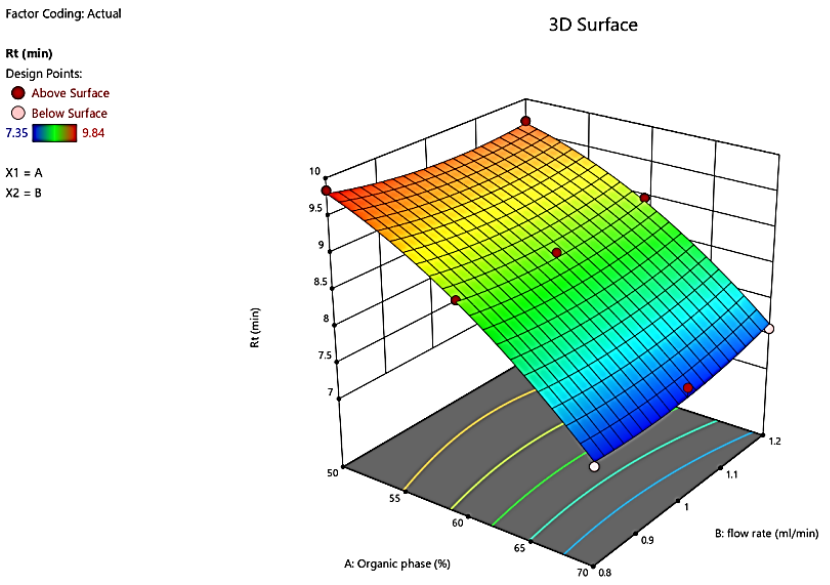

Figure 2: Response surface plot of retention time.

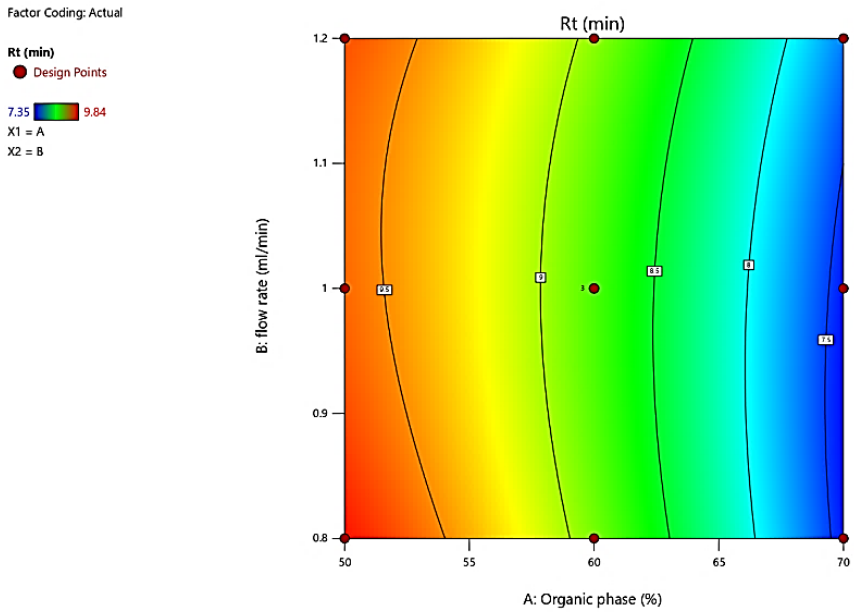

Figure 3: Contour plot of retention time.

Table 3: Parameters of the Analytical Target Profile for the method development.

Parameters	Target	Justification
Sample	API	Developing an analytical method for the estimation of Darunavir in API
Instrument	HPLC	Darunavir is nonvolatile and exhibit UV absorption. Consequently, the HPLC method with a UV detector was selected.
Type of method	Reveres phase- HPLC	The retention of molecules is typically improved by nonpolar stationary phase.
Standard and sample preparation	Methanol	Based on the pKa and solubility of Darunavir, Formic acid and methanol were selected as the diluent.

