Available online on 15.07.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Development and Validation of Stability Indicating HPLC Method for Estimation of Darunavir

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ABSTRACT

To develop a new simple, precise and accurate HPLC method for estimation of Darunavir in tablet formulation. An isocratic, HPLC method was developed using C-18, 50 x 3 mm, 3.5 µm (X-Terra MS, Waters) column 0.1 % Acetic acid in water: ACN (63:37 v/v) as mobile phase at flow rate of 1.0 ml/min at detection wavelength of 267 nm. The retention time (RT) of drug was 3.123± 0.034 min . The method was validated with respect to linearity, precision, assay, accuracy and robustness. The data of linear regression analysis indicated a good linear relationship over the range of 5-30 µg/ml concentrations with a correlation coefficient(R^2) of 0.9995.The developed method was found to be simple, sensitive, selective, accurate, and precise for analysis of Darunavir and can be adopted for routine analysis of drug in bulk and pharmaceutical dosage form.

Keywords: High performance liquid chromatography (HPLC), Darunavir, method development, Validation.

Article Info: Received 04 May 2019; Review Completed 12 June 2019; Accepted 21 June 2019; Available online 15 July 2019

Cite this article as:

Gadekar AS, Chabukswar AR, Development and Validation of Stability Indicating HPLC Method for Estimation of Darunavir Journal of Drug Delivery and Therapeutics. 2019; 9(4):65-71 **http://dx.doi.org/10.22270/jddt.v9i4.3148**

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INTRODUCTION:

Darunavir is chemically [(3aS,4R,6aR)-2,3,3a,4,5,6a-hexahydrofuro[2,3-b]furan-4-yl]N-[(2S,3R)-4-[(4-aminophenyl)sulfonyl-(2-methylpropyl)amino]-3-hydroxy-1-phenylbutan-2-yl]carbamate it is HIV protease inhibitor that is used in the treatment of AIDS and HIV infections. Darunavir is an antiretroviral protease inhibitor that is used in the therapy and prevention of human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS). Darunavir can cause transient and usually asymptomatic elevations in serum aminotransferase levels and has been linked to rare instances of clinically apparent, acute liver injury. In HBV or HCV coinfected patients, highly active antiretroviral therapy with darunavir may result of an exacerbation of the underlying chronic hepatitis B or C. Literature survey reveals that few analytical methods have been reported for the estimation of Darunavir including UV-Vis spectroscopy[1,2], high performance liquid chromatography (HPLC) [3,4], high performance thin layer chromatography(HPTLC) [5,6], Infrared Spectroscopy(IR) [7] , Capillary Electrophoresis [8] , LC-MS/MS[9,10] .

MATERIALS AND METHODS:

Reagents and chemicals

20 tablets each containing 300 mg of Darunavir (Daruvir, Cipla Ltd.) was procured from local market. Acetonitrile (HPLC grade), Acetic acid in water, Hydrochloric acid (HCl), acetic acid (CH_3COOH), hydrogen peroxide (H_2O_2), and sodium hydroxide (NaOH).

Chromatographic condition

HPLC system used was Agilent 1260 series system equipped with model 1260 Qyat Pump VL, Rheodyne sample injection port (20 µl), 1260 VWD detector and OpenLAB EZ chrom software. A chromatographic column C-18, 50 x 3 mm, 3.5 µm (X-Terra MS, Waters) was used, for separation at a flow rate of 1.0 ml/min using 0.1 % Acetic acid in water: ACN (63:37 v/v) as mobile phase and detection at 267 nm. The representative chromatogram is shown in Fig. 1.

