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Validated RP - HPLC Method for the Determination of Darunavir in Bulk and Pharmaceutical Formulation.

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Darunavir in bulk and pharmaceutical formulation. Isocratic elution at a flow rate of 1.0ml/min was employed on symmetry C18 (250 mm x 4.6 mm, 5µm) column at ambient temperature. The mobile phase consisted of acetonitrile: Methanol in the ratio of 90:10 v/v. The UV detection wavelength was 271nm and 20µl sample was injected. The retention time for Darunavir was 2.59 min. The percentage RSD for precision and accuracy of the method was found to be 0.48%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Darunavir in the rapid and reliable determination of Darunavir in pharmaceutical formulation.

Keywords: Darunavir, RP-HPLC, UV detection, recovery, precise.

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INTRODUCTION

Systematic (IUPAC) name for Darunavir is (1*R*, 5*S*, 6*R*)-2, 8-dioxabicyclo [3.3.0] oct-6-yl] *N* [(2*S*, 3*R*)-4- [(4-aminophenyl) sulfonyl- (2 methylpropyl) amino]-3-hydroxy-1-phenyl- butan-2 yl] carbamate. It works by slowing the spread of HIV in the body. It was approved by FDA on June 23, 2006.[1]

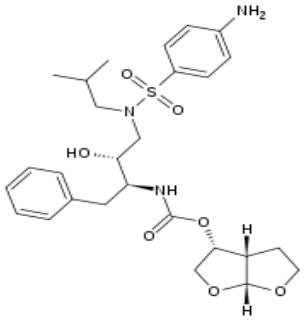

Figure 1: Darunavir

Several analytical methods have been reported for the determination of Darunavir in pure drug, pharmaceutical dosage forms and in biological samples using spcetrophotometry liquid chromatography, electro kinetic chromatography high performance thin layer chromatography either in single or in combined forms. Darunavir was designed to form robust interactions with the protease enzyme from many strains of HIV, including strains from treatment-experienced patients with multiple resistance mutations to PIs.

It was discovered by an Indian chemist, Arun K Ghosh at the University of Illinois, Chicago [2-3]. It blocks HIV protease, an enzyme which is needed for HIV to multiply. Raveendra B.Ganduri et al [4] were developed that Waters, Symmetry shield RP18 (250X4.6mm, 5µm) column0.1% orthophosphoric acid and acetonitrile (50:50 % v/v) as mobile phase, detection wavelength of 265 nm, flow rate of 1.0 mLmin-1. The method is linear from 25µg mL-1 to 100µg mL-1, accuracy was found to be 99.54%, mean inter and intraday assay relative standard deviation (RSD) were less than 1.0%. Masaaki Takahashi et al [5] were emphasized the calibration curve was linear (range of 0.13 to 10.36 µg/ml). The average accuracy ranged from 100.7 to 105.6%. Both the interday and intraday coefficients of variation were less than 6.7%. Lauriane Goldwirt et al [6] were described that internal standard, methylclonazepam, was added to 100 µL of plasma before a solid-phase extraction on C18 Bond Elute column. The separation was performed on a C8 column using an acetonitrile and ultrapure water mixture (40:60, v/v). All compounds were detected at a wavelength of 266 nm. The method was linear and validated over a concentration range of 0.25–20 mg/L. Precision ranged from 3.0-7.9%, while the accuracy ranged from −11.4 to 0.5%. This method provides a useful tool for therapeutic drug monitoring in HIV patients.

Ivy Song et al[7] were conducted a pharmacokinetic work to determine the effect of Lopinavir/Ritonavir and Darunavir/Ritonavir on the HIV Integrase Inhibitor S/GSK1349572 in Healthy Participants. Studies demonstrated that co administration of lopinavir/ritonavir had no

significant effect on steady-state PK of S/GSK1349572. D'Avolio A et al[8] has developed that Darunavir and other 11 antiretroviral agents. A simple chromatographic separation of drugs and Internal Standard was achieved with a gradient (acetonitrile and water with formic acid 0.05%) on a C-18 reverse phase analytical column with 25 min of analytical run. Mean intra- and inter-day precision for all compounds were 8.4 and 8.3%, respectively, and mean accuracy was 3.9%. Extraction recovery ranged within 93 and 105% for all drugs analyzed. Bhavini N. Patel et al [9] has developed Darunavir ethanolate (DRV) in tablets. DRV from the formulations was separated and identified on silica gel 60 F_{254} HPTLC plates with toluene-ethyl acetate-methanol 7.0:2.0:1.0 (*v/v*) as mobile phase. The calibration plot for DRV standard was linear in the range 250–1750 ng per band with r = 0.9994, slope = 0.4253, and intercept = 44.81. Jose Molto et al[10] has described a Open-label, randomized pilot study in HIV-infected patients on Darunavir/ritonavir 600/100 mg twice daily.

