Asian J. Pharm. Res. Vol 1, Issue 1, 10-14, 2011.

Asian Journal
of
PHARMACEUTICAL RESEARCH
Journal homepage: - www.ajprjournal.com

NEW RP-HPLC METHOD FOR THE DETERMINATION OF DARUNAVIR IN TABLET DOSAGE FORM

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ABSTRACT

To develop a simple, rapid, sensitive, accurate, precise and reproducible high performance liquid chromatographic method for the determination of Darunavir in tablet dosage form. Waters, Symmetry shield RP18 (250X4.6mm, 5µm) column, 0.1% orthophosphoric acid and acetonitrile (50:50 % v/v) as mobile phase, detection wavelength of 265 nm, flow rate of 1.0 mL min^{-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%. The method is simple, accurate, specific and precise, can be used for the determination of Darunavir.

Key words: Darunavir, antiretroviral, reversed-phase and validation

INTRODUCTION

Darunavir (DAR) is chemically [(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, a new protease inhibitor , is used to treat human immunodeficiency virus (HIV) infection. Darunavir is an inhibitor of the HIV-1 protease. It selectively inhibits the cleavage of HIV encoded Gag-Pol polyproteins in infected cells, thereby preventing the formation of mature virus particles [1-5].

Bouche et al. determined plasma DAR concentrations using liquid chromatography-tandem mass spectrometry (LC/MS/MS) [6]. Goldwirt et al. reported a method for quantification of Darunavir (TMC114) in human plasma by high-performance liquid chromatography with ultra-violet detection [7]. Takahashi, M et al.reported the validation of Plasma Darunavir Concentrations Determined by the HPLC Method for Protease Inhibitors [8]. So, far no assay method has been reported for the estimation of DAR in pharmaceutical formulations and DAR is not yet official in any pharmacopeia. The aim of the present study was to develop a simple, specific reversed phase HPLC method for the estimation of DAR in pharmaceutical tablet dosage form.

Materials and Methods

Chemicals and Reagents

All the reagents were of analytical-reagent or HPLC grade unless stated otherwise. Milli-Q-water was used throughout the experiment. Acetonitrile (S.D. Fine Chem., Mumbai, India) and orthophosphoric acid (Merck, Mumbai, India) were used. DAR standard and sample tablet dosage forms were obtained from Hetero drugs Ltd (Hyderabad, India).

Instrumentation

The HPLC system was composed of 2695 water alliance system fitted with 2996 PDA detector with empower software. Analytical column used for this method is Waters, Symmetry shield RP18 (250X4.6mm, 5µm).

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Mobile phase preparation

0.1% V/V solution of orthophosphoric acid in water and acetonitrile were mixed in equal volumes and degassed the resultant solution.

Standard Preparation

50 mg of DAR working standard was accurately weighed and transferred into a 100 mL volumetric flask. 60 mL of the mobile phase was added to the above flask and sonicated to dissolve and the volume was made up to the mark with mobile phase. 5.0 mL of the above solution was transferred into a 50 mL volumetric flask and made up the volume with mobile phase.

Sample Preparation

Ten tablets of Darunavir were separately weighed and grounded to fine powder. An amount equivalent to 1000 mg of Darunavir was transferred into a 200 mL volumetric flask and about 150 mL of mobile phase was added, sonicated for not less than 30 min with occasional stirring (Sonicator bath temperature was maintained between 20-25^{0}C) and made up the volume with mobile phase. The above solution was filtered through 0.45 µm membrane filter. 2.0 mL of the above solution was transferred in to a 200 mL volumetric flask and diluted to volume with mobile phase.

Chromatographic conditions

The mobile phase was filtered through 0.45 µm PVDF membrane filter and degassed using vacuum before delivered into the system. The chromatographic conditions used for the analysis were given below.

Column	: Waters, Symmetry shield RP18 (250X4.6mm, 5 µm)
Wavelength	: 265 nm
Injection volume	: 20 µl
Flow rate	: 1.5 mL min^{-1}
Column temperature	: 25^{0}C
Run time	: 10 min

Method validation

Method validation was conducted according to published guidelines [9-10]. Assay performance was evaluated by intraday and inter day (two different days) precision and determined from replicate analysis of samples (50µg mL^{-1}) in two analytical runs. Analysis of six different sample solutions was performed in the same day for intraday precision. Accuracy of the method was tested by adding a known amount of Darunavir standard (25, 50 and 100 µg mL^{-1}) in three sample solutions. The precision and accuracy were expressed in terms of RSD from mean intra and inter day assays and recovery of the theoretical concentration.

Robustness was tested by analysis of variations in analytical condition. Influence of mobile phase composition and different column brands were evaluated. The chromatographic parameters monitored were peak retention time, tailing factor and theoretical plate number.