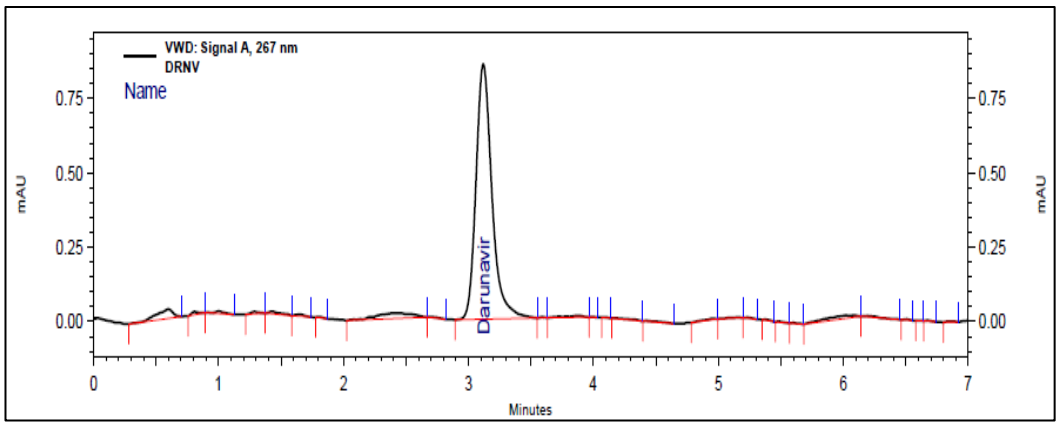

Fig 1 : Chromatogram of Darunavir (10 µg/ml)

Preparation of Standard stock solution:

Standard stock solution of drug was prepared by dissolving 10 mg of drug in 10 ml of Acetonitrile to get concentration of 1000 µg/ml (A). From this working standard solution was prepared containing 100 µg/ml of Darunavir in acetonitrile (B). From this further dilution was made in acetonitrile to get final solution of Darunavir.

Selection of detection wavelength:

From the standard stock solution further dilutions were done using ACN and scanned over the range of 200 - 800 nm and the spectrum was obtained. It was observed that the drug showed considerable absorbance at 267 nm. Representative UV Spectrum of Darunavir is shown in Fig 2.

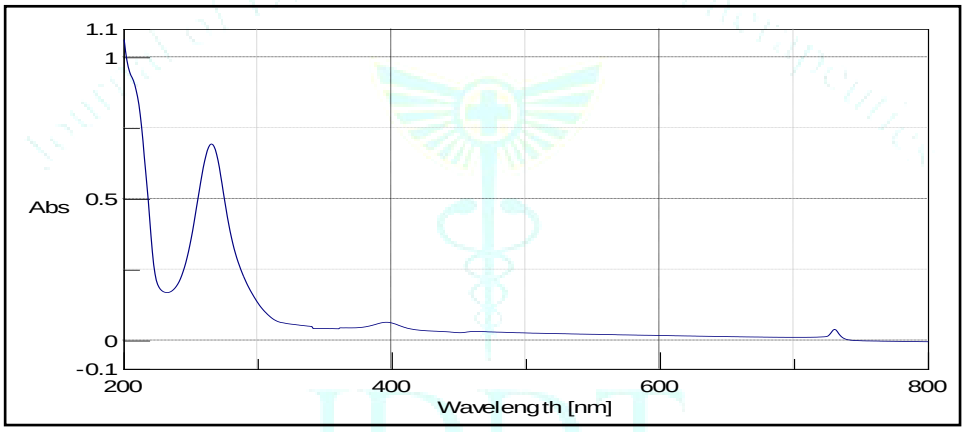

Fig 2 : UV-VIS Spectra of DARUNAVIR (10 µg/ml)

Selection of mobile phase:

Chromatographic separation studies were carried out on the working standard solution of Darunavir 10 µg/ml. Initially, trials were carried out using Methanol and Acetonitrile in various proportions along with buffer of varying pH, to obtain the desired system suitability parameters. After few trials, 0.1 % Acetic acid in water: ACN (63:37 v/v) was chosen as the mobile phase, which gave good resolution and acceptable peak parameters.

Preparation of sample solution

20 tablets each containing 300 mg of Darunavir (Daruvir, Cipla Ltd.) was weighed and powdered. Powder equivalent to 10 mg of drug was transferred to 10 ml volumetric flask and volume was made up with acetonitrile to get concentration (1000 µg/ml) and was sonicated for 10 min. Solution was filtered, from this solution 1 ml of solution was taken in 10 ml volumetric flask and volume was made up with acetonitrile. Further dilution was done to get concentration 10 µg/ml.

