Research Article

RP-HPLC Method for Determination of Darunavir in Bulk and Pharmaceutical Preparations

Manisha B. Mane*[1], Pranali J. Gaikawad[1], Anuja V. Patil[2], Aashish S. Mogale[3]
[1]Department of pharmaceutical Chemistry, Government College of Pharmacy, Karad, Maharashtra, India.
[2]Department of Biopharmaceutics, Government College of Pharmacy, Karad, Maharashtra, India.
[3]Department of Pharmaceutical Chemistry, Sahyadri College of Pharmacy Methwade, Sangola, Maharashtra, India.
***Corresponding author's E-mail:** manisha.mane09@gmail.com

Accepted on: 30-04-2013; **Finalized on:** 31-07-2013.

ABSTRACT

A Simple, economic, sensitive and reliable reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for Darunavir in tablets. Isocratic chromatography was performed on a C18 column with methanol-acetonitrile 95:5 (v/v) as a mobile phase at a flow rate 0.7ml/min. The effluent was monitored by UV- detector at 264 nm and total run time was 10 min. Calibration curve was linear over the concentration range of 3-27 μg/ml. The method was validated with respect to accuracy, linearity, precision, selectivity and robustness; these parameters examined met the current recommendations of U.S.P for analytical method validation. The developed RP-HPLC method was successfully applied for the quantitative determination of Darunavir in pharmaceutical dosage forms.

Keywords: Calibration curve, Darunavir, Isocratic, quantitative, RP-HPLC, Validation.

INTRODUCTION

Darunavir[1] is an Antiretroviral drug from the protease inhibitor class used to treat HIV infection and AIDS. Nomenclature:- [(1*S*,2*R*)-3-[[(4-Aminophenyl)sulfonyl] (2-methylpropyl)amino]-2-hydroxy -1-(phenylmethyl) propyl] carbamic acid (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-*b*]furan-3-yl ester. Molecular formula is $C_{27}H_{37}N_3O_7S$. Molecular weight is 547.66. Melting point of drug is 74ºC.[2] It is a amorphous white, solid, freely soluble in methanol, acetonitrile and soluble in ethanol. Darunavir contains a bis-tetrahydro-furnanyl (bis-THF) moiety and sulfonamide isostere; the drug is administered as its ethanolate salt. The chemical structure of Darunavir is (figure 1)

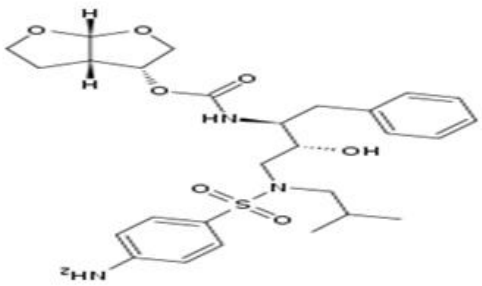

Figure 1: Chemical structure of Darunavir

Literature survey reveals that several analytical methods have been reported for the estimation of Darunavir by UV spectroscopy[3], Infrared spectroscopy,[4] LC-MS,[5] HPLC,[6-10] HPTLC.[11-12] HPLC methods are developed on Darunavir ethanolate but not developed on Darunavir. So aim of the present study to develop a simple, rapid method on Darunavir. Therefore, in present investigation an attempt has been made to determine Darunavir in dosage form and in bulk using RP-HPLC without internal standard but using a simple mobile phase composition.

MATERIALS AND METHODS

Chemicals and Reagents

An analytically pure sample of Darunavir was obtained as a gift sample from Cipla lab Pvt. Ltd., Mumbai, India. High purity water was prepared by using Millipore Milli-Q plus water purification system. HPLC grade methanol and acetonitrile was procured from Loba Chemie. Tablet formulation Daruvir (Cipla) were obtained from a local pharmacy market with labeled amount 300 mg per tablet.

Instrumentation

The instrument used were HPLC, pH meter, Electronic analytical balance (AND) and Sonicator. The HPLC System details are as follows: Instrument- High Performance Liquid Chromatography JASCO- LC Net II / ADC; Injector- Rheodyne; Column- GraceSmart C_{18} RP Column (250 × 4.6 mm, 5μm particle diameter); Mixing Module- JASCO MX-2080-31 PLUS; Detector- JASCO UV 2075 PLUS Intelligent UV/Vis; Pump- JASCO PU- 2080 PLUS Intelligent HPLC Pump. Data acquisition was performed by the Browin software.

