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Research Article

NEW VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF DARUNAVIR IN BULK AND ITS DOSAGE FORM

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ABSTRACT
Present study describes the development and subsequent validation of reverse phase high performance liquid chromatographic (RP-HPLC) method for estimation of Darunavir (Figure 1) a retroviral drug in bulk and its formulation with greater precision and accuracy. Separation was achieved on C_{18} column (250 x 4.6 mm id.5 µm) in isocratic mode using water: methanol (0.2 % TEA) in the ratio of 30 : 70 (v / v) pH adjusted to 3 as mobile phase, pumped into column at flow rate 1.0 ml / min and the detection of eluent was carried out at 262 nm. Retention time was obtained at 5.2 minutes; total run time was set to 8 minutes. The standard curves were linear over the concentration range of 5-50 µg / ml with correlation coefficient 0.9995 and the LOD and LOQ values were 0.48 µg / ml and 1.5 µg / ml respectively. The percentage recovery was found to be within 98.2 % - 101.2 %. The % RSD of intra-day and inter-day precision was found to be 0.69 and 1.3 for the assay concentration respectively. The percentage amount of marketed tablet formulation of Darunavir was found to be 99.66 %. The robustness of method has been studied by slightly varying the chromatographic conditions. Validation studies demonstrated that proposed RP-HPLC method is simple, specific, rapid, reliable and reproducible.
Keywords: Darunavir (DRN), RP-HPLC, validation, pharmaceutical dosage form.

INTRODUCTION

Darunavir is chemically (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl N-[(2S,3R)-3-hydroxy-4-[N-(2-methylpropyl)(4 aminobenzene)sulfonamido]-1-phenylbutan-2-yl]carbamate.
It is white to off-white powder that is very slightly soluble in water and soluble in methanol[1,2]. DRN is generally co-administered along with Ritonavir (100 mg)[3]. Darunavir is an inhibitor of Dimerisation and the catalytic activity of the HIV-1 protease. It selectively inhibits the cleavage of HIV encoded Gag-Pol polyproteins in the virus infected cells, thereby preventing the formation of infectious virus particles[4,5]. Proposed method is validated as per ICH guideline for Analytical Procedures[6]. Recent studies suggested that protease inhibitors like Darunavir, Ritonavir, Nelfinavir showed no evidence of antagonism when used with TMC114[7,8]. Literature survey reveals that there are reports describing the determination of Darunavir in Plasma using liquid chromatography coupled with Tandem Mass Spectroscopy[9], few HPTLC method for determination of Darunavir in rat plasma and in tablet dosage form its application to pharmacokinetic studies[10,11], infrared spectroscopy method for determination of Darunavir in tablets[12], and few RP-HPLC and Spectrophotometric methods[13,14]. The focus of present study is to develop and validate a rapid, stable and economic High performance Liquid Chromatographic method for Quality Control of DRN in tablet dosage form.

Experimental

Chemicals and Reagents

Working standard pure sample (99.97 %) was obtained as a gift sample from Hetero Drugs Ltd (Hyderabad, India) and commercial dosage form was obtained from local market. HPLC Grade methanol and water are from E. Merck, Mumbai, India. All other chemicals and reagents used were of AR grade.

HPLC instrumentation and Chromatographic Conditions

Proposed method was performed on Agilent LC 1200 Equipment comprising quaternary pump, degasser and Diode Array detector. Chromatographic separation was achieved at ambient temperature on column C_{18} (250 x 4.6, 5 µm). A Rheodyne injector fitted with a 20 µl loop and data recorded on EZ CHROME ELITE software were used. The flow rate and Run time was set to 1ml / min and 8 minutes respectively. Analytical balance Keroy and pH meter systronics 802 were used.

Mobile Phase Preparation

The mobile phase was a mixture of water: methanol (0.2 % TEA) 70 : 30 v / v and pH adjusted to 3 was filtered and degassed for 15 minutes prior to use.

Preparation of Standard Solution

10 mg of DRN working standard was accurately weighed and transferred into 10 ml volumetric flask, and then small amount methanol was added and ultrasonicated for 5 minutes and diluted up to the mark with methanol to get a stock solution of 1000 µg / ml. From this 5 ml was transferred into 50 ml volumetric flask and made up the volume with mobile phase (concentration 100 µg / ml). From this dilutions were made to get concentrations 5-50 µg / ml. 20 µl of each solution was injected to column. The calibration curve was constructed by plotting peak area of analytes versus their respective drug concentrations.

Sample Preparation

An accurately weighed portion of powder equivalent to 10 mg of Twenty tablets of PREZISTA (containing 300 mg of DRN) were weighed and was transferred to 10 ml standard

volumetric flask and small amount methanol was added. The solution was sonicated for 15 minutes and the final volume was made with methanol to obtain solution of DRN (1000 µg / ml). The mixture was then filtered through a nylon 0.45 mm membrane filter. The above solution was suitably diluted with mobile phase to obtain final dilution of DRN (20 µg / ml).