Table 4: **CMP and CAA and Their Relationship in the Method Development.**

Sl. No.	Critical Method Parameters (CMP)	Critical Analytical Attributes (CAA)
1	Column flow rate	RT
2	HPLC column	Peak area
3	Column oven temperature	Tailing factor and RT
4	Buffer of mobile phase	Plate counts

Table 5: **Results of linearity.**

Sl. No.	Validation parameter	Darunavir
1.	Linearity range (µg/mL)	10-50
	Correlation Coefficient	0.998
2	LOD	5.712
	LOQ	17.311

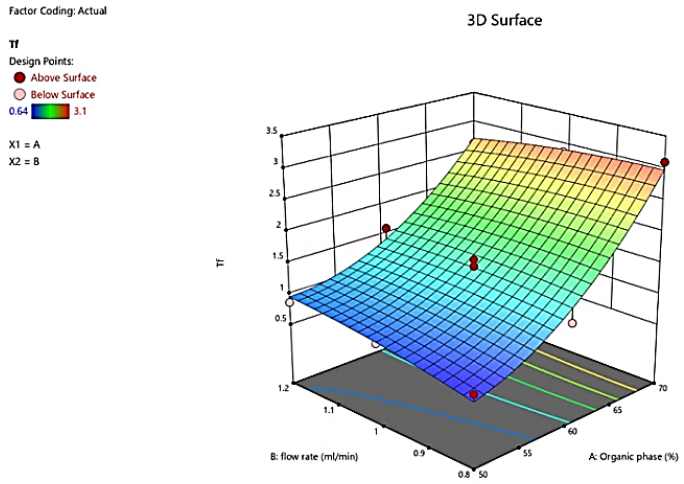

Figure 4: Response surface plot of tailing factor.

Oxidative degradation (30% H_2O_2)

1 mL of the sample stock solution, 1 mL of 30% H_2O_2, and a paraffin seal were combined in a 10 mL volumetric flask. The flask was then heated at 80°C for 2 hr using a water bath. Once removed from the water bath, the flask was allowed to reach ambient temperature before being diluted and thoroughly mixed. Chromatograms were then recorded by injecting the solution into the apparatus to evaluate the sample's stability.

Thermal degradation

2 mL of the sample stock solution is poured into a volumetric flask, followed by the addition of 2 mL of the mobile phase. The flask is sealed with paraffin and the mixture is heated to 80°C for 2 hr in a water bath. The flask is then allowed to cool to room temperature before the solution is diluted to a total volume of 10 mL and thoroughly mixed. Chromatograms were then obtained by injecting the solution into the device to assess the stability of the sample.

Statistical analysis

Each measurement was conducted in triplicate. The data obtained from this study were analyzed using the relative standard deviation method. The results were presented as the mean value accompanied by the Standard Deviation (SD).

RESULTS

Preliminary method development studies

The objective of this research was to assess the starting feasibility and pertinence under various chromatographic parameters. The mobile phase chosen was a mixture of methanol as the organic phase and 0.1% formic acid dissolved in water as the aqueous phase, in a volumetric ratio of 60:40% v/v. Initial experiments were conducted using the specified solvent system, with a sample injection volume of 10 µL, a column oven temperature set at 30°C, and a flow rate of 1 mL/min. The results indicated the appearance of distinct peaks for medications. Extensive investigation of several key method variables was undertaken to address these

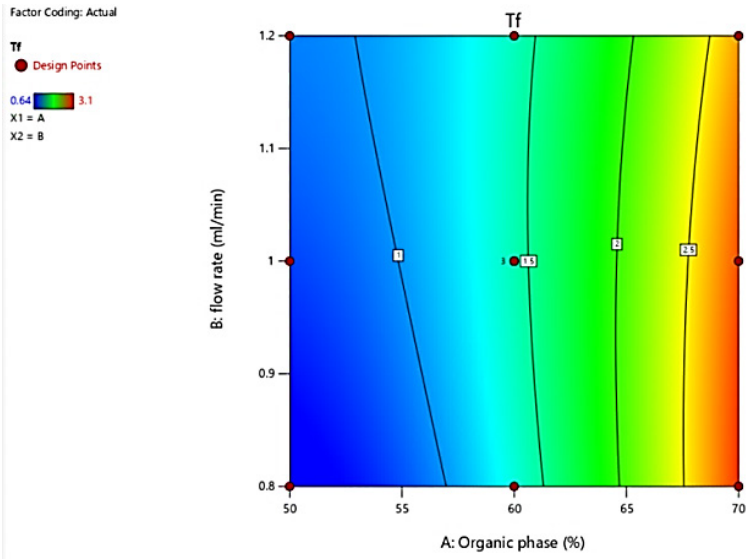

Figure 5: Contour plot tailing factor.