Chromatographic Conditions

The mobile phase consists of methanol:acetonitrile (95:5v/v) and set at a flow rate of 0.7ml/min. The mobile phase was filtered through 0.45μm membrane filter and then sonicated as degassed for 15 min. After that it can be set for pumping into HPLC system. The effluent was monitored by UV detector at 264 nm.

Preparation of Solutions

Preparation of working stock solution

Stock solution of Darunavir was prepared by dissolving 10mg drug in sufficient HPLC grade methanol, sonicated

and then make the volume upto 100 ml. The concentration of stock solution was found to be 100µg/ml.

Preparation of Standard drug solution

The working standard solutions of Darunavir was prepared by taking suitable aliquots of drug solution from the working stock solution and the volume was made up to 10 ml with mobile phase to get concentrations of 5 to 35 µg/ml (5,10,15,20,25,30,35). The solutions were filtered through 0.45 µ membrane filter before injection and 20 µl of solution was injected to the chromatographic system.

Preparation of pharmaceutical sample solution

For the preparation of sample solutions, twenty tablets were weighed, powder was collected and mixed. A quantity equivalent to 10mg of Darunavir was transferred into 100ml volumetric flask, HPLC Grade methanol was added and shaken to dissolve the contents. The flask was subjected to sonication for 15 minutes. The solution was filtered using filter paper 0.41µ. From this, different aliquots were taken in separate 10ml volumetric flasks. The contents of the flasks were made up to the volume with methanol to get 20 µg / ml concentration and mixed well. The solutions were filtered through 0.45 µ membrane filter before injection and 20 µl of solution was injected to the chromatographic system

RESULTS AND DISCUSSION

Optimization of the chromatographic condition

In order to develop a suitable and robust liquid chromatography method for the determination of Darunavir, various chromatographic conditions were employed using different mobile phases. The method involved a mobile phase consisting of methanol: acetonitrile (95:5v/v) at a flow rate of 0.7ml/min was found to be satisfactory and gave well resolved peak for Darunavir. A UV scan was performed and 264 nm was selected as a detection wavelength and it is a λ_{max} of the drug. The retention time was 3.45 min and total run time for an assay was 10 min.

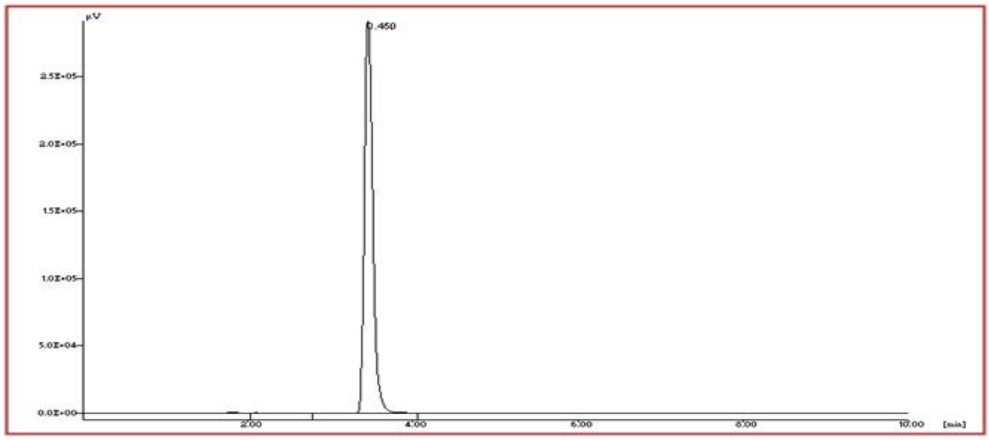

Figure 2: HPLC Chromatogram of Darunavir at 264 nm

Method Validation of Drug

System Suitability

System suitability was performed before each validation run, five replicate injections of system suitability were performed. Retention time, area, asymmetry, theoretical plates and tailing factor for the five suitability injections were determined (Table 1).

Table 1: System Suitability for RP-HPLC method

Parameters	Observed value (Mean)	Expected value
Retention time [Rt]	3.45	
% RSD for Rt	0.7936	% RSD ≤ 1
Theoretical plates [n]	8078.66	n > 2000
Theoretical plates per meter [N]	1,24,850	N > 13333.33
Height equivalent to theoretical plates [HETP] (mm)	0.0002 mm	HETP < 0.075 mm
Tailing factor [T]	1.0	T ≤ 2

Linearity

The linearity of response for Darunavir assay method was determined by preparing and injecting solution with a concentration of about 5, 10, 15, 20, 25, 30 and 35µg/ml of the drug. The linearity of peak area responses Vs concentration was studied and a calibration curve was plotted. It shows that Darunavir have linearity in the range of 5-35 ppm. The results have been shown in table 2, 3 and figure 3.