Table 1: Linearity of Darunavir

S. No	Concentration	Absorbance
1.	5	252605
2.	10	550598
3.	20	1105544
4.	30	1631730
5.	40	2244120
6.	50	2827423

Table 2: Results from Accuracy Studies

Concentration taken (pre analysis)	Recovery level	Amount added (µg / ml)	Amount found (µg / ml) mean*	% Recovery
20 µg / ml	80 %	16	35.8	98.7
20 µg / ml	100 %	20	40.2	101
20 µg / ml	120 %	24	43.9	99.5

*Average of three determinations (n = 3)

Table 3: Assay of Darunavir

Formulation Name	Labelled amount in mg	Assay concentration (µg / ml)	Amount found (%) Mean*	% RSD
Prezista	300	20	99.66	0.76

*Average of six determinations (n=6)

Table 4: Results from Precision Studies

Concentration (µg / ml)	Intra-day precision		Inter-day precision	
	*mean ± SD	% RSD	*mean ± SD	% RSD
10	540982.26 ± 2467.78	0.46	550965.21 ± 4498.23	0.81
20	1119196.5 ± 7772.46	0.69	1128637.16 ± 15298.42	1.3
30	1633845.16 ± 7490.82	0.45	1733845.45 ± 10981.2	0.63

*Average of six determinations (n=6)

Table 5: LOQ and LOQ of Darunavir

STD Solution	LOD (µg / ml)	LOQ (µg / ml)
Darunavir	0.48	1.5

Table 6: Robust Values of Darunavir

Parameter	Condition	Rt	Area	% Assay
Flow rate	0.9	5.7	2010431	105 %
	1.1	4.7	1820734	95.4 %
Mobile phase	28:72	4.8	1935624	101.4 %
	32:68	5.8	1899629	99.5 %
Wavelength	265	5.2	1912204	100.2 %
	258	5.2	1938762	101.6 %

Table 7: Results from Ruggedness Studies

S. No.	Assay concentration	Amount found (%)	Mean* ± % RSD
Analyst 1	20 µg / ml	99.66 %	0.76
Analyst 2	20 µg / ml	101.4 %	0.78

*Average of three determinations (n = 3)

Table 8: System Suitability Parameters

S. No.	Parameter	Values*
1.	Retention time	5.238
2.	Theoretical plates (N)	6498
3.	Tailing factor (T)	1.12
4.	Asymmetry factor(As)	1.09
5.	LOD (µg / ml)	0.46 µg / ml
6.	LOQ (µg / ml)	1.65 µg / ml

*Average of six determinations (n = 6)

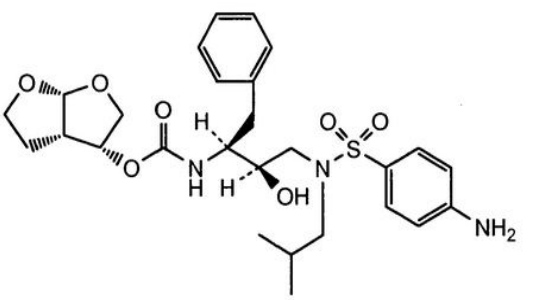

Figure 1: Structure of Darunavir

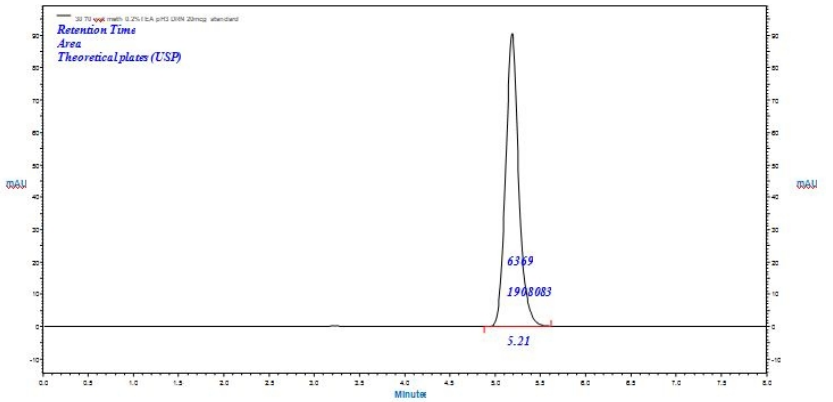

Figure 2: HPLC Chromatogram of Darunavir (standard)

