Original Research Article

DEVELOPMENT & VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF DORAVIRINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

ABSTRACT

A simple, accurate, rapid and precise isocratic reverse-phase high-performance liquid chromatographic method has been developed and validated for determination of Doravirine in tablets. The chromatographic separation was carried out on Dionex C_{18} (250 x 4.6mm, 5µ) with a mixture of methanol: potassium di hydrogen phosphate (40:60%v/v) as a mobile phase at a flow rate of 1.5 mL/min. UV detection was performed at 306 nm. The retention time was 5.24 min for Doravirine. Calibration plot was linear (r^2=0.999) over the concentration range of 200-600 µg/mL. The method was validated for accuracy, precision, specificity, linearity, robustness, LOD and LOQ. The proposed method was successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that method is specific, rapid, reliable, and reproducible. The high recovery and low relative standard deviation confirm the suitability of the method for routine determination of Doravirine in bulk and tablet dosage form.

Keywords: Doravirine, RP-HPLC, Tablets.

INTRODUCTION

Doravirine is an HIV-1 non-nucleoside reverse transcriptase inhibitor (NNRTI) intended to be administered in combination with other antiretroviral medicines. Chemically it is 3-chloro-5-({1-[(5-hydroxy-4-methyl-4H-1,2,4-triazol-3-yl) methyl]-2-oxo-4-(trifluoromethyl)-1,2dihydro pyridin-3-yl} oxy) benzonitrile[1-6].

Doravirine is subsequently available by itself or as a combination product of doravirine (100 mg), lamivudine (300 mg), and tenofovir disoproxil fumarate (300 mg). Doravirine is formally indicated for the treatment of HIV-1 infection in adult patients with no prior anti retroviral treatment experience, further expanding the possibility and choice of therapeutic treatments available for managing HIV-1 infection or AIDS. Doravirine should be kept in a well closed container, protected from light.

Literature survey[7-9] reveals that few spectrophotometric and chromatographic methods were reported for estimation of Doravirine in single and combination with other drugs. In this study, an attempt has been made to develop an accurate, rapid and reproducible reverse phase HPLC method for determination of Doravirine in tablet dosage form and validated[10-12] in accordance with International Conference on Harmonization (ICH) guidelines. No reference

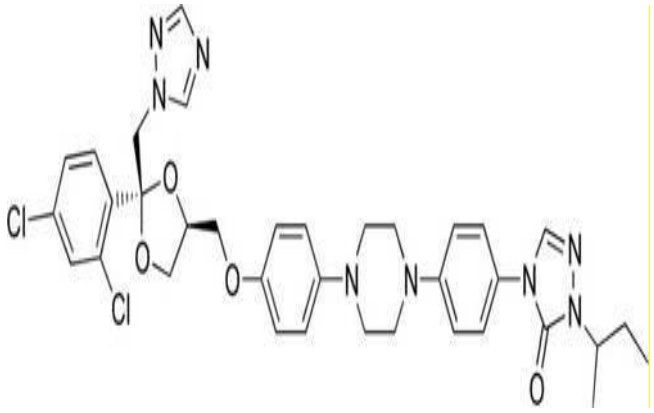

Fig. 1: Molecular structure of Doravirine

MATERIALS AND METHODS

Chemicals and reagents

The reference sample of Doravirine (API) was obtained from RA Chem labs, Hyderabad. The branded formulation PIFELTRO was procured from the market. Tablet claimed to contain 0.1% Doravirine have been utilized in the present work. All chemicals and reagents used were HPLC grade and purchased from Merck chemicals, India.

Chromatographic conditions

Separation was performed on an isocratic waters HPLC 2965 system instrument equipped with a binary pump and variable wavelength PDA detector with auto injector. Data was analyzed by using Empower2 software. Degassing of the mobile phase was done by using bath sonicator. A Shimadzu balance was used for weighing the materials. The separation was achieved on a Dionex C_{18} (250 x 4.6 mm, 5µ) analytical column. The mobile phase consisted of potassium dihydrogen phosphate buffer: methanol (60:40%v/v). The flow rate was 1.5 mL/min and UV detection was performed at 306 nm. The mobile phase was shaken on an ultrasonic bath for 30 min. The resulting transparent mobile phase was filtered through a 0.45 µ membrane filter (Millipore, Ireland). The injection volume was 10 µL and all the experiments were performed at ambient temperature.

Preparation of Standard stock solution:

Accurately weighed and transferred 100mg of Doravirine pure drug into 100ml clean & dry volumetric flask. 3/4th volume of diluent was added to the flask and sonicated for 30 minutes. Flask was made up with diluent and filtered through 0.45 µ Millipore PVDF filter and labeled as standard stock solution.

