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Der Pharmacia Lettre, 2016, 8 (5):291-296
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

RP-HPLC method development and validation for quantification of darunavir ethanolate in tablet dosage form

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for quantification of Darunavir ethanolate in bulk and its tablet dosage form. The separation was carried out on Shiseido C8 (250 × 4.6mm; 5µm) column at ambient temperature using phosphate buffer pH 3: acetonitrile (40:60) as eluent. The flow rate was 1.0 ml/min and effluent was detected at 270 nm. The retention time of Darunavir ethanolate was 4.30 min. The percentage recovery was within the range between 99.46% and 100.17% for Darunavir ethanolate. The linear ranges were found to be 5-15µg/ml (r^2 = 0.9997) for Darunavir ethanolate. The percentage relative standard deviation for accuracy and precision was found to be less than 2%. Hence, the method could be successfully applied for routine analysis of Darunavir ethanolate in pharmaceutical formulations.

Keywords: Darunavir ethanolate, RP-HPLC, Tablets, Quantification

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INTRODUCTION

Darunavir ethanolate (Fig.1), 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, is a protease enzyme inhibitor [1] used to treat human immunodeficiency virus (HIV) type-1 in adults and children 6 years of age and older [2]. Darunavir ethanolate has forceful interaction with the protease enzyme from many strains of HIV. It blocks HIV protease enzyme which is needed for HIV to multiply.

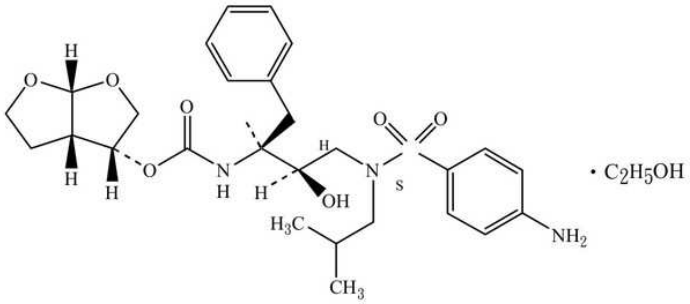

Figure 1: Structure of Darunavir ethanolate

Literature survey revealed that some methods had been developed for determination of Darunavir ethanolate by HPLC [3-5], HPTLC [6] and Spectrophotometric method [7]. The reported HPLC methods require more time for analysis of one sample and less number of samples may be analyzed. Hence, the present work involves the

development of a simple, selective, linear, precise and accurate RP-HPLC method for quantification of Darunavir ethanolate bulk and tablet dosage form.

MATERIALS AND METHODS

Experimental

Chemicals and reagents

Acetonitrile of HPLC grade was purchased from E.Merck (India) Ltd., Mumbai. Orthophosphoric acid and Potassium dihydrogen phosphate of AR grade were obtained from Qualigens Fine Chemicals Ltd., Mumbai. Darunavir ethanolate was a gift sample by Sai Mirra Innopharm Pvt. Ltd., Chennai – 600 098, Tamil Nadu, India. The commercially available tablets containing Darunavir ethanolate were procured from the local market.

Instrumentation and chromatographic conditions

The chromatographic separation was carried out on HPLC system (Shimadzu 1100 Series, Germany) with UV-Visible dual absorbance detector (PDA), Shiseido C8 (250 × 4.6mm; 5µm). The mobile phase consisting of phosphate buffer pH 3 and acetonitrile was filtered through 0.45µ membrane filter before use, degassed and was pumped from the solvent reservoir in the ratio of 40:60 v/v was pumped into the column at a flow rate of 1.0 ml/min. The detection was monitored at 270nm. The volume of injection loop was 20 µl prior to the injection of the drug solution; the column was equilibrated for at least 30 min. with the mobile phase following through the system.

Preparation of Standard solutions

25 mg of Darunavir working standard was weighed and transferred carefully in 50 ml volumetric flask. About 30 ml of mobile phase was added, sonicated to dissolve the drug completely and the volume was made up with mobile phase. 2 ml of above solution was diluted to 100 ml with mobile phase. The resulting solution was mixed and filtered through 0.45 µm filter.