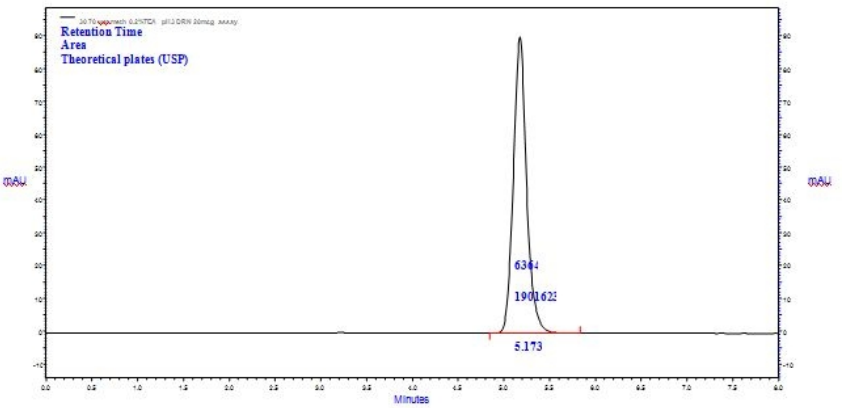

Figure 3: HPLC Chromatogram of Darunavir (formulation)

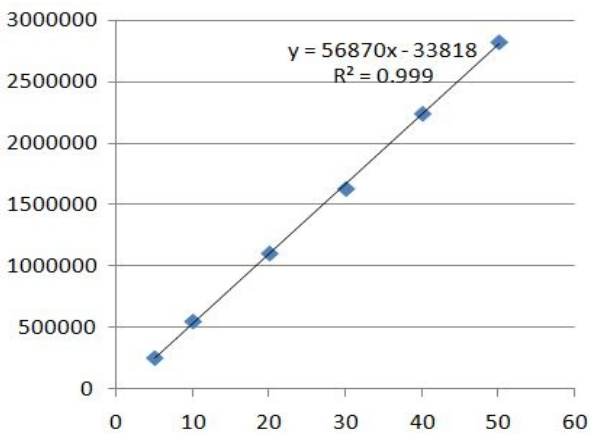

Figure 4: Calibration curve of Darunavir

RESULT AND DISCUSSION

In this RP-HPLC method, chromatographic conditions were optimized to obtain best peak shape and resolution. Mobile phase selection of is based on the peak parameters like symmetry, theoretical plates, capacity factor tailing factor and ease of preparation and cost. Chromatograms of Standard and Formulation are given below in Figure 2 and Figure 3 respectively. The optimum wavelength for detection and quantification was set at 262 nm.

Method Validation

Method was validated according to ICH guidelines for validation of analytical procedures[6].

Calibration curve and linearity

Calibration curve was plotted over the concentration range of 5-50 µg / ml for DRN with regression coefficient [r^2] 0.999. Linearity was calculated by least square linear regression analysis of calibration curve. The constructed calibration curve was linear over the concentration range of 5-50 µg / ml. The linear regression equation was

$$y = 56870x - 33818.$$

Accuracy

Accuracy of the method was checked by Recovery studies by adding known amount of standard to pre-analyzed sample. Studies were carried out at three different levels (80 %, 100

%, 120 %). The proposed method affords recovery values within 98-102 % shown in Table 2.

Precision

The proposed method was tested by performing intra-day and inter-day studies. Data is summerized in Table 4. The intra-day was carried out by analyzing standard solution of 20 µg / ml for six times on the same day and inter-day was carried out over a period of six days. Value of % RSD for inter-day and intra-day were found to be within limits.

LOD and LOQ

The sensitivity of the method was estimated in terms of Limit of Detection and Limit of Quantification by using formulae LOD= 3.3σ/S and LOQ= 10σ/S. where, S is average of slope corresponding to calibration plot and σ is standard deviation of intercept in calibration plot, and the obtained values were tabulated below in Table 5.

Robustness

The robustness of the proposed method was checked by making small delibrate changes in mobile phase, flow rate and wavelength. By making these small changes showed no significant change in peak arae for estimation of Darunavir. The values given in Table 6 indicates the method is robust.

Ruggedness

It is the reproducibility of a test result under operating condition from instrument to instrument and from analyst to analyst. The ruggedness of the method was performed by comparing the assay results by two analysts in the same laboratory and the results presented in Table 7.

System Suitability

It is to ensure the adequate performance of the chromatographic system. System suitability parameters like Retention time, number of theoretical plates (N), tailing factor (T), and peak asymmetry (As) were evaluated for six replicate injections on assay concentration 20 µg / ml. Results are presented in below Table-8.

CONCLUSION

A simple, precise, selective and sensitive RP- HPLC assay method with DAD detection for Darunavir in pharmaceutical dosage form has been developed and validated. Hence the proposed method can be extensively applied for general quality control analysis of Darunavir in bulk and tablet dosage form.

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