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Research Article

Stability Indicating Rp-Hplc Method for Estimation of Doravirine in Tablet Dosage Form

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ABSTRACT

The estimation of Doravirine was done by RP-HPLC. The assay of Doravirine was performed with tablets and the % assay was found to be 100.50 which show that the method is useful for routine analysis. The linearity of Doravirine was found to be linear with a correlation coefficient of 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.6 for Doravirine which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 1.0 for Doravirine which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 98.0% - 102.0%. The total recovery was found to be 100.02% for Doravirine. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ are 3 and 10. The LOD and LOQ for Doravirine was found to be 2.98 and 9.97. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions. The acceptance criteria for degradation studies are less than 15%. The degradation results are within the limit. Thus the proposed method was found to be accurate, precise, reproducible and specific and can be used for estimation of doravirine in tablet dosage form.

Keywords: Stock solution; Doravirine; Sulphonic acid buffer; Rheodyne injector

INTRODUCTION

Doravirine has been used in trials studying the treatment of HIV-1, HIV-1 Infection, Renal Impairment, and Human Immunodeficiency Virus (HIV) Infection. In particular, doravirine is an HIV-1 Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) intended to be administered in combination with other antiretroviral medicines. Doravirine is subsequently available by itself or as a combination product of doravirine (100 mg), lamivudine (300 mg), and tenofovir disoproxil fumarate (300 mg) [1].

Doravirine is formally indicated for the treatment of HIV-1 infection in adult patients with no prior antiretroviral treatment experience, further expanding the possibility and choice of therapeutic treatments available for managing HIV-1 infection or AIDS. Its Systematic IUPAC name is 3-chloro-5-({1-[(5-hydroxy-4-methyl-4H-1,2,4-triazol-3-yl)methyl]-2-oxo-4-(trifluoromethyl)-1,2-dihydropyridin-3-yl}oxy)benzonitrile.its molecular formula is C17H11ClF3N5O3. Its molecular weight is 425.749 g/mol. The present study is to develop a new, simple, precise and accurate method for its analysis in formulation after a detailed study a new RP-HPLC method was decided to be developed and validated.

MATERIALS AND METHODS

A gradient HPLC system (waters) consisting of LC10AT liquid pump, Rheodyne injector (2E, 7725; 20 µl-loop), 2487 UV/Vis detector, Xterra C18(150 mm × 4.6 mm, 5 µm)column, 20 µl Hamilton injecting syringe and empower software, 2695 separation module was used. Pure drug samples of Doravirine were procured Pharm train Ltd. The solvents Octane sulphonic acid and Water and Methanol for HPLC used in the investigation were of HPLC grade (FINAR chemical LTD). Acetonitrile for HPLC Standard solutions Ltd) was used. Hydrochloric acid, sodium hydroxide, (Merck) are also used.

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The HPLC method was developed, first by Wave length selection. UV spectrum of 10 µg/ml Doravirine in diluents (mobile phase composition) was recorded by scanning in the range of 200 nm to 400 nm. From the UV spectrum wavelength selected as 260. At this wavelength both the drugs show good absorbance. Then optimized chromatographic conditions like, High performance liquid chromatography equipped with, Auto Sampler and DAD or UV detector ,with ambient temperature maintenance, using Xterra C18(150 mm × 4.6 mm, 5 µm) Column and 0.1% Octane Sulphonic acid Buffer 0.1% Octane Sulphonic acid: Acetonitrile (40:60) used as a mobile phase with 1.0 ml per min flow rate at 260 nm wavelength. Its runtime done for 10 min using 20 µl injection volume (Figure 1).

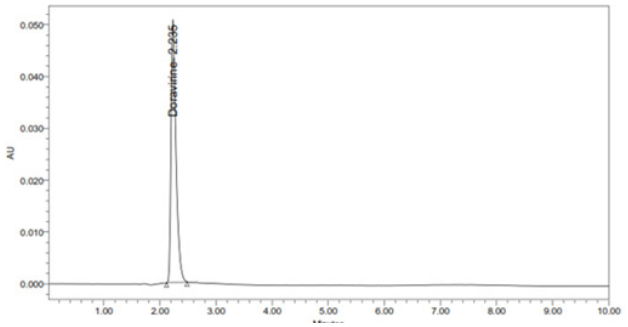

Figure 1: Optimized chromatographic conditions, peak was good, plate count is less.

Accurately weighed 1 gram of Octa sulphonic acid was taken in a 1000 ml volumetric flask, dissolved and diluted to 1000 ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid Mix a mixture of above buffer 400 ml (40%) and 600 ml Methanol HPLC (60%) and degas in ultrasonic water bath for 5 minutes. Filter through 045 µ filter under vacuum filtration [2].