Accuracy

Recovery study was employed to determine the accuracy of the proposed methods. Recovery experiment was carried out at different levels of drug concentrations (50 %, 100 % and 150 % of targeted drug concentration) which were analyzed six times. Accuracy was assessed as the % Recovery at each concentration level with mean % Recovery. Data obtained from accuracy study is given in Table 4. The results obtained indicated that recovery was excellent, not less than 100%± 2.

Table 2: Data of Calibration Curve for DAR by RP-HPLC method

Conc. (µg / ml)	AUC
5	71041.8
10	432683.5
15	813361.3
20	1215585.5
25	1632082.00
30	2060236.00
35	2408629.00

Table 3: Statistical data of linearity for Darunavir by RP-HPLC method

Parameters	RP-HPLC Method
Detection wavelength (nm)	264
Beer's law limits (µg / ml)	05 – 35
Regression equation [Y]	Y= 79190X - 35042
Slope [b]	79190
Intercept [a]	35042
Correlation coefficient [r^2]	0.999

Table 4: Accuracy result for Darunavir

No of Preparation				Statistical Analysis		
Recovery level	Formulation	Pure Drug	% Recovery	Mean	SD	%RSD
50%	10	5	101.4	100.67	0.550	0.545
50%	10	5	100.4			
50%	10	5	100.5			
100%	10	10	100.6	100.64	0.115	0.1144
100%	10	10	100.4			
100%	10	10	100.4			
150%	10	15	100.2	100.68	0.665	0.659
150%	10	15	100.3			
150%	10	15	100.1			

Table 5: Inter-day and intra-day Precision results for Darunavir

Method Precision	Conc. (µg /ml)	Day	Amount found in µg / ml (Mean)	% Recovery (Mean)	SD	% RSD
Interday Precision*	20	1st	20.14	100.72	0.3652	0.3626
		2nd	20.17	100.85	0.3357	0.3329
		3rd	20.18	100.91	0.3210	0.3181
		Mean	20.16	100.82	0.3406	0.3378
Intraday Precision#	20	-	20.16	100.86	0.3280	0.3261

*Mean SD are obtained from 6 determinations (6 determinations per day).
#Mean SD are obtained from 18 determinations

Table 6: Ruggedness studies of Darunavir by RP- HPLC method

Analyst- 1				Analyst -2			
Conc. (µg/ml)	AUC	Calc. Amt.	Statistical Analysis	Conc. (µg/ml)	AUC	Calc. Amt.	Statistical Analysis
20	1548585.5	19.99	Mean= 19.90 S.D= 0.26 %RSD= 1.30	20	1556511.3	20.09	Mean= 20.08 S.D= 0.045 %RSD= 0.22
20	1516550.2	19.59		20	1556600.0	20.09	
20	1515600.8	19.58		20	1560551.1	20.14	
20	1546567.3	19.97		20	1549516.4	20.00	
20	1556510.5	20.09		20	1556510.9	20.09	
20	1565585.5	20.21		20	1556600.5	20.09	

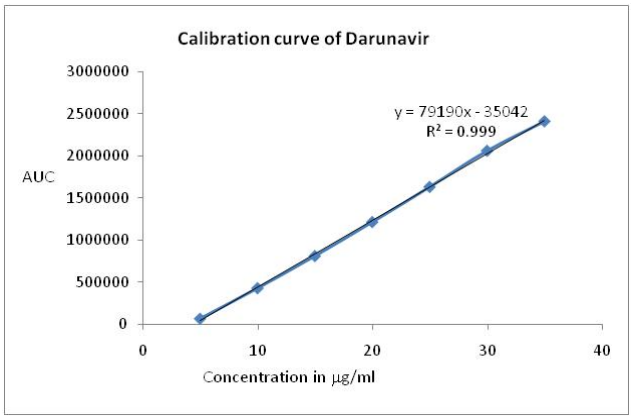

Figure 3: Calibration curve of Darunavir at 264 nm

Precision

The precision of the method was obtained by inter-day and intra-day variation studies. In the intra- day studies, six injection of standard solution were injected into the chromatographic system in different time interval within a day. In the inter-day variation studies, six injections of standard solution were injected at different days. %RSD was calculated presented in Table 5.

