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Original Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF DARUNAVIR ETHANOLATE IN BULK AND TABLETS

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ABSTRACT

Objective: A new precise, accurate, sensitive and robust RP-HPLC method was developed for estimation of darunavir ethanolate in bulk and tablets.

Methods: The chromatographic separation was achieved on Enable C_{18} column (250 × 4.6 mm, 5 µm) at an ambient temperature. The mobile phase consists of acetonitrile and 0.01M potassium acetate buffer, pH 5.1 (75:25 v/v) was at the flow rate 1 ml/min and UV detection was done at 268 nm.

Results: The method was linear over the concentration range of 40-90 µg/ml (r^2= 0.998) of the drug. The percentage content was found in darunavir ethanolate 99.19±0.58 in tablets. The low value of the drug %RSD (0.11) indicates that reproducibility of this method. Low value of LOD and LOQ suggests the sensitivity of the method.

Conclusion: It can be concluded from the results that the proposed RP-HPLC method was found to be rapid, simple, accurate, robust and precise for the analysis of darunavir ethanolate in bulk and tablet dosage form. The developed method can be applied in routine analysis of this drug in the pharmaceutical industry.

Keywords: Darunavir ethanolate, Validation and RP-HPLC.

INTRODUCTION

Darunavir ethanolate is an oral anti-retroviral agent which selectively inhibits the cleavage of Human immunodeficiency virus (HIV-1) encoded Gag-polyproteins in infected cell, thereby preventing the formation of mature virus. Darunavir ethanolate has robust interaction with the protease enzyme from many strains of HIV. It blocks HIV protease enzyme which is needed for HIV to multiply. Darunavir Ethanolate is chemically [(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 [1] (fig. 1). Literature survey revealed that some methods had been developed for determination of darunavir ethanolate by HPLC [2-4], HPTLC [5] and Spectrophotometric method [6]. Reported HPLC methods require more time for analysis of one sample and less number of samples may be analyzed. Therefore, the present work involves the development of a rapid RP-HPLC method for estimation of darunavir ethanolate in bulk and tablet dosage form and may be suitable for an industry as more number of samples can be analyzed in the given time period.

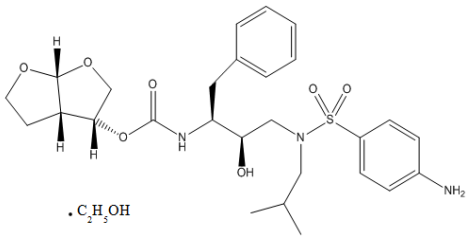

Fig. 1: Chemical structure of darunavir ethanolate

MATERIALS AND METHODS

Chemicals and reagents

The reference standard of darunavir ethanolate was provided by Cipla Ltd, Aurangabad, India "Daruvir®-300 mg" tablets were procured from local pharmacies. The acetonitrile (HPLC grade) and potassium acetate of (AR grade) was provided by hind media Lab Pvt. Ltd, Mumbai, India.

Instruments

Shimadzu LC2010 HPLC system, Elga Lab water (PURE LAB UHQ-II) water purification system for HPLC study. Shimadzu model AY-120 balance, Enable C_{18} column, LC solution software was used.

Preparation of standard stock solutions

Darunavir ethanolate (100 mg) was transferred to 100 ml of the volumetric flask, dissolved in acetonitrile and diluted up to the mark. The standard stock solution was further diluted to obtain six different concentrations between 40-90µg/ml. The 20 µl volume of each solution was injected into the HPLC system under the optimized chromatographic conditions.

Assay of tablet formulation

20 tablets of "Daruvir®-300" were weighed correctly and crushed to fine powder using a glass mortar and pestle. A portion equivalent to about 100 mg of darunavir ethanolate was correctly weighed and transferred to 100 ml volumetric flask. The powder was dissolved in acetonitrile, sonicated for 10 min and diluted up to the mark. The solution was further diluted with acetonitrile and filtered using what man filter paper no. 41. The filtrate was diluted to obtain 70µg/ml of drug and injected (in triplicate) to HPLC system.

RESULTS AND DISCUSSION

Selection of detection wavelength

Analytical wavelength was selected by taking the absorption spectrum of darunavir ethanolate which gave λmax of darunavir ethanolate. A Stock solution of the drug was prepared in acetonitrile and a UV spectrum of 100 µg/ml solution of darunavir ethanolate was scanned in the range 200-400 nm. UV spectrum (fig. 2) showed maximum absorbance at 268 nm. Hence, wavelength 268 nm was selected for the further studies.

