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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF DARUNAVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

An RP-HPLC method has been developed and validated for the determination of Darunavir in bulk and tablet formulation. The RP-HPLC analysis was performed on the Fortis C18 column (150 mm x 4.6 mm i.d., 2.5µm) in isocratic mode, at 300c using methanol: water (75:25*v/v*) pH adjusted to 8.0 with triethylamine as the mobile phase**;** flow rate was set at 1.0 mL/min. The detection was carried out at 268nm. The retention time for Darunavir was found to be found to be 3.840± 0.02 min. Darunavir followed linearity in the concentration range of 30 – 70µg/mL (r2 = 0.999). The method has successively been applied for the determination of Darunavir in marketed formulation. There was no interference from the excipients routinely present in the tablet. The drug content for Darunavir was found to be 99.28 % ± 0.48. The accuracy of the method was studied by the recovery studies at three different levels i.e. 80 %, 100 %, and 120 % level. The % recovery was found to be within the limits of the acceptance criteria within the range of 98.46 – 100.18 %. The precision of the method was studied as the repeatability of the sample application, intra-day, and inter-day precision. The results were examined as %RSD values of the concentration of drugs determined. The low value of %RSD (less than

2) indicates the high precision of the method. The method proved to be adequately sensitive as indicated by low values of LOD and LOQ.

KEYWORDS: Darunavir, HIV, AIDS, Analytical method development, HPLC, Method validation.

INTRODUCTION

Darunavir is an antiretroviral protease inhibitor that is used in the therapy and prevention of human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS).[1] 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.[2] In HBV or HCV-coinfected patients, highly active antiretroviral therapy with darunavir may result in an exacerbation of the underlying chronic hepatitis B or C.[2] [3] Darunavir is used with a pharmacokinetic booster (a medication that increases the number of other medications in the body) such as ritonavir (Norvir) or cobicistat (Tybost), and other medications to treat human immunodeficiency virus (HIV) infection in adults and children 3 years of age and older.[1][3] Darunavir is in a class of medications called protease inhibitors.[4][1] It works by decreasing the amount of HIV in the blood.[4][5] Although darunavir does not cure HIV, it may decrease the chance of developing acquired immunodeficiency syndrome (AIDS) and HIV-related illnesses such as serious infections or cancer.[3][6][7] Taking these medications along with practicing safer sex and making other lifestyle changes may decrease the risk of transmitting the HIV virus to other people.[6][7]

As very few analytical methods are available in the literature for analysis of Darunavir in bulk and in tablet formulation; therefore, an objective of the present work is to develop and validate the RP-HPLC method for determination of Darunavir in bulk and pharmaceutical formulation using ICH guidelines.

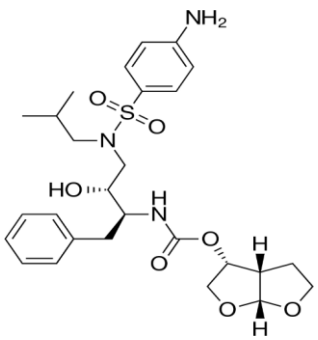

Figure 1: Chemical Structure of Darunavir.[5]

MATERIALS AND METHODS

Reagent and Chemicals

Pure standard Darunavir was provided by Cipla Pharmaceuticals Pvt Ltd, Mumbai. HPLC grade Methanol was purchased from Merck, Mumbai (India). Darunavir Tablets Daruvir® (label claim 300 mg) was purchased from a local market. Double distilled water was prepared by distillation assembly.

Optimization of Chromatographic conditions

The Fortis C18 column (150 mm x 4.6 mm i.d., 5 µ) was used for the separation of the drug, which gives satisfactory resolution and run time. The mobile phase was optimized with a view to separate Darunavir. Initially, methanol and water in various proportions were tried as a mobile phase but the tailing of the peak was observed. Adjustment of pH of aqueous phase combination of methanol and water was tried for resolution of the drug as well as the tailing was reduced. Finally, good resolution and symmetric peak were obtained for the drug when the pH of the aqueous phase of the mobile phase was adjusted to 8.0 ± 0.2. The flow rate of the mobile phase was 1.0 mL/min. Under optimum chromatographic conditions, the retention time for Darunavir was found to be 3.840minand the detection was carried out at 268 nm. The chromatographic conditions are shown in **Table 1** while the typical chromatograph is shown in **Figure 5.**

Preparation of Stock Standard Solution

The stock standard solution was prepared by dissolving 10 mg of Darunavir in 100 mL of methanol to obtain a concentration of 100µg/mL.