Validation of Analytical Method: [11]

Validation of Analytical Method

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 996 [Purity front 997.54 and Purity tail 996.12] indicating the no interference of any other peak of degradation product, impurity or matrix.

Linearity

From the standard stock solution (1000 µg/ml) of Darunavir, solution was prepared containing 100 µg/ml of Darunavir with acetonitrile. This solution was further used to prepare range of solution containing six different concentrations. The linearity (relationship between peak area and concentration) was determined by analyzing six solutions over the concentration range of 5-30 µg/ml of Darunavir. The results obtained are shown in Table 1. The peak area of drug was plotted against the corresponding concentrations to obtain the calibration curve as shown in Figure 3.

Table 1: Linearity study of Darunavir

Replicates	Concentrations of DARUNAVIR					
	5µg/ml	10µg/ml	15µg/ml	20µg/ml	25µg/ml	30µg/ml
	Peak Area					
1	72301.4	184904.2	235901.5	300842.5	383554.8	425349.1
2	72633.2	184087.2	235864.9	306577.3	378338.4	424821.6
3	72035.82	183800.4	235791.9	304596.3	381071	426413.8
4	72356.3	186522.4	235882.7	303566.2	385172.9	424653.7
5	72725.53	181562.4	236859.6	306788.2	381573	424657.8
6	71464.99	184981.3	235119.6	300756.3	380427.1	424053.8
Mean	72252.87	184309.7	235903.4	303854.5	381689.5	424991.6
Std. Dev.	458.03	1646.36	555.51	2657.99	2401.3	810.47
%RSD	0.63	0.89	0.23	0.87	0.62	0.19

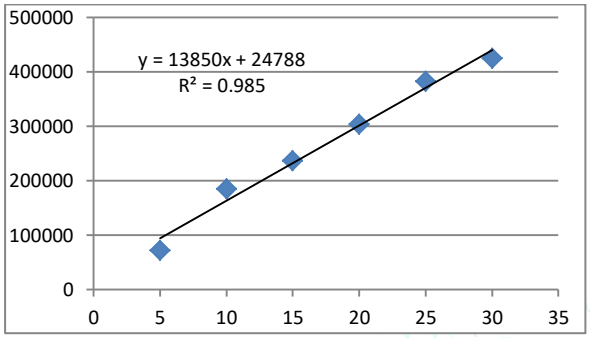

Figure 3: Calibration curve of Darunavir (5-30 µg/ml)

Range:

Darunavir = 5-30 µg/ml.

Precision:

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the Intra-day studies, 3 different concentrations were analyzed in a day and percentage RSD was calculated. For the inter day variation studies, 3 different concentrations were analyzed on 3 consecutive days and percentage RSD were calculated. The results obtained for Intraday and Inter day variationsare shown in Table 2 and Table 3 respectively.

Table2: Intra-day precision study of Darunavir

Replicates	Conc. (µg/ml)		
	5	10	15
1	72301.4	184904.2	232901.3
2	72633.2	184087.2	231867.6
3	72035.8	183800.4	235791.9
Mean	72323.4	184263.9	232520.3
SD	299.30	572.72	2034.02
%RSD	0.41	0.31	0.87

Table 3: Inter-day precision of Darunavir

Replicates	Conc. (µg/ml)		
	5	10	15
1	72301.4	185004.3	235901.5
2	72725.3	184178.3	236859.6
3	71464.9	183810.3	235119.6
Mean	72163.9	185084	235960.2
SD	641.44	247.45	871.48
%RSD	0.88	0.13	0.36

Limit of Detection (LOD) andLimit of Quantification (LOQ)

LOD and LOQ are calculated from the formula: -

$$LOD = \frac{3.3\ \sigma}{S} \qquad LOQ = \frac{10\ \sigma}{S}$$

Where,

σ = standard deviation of Y intercept

S = slope of the calibration curve

Table 4: LOD and LOQ of Darunavir

Method	Avg. slope	S.D	LOQ (µg/ml)	LOD (µg/ml)
S.D of y-intercept	13851.83	1886.22	0.44	1.36

Assay

Daruvir tablet formulation analysis was carried out as mentioned under section preparation of sample solution. Procedure was repeated for six times. Sample solution was injected and area was recorded for each drug. Concentration and % purity was determined from linear equation. The results obtained are shown in Table 5.