Optimization of chromatographic conditions

The HPLC method was optimized with a view to develop an assay method for darunavir ethanolate. Initially, different columns,

different combinations of mobile phases such as methanol: water and Acetonitrile: Potassium Acetate in different proportions, were tried at different flow rates. Finally, A mobile phase consisting acetonitrile and 0.01M potassium acetate buffer, pH 5.1 (75:25 v/v) at the flow rate 1 ml/min was selected. The injection volume was 20 µl. The analysis was performed at an ambient temperature with UV detection at 268 nm. The Chromatogram of darunavir ethanolate is shown in fig. 3 and the optimized chromatographical conditions are mentioned in table 1. The retention time of the drug is 3.85 min under optimized chromatographical conditions.

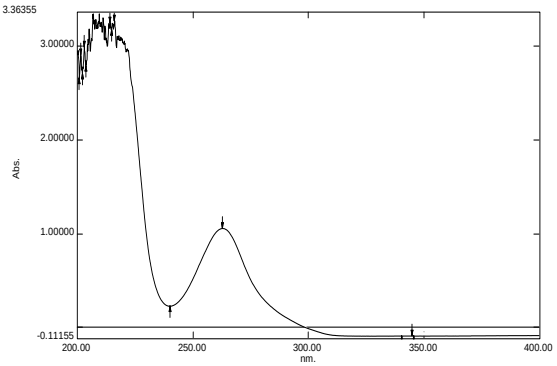

Fig. 2: UV-Spectrum of darunavir ethanolate in acetonitrile (100µg/ml)

Table 1: Optimized chromatographic conditions

Parameters	Details
Mobile phase	Acetonitrile: potassium acetate (75:25 v/v) (pH 5.1)
Column	Enable C_{18}, 250 mm x 4.6 mm, 5 µm
Flow rate	1.0 ml/minute
Detection	UV at 268 nm
Column oven temperature	Ambient
Injection volume	20 µl
Run time	10 min
Retention time (min.)	3.85+0.02
Diluent	Acetonitrile: potassium acetate (75:25 v/v) (pH5.1)

Assay of tablet formulation

The value of % drug found to be 100.3±0.58 in assay study which is satisfactory. The results of assay study are shown in table 3.

Method validation [7-9]

Validation of an analytical procedure is the process by which it is established by laboratory studies that the performance characteristics of the procedure meet the requirements for the intended analytical application. The developed chromatographic method was validated for system suitability, linearity and range, accuracy, precision, and robustness as per ICH guidelines [10].

Linearity and range

Linearity was evaluated on a set of six standard solutions containing 40-90 µg/ml, for darunavir ethanolate. The relationship between peak area (as a dependent variable) and concentration of the drug in the solution (as an independent variable) was established by the simple linear regression method. Calibration curve was constructed by plotting an average peak area vs concentration as shown in fig. 4. The regression equation was obtained as a calibration curve.

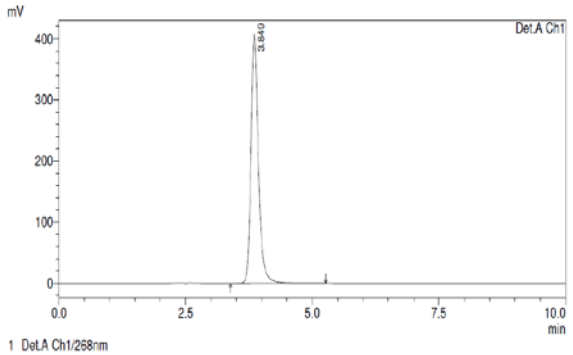

Fig. 3: Chromatogram of darunavir ethanolate

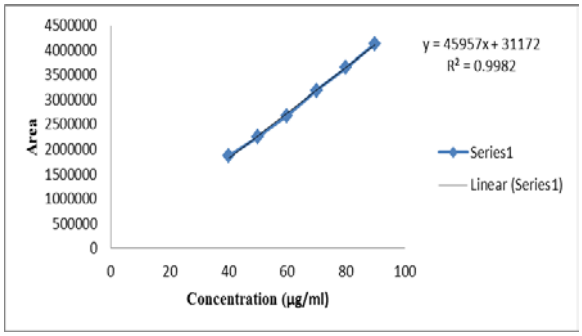

Fig. 4: Calibration curve of darunavir ethanolate

Table 3: Results of assay of darunavir ethanolate

S. No.	Sample solution concentration (µg/ml)	Sample solution area	Mean sample solution area	%Drug found
1	70	3188057	3182058	100.3±0.58
2	70	3180406		
3	70	3177712		

The value of R^2 is found to be 0.9982, which indicates that the calibration curve is linear in the concentration range of 40-90 µg/ml.