Sensitivity

Limit of detection (LOD) and limit of quantification (LOQ) were estimated from the signal-to-noise ratio. The LOD was defined as the concentration level corresponding to peak area of three times the baseline noise. The LOQ was defined as the lowest concentration level of a peak area with a signal-to-noise ratio higher than 10. LOD and LOQ values were found to be 0.00161 and 0.00487µg/ml respectively.

Reproducibility (Ruggedness)

Ruggedness study was performed to establish the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions. In ruggedness study of the proposed method, assay of the branded DAR tablets were performed by two different analysts on two different days. % Recovery with SD and % RSD for tablet were calculated and illustrated in Table 6. Result of this study also represents the assay results of DAR tablet, Daruvir (Cipla).

CONCLUSION

A rapid, accurate, fast and precise isocratic RP-HPLC method has been developed. The developed method was found to be simple and have short run time which makes the method rapid. The chromatographic run time of 10 min allow analysis of lot of samples in a short period of time. Therefore, the method is suitable for analysis of large samples during routine analysis of formulations and raw materials. Nevertheless, the results of the study indicate that the developed HPLC method is simple, precise, accurate and less time consuming.

Acknowledgment: Authors are thankful to Cipla Pvt. Ltd., Mumbai for providing gift sample of Darunavir. Authors are also thankful to Government College of Pharmacy, Karad for their help and support.

REFERENCES

1. http://www.rxlist.com/ norvir-drug.htm.
2. Goldwirt L, Chhuna S, Rey E, Launay O, Viard P, Pons p, Jullien V, Quantification of Darunavir (TMC114) in human plasma by high-performance liquid chromatography with ultra-violet detection, Journal of Chromatography B, 857, 2007, 327-331.
3. Shinde VR, Gosavi SA, Pawar SS, Kasture VS, Musmade DS, Development and validation of uv spectroscopic method for determination of Darunavir in bulk and tablet formulation, Inventi Rapid: Pharm Analysis & Quality Assurance, 1, 2013, 586-587.
4. Ana Carolina Kogawa, Hérida Regina Nunes Salgado, Development and Validation of Infrared Spectroscopy Method for the Determination of Darunavir in Tablets, Journal of Physical Chemistry, 3, 2013, 1-6.
5. Kanneti R, Jaswanth KL, Neeraja KR, Bhat PA, Development and validation of lc-ms/ms method for determination of Darunavir in human plasma for application of clinical pharmacokinetics, International Journal of Pharmacy and Pharmaceutical Sciences, 3(5), 2011, 0975-1491.
6. Patel BN, Suhagia BN, Simultaneous Determination And Validation Of Darunavir Ethanolate And Ritonavir In Binary Mixture By Liquid Chromatography, International Journal of PharmTech Research, 4, 2012, 1450-1456.
7. Patel BN, Suhagia BN, Patel CN, RP-HPLC method development and validation for estimation of Darunavir ethanolate in tablet dosage form, International Journal of Pharmacy and Pharmaceutical Sciences, 4(3), 2012, 270-273.
8. Ganduri RB, Lanka RA, Pamidi, Peddareddigari JR, Rao JVLNS, New RP-HPLC method development of Darunavir in tablet dosage form, Asian Journal of Pharmaceutical Research, 1(1), 2011, 10-14.
9. Satyanarayana L, Naidu SV, Rao MN, Kumar A, Suresh K, The Estimation of Darunavir in Tablet dosage form by RP-HPLC, Asian Journal of Research pharmaceutical Science, 1(3), 74-76.
10. Rami BV, Reddy G. Jyothi , Reddy BS, Raman N.V.V.S.S, K. Reddy SC , Rambabu C, Stability-Indicating HPLC Method for the Determination of Darunavir Ethanolate, Journal of Chromatographic Science, 2012, 471-476.
11. Ramesh, Bokka, Ramakrishna, Sisla, Reddy, Rentam Kiran Kumar, babu, Kothapalli Hari, Sarma, Vanka Uma Maheswara Devi, Potturi Sita, HPTLC method for determination of Darunavir in rat plasma and its application in pharmacokinetic studies, Journal of Liquid Chromatography & Related Technologies, 36(2), 2013, 167-179.
12. Kiado A, A simple and sensitive HPTLC method for quantitative analysis of Darunavir ethanolate tablets, Journal of Planar Chromatography - Modern TLC, 24(3), 2011, 232-235.
13. International Conference on Harmonization, Topic Q2B, Validation of Analytical Method: Methodology, ICH topic Q2B, the European Agency for the Evaluation of Medical Products, 1996.

Source of Support: Nil, **Conflict of Interest:** None.