Assay was performed by using standard solution prepared by, accurately weigh and transfer 25 mg of Doravirine working standard into a 25 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Further pipette 0.3 ml of Doravirine of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with Diluents.

Sample solution was prepared by, accurately weigh and transfer equivalent to 25 mg of Doravirine equivalent weight of the sample(synthetic mixture) into a 25 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution) [3-5].

Further pipette 0.3 ml of Doravirine of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with Diluents.

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for the Doravirine peaks and calculate the % Assay by using the formulae (Figures 2 and 3).

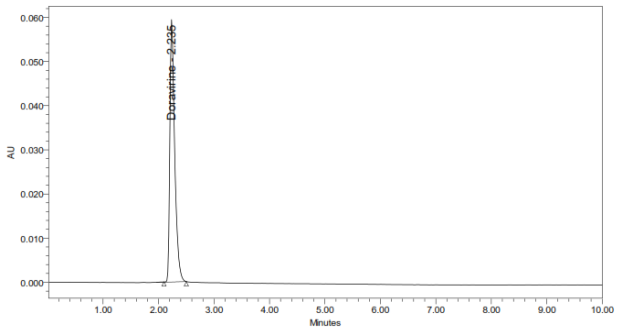

Figure 2: Chromatogram for standard.

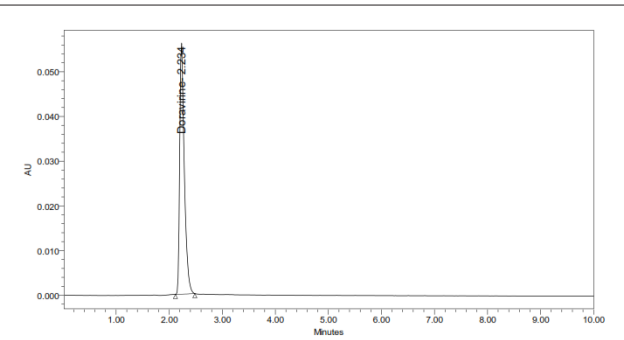

Figure 3: Chromatogram for sample.

RESULTS AND DISCUSSION

The above assay method is validated by using validation parameters like linearity, found by preparing stock solution and its different dilutions. Accurately weigh and transfer 25 mg of Doravirine working standard into a 25 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). 0.1 ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with Diluents to prepare Level–I(10 ppm of Doravirine.) 0.2 ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with Diluents to prepare Level–II(20 ppm of Doravirine) 0.3 ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with Diluents to prepare Level–III (30 ppm of Doravirine) 0.4 ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with Diluents to prepare Level–IV 40 ppm of Doravirine 0.5 ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with Diluents to prepare Level–V 50 ppm of Doravirine. Then inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient (Figure 4, Tables 1 and 2).

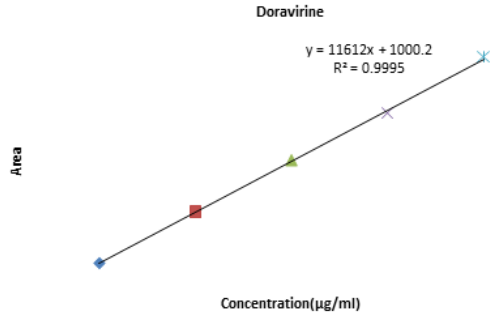

Figure 4: Calibration graph for Doravirine.

Table 1: Area of different concentration of Doravirine.

S. No	Doravirine	
	Concentration (µg/ml)	Area
1	10	117116
2	20	234231
3	30	351347
4	40	458463
5	50	585578

Table 2: Analytical performance parameters of Doravirine.

Parameters	Doravirine
Slope (m)	11612
Intercept (c)	1000
Correlation coefficient (R2)	0.999

Precision validation parameter is done by preparing stock Solution. Accurately weigh and transfer 25 mg of Doravirine working standard into a 25 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent(Stock solution). Further pipette 0.3 ml of Doravirine the above stock solution into a 10 ml volumetric flask and dilute up to the mark with Diluents. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day within the laboratory. The stock solution is prepared by accurately weigh and transfer 25 mg of Doravirine working standard into a 25 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent(Stock Solution). Further pipette 0.3 ml of Doravirine the above stock solution into a 10 ml volumetric flask and dilute up to the mark with Diluents. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits (Table 3).

Table 3: Results of precision for Doravirine.

Injection	Area
Injection-1	347358
Injection-2	345898
Injection-3	349624
Injection-4	351347
Injection-5	345567
Injection-6	349045
Average	348139.8
Standard deviation	2261.2
%RSD	0.6

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same. The Standard stock solution was prepared by, accurately weigh and transfer 25 mg of Doravirine working standard into a 25 ml clean dry volumetric flask add diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Further pipette 0.3 ml of Doravirine the above stock solution into a 10 ml volumetric flask and dilute up to the mark with Diluents [6-8].