Analysis of Sample Preparation

Twenty tablets containing Darunavir ethanolate were accurately weighed and crushed to fine powder using a glass mortar and pestle. A portion of the powder equivalent to about 100 mg of Darunavir ethanolate was weighed and transferred to 100 ml volumetric flask. 30 ml of mobile phase was then added and sonicated to dissolve the powder completely and the volume was made up with mobile phase. 5 ml of the above stock solution was taken in a 50 ml volumetric flask and diluted up to the mark with mobile phase. Further, 5ml was taken from the above solution and diluted to 50 ml. The final solution was mixed well and filtered through 0.45 µm filter.

Procedure: About 20 µl each of the test and the standard solutions were injected separately into the chromatograph and the chromatograms were recorded and the responses for the major peaks were then measured. The quantity of Darunavir ethanolate equivalent to Darunavir in mg/ tablet was calculated by using the formula:

$$\frac{\text{Test area}}{\text{Std. area}} \times \frac{\text{Std. weight in mg}}{50} \times \frac{2}{100} \times \frac{100}{\text{TW}} \times \frac{50}{5} \times \frac{50}{5} \times \frac{P}{100} \times \text{Average weight in mg}$$

Where, P= Purity of Darunavir working reference standard;
547.66 = Molecular weight of Darunavir; 593.73 = Molecular weight of Darunavir ethanolate

RESULTS AND DISCUSSION

All of the analytical validation parameters for the proposed method were determined according to International Conference on Harmonization (ICH) guidelines [8].

System Suitability

It is essential for the assurance of the quality performance of chromatographic system. Five injections of standard drug solutions were given separately to the system. The system suitability parameters such as retention time, peak area response, number of theoretical plates, tailing factor and their respective mean, standard deviation & %RSD were calculated for the standard drug solutions and mentioned in Table 1. It was observed that all the values are with in the limits.

Table 1: System suitability for Darunavir ethanolate

S.No.	Standard	System suitability parameters			
		Retention time (min)	Area	Number of theoretical plates	Tailing factor
1.	Standard -1	4.313	354232	5423	1.435
2.	Standard -2	4.322	354229	5425	1.439
3.	Standard -3	4.330	354374	5422	1.439
4.	Standard -4	4.333	353981	5405	1.443
5.	Standard -5	4.336	354418	5412	1.440
Mean		**4.327**	**354247**	**5417**	**1.4392**
Standard deviation		**0.0093**	**170.79**	**8.56**	**0.0028**
RSD in %		**0.22**	**0.05**	**0.16**	**0.20**

Specificity

The specificity of the HPLC method is illustrated in Fig. 2, where complete separation of Darunavir ethanolate was noticed in presence of other inactive excipients used in tablet dosage form. In addition, there was no any interference at the retention time of in the chromatogram of placebo solution. In peak purity analysis with PDA, purity angle was always less than purity threshold for the analyte. This shows that the peaks of analyte were pure and excipients in the formulation does not interfere the analyte. The data were presented in the Table 2.

Table 2: Specificity for Darunavir ethanolate

S.No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	354232
4.	Sample	1	354146

