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Research Article

DEVELOPMENT AND VALIDATION OF NOVEL HPLC METHOD FOR ESTIMATION OF DARUNAVIR IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, rapid, accurate and precise RP-HPLC method was developed for the determination of Darunavir in pure and tablet dosage forms. Separation of the drug was achieved on a reverse phase Hypersil BDS C18 Column (250 mm x 4.6 mm, 5µm). The method showed a linear response for concentration in the range of 5-25 µg/mL using phosphate buffer pH 3:acetonitrile as the mobile phase in the ratio of 40:60, v/v with detection at 270 nm with a flow rate of 1 mL/min and retention time was 2.711 min. The method was statistically validated for linearity, accuracy, precision and selectivity. Quantitative and recovery studies of the dosage form were also carried out and analyzed, the %RSD from recovery studies was found to be less than 1. Due to simplicity, rapidity and accuracy of the method, we believe that the method will be useful for routine quality control analysis of Darunavir in pharmaceutical formulations.

Keywords: Darunavir, Estimation, Tablets, RP-HPLC.

INTRODUCTION

Darunavir ethanolate (Figure 1) is an antiviral drug and inhibitor of the human immunodeficiency virus protease[1]. Chemically it is [(1S,2R)-3-[[(4-aminophenyl)sulfonyl](2-methylpropyl) amino]-2-hydroxy-1-(phenylmethyl)propyl]-carbamic acid (3R,3aS,6aR) hexahydrofuro[2,3-b] furan-3-yl ester monoethanolate. Darunavir selectively inhibits the cleavage of HIV-1 encoded Gag-Pol polyproteins in infected cells, thereby preventing the formation of mature virus particles[2]. Darunavir is co-administered with ritonavir and with other antiretroviral agents, is indicated for the treatment of human immunodeficiency virus (HIV-1) infection[3]. A few spectrophotometric[4], HPLC[5-8], LC-MS[9-11], HPTLC[12-13] and electrophoresis[14] methods were reported earlier for the determination of Darunavir in bulk and pharmaceutical dosage forms. In the present study the authors report a rapid, sensitive, accurate and precise HPLC method for the estimation of Darunavir in bulk samples and in tablet dosage forms.

MATERIALS AND METHODS

Instrumentation

The author had attempted to develop and validate a liquid chromatographic method for the determination of Darunavir using an isocratic Waters HPLC system on Hypersil BDS C18 column (250 mm x 4.6 mm; 5 µm). The instrument is equipped with a 2695 binary pump with inbuilt degasser, 2487 Dual absorbance detector and Rheodyne injector with 20 µL sample loop. A 20 µL Hamilton syringe was used for injecting the samples. Data was analysed using Waters Empower 2 software. A double-beam Elico SL-159 UV-Visible spectrophotometer was used for spectral studies. Degassing of the mobile phase was done by using an ultrasonic bath sonicator. A Shimadzu balance was used for weighing the materials.

Chemicals and Solvents

The reference sample of Darunavir was provided as gift sample from Pharma Train, Hyderabad, India. The branded formulation

(tablets) (DARUVIR tablets containing 300 mg of Darunavir) was procured from the local market. HPLC grade acetonitrile were purchased from E. Merck (India) Ltd., Mumbai, India. Potassium dihydrogen phosphate and orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai, India. Milli-Q water was used throughout the experiment was dispensed through 0.22 µ filter of the Milli-Q water purification system from Millipore, Merck KGaA, Darmstadt, Germany.

Preparation of phosphate buffer

2.72 grams of potassium dihydrogen phosphate was weighed and transferred into a 1000 mL beaker, dissolved and diluted to 1000 mL with HPLC water. pH adjusted to 3.0 with orthophosporic acid.

Preparation of mobile phase and diluents

400 mL of the phosphate buffer was mixed with 600 mL of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum. The same mobile phase was used as diluent.

Preparation of standard solution

Accurately weigh and transfer 10 mg of Darunavir working standard into a 10 mL volumetric flask, add about 7 mL of diluent, sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.15 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter.

Preparation of sample solution

Weigh 20 Darunavir tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Darunavir into a 10 mL volumetric flask. Add about 7 mL of diluent, sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 0.15 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter.

Calibration Plot

About 10 mg of Darunavir was weighed accurately, transferred into a 10 mL volumetric flask and dissolved in 7 mL of a 40:60 v/v mixture of phosphate buffer pH 3.0 and acetonitrile. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluents. From this, a working standard solution of the drug (15 µg/mL) was prepared by diluting 0.15 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent. Further dilutions ranging from 5-25 µg/mL were prepared from the stock solution in 10 mL volumetric flasks using the above diluent. 20 µL of each dilution was injected six times into the column at a flow rate of 1.0 mL/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area was found to be linear in the concentration range of 5-25 µg/mL of the drug. The relevant data are furnished in Table-1. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of Darunavir in tablet dosage forms.

Procedure

A mixture of phosphate buffer pH 3.0 and acetonitrile in the ratio of 40:60, v/v was found to be the most suitable mobile phase for ideal separation of Darunavir. The solvent mixture was filtered through 0.45 µm membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 mL/min. The column was maintained at ambient temperature. The pump pressure was set at 800 psi. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. Inject 20 µL of the standard, sample solutions into the chromatographic system and measure the area for the Darunavir peak. The detection of the drug was monitored at 270 nm. The run time was set at 5 min. Under these optimized chromatographic conditions the retention time obtained for the drug was 2.711 min. A typical chromatogram showing the separation of the drug is given in Figure 2.