The Sample solutions are prepared by, accurately weigh and transfer 12.5 mg of Doravirine equivalent weight of synthetic mixture into a 25 ml clean dry volumetric flask add and sonicate to dissolve it completely and make volume up to the mark with the same solvent gives for preparation of 50% solution (With respect to target Assay concentration). Further pipette 0.3 ml of Doravirine the above stock solution into a 10 ml volumetric flask and dilute up to the mark with Diluents. Accurately weigh and transfer 25 mg of Doravirine equivalent weight of synthetic mixture into a 25 ml clean dry volumetric flask add and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3 ml of Doravirine the above stock solution into a 10 ml volumetric flask and dilute up to the mark with Diluents for preparation of 100% solution (With respect to target Assay concentration). Accurately weigh and transfer 37.5 mg of Doravirine equivalent weight of synthetic mixture into a 25 ml clean dry volumetric flask add and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution) for preparation of 150% solution (With respect to target Assay concentration). Further pipette 0.3 ml of Doravirine the above stock solution into a 10 ml volumetric flask and dilute up to the mark with Diluents Now Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Doravirine and calculate the individual recovery and mean recovery values (Table 4).

Table 4: Accuracy (recovery) data for Doravirine.

%Concentration (at specification level)	Area*	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	174573	12.5	12.6	100.49	100.02
100%	347420	25	25.00	99.99	
150%	518990	37.5	37.34	99.58	

Note: * Results for actual flow (1.0ml/min) have been considered from assay standard.

To find limit of detection, Accurately weigh and transfer 25 mg of Doravirine working standard into a 25 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3 ml Doravirine the above stock solution into a 10 ml volumetric flask and dilute up to the mark with Diluent. Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with Diluent. Further pipette 0.34 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with Diluent (Table 5).

Table 5: Results of LOD.

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Doravirine	64	191	2.98

To find limit of quantification, accurately weigh and transfer 25 mg of Doravirine working standard into a 25 ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3 ml Doravirine the above stock solution into a 10 ml volumetric flask and dilute up to the mark with Diluent. Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with Diluent. Further pipette 1.1 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with Diluent (Table 6).

Table 6: Results of LOQ.

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Doravirine	64	638	9.97

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.9 ml/min to 1.1 ml/min. Standard solution 30 µg/ml of Doravirine prepared and analysed using the varied flow rates along with method flow rate. The Organic composition in the Mobile phase was varied from 54% to 66%. Standard solution 30 µg/ml of Doravirine was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method (Tables 7-9).

Table 7: Results for variation in flow for Doravirine.

S. No	Flow rate (ml/min)	System suitability results	
		USP plate count	USP tailing
1	0.9	3639.37	1.55
2	1.0	3248.37	1.53
3	1.1	3386.38	1.54

Table 8: Results for variation in mobile phase composition for Doravirine.

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP plate count	USP tailing
1	10% less	3674.67	1.55
2	*Actual	3248.37	1.53
3	10% more	3465.33	1.53

Table 9: Degradation results for Doravirine.

Sample name	Doravirine	
	Area	% Degraded
Standard	346387	
Acid	316528	8.62
Base	338212	2.36
Peroxide	324461	6.33
Thermal	340602	1.67
Photo	334402	3.46

Degradation Studies are done as per International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Doravirinee using the proposed method. Accurately weigh and transfer 25 mg of Doravirine equivalent weight of tablet powder into a 25 ml clean dry volumetric flask add and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution) Hydrolytic degradation under acidic condition was done by, Pipette 0.3 ml of above solution into a 10 ml volumetric flask and 3 ml of 0.1 NHCl was added. Then, the volumetric flask was kept at 60°C for 6 hours and then neutralized with 0.1 N NaOH and make up to 10 ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials. Hydrolytic degradation under alkaline condition was done by, Pipette 0.3 ml of above solution into a 10 ml volumetric flask into a 10 ml volumetric flask and add 3 ml of 0.1 N NaOH was added in 10 ml of volumetric flask. Then, the volumetric flask was kept at 60°C for 6 hours and then neutralized with 0.1 NHCl and make up to 10 ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials. Oxidative degradation was done by, Pipette 0.3 ml above stock solution into a 10 ml volumetric

flask solution into a 10 ml volumetric flask 1 ml of 3% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent [9,10].

CONCLUSION

The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials. Thermal induced degradation was done by; Doravirine sample was taken in Petri dish and kept in Hot air oven at 110°C for 24 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed. Photo degradation was done by, Pipette 0.3 ml above stock solution into a 10 ml volumetric flask and expose to sunlight for 24 hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45 micron.

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