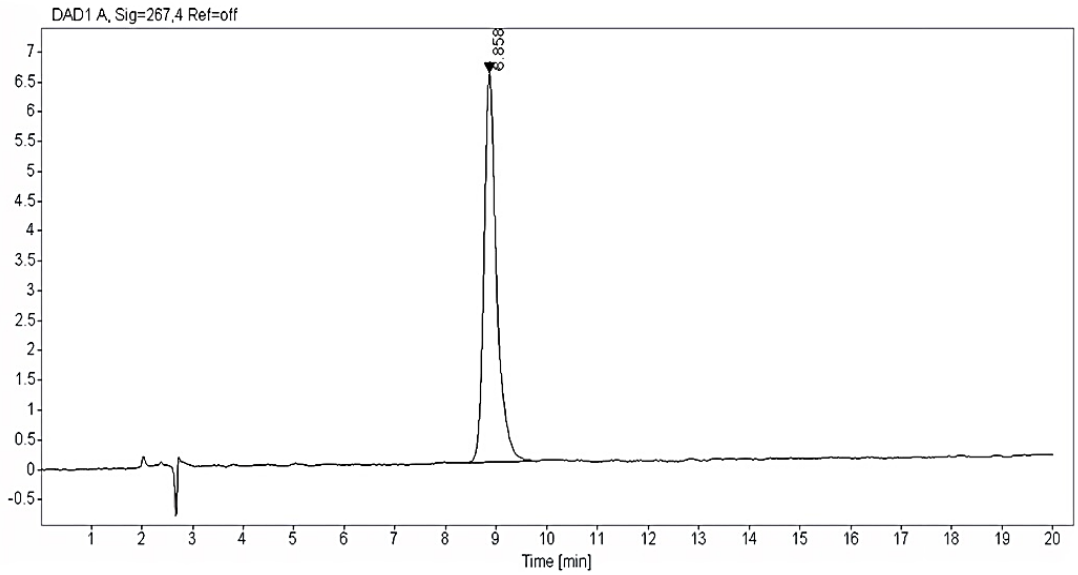

Figure 6: HPLC chromatogram of Darunavir.

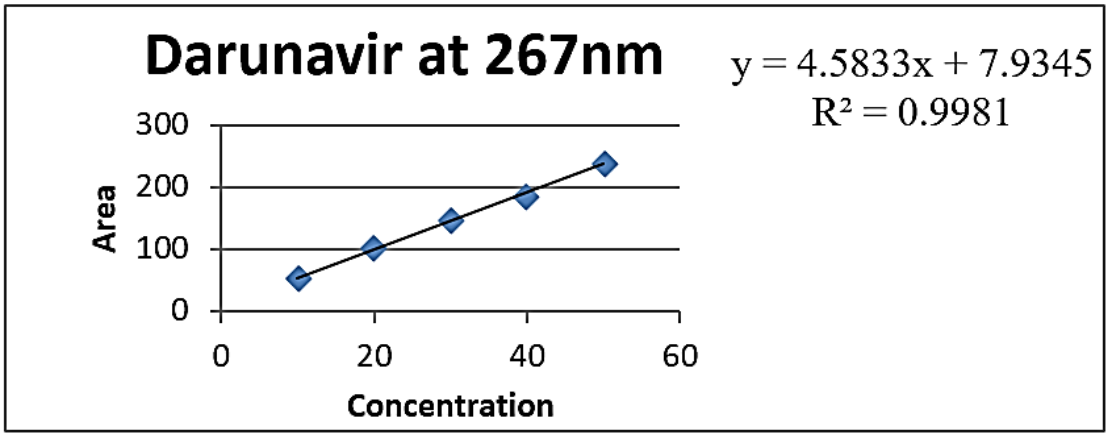

Figure 7: Calibration curve of Darunavir.

Table 6: System suitability parameter.

Parameter	Mean	SD	%RSD
RT (min)	8.944	0.123	1.385
Peak area	103.05	0.0372	0.4163
Plate count	7111.833	130.134	1.829
Telling factor	1.369	0.00862	0.629

Table 7: Robustness parameter.

Absorbance	58:42%	60:40%	62:38%	0.9 (mL/min)	1 (mL/min)	1.1 (mL/min)
			MP Change FR Change			
mean	137.366	147.5	156.366	147.7	157.2	135.233
SD	0.1527	0.1527	1.877	0.435	0.1527	1.0115
%RSD	0.1112	0.0135	1.2004	0.295	0.0971	0.7480

issues, as they are significantly impacted the effectiveness of the method.