Validation of the proposed method

The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method as per the ICH guidelines for the estimation of Darunavir[15]. Solution containing 15 µg/mL solution of Darunavir was subjected to the proposed HPLC analysis to check system precision, method precision and intermediate precision of the method and the results are furnished in Table-2 to Table-4. The accuracy of the HPLC method was assessed by analyzing solutions

of Darunavir at 50, 100 and 150% concentration levels by the proposed method. The results are furnished in Table-5. The system suitability parameters are given in Table-6.

Estimation of Darunavir in tablet dosage forms

Commercial formulation of tablets was chosen for testing the suitability of the proposed method to estimate Darunavir in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 10 mg of Darunavir was transferred into a 10 mL volumetric flask and dissolved in 7 mL of a 40:60, v/v mixture of phosphate buffer pH 3.0 and acetonitrile. The contents of the flask were sonicated for 15 min and a further 3 mL of the diluent was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45 µm membrane filter. This solution containing 15 µg/mL of Darunavir was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table-5.

RESULTS AND DISCUSSION

In the proposed method, the retention time of Darunavir was found to be 2.711 min. Quantification was linear in the concentration range of 5-25 µg/mL. The regression equation of the linearity plot of concentration of Darunavir over its peak area was found to be $Y=4754253+129878.16X$ ($r^2=0.999$), where X is the concentration of Darunavir (µg/mL) and Y is the corresponding peak area. The number of theoretical plates calculated was 2533, which indicates efficient performance of the column. The limit of detection and limit of quantification were found to be 0.003 µg/mL and 0.009 µg/mL respectively, which indicate the sensitivity of the method. The use of phosphate buffer pH 3.0 and acetonitrile in the ratio of 40:60, v/v resulted in peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug Darunavir by the proposed HPLC method.

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of Darunavir and can be reliably adopted for routine quality control analysis of Darunavir in its tablet dosage forms.

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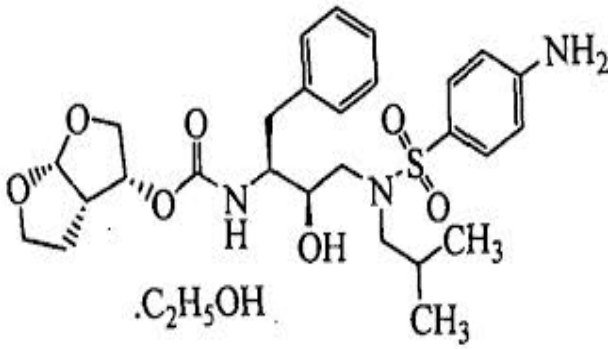

Fig. 1: Chemical structure of Darunavir ethanolate

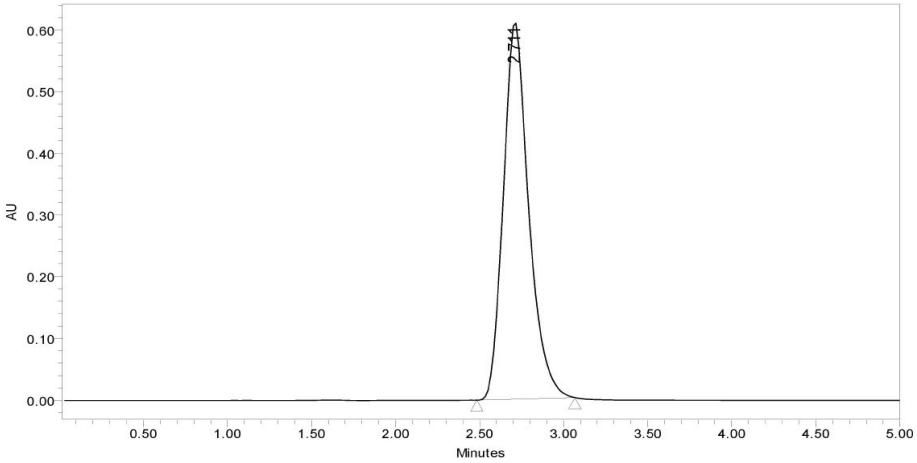

Fig. 2: Typical chromatogram of Darunavir

Table 1: Calibration data

Concentration (µg/mL)	Peak area
5	5392733
10	6078228
15	6672839
20	7379052
25	7989275

Table 2: System precision

Injection number	Peak area of Darunavir
1	6519984
2	6503956
3	6577107
4	6558442
5	6498691
6	6476714
Mean	6522483
SD	38176.4
%RSD	0.59

Table 3: Method precision

Injection number	Peak area of Darunavir
1	6476689
2	6484057
3	6507422
4	6502419
5	6504297
6	6503069
Mean	6496326
SD	12691.5
%RSD	0.20

Table 4: Intermediate precision

Injection number	Peak area of Darunavir
1	6526851
2	6521459
3	6538456
4	6543298
5	6544712
6	6555877
Mean	6538442
SD	12567.0
%RSD	0.19

Table 5: Recovery studies

%Concentration(at specification Level)	Mean peak area	Amount of Darunavir added (mg)	Amount of Darunavir found (mg)	%Recovery	Mean Recovery
50%	3173445	5.0	5.04	100.8%	99.9%
100%	6303182	10.0	10.01	100.1%	
150%	9339052	15.0	14.84	98.9%	

Table 6: System suitability parameters

Parameter	Result
Linearity ((µg/mL)	5-25
Correlation coefficient	0.999
Theoretical plates (N)	2533
Tailing factor	1.24
LOD (µg/mL)	0.003
LOQ (µg/mL)	0.009

Table 7: Assay studies

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
DARUVIR	10	9.93	99.38

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