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A Novel RP- HPLC Method with Core Shell Tecnology Column for Assay Analysis of Darunavir Ethanolate in Tablet Dosage Form

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ABSTRACT

High performance liquid Chromatography method was developed and validated for the detection of Darunavir Ethanolate in tablet dosage form. The method was carried out on a Sunshell C18 Column (100 × 4.6 mm id, 2.6µ) maintained at 30ºC. The mobile phase consisted of water-acetonitrile (60 + 40, vol./vol.) pumped at a flow rate 1.0 mL/min. PDA detection was at 265 nm. The chromatography separation was obtained with a retention time of 2.4 min, and the Method was linear in the range of 1-25 mg/mL (r2 = 0.9997). Specificity which also showed that there was no interference of the excipients. The method was validated for linearity, precision, accuracy, robustness, The developed method, after being validated was successively applied to the analysis. of tablet formulations. Hence the method can be used for routing analysis.

Keywords: Darunavir Ethanolate, ICH guidelines, Validation, RP-HPLC, Core shell Technology

I. INTRODUCTION

A novel nonpeptidic human immunodeficiency virus type 1 (HIV-1) protease inhibitor (PI)
Darunavir Ethanolate is [(1S,2R)-3-[[(4-aminophenyl)sulfonyl] (2-methylpropyl)amino]-2-hydroxy-1-(phenylmethyl) propyl]-carbamicacid (3R,3aS,6aR)-hexahydrofuro[2,3-b] furan-3-yl ester monoethanolate [Figure 1].[1]Themolecular weights of darunavir base and Darunavir ethanolate are 547.73 and 593.73g/mL, respectively. DRV (Darunavir Ethanolate) was active against HIV-1 with PI-resistancemutations and against PI-resistant clinicalisolates [2, 3, 4]. This drug is expected to beeffective in patients experienced inantiretroviral treatment, such as those carrying HIV-1 strains which are resistant to more thanone PI[5].

From the literature it is revealed that various analytical methods for the determination of DRV have been reported, which include highperformance liquid chromatography (HPLC) with UV detection (HPLC-UV) to determine DRV in tablet dosage form and in human plasma [5,6];HPTLC method [7];a novel LC–ESI-MS method [8]; RP HPLC–MS method for the simultaneousdetermination of DRV and 11 otherantiretroviral agents in plasma of HIV infected patients [9]; LC-tandem MS assay [10]; andvalidation of plasma DRV concentrations by the HPLC for PIs [11].

The development of assays, using the approach of Short run time testing as determined by the International Conference on Harmonization (ICH) guidelines [12], is highly recommended for the QC of pharmaceutical formulations[13,14]. Darunavir Ethanolate is commercially available but at the moment is not available in any pharmacopoeia the aim of the present research was to develop and validate a simple and fast high performance liquid chromatographic method for the quantitative analysis of DRV in tablet dosage form.

II. METHODS AND MATERIAL

Reagents and Chemicals

Darunavir Ethanolate pure powder as reference standard with 99.96% purity was obtained as a gift sample from Raks Pharma Ltd (Ahmedabad , India). DRV tablets (300.0 mg/tablet) were purchased from the local market. HPLC grade cetonitrile, methanol and water (Finar chemicals Ltd., Ahmedabad, India) and nylon filter (Millipore Pvt. Ltd., Bangalore, India) were used for study.

Apparatus and Chromatographic Conditions

The method was performed on a Agilent 1200 quaternary pump HPLC system with autosampler and inbuilt degasser . Chromanik Technologies Inc, Japan Sunshell C18 Column (100 × 4.6 mm id, 2.6µ); DAD detector and rheodyne injector with 100µL sample loop. The peak areas were integrated automatically by computer using a EZChrom software program. All analyses were done at ambient temperature (30°C) using mobile phase water-acetonitrile (60 + 40, v/v). The flow rate was 1.0 mL/min and the injection volume was 20 µL. DAD detection was performed at 265 nm. All weighing were done on analytical balance (Shimadzu balance Japan).For sonication purpose sonicator (EN 30 US Enertech Fast Clean, Mumbai, India) was used. All solutions were prepared fresh daily.

Preparation of Standard Solution

Accurately weighed amount of standard DRV (10mg) were transferred to a 10 mL volumetric flask, dissolved and diluted to the mark with methanol to obtain a standard stock solution (1000 µg/mL). An aliquot (1.0 mL) was diluted to 100 mL with water to obtain a working standard solution of DRV (10 µg/mL).