Preparation of Standard working solution (100% solution)

4ml from the standard stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. The resulting chromatogram is shown in Fig 2.

Preparation of Sample solution:

20 tablets were weighed and the average weight of the tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluent was added and sonicated for 30 min, further the volume was made up with diluent and filtered by HPLC filter. It was further diluted to within the calibration range. All the determinations were performed six times to ensure repeatability of the method.

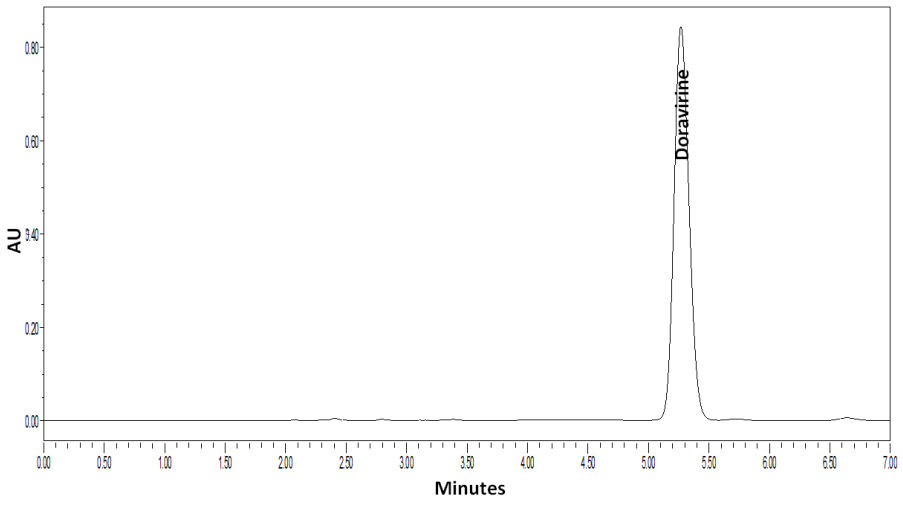

Fig 2. Standard chromatogram of Doravirine

Method validation:

The developed method was validated according to ICH guidelines. The system suitability was evaluated by five replicate analysis of Doravirine by injecting blank, standard and sample solutions and ensures that there is no interference with the main peak.

Table 1: System suitability parameters of proposed method

Parameters	Doravirine	Acceptance Criteria
Retention time (min)	5.278	-
No. of theoretical plates	8609	NLT 2000
Tailing factor	1.138	NMT 2.0
Resolution	-	NLT 2.0

Linearity

Different linearity levels were prepared and injected into the HPLC system keeping the injection volume constant. Standard calibration curve was plotted against the concentration ranging from 200-600 µg/mL for Doravirine through which slope, intercept and the correlation coefficient were determined.

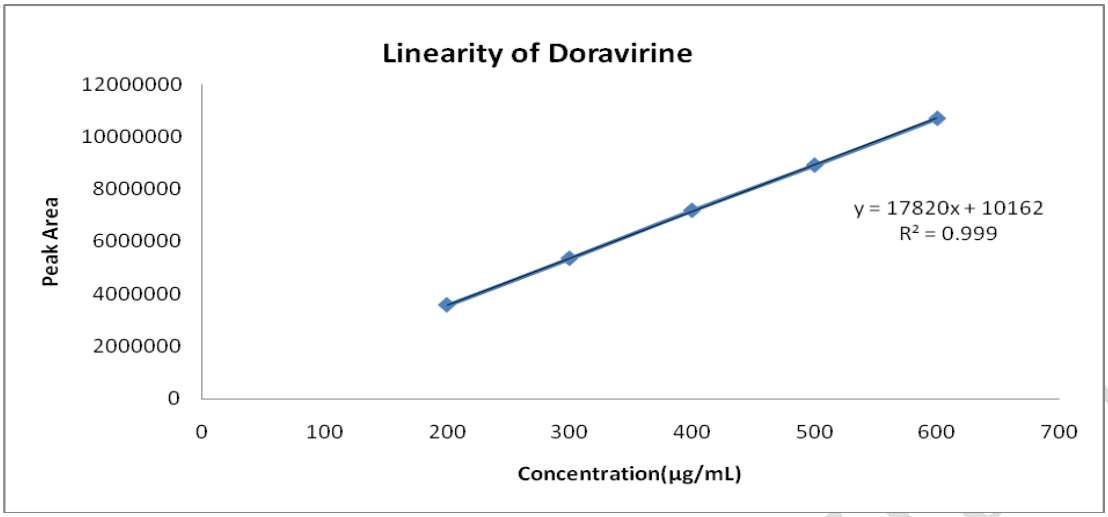

Fig 3. Calibration curve of Doravirine

Precision:

Precision of assay was determined by System and Method Precision. Every sample was injected six times. The repeatability of sample application and measurements for peak area were expressed in terms of %RSD.