Evaluation of the analytical procedure utilizing the QbD methodology

The improvement of an analytical method is greatly influenced by the Analytical Target Profile (ATP), especially when crucial method parameters, as outlined in Table 3, are being evaluated. The ATP provides a clear understanding of the objective and intended application of an analytical method, along with defining the desirable performance characteristics of the method. It assists in specifying the specific conditions that need to be met for the method to be successful, taking into account crucial method factors. The primary technical factors influencing the HPLC process include column temperature, concentration of the organic phase, and flow rate. These factors, for instance, can impact the tailing factor and plate counts, which are considered as CAAs. In this experimental setup, theoretical plates, Retention Time (RT), and tailing factor were identified as important analytical attributes (CAAs), as shown in Table 4.

Development of the RP-HPLC method with the assistance of chemometrics

The trial runs were optimized with CCD to obtain 3D graphics that illustrate the effects of the factors. Clear variations in values across all responses were observed. By using a polynomial equation, the relationship between the variables and responses has been anticipated. In this case, a positive value indicates an influence that facilitates optimization, whereas a negative value indicates an interaction between the components that is counterproductive. The equations for each response are shown below, and a tight correlation between each value is observed.

RT: +8.78-1.09*A+0.0250*B+0.1000*AB-0.2811*A2+0.1339*B2

Tf: +1.43+1.05*A+0.0400*B-0.1725*AB+0.4211*A2-0.0589B2

In relation to the 3D plots, two unique factors were analyzed. Of these two elements, the impact of two was focused on, while the third was left at its usual level. The substantial influence of the organic phase and flow rate on the theoretical plates is greatly observed.

Figures 2 and 3 depict how the RT of the analyte was affected by the independent variable. The RT of the analyte was clearly influenced by changes in the flow rate and organic phase ratio. Increases in the organic phase ratio and flow rate resulted in increases in the analyst's RT, while decreases in these parameters led to decreases in RT. Figures 4 and 5 illustrate the effect of the independent variable on the tailing factor. The findings clearly demonstrated that the organic phase ratio and flow rate positively impacted the analyst's tailing factors. The tailing factor increased with an increase in the volume of the organic phase and flow rate. Conversely, reducing the volume of the organic phase and flow rate caused the tailing factor to decrease.Using Stat-Ease, Inc.'s Design-Expert software (version 13.0), it was determined that every response yielded acceptable results. At these specific concentrations, the program facilitated the determination of the ideal values for the independent parameters: organic phase (60%) and flow rate (1 mL/min). The remarkably low prediction error (5%) between the observed values and the expected values demonstrated the model's exceptional predictive capability for the response variables. As shown in Figure 6, the approach demonstrated remarkable chromatographic separation of the drug under optimal conditions.

Method validation

Linearity

Table 5 presents the conformational results of the proposed RP-HPLC method in a clear format. To verify the appropriateness of the developed method for the system, the percent Relative Standard Deviation (RSD) of various parameters, including peak area, RT, and tailing factor, was calculated. The percent RSD for

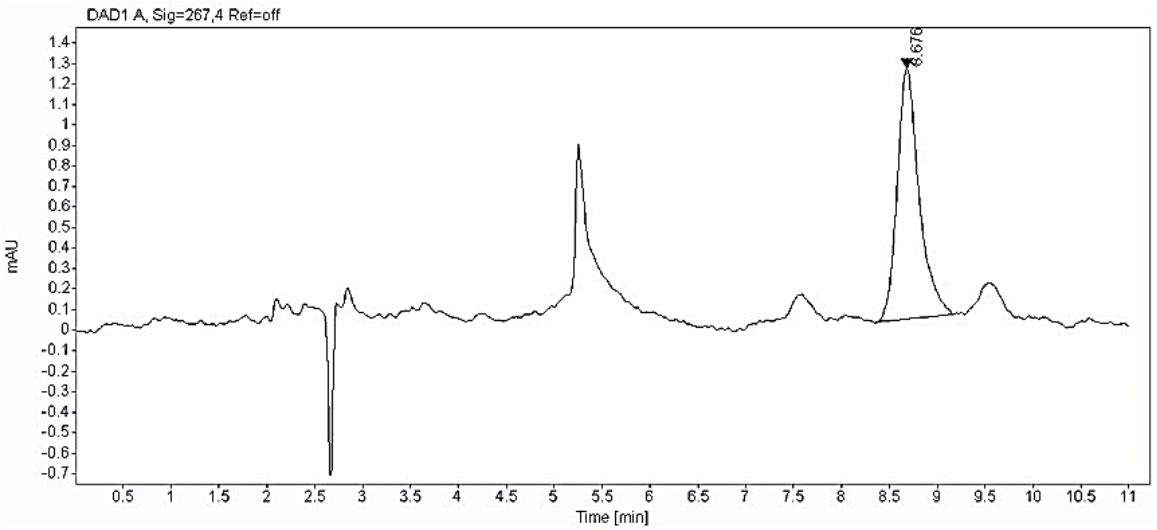

Figure 8: Acid degradation.