RESULT AND DISCUSSION

Optimization of Detection Wavelength

Detection using a PDA detector at different wavelengths was performed. Finally, 268 nm wavelength were selected as detection wavelengths as shown in the **figure.2**

System suitability test

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing. Results are shown in **Table 10.**

Linearity Studies

From the stock standard solution, an appropriate volume in the range of 1.0 – 7.0 mL was transferred into seven separate 10mL volumetric flask, and volume was made up to the mark to obtain concentration in the range of 10- 70 µg/mL. From each volumetric flask, a volume of 20µL solution was manually injected with the help of Hamilton Syringe. All measurements were repeated six times for each concentration and calibration curves were constructed by plotting the peak area *versus* the corresponding drug concentration. Calibration curves are shown in **Figure 4** and results for linearity are shown in **Table 2.**

Analysis of Bulk Material

An accurately weighed quantity of 10 mg of Darunavir was transferred into a 100 mL volumetric flask. It was dissolved in methanol and volume was adjusted to mark. The solution was further diluted to get a concentration 30 µg/ml which was subjected to the proposed method and the amount of Darunavir was determined. The procedure was repeated for six times; results are shown in **Table 3.**

Analysis of marketed Formulation

Twenty tablets of Daruvir® (Label claim 300 mg) were weighed accurately and powdered. An amount of the powder equivalent to 10mg of Darunavir was transferred to 100 mL volumetric flasks containing 50mL of methanol, shaken manually for 25 min and volume was made up to the mark using the same solvent and filtered through Whatman filter no. 41. From it, 3.0 mL was diluted to 10 mL to get 30µg/mL solutions and injected into the system for analysis. Results are shown in **Table 4.** The Chromatogram of Darunavir extracted from tablets is shown in **Figure 5.**

Method Validation

The proposed method was validated as per ICH guidelines. The solutions of the drug were prepared as per the earlier adopted procedure given in the experiment.

Accuracy (Recovery Study)

Accuracy was determined by performing recovery studies by spiking different concentrations of pure drug in the pre-analyzed sample solution. To analyze the sample solution (30 µg/mL), a known amount of stock standard solution was added at different levels i.e. 80%, 100%, and 120%. The solutions were re-analyzed by the proposed method; the results are shown in **Table 5.**

Precision

The precision of the method was studied as intra-day and inter-day variations and also as repeatability.

Intra – day and Inter – day Precision

Intra-day precision was determined by analyzing 20, 30, and 40µg/mL of Darunavir solutions for three times in the same day. Inter-day precision was determined by analyzing the same concentration at three different days over a period of a week; the results are shown in **Table 6.**

Sensitivity

The sensitivity of measurement of Darunavir by the use of the proposed method was determined in terms of the LOD and LOQ. The LOD and LOQ were calculated using equation LOD = 3.3 X N/B and LOQ = 10 X N/B; Where, 'N' is the standard deviation of the peak areas of the drug (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve. It was performed in the range of 10-20 µg/mL.

Specificity and Selectivity

The analyte should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in the presence of components that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in the presence of components that may be expected to be present in the sample matrix. The method is quite selective. There was no other interfering peak around the retention time of Darunavir; also, the baseline did not show any significant noise.

Ruggedness

The appropriate volume of sample solution, 30µg/mL was prepared and analyzed by two different analysts using similar operational and environmental conditions. The area was measured for the same concentration solutions, six times. The results are shown in **Table 8.**

Robustness

The robustness of the method was studied by making deliberate variations in the chromatographic conditions and effects on the peak areas were recovered. Different chromatographic parameters such as variations in flow rate, mobile phase composition,

change in temperature, and mobile phase pH were made. It was performed using 30mg/mL of Darunavir and the effects on the peak areas were recorded. Each parameter was repeated six times. The results are shown in **Table 9.**

Table 1: Finalized Chromatographic Conditions for analysis of Darunavir.