MATERIALS AND METHODS

Instrumentation

HPLC containing LC 20AT pump and variable wavelength programmable UV-Visible detector and Rheodyne® injector was employed for investigation. The chromatographic analysis was performed on a Kromosil C18 column (250 mm × 4.6 mm, 5µm). Degassing of the mobile phase was done using a Loba® ultrasonic bath sonicator. A Denwar® Analytical balance was used for weighing the materials.

Chemicals and Solvents

The reference sample of Darunavir (API) was obtained from Jolly company. The Formulation PREZISTA™ (Darunavir) was purchased from the local market. Methanol, Water used was of HPLC grade and purchased from Merck Specialties Private Limited, Mumbai, India.

Chromatographic Conditions

Mobile phase**:** Acetonitrile (90%), Methanol (10%)
P^H: 6.2
Analytical Column: Kromosil C18 column (250 mm × 4.6 mm, 5µm)
UV Detection wave length: 271nm
Flow rate: 1.0ml/min
Injection volume: 20 µl
Temperature: Ambient
Runtime: 5Min
Retention time: 2.59Min
HPLC Chromatogram was shown in Fig: 2

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Standard solution of the drug

For analysis 100 ppm standard solution was prepared, required concentrations were obtained from 100 ppm solution by appropriate dilution.

Sample solution

The formulation tablets of Darunavir (PREZISTA™ - 75 mg) were crushed to give finely powdered material. From the powder prepared a 30 ppm solution with mobile phase and then filtered through Ultipor™ N_{66} Nylon 6, 6 membrane sample filter paper.

Method Development

For developing the method [11-17], a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choice of stationary and mobile phases. The following studies were conducted for this purpose.

Detection of wavelength

The spectrum of 10 ppm solution of the Darunavir in methanol was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength was observed. The spectra of Darunavir were showed maximum absorbance at 271nm.

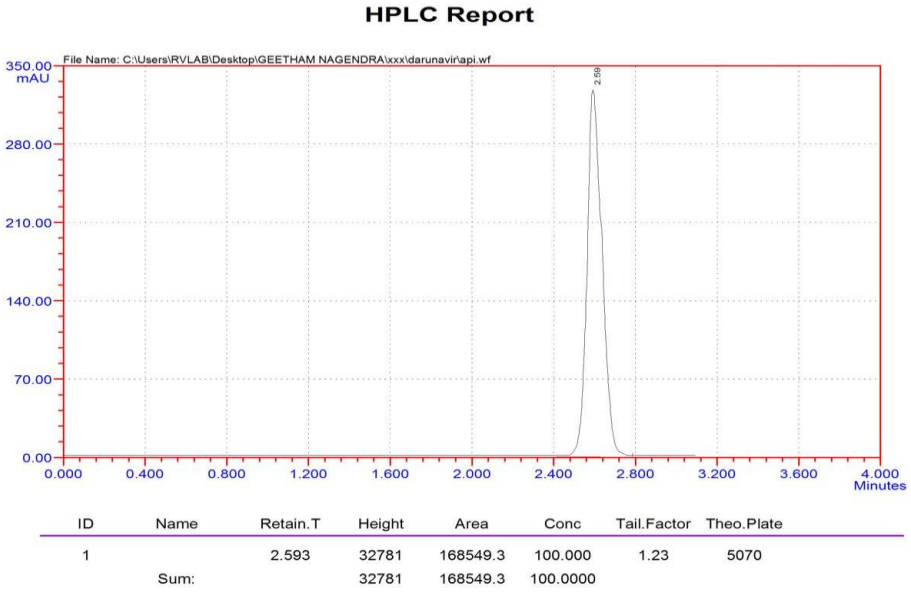

Figure 2: Chromatogram of standard solution

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Validation of Proposed Method

The proposed method was validated [18-26] as per ICH Quality guidelines [18]. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

Specificity:

The specificity of method was performed by comparing the chromatograms of blank, standard and sample (Prepared from Formulation). It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention times of standard and sample. The specificity results are shown in Table.1.