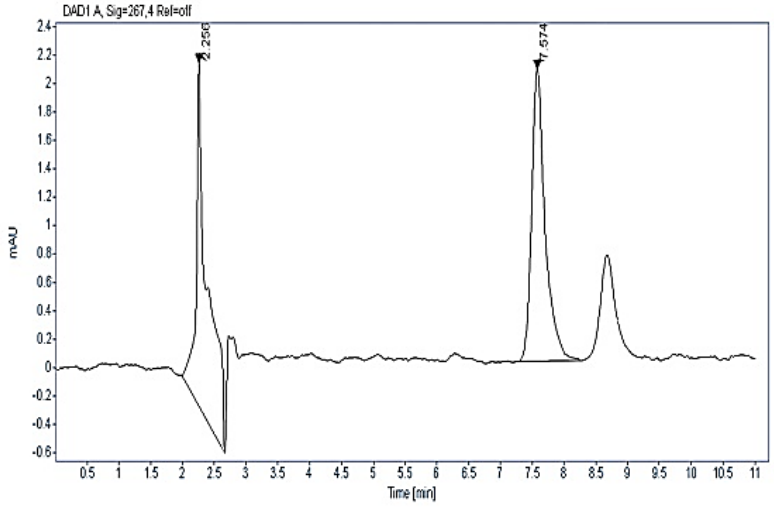

Figure 9: Base degradation.

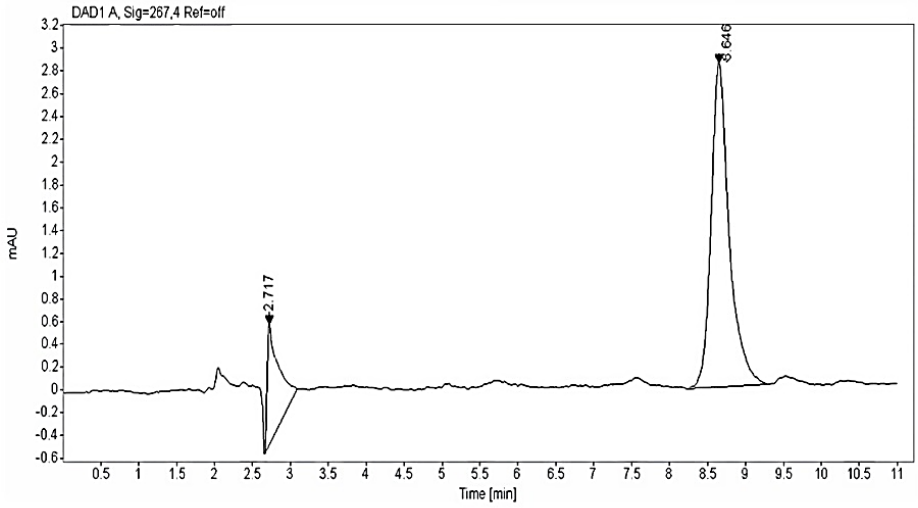

Figure 10: Oxidative degradation.

the tailing factor, peak area, and retention time were found to be less than two, indicating the adequacy of the proposed approach. A calibration curve was constructed by plotting the average peak areas of the analyte (DRV) against their corresponding concentrations ranging from 10 to 50 µg/mL, as shown in Figure 7, to establish the linear correlation. The LOD and LOQ were determined using the data obtained from the linear regression equation of the calibration curve.

System suitability

To assess the appropriateness of the chromatographic system, the peak area was analysed by conducting six sample runs. % SD should found be less than 2%. As shown in Table 6.

Robustness

In order to evaluate the robustness of the analytical process, it was necessary to slightly modify the parameters of HPLC. These modifications included shifts in variables such as flow rate and mobile phase ratio. The results showed that the %RSD of the analyte for both Tf and RT were well within the permitted range of ≤2%, as shown in Table 7.

Precision-The great precision of the devised approach was revealed by the percentage RSD values of the intra-day and inter-day precisions, as shown in Tables 8 and 9.