Preparation of Sample Solution

To prepare the sample solution, twenty tablets were accurately weighed and crushed to a fine powder. The accurately weighed powder equivalent to 10.0 mg DRV was transferred to 10 mL volumetric flask and methanol (5.0 mL) was added. The solution was sonicated for 15 min. The flask was allowed to stand at room temperature for 5 min and the volume was diluted to the mark with methanol to obtain the sample stock solution (1000 µg/mL). The solution was filtered and suitably diluted with diluents to obtain sample solution of DRV 10 µg/mL.

Method validation

This optimized HPLC method was validated for the parameters listed in the International Conference on Harmonization (ICH Q2 (R1)) guidelines. [15]

Linearity and range

Five different concentration levels for DRV were prepared in the range of 1-30 µg/mL. 20 µL of each solution was injected duplicate into the HPLC system (n = 2) and mean values of peak areas were plotted against concentrations. The curves were constructed by linear regression with least squares method. The linearity of the proposed method was evaluated by calculating the r2, slope and intercept values of the calibration curve.

Sr. No.	Level	Concentration of Darunavir Ethanolate µg/ mL	Peak Area-1	Peak Area-2	Mean
1	10%	1	94352	96452	95402
2	50%	5	639982	625641	632811.5
3	100 %	10	1236511	1238434	1237473
4	120 %	20	1870996	1861098	1866047
5	130 %	30	2455987	2456087	2456037
Slope					595451
Intercept					-528798
Correlation Coefficient					0.9993

System Precision

The precision of proposed method was evaluated by the repeatability was checked by repeatedly injecting (n=5) solutions of DRV (10 µg/mL).The precision was expressed as RSD of the responses in each case.

Sr.No.	Injection No.	Area of Darunavir Ethanolate	Acceptance Criteria
1	Injection - 1	1236742	The %RSD of peak area of
2	Injection - 2	1235342	
3	Injection -	1240981	

	3		Darunavir Ethanolate should not be more than 2.0
4	Injection - 4	1240751	
5	Injection - 5	1246261	
6	Mean	1240015	
7	SD	4273.079	
8	%RSD	0.34	

Intermediate Precision

The intermediate precision study was carried out using different columns, different reagents using different HPLC systems from the same tablet of Darunavir Ethanolte and the peak area of Darunavir Ethanolate was calculated. The %RSD of the peak areas of five preparations in intermediate precision

Sr.No.	Injection No.	Area of Darunavir Ethanolate	Acceptance Criteria
1	Injection - 1	1234523	The %RSD of peak area of Darunavir Ethanolate should not be more than 2.0
2	Injection - 2	1245624	
3	Injection - 3	1237981	
4	Injection - 4	1234671	
5	Injection - 5	1256254	
6	Mean	1241811	
7	SD	9245.305	
8	%RSD	0.74	

Accuracy

To evaluate the accuracy of the proposed method, recovery tests were carried out. Recovery tests were performed by adding known amounts of standard solutions to samples, followed by analysis using the proposed method. The study was done at three different concentration levels (50%, 100%,and 150%). The accuracy was calculated as the percentage of the drug recovered from the formulation.

% Concentration	Mean Peak area	Amount of Darunavir	% Recovery	Mean	Acceptance Criteria
(At Specification Level)		Ethanolate Spiked in (µg/mL)			
50 %	621653	5.1	98.5	100.1	Between 98.0 to 102.0
100 %	1240971	10.2	100.1		
150 %	1871467	15.6	101.6		

Robustness

Flow rate of the mobile phase and composition of the mobile phase were varied slightly to conduct robustness study for the method. Samples of Darunavir Ethanolate at 10 µg/mL concentration were analyzed under these changed experimental conditions. No marked changes in chromatograms were observed, which indicated that the developed method was robust in nature. depicts the results of robustness study.

Condition	% Assay
Flow rate with 1.05 mL/min.	99.2 %
Flow rate with 0.95 mL/min.	100.7%
Mobile phase ratio 62:38	101.4%
Mobile phase ratio 58:42	99.9%

III. CONCLUSION

The proposed stability-indicating RP-HPLC method was simple, fast, specific, sensitive, accurate and precise and can be used for analysis of Darunavir Ethanolate in bulk samples and its tablet dosage forms.

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