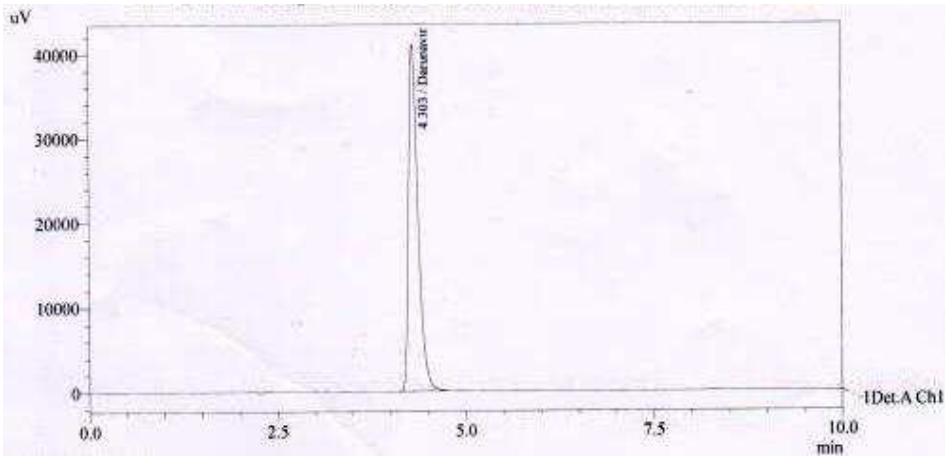

Figure 2 : Typical HPLC Chromatogram of Darunavir ethanolate Tablets

Linearity and Range

The Linearity of this method was determined at five levels from 50%– 150% of operating concentrations for Darunavir ethanolate and it was shown in Table 3. The plot of peak area of each sample against respective concentration of Darunavir ethanolate was found to be linear (Figure 3) in the range of 50%– 150% of operating concentrations such as 5 µg/ml to 15 µg/ml. Beer's law was found to be obeyed over this concentration range. The linearity was evaluated by linear regression analysis using least square method. The regression equations were found to be Y= 34758.34x – 6791.35 for Darunavir ethanolate and correlation coefficient of the standard curve was found to be 0.9997 for Darunavir ethanolate. It observed that correlation coefficient and regression analysis are with in the limits.

Table 3: Linearity of response for Darunavir ethanolate

S.No	Linearity Level (%)	Concentration (µg/ml)	Area
1.	50	5	181482
2.	80	8	280186
3.	100*	10	358571
4.	120	12	424803
5.	150	15	526832

** Operating concentration*

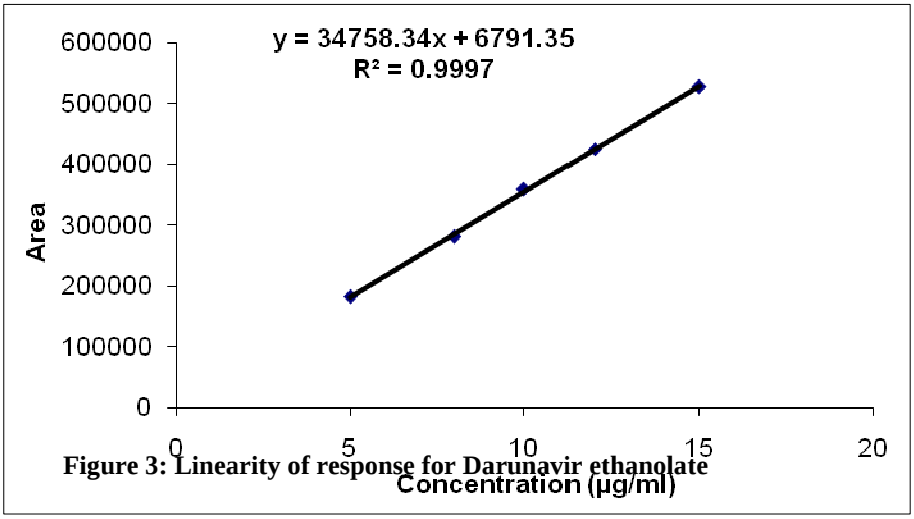

Figure 3: Linearity of response for Darunavir ethanolate

Accuracy

Accuracy of the method was found out by recovery study by standard addition method. The known amounts of standard, Darunavir ethanolate was added to pre-analysed samples at a level from 50% up to 150% and then subjected to the proposed HPLC method individually. The results of recovery studies were shown in Table 4. It was observed that the mean percentage recovery was found to be for Darunavir ethanolate which demonstrated that the method was highly accurate.