RESULTS AND DISCUSSION

Changes in the analytical procedure were tested. Different mobile phases with different proportions of organic modifier (acetonitrile) were tried. Chromatographic run was evaluated using Waters, Symmetry shield RP18 (250X4.6mm, 5µm) column. After selecting the best conditions based on peak performance, the run time of the proposed assay was 10 min with isocratic elution. During injection of solutions of standard, sample and prezista (Darunavir ethanolate) commercially available tablets, the retention times were 4.951, 4.954 and 4.981 respectively (Fig.1).

Robustness

Typical variations in analytical conditions were tested. Influence of flow rate, mobile phase composition and filter variability were studied. The results were shown in the Table 1.

Linearity

The curve proved to be linear over a concentration range of 20-100 µg mL^{-1} (Fig 2).Standard solution were prepared at six concentrations (20, 40, 50, 60, 80 &100 µg mL^{-1}) were injected in duplicate. Linear regression of concentration Vs peak area resulted in an average coefficient of determination (R^2) 0.999. The Regression equation is Y= 26472x-719.29 (Fig.3).

Precision

The intra and inter day precision were estimated from duplicate injection of six sample solutions prepared at 50 µg mL^{-1} of Darunavir analyzed on two different days. Mean and RSD were obtained from calculated Darunavir concentration (Table 2). The results indicate that the method is reproducible.

Accuracy

Accuracy was calculated as the percentage recovery of the known added amount of Darunavir reference substance in the sample solutions using three concentration levels covering the specified range (20, 50,100 µg mL^{-1}) was added in the sample solutions (equivalent to 50 µg mL^{-1}). The accuracy of the method ranged from 98.7 to 100.3 %, indicating that this assay is reliable (Table 3) and meeting the acceptance criteria 98.0 to 102 %.

Fig 1. [A] Chromatogram of the standard solution

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[B] Chromatogram of the sample solution
[C] Chromatogram of the prezista (innovator) solution