Table 2: Precision data of proposed method

S. No	System Precision	Method Precision
1.	7139718	7126360
2.	7141270	7148338
3.	7180014	7173736
4.	7189738	7137998
5.	7199631	7163444
6.	-	7140485
Mean	7170074	7148393
Std. dev	27885	17463
%RSD	0.4	0.2

ACCURACY:

Accuracy was performed by following standard addition method. In this standard was added to pre-analyzed sample solution at three different concentrations.

Table 3: Accuracy data for proposed method

Drug	Accuracy	Peak area	% Recovery	Mean % recovery	Overall mean % recovery
Doravirine	50 %	3578856	100	Mean=99.33 SD=0.577 % RSD=0.58	Mean=99.33 SD=0.577 % RSD=0.58
	50 %	3547046	99		
	50 %	3573014	99		
	100 %	7114998	99	Mean=99.66 SD=0.577 % RSD=0.58	
	100 %	7165041	100		
	100 %	7171581	100		
	150 %	10680557	99	Mean=99.33 SD=0.577 % RSD=0.58	
	150 %	10681963	99		
	150 %	10683103	100		

Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) were estimated from signal-to-noise ratio. LOD and LOQ were calculated using 3.3 σ/s and 10 σ/s formulae, respectively. Where, σ is the standard deviation of the peak areas and S is the slope of the corresponding calibration curve. The LOD and LOQ of Doravirine was found to be 1.85 µg/ml and 6.19 µg/ml, respectively.

Robustness

In order to demonstrate the robustness of the method, a few parameters were deliberately varied. The parameters included are variation of flow rate and Detection Wavelength.

Table 4: Robustness for flow rate variation of Doravirine

S.No	Robustness Conditions	% RSD of Peak Area
1	Flow rate- 1.4ml/min	0.9
2	Flow rate-1.6 ml/min	0.9
3	Temperature-43°c	0.8
4	Temperature-47°c	0.6

ASSAY

Standard and sample solution injected as described under experimental work. The corresponding chromatograms and results are shown below. Results obtained are tabulated in table 5.

Table 5: Analysis of marketed formulation by proposed method

Brand Name	Drug	Amount found*	% Assay*
PIFELTRO	Doravirine	9.95mg	99.50

***Avarage of six determinations**

RESULTS AND DISCUSSION

During the optimization of HPLC method, C-18 analytical column (4.6×250 mm; 5 µm) organic solvent (methanol), one buffer (phosphate) was tested. Initially Water: Acetonitrile and Phosphate buffer were tried in different ratios. Finally, mobile phase consisting of mixture of Methanol: Potassium phosphate buffer in ratio 40:60% v/v was selected as mobile phase to achieve clear separation and sensitivity. A flow rate of 1.5 mL/min gave an optimum signal to

noise ratio with reasonable separation time using a C_{18} Dionex column (4.6×250 mm; 5 µm), the retention time for Doravirine was observed at 5.27 min. Total run time was less than 7 min. The chromatogram at 306 nm showed a complete resolution for all peaks (Fig.1). Validity of the analytical procedure as well as the resolution between different peaks of interest is ensured by the system suitability tests and the results are tabulated in Table 1. All critical parameters tested meet the acceptance criteria on all days. Linearity was obtained for Doravirine in the range of 200-600 µg/mL. The correlation coefficient (r^2) was found to be greater than 0.999 in all instances (Fig.2). As can be seen in Table 2 the %RSD values were less than 2 for system & method precision. Hence, the method was found to be more precise.

The proposed method afforded high recoveries for Doravirine in dosage form. Results obtained from recovery studies presented in Table 3 indicated that the assay procedure can be used for routine quality control analysis of Doravirine in sample.

LOD and LOQ were found to be 1.85µg/mL and 6.19µg/mL for Doravirine. In all deliberately varied conditions, the %RSD for replicate injections of Doravirine was found to be within the acceptable limit. The tailing factor was found to be less than 1.5 and the results are shown in Table 4. The validate method was used in analysis of marketed tablet dosage form. The results for the drugs assay showed good agreement with label claims and the results are shown in Table 5.

CONCLUSION

The developed stability indicating RP-HPLC method is simple, specific, accurate and precise for the determination of Doravirine in dosage form. It was successfully validated in terms of system

suitability, linearity, precision, accuracy, specificity, LOD, LOQ and robustness in accordance with ICH guidelines. Thus, the described method is suitable for routine analysis and quality control of pharmaceutical preparations.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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