Table 5: Assay of marketed formulation

Sr. No.	Peak area	Amount Recovered (µg/ml)	% Recovery
1	156358.5	9.80	98.05
2	156531.6	9.81	98.18
3	157092.4	9.85	98.58
4	158555.4	9.96	99.64
5	157868.3	9.91	99.14
6	157986.3	9.92	99.23
Mean	157398.7	9.88	98.80
SD	875.38	0.063	0.630
% RSD	0.55	0.63	0.638

Stress Degradation Studies of Bulk Drug:

Stress degradation studies were carried under condition of acid as well base hydrolysis, oxidation and dry heat. Dry heat and photolytic degradation were carried out in solid state.

Preparation of standard stock solution

Accurately weighed 5 mg of Darunavir was transferred to the 5 ml pre-calibrated volumetric flask. Darunavir was dissolved in small quantity of water. Volume was made up to 5 ml with water to achieve a stock solution of 1000 µg/ml (Stock-1) which was further diluted to get 100 µg/ml (Stock-2)

Preparation of sample:

Acid hydrolysis

2.5 ml working standard solution of Darunavir (100 µg/ml) was mixed with 1 ml of 0.5 N HCl and kept aside for 4 hours at room temperature. After exposure the solution was neutralized with 0.5 N NaOH and volume was made up to 10 ml with Mobile phase and injected. After acid hydrolysis, Darunavir showed no peak of degradation product. The percent recovery of Darunavir was found to be. 17.10 %

Alkaline hydrolysis

2.5 ml of working standard solution of Darunavir (100 µg/ml) was mixed with 1 ml of 0.5 N NaOH and kept aside for 4 hours at room temperature. After exposure the solution was neutralized with 0.5 N HCl and the volume was made up to 10 ml with mobile phase and injected; After Alkaline hydrolysis, Darunavir showed no peak of degradation product. The percent recovery of Darunavir was found to be 13.28 %

Oxidation

2.5 ml working standard solution of Darunavir (100 µg/ml) was mixed with 1 ml of 10 % solution of H_2O_2 and was kept aside for 4 hours at room temperature. After exposure the volume was made upto 10 ml with mobile phase and injected. In the oxidative condition, percent recovery obtained for Darunavir was 29.29 % with no peaks of degradant.

Degradation under dry heat

Dry heat studies were performed by keeping drug sample in oven (80^0 C) for a period of 4 hours. A sample was withdrawn after 4 hour, dissolved in acetonitrile to get solution of 1000 µg/ml and further diluted with acetonitrile to get 25 µg/ml as final concentration and was injected. In the dry heat degradation condition, percent recovery obtained for Darunavir was 69.73 % with no peak of degradant.

Photo-degradation studies.

Photolytic studies were carried out by exposure of drug to UV light up to 200 watt hours/square meter Sample was weighed, dissolved in and diluted with acetonitrile to get 25 µg/ml. After the photo degradation study for UV light Darunavir was 51.54 % recovered, with no peak of degradant.