Table 4: Accuracy for Darunavir ethanolate

S.No.	Spike Level (%)	Amount added (mg)	Amount recovered (mg)	Recovery (%)
1.	50	50.12	50.18	100.13
2.	50	50.02	50.16	100.28
3.	50	50.12	50.17	100.11
4.	100	98.76	98.66	99.90
5.	100	98.85	98.49	99.63
6.	100	98.66	98.50	99.84
7.	150	146.31	145.03	99.12
8.	150	146.02	145.05	99.34
9.	150	145.23	145.09	99.90
			Mean	**98.23**
			Standard deviation	**41.47**
			RSD in %	**42.22**

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions.

Reproducibility

Examines the precision between laboratories and is often determined in collaborative studies. Reproducibility data for Darunavir ethanolate was shown in Table 5. This indicated that method was highly precise.

Table 5: Precision - Reproducibility for Darunavir ethanolate

S.No.	Sample Name	Amount (µg/ml)	Area	Drug Content (%)
1.	Standard -1	10	354319	100.01
2.	Standard -2	10	354710	99.79
3.	Standard -3	10	355379	100.18
4.	Standard -4	10	355892	100.17
5.	Standard -5	10	356568	100.30
6.	Standard -6	10	356901	100.09
Mean				**100.09**
Standard deviation				**0.1760**
RSD in %				**0.18**

Repeatability

Repeatability is the precision of a method under the same operating conditions over a short period of time. One aspect of this is instrumental precision. A second aspect is sometimes termed intra-assay precision and involves multiple measurements of the same sample by the same analyst under the same conditions. Repeatability data for Darunavir ethanolate was shown in Table 6. This indicated that method was highly precise.

Table 6: Precision - Repeatability for Darunavir ethanolate

S.No.	Sample Name	Amount of preparation (mg)	Area	Drug Content (%)
1.	Sample -1	214.8	358189	99.98
2.	Sample -2	214.3	358404	100.28
3.	Sample -3	215.1	357694	99.71
4.	Sample -4	230.1	357271	100.19
5.	Sample -5	232.4	358182	100.12
6.	Sample -6	232.9	359043	99.99
Mean				**100.04**
Standard deviation				**0.2006**
RSD in %				**0.20**

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), the composition of mobile phase, buffer: acetonitrile (42:58 and 38:62) and the wavelength (268nm and 272nm). The results obtained by changing these conditions were obtained in terms of % RSD values. The values % RSDs were given in Table 7. These values were within acceptance a criterion which indicates that the developed method is robust.

Table 7: Robustness data for Darunavir ethanolate

Condition	Level	RSD in %
A: *Change in flow rate*		
0.9 ml/min	-1	0.15
1.0 ml/min	0	0.20
1.1 ml/min	+1	0.22
B: *Change in composition of mobile phase (buffer: acetonitrile)*		
38.62	-1	0.22
40:60	0	0.18
42.58	+1	0.16
C: *Change in wavelength*		
268nm	-1	0.17
270nm	0	0.22
272nm	+1	0.10

Ruggedness

Six sample preparations were analyzed as per the methodology by a different analyst on a different instrument on a different day. The Ruggedness data Darunavir ethanolate was shown in Table 8. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was rugged.

Table 8: Ruggedness data for Darunavir ethanolate -Change of analyst

S.No.	Sample Name	Wt. taken (mg)	Area	Drug Content (%)
1.	Sample -1	214.8	358375	100.04
2.	Sample -2	214.7	358607	100.15
3.	Sample -3	215.6	362162	100.72
4.	Sample -4	214.8	359025	100.22
5.	Sample -5	214.5	357408	99.90
6.	Sample -6	214.1	356919	99.95
Mean				**100.16**
Standard deviation				**0.2977**
RSD in %				**0.30**

CONCLUSION

The proposed study describes new and simple RP-HPLC method for the quantification of Darunavir ethanolate in tablet dosage form. The method was validated as per ICH guidelines and found to be simple, sensitive, accurate and

precise. Therefore the proposed method can be successfully used for the routine analysis of Darunavir ethanolate in pharmaceutical dosage form without interference.

Acknowledgements
The authors are thankful to the management of Sai Mirra Innopharm Pvt. Ltd., Chennai – 600 098, Tamil Nadu, India for providing the necessary facilities to carry out for the research work.

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