Chromatographic Parameter	Chromatographic Condition
Standard solution	100µg/mL in Methanol
HPLC System	Agilent Tech. Gradient System (1100)
Detector	UV (DAD) G13148
Data processor	CHEMSTATION 10.1
Stationary phase	Fortis C18 column (150 mm x 4.6 mm i.d., 5µm)
Mobile phase	methanol: water(75:25% *v/v*) pH adjusted to 8.0 with triethylamine
Detection wavelength	268 nm
Flow rate	1 mL/min
Sample size	20 µL

Table 2: Linearity Study of Darunavir.

Concentration of Darunavir [µg/mL]	Peak Area [Mean ± SD; n = 5]	% RSD
10	32145±220.19	0.685
20	63784±348.89	0.547
30	96823±458.94	0.474
40	127262±716.48	0.563
50	158501 ±733.85	0.463
60	195640±692.56	0.354
70	227479±1694.72	0.745

Table 3: Analysis of bulk material.

Component	Amount taken in[µg/mL]	Amount Found [µg/mL]± SD	% Amountfound	%RSD [n=6]
Darunavir	30	29.87	99.57	0.53

Table 4: Analysis of Tablet formulation

Brand Name: Daruvir® Mfg. By: Cipla Pharmaceuticals Pvt. Ltd.

Batch No.: DRA210801Average weights: 440.36 mg

Marketed by: Cipla Pharmaceuticals Pvt. Ltd.

Component	Amount taken in [µg/mL]	Amount Found[µg/mL] ± SD [n = 6]	% Amount found ± SD	% RSD
Darunavir	30	29.78± 0.14	99.28 ± 0.48	0.48

Table 5: Accuracy studies.

Drug	Initial amount [μg/mL]	Excess drug added to the analyte [%]	Amount recovered ± S.D.[μg/mL]	Recovery [%]	%RSD [n = 3]
Darunavir	30	80	53.63± 0.35	98.46	0.66
	30	100	60.05± 0.31	100.18	0.52
	30	120	65.86± 0.48	99.61	0.74

Table 6: Precision studies.

Drug	Conc. [μg/mL]	Intra –day Amount Found [%] [n = 3]		Inter- day Amount Found [%] [n = 3]	
		Mean	% RSD	Mean	% RSD
Darunavir	20	100.13	0.75	101.04	0.98
	30	99.22	0.62	99.87	0.79
	40	98.86	0.48	99.46	0.64

Table 7: Repeatability studies.

Drug	Concentration [μg/mL]	Amount found Mean ± SD, [n = 6]	% RSD
Darunavir	30	30.28	0.56

Table 8: Ruggedness study.

Drug	Amt in μg/mL	% Amount Found [n = 6]		% RSD	
Darunavir		Analyst I	Analyst II	Analyst I	Analyst II
	30	99.95	99.36	0.84	0.72

Table 9: Robustness Studies.

Sr. No.	Parameter	% RSD
1.	**Mobile phase composition** a) Methanol :Water (73: 27*v/v*) b) Methanol :Water (77: 23*v/v*)	 0.35 0.43
2.	**Column Temperature (^{0}C)** a) 32 b) 28	 0.61 0.59
3.	**Change in Flow Rate** a) 0.9 b) 1.1	 0.23 0.49
4.	**Change in pH flow rate** a) 8.1 b) 7.9	 0.83 0.74

Table 10: System Suitability Test.

Syatem suitability parameters	Darunavir
Retention time (min)	3.840
Theoretical Plates (N)	4568
Tailing Factor	0.82
Capacity factor	1.56