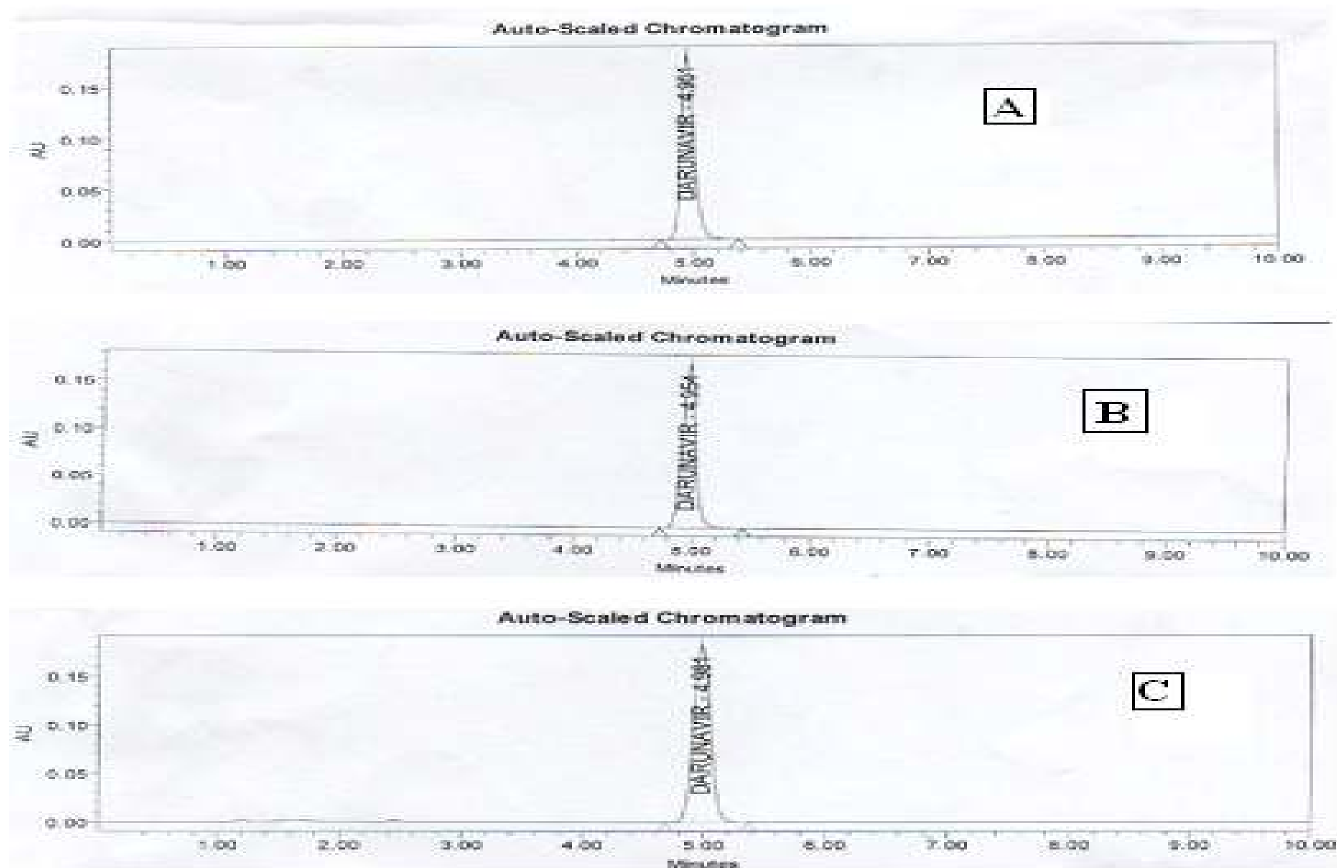

Table 1. Robustness study of Darunavir

Robustness Parameter	Tailing factor	Theoretical plate number (n)	RSD (%)
	Mobile phase Composition		
55:45 50:50 (Optimized) 60:40	1.3 1.1 1.3	9568 13247 11053	0.11 0.05 0.12
	Variation in flow rate		
1.3 mL min^{-1} 1.5 (Optimized) 1.7	1.2 1.1 1.0	14560 13247 11221	0.08 0.05 0.05
	Filter variability		
Nylon Centrifuged PVDF	1.3 1.1 1.2	15938 13247 14009	0.11 0.05 0.08

Fig 2. Calibration curve of Darunavir

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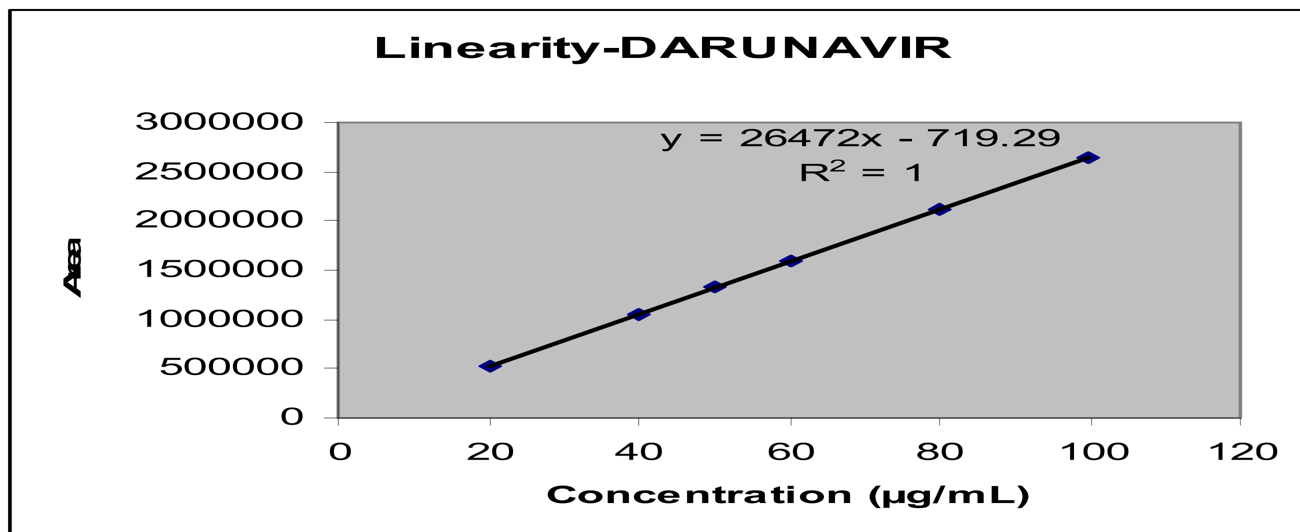

Table 2. Intra and inter day precision for Darunavir

S.No	Intraday	Interday
1	99.6	99.2
2	101.2	100.7
3	98.5	99.1
4	100.2	99.5
5	99.1	98.8
6	99.8	100.2
Mean	99.7	99.6
SD	0.93	0.88
% RSD	0.93	0.89

Table 3. Accuracy of the analysis of Darunavir

Percent level	Added amount[a]	Found amount[a]	% Recovery[a]	RSD (%)[a]
25	25.26	25.05	99.17	0.71
50	50.11	50.90	101.5	0.60
100	99.82	99.14	99.32	0.55

a: Average of three determinations.

CONCLUSION

The HPLC method developed and validated allows a simple and fast quantitative determination of Darunavir from its formulation. A mobile phase composed of 0.1% ortho phosphoric acid and acetonitrile with a short run time (10 min) and isocratic elution used are advantageous and made the routine analysis easy. Among the significant advantages of this method are simplicity, selectivity, accuracy and precision ensuring that it is suitable for determining the content of Darunavir in tablet dosage form. The developed method can also be used for the estimation of darunavir in in-process stages like pre-lubrication, granulation and lubrication.

ACKNOWLEDGEMENTS

The authors are thankful to Hetero Drugs Limited, Hyderabad, India for providing all the necessary facilities to complete this research work.

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