Intra Day-The analysis was performed at three distinct time points on the same day.

Inter Day-The analysis was carried out for three consecutive days.

Accuracy

The percentage of recovery, which expresses the degree of accuracy, is regarded as an indicator of how closely the measured values match the genuine values. The trial results for the 50%, 100%, and 150% concentrations demonstrated a high degree of accuracy for the recently created technique, as shown in Table 10.

Table 8: Intraday results.

Absorbance	10 (µg/mL)	30 (µg/mL)	50 (µg/mL)
Morning			
mean	52.151	52.822	54.136
SD	0.00556	0.5765	0.00953
%RSD	0.0106	1.0915	0.01762
Afternoon			
mean	147.6	156.566	166.33
SD	0.100	2.1031	2.055
%RSD	0.0677	1.3433	1.2355
Evening			
mean	239.075	249.051	259.035
SD	0.011	0.01604	0.00916
%RSD	0.00465	0.00644	0.00353

Table 9: Inter day Results.

Absorbance	10 (µg/mL)	30 (µg/mL)	50 (µg/mL)
Day 1			
mean	52.13767	52.136	53.136
SD	0.0135	0.00953	0.009539
%RSD	0.0260	0.0182	0.0179
Day 2			
mean	147.366	146.63	156.33
SD	0.1527	1.9035	2.0550
%RSD	0.10366	0.1073	1.3145
Day 3			
mean	239.051	239.185	249.051
SD	0.0325	0.01073	0.0285
%RSD	0.0136	0.0448	0.0114

Forced degradation

Following the guidance of ICH Q1A (R2), the forced degradation study was conducted under different stress conditions, encompassing hydrolytic (acidic, basic), oxidative, thermal, and photolytic conditions, as shown in Figures 8-12. The obtained results show declines in basic, oxidation, and photolytic conditions, but after 2 hr, a high degree of stability was demonstrated in thermal and acidic environments. The discovery in this study, that there was no change in the RT after applying the stress conditions, suggests that the strategy was selective, A thorough evaluation of the analyst's peak purity was conducted during the forced degradation experiment. The results demonstrated that the purity degree was consistently less than the purity standards for all stressed samples. This indicates that the process is selective and that the lack of significant co-eluting breakdown products assures the validity of the test.

Pharmaceutical implementation of the Developed optimized method

The amount of Darunavir in solid supersaturated SEDDS was successfully measured using the optimized method, demonstrating excellent peak characteristics and superior performance in terms of sensitivity, accuracy, and precision, ensuring trustworthy results. The primary peak of the chromatogram, which identified the API, appeared at a retention time of 8.85. This retention time shows that the molecule has been correctly encapsulated within the solid SEDDS matrix because it is consistent with the standard solution of the pure medicine. Plate count of less than 2000 in theoretically suggested effective separation. The retention time did not change significantly between the chromatograms of the SS-SEDDS and the pure drugs, suggesting that the SS-SEDDS had no effect on the chromatographic behavior of the API. Following extensive testing and validation, the suitability of this method for routine analysis has been confirmed, as depicted in Figure 13.

DISCUSSION

High-Performance Liquid Chromatography (HPLC) analysis of solid supersaturated Self-Emulsifying Drug Delivery Systems (SEDDS) containing Darunavir is essential to evaluate the drug's stability and concentration in the formulation. It is especially novel to use a 60:40 methanol: water mobile phase, as different solvent systems were frequently used in earlier research. Darunavir, a hydrophobic antiretroviral medication, is better separated and quantified when methanol, the organic phase, has strong elution strength. For accurate detection and analysis, this mobile phase composition with a 1 mL/min flow rate provides the best resolution and peak form. The reduced viscosity of methanol in comparison to solvents such as acetonitrile prolongs column life and lowers back pressure. Peak purity is within permitted limits, suggesting that the process is dependable even under stress. Additionally, the use of methanol promotes

Table 10: Accuracy parameter.