Limit of detection (LOD) and limit of quantification (LOQ)

Five sets of known concentrations (40-90 µg/ml) were prepared. Calibration curves were plotted for each set. LOD and LOQ were calculated using the following formulae.

$$LOD = 3.3\frac{SD}{S}$$

$$LOQ = 10\frac{SD}{S}$$

Where, SD = Standard Deviation of response

S = Average of the slope of the calibration curve

The LOD and LOQ were calculated as 0.234 µg/ml and 0.734 µg/ml, respectively, which indicate the method is sensitive.

Precision

(i) Repeatability

In repeatability, six standard solutions were prepared each having a concentration of 70µg/ml of darunavir ethanolate. The response of each of these solutions was measured and percentage relative

standard deviation (% RSD) was calculated. The results are mentioned in table 4.

(ii) Intermediate precision

For intraday precision, nine different solutions were prepared across the intended range (60, 70 and 80 µg/ml) with three replicates of each, and their area was measured on the same day. For interday precision, nine different solutions were prepared across the intended range (60, 70 and 80 µg/ml) with three replicates of each, and their corresponding area was measured on three subsequent days. The results were reported in terms of the relative standard deviation (RSD) table 5(a) & 5(b).

Table 4: Results for method precision (repeatability)

Precision	Amount (µg/ml)	Area	Mean area±SD	%RSD
	70	3144329		
Repeatability	70	3179660	3169144+3289.592	0.0445
	70	3173584		
	70	3174938		
	70	3181435		
	70	3160917		

Table 5 (a): Intraday precision studies

Precision	Amount (µg/ml)	Area	Mean area±SD	%RSD
Intra-day	60	2795891	2788966±6176.946	0.2214
(n=3)	60	2786981		
	60	2784025		
	70	3188057	3182058±5366.788	0.1686
	70	3180406		
	70	3177712		
	80	3594261		0.336
	80	3571863	3585630±12049.74	
	80	3590765		

Table 5 (b): Inter-day precision studies

Precision	Amount (µg/ml)	Area	Mean area±SD	%RSD
Inter-day	60	2795890	2788985±6152.44	0.2205
(n=3)	60	2786981		
	60	2784085		
	70	3144329	3165858±18890.26	0.5966
	70	3179660		
	70	3173584		
	80	3590352		
	80	3575779	3585867±8754.01	0.2441
	80	3591469		

The value of % RSD in repeatability, intraday precision and intraday precision indicate that method is precise.

Table 6: Results of recovery study for darunavir ethanolate

Spike level	Amount added (µg/ml)	Area	Amount recovered (µg/ml)	% Recovery	Mean % recovery±S. D	% RSD
		3373533	72.72	101.01	100.33+0.583124	0.58
80%	72	3351109	72.24	100		
		3345048	72.10	100		
		3579287	77.20	96.5	96.60+0.115902	0.11
100%	80	3587541	77.38	96.73		
		3582467	77.27	96.59		
		3973756	85.78	97.47	97.19+0.275015	0.27
120%	88	3962042	85.53	97.19		
		3951109	85.29	96.92		

Table 7: Robustness data for darunavir ethanolate

Factor	Level	%RSD
A: Change in flow rate		
0.9	-1	0.3149
1	0	0.3147
1.1	+1	0.6244
B: Change in temperature		
24 ° C	-1	0.2501
25 ° C	0	0.2502
26 ° C	+1	0.2471
C: Change in wavelength		
267 nm	-1	0.7322
268 nm	0	0.7279
269 nm	+1	0.7229

Robustness

Robustness of the above method was carried out by purposefully varying some chromatographic method parameters. These parameters include changes in the flow rate (0.9 ml and 1.1 ml), temperature (24 ˚C and 26 ˚C) and wavelength (267 nm and 269 nm). The results obtained by changing these conditions are obtained in terms of % RSD values. The values % RSDs are given in table 7. These values are within acceptance an criterion which indicates that the developed method is robust.

Accuracy

Recovery studies were performed at 80.0%, 100.0% and 120.0% levels using the standard addition method. Standard drug solution of darunavir ethanolate was spiked at 80.0%, 100.0% and 120.0%. For each level, three replicate were prepared and injected in the column under optimized HPLC conditions.

The calculation for accuracy of darunavir ethanolate was performed and the results are expressed in terms of % recovery±SD and % R. S. D values (table 6). The values of % recovery at each level are found within acceptance criteria that indicate the method is accurate.

CONCLUSION

The present work represents the report that deals with analysis of darunavir ethanolate in bulk and tablet dosage forms using RP-HPLC. It can be concluded from the results that the proposed method is simple, accurate, robust and precise.

This method was validated as per ICH guidelines. Thus, it can be used for routine quality control studies for assay of darunavir ethanolate.

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CONLFICT OF INTRESTS

Declared None

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