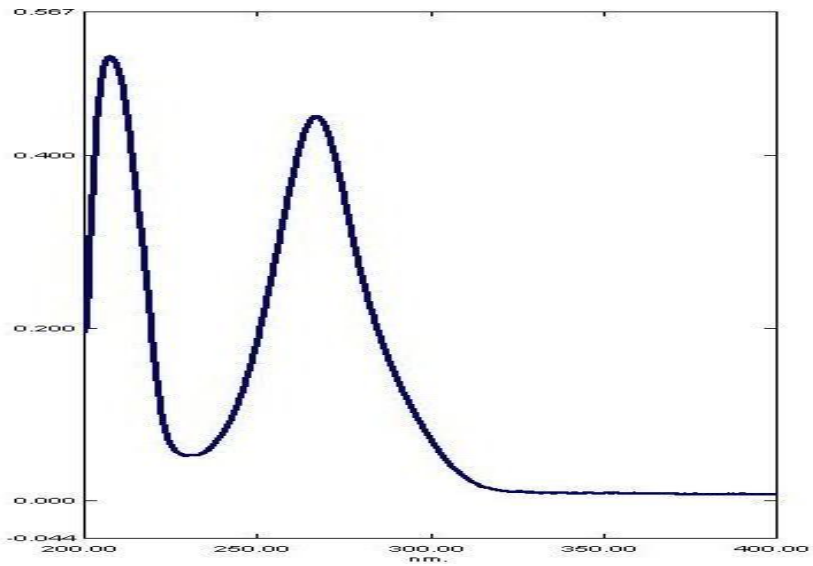

Figure 2: UV Spectrum for Darunavir showing maximum absorbance at 268 nm.

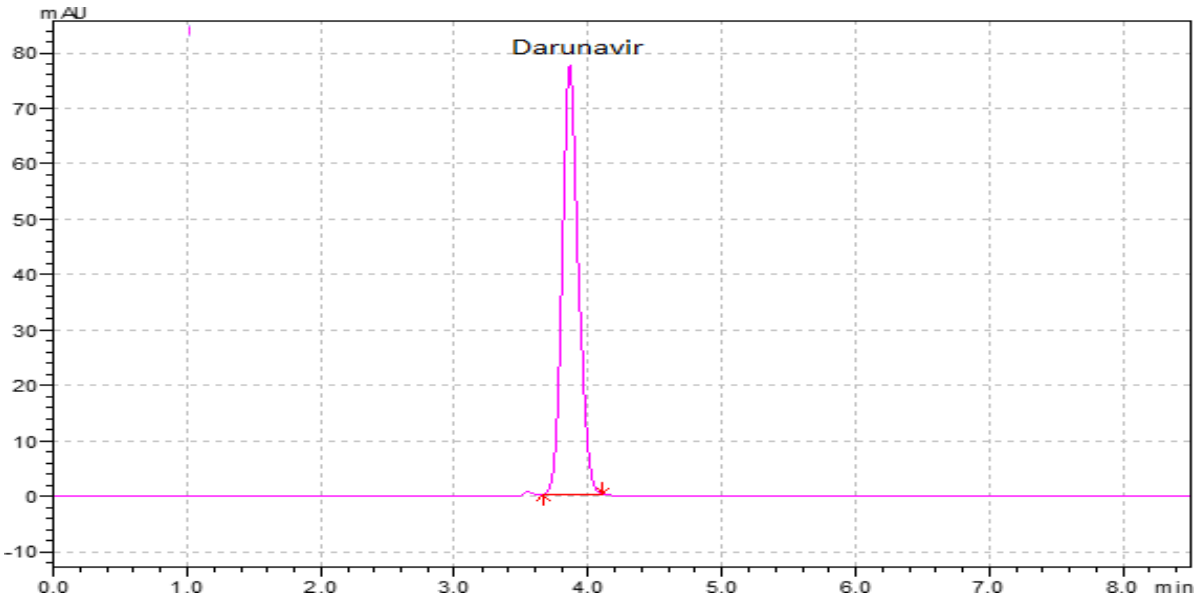

Figure 3: RP-HPLC chromatogram of Darunavir bulk in methanol: water (75:25 % *V/v*) pH adjusted to 8.0 with triethylamine at 268 nm; showing retention time 3.840min.