Conc added (µg/mL)	Level	Mean	Concentration found in percentage	% recovery
25	50%	122.73	25.046	100.185
50	100%	237.50	50.087	100.175
75	150%	355.842	75.906	101.210

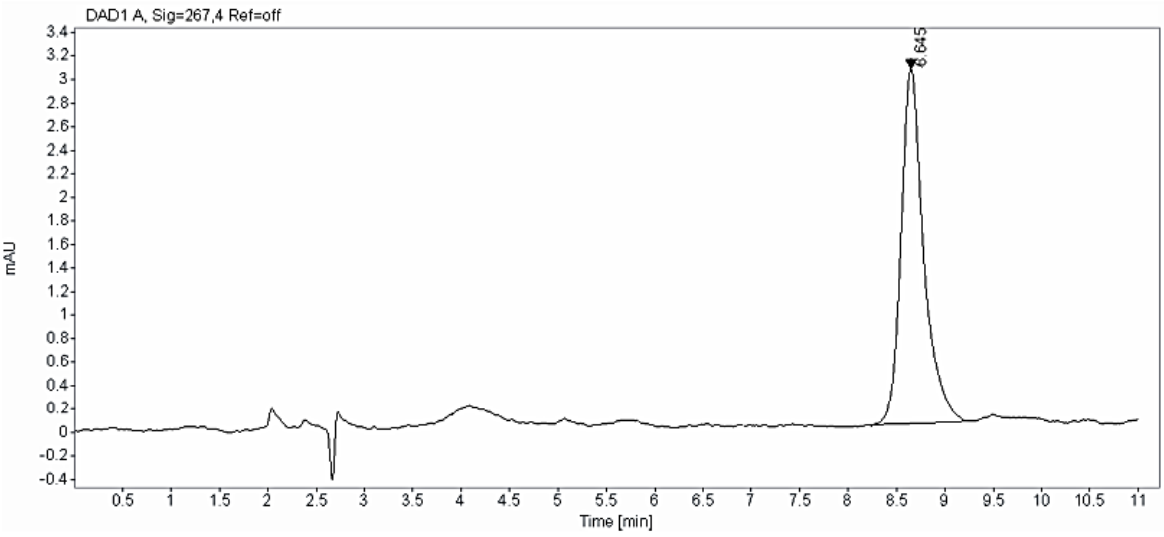

Figure 11: Thermal degradation.

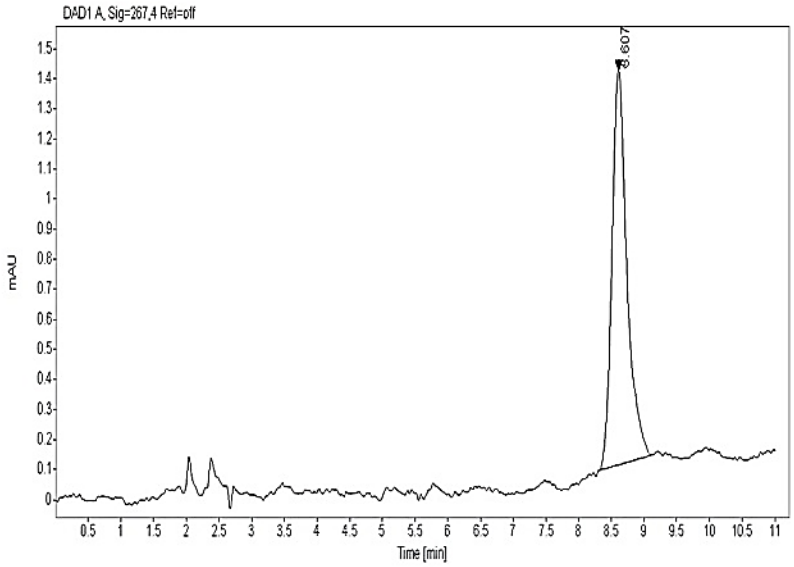

Figure 12: Photolytic degradation.