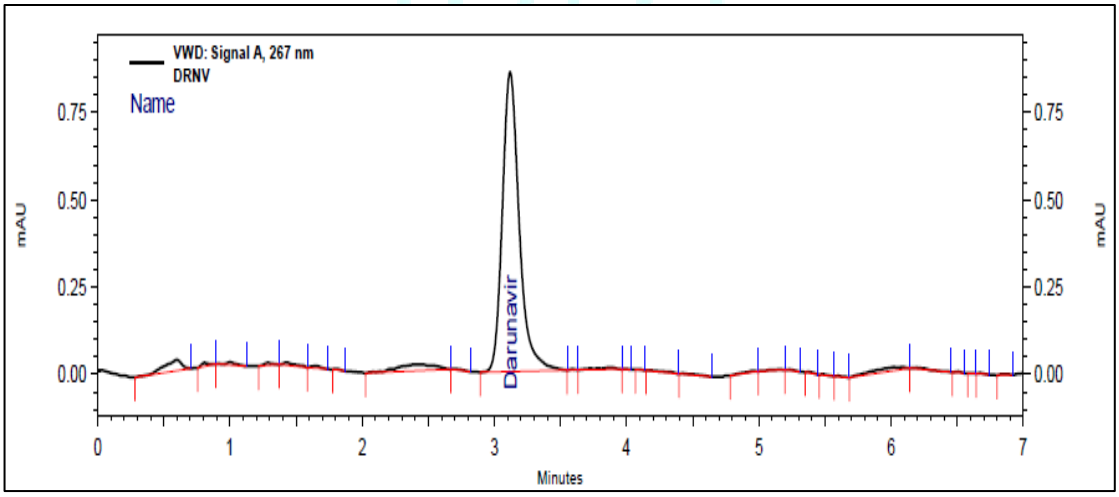

Figure 4: Chromatograph of Darunavir standard

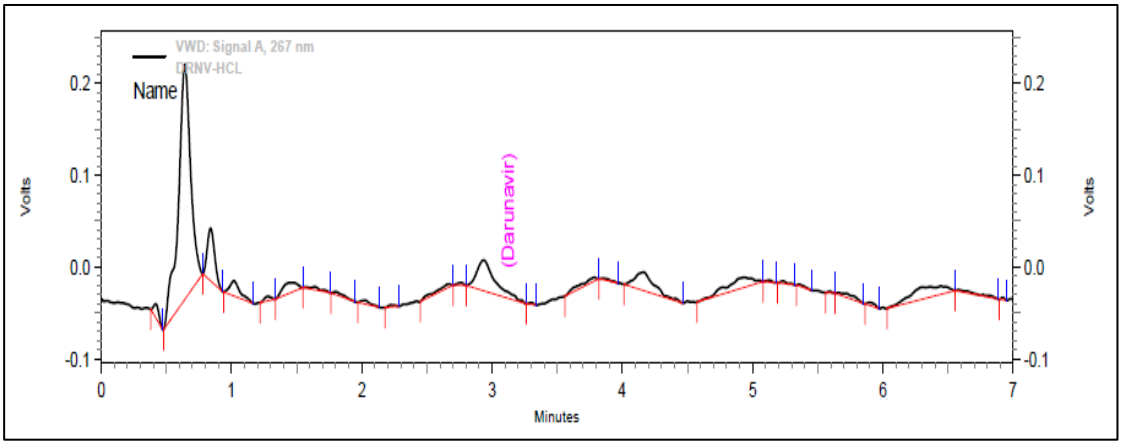

Figure 5: Chromatograph of HCl mediated degradation of Darunavir

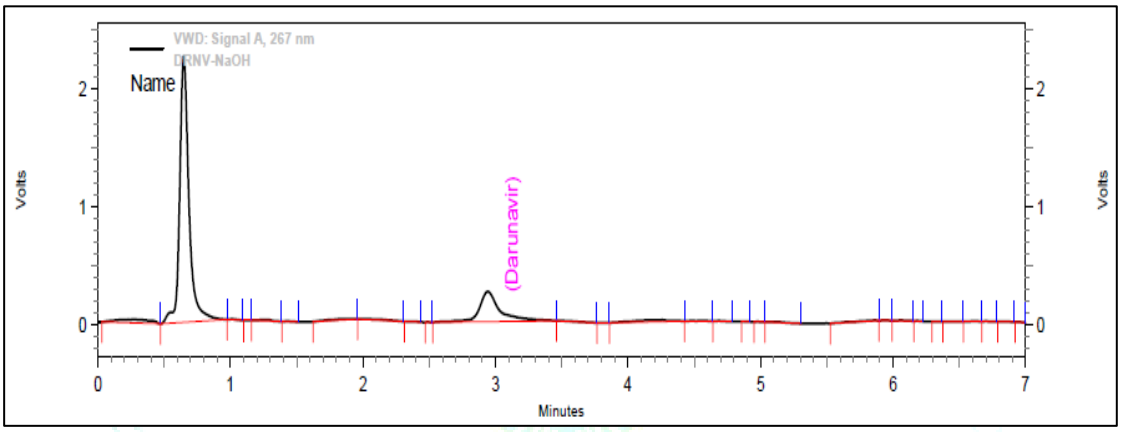

Figure 6: Chromatograph of NaOH mediated degradation of Darunavir

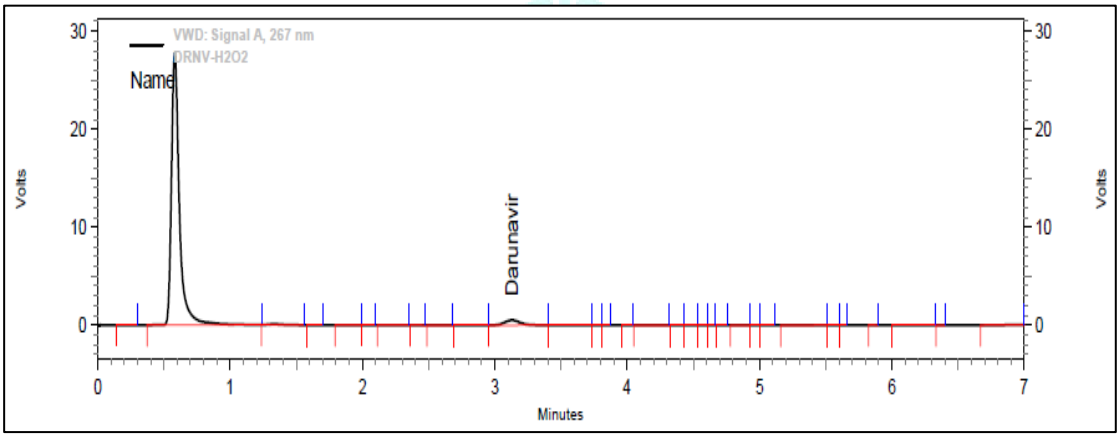

Figure 7: Chromatograph of Hydrogen peroxide mediated degradation of Darunavir

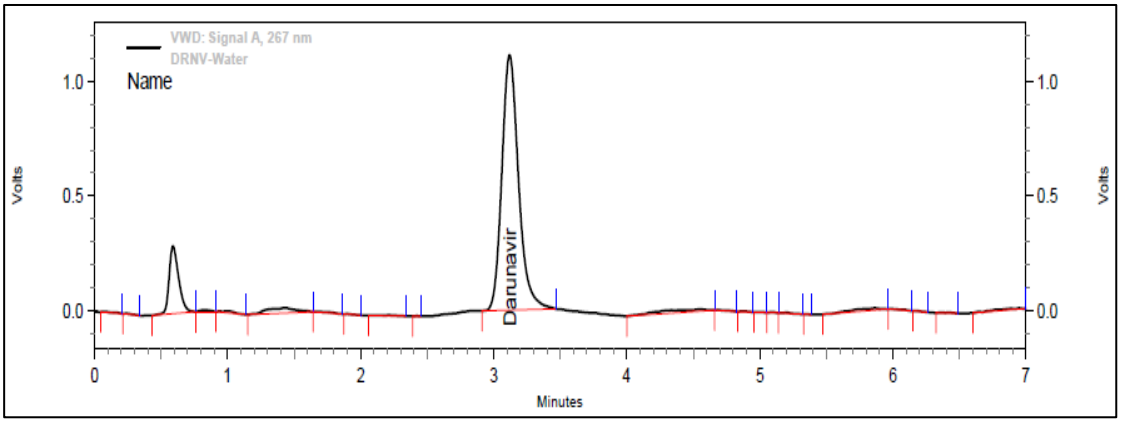

Figure 8: Chromatograph of heat mediated degradation of Darunavir

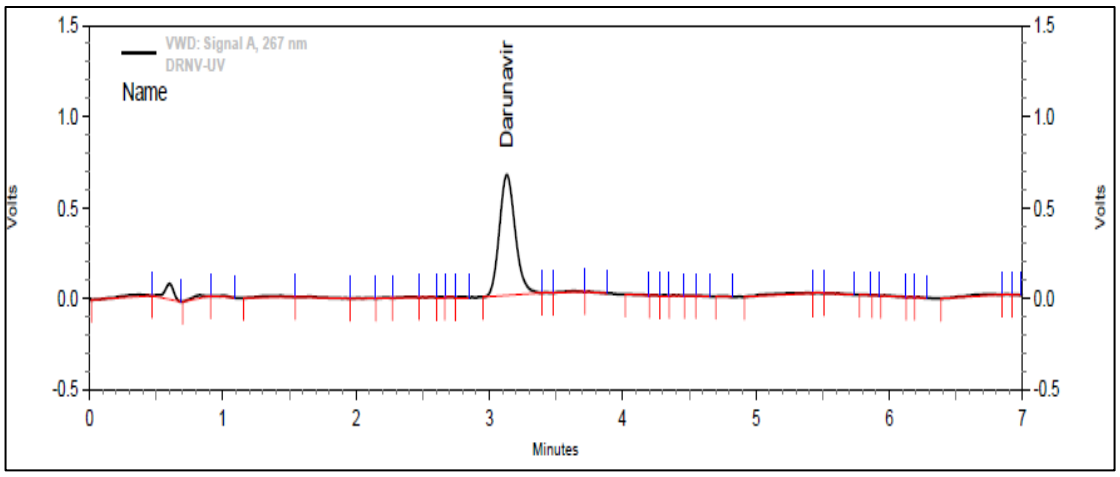

Figure 9: Chromatograph of Degradation under UV light of Darunavir

Table 6: Summary of degradation parameters:

Sr.	Stress Condition	% Assay of Darunavir
1.	Acid (0.1 N HCl)	17.10
2.	Alkali (0.1 N NaOH)	13.28
3.	Oxidation (10 % H_2O_2)	29.29
4.	Heat (80°C)	69.73