Table 1:

Name Of The Solution	Retention Time in Min
Blank	No peaks
Darunavir (Standard)	2.59
Darunavir (Sample)	2.56

Linearity

Linearity was performed by preparing mixed standard solutions of Darunavir at different concentration levels including working concentration mentioned in experimental condition i.e. 30ppm. Twenty micro liters of each concentration was injected in duplicate into the HPLC system. The response was read at 271 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually. The regressions of the plots were computed by least square regression method. Linearity results were presented in Table.2.

Table 2:

Level	Concentration of Darunavir in ppm	Mean peak area
Level -1	10	66073
Level -2	20	112573.0
Level -3	30	169196.8
Level -4	40	214545.7
Level -5	50	281291.2
Range: 10-30ppm	Slope Intercept Correlation coefficient	5324.09 9013.21 0.997

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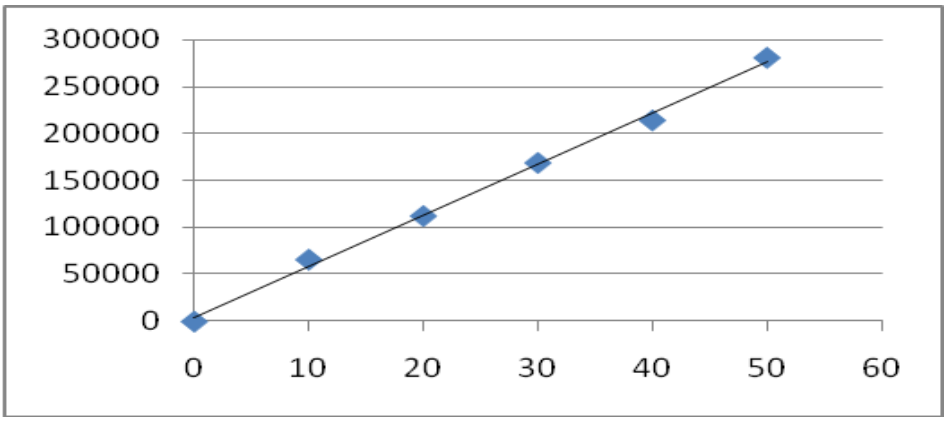

Figure 3: Linearity Graph of Darunavir (On X axis concentration of sample, On Y axis peak area response)

Precision

Precision of the method was performed as intraday precision, Inter day precision. To study the intraday precision, six replicate standard solutions (50ppm) of Darunavir were injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 1.068, which are well within the acceptable. For inter Day precision the percent relative standard deviation (% RSD) was calculated and it was found to be 0.560. Results of system precision studies are shown in Table.4 and Table.5.

Table 4

SAMPLE	CONC(PPM)	INJECTION No.	PEAKS AREA	R.S.D (Acceptance criteria ≤ 2.0%)
Darunavir	50	1	280344.9	1.068
		2	281291.2	
		3	287555	
		4	286832.8	
		5	286435.8	
		6	284917.2	

Table 5

SAMPLE	CONC(PPM)	INJECTION No.	PEAKS AREA	R.S.D (Acceptance criteria ≤ 2.0%)
Darunavir	50	1	288032.7	0.560
		2	287629.1	
		3	290361.9	
		4	290419.7	
		5	291211.5	
		6	287647.5	

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Accuracy

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at 50%, 100% and 150% level of 20ppm. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD was calculated and results are presented in Table.6. Satisfactory recoveries ranging from 99.0 to 102.0 were obtained by the proposed method. This indicates that the proposed method was accurate.

Table 6

Level	Amount of Darunavir spiked (ppm)	Amount of Darunavir recovered(ppm)	% Recovery	% RSD
50 %	30	29.95	99.83	0.440
	30	29.9	99.66	
	30	29.7	99.00	
100%	40	39.8	99.5	0.318
	40	40.02	100.05	
	40	39.8	99.5	
150%	50	49.5	99.0	0.696
	50	49.5	99.0	
	50	50.1	100.2	
			% recovery = 99.52	Mean RSD = 0.484

Robustness

The robustness study was performed by slight modification in flow rate of Mobile phase, pH of the buffer and composition of the mobile phase. Darunavir at 40 ppm concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The results of robustness study are shown in Table.7.

Table 7

Condition	Mean area	% assay	% difference
Unaltered	214545.7	100.0	0.0
Flow rate at 0.8 mL/min	215328.6	100.36	0.36
Flow rate at 1.2mL/min	215124.8	100.26	0.26
Mobile phase: MEOH: Water			
75% 25%	213845.5	99.67	0.33
85% 15%	214853.0	100.14	0.14
pH of mobile phase at 7.3	2140537.5	99.77	0.23
pH of mobile phase at 7.7	213452.9	99.49	0.51

System suitability

System suitability was studied under each validation parameters by injecting six replicates of the standard solution 2 ppm). The results obtained were within acceptable limits (Tailing factor ≤2 and Theoretical plate's ≥2000) and are represented in Table.8.

Table 8

Parameter	Tailing factor	Theoretical plates
Specificity study	1.23	5069.89
Linearity study	0.95	5368.86
Precision study	1.21	4709.73

Limit of detection and Limit of quantification:

Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of quantification (LOQ) is defined as the lowest Concentration that can be quantified reliably with a specified level of accuracy and Precision. For this sample was dissolved by using Mobile Phase and injected until peak was disappeared. After 0.6ppm dilution, Peak was not clearly observed. So it confirms that 0.6ppm is limit of Detection and 0.198ppm dilution is Limit of Quantification. For this study six replicates of the analyte at lowest concentration were Measured and quantified. The LOD and LOQ of Darunavir are given in Table.9.

Table 9

Parameter	Measured volume
Limit of Quantification	0.20 ppm
Limit of Detection	0.60 ppm

Formulation

For assay Darunavir (PREZISTA™ - 75 mg) 20 tablets were weigh and calculate the average weight. Transfer the sample equivalent to 10mg of Darunavir in to a 10ml volumetric flask. Add diluent and sonicate to dissolve it completely and make volume up to the mark with diluents. Mix well and filter through 0.45um filter. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to mark with diluents and finally 10 ppm were prepared. Mix well and filter through 0.45um filter. An aliquot of this solution was injected into HPLC system. Peak area of Darunavir was measured for the determination.

DISCUSSION

Having optimized the efficiency of a chromatographic separation the quality of the chromatography was monitored by applying the system suitability tests. The acceptance criterion was ± 2% for the percent coefficient of variation for the peak area and retention times for Darunavir. The number of theoretical plates should not be less than 2500 and the tailing

factor should not be more than 2.0. The peak purity of Darunavir was assessed by comparing the retention time (Rt) of standard and the sample. Good correlation was obtained between the Rt of std. and sample. A chromatogram obtained from reference substance solution is presented.

The linearity ranges for Darunavir found to be 10-50ppm. The regression equation for LP and AT were found to be y = 9013x+5324 with coefficient of correlation, (r) 0.997. The results of Linearity studies are shown in Table.2 and the linearity curve were shown in Figure.3. Percentage relative standard deviation (% RSD) was (for Intraday-1.068, For Inter day-0.560) found to be less than 2% for within a day and day to day variations.

Results of recovery studies are shown range 99.00-101.45%. The mean recovery data obtained for each level as well as for all levels combined were within 2.0% satisfied the acceptance criteria set for the study.

To evaluate the robustness of the developed RP-HPLC method, small deliberate Variations in the optimized method parameters were done. The effect of change in flow rate, pH and mobile phase ratio on the regent ion time and tailing factor were studied. The values for proposed method are well within acceptance limits of 98-102 %, with a RSD of less than 2.0%. Above experiments indicated that the method is rugged and provides consistent and reliable results. Limit of detection (LOD) and Limit of quantification (LOQ) were 0.65ppm and 24 ng/ml respectively. Efficient UV detection at 271 nm was found to be suitable without any interference from injectable solution excipients or solvents.

CONCLUSION

Statistical analysis of the results has been carried out revealing that the proposed method is simple, sensitive and reproducible and hence can be used in routine for simultaneous determination of Darunavir in bulk as well as in pharmaceutical preparations.

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