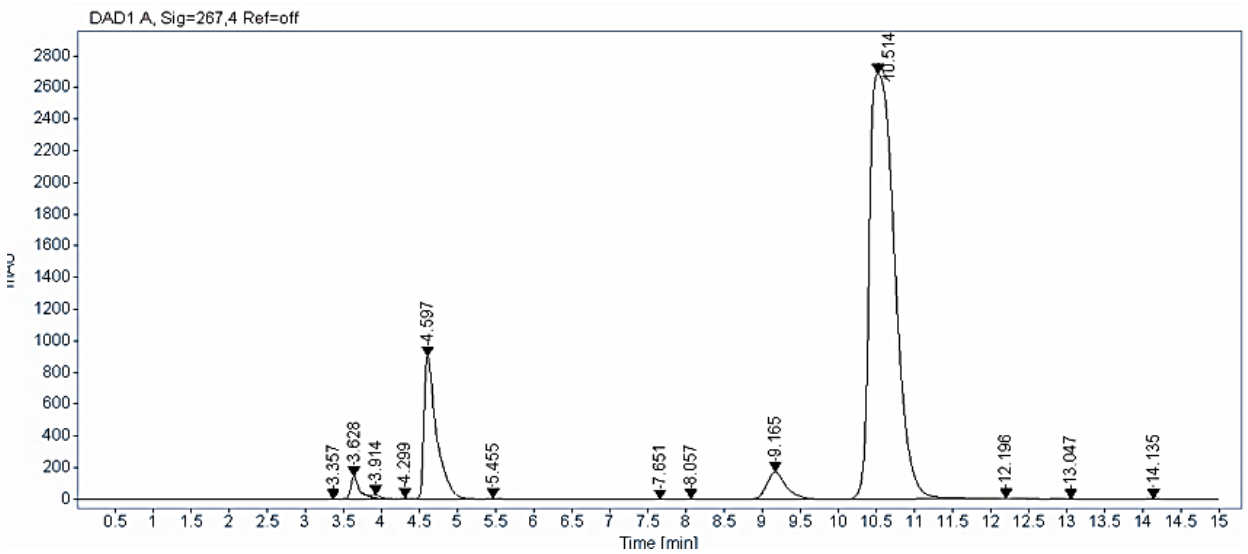

Figure 13: HPLC chromatogram of solid SEDDS.

the advancement of more efficient oral delivery methods by encouraging the development of repeatable and trustworthy analytical methodologies for SEDDS formulations.

CONCLUSION

A new RP-HPLC method has been successfully developed and validated in the current study for quantifying Darunavir in both its pure drug form and within SS-SEDDS formulations. The efficient separation and quantification of the compound were achieved through the application of Analytical AQbD principles, coupled with the CCD. This systematic approach significantly enhanced both the efficiency and the reliability of the method. The experimental process was streamlined by utilizing Design Expert software from Stat-Ease, Inc. (version 13), which reduced the number of required trial runs. Subsequent validation procedures followed the guidelines outlined in ICH Q2 R (1), considering all parameters to ensure the method's affordability, accuracy, robustness, and sensitivity within acceptable limits. This method offers a valuable choice for the regular assessment of DRV in both bulk and SS-SEDDS formulations. In the study for the Force degradation, the acceptable limit was reached by the peak purity index.

ACKNOWLEDGEMENT

The facilities required to conduct the research were provided by KLE College of Pharmacy in Belagavi, Karnataka, India, for which the authors are grateful.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RP-HPLC: Reverse phase-high performance liquid chromatography; **HIV:** Human immunodeficiency viruses; **DRV:** Darunavir; **Nm:** Nanometer; **SEDDS:** Self emulsifying drug delivery system; **µL:** Microliter; **QbD:** Quality by design; **mL/min:** Mililitre per minute; **%:** Percentage; **LC/MS:** Liquid chromatography-mass spectrometry; **AIDS:** Acquired Immunodeficiency Syndrome; **PI:** Protease inhibitor; **HPLC-MS:** High performance liquid chromatography-Mass spectrometry; **UV:** Ultra violet; **v/v:** Volume by volume; **DOE:** Design of Experiments; **HCl:** Hydrochloric acid; **NaoH:** Sodium hydroxide; **Milli-Q:** Millipore quality; **pH:** Potential of Hydrogen; **Mm:** Millimetre; **US:** United State; **MeOH:** Methanol; **mL:** Mililiter; **QTPP:** Quality target product profile; **ICH:** International Council for Harmonisation; **PDI:** Polydispersity index; **EE:** Entrapment Efficiency; **CQA:** Critical quality attributes; **CMPs:** Critical method parameters; **CCD:** Central Composite Design; **BBD:** Box-Behnken Design; **ANOVA:** Analysis of Variance; **LOD:** Limits of detection; **LOQ:** limits of quantification; **ºC:** Degree Celsius; **Mg:** Miligram; **SD:** Standard Deviation; **ATP:** Analytical Target Profile; **RT:** Retention time; **RSD:** Relative standard deviation; **AQbD:** Analytical Quality by Design.

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Cite this article: Sharannavar B, Deo A, Suryawanshi S, Bhandurge P, Darbha NS. Stability-Indicating RP-HPLC Method Development and Validation of Darunavir Using a Quality by Design (QbD) Approach, with Estimation in Prepared Solid Supersaturated Self-Emulsifying Drug Delivery Systems (SEDDS). Int. J. Pharm. Investigation. 2026;16(1):357-70.