5.	UV Exposure	51.54

Accuracy

To check accuracy of the method, recovery studies were carried by spiking the standard drug to the Daruvir Tablet sample solution, at three different levels around 50, 100 and 150 %. Basic concentration of sample solution chosen was 10 µg/ml of Darunavir and recovery was determined from linearity equation. The results obtained are shown in Table 7.

Table 7: Recovery studies of Darunavir

Level	Conc. of Sample solution (µg/ml)	Conc. of Standard solution spiked (µg/ml)	Area	Amount recovered(µg/ml)	% recovery
50 %	10	5	226920.4	14.88	98.56
			225013.3	14.75	
			224523.5	14.71	
100 %	10	10	292556.3	19.61	98.22
			293655.2	19.69	
			292655.53	19.62	
150 %	10	15	364552.3	99.21	99.58
			365875.3	99.59	
			367052.3	99.93	

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition, detection wavelength, flow rate were altered and the effects on the area were noted. The method was found to be robust. The results obtained are shown in Table 8.

Table 8: Robustness study

% RSD Found For Robustness Study(peak area)								
MP COMPOSITION			DETECTION WAVELENGTH (± 1 nm)			FLOW RATE (± 0.05 ml/min)		
61:39	63:37	65:35	266	267	268	0.95	1	1.05
0.35	0.90	0.49	1.949	1.169	0.358	1.877	1.274	1.038

Summary of validation study:

Table 9: Summary of validation study by HPLC method

Sr. No.	Validation Parameter	Darunavir
1.	Linearity equation R^2	y = 13850 x+ 24788 R^2 = 0.985
2.	Range	5-30 µg/ml
3.	Precision	(%RSD)
	A) Intraday precision	0.53
	B) Interday precision	0.45
4.	Limit of Detection	0.44
5.	Limit of Quantitation	1.36
6.	Assay±RSD	98.80± 0.63
7.	Accuracy±RSD	98.79± 0.40
8.	Robustness	Robust
9.	Specificity	Specific

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