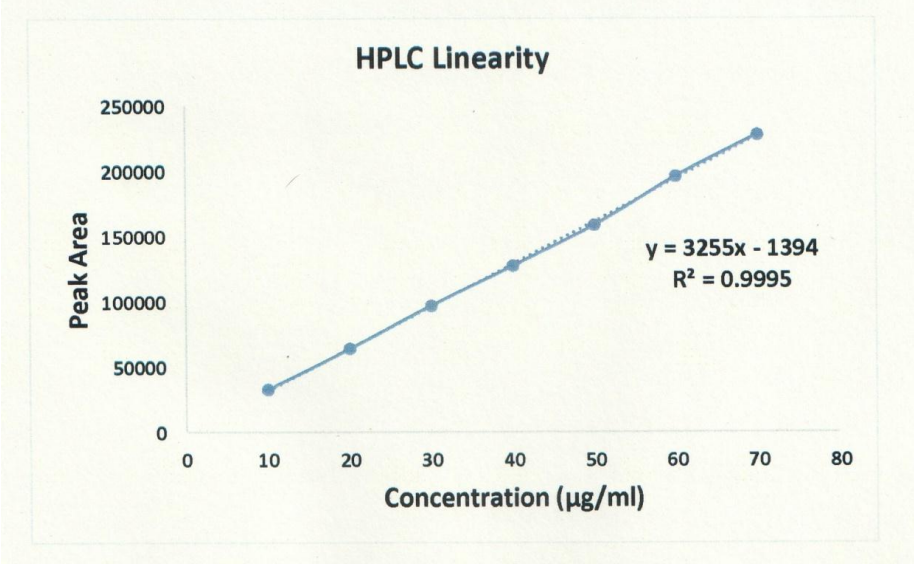

Figure 4: Calibration curve for Darunavir.

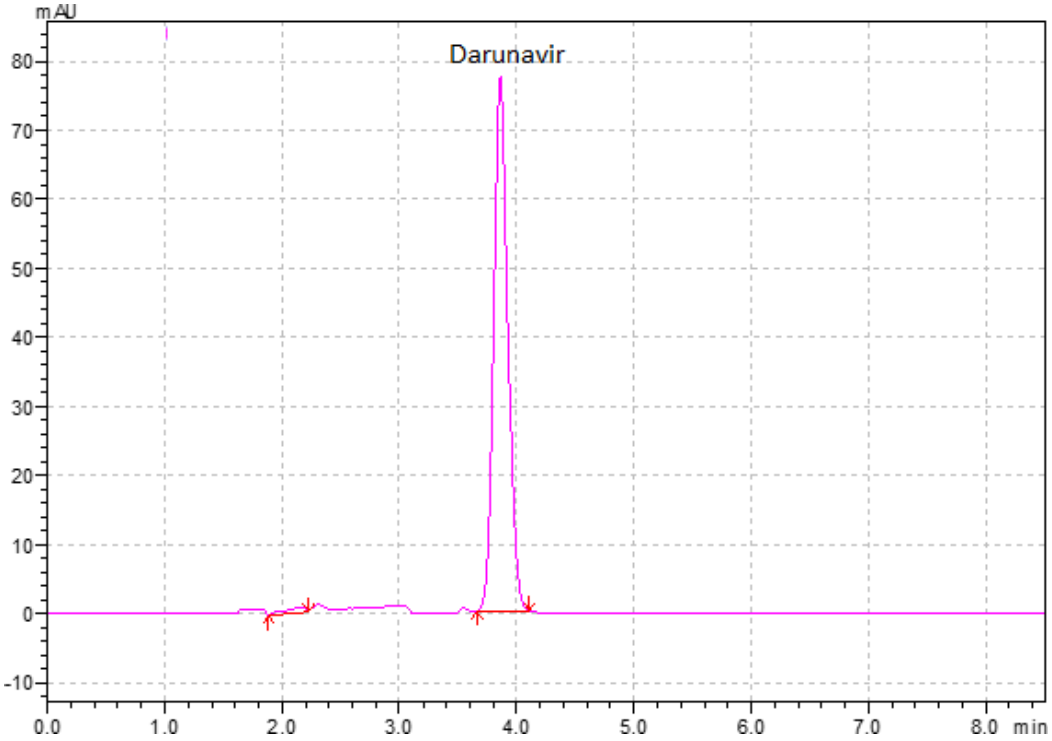

Figure 5: Chromatogram of Darunavir extracted from tablets.

CONLCUSION

RP-HPLC has been developed for the determination of Darunavir in bulk and tablets. Developed RP-HPLC method validated as per ICH guidelines and found to be accurate, precise, rugged, and robust. RP-HPLC method for estimation of Darunavir is simple and can be used for routine analysis of Darunavir in bulk and